Volume 8, Number 3, (Cumulative No.26) Part 3 September 25, 2011 ISSN:1097-8135

Life Science Journal

Marsland Press

PO Box 180432, Richmond Hill, New York 11418, USA

Website:
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

Phone: (347) 321-7172

Life Science Journal 2011 Volume 8, Number 3, Part 3 ISSN:1097-8135

Volume 8, Number 3, Part 3 September 25, 2011 ISSN:1097-8135

Life Science Journal

ISSN 1097-8135

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Websites:
http://www.lifesciencesite.com
http://www.sciencepub.net

Emails:
lifesciencej@gmail.com
editor@sciencepub.net

Volume 8, Number 3, Part 3 September 25, 2011 ISSN:1097-8135

Life Science Journal

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Websites:
http://www.lifesciencesite.com
http://www.sciencepub.net

Emails:
lifesciencej@gmail.com
editor@sciencepub.net

ISSN: 1097-8135

Life Science Journal

Acta Zhengzhou University Oversea Version

(*Life Sci J*)

Life Science Journal, the Acta Zhengzhou University Oversea Version, is an international journal with the purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. The journal is calling for papers from all who are associated with Zhengzhou University-home and abroad. Any valuable papers or reports that are related to life science - in their broadest sense - are welcome. Other academic articles that are less relevant but are of high quality will also be considered and published. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings. Let's work together to disseminate our research results and our opinions.

Introductions to Authors

1. General Information

(1) Goals: As an international journal published both in print and on internet, **Life Science Journal** is dedicated to the dissemination of fundamental knowledge in all areas of nature and science. The main purpose of **Life Science Journal** is to enhance our knowledge spreading in the world under the free publication principle. It publishes full-length papers (original contributions), reviews, rapid communications, and any debates and opinions in all the fields of nature and science.

(2) What to Do: The **Life Science Journal** provides a place for discussion of scientific news, research, theory, philosophy, profession and technology - that will drive scientific progress. Research reports and regular manuscripts that contain new and significant information of general interest are welcome.

(3) Who: All people are welcome to submit manuscripts in life science fields. Papers of other fields are also considered.

(4) Copyright and Responsibility of Authors to their Articles: When the manuscript(s) is submitted to the journal, the authors agree the following: All the authors have participated sufficiently in this work; The article is not published elsewhere; Authors are responsibility on the contents of the article; The journal and author(s) have same right for the copyright of the article and either of the journal or author(s) can use it by anyway without noting the other party.

(5) Publication Costs: US$500 per article to defray costs of the publication will be paid by the authors when it is received. Extra expense for color reproduction of figures will be paid by authors (estimate of cost will be provided by the publisher for the author's approval).

(6) Advertisements: The price will be calculated as US$400/page, i.e. US$200/a half page, US$100/a quarter page, etc. Any size of the advertisement is welcome.

2. Manuscript Preparation

Each manuscript is suggested to include the following components but authors can do their own ways:

(1) Title: including the complete article title; each author's full name; institution(s) with which each author is affiliated, with city, state/province, zip code, and country; and the name, complete mailing address, telephone number, facsimile number (if available), and at least one email address for author(s). **(2) Abstract**: including Background, Materials and Methods, Results, and Discussions. **(3) Key Words**. **(4) Introduction**. **(5) Materials and Methods**. **(6) Results**. **(7) Discussions**. (8) **Acknowledgments**. **(9) References**.

3. Manuscripts Submission

(1) Submission Methods: Submission through email (editor@sciencepub.net) is encouraged.

(2) Software: The Microsoft Word file will be preferred.

(3) Font: Normal, Times New Roman, 10 pt, single space.

(4) Indent: Type 2 space in the beginning of each new paragraph.

(5) Manuscript: Don't use "Footnote" or "Header and Footer".

(6) Email: At least one author's email must be put under title.

(7) Title: Use Title Case in the title and subtitles, e.g. "**Debt and Agency Costs".**

(8) Figures and Tables: Use full word of figure and table, e.g. **"Figure 1.** Annul Income of Different Groups**", Table 1. Annual Increase of Investment".**

(9) References: Cite references by "last name, year", e.g. "(Smith, 2003)". References should include all the authors' last names and initials, title, journal, year, volume, issue, and pages etc.

Reference Examples:

Journal Article: Hacker J, Hentschel U, Dobrindt U. Prokaryotic chromosomes and disease. Science 2003;301(34):790-3. **Book**: Berkowitz BA, Katzung BG. Basic and clinical evaluation of new drugs. In: Katzung BG, ed. Basic and clinical pharmacology. Appleton & Lance Publisher. Norwalk, Connecticut, USA. 1995:60-9.

(10) Submission Address: editor@sciencepub.net, Marsland Press, PO Box 180432, Richmond Hill, New York 11418, USA, 347-321-7172.

Marsland Press / Zhengzhou University
PO Box 180432, Richmond Hill, New York 11418, USA
http://www.lifesciencesite.com; http://www.sciencepub.net
lifesciencej@gmail.com; editor@sciencepub.net

CONTENTS

Symbol of lotus in ancient world

Pedram Rezania

PhD Student, Department of Cultures and languages of ancient Iran, Science and Research branch, Islamic Azad University, Tehran, Iran. pedramrezania@yahoo.com

Abstract: This research has been performed in purpose of definition and explanation of Total quality management advanced model and investigating its role in organizations' strategic evolution. This article is in type of developmental researches and its performance method is survey and library studies. Moreover information collecting tool is taking notes. In literature of subject of this article initially the principles of total quality management has been discussed, so that we can obtain better perception in order to applying this system in business and improvement of organizations' performance. In order to apply total quality management in organizations strategically, we have examined TQM movement in Japan and some cases about using of this method have been mentioned. In fact, in this article the model which has been named Total Quality Management Advanced Model (TQMEX) is based on TQM facts and has been modeled according to instructions of this model. A referendum which has been done in Japan, Hong Kong and South Korea indicates the importance of Japanese system of total quality management advanced model in order to implement total quality management system in organizations. Finally, results and information obtained from this research provide criteria and methods for which are the result of the large organizations' experiences that not only have passed two world's oil crisis and Asian financial crisis successfully, but also have continued their growth while these two crises.
[Pedram Rezania. **Symbol of lotus in ancient world**. Life Science Journal. 2011;8(3):309-312] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Mythos of lotus, ancient world, symbol of lotus

1. Introduction

Myths have formed very most important part of believes and routine life of ancient people. Since the plant has had very important role in human life and also now it has, some of myths have formed around plants and gradually enjoyed of high strength. These mythos believes could be traced through symbols in art of ancient nations. Symbolism is a tool of old knowledge and the eldest and most fundamental means for explaining conceptions. Symbolism has placed during the times and centuries and in thoughts and dreams of different races. Symbol, motivates thought and leads human to spread thought without speech. Symbol operates in art in different levels and according to believes and social customs which induces artist. In another level, there are thoughts that are related to worship. In this way, artist by giving substance and identification to existents which their form is unknown, gives a symbolic imagine (Hall, 2008, preface). These symbols mostly are formed in one cause and are transferred to another nation and during this transferring are evolved and / or partly are caused a little change in nature. Investigation of this trend of transfer and changes can give us worthy information about art and architect.

2. Investigation and Analyzing

Lotus with 12 rose leaves in square form is portrayed in ancient designs and specially, in Achemenian art. We know that 12 has been a holy figure in ancient world and indicates full round and in a type is perfection and may be for this reason the figure 13 had been unlucky, because caused exit of this evolution. Lotus means spiritual bloom because its roots is in lack mud and although grow up in the direction of sky.

Figure 1. It goes out of dark waters but its flowers grow under sun and sky light. Lotus was also the beauty transcendency. Roots of lotus are the symbol of eternity and its peduncle is the symbol of the umbilical cord, which connected human to his origin and its flower symbolizes sun lights.

Ra', sun-god and the creator of the ancient Egypt is represented in the form of a child who sleeps on the Lotus or his head goes out of the lotus. Nefertum, the god relating to Ra', has a crown of the lotus on his head with two feathers on that. Hathur, Egyptian sky-goddess, in one of her faces, which was related to Ra', had sometimes a lotus in hand. Capitals of Egyptian temples, were decorated so that

Lotus, on them sometimes were curved in the form of bud and sometimes open and the form of bell. (Hall, 2008, 310).

Lotus in ancient East is as important as Rose in the West. In the 8th BC the image of lotus is transferred from Egypt into Phoenicians and then to Mesopotamia and Iran. The Phoenicians goddess have lotus in hands as the symbol of their creation power. This plant was worshipped in ancient Egypt and in many parts of Asia. The holiness of Lotus goes back to its aquatic environment because the water was the ancient symbol of an old ocean, which the world has created from that and the floating lotus on that was the symbol of uterus. Since Lotus is opened at the rising of the sun and closed at sunset, seems to be like the sun. In the art of ancient Egypt lotus is the symbol of north Egypt.

In a relief of pillar column bellow of huge statutes of Rameses II, in front of big temple of Abosimbel in 1200 to 1300 BC, two gods of Nile are seen who tie ropes of Lotus and Papyrous as symbol of union of north and south of Egypt. The god of the left side is as north Egypt and has a crown of Lotus on head (View, 2005, 129). In Amun's temple located in Karnak, there are columns decorated by Lotus and Papyrous. In a scene of the dead book, Oziris has sat on a bed that a lotus containing four Horous, is grown of that bed (the same 2005, 147).

According to Georg Hart "it seems that priests of Heliopolis used the allegory of lotus to make clear the birth of Amon, the sun-god. From one of masculine gods in Egyptian creation fable grew a lotus and the sun-god, who was yet developing himself, came out like a baby. Lotus was recognized later as the god Nephertum and worshiped in Memphis, one of the important cities of ancient Egypt. Therefore there are some incantations in the book of the dead which transferred the dead to Nephertum because he is lotus in the nose of sun. In Cairo museum the most beautiful image of this concept in a wooden painting lotus can be found, in which the head of sun-god baby comes out (Hart, 2005, 14).

In Assyrian-Phoenician and in Greek-Roman art Lotus means burial and funeral ceremony and indicates death and second born, resurgence and eternity life and new-born forces of the nature and in Greek and Roman myths is marked sign of Afrodite-Venus. Lotus myth after arising from Egypt in its root to Iran, first reaches to Mesopotamia. Mortgat nominates two cylindrical seal belong to Mesopotamia one of which is London museum and another one in Berlin Museum and shows similar subject with different combination. In both two pictures ruler in a trimming cloth is feeding two holy ships Innin. Seal of London Museum shows a mythos and religious scene. Ruler is approaching to two ships and by his hand has taken Second seal also has these holy pictures. The king is wearing a trimming cloth and has branches of rose in hand and winged ship is seen between two Innin ring flags (Mortgat, 2008, 28). May be this is for the first time in

Figure 2. Mesopotamia art that a holy plant is placed in king's hand (which itself also is holy) in a holy space doing custom practice to inspire more holiness giving aspect.

In Assyrian reliefs we encounter a scene that has pictured a holy marriage in Takistan. In this relief the king is rested in bed and near him queen is sitting in a chair and attendants behind them while have taken up fly flaps are standing. Both king and queen are drinking wine. The king takes in his hand a bud of flower very similar to lotus in Achaemenid's designs. This relief which belongs to northern palace of Ashurbanipal from white marble which now is maintained in Britain (same, 2008, 287). In another Assyrian relief belonging to Sargon II, he has been shown with a court authority. The king while is sitting and takes in his right hand the royal stick has fastened to the same hand a bracelet adorned with a picture of louts (Mac Call, 2007, 31).

Figure 3. Sample of picture

Samples of these pictures in them high rank officials have fastened to their wrist lotus symbol is found plentifully which we just refer to some samples

of them: In famous picture of Gilgamesh[1] hero which has taken his hunted lion in hand and has gained of a relief in Khorsabad[2] belonging to 8th BC, the hero has fastened to his two wrist a lotus bracelet. This picture now is maintained in Louver museum (Zhiran, 2003, picture 31). And or in relief of north western of Namrood place belonging to 900 BC two feminine winged figure are seen in two sides of holy tree who they also in the same manner has fastened around the wrist a lotus bracelet (Zhiran, 2003, picture 36). As it was referred, above samples are only a little part of all reliefs which in Mesopotamia, the picture of louts is used in them and indicate importance of this picture in that land.

Figure 4. In ancient Iran, the first time trace of lotus symbol.

In ancient Iran, the first time trace of lotus symbol is seen in Elam art; Central dome indicates a glazed tile belonging to 2nd BC shows design of a 12 rose leaf flower which is very similar to designs of lotus of Achaemenid' period. Color used in decoration of this tile is white and yellow. This tile is keeping in Louver museum. In addition, on a crockery dish in form of cylindrical box belonging to 7th or 8th BC, rose flowers and twin bloom of 12 rose leaves Lotus, has been designed on it (Majidzadeh, 1991, 82). These two samples of application of lotus role in Elamite art, is introduction of presence of this ancient symbol in Iran land which plentifully has been used in Achaemenid' art and after that in Sassanid designs. In Achamenid palaces both in Susa and Takht-e-Jamshid, the Lotus has pictured so that statistically more includes visual symbol. Lotus holds in hands of kings and his crown prince (successor) in formal ceremonies, also its picture in hands of greats of court in stairs of Achaemenid palaces is inducing a type of spiritual and holy condition. In addition in border of many designs and or on neck of guardian winged caws we are witnessed of in entrances and capitals application of these designs.

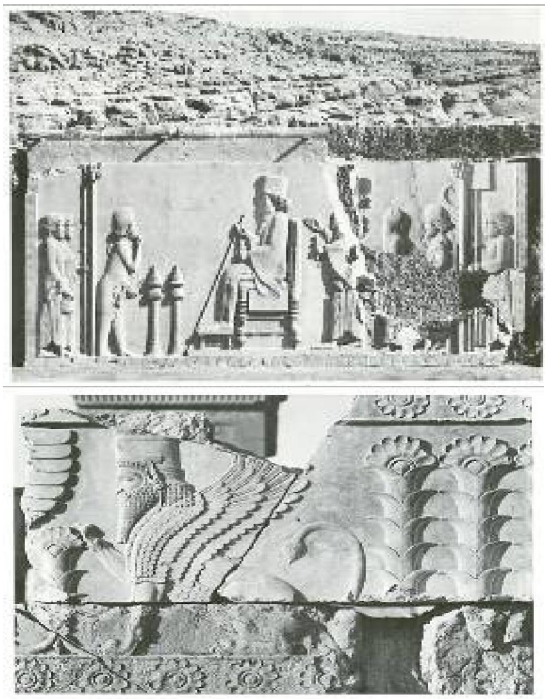


Figure 5. Lotus in feasts of the Near East

According to William Kalikan believe, Lotus in feasts of the Near East, before beginning the feast offered to each other or whom they are accepted in his presences and this itself has been of old tradition of these areas (Kalikan, 1979, 97).

One of the famous tablets of Daryoush about excavation of Suez Canal was discovered in 1869 near of this canal. In face of this tablet an inscription in three characters of ancient Persia, Babel and Elami and in its overleaf an inscription in Egyptian Hieroglyph has engraved. In upper part of Hieroglyph inscription, the design of Ra' God (winged disc) and design of Gods in knotting ceremonies of Lotus and Papyrus flowers around oval frame of pharaoh is seen. (Mohammadpanah, 2009, 89). Symbol of Lotus is entered through Iran to India and Buddha custom. In India Lotus was related to a sun-God. Souria vedaic God, who is personification of sun, has a Lotus in each of its hand. Lotus also is related to mother of Indian Gods and has been as a symbol of universal uterus. After victory of Arians, Indian Gods mainly became in the form of man that in head of them there was Brahma, Egyptian Creator God. In a recent myth in Rig Veda has been referred,

[1] One of the mythos heroes of Mesopotamia which on the basis of texts about 2600 AC has been one of young rulers and has semi-divine characteristic. Story of his famous epic poem in 12 chapter as several inscription has been reached to our hand (Mac Call, 2007, 51).

[2] One of the cities of Mesopotamia.

it is mentioned that how the universe created from a gold Lotus which was flowing on universe waters and from it Brahma was born. When his ceremonies replaced with Vishnu ceremonies, later he was imagined in a form that is sitting on a Lotus which is growing from Vishnu's umbilicus. Ceremonies of Mother-God in India never abolished. This goddess appears as Hinduic goddess and in the name of Padmapani which its lexical meaning is a woman how has Louts in hand. Shaketies i.e. spouses of Gods who are representative of creator force who shifts universe, nearly always among their numerous features have Lotus with themselves. Lotus was main Goddess of Buddhism Prajna-paramite which has keep Louts on a book. In Buddha instructions, the Lotus to the extent enters into metaphysics territory. Thus Louts is shown in Buddhism temples among decorated ceilings specially, in Central Asia. Statues of sitting Buddha on a bed of Lotus flower shows him as ruler of universe. Later, nearly, all Buddhas and follower of Buddha are sitting or standing on Lotus. Lotus is a part of eight symbol of auspiciousness in sole of Budha (Hall, 2008, 310).

Lotus in Chinese culture is symbol of innocence, spiritual elegance, peace, fertility and womanish personification and sign of one the four season of year i.e. summer. Chinese know Louts appearance of past, present and future, because it is a plant that simultaneously blooms, flowers and seeds. Thus is appearance of decency because appears of contaminated waters but doesn't accept its contamination.

Conclusion:

Symbol of Lotus because of its natural characteristics is considered by ancient people and gradually a halo of holiness and innocence has embodied it. Arising this symbol of ancient Egypt and its movement through Mesopotamia to Iran and India and then China, is a symbol of cultural exchanges and even believing exchange of ancient world. Believes around myths and symbols, for long years has remained in mind of people. For example in Ancient Iran symbol of Lotus even was remained up to Sassanid period and individual together with Sassanid king during receiving monarchy ring from Ahora Mazda, is sitting on Lotus flower (Hinler, 2002, 152). Evolution of symbolic myth of Lotus is indicating multilateral communication between last nations in one side and staying their religious and mythos believes in another side.

Resources:

[1] Zhiran, F. (2007). *Myth of Assyria and Babol,* translated by Abolghasem Esmaeilpour, Tehran: Karvan publication, 3rd edition.

[2] Kalikan, William, (2006), *archeology and history of art in Median and Persian,* translated by Goodarz Asaad Bakhtiar, Tehran: Parineh publication, 3rd edition.

[3]Majidzadeh, Yousef, (1991), *history of civilization of Elam,* Tehran: Center of university publication, 1st edition.

[4]Mohammadpanah, Behnam, (2001), *Ancient country,* 1st volume, Tehran: Sabzan publication, 6th edition.

[5]Mack Kall, Henrita, (2007), *Mesopotamian myths,* translated by Abbass Mokhber, Tehran: Markaz publication, 4th edition.

[6] Mortgat, Anton, (2008), *Ancient Mesopotamia Art, translated by Mohammadrahim Sarraf, Tehran: Samt publication, 3rd edition.*

[7] Vio, J, (2005), *Egypt myths,* translated by Abolghasem Esmaeilpour, Tehran: Karvan publication, 2nd edition.

[8] Hart, Jorge, (1991), *Egyptians Myths,* translated by Abbas Mokhber, Tehrna: Markaz Publication, 2nd edition.

[9] Hall, James, (2008), *Pictorial Culture of Symbols in Art of East and West,* Translated by Roghayeh Behzadi, Tehran: Farhang Moaser Publication, 3rd edition.

[10] Hinlz, John, (2002), *Recognition of Iran Myths,* translated by Zhaleh Amouzgar-Ahmad Tafazoli, Cheshmeh Publication, 7th edition.

[11] Hall, James, (1996). *IllusTRATED DICTIOINARY OF SYMBOLS*, San Diego, Wets View Press.

[12] Hart, George, (1990). *EGYPTIAN MYTHS*, London , British Museum Publications.

[13] MC. Call, Henrietta, (1990). *MESOPOTAMIAN MYTHS* , Texas , University of Texas press.

[14] Moortgat, Anton, (1996). *THE ART OF ANCIENT MESOPOTAMIA*, New York, Phaidan Press Ltd.

7/27/2011

Identification and Prioritization of Factors Affecting the Dropouts of the Employees: A case study of Fuzzy Analytic Hierarchy-Based Approach from Tehran

Davood Vahdat [1], Sevil farshid [2]

[1.] Lecturer, IT and Computer Engineering Department, Payame Noor University, 19395-4697, Tehran, Iran.
[2.] MSc. in Management of Information Technology, IT and Computer Engineering Department, Payame Noor University, 19395-4697, Tehran, Iran
se_farshid@yahoo.com

Abstract: If organizations wish to achieve their goals, they should have the capacity to attract, maintain and preserve their human resources. The desire to quit the job is one of the factors that make the organization face problems in achieving its goals. In this research, after the literature review and the background of the study, the dimensions and the components which force the employees to leave their jobs are identified. Then with the use of the fuzzy analytic hierarchy process (FAHP), the degree of importance of each of the components is specified. This research aims to determine the degree of importance of each of the factors causing the nurses to quit their jobs. The statistical population of this research consists of the nurses from the governmental hospitals of Tehran province. The findings of this study indicate that such factors as supervision style, job identity, job significance, job security, and salary have great effects on the decision of the nurses in the governmental hospitals of Tehran province to leave their jobs. It has also become known that the availability of resources, age and role conflict as well as role ambiguity have no significant influence on their decisions.
[Davood Vahdat, Sevil farshid. Identification and Prioritization of Factors Affecting the Dropouts of the Employees: A case study of Fuzzy Analytic Hierarchy-Based Approach from Tehran. Life Science Journal. 2011;8(3):313-317] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Dropout; Hospital, fuzzy analytic hierarchy process (FAHP)

1. Introduction

One of the fundamental problems of organizations is the dropout of the employees especially the specialists. According to the organization managers and the human resources researchers, dropout is costly and problematic and this reduces the efficiency of the overall service of the organization. Thus, the topic of dropout of the employees has drawn the attention of researchers and managers in such a way that a huge number of studies have been conducted on the identification of the factors affecting the dropout of the employees. Quitting the job can represent a great social loss in terms of provision, training, social capital, replacement of the human workforce and many other side expenditures. Following these are the adverse effects on the morale and the efficiency of the remaining individuals in the organization (Mitchell, 1982).

Dropouts turn into a critical issue in a system of offering health and treatment services like in the nursing positions which bear commitment and liability just like other medical professions do for life settlement, securing health, prevention, controlling diseases, treatment and rehabilitation of personal, familial and social problems (Fletcher, 2001). One of the issues related to the dropout of the nurses is the high cost of training nurses. Nowadays, one of the points which is taken into consideration in planning and budget allocation is whether it is economical to design and run a plan. If a project for the society is not economically and socially efficient, it is obvious that the planning department will never include it in its list of projects. However, there are some plans and programs which lack the economic outputs, but bear some social necessity. When the government invests in the nursing sector and trains nurses who should serve the country for 30 years, but the nurse quits her job, it clear that the economic resources are wasted in this way. Another issue of significant importance is that the ratio of the nurses to the hospital beds is low. This imposes an additional workload on the shoulders of the nurses and they choose to quit.

This research aims to study and determine the degree of importance of the factors which affect the dropouts in the employees. Considering the above-mentioned points and the importance of the nursing profession, this study reviews the effective factors in replacement and dropouts of the nurses in the governmental hospitals of Tehran province. The degree of importance of each of the factors will individually be specified. With respect to the determining factors in this study, it is expected that the people in charge will take the necessary measures to reduce the turnover among the nurses and increase the satisfaction level so that they will continue their service.

In order to investigate this issue, we need to make a series of comparisons for the different stages of the research. In order to make these comparisons among the various decision choices according to an index and in order to assess the importance of the decision index through the paired comparisons after the establishment of the hierarchy of the decision issue, the decision maker must determine the set of matrices which numerically measure the importance or the relative priority against each other and he should also determine each choice of decision based on the indices in comparison to other choices. This can be done through a paired comparison between the decision components (paired comparison) and through the allocation of numerical values which represent the priority or importance between the two components of decision. To do so, the choices comparisons with the indices of "i" against the choices with the indices of "j" are used. In Table 1, the method of valuing the indices has been comparably presented.

Table 1. Identification if the Fuzzy linguistic preference scale.

Verbal expressions for determination of priority	Fuzzy triangular number
Absolute importance or priority	(5.2, 3, 7.2)
Much higher importance or priority	(2, 5.2 , 3)
Higher importance or priority	(3.2, 2, 5.2)
Low importance or priority	(1, 3.2, 2)
Almost equal importance or priority	(1.2, 1, 3.2)
Absolutely equal importance or priority	(1, 1, 1)

2. Material and Methods

This research is an applied study in terms of the goal and a descriptive study in terms of the nature. The statistical population of this research consists of all the nurses in the governmental hospitals of Tehran province. As the size and the variance of the statistical population are not definite, the sample size has been estimated to be 1200 according to the sampling from an unlimited population and based on the following equation:

$$n = \frac{t_{\alpha/2}^{2} S^{2}}{\varepsilon^{2}}$$ and

$$\varepsilon = Z_{\alpha/2} \frac{\sigma}{\sqrt{n'}}$$

After reviewing the articles and the literature on this subject in order to determine the factors affecting the dropouts of the employees, four dimensions and thirty components were identified. Foloowing the interviews with the university professors and the nursing experts, the final research questionnaires were prepared based on four dimensions and twenty four components. The structure of the survey is based on Mqaysat paired value of analytic hierarchy process in which the paired comparisons were made between the four dimensions and also between each of the components of the four dimensions. To collect the views of the respondents about each of the paired comparisons, the geometric mean of the respondents' views was used. In addition, for the analysis of the data, Excel 2007 was applied. The dimensions and the components which affect the dropout of the employees according to the literature review and the background of the study are shown in figure 1. In fact, this figure represents the hierarchy.

3. Results

3.1 The calculation of the weight of each dimension

The paired comparison between the four organizational dimensions such as the organizational, professional, individual, and environmental factors has been calculated in order to determine the degree of importance for each of the components affecting the dropout of the employees with Fuzzy AHP Group Decision and by considering the method of Chang's extent analysis. Based on the points mentioned in the section of fuzzy AHP and after measuring the geometric mean from the fuzzy numbers of all the samples, following weights (Table 2) were derived.

Table 2: The geometric mean of the paired comparison for each of the four components

Aim: Determining the degree of importance for each dimension	Professional factors	Individual factors	Environmental factors	Organizational factors
Professional factors	(1,1,1,)	(0,97,1,26,1,61)	(1,1,5,2)	(0,74,1,14,1,59)
Individual factors	(0,62,0,79,1,04)	(1,1,1)	(0,72,0,96,1,36)	(0,63,0,87,1,26)
Environmental factors	(0,50,0,67,1)	(1,0,4,1,38,0,74)	(1,1,1)	(0,50,0,67,1)
Organizational factors	(0,63,0,87,1,26)	(1,14,1,59,0,74)	(1,1,5,2)	(1,1,1)

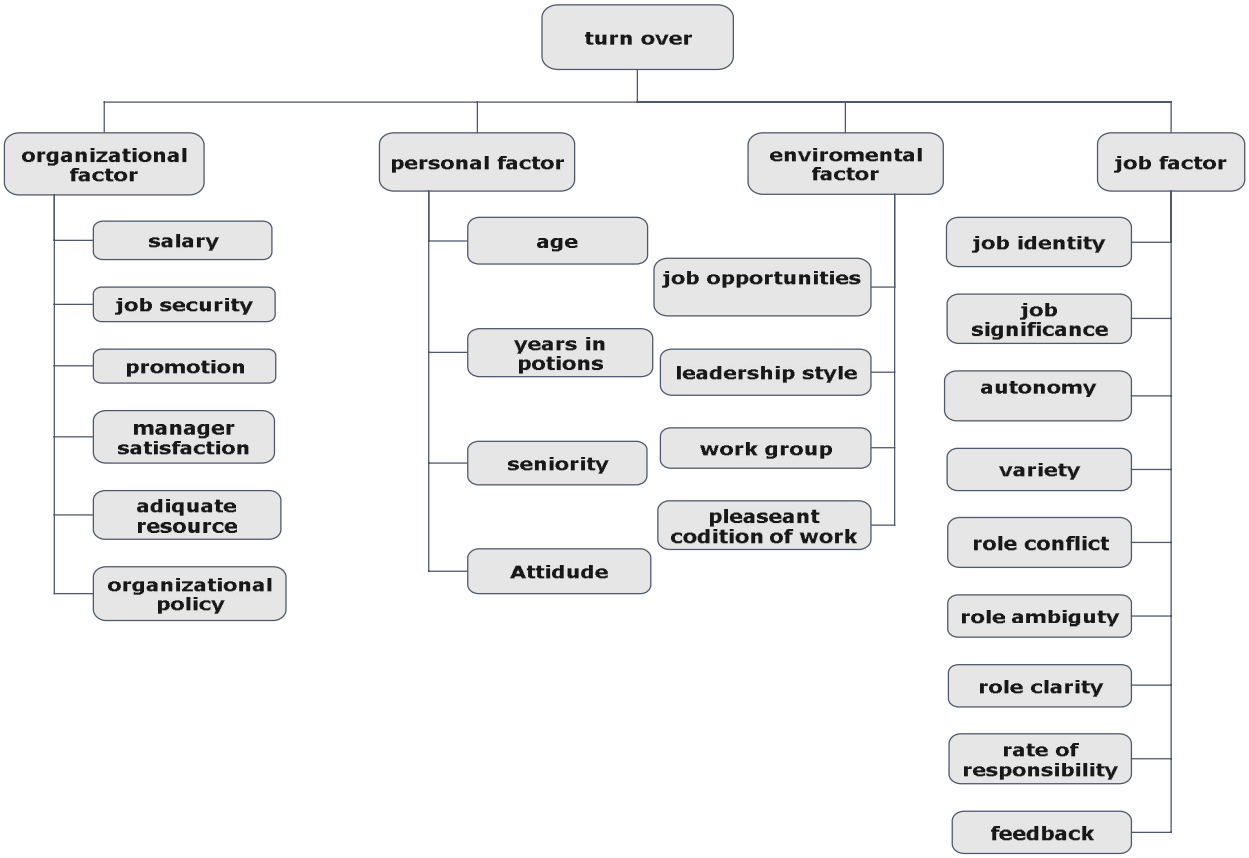


Figure 1. The dimensions and the components which affect the dropout of the employees.

Based on the results given in Table 2 and the process of Fuzzy AHP, we continued to calculate the weight of each of the four dimensions as presented below:

Step 1. The calculation of the fuzzy compound expansion for each of the dimensions:

$$\Sigma_{j=1}^{j} M_{g_i}^{j} = (l_{i1}, m_{i1}, u_{i1}) \oplus (l_{i2}, m_{i2}, u_{i2}) \oplus \ldots \oplus (l_{im}, m_{im}, u_{im})$$

In the following equation, S1 to S4 are related to the professional, individual, environmental, and organizational factors respectively:

$$S_i = \Sigma_{j=1}^{m} M_{g_i}^{j} \otimes [\Sigma_{i=1}^{n} \Sigma_{j=1}^{m} M_{g_i}^{j}]^{-1}.$$

Step 2. The calculation of the degree of possibility: S_i over S_k

$$V(S_i \geq S_k) = \sup_{x \geq y} (\min\{\alpha_{S_i}(x), \alpha_{S_k}(y)\})$$

Step 3. The calculation of the degree of preference (the degree of possibility) of convex fuzzy number S which is greater than convex fuzzy number k $S_i : i = 1,2,\ldots,k$:

$$V(S \geq S_1, S_2, \ldots, S_K) = (V((S \geq S_1), (S \geq S_2), \ldots, (S, S_K))$$

$$= \min(V((S \geq S_1), (S \geq S_2), \ldots, (S, S_K)) = \min V(S \geq S_i)$$

$$i = 1,2,..., k$$

Step 4. The normalization of the vector W' and the calculation of the vector of the normalized weight W:

If we suppose $k = 1,2,..., n \; k \neq i$ where $d'(A_i) = \min V(S_i, S_k)$, Then the vector of weight will be as follows:

$$W' = (d'(A_1), d'(A_2),..., d'(A_n))$$

The normalization of the vector W' and the calculation of the vector of the normalized weight W:

$$W = (d(A_1), d(A_2),..., d(A_n))$$

3.2 The calculation of the weight of each of the components of the various dimensions

Likewise, after the collection of the respondents' views about the paired comparison between each of the components of the various dimensions by means of geometric mean, the weight of each of the components was calculated as presented below. It is necessary to mention that the order of the components of the normalized weight in each of the dimensions is given according to the order given for each component:

The vector of the normalized weight for the dimension of the professional factors:
(0.202,0.199,0.108,0.101,0.052,0.042,0.071,0.124,0.101)

The vector of the normalized weight for the dimension of the individual factors:
(0.133,0.279,0.371,0.127)

The vector of the normalized weight for the dimension of the environmental factors:
(0.237,0.369,0.185,0.209)

The vector of the normalized weight for the dimension of the organizational factors:
(0.201,0.211,0.124,0.144,0.129,0.052,0.139)

3.3 The Calculation of the final weight of each of the components affecting the dropouts

By multiplying the weight of each of the dimensions by the weight of the components of the same dimension, the final weight of each of the components is derived.

4. Discussions

The findings of this research indicate that among the four dimensions affecting the dropout of the nurses, the professional factor has the highest significance. The organizational, environmental, and individual factors are the next priorities respectively. Of all the various components studied in this research, the supervision style as one of the organizational factors has the highest impact on the dropout of the nurses. Meanwhile, the job identity, job importance, job security, and salary and wage are some of the items that greatly influence the dropout of the workers in this profession. In this research, it has been made clear that factors such as the sufficiency of the resources, age, and sex conflict and job ambiguity do not have a significant effect on the dropout of the nurses.

Due to its high degree of importance, the issue of employee dropouts has been numerously studied so far (Shaw et al, 1998). Iverson (1999) says that there have been over 800 researches in this regard. Despite the various researches conducted in this field, unfortunately, it is still not clear what the main causes of dropouts of the employees are (Lee and Mitchell, 1994). Voluntary dropouts have considerable cost for the organization. These expenses may be either part of the direct expenditures such as selection, and the temporary employment of the workers and the time management or they may be related to the indirect costs such as the cost of education, the costs of weakened spirit of the workers, and extra workload on other employees and the reduction in the quality of the products and the services given by the organization (Dess and Shaw, 2001). When there are some changes in the organization for example, the organization decides to become smaller in size; some of the competent employees quit their jobs to earn a better job opportunity in another organization (Jackofsky et al., 1986).

Although there is no definite and structured pattern for the dropout, some factors have been identified that affect it. Many organizations have made use of these factors in order to reduce the rate of the dropout of their own employees (Kevin, 2004). These factors include job satisfaction (Hom and Kinicki, 2001), work market variables (Kirschenbaum and Mano-Negrin, 1999), organizational commitment (Meyer, 2001), justness and fairness (Aquino et al., 1997, 1).

Various projects have identified the factors which affect the job satisfaction of the employees and prepared the ground for their retention in the organization or encouraged them to quit their jobs. These factors are generally classified into four groups: organizational factors, environmental factors, the individual factors, and the nature of the work which we have discussed. According to the studies conducted by the experts and the researchers of management science, the organizational factors which bring about the satisfaction and the retention of the employees in the organization include such items as salary and wage, job promotion and the policy of the organization. The studies show that among all the organizational factors, the salary is a determining element for job satisfaction especially when the payment is fair in the view of the employees. In addition, promotion and the organization's policy are considered as the other effective organizational factors. The promotions include such items that boost the employee's spirit like a positive change in salary, receiving less support, more job challenges, more responsibilities, freedom in decision-making because of his personality promotion in the eyes of the organization. The inflexible policies cause negative job attitudes in the employees while the flexible policies create positive job attitudes in them (Morrell, 2002; McKnight et al, 2009).

Based on the findings of this research, it is suggested a proper supervision style be followed in a participatory and supportive manner, but despotic styles be avoided. These can encourage the nurses to continue their services. Considering the hardship of the nursing profession, it is suggested that the salary and the payment to the employees in this profession be increased. Furthermore, appreciation of this valuable profession and determination of its real position in the group of the medical professions can help to retain the nurses in their workplaces.

Acknowledgements:

The authors thank Mr. Davood Vahdat (Lecturer of IT and Computer Engineering Department, Payame Noor University, Tehran, Iran) for providing technical supports.

Corresponding Author:
Sevil Farshid
IT and Computer Engineering Department
Payame Noor University, 19395-4697, Tehran, Iran
Tell: +98.9143458184

E-mail: se_farshid@yahoo.com

4/4/2011

References

1. Aquino, K., Griffeth, R.W., Allen, D.G. and Hom, P.W. (1997), "Integrating justice constructs into the turnover process: a test of a referent cognitions model", Academy of Management Journal, Vol. 40 No. 5, pp. 1208-27.
2. Dess, G.D. and Shaw, J.D. (2001), "Voluntary turnover, social capital, and organizational performance", Academy of Management Review, Vol. 26 No. 3, pp. 446-56.
3. Fletcher, C.E. (2001), Hospital Job Satisfaction and dissatisfaction, JONA, 31(16).
4. Hom, P.W. and Kinicki, A.J. (2001), "Toward a greater understanding of how dissatisfaction drives employee turnover", Academy of Management Journal, Vol. 44 No. 5, pp. 975-87.
5. Iverson, R.D. (1999), "An event history analysis of employee turnover: the case of hospital employees in Australia", Human Resource Management Review, Vol. 9 No. 4, pp. 397-418.
6. Jackofsky, E.F., Ferris, K.R. and Breckenridge, B.G. (1986), "Evidence for a curvilinear relationship between job performance and turnover", Journal of Management, Vol. 12, pp. 105-11.
7. Kirschenbaum, A. and Mano-Negrin, R. (1999), "Underlying labor market dimensions of 'opportunities': the case of employee turnover", Human Relations, Vol. 52 No. 10, pp. 1233-55.
8. Lee, T.W. and Mitchell, T.R. (1994), "An alternative approach: the unfolding model of voluntary employee turnover", Academy of Management Review, Vol. 19 No. 1, pp. 51-89.
9. McKnight D. Harrison, Brandis Phillips, Bill C. Hardgrave, (2009), Which reduces IT turnover intention the most: Workplace characteristics or job characteristics?, Information & Management 46, pp.167–174
10. Meyer, J.P. (2001), "Organisational commitment", in Robertson, I. and Cooper, C. (Eds), Personnel Psychology and HRM, Wiley, London.
11. Mitchell, R, (1982), People in organizations, 2nd edition. London, M.C. Graw Hill Internatioal Book Company.
12. Morrell, K.M. (2002), "Modelling employee turnover", unpublished doctoral dissertation, University of Loughborough, Loughborough.
13. Shaw, J.D., Delery, J.E., Jenkins, G.D. and Gupta, N. (1998), "An organisation-level analysis of voluntary and involuntary turnover", Academy of Management Review, Vol. 41 No. 5, pp. 511-25.

Control of Experimental Colisepticaemia in Broiler Chickens Using Sarafloxacin

*Wafaa A. Abd El-Ghany and K. Madian

Faculty Veterinary Medicine, Cairo University, Cairo, Egypt
*Wafaa.ghany@yahoo.com

Abstract: This work was conducted to detect the effect of using sarafloxacin (5 mg/kg body weight) in the drinking water of broiler chickens to control experimental colisepticaemia in broiler chickens. One hundred and seventy, day old broiler chicks were used in the study. Twenty chicks at the day of arrival were sacrificed and cultured to ensure absence of *E. coli* infection. One hundred and fifty chicks were divided into three equal groups, each consists of 50 birds. Group (1) was challenged with *E. coli* and not treated with sarafloxacin (control positive), group (2) was challenged with *E. coli* and treated with sarafloxacin, while group (3) was neither challenged with *E. coli* nor sarafloxacin treated (blank control). Challenge was done intramuscularly (I/M) at 2 weeks of age in groups (1 and 2) as each bird received 0.5 ml of the nutrient broth culture containing 10^8 colony forming unit (CFU) *E. coli* O78 / ml. One appearance of signs, sarafloxacin was added to the drinking water for 3 successive days. All the birds were kept under complete observation for 6 weeks for estimating the bird's performance (body weight and feed conversion rate) and recording signs, mortalities, gross lesions, re-isolation of the organism and microscopical examination of the organs. The obtained results indicated significant ($P<0.05$) improvement in chickens performance in chickens challenged with *E. coli* and treated with sarafloxacin than those challenged and not treated. On the other hand, significant ($P<0.05$) decrease in morbidity and mortality rates, gross organs lesion score and re-isolation of *E. coli* O78 from the internal organs of chickens treated with sarafloxacin when compared with *E. coli* challenged non treated birds. Also, improvement of the microcscopical lesion scores was also detected in sarafloxacin treated group. It could be concluded from the above results that sarafloxacin used in a dose of 5 mg/kg body weight in the drinking water for 3 consecutive days is very effective in controlling of colisepticaemia in broiler chickens.
[Wafaa A. Abd El-Ghany and K. Madian, Control of Experimental Colisepticaemia in Broiler Chickens Using Sarafloxacin. Life Science Journal. 2011;8(3):318-328] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: Sarafloxacin, *E. coli*, Chickens, Treatment

1. Introduction

Escherichia coli (*E. coli*) is a normal inhabitant chicken's microflora. Some avian *E. coli* serotypes are pathogenic and induce significant economic problems in broiler chickens (Goodwin *et al.*, 1993, Yogaratnam, 1995, Jakob *et al.*, 1998, Dho-Moulin and Fairbrother, 1999 and Russell, 2003). Serogroups O78, O2 and O8 are common serotypes usually associated with colisepticaemia in poultry (Wray and Carroll**,** 1993). Colisepticaemia is the primary cause of death associated with an early respiratory disease complex (RDC) characterized by depression, respiratory distress and increased mortality in broiler chickens (Tablante *et al.*, 1999 and Barnes *et al.*, 2008). Typical lesions among birds with field and experimentally induced colisepticemia are airsacculitis, pericarditis and perihepatitis (Wray *et al.*, 1996). The response of coliform infections to various medications is erratic and often difficult to evaluate. Significant increase in appearance of drug resistant strains of *E. coli* isolated from poultry has complicated the problem (Scioli *et al.*, 1983, Alimehr *et al.*, 1999 and Georgnaras *et al.*, 2001). Laboratory tests to determine the sensitivity of *E. coli* to the various drugs are useful to select the most beneficial drugs (Vandemaele *et al.*, 2002).

Fluoroquinolones are broad spectrum antimicrobial agents that are effective in the treatment of wide range of infections (Medders *et al.*, 1998). Norfloxacin, enrofloxacin, ciprofloxacin, pefloxacin and sarafloxacin are examples of synthetic antimicrobials belonging to fluoroquinolone class of compounds (Hooper, 1998). The efficacies of different members of fluoroquinolone group against *E. coli* infections when the medication was administered in drinking water have been reported in several avian species (Bauditz, 1987, Copeland *et al.*, 1987, Behr *et al.*, 1988, Hafez *et al.*, 1990, Ter Hune *et al.*, 1991, Kempf *et al.*, 1995, Glisson, 1996, Gautrais and Copeland, 1997, Sumano *et al.*, 1998, Glisson *et al.*, 2004, Marien *et al.*, 2007, Da Costa *et al.*, 2009 and Garmyn *et al.*, 2009).

Sarafloxacin is a fluoroquinolone antibacterial drug which was approved in 1996 in United States for veterinary use to control morbidity and mortality associated with avian colibacillosis infections (Jones and Erwin, 1998 and Medders *et al.*, 1998). Like other fluoroquinolones, it acts by inhibiting the structure and function of DNA gyrase, a bacterial topoisomerase II which is an essential enzyme for DNA replication and transcription (Wolfson and Hooper, 1985 and Martinez *et al.*,

2006). The chemical structure of sarafloxacin hydrochloride is 6-Fluoro-1-(4-fluorophenyl)-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid hydrochloride. *In vitro* activities of sarafloxacin against avian *E. coli* and other infections were studied previously (Soussy *et al*., 1987, Jones and Erwin, 1998, Medders *et al*., 1998, Wang *et al*., 2001 and Smith *et al*., 2007) and the drug proved its efficacy in comparison with other fluoroquinolones. Sarafloxacin had been proposed successfully for the use in the drinking water of chickens to treat bacterial infections caused by *E. coli* (McCabe *et al*., 1993, Charleston *et al*., 1998, Medders *et al*., 1998, Hofacre *et al*., 2000 and Chansiripornchai and Sasipreeyajan, 2002), *Salmonella spp* (Jiang *et al*., 2000 and Roy *et al*., 2002) and *Campylobacter jejuni* (McDermott *et al*., 2002) and to prevent spiking mortality in turkeys (Vukina *et al*., 1998).

The pharmacokinetics of sarafloxacin in broiler chickens following single-dose applications was determined (Ding *et al*., 2000, 2001) and the results indicated that sarafloxacin was rapidly absorbed, extensively distributed, and quickly eliminated in broilers. Moreover, a dosage of 10 mg/kg administered orally every 8 hours in broilers could maintain effective plasma concentrations with bacteria infections. Also, Zhang *et al*. (2011) measured the inhibitory effects of sarafloxacin in comparison with enrofloxacin and marbofloxacin on the enzyme activity, protein levels and mRNA expression of liver cytochrome P450 (CYP) 1A and 3A in broilers and the results revealed that sarafloxacin didn't inhibit CYP in chick liver raising the possibility of drugs interaction when using those compounds.

Sarafloxacin tissue residues in different tissues and eggs of birds were comprehensively examined by Maxwell *et al*. (1999), Chu *et al*. (2000), Posyniak *et al*. (2001), Barrón *et al*. (2002), Schneider and Donoghue (2002), Christodoulou *et al*. (2007), Durden and MacPherson (2007), Herranz *et al*. (2007), Zhao *et al*. (2007), Guo *et al*. (2009), Lin (2009), Rodríguez Cáceres *et al*. (2009), Zhao *et al*. (2009), Anadón *et al*. (2010), Cho *et al*. (2010), Pena *et al*. (2010) and Rodríguez Cáceres *et al*. (2010) and all of them proved that sarafloxacin has very low tissue and egg residual effect indicating its safety which will directly reflect on the health hazard of human.

So, the objective of this study was to evaluate the efficacy of using sarafloxacin in the drinking water of broiler chickens for the treatment of experimental *E. coli* infection.

2. Material and Methods

Experimental birds:

One hundred and seventy, day old Hubbard broiler chicks of mixed sex were taken from a commercial hatchery. The birds were kept in separately thoroughly cleaned and disinfected houses and provided with feed and water adlibitum during the course of the experiment. All the birds received vaccination against Newcastle disease (ND) using Hitchner B1 and La Sota vaccines and against infectious bursal disease (IBD) using D78 vaccine at 6, 20 and 14 days of age; respectively through eye-drop instillation method. Also avian Influenza (H5N2) vaccine was given to the birds at 7 days old via intramuscular route. At day old, twenty random birds were collected and the internal organs (yolk sac, liver and heart) were cultured to certain absence of *E. coli* infection in experimental chicks.

The challenge inoculum:

The strain of *E. coli* that used for experimental challenge of the birds was serotype O78 and it was obtained kindly from Microbiology Department, Faculty of Veterinary Medicine, Cairo University. That serotype was isolated from a farm with an outbreak of avian colisepicaemia. The challenge inoculum was prepared according to the method of Quinn *et al*. (1994). At 2 weeks old, each chicken in the infected groups was intramuscularly (I/M) inoculated with 0.5 ml of the nutrient broth culture containing 10^8 colony forming unit (CFU) *E. coli*/ ml (Fernandez *et al*., 2002).

Sarafloxacin treatment:

Sarafloxacin hydrochloride (white to light yellow crystalline powder) was obtained from Vetchem Biochemistry Science (batch number, 91296-87-6). Sarafloxacin was dissolved in the drinking water to prepare sarafloxacin 10% solution (according company's recommendation) at the dose level of 5 mg/kg live body weight for 3 days. Prior treatment, the daily water consumption of birds was monitored for 24 hours. The daily drug dose was administered continuously (continuous dosing regimen) during 24 hours period in an amount of water that was consumed in the same period. Identical dosing regimen was repeated during two subsequent days for a total of 3 consecutive days. Daily fresh drug solution was mixed with drinking water and replaced at the same time each day. Just before treatment, all birds in the treated groups were weighed to calculate the required daily amount of sarafloxacin (5 mg/kg body weight).

In vitro antimicrobials sensitivity test:

To measure the sensitivity of the used *E. coli* strain to sarafloxacin, the antibiotic sensitivity test was done using disc diffusion method (Prasad *et al*.,

1997). Other fluoroquinolones like enrofloxacin, norfloxacin, ciprofloxacin and pefloxacin discs (Oxoid, UK) were used to compare their zones of inhibition with sarafloxacin. The diameters of inhibition zones were interoperated by referring to the table which represents the National Committee for Clinical Laboratory Standards (NCCLS sub-Committee's recommendation, 2001).

Experimental design:

One hundred and seventy, day old Hubbard broiler chicks of mixed sex were randomly divided into three equal groups, each consists of 50 birds. Twenty chicks at the day of arrival were sacrificed and the yolk sac, liver and heart were cultured to ensure absence of *E. coli* infection in them. Group (1) was *E. coli* challenged and not treated with sarafloxacin (control positive), group (2) was *E. coli* challenged and treated with sarafloxacin, while group (3) was neither *E. coli* challenged nor sarafloxacin treated (blank control). At 2 weeks old, each bird in the infected groups (1 and 2) were intramuscular (I/M) inoculated in the thigh muscles with 0.5 ml of the nutrient broth culture containing 10^8 colony forming unit (CFU) *E. coli* O78 / ml. Sarafloxacin treatment in the drinking water began 3 days after experimental infection (onset of signs appearance) and continued for 3 consecutive days in the treated groups (1 and 2). All the birds were kept under complete observation for 6 weeks (experimental period).

Drug evaluation parameters:

1- Performance:

Along the whole period of the experiment (6 weeks), randomly selected birds in each group were weighed each week. Also the feed consumption of each group was determined to calculate the feed conversion rate and consequently the European Production Efficiency Factor (EPEF) (Sainsbury, 1984).

2- Clinical signs and mortalities:

Four weeks after experimental infection, all the birds in the infected and treated groups were monitored daily for clinical signs and deaths of *E. coli* infection. Dead birds were subjected to post-mortem examination.

3- Post-mortem lesions:

Sacrificed chickens as well as dead birds at the 1st, 2nd, 3rd and 4th week post challenge were subjected to post-mortem examination to determine the lesion score. Serous membranes (air-sacs, pericardium and perihepatic capsule) were examined for lesions and the lesion score were scaled from 0 to 3 as the following criteria; 0= no lesions, 1= mild, 2= moderate and 3= severe (Nakamura *et al*., 1985, 1992 and Fernandez *et al*., 2002). The severity index of the lesions was calculated as Nakamura *et al*. (1990).

Lesions of colisepticemia were scored as follows; For air sacs, 0 indicated no lesions, 1 indicated cloudiness of air sacs, 2 indicated that air sac membranes were thickened, 3 indicated "meaty" appearance of membranes, with large accumulations of a cheesy exudate confined to one air sac, and 4 was the same as a score of 3 but with lesions in two or more air sacs. For the pericardial lesions, 0 indicated no visible lesions, 1 indicated excessive clear or cloudy fluid in the pericardium, and 2 indicated extensive fibrination in the pericardial cavity. For perihepatic lesions, 0 indicated no visible lesions, 1 indicated definite fibrination on the surface of the liver, and 2 indicated extensive fibrination, adhesions, liver swelling and necrosis.

Birds with severe lesions were characterized as having an air sac lesion score of 4 and pericarditis and perihepatitis scores of either 1 or 2.

4- Re-isolation of the challenge organism:

Swabs from the trachea, heart, liver and air-sacs were collected from sacrificed chickens at the 7, 14, 21 and 28 days following the beginning of the treatment regimens. The swabs were streaked onto MacConkey agar and then incubated at 37ºC for 24 hours. Grown colonies were further identified biochemically and serologically according to Cruickshank *et al*. (1975).

5- Histopathological examination:

Specimens from the liver, heart and lungs of birds in each group at the end of the study were collected, fixed in 10% formol saline for 24 hours, washed in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by slidge microtome. The obtained tissue sections were collected on glass slides, deparffinized and stained by hematoxylin and eosin stains (Bancroft *et al*., 1996) for histopathological examination through the electric light microscope.

Statistical analysis:

The data were analyzed using ANOVA test and the Least Significant Differences (LSD) test was also detected between different treatments groups as Snedecor and Corchran (1980).

3. Results and Discussion

Infection of broiler chickens with *E. coli* usually happens at 2-8 week old with colisepticaemia and high mortalities (Leitnes and Heller, 1992). Infection with *E. coli* could be controlled using antimicrobials but gens present on the bacterial plasmids usually encode resistance to these antibiotics. Also, these plasmids transfer from one bacterial population to another rendering drug resistance (Chansiripornchai *et al*., 1995 and Chansiripornchai and Sasipreeyajan, 2002). Recently introducing third generations of fluoroquinolones (sarafloxacin) can overcome the problem of drug fastness.

Avian pathogenic *E. coli* is frequently found to be resistant to commonly used antibacterial agents such as ampicillin, amoxicillin, tetracyclines, sulphonamides + trimethoprim and flumequine. Also, resistance to enrofloxacin is commonly encountered (Vandemaele *et al*., 2002).

The results of *in-vitro* antibiotic sensitivity test showed that the used *E. coli* challenge strain (O78) was sensitive to sarafloxacin than the other antibiotics discs (enrofloxacin, norfloxacin, ciprofloxacin and pefloxacin). Our result coincide with these recorded by Jones and Erwin (1998) who found that sarafloxacin was very active and comparable to ciprofloxacin and enrofloxacin for inhibiting 823 strains from a wide variety of *E. coli* species. The *in vitro* studies to determine the rates of mutation of avian isolates of *E. coli* following nalidixic acid, sarafloxacin, or enrofloxacin pressure was done by Medders *et al*. (1998) and detected that lower rate of mutation was seen after sarafloxacin pressure. Moreover, Smith *et al*. (2007) demonstrated high sensitivity of *E. coli* broiler chickens strain to sarafloxacin when compared with enrofloxacin, sulfonamides and oxytetracycline.

Data present in Table (1) represents the performance parameters (Average body weight, cumulative feed conversion and European Production Efficiency Factor) that were measured along the 6 weeks course of the experiment. The performance parameters were the best and were significantly ($P<0.05$) higher in birds that were non challenged or treated than those in challenged - non treated or challenged - treated groups. Sarafloxacin treated chickens showed higher significant ($P<0.05$) parameters than challenged - non treated group. Parallel results to this study was obtained by McCabe *et al*. (1993), Joong Kim (1995), Chansiripornchai and Sasipreeyajan (2002) and Zhenling *et al*. (2002) who detected significant increase in the average daily gain and feed conversion ratio with reduction in mortalities of broilers treated with sarafloxacin than those not received treatment after infection with *E. coli* serogroup O78. The improvement of the performance of the medicated group may be indirectly related to the bactericidal effect sarafloxacin on *E. coli* and accordingly the enhancement in the bird's health conditions.

Non *E. coli* challenged and non sarafloxacin treated (blank control) group showed no signs. While *E. coli* challenged chickens revealed signs of depression, off food, difficult breathing a day after *E. coli* challenge and these signs were estimated as an incidence of 30-70%. Twenty four hours after treatment with sarafloxacin in the drinking water, the clinical signs were declined and showed continuous reduction at the next two days of treatment (treatment course). No clinical signs were observed 5 days after treatment with sarafloxacin. However, survived chickens in the challenged non treated group estimated incidence of signs between 25-45% a week post-challenge.

The results of mortality rate, post mortem lesions and the mean of macroscopic lesion score are tabulated in Table (2). Blank control (non challenged or treated) chickens showed no mortalities along the course of experiment. In *E. coli* challenged groups, mortalities started at the 3rd day post-challenge then gradually reduced by sarafloxacin treatment and completely disappear at the 7th day of treatment. Challenged birds with *E. coli* showed cumulative mortality rate of (38%) which were significantly ($P<0.05$) higher than birds in sarafloxacin treated group (10%). Sekizaki *et al*. (1989) and Frenandez *et al*. (2002) found that *E. coli* serotype (O78) is highly pathogenic for chickens and can induce mortalities within short time. The finding of this work is in agreement with these reported by McCabe *et al*. (1993) and Joong Kim (1995) on sarafloxacin treatment of *E. coli* infected chickens. Also, our results are constant with this published by Chansiripornchai and Sasipreeyajan (2002) who reported that sarafloxacin treatment of broiler chickens could significantly ($P<0.05$) reduced mortalities from 75% in *E. coli* infected birds to 27% in infected medicated ones.

Infected groups with *E. coli* (O78) showed lesions at the 3rd day post challenge including septicaemia and serous to fibrinous air-sacculitis, pericarditis and perihepatitis either in dead or sacrificed birds. Administration of sarafloxacin significantly ($P<0.05$) reduced the macroscopic lesion score in the medicated birds than non medicated infected ones. The mean macroscopic gross lesion score in different organs of *E. coli* infected birds were varied from 2-4, however, it was not exceed 1 in sarafloxacin treated chickens. The lesions were completely absent a week after sarafloxacin medication. No lesions were observed in non infected

and non treated group. The necropsy findings of this experiment are supported by these reported by Sasipreeyajan and Pakpinyo (1992) and Gross (1999) who observed lesions of fibrinopurulent air-sacculitis, pericarditis and perihepatitis after systemic inoculation of *E. coli* serogroup (O78) in chickens. Prabhavathi *et al*. (1986) gave sarafloxacin at 4 times the minimum inhibitory concentration to the mice and found that the highest efficacy against *E. coli* (99.9%) occur within 2 hours after giving the drug. In addition, similar lesions score in serous membranes of broiler chickens were observed by Chansiripornchai and Sasipreeyajan (2002) after infection with *E. coli* and medication with sarafloxacin.

Table (3) reveals the percentages of the re-isolation rate of *E. coli* (O78) from the trachea, heart, liver and air-sacs in different groups. No re-isolation of the organism was detected in non infected non medicated group. The re-isolation rate was significantly ($P<0.05$) higher in *E. coli* infected group than sarafloxacin treated one. The organism couldn't be re-isolated after seven days of the treatment beginning. In the study of Chansiripornchai and Sasipreeyajan (2002), *E. coli* was re-isolated only from the liver in the rate of 60% in the infected birds while it was 14% (significantly $P<0.05$ lower) in birds treated with sarafloxacin in the drinking water.

Regarding the results of performance, morbidities, mortalities, organs lesion scores and re-isolation of the organism that are used as criteria for evaluation of *E. coli* infection in birds in this work, Piercy and West (1976), Nakamura *et al*. (1992), Mognet *et al*. (1997) and Glisson *et al*. (2004) observed nearly similar results. On the other hand, Charleston *et al*. (1998) made a comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *E. coli* model of colisepticaemia in chickens and found that enrofloxacin was more efficacious than either danofloxacin or sarafloxacin for the treatment of colisepticemia in chickens by medication in drinking water. Similarly, danofloxacin appeared to be more effective than sarafloxacin in treating colisepticemia.

Unfortunately, the literatures concerning using of sarafloxacin to treated *E. coli* or other infections in poultry are scarcely, but all published data (McCabe *et al*., 1993, Charleston *et al*., 1998, Medders *et al*., 1998, Hofacre *et al*., 2000, Jiang *et al*., 2000, Chansiripornchai and Sasipreeyajan, 2002 and Roy *et al*., 2002) agreed that sarafloxacin is effective in reducing signs, mortalities, lesions and the organism shedding as well as improving the performance.

Improving the health status of the birds caused by sarafloxacin treatment may be related to several aspects such its bactericidal broad spectrum effect as a result of inhibiting the structure and function of DNA gyrase, a bacterial topoisomerase II which is an essential enzyme for DNA replication and transcription (Martinez *et al*., 2006), good result of sarafloxacin antibiogram *in vitro* (Wang *et al*., 2001 and Smith *et al*., 2007) and sarafloaxcin rapid absorption, extensive distribution, quick elimination and effective maintenance of plasma concentrations with bacterial infections (Ding *et al*., 2001).

The histopathological alterations in the liver, heart and lungs in non *E. coli* infected, infected as well as sarafloxacin treated groups are seen in Table (4) and Figures (1-9). Non infected or treated group showed no histopathological alterations with normal histological structure of the central veins, sinusoids and surrounding hepatocytes of the liver (Figure 1), there were no microscopical alterations in the lungs lobules (Figure 2) and also no changes were recorded in the pericardium and myocardium (Figure 3). Nevertheless, *E. coli* infected bird's revealed severe microscopic lesions as there were congestion and dilatation in the portal veins and sinusoids associated with inflammatory cells infiltration in the portal area as well as focal aggregation in circumscribed manner in the heapatic parenchyma (Figure 4), the lining epithelial cells of the lungs bronchiols showed hyperplastic activation with polyps formation while the underlying lamina popria had focal circumscribed round aggregation of lymphoid cells with oedema and congested blood capillaries (Figure 5), fibrinonecrotic reaction with inflammatory cells infiltration, oedema and dilated blood capillaries were also detected in the pericardium while the myocardium showed leucocytes inflammatory cells infiltration (Figure 6). Sarafloxacin treatment alleviated the severity of lesions where the liver showed dilatation in the portal vein and sinusoids associated with few inflammatory cells infiltration in the portal area (Figure 7), congestion in the blood vessels and capillaries of the lungs lobules was detected (Figure 8) as well as oedema in the myocardium with congestion in the blood vessels were detected (Figure 9).

These observations were similar to those detected by Nakamura *et al*. (1985, 1992), Kutkat *et al*. (2002) and Sahar and El-shazly (2002) who observed that (O78) serotype of *E. coli* induced peri-hepatitis, vascular degeneration of the hepatocytes as well as mononuclear leucocytes inflammatory cells infiltration and dilatation of the portal veins. Also, they found severe pericarditis and myocardial heterophilic cells infiltration.

From the above mentioned results in this study, it could be concluded that sarafloxacin (3rd generation of flouroquinolnes) when used in a dose of

5 mg/kg body weight in the drinking water for 3 consecutive days is very effective in controlling of colisepticaemia in broiler chickens.

Table (1): The average body weight, cumulative feed conversion and EPEF in sarafloxacin treated and *E. coli* challenged and non challenged groups of broiler chickens

Group	Average body weight/gm						CFC	EPEF
	Age/week							
	Before *E. coli* challenge		After *E. coli* challenge					
	1	2	3	4	5	6		
Challenged-not treated	120.21±5.44[a]	290.30±8.76[a]	478.20±22.1[b]	705.41±24.84[b]	810.30±35.72[b]	1456.12±13.15[b]	2.40	145.22
Challenged-treated	125.29±2.99[a]	300.51±8.31[a]	610.30±10.12[c]	804.66±25.20[c]	1256.30±60.31[c]	1741.21±21.56[c]	1.89	198.41
Not challenged-not treated	129.40±2.00[a]	315.90±8.78[a]	623.4±15.61[a]	810.70±22.9[a]	1297.19±55.70[a]	1808.15±31.50[a]	1.78	203.09
LSD	18.65	30.10	28.29	70.51	121.92	132.65		

CFC= Cumulative feed conversion EPEF= European Production Efficiency Factor.
The higher the value, the better the performance LSD= Least significant difference as determined by Fisher's protected LSD procedures.
Means within the column with no superscripts are significantly different (P<0.05).

Table (2): The mortality rate and the mean gross lesion score in sarafloxacin treated and *E. coli* challenged and non challenged groups of broiler chickens

Groups	Examined birds		Cumulative mortality rate	Mean gross lesion score		
	Sacrificed	Dead		Pericarditis	perihepatitis	Airsaculitis
Challenged-not treated	10	19	19/50 (38%)	2.42±0.21[a]	2.33±0.19[a]	2.67±0.15[a]
Challenged-treated	10	5	5/50 (10%)	0.18±0.32[c]	0.16±0.54[c]	0.20±0.11[c]
Not challenged-not treated	10	0	0/50 (0.0%)	0	0	0
L.S.D				1.51	1.43	1.17

Values within a column represent means ± SEM. L.S.D: least significant difference.
Values in a column not sharing a common letter are significantly (P<0.05) different.

Table (3): Re-isolation rate of *E. coli* (O78) in sarafloxacin treated and *E. coli* challenged and non challenged groups of broiler chickens

Groups	**Examined birds**	**Re-isolation rate of *E. coli* (O78) from different organs**			
		Trachea	**Heart**	**Liver**	**Air-sacs**
Challenged-not treated	10	5/10 (50%)[b]	7/10 (70%)[b]	6/10 (60%)[b]	8/10(80%)[b]
Challenged-treated	10	1/10 (10%)[a]	0/10 (0%)[a]	2/10 (20%)[a]	1/10 (10%)[a]
Not challenged-not treated	10	0/10 (0.00%)[a]	0/10 (0.00%)[a]	0/10 (0.00%)[a]	0/10(0.00%)[a]

Values in a column not sharing a common letter are significantly (P<0.05) different.

Table (4): The severity of reactions in different tissues according to histopathological alterations in sarafloxacin treated and *E. coli* challenged and non challenged groups of broiler chickens

Organ	Lesion	Groups		
		Challenged-not treated	Challenged-treated	Not challenged-not treated
Liver	Congestion of portal veins and sinusoids	+++	++	_
	Inflammatory cells infiltration in portal area	++	+	_
	Focal circumscribed inflammatory cells aggregation in parynchyma	+++	_	_
Lungs	Hyperplasia with polyps in the lining epithelium	++	_	_
	Peribronchiolar focal leucocytic inflammatory cells aggregation	+++	_	_
	Oedema in the peribronchiolar tissues	++	_	_
	Congestion and dilation of peribronchiolar blood capillaries	++	_	_
Heart	Fibrino necrotic reaction with oedema and inflammatory cells in the pericardium	++++	_	_
	Inflammatory cells infiltration in the mycordium	++++	_	_
	Dilated and congested blood vessels in myocardium	++	+	_
	Oedema in myocardium	++	+	_

++++= Very severe +++= Severe ++= Moderate += Mild -= Nil

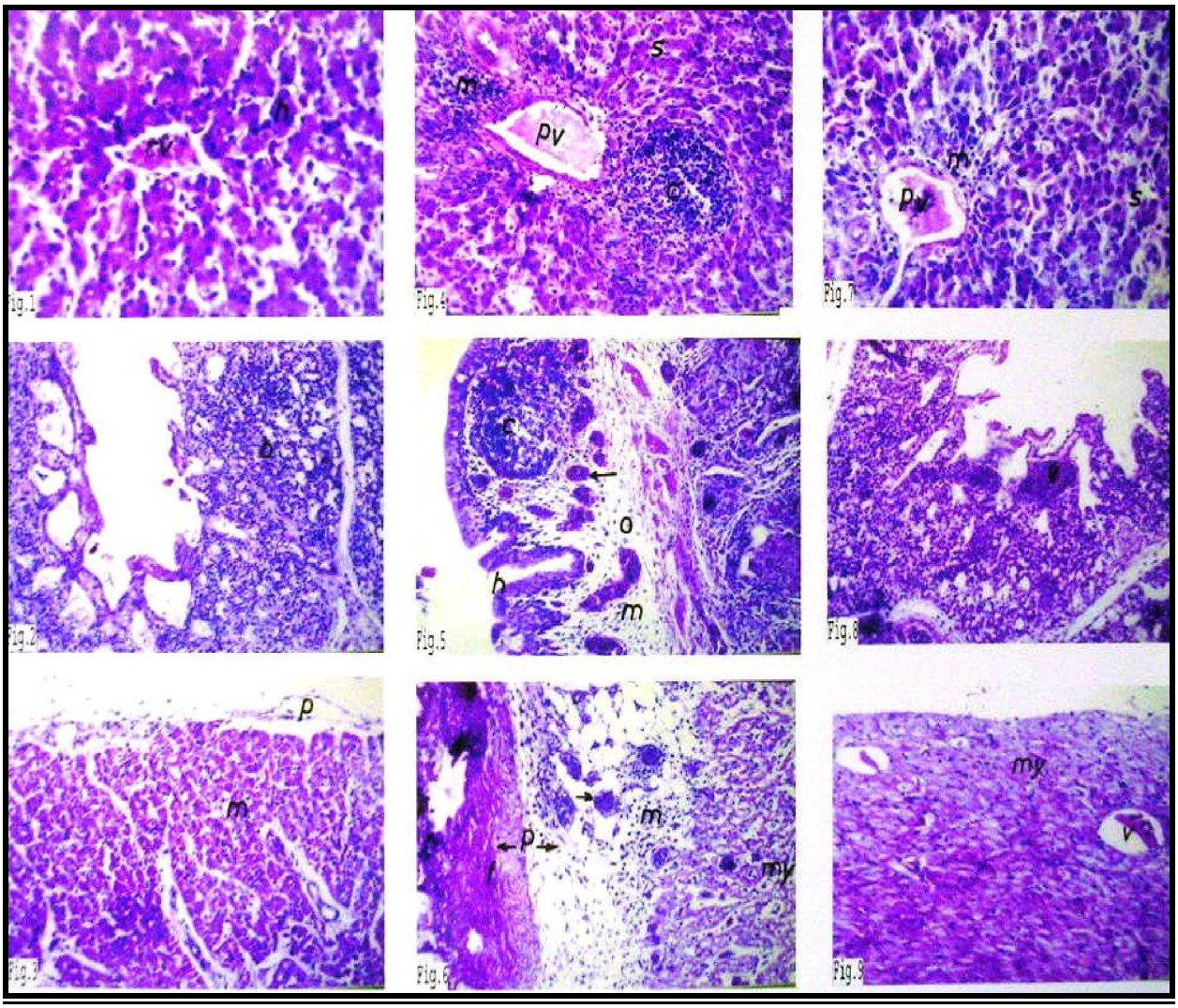


Fig. (1). The liver of non *E. coli* infected or treated group showing normal histological structure of the hepatic cells (h), central veins (cv) and sinusoids (s). H & E (X 80).

Fig. (2). The lungs of non *E. coli* infected or treated group showing normal histological structure of the lobules (b). H & E (X 40).

Fig. (3). The heart of non *E. coli* infected or treated group showing normal histological structure of the pericardium (p) and myocardium (m). H & E (X 64).

Fig. (4). The liver of *E. coli* infected group showing congestion and dilatation of portal vein (pv) and sinusoids (s) with inflammatory cells infiltration in the portal area (m) and focal aggregation in circumscribed manner of hepatic paryncyma (c). H & E (X 64).

Fig. (5). The lungs of *E. coli* infected group showing hyperplasia with polyps formation in the bronchiolar lining epithelium (h) with peribronchiolar focal leucocytic inflammatory cells aggregation (c), oedema (o) and dilated capillaries (arrow). H & E (X 40).

Fig. (6). The heart of *E. coli* infected group showing fibrinonecrotic reaction (f) with oedema (o), inflammatory cells infiltration (m) and dilated blood capillaries (m) in the pericardium (p) as well as inflammatory cells infiltration in myocardium (my). H & E (X 64).

Fig. (7). The liver of sarafloxacin treated and *E. coli* infected group showing dilated portal veins (pv) and sinusoids (s) with few inflammatory cells infiltration (m) in portal area. H & E (X 80).

Fig. (8). The lungs of sarafloxacin treated and *E. coli* infected group showing congestion of the blood vessels of the lobules (v). H & E (X 40).

Fig. (9). The heart of sarafloxacin treated and *E. coli* infected group showing myocardial oedema (my) with dilatation and congestion in blood vessels (v). H & E (X 64).

Corresponding author
Wafaa A. Abd El-Ghany
Faculty Veterinary Medicine, Cairo University,
Cairo, Egypt
*Wafaa.ghany@yahoo.com

References

Alimehr, M., G. Sadeghi-Hashjin, S. A. Poorbakhsh and K. Nofoozi, (1999): Isolation, identification and *in vitro* susceptibility of avian *Escherichia coli* to selected fluoroquinolones. Ach Razi Ins., 50: 77-82.

Anadón, A., F. H. Suárez, M. A. Martínez, V. Castellano, M. Martínez, I. Ares, E. Ramos, F. Gamboa and M. R. Martínez-Larrañaga, (2010): Plasma disposition and tissue depletion of difloxacin and its metabolite sarafloxacin in the food producing animals, chickens for fattening. Food Chem Toxicol., Nov. 23.

Bancroft, J. D., A. Stevens and D. R. Turner, (1996): Theory and practice of histopathological techniques. 4th Ed., Churchill Livingstone, New York.

Barnes, H. J., K. N. Lisa and J. P. Vaillancourt, (2008): Colibacillosis. Pages 691–737 in Diseases of Poultry. 12th ed. Y. M. Saif, H. J. Barnes, A. M. Fadly, J. R. Glisson, L. R. Mc Dougald, L. K. Nolan, and D. E. Swayne, ed. Iowa State University Press, Ames.

Barrón, D., E. Jiménez-Lozano, S. Bailac and J. Barbosa, (2002): Determination of difloxacin and sarafloxacin in chicken muscle using solid-phase extraction and capillary electrophoresis. J Chromatogr B Analyt Technol Biomed Life Sci., 767 (2): 313-319.

Bauditz, R. (1987): Results of clinical studies with Baytril in poultry. Vet Med. Rev., 2: 130-136.

Behr, K. P., M. Friederichs, K. H. Hinz, H. Lüders and O. Siegmann, (1988): Klinische Erfahrungen mit dem Chemotherapeutikum Enrofloxacin in Hühner- und Putenherden. Tierärztl. Umschau, 43: 507-515.

Chansiripornchai, N. and J. Sasipreeyajan, (2002): Efficacy of sarafloxacin in broilers after experimental infection with *Escherichia coli*. Vet Res Commun., 26: 255-262.

Chansiripornchai, N., J. Sasipreeyajan and S. Pakpinyo, (1995): The *in vitro* antimicrobial sensitivity testing of *Escherichia coli* isolated from commercially reared chickens. Thia J Vet Med., 25: 275-283.

Charleston, B., J. J. Gate, I. A. Aitken, B. Stephan and R. Froyman, (1998): Comparison of the efficacies of three fluoroquinolone antimicrobial agents, given as continuous or pulsed-water medication, against *Escherichia coli* infection in chickens. Antimicrob Agents Chemother., 42 (1): 83-87.

Cho, H. J., H. Yi, S. M. Cho, D. G. Lee, K. Cho, A. M. Abd el-Aty, J. H. Shim, S. H. Lee, J. Y. Jeong and H. C. Shin, (2010): Single-step extraction followed by LC for determination of (fluoro) quinolone drug residues in muscle, eggs, and milk. J Sep Sci., 33 (8):1034-1043.

Christodoulou, E. A., V. F. Samanidou and I. N. Papadoyannis, (2007): Validation of an HPLC-UV method according to the European Union Decision 2002/657/EC for the simultaneous determination of 10 quinolones in chicken muscle and egg yolk. J Chromatogr B Analyt Technol Biomed Life Sci., 859 (2): 246-255.

Chu, P. S., D. J. Donoghue and B. Shaikh, (2000): Determination of total 14C residues of sarafloxacin in eggs of laying hens. J Agric Food Chem., 48 (12): 6409-6411.

Copeland, D. D., R. H. Schulz and J. N. Davidson, (1987): The efficacy and optimum dosage of Bay Vp 2674 for the control of *E. coli* infections in chicken. Poult Sci., 66 (Suppl. 1): 85.

Cruickshank, R., J. P. Duguid, B. P. Marmion and R. H. A. Swain, (1975): Med. Microbiology Vol. II 12th edn.

Da Costa, P. M.., A. Belo, J. Goncalves and F. Bernardo, (2009): Field trial evaluating changes in prevalence and patterns of antimicrobial resistance among *Escherichia coli* and *Enterococcus spp.* isolated from growing broilers medicated with enrofloxacin, apramycin and amoxicillin. Vet Microbiol., 139: (3-4): 284-292.

Dho-Moulin, M. and J. M. Fairbrother, (1999): Avian pathogenic *Escherichia coli* (APEC). Vet Res., 30: 299-316.

Ding, H. Z., Q. H. Fing, Z. L. Zeng and Z. L. Chen, (2000): Pharmacokinetic studies of saafloxacin in chickens. Chinese J Vet Sci., 20 (5): 493-496.

Ding, H. Z., Z. L. Zeng, K. F. Fung, Z. L. Chen and G. L. Qiao, (2001): Pharmacokinetics of sarafloxacin in pigs and broilers following intravenous, intramuscular, and oral single-dose applications. J Vet Pharmacol Ther., 24: 303-308.

Durden, D. A. and T. MacPherson, (2007): Quantitation and validation of fluoroquinolones in eggs using liquid chromatography/tandem mass spectrometry. J AOAC Int., 90 (2): 613-625.

Fernandez, A., C. Lara, A. Loste and M. C. Marca, (2002): Efficacy of calcium fosfomycin for the treatment of experimental infection of broiler chickens with *Escherichia coli* O78:K80. Vet Res Commun., 26: 427-436.

Garmyn, A., A. Martel, R. Froyman, H. Nauwynck, L. Duchateau, F. Haesebrouck and F. Basmans, (2009): Effect of multiple- and single-day enrofloxacin medications against dual experimental infection with avian pneumovirus and *Escherichia coli* in turkeys. Poult Sci., 88 (10): 2093-3000.

Gautrais, B. and D. Copeland, (1997): Use of enrofloxacin in turkeys; a worldwide experience. Page 79 in Proc. 46th West Poult Dis Conf., Sacramento, CA.

Georgnaras, I., J. W. Hastings and A. Holy, (2001): Genotypic analysis of *Escherichia coli* strains from poultry carcasses and their susceptibilities to antimicrobial agents. Appl Environ Microbiol., 67 (4): 1940-1944.

Glisson, J. R. (1996): The efficacy of enrofloxacin (Baytril) for the treatment of colibacillosis in chickens and turkeys and fowl cholera in turkeys, p. 22. In Proceedings of the 45th Western Poultry Disease Conference.

Glisson, J. R., C. L. Hofacre and G. F. Mathis, (2004): Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the control of morbidity and mortality caused by *Escherichia coli* in broiler chickens. Avian Dis., 48*:* 658-662.

Goodwin, M. A., J. Brown and L. M. Rowland, (1993): Disease prevention and control in broilers, p. 140-175. In M. Pattison (ed.), The health of poultry. Longman Scientific and Technical, Harlow, United Kingdom.

Gross, W. B. (1999): Colibacillosis: In Calnek, B.W., C.W. Beard, W.M. Rie, and S.H. Yoder, Jr. (ed), Diseases of Poultry, 9th edn, (Iowa State University Press, Ames, IA), 138-144.

Guo, W., Y. Liu and N. Liu, (2009): Simultaneous analysis of 7 fluoroquinolone residues in chicken muscle by ultra performance liquid chromatography-electrospray ionization tandem mass spectrometry. Se Pu., 27 (4): 406-411.

Hafez, H. M., J. Emele and H. Woernle, (1990): Turkey rhinotracheitis (TRT). Serological flock profiles and economic parameters and treatment trials using enrofloxacin (Baytril). Tierärztl. Umschau, 45:111-114.

Herranz, S., M. C. Moreno-Bondi and M. D. Marazuela, (2007): Development of a new sample pretreatment procedure based on pressurized liquid extraction for the determination of fluoroquinolone residues in table eggs. J Chromatogr A., 1140 (1-2): 63-70.

Hofacre, C. L., A. R. de Cotret, J. J. Maurer, A. Garritty and S. G. Thayer, (2000): Presence of fluoroquinolone-resistant coliforms in poultry litter. Avian Dis., 44 (4): 963-967.

Hooper, D. C. (1998): Clinical applications of quinolones. Biochem Biophys Acta., 1400: 45-61.

Jakob, H. P., R. Morgenstern, P. Albicker and R. K. Hoop, (1998): Reasons for condemnation of slaughtered broilers from two large Swiss producers. Schweiz Arch Tierheilkd., 140: 60-64.

Jiang, Z. Q., L. Y. Fang, W. Zong, Y. P. Wang and X. Ye, (2000): Studies on acute toxicity and efficacy of sarafloxacin against experimental salmonellosis in chicks. Acta Agri Zhejiangensis, 12 (1): 38-41.

Jones, R. N. and M. E. Erwin, (1998): *In vitro* susceptibility testing and quality control parameters for sarafloxacin (A-56620): a fluoroquinolone used for treatment and control of colibacillosis in poultry. Quality Control Study Group. Diagn Microbiol Infect Dis., 32 (1): 55-64.

Joong Kim, S. (1995): Efficacy of floxacin for the control of poultry bacterial diseases. Samji Vet., 10: 2-23.

Kempf, I., F. Gesbert, M. Guittet, R. Froyman, J. Delaporte and G. Bennejean, (1995): Dose titration study of enrofloxacin (Baytril) against respiratory colibacillosis in Muscovy ducks. Avian Dis., 39:480-488.

Kutkat, M. A., S. Nagwa and A. Ebtehal, (2002): Effect of *Lactobacillus acidophilus* on controlling of *clostridium perfringens* and *E. coli* infections in native breed chickens. J Egypt Vet Med Associ., 62 (6): 89-101.

Leitnes, G. and E. D. Heller, (1992): Colonization of *Escherichia coli* in young turkeys and chickens. Avian Dis., 36: 211-220.

Lin, B. (2009): Multi-residue determination of 11 quinolones in chicken muscle by high performance liquid chromatography with fluorescence detection. Se Pu., 27 (2): 206-210.

Marien, M., A. Decostere, L. Duchateau, K. Chiers, R. Froyman and H. Nauwynck, (2007): Efficacy of enrofloxacin, florfenicol and amoxicillin against *Ornithobacterium rhinotracheale* and *Escherichia coli* O2:K1 dual infection in turkeys following APV priming. Vet Microbiol., 121: 94-104.

Martinez, M., P. McDermott and R. Walker, (2006): Pharmacology of the fluoroquinolones: A perspective for the use in domestic animals. Vet J., 172: 10-28.

Maxwell, R. J., E. Cohen and D. J. Donoghue, (1999): Determination of sarafloxacin residues in fortified and incurred eggs using on-line microdialysis and HPLC/programmable fluorescence detection. J Agric Food Chem., 47 (4): 1563-1570.

McCabe, M. W., R. H. Rippel and R. E. Miller., (1993): Efficacy of sarafloxacin for the control of mortality associated with *E. coli* infections in broiler chickens and turkeys, p. 75. In Proceedings of the 42nd Western Poultry Disease Conference (Sacramento, California).

McDermott, P. F., S. M. Bodeis, L. L. English, D. G. White, R. D. Walker, S. Zhao, S. Simjee and D. D. Wagner, (2002): Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. J Infect Dis., 185 (6): 837-40.

Medders, W. M., R. E. Wooley, P. S. Gibbs, E. B. Shotts and J. Brown, (1998): Mutation rate of avian intestinal coliform bacteria when pressured with fluoroquinolones. Avian Dis., 42: 146-53.

Mognet, L., P. Bezille, J. Guyonett and H. Karembe, (1997): comparison de la flumequine á l'amoxicilline dans deux modes d'adinistration par voie orale, en traitement de la colibacillose du poulet: approche phamacodynamique et clinique. Revue Medicine Veterinaire, 148: 793-804.

Nakamura, K., N. Youasa, H. Abe and M. Narita, (1990): Effect of infectious bursal disease virus on infections produced by *Escherichia coli* of high and low virulence in chickens. Avian Pathol., 19: 713-712.

Nakamura, K., K. A. Cook, A. Frazier and M. Narita, (1992): *Escherichiacoli* multiplication and lesions in the respiratory tract of chickens inoculated with infectious bronchitis virus and / or *E. coli*. Avian Pathol., 36: 881-890.

Nakamura, K., M. Maeda, Y. Imada, T. Imada and K. Sato, (1985): Pathology of spontaneous colibacillosis in broiler flock. Vet Pathol., 22: 592-597.

National Committee for Clinical Laboratory Standards, (2001): Performance Standards for antimicrobial susceptibility testing- Eleventh information. Supplement M100-S11. NCCLS Villanova, PA, USA.

Pena, A., L. J. Silva, A. Pereira, L. Meisel and C. M. Lino, (2010): Determination of fluoroquinolone residues in poultry muscle in Portugal. Anal Bioanal Chem., 397 (6): 2615-2621.

Piercy, D. W. T. and B. West, (1976): Experimental *Escherichia coli* infection in broiler chickens: course of the disease induced by inoculation via the air-sac route. J Comp Pathol., 86: 203-210.

Posyniak, A., J. Zmudzki and S. Semeniuk, (2001): Effects of the matrix and sample preparation on the determination of fluoroquinolone residues in animal tissues. J Chromatogr A., 914 (1-2): 89-94.

Prabhavathi, B. F., T. W. Danial, R. B. Robert, P. J. Kenneth, R. R. Nancy and S. Nathon, (1986): *In vitro* evaluation of A-56619 (Difloxacin) and A-4552: new aryl fluroquinolones. J Antimicrob Agent Chemotherap., 29: 70-77.

Prasad, V., K. Krishnamurlthy and T. V. Janardhana Rao, (1997): *In vitro* antibiogram studies of *Escherichia coli* in chickens. Indian Vet J., 74: 616-617.

Quinn, P. J., M. E. Carte, B. Markeryo and G. R. Carter, (1994): Clinical Vet Microbiol. Year book-wolf publishing- Europe Limited.

Rodríguez Cáceres, M. I., A. Guiberteau Cabanillas, D. Bohoyo Gil and M. A. Martínez Cañas, (2009): Quantification of danofloxacin and difloxacin in chicken tissues in the presence of sarafloxacin as interference. J Agric Food Chem., 57 (17): 7627-7633.

Rodríguez Cáceres, M. I., A. Guiberteau Cabanillas, T. Galeano Díaz and M. A. Martínez Cañas, (2010): Simultaneous determination of quinolones for veterinary use by high-performance liquid chromatography with electrochemical detection. J Chromatogr B Analyt Technol Biomed Life Sci., 878 (3-4): 398-402.

Roy, P., A. S. Dhillon, L. H. Lauerman, D. M. Schaberg, D. Bandli and S. Johnson, (2002): Results of Salmonella isolation from poultry products, poultry, poultry environment, and other characteristics. Avian Dis., 46 (1): 17-24.

Russell, S. M. (2003): The effect of airsacculitis on bird weights, uniformity, fecal contamination, processing errors, and populations of *Campylobacter* spp. and *Escherichia coli*. Poult Sci., 82: 1326-1331.

Sahar, A. and M. El-shazly, (2002): Effect of probiotic and prebiotic combination (Biomin IMBO and Biomin C-EX) on *E. coli* infection in broiler chickens. J Egypt Vet Med Associ., 62 (6): 113-119.

Sainsbury, D. (1984): Systems of management. Ch.9 P.102. In Poultry Health and Management. 2nd Ed. By Sainsbury. Granada Publishing LTD. 8 Grafton Street, London WIX3 LA.

Sasipreeyajan, J. and S. Pakpinyo, (1992): The result of using enrofloxacin in broiler. Thai J Vet Med., 22: 95-104.

Schneider M. J. and D. J. Donoghue, (2002): Multiresidue analysis of fluoroquinolone antibiotics in chicken tissue using liquid chromatography-fluorescence-multiple mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci., 780 (1): 83-92.

Scioli, C., S. Esposito, G. Anazilotti, A. Parone and C. Pennucci, (1983): Transferable drug resistance in *Escherichia coli* isolated from antibiotic fed chickens. Poult Sci., 382-384.

Sekizaki, T.; L. Nonomura and Y. Imada, (1989): Loss of virulence associated with plasmid curing

of chickens pathogenic *Escherichia coli*. J Japanese Vet Sci., 51: 659-661.

Smith, J. L., D. J. Drum, Y. Dai, J. M. Kim, S. Sanchez, J. J. Maurer, C. L. Hofacre and M. D. Lee, (2007): Impact of antimicrobial usage on antimicrobial resistance in commensal *Escherichia coli* strains colonizing broiler chickens. Appl Environ Microbiol., 73 (5): 1404-1414.

Snedecor, G. W. and W. G. Corchran, (1980): Statistical Methods. 7th Ed., (Iowa State College Press, Ames, IA), 39-63.

Soussy, C. J., L. Deforges, J. Le Van Thoi and J. Duval, (1987): *In vitro* antibacterial activity of 2 new quinolones: A 56619 (difloxacin) and A 56620. Comparison with pefloxacin, ofloxacin and ciprofloxacin. Pathol Biol., 35: 759-67.

Sumano, L. H., C. L. Ocampo, G. W. Brumbaugh and R. E. Lizarrage, (1998): Effectiveness of two fluoroquinolones for the treatment of chronic respiratory disease outbreak in broilers. Br Poult Sci., 39 (1): 42-46.

Tablante, N. L., P. Y. Brunet, E. M. Odor, M. Salem, J. M. Harter-Dennis and W. D. Hueston, (1999): Risk factors associated with early respiratory disease complex in broiler chickens. Avian Dis., 43 (3): 424-428.

Ter Hune, T. N., P. W. Wages, W. S. Swafford and S. T. Tolling, (1991): Clinical evaluation of efficacy of danofloxacin treatment of *E. coli* airsacculitis, p. 270. In Proceedings of the 40th Western Poultry Disease Conference.

Vandemaele, F., M. Vereecken, J. Derijcke and B. M. Goddeeris, (2002): Incidence and antibiotic resistance of pathogenic *Escherichia coli* among poultry in Belgium. Vet Rec., 151: 355-356.

Vukina,T., H. J. Barnes, and M. N. Solakoglu, (1998): Intervention decision model to prevent spiking mortality of turkeys. Poult Sci., 77 (7): 950-955.

Wang, C., M. Ewing and S. Y. Aarabi, (2001): *In vitro* susceptibility of avian mycoplasmas to enrofloxacin, sarafloxacin, tylosin, and oxytetracycline. Avian Dis., 45: 456-460.

Wolfson, J. S. and D. C. Hooper, (1985): The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity *in vitro*. Antimicrob Agents Chemother., 28: 581-586.

Wray, C. M. and P. J. Carroll, (1993): *Escherichia coli* isolated from farm animals in England and Wales between 1986 and 1991.Vet Rec., 133.

Wray, C., R. H. Davies and J. D. Corkish, (1996): Enterobacteriaceae, p. 9-43. In F. T. W. Jordan, and M. Pattison (ed.), Poultry diseases. W. B. Saunders, London, United Kingdom.

Yogaratnam, V. (1995): Analysis of the causes of high rates of carcase rejection at a poultry processing plant. Vet Rec., 137: 215-217.

Zhang, L. L., J. R. Zhang, K. Guo, H. Ji, Y. Zhang and S. X. Jiang, (2011): Effects of fluoroquinolones on CYP4501A and 3A in male broilers. Res Vet Sci., 90 (1): 99-105.

Zhao, S., H. Jiang, X. Li, T. Mi, C. Li and J. Shen, (2007): Simultaneous determination of trace levels of 10 quinolones in swine, chicken, and shrimp muscle tissues using HPLC with programmable fluorescence detection. J Agric Food Chem., 55 (10): 3829-3834.

Zhao, S., X. Li, Y. Ra, C. Li, H. Jiang, J. Li, Z. Qu, S. Zhang, F. He, Y. Wan, C. Feng, Z. Zheng and J. Shen, (2009): Developing and optimizing an immunoaffinity cleanup technique for determination of quinolones from chicken muscle. J Agri Food Chem., 57 (2): 365-371.

Zhenling, Z., S. Yongxue, F. BingHu, C. Zhangliu, Z. Zl and B. Fang, (2002): Therapeutic efficacy of sarafloxacin against mixed infection of *Mycoplasma gallisepticum* and *Escherichia coli* in chickens. Chinese J Vet Med., 28: 17-19.

7/12/2011

Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus*

Wagdy K. B. Khalil[1], Wafaa S. Hasheesh[2], Mohamed- Assem S. Marie[2], Hossam H. Abbas[*3] and Eman A. Zahran[4]

[1]Cell Biology Department, National Research Center, 12622 Dokki, Giza, Egypt
[2]Zoology Department, Faculty of Science, Cairo University, Giza, Egypt
[3]Department of Hydrobiology, National Research Center, 12622 Dokki, Giza, Egypt
[4]Egyptian Environmental Affairs Agency
*h3abbas@yahoo.com

Abstract: The present study was designed to explain clearly why methyltestosterone is widely used by the Egyptian producers of farmed tilapia and also to investigate its fate in treated fish to guarantee that no hazards on consumers, producers and on the environment. In this study, samples of untreated and treated Nile tilapia were collected at several time intervals. Water quality parameters were within the acceptable range for fish growth. The present analyses showed highly significant increase in body weight, body length, condition factor (K), HSI and GSI, between different time intervals (April - November, 2009) in the untreated control and treated groups. On the other hand, plasma testosterone and residual concentration of testosterone hormone in muscle showed highly significant differences between the studied months in untreated control and treated groups. Molecular biological analyses revealed that methyltestosterone was able to induce DNA fragmentation and molecular genetic variability (using RAPD- PCR fingerprinting pattern) in the testis tissues of the treated Nile tilapia; *Oreochromis niloticus*, which was higher in the first four studied months than the untreated control tilapia. Additionally, histopathological examination showed no changes and no traces of hormone accumulation in the muscle structure. Testis showed moderate number of spermatozoa followed by increasing in number of spermatozoa at the end of the study.
[Wagdy K. B. Khalil, Wafaa S. Hasheesh, Mohamed-Assem S. Marie, Hossam H. Abbas and Eman A. Zahran, Assessment the impact of 17α-methyltestosterone hormone on growth, hormone concentration, molecular and histopathological changes in muscles and testis of Nile tilapia; *Oreochromis niloticus*. [Life Science Journal, 2011; 8(3): 329-342] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Nile tilapia, 17α- methyltestosterone, Sex reversal, Growth indices, Plasma testosterone, DNA damage, RAPD-PCR.

1. Introduction

Tilapia species constitute a major and important item in the Egyptian fish farming. Tilapias are among the important fishes for aquaculture because of many positive characteristics and have been cultured in more than 100 countries (Altun *et al*., 2006).

The sex of fish can be significant in aquaculture because of differences between males and females in growth rate, size, behavior patterns, and breeding time. Administration of exogenous steroids can be effective in controlling sexual development (Al-ablani and Phelps, 2002). The synthetic steroid 17α- methyltestosterone is a male-specific hormone commonly used to induce sex reversal in teleost fish.

17α-Methyltestosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and the development of male sexual characters. Hanson *et al*. (1984) reported that 10- 60 MT-treatment showed the best growth than control. On the other hand, Dan and Little (2000) who compared the culture performance of different species of stains of *O. niloticus* found that, MT treatment resulted a final size of fish 10.7 % larger than the mixed sex fish.

In a previous study, different doses of 17 α–methyltestosterone hormone (MT) used as a growth promoter was administrated to Nile tilapia; *Oreochromis niloticus* (L.) in fishmeal based pelleted diet for 90 days (Ahmad *et al*., 2002). The applied doses were 0.5, 1.0, 2.5, 5, 10, 20 and 40 mg MT/kg feed. The obtained results showed that only the dose of 5 mg/kg was the optimum effective dose in promoting significant final weight, weight gain and SGR of Nile tilapia (Ahmad *et al*., 2002).

In addition, Marjani *et al*. (2009) studied the effect of different doses of synthetic androgen 17α-Methyl Testosterone (MT), i.e., 50, 75 and 100 mg of hormone per kg of feed, on sex reversal and growth performance of Mozambique tilapia. They found that,

the dose rate 75mg/kg MT feed gave the maximum gain in body weight, i.e. 11.8 g, which is 1.2 times greater than the control.

On the other hands, Curtis *et al.* (1991) and Ahmad *et al*. (2002) found that plasma testosterone concentration is rapidly metabolized and excreted. Also, Rizkalla *et al*. (2004) found that whole body samples of normal fish and those treated for 28 days with 17 α-methyltestosterone (17 α-MT) contained detectable amounts of testosterone only in the first five months after the termination of feeding. Moreover, Rizkalla *et al*. (2004) found that, muscle samples taken from the monosex fish at marketable size, did not differ from the untreated controls and testosterone concentrations were below the detectable level (3ng/g).

Due to the misuse of hormonal treatments in sex reversal of tilapia especially in the Egyptian private sector hatcheries, the main objective of this study is to evaluate human food safety associated with sex reversal of Tilapia and may provide supporting data to approve the use of this hormone in aquaculture.

So, the present study aims to evaluate the effect of MT and its environmental impacts on the Nile tilapia in several aspects: (a) study its effect on the sex reversal ratio; (b) assessment its role as growth promoter on growth performance; (c) determination its concentration in the blood and the different vital organs ; (d) study its effect on the histological alterations; and (c) evaluation of its effect on the DNA damage which lead to abnormal changes of DNA fingerprints.

2. Materials and Methods

Fish

Nile tilapia was collected from World Fish Center Farm (WFC) in El- Abbassa, El-Sharkeya governorate and El- Nubaria fish farm, El-Behera governorate Egypt.

Experimental design and fish and water sampling

The present work was carried out on two groups of Nile tilapia fish; *Oreochromis niloticus* collected from natural condition and controlled farms, respectively a follows: (a) First group: (untreated Control tilapia growing in natural condition away from the hormonal effect) was taken from World Fish Center Farm (WFC) in El- Abbassa, El-Sharkeya governorate. Nile tilapia in this group ranged in length between 10.80 ± 0.26 cm and 22.77± 0.49 cm and ranged in weight between 19.2± 1.1 g and 256.7 ± 12.9 g. (b) second group (treated group): samples of tilapia fish were taken from El- Nubaria fish farm belongs to National Research Center (NRC) farm which is previously used the oral administration of the synthetic androgen (17 α-Methyl testosterone) hormone to produce all male tilapia at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days which results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). This group was with range length between (11.53± 0.23 cm and 25.93 ± 0.78 cm) and range weight between was (25.4 ± 1.9 g and 287.4 ± 10.5 g). Samples of fish were taken through different months (April till November 2009) and also water samples will be taken to analyze all possible parameters to know the water quality using in the two sources and its contents.

Water samples, collected from the different studied sites, were analyzed for water temperature, oxygen content, pH, water hardness, total alkalinity, ammonia, nitrite and electric conductivity according to the method described by Association standard methods American Public Health (APHA, 1995).

Growth indices:

Body weight was recorded to the nearest gram and the total body length was measured to the nearest 0.1 cm for Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

a) Relative growth (RG) and Relative growth rate (RGR) (Busacker *et al*., 1990)**:**

Relative growth (%RG) = $[(W_2-W_1)/ W_1]$ x 100 (% / fish)

Relative growth rate (%RGR) = $[(W_2 –W_1) / (W_1$ x T)] x 100 (% / day)

Where W_1 : Initial weight at the start of the studied period (g).

W_2 : Final weight at the end of the studied period (g).

T : Time of the studied period.

b) Specific growth rate calculations (SGR) (Ahmad *et al*., 2002)**:**

Specific growth rate (SGR) = (ln W_2 – ln W_1) / T x 100 (% / day)

Where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days of the feeding period.

c) Condition factor (k) (Schreck and Moyle, 1990):

K = (W / L^3) X 100

Where W : is the wet weight in g.

L : is the total length in cm.

d) Hepatosomatic index (HSI) (Schreck and Moyle, 1990):

H S I = (Weight of the liver / Total fish weight) X 100

e) Gonadosomatic index (GSI) (Abbas *et al*., 2008)**:**

G S I = (Weight of the gonads / Total fish weight) X 100

Tissue sampling:

Fish were dissected to get male testis and muscles which were kept frozen (- 20 ^{0}C) for determination of residual testosterone in muscle and DNA analyses of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

Determination of plasma testosterone and residual concentration of testosterone in muscle by Coat – A-Count Total Testosterone, Radioimmunoassay procedure:

The Coat-A-Count procedure is a solid-phase radioimmunoassay (RIA), based on testosterone- specific antibody immobilized to the wall of a polypropylene tube. ^{125}l-lable testosterone competes for a fixed time with testosterone in the sample for antibody sites. The tube is then decanted, to separate bound from free, and counted in a gamma counter. The amount of testosterone present in the sample is determined from a calibration curve.

Molecular biological analyses:

I- Quantitative analysis of DNA fragmentation

1-1 Diphenylamine reaction procedure

According to Burton (1956) testis tissues were used to determine the quantitative profile of the DNA fragmentation. The DNA fragmentation was determined in the pellets (P) and the supernatants of the samples. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm wave length using the formula:

$$\%\text{Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} X\ 100$$

1-2 DNA gel electrophoresis laddering assay

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu *et al*., (2002).

2. Random Amplification of Polymorphic DNA (RAPD-PCR) analysis

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook *et al.*, 1989).

To generate RAPD profiles from the rat DNA, two random primer kits (A and C) from Operon Technologies (Operon, Almeda, CA, USA) were used. DNA amplification reactions were performed under conditions reported by Williams *et al*. (1990) and Plotsky *et al*. (1995). PCR amplification was conducted in 50 µl reaction volume containing 100 ng genomic DNA, 100 µM dNTPs, 40 nM primer, 2.5 units of Taq DNA polymerase and 5 µl promega 10X Taq DNA polymearse buffer.

Histopathological Examination:

Muscle and testis of Nile tilapia; *Oreochromis niloticus* , collected from different studied sites were fixed in neutralized formalin, dehydrated, embedded in paraffin wax and sectioned at 5 µm then stained with Haematoxylin and Eosin according to Carleton *et al*. (1967). Testes were classified by developmental stage based on histological criteria adapted from Grier (1981) and Bancroft *et al*., (1996).

Statistical analyses:

The results were statistically analyzed using Duncan's multiple range tests to determine difference in means Statistical Analyses System (SAS, 2000) and Software Program of Statistical Analysis (SPSS, 2008). One way ANOVA test (Analysis of variance) comparing the treated and untreated control groups in all months. Differences in all the studied parameters were assessed by one way ANOVA.

3. Results and Discussion

There is growing attention being given to the impact of pharmaceutically active compounds, including hormones released into the environment via wastewater discharge (Heberer, 2002). Anabolic steroids are potentially useful compounds in aquaculture due to their ability to increase weight gains and muscle deposition of treated fish.

In Egypt, there is a considerable interest in extending the culture of the Nile tilapia; *Oreochromis niloticus*, which gives a good quality fish with a high marketability and excellent growth rates (Kheir *et al*., 1998). Hanson *et al*. (1984) reported that 10- 60 ppm methyltestosterone treatment showed the best growth than control, these are also in line with Dan and Little (2000), who compared the culture performance of different strains of *Oreochromis niloticus* and found that considering all strains, MT treatment resulted in a final size of fish 10.7% larger than mixed sex fish.

Oral administration of the synthetic androgen 17 α-Methyltestosterone at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). Romerio *et al*. (2000) obtained 98% male population in *Oreochromis sp*., at dose rate of 60 mg kg-1 MT of feed. The results of this study showed a significantly lower male proportion (84.3%) for highest dose rate of MT (100 mg kg-1 of feed). These results are in line with the findings of Okoko (1996), who obtained 71.9% males at the dose rate of 120 mg kg-1 MT of feed.

The water quality of the aquatic habitat is considered the main factor controlling the state of health and disease in both cultured and wild fish. Nowadays, deterioration of the natural water resources conditions by the action of effluents discharged from various industries affect the quality and quantity of the fish (Elghobashy *et al*., 2001 and Zaghloul *et al*., 2005). In the current study, physicochemical properties of the water samples from the locations of the different sites were normal and there has been no change during the studied period (Table 1). There is no any negative impact on health and growth of fish naturally in the sampling areas of untreated control and treated fish during the studied period. The present results of water quality in El- Abbassa and El- Nubaria (Table 1) were within the acceptable range for fish growth (Boyd, 1984) and in agreement with Abdel-Tawwab and Ahmad (2009), who found that dissolved oxygen concentrations ranged from 6.6 to 7.4 mg L-1, the ambient water temperature range was 24.5-26.8 ºC, the pH range was 7.8-8.1, unionized ammonia concentration ranged from 0.11 to 0.19 mg L-1 and total alkalinity and total hardness ranges were 250-285 and 235- 290 mg L-1 as CaCO3 respectively.

Table (1) Water Quality Criteria for Water Samples collected from each fish farm for untreated control and treated sampling sites with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* during April till November (2009).

Parameters	**Water quality for untreated control Site (El-Abbassa)**	**Water quality for treated site (El-Nubaria)**
Temperature (^{0}C)	(19-24) 22.00 ± 0.68	(19-27) 23.3 ± 1.23
Dissolved Oxygen (mg/L)	(6.11-8.2) 6.86 ± 0.32	(5.8-7.3) 6.6 ± 0.24
pH	(6.82 – 7.8) 6.85 ± 0.26	(6.45-7.8) 7.3 ± 0.14
Ammonia (mg/L)	(1.1 – 1.4) 1.27 ± 0.05	(1.3- 2.4) 1.9 ± 0.14
Total Hardness (mg/L as CaCO3)	(111 – 132) 120.00 ± 3.24	(129 – 161) 147.2 ± 4.48
Total Alkalinity (mg/L as CaCO3)	(186 -206) 189.33 ± 4.10	(196 – 228) 216.8 ± 4.53

- Data are represented as means of six samples ± SE.
- Student's t-Test between the two groups of the same parameter in the two studied sites for the whole studied period.

The obtained results in the present study revealed that administration of 17 α–methyltestosterone (MT) induced significant increase in fish growth of treated Nile tilapia (Table 2). These results are in agreement with the previous studies (Woo *et al*., 1993; Satpathy *et al*., 1995; Sambhu and Jayaprakas, 1997; Ahmad *et al*., 2002). In addition, methyletestosterone has been reported to enhance growth of various fish species such as Nile tilapia; *Oreochromis niloticus* (Tayamen and Shelton, 1978). The increase in body weight gain (Table 2) may attribute to that androgenic steroids enhance the release of growth hormone from the pituitary somatotrops of fish and/or induce the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976).

The values of the condition factor "k" are estimated for comparative purposes to assess the impact of environmental alterations on fish performance (Clark and Fraser, 1983). Therefore, the fluctuation in "k" may reflect the health condition of the fish as well as their protein and lipid contents (Weatherley and Gill, 1983). In the present study, the condition factor of the Nile tilapia, *Oreochromis niloticus*, collected from the different studied sites (Table 3) showed a significant difference in k values of fish collected from the different studied sites throughout the studied period. Steroid treatments have increased the condition factor of some salmonids (Fagerlund and McBride, 1975& 1977; Saunders *et al*., 1977) suggesting the increase in the percentage muscle of the body (Ahmad *et al*., 2002). The present results were also nearly similar with the

results which obtained by Winfree and Stickney (1981) who found the K values ranged from 1.8 to 2.85 in *Tilapia aurea*. The differences may be due to differences in season. We found that the significant gradual increase in (k) values of treated fish in September and that of untreated control fish collected in August and November may be due to the natural increase in the growth parameters of untreated control and treated fish as a result of natural growth during the studied period.

Hepatosomatic index (HSI) is another biological parameter that helps in studying growth of fish (Weatherley and Gill, 1987). Hepatosomatic index of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3) showed progressive natural increase appeared in the untreated control and treated fish that amounted to a significant increase in the treated fish in the last three months of the study. This is in agreement with Ahmad *et al*. (2002) who found that, HSI was significantly changed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed) and slightly increase at high MT doses (5, 10, 20 and 40 mg MT/kg feed).

Gonadosomatic index (GSI) of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3) showed a progressive natural increase in the untreated control and treated fish, which amounted to a significant increase in the treated fish in the last month of the study (marketing season) and this is may be due to the effect of using 17α-methyltestosteronehormone.

The effect of 17α-methyltestosterone on the gonads appears to be complex (Ahmad *et al*., 2002). Macintosh *et al*. (1988) showed the higher level of 17-alpha methyltestosterone (60 mg/Kg of feed) produced some testicular degeneration which lowered the GSI value. Ahmad *et al*. (2002) found that male and female GSI was significantly decreased at high MT doses (5, 10, 20 and 40 mg MT/kg feed), while non-significant change were observed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed).

Table (2): Relative growth rate (RGR) and specific growth rate (SGR) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El-Nubaria fish farms during April till November (2009).

Parameter / Months	Mean weight (g) of Untreated control samples (El- Abbassa)	Mean weight (g) of treated samples (El- Nubaria)
April (Initial Mean)	19.2 ±1.1	20.1 ± 0.1
November (Final Mean)	256.7±12.9 **	287.4 ±10.5 **
Weight gain (g)	237.5	267.3
Relative growth (%RG)	206 % per fish	222 % per fish
Relative growth rate (%RGR)	5.2 % per day	5.5 % per day
Specific growth rate (SGR)	1.08 % per day	1.11 % per day

** Highly significant difference at P<0.01 between initial and final weight.

Table (3): Condition factor (k) , Hepatosomatic index (HSI) and Gonadosomatic index (GSI) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	(K) in control samples (El- Abbassa)	(K) in treated samples (El- Nubaria)	(HSI) in control samples (El-Abbassa)	(HSI) in treated samples (El- Nubaria)	(GSI) in control Samples (El- Abbassa)	(GSI) in Treated Samples (El- Nubaria)
April	1.52 ±0.05 ^{c}	1.64 ±0.05 ^{cd}	0.64 ±0.16 ^{c}	0.74 ±0.03^{b} *	0.16±0.01 ^{b}	0.17±0.03 ^{b}
May	1.88 ±0.10 ^{b}	1.55 ±0.08 ^{d} *	1.16 ±0.15 ^{b}	0.69 ±0.10 ^{b} **	0.19±0.01 ^{b}	0.1±0.02 ^{b}
June	1.86 ±0.06 ^{b}	1.69 ±0.06 ^{cd}	1.67 ±0.15 ^{b}	0.97 ±0.08 ^{b} **	0.17±0.02 ^{b}	0.13±0.01 ^{b}
July	1.99 ±0.06 ^{ab}	1.93 ±0.03 ^{bc}	1.71 ±0.25 ^{b}	0.83 ±0.12 ^{b} **	0.19±0.01^{b}	0.13±0.01^{b}
August	2.18 ±0.05 ^{a}	1.78 ±0.08 ^{bcd} **	1.51 ±0.15 ^{b}	0.59 ±0.04 ^{b} **	0.25±0.07 ^{b}	0.14 ±0.01^{b} **
September	1.98 ±0.05 ^{ab}	2.28 ±0.13 ^{a}	1.0 ±0.18 ^{bc}	2.44 ±0.36^{a} **	0.33±0.05 ^{c}	0.18±0.02^{b}
October	1.99 ±0.05 ^{ab}	2.03 ±0.08 ^{ab}	1.88 ±0.31 ^{bc}	2.50 ±0.32 ^{a} **	0.41±0.05 ^{b}	0.28 ±0.05 ^{c} **
November	2.17 ±0.10 ^{a}	1.68 ±0.14 ^{cd} *	2.34 ±0.23 ^{a}	2.44 ±0.31 ^{a}	0.67±0.14 ^{a}	1.01±0.45 ^{a}
F- Values	8.093**	7.057**	4.583**	15.896**	6.788**	3.594**

Data are represented as means of six samples ± SE.
Means with the same letter for each parameter in the same column between all months are non-significant different (P > 0.05); otherwise they do (SAS, 2000).
Student's t-Test between the two groups in the same month for the whole studied period.
One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
* Significant difference at P<0.05 ** Highly significant difference at P<0.01.

Regarding to the concentration of the plasma testosterone hormone of the Nile tilapia collected from the different studied sites (Table 4) there is a significance difference in the concentrations of the untreated control and treated fish. There is a significant increase in the hormone concentrations of untreated control fish during June and July. This is may be due to male pairing with several females during mating season which starts in April or May and continue into September and October depending on water temperature as well as on the length of lighting period. This result is in agreement with the examination of the histopathological section of testis in the present study through these studied months. However, in treated fish the hormone concentration was high only in the first month of treatment, then gradually decreased till the end of the studied period. The plasma testosterone concentration seems not existing (detectable amounts), which indicates that, there is no effect of the used hormone at the end of the study period. The rapid metabolism and excretion of MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001). Ahmad *et al*. (2002) also reported that digested MT is rapidly metabolized and excreted. These results are in accordance with Rizkalla *et al*. (2004), who reported that whole body samples of normal fish and those treated for 28 days with 17 α-methyltestosterone (17 α-MT) contained detectable amounts of testosterone only on the first five months after the termination of the feeding with 17 α- methyltestosterone.

Table (4) Plasma concentration of the testosterone hormone (ng/ml) and Residual concentration of testosterone in muscles (ng/g) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma testosterone in control samples (El- Abbassa)	Plasma testosterone in treated samples (El- Nubaria)	Residual testosterone in muscles in control samples (El- Abbassa)	Residual testosterone in muscles in treated samples (El- Nubaria)
April	7.16 ±0.58 ^{c}	16.42 ±0.71 ^{a} **	0.95 ±0.07 ^{e}	2.05 ±0.20 ^{ab} **
May	6.35 ±0.29 ^{cd}	7.26 ±0.59 ^{b}	0.64 ±0.04 ^{e}	0.86 ±0.03 ^{c} **
June	30.39 ±0.63 ^{a}	6.50 ±0.31 ^{bc} **	2.60 ±0.58 ^{cd}	1.78 ±0.29 ^{b}
July	20.64 ±0.86 ^{b}	5.31 ±0.36 ^{cd} **	4.09 ±0.60 ^{b}	1.91 ±0.13 ^{ab} **
August	4.94 ±0.32 ^{de}	5.55 ±0.35 ^{cd}	4.44 ±0.33 ^{b}	0.83 ±0.05 ^{c} **
September	3.42 ±0.76 ^{ef}	4.41 ±0.39 ^{d}	5.61 ±0.33 ^{a}	1.38 ±0.36 ^{bc} **
October	2.09 ±0.06 ^{fg}	2.56 ±0.31 ^{e}	3.63 ±0.32 ^{bc}	2.67 ±0.41 ^{a}
November	1.10 ±0.07 ^{g}	1.29 ±0.15 ^{e}	1.52 ±0.30 ^{ed}	1.44 ±0.33 ^{bc}
F-Values	** **381.804**	** **112.483**	** **22.762**	** **5.438**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

It is apparent from the previous literatures that 17 α- methyltestosterone has an important economic potential for use in fish culture. However, before this synthetic steroid is used on a large scale in Egypt, and although there is a very long interval between treatment and harvest, it is necessary to investigate its fate in treated fish to guarantee that no hazards for consumer if residues remain in the destined for marketing as reported by Rizkalla *et al*. (2004). Literature regarding steroid residues in tissues of fish treated with androgenic steroids has been reviewed by Donaldson *et al*. (1979) and Higgs *et al*. (1982). They indicated that steroids are rapidly metabolized and /or excreted from fish tissues. 17 α-methyltestosterone was eliminated from tissues more slowly than testosterone (Fagerlund and McBride, 1978 and Fagerlund and Dye, 1979), and it was postulated that the presence of the 17 α- methyl group enhance efficacy of the synthetic steroid over endrogenous steroids (Donaldson *et al*., 1979). Hormone elimination is rapid in fish because it is believed to occur mainly via excretion in the faeces and via the gills (Cravedi *et al*., 1993).This rapid metabolism and excretion of methyltestosterone by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001).

In the current work, residual concentration of the testosterone hormone in muscles of the Nile tilapia collected from the different studied sites (Table 4), there is a significance difference in the residual concentration of testosterone hormone in muscles in the untreated and treated fish. The

presence of hormone residues is not high in muscle tissue of the untreated and treated fish and decreased in the last month of the study which is the marketing season for this fish. This indicates that there is no effect of the used hormone at the end of the studied period. These findings were in accordance with Johnstone *et al*. (1983), who found > 95% of the radio-labeled MT in the viscera and no radioactivity could be found 50 h post-treatment. They were also in agreement with Goudie *et al*. (1986), who reported that tilapia fry fed for 30 days a feed containing radioactive labeled methyltestosterone showed a rapid depletion of radioactive methyltestosterone from tilapia muscle with only a trace of methyltestosterone could be found.

El- Nemr *et al*. (1999) recorded that the residual value of 17 α- methyltestosterone in *Oreochromis niloticus* fry muscle was significantly dropped after 6 weeks withdrawal period and still higher than control group. Our results agree also with Rizkalla *et al*. (2004), who reported that muscle samples taken from the monosex fish did not differ from the untreated controls and testosterone concentrations were below the detectable level (3ng/g). Based on the scientific evidence that methyltestosterone is rapidly eliminated from fish, and therefore, there is no possibility that methyltestosterone will persist in adult fish after the several months required for farmed tilapias to reach marketable size.

The molecular genetic variability of the treated tilapia genomes and their control were evaluated using two primer kits (A and C). Only five of these primers (10-mer random primers: (A04: 5'-AAT CGG GCTG-3', A08: 5'-GTG ACG TAGG-3', A10: 5'-GTG ATC GCAG-3', C09: 5'-CTC ACC GTCC-3', C12): 5'-TGT CAT CCCC-3') gave positive and detectable bands (Fig. 3). PCR-based techniques, such as RAPDs, have previously allowed the discrimination as well as estimation of genetic variation attributed to genotoxic elements. The exposure to genotoxic agents will give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprints. Therefore, we have applied the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects.

Methyltestosterone usage in tilapia farming is expected to continue to increase rapidly as the global demand for large whole tilapia and tilapia fillets grows. Despite widespread use of the androgen 17α-methyltestosterone (MT) in tilapia farming, the implications of tilapia hormone treatment in relation to human health and the environment have not been well articulated to the fish trade or the general public.

The molecular biological results of the present study (Table 5 and Figs. 1, 2 &3) revealed that methyltestosterone was able to induce DNA fragmentation in testes of Nile tilapia in the first four studied months after MT treatment to induce sex reversal in farmed tilapias compared to the untreated control tilapia. In addition, the molecular genetic variability (using RAPD fingerprinting pattern) among the treated tilapia (in liver and testes tissues) was higher in the first four studied months after treatment than the untreated control tilapia.

For our knowledge, there are no data regarding the effect of methyltestosterone on the DNA damage in fish especially Nile tilapia. However, it could be postulated that the methyltestosterone residues were still existed in the fish tissues and/or in the fish environment up to the first four months after treatment and then began to be disappeared, whereas the DNA fragmentation decreased after the first four studied months.

The action mechanism of testosterone treatment inducing genetic toxicity during the first months of age in tilapia (tilapia fry) is not investigated yet. In the present study, the negative effect of testosterone induced DNA damage may be attributed to the weakness in the immune system which may not be completed in growth yet. The main way in which steroid hormones interact with cells is by binding to proteins called steroid receptors. When steroids bind to these receptors, the proteins move into the cell nucleus and either alter the expression of genes (Lavery and McEwan, 2005) or activate processes that send signals to other parts of the cell (Cheskis, 2004) cause genetic toxicity. Beg *et al* (2008) reported that the possible genotoxicity of testosterone is depend on the metabolic activation.

The results of the present study (Figs. 1, 2 &3) revealed that the DNA damage attributed to methyltestosterone treatment was markedly disappeared after the first four studied months until it reached a relative stability rate similar to control untreated tilapia. It could be explained that the methyltestosterone residues in the fish tissues and/or in the fish environment were removed. Furthermore, disappearance of the DNA damage may be attributed to the increase of the immunity defense in the growing fish.

In agreement with our results, Hana *et al* (2008) reported that there were no significant differences in the frequency of total chromosomal aberrations between control and testosterone propionate-treated adult mice. In addition, they found that the molecular genetic variability using RAPD-PCR among the testosterone-treated adult mice was similar to control untreated mice. Whereas, all of the oligodecamers used revealed monomorphic bands in

the control samples and those treated with testosterone propionate.

Additionally, histopathological examination or biomarkers have been increasingly recognized as a valuable tool for field assessment of the impact of using 17α-methyltestosterone hormone on fish organs (Heath, 1995; Schwaiger *et al*., 1996 and Teh *et al*., 1997). The investigated biochemical and physiological changes were confirmed by histopathological alterations of muscle, liver and testis of the Nile tilapia collected from the two fish farms.

Table (5): Effect of the 17 α methyl testosterone hormone on the DNA fragmentation ratio in testis tissues collected from Nile tilapia; *Oreochromis niloticus,* for several time intervals (April - November, 2009).

Parameter / Months	DNA fragmentation % in testis tissue for control samples (El- Abbassa)	DNA fragmentation % in testis tissue for treated samples (El- Nubaria)
April	10.66 ±0.33^{a}	12.66 ±0.33^{a}
May	10.00 ±0.57^{a}	12.66 ±0.33^{a}
June	10.66 ±0.33^{a}	11.66 ±0.33^{ab}
July	10.33 ±0.33^{a}	11.66 ±0.33 ^{ab}
August	10.33 ±0.33^{a}	10.66 ±0.33^{b}
September	10.66 ±0.33^{a}	10.66 ±0.33^{b}
October	10.66 ±0.33^{a}	10.66 ±0.33^{b}
November	10.66 ±0.33^{a}	10.66 ±0.33^{b}
F-Values	6.843 **	6.614 **

- Data are represented as means of six samples ± SE. Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000). Student's t-Test between the two groups in the same month for the whole studied period. One way ANOVA test (F-value) between all months in each group separately for the whole studied period. * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

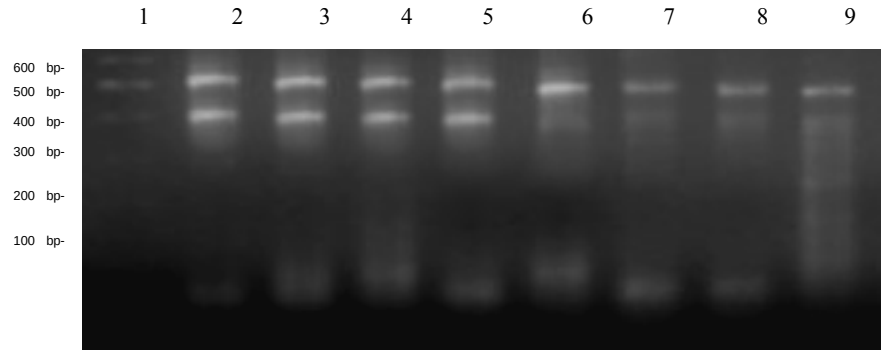


Figure (1): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from testis exposed to testosterone in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent testis tissues collected from April to November (2009).

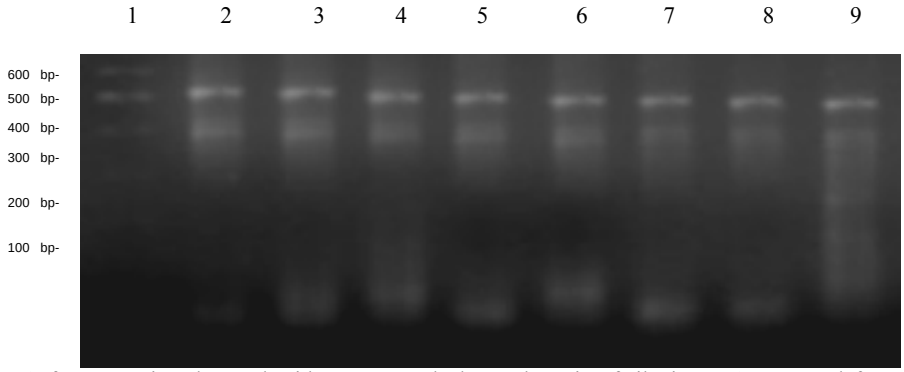


Figure (2): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from untreated testis in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent testis tissues collected from April to November (2009).

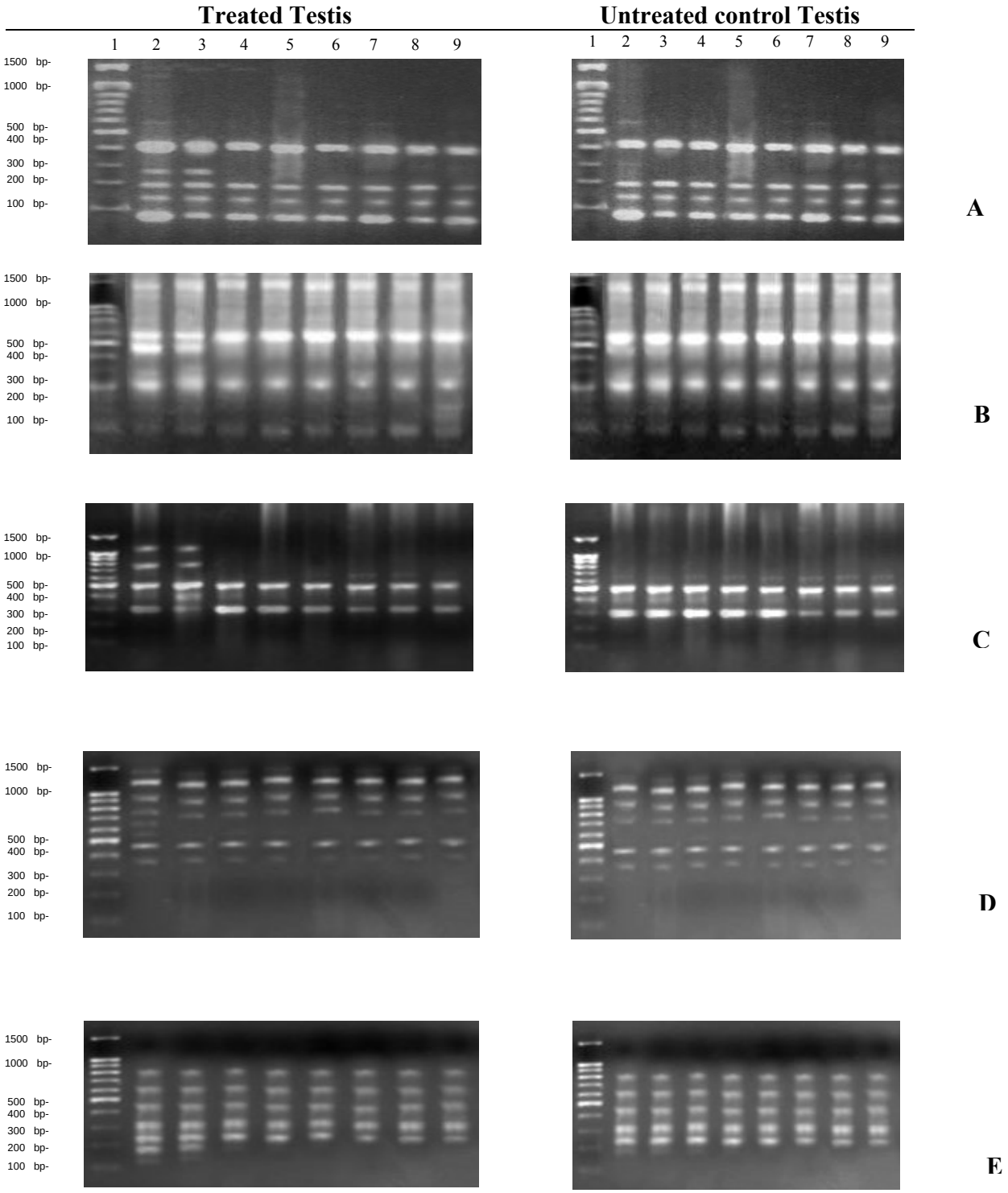


Figure (3). Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA. (A) Represents PCR products with primer A04, (B) Represents PCR products with primer A08, (C) Represents PCR products with primer A10, (D) Represents PCR products with primer C09, (E) Represents PCR products with primer C12. The DNA marker is in lane 1. Lane 2 to Lane 9 represent months of collection (April till November, respectively) of fish testis tissue samples treated with 17 α- methyltestosterone throughout the period of study.

The histopathological examination revealed that muscles sections of control and treated fish showed normal structure throughout the experimental period (Photomicrograph 1). There was no change in the composition of the muscle, and also there were no traces of hormone accumulation in the muscle tissue of fish during the studied period. These results are in agreement with Curtis *et al*. (1991), who reported that digested methyltestosterone is rapidly metabolized and excreted. This rapid metabolism and excretion of methyltestosterone by a fish treated early in its life history combined with the extended period needed to produce a marketable size fish results in a safe consumer product (Phelps, 2001).

The histopathological finding showed that the effect of 17 ∝-methyltestosterone on the gonads appears to be complex. Testes shown to be bilobed with spermatogonia dispersed throughout the gametogenic epithelium of the seminiferous tubules. Each testicular lobe covered luminally by an epithelium consisting of primary germ cells and Sertoli cells. The highest number of spermatogonia was found at the apical ends of the tubules. During maturation extensions of Sertoli cells surround single or small groups of B-spermatogonia, forming the spermatocysts, the final dimensions of which reflect the final number of contained spermatozoids (Fishelson *et al*., 2006).

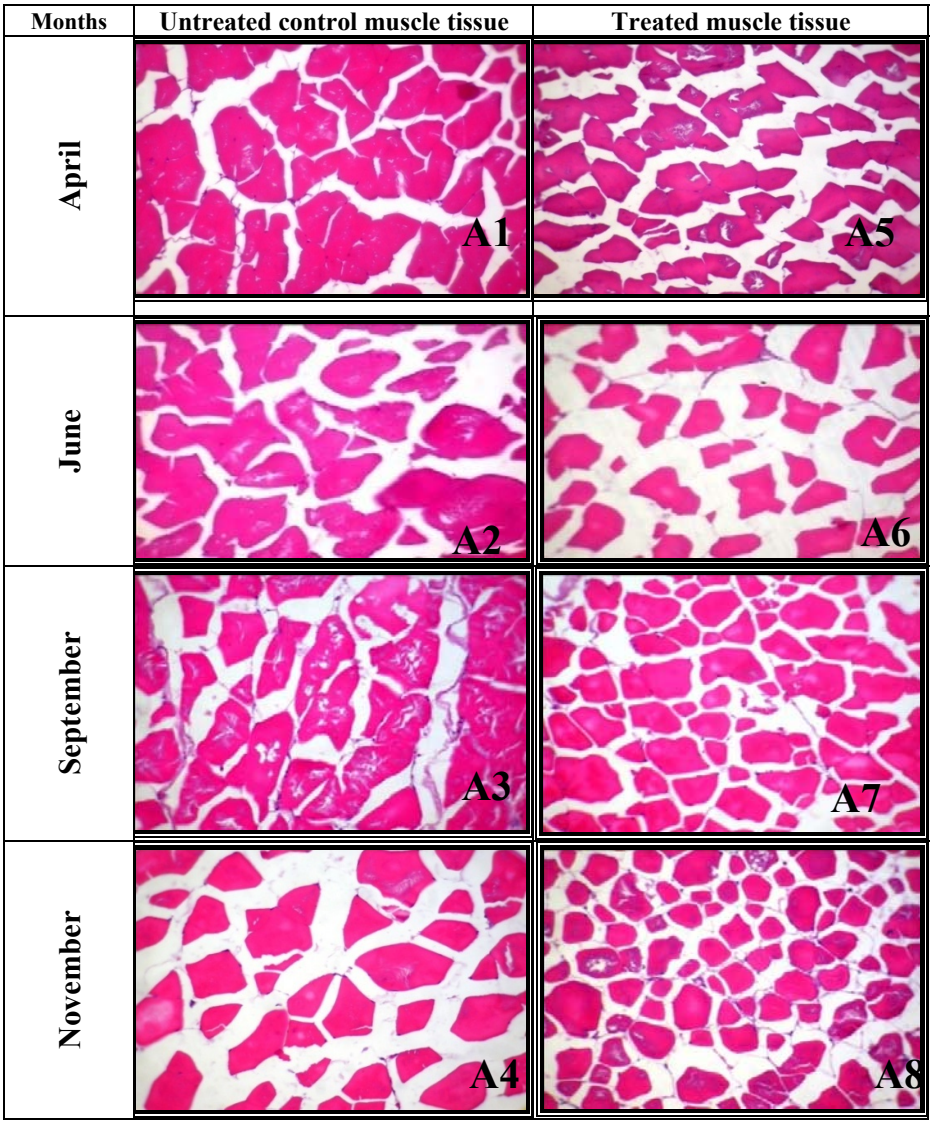
Regarding the control samples the histopathological examination showed moderate number of spermatozoa inside the seminiferous tubules and which followed by increasing in number of spermatozoa inside seminiferous tubules this appear especially in June and July (Photomicrograph 2). This confirmed by the present results of the plasma testosterone analysis.

Testis sections of treated fish collected from the different studied sites showed moderate number of spermatozoa inside seminiferous tubules which followed by high density of spermatozoa inside seminiferous tubules till the end of the studied period (Photomicrograph 2). Generally this increase reflects the enhancing effect of using 17 α-methyltestosterone hormone.

Our results were in agreement with Yamazaki (1972) in pink and chum salmon and by Hirose and Hibiya (1968a & 1968b) in goldfish and rainbow trout, who reported that MT administration of 2.5 mg/kg induced the degenerative changes in the ovaries and testes. Furthermore, Higgs *et al*. (1977) found clear signs of gonads degeneration in coho salmon affected by MT causing fish sterility, which might be considered advantageous in fish culture because less food energy would be channeled into gonodal development and therefore more would be available for body growth.

The above results demonstrated that 17α-methyltestosterone has an important economic potential for use in fish culture. Also, methyltestosterone treatment in tilapia farming is considered to be entirely safe provided the following recommended best practices are adopted by producers: (1) They restrict tilapia methyltestosterone treatment to the early fry stages. (2) They limit the dosage of methyltestosterone used to a maximum of 60 mg methyltestosterone /kg fry feed. (3) They rear methyltestosterone treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish. (4) As a precautionary measure, adopt safe handling protocols when preparing and administering methyltestosterone treated tilapia feed; use latex gloves and a protective face mask to avoid dermal contact or inhalation of methyltestosterone. (5) They keep a careful inventory of the amounts of methyltestosterone supplied to and used in each tilapia hatchery, and ensure that access of the hormone supply and record-keeping are controlled by the farm manager or hatchery supervisor. (6) They avoid direct release of hatchery water used for methyltestosterone treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

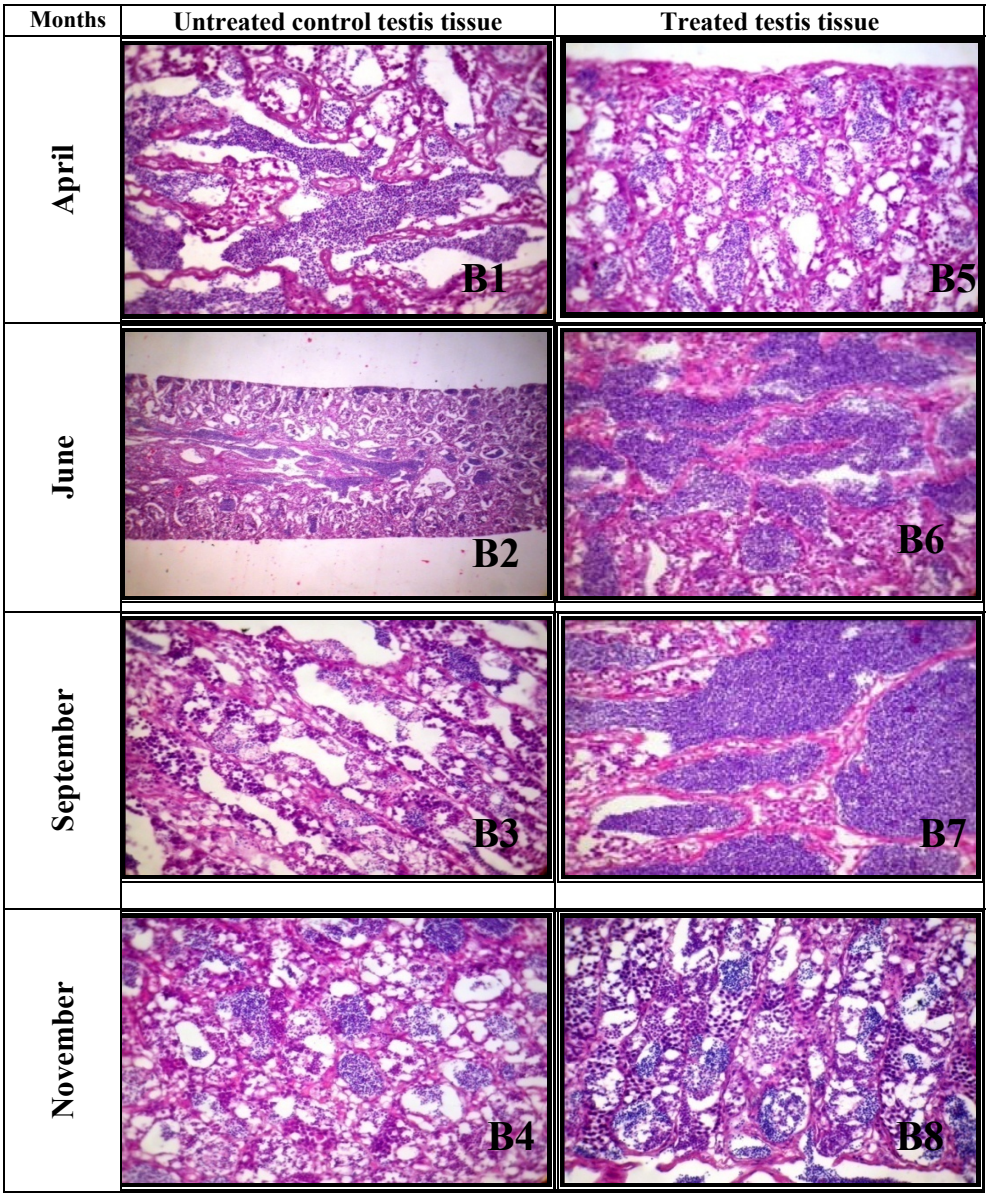
Corresponding author
Hossam H. Abbas
Department of Hydrobiology, National Research Center, 12622 Dokki, Giza, Egypt
h3abbas@yahoo.com



Photomicrograph (1):
Histological sections in skeletal muscle tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(A1) Skeletal muscles showing normal structure (H&E 400X).
(A2) Skeletal muscles showing normal structure (H&E 400X).
(A3) Skeletal muscles showing normal structure (H&E 400X).
(A4) Skeletal muscles showing normal structure (H&E 400X).

Treated
(A5) Skeletal muscles showing normal structure (H&E 400X).
(A6) Skeletal muscles showing normal structure (H&E 400X).
(A7) Skeletal muscles showing normal structure (H&E 400X).
(A8) Skeletal muscles showing normal structure (H&E 400X)



Photomicrograph (2): Histological sections in testis tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(B1) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B2) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 200X).
(B3) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B4) Testis showing increased number of spermatozoa inside seminiferous tubules (H&E 400X).

Treated
(B5) Testis showing moderate number of spermatozoa inside seminiferous tubules (H&E 400X).
(B6) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).
(B7) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).
(B8) Testis showing high density of spermatozoa inside seminiferous tubules (H&E 400X).

References

Abbas, H. H.; Ali, F. K. and Kenawy, A. M. (2008): Assessment Study about Using Underground Water for Tilapia Culture for the First Time in El-Bahria Oasis desert, Egypt, Nutrition and Health., 19: 237–256.

Abdel-Tawwab, M. and Ahmad, M. H. (2009): Effect of dietary protein regime during the growing period on growth performance, feed utilization and whole-body chemical composition of Nile Tilapia; *Oreochromis niloticus* (L.) Aquacul. Res., 40: 1532-1537.

Ahmad, M.H.; Shalaby, A.M.E.; Khattab, Y.A.E. and Abdel-Tawwab, M. (2002): Effects of 17 α–methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus* L.). Egypt. J. Aquat. Biol. Fish, 4(4): 295-311.

Al-ablani, S.A. and Phelps, R.P. (2002): Paradoxes in exogenous androgen treatments of bluegill. *J. Appl. Ichthyol.*, 18:61-64.

Altun,T. ; Tekelioğlu,N. and Danabaş,D.(2006): Tilapia Culture and Its Problems in Turkey. E.U. J. Fish.Aquat. Sci., 23 (3-4): 473–478.

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation (1995): Standard Methods for the Examination of Water and Wastewater. 19th edition, New York, N.Y.

Bancroft, D.; Stevens, A. and Turner, R. (1996): Theory and Practice of Histological Techniques, 4th edn. Churchill Livingstone: Edinburgh,London, Melbourne.

Beg, T.; Siddique, Y. H.; Ara, G. ; Afzal, M. and Gupta, J. (2008): Antioxidant Effect of ECG on Testosterone Propionate Induced Chromosome Damage. Human Genetics and Toxicology Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh 202002 (UP), India.Interna.J.Phamacol. 4 (4): 258-263.

Boyd, C.E. (1984): Water Quality in Warm water Fishponds. Auburn University, Agricultural Experimental Station, Auburn, AL, USA.

Burton, K. (1956): The study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62: 615–623.

Busacker, G.P.; Adelman, I.R. and Goolish, E.M. (1990): Growth. In: Methods for Fish Biology. Schreck, C.B. and P.B. Moyle (Eds.), American Fisheries Society, USA., pp: 363-387.

Carleton, M.; Drary, Y.; Wallington, E.A. and Cammeron, H. (1967): Carleton's Histological Technique, 4th ed., Oxford Univ. Press, New York, Toronto.

Cheskis, B. (2004): Regulation of cell signaling cascades by steroid hormones. J. Cellular Biochem., 93 (1): 20–7.

Clark, E.R. and Fraser, J.A.L. (1983): The survival and growth of six species of freshwater fish, in tap water and diluted and undiluted effluents from sewage percolating filters.J.Fish Biol., (22): 431-445.

Cravedi, J.P.; Delous, G.; Debrauwer, L. and prome, D. (1993): Biotransformation and branchial excretion of 17-alpha methytestosterone in trout. Drug Meta.Disposition, 21: 377-385.

Curtis, L.R.; Diren, F.T.; Hurley, M.D.; Seimand, W.K. and Tubb, R.A. (1991): Disposition and elimination of 17 ∝-testosterone in Nile tilapia (*Oreochromis niloticus*). Aquacul., 99: 193-201.

Dan, N.C. and Little, D.C. (2000): The culture performance of mono-sex and mixed-sex new season and overwintered fry in three strains of *Nile tilapia in* North Vietnam. Aquacul., 184: 221-231.

Donaldson, E.M.; Fagerlund, U.H.M.; Higgs, D.A. and McBride, J.R. (1979): Hormonal enhancement of gorwth in fish In: "Fish Physiology", Vol. 8, pp. 455-598 Edited by W.S. Hoar. D.J. Randall and J.R. Brett Academic Press Inc, New York and London.

Elghobashy, A.H.; Zaghloul, K.H. and Metwally, M.A. (2001): Effect of some water pollutants on the Nile tilapia; *Oreochromis niloticus* collected from the river Nile and some Egyptian Lakes.Egypt.J.Aquat. Biol. Fish., 5(4): 251- 279.

El-Nemr, I.Z; Haleem, H.H. and Abdou, K.A.H. (1999): Evaluation of the effect of 17 α-methyltestosterone on monosex, mortality rate and residual values in tilapia (*Oreochromis niloticus*). Beni- Suef Vet.Med. J., 9(2):25-33.

Fagerlund, U.H.M. and McBride, J.R. (1975): Growth increments and some flesh characteristics of juvenile coho salmon receiving diets supplemented with 17 α-methyltestosterone. J. Fish Biol., 7: 305-314.

Fagerlund, U.H.M. and McBride, J.R. (1977): Effect of 17 α-methyltestosterone on growth, gonad development, external features and proximate composition of muscle of steelhead trout, coho and pink salmon. Fish. Mar. Serv. Tech. Rep., 716: 36-39.

Fagerlund, U.H.M. and McBride, J.R. (1978): Distribution and disappearance radioactivity in blood and tissues of coho salmon (*Oncrhynchus kisutchi*) after oral administration of ^{3}H-testosterone. J.Fish Res. Bd. Can., 35: 893-900.

Fagerlund, U.H.M.; Dye, H.M. (1979): Depletion of radioactivity from yearling coho salmon (*Oncrhynchus kisutchi)* after extended ingestion of anabolically effective doses of 17a-methyltestosterone-1,2-^{3}H. Aquacul., 18: 303–315.

Fishelson, L.; Delarea, Y. and Gon, O. (2006): Testis structure, spermatogenesis, spermatocytogenesis, and sperm structure in cardinal fish (Apogonidae, Perciformes). Department of Zoology and Electronmicroscopy Unit, George S. Wise Faculty of Life Sciences, Tel Aviv University, 69978 Tel Aviv, Israel. fishelv@post.tau.ac.il. Anat Embryol (Berl). 211(1):31-46.

Goudie, C.A.; Shelton, W.L. and Parker, N.C. (1986): Tissue distribution and elimination of radiolabelled methyltestosterone fed to sexually undifferentiated blue tilapia. Aquacul., 58: 215-226.

Grier, H.J. (1981): Cellular organization of the testis and spermatogenesis in fishes. *Am.Zool.*, 21:345-357.

Hana, Y.H.; Khalil, W. K.B.; Elmakawy, A. I. and Elmegeed, G. A. (2008): Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. J. Steroid Biochem. Mol. Biol., 110: 284–294.

Hanson, R.; Sinith Evmanm, R.O.; Shelton, H.W. and Dunham, R.A. (1984): Growth comparison of monosex tilapia produced by separation of sexes hybridization and sex reversal. M.Sc. Thesis, Auburn University, pp: 570.

Heath, A.G. (1995): Water Pollution and Fish Physiology. CRC. Press. Inc. Boca Raton, Florida. 359pp.

Heberer, T. (2002): Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.,* 131: 5–17.

Higgs, D.A.; Fagerlund, U.H.M.; Eales, J.G. and McBride, J.R.; (1982): Application of thyroid and steroid hormones as anabolic agents in fish culture. Comp.Biochem.Physiol., 73 B (1):143-176.

Higgs, D.A.; Fagerlund, U.H.M.; McBride, J.R.; Dye, H.M. and Donaldson, E.M. (1977): Influence of combinations of bovine growth hormone, 17 α-methyltestosterone and L-thyroxine on growth of young coho salmon (*Oncorhynchus kisutch*). Can. J. Zool., 55: 1048-1056.

Hirose, K. and Hibiya, T. (1968 a): Physiological studies on growth promoting effect of protein-anabolic steroids on fish. I. Effects on goldfish. Bull. Jap. Soc. Sci. Fish., 34: 466-472.

Hirose, K. and Hibiya, T. (1968 b): Physiological studies on growth promoting effect of protein-anabolic steroids on fish. I. Effects of 4-chlorotestosterone acetate on rainbow trout. Bull. Jap. Soc. Sci. Fish., 34: 473-481.

Johnstone, R.; Macintosh, D.J. and Wright, R.S., (1983): Elimination of orally administered 17α-Methyltestosterone by *Oreochromis mossambicus* (Tilapia) and Salmo gairdneri (rainbow trout) juveniles. Aquacul., 35: 249-257.

Kheir, M.T.; Mechail, M.M. and Abo-Hegab, S. (1998): Growth of adult and newly hatched fry of Oreochromis niloticus reared at different saline concentrations. Egypt. J. Zool., (30):107- 115.

Lavery, D.N. and McEwan, I.J. (2005): Structure and function of steroid receptor AF1 transactivation domains: induction of active conformation. Biochemical J., 391 (Pt 3): 449–64.

Lu, T.; Xu, Y. and Mericle, M.T. (2002): Quantitative analysis of DNA fragmentation. Biochimica. et Biophysica. Acta., 1590: 16-20.

Macintosh, D.J. and Vargheso, G.P.S. (1985): Rao hormonal sex reversal of wild spawned tilapia in India. J. Fish Biol., 28: 87-94.

Macintosh, D.J.; Singh,T.B.; Little, D.C. and Edwards, P.(1988): Growth and sexual development of 17 ∝-methyltestosterone and progesterone- treated Nile tilapia (*Oreochromis niloticus*) reared in earhen ponds. In R.S.V. pullin, T. Bhukaswan, K. Tongothai and J.L.Maclean (Editors), The second International Symposium on Tilapia in Aquaculture, Bangkok, Thailand,Manila, Philippines.pp. 457- 463.

Marjani, M.; Jamili, S.; Mostafavi, P.G.; Ramin, M. and Mashinchian, A. (2009): Influence of 17-alpha methyl testosterone on masculinization and growth in Tilapia (*Oreochromis mossambicus*). J. Fish. Aquat. Sci., 4(1): 71-74.

Okoko, M. (1996): Effect of 17-alpha methyltestosterone concentrations on the sex ratio and gonadal development of Nile tilapia; *Oreochromis niloticus*. M.Sc. Thesis. Auburn University, Alabama, US.

Phelps, R.P. (2001): Sex reversal: the directed control of gonodal development in tilapia. Pages 35-60. In: D.E. Meyer (ed.). Proceedings for Tilapia Sessions from the 6th Central American Aquaculture Symposium. 22-24 August 2001, Tegucigalpa, Honduras.

Plotsky, Y.; Kaiser, M.G. and Lamont, S.J. (1995): Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics, 26: 163-170.

Popma, T.J. and Green, B.W. (1990):. Sex reversal of tilapia in earthen ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35, Auburn University, Alabama, pp: 15.

Rizkalla, E.H.; Haleem, H.H.; Abdel-Halim, A.M.M and Youssef, R.H. (2004): Evaluation of using 17α-methyl testosterone for monosex *Oreochromis niloticus* fry production. .Egypt.Ger.Soc.Zool., 43(a): 315-335.

Romerio, M.P.; Fencrich-Verani, C.S.N.; Santo De-Copmus, B.E. and Pasilva, A.S. (2000):

Masculinization of Nile tilapia, using different diets and different doses of MT. Revista Brasileira de Zootecnia, 29: 654-659.

Sambhu, C. and Jayaprakas, V. (1997): Dietary supplementation of testosterone propionate on growth performance of white prawn; *Panaeus indicus* (M. Edwards). Indian J. Experimental. Biol., 35(12): 1353-1358.

Sambrook, L.; Fritsch, E.F. and Manitatis, T. (1989): Molecular cloning: A laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Satpathy, B.B.; Mukhopadhyay, P.K. and Ray, A.K. (1995): Nutritive utilization and growth bioenergetics of *Labeo rohita* fry in relation to different dietary protein sources and dietary inclusion of 17 α-methyl testosterone. Pages 262-265. In: M.K. Dasgupta, D.C. Ghosh, D. Das-Gupta, D.K. Majumdar, G.N. Chattopadhyay, P.K. Ganguli, P.S. Munsi and D. Bhattacharya (eds.). Proceedings National Symposium on Sustainable Agriculture in Subumid Zone, March 3-5, Institute of Agriculture, Sriniketan, India.

Saunders, R.L., Fagerlund, U.H.M. and McBride, J.R. (1977): 17 α-methyltestosterone. A potential anabolic hormone in Atlantic salmon culture. Int. Council Explo. Sea, C.M. 1977/E.50, pp 8.

Schreck, C.B. and Moyle, P.B.(1990): Method of fish Biology. American Fisheries Society, Bethesda, Mavyland, USA.

Schwaiger, J.; Fent, K.; Stecher, H.; Ferling, H. and Negele, R. D. (1996): Effect of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorchynchus mykiss)*. Arch. Environ. Contam. Toxicol., (30): 327- 334.

Software Program of Statistical Analysis, SPSS. (2008): Software Program of Statistical Analysis, Version 17.0 Edition for Windows. SPSS Inc., Chicago, IL, US.

Statistical Analyses System S.A.S. (2000): Statistical Analyses System, Version 6.2, SAS institution in corporation. Carry, NC27513 USA.

Tayamen, M.M. and Shelton,W.L. (1978): Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquacul., 14:349-354.

Teh, S. J.; Adams, S. M. and Hinton, D. E. (1997): Histopathologic biomarker in feral freshwater fish populations exposed to different types of contaminant stress. Aquat. Toxicol., (37): 51- 70.

Weatherley, A.H. and Gill, H.S. (1983): Relative growth of tissues at different somatic growth rates in rainbow trout; *Salmo gairdneri* Richardson. J. Fish Biol., 22: 43-60.

Weatherley, A.H. and Gill, H.S. (1987): Tissues and growth. In "The biology of Fish Growth". St. Edmundsbury Press, Great Britain. Pp. 147- 173.

Williams, J.K.G.; Kubelik, A.R.; Livak, K.J.; Rafalsky, J.A. and Tyngey, S.V. (1990): Molecular changes during gonad masculinization. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Winfree, R.A. and Stickney, R.R.(1981): Effect of dietary protein and energy on growth, feed conversion, feed effiecncy and body composition of tilapia aurea. J.Nutr.III: 1001-1012.

Woo, N.Y.S.,Chung, A.S.B. and Ng, T.B.(1993): Influence of oral administration of 17 β–estradiol and testosterone on growth, digestion, food conversion and metabolism in the underyearling red sea bream, *Chrysophrys* major. Fish Physiol. Biochem., 10(5): 377-387.

Yamazaki, F. (1972): Effects of methyltestosterone on the skin and the gonads of salmonids. Gen. Comp. Endocrinol. Suppl., 3: 741-750.

Yamazaki, F. (1976): Application of hormones in fish culture. J. Fish. Res. Board Can., 33: 948-958.

Zaghloul, K.H.; Omar, W.A. and Abo-Hegab, S. (2005): Environmental hazard risk assessment on *Oreochromis niloticus* and *Tilapia zilli* fsh. J. Egypt. Ger. Soc. Zool., 46(A): 105- 139.

An Evaluation of the Effect of 17α-Methyltestosterone Hormone on some Biochemical, Molecular and Histological Changes in the Liver of Nile Tilapia; *Oreochromis niloticus*

Wafaa S. Hasheesh [1], Mohamed-Assem S. Marie [1], Hossam H. Abbas[*2], Mariam G. Eshak [3] and Eman A. Zahran [4].

[1] Zoology Department, Faculty of Science, Cairo University, Egypt
[2] Hydrobiology Department, National Research Center, Dokki, Giza, Egypt
[3]Cell Biology Department, National Research Center, Dokki, Giza, Egypt
[4]Egyptian Environmental Affairs Agency, Egypt
*h3abbas@yahoo.com

Abstract: The present field investigation was designed to explain clearly why methyltestosterone is widely used by the producers of farmed tilapia. Also to demonstrate why there are no known risks to consumers, producers and on the environment from using this hormone provided the recommended best practices for methyltestosterone used in aquaculture of fish. In this study, all water quality parameters were within the acceptable range for fish growth. The present analyses showed no significant differences in plasma total protein, albumin, globulin, A/G ratio, AST, ALT, LDH, it showed highly significant differences in plasma CPK activities. Molecular biological analyses revealed that using of methyltestosterone was able to induce DNA fragmentation and molecular genetic variability (using RAPD-PCR fingerprinting pattern) in the liver tissues of the treated Nile tilapia; *Oreochromis niloticus*, which was higher in the first four studied months than the untreated control tilapia. Additionally, histopathological examination in liver sections of control fish showed normal structure followed by diffuse severe hepatocytic vacuolations, the treated fish showed diffuse vacuolar degeneration followed by mild and severe hepatocytic vacuolations .
[Wafaa S. Hasheesh, Mohamed- Assem S. Marie, Hassam H. Abbas, Mariam G. Eshak and Eman A. Zahran. **An Evaluation of the Effect of 17α-Methyltestosterone Hormone on some Biochemical, Molecular and Histological Changes in the Liver of Nile Tilapia; *Oreochromis niloticus***]. [Life Science Journal,. 2011; 8(3): 343-358] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Nile tilapia, 17α- methyltestosterone, sex reversal, plasma constituents, DNA alteration.

1. Introduction

Tilapias are among the most resistant fishes known against to diseases and relatively bad environmental conditions such as high stocking density of fish, lower water quality, organically pollutant water, and low dissolved oxygen level of the water (less than 0.5 mg/l). They have tolerance to salinity in wide range and are suitable for maintaining and feeding conditions in culture (Cruz and Ridha, 1994). Tilapia is a delicious, mild flavored fish that has become very popular because of its low price. This low price is achieved by converting the young females to males through the use of the hormone drug 17α-methyltestosterone.

Methyltestosterone treatment of tilapia fries is the most simple and reliable way to produce all male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female tilapias. It is highly effective on the Nile tilapia; *Oreochromic niloticus*, the main species farmed commercially worldwide, thus methyltestosterone treatment has become the standard technique to produce all-male tilapias.

Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. Ahmad *et al*. (2002) found a significant reduction of plasma total protein was in fish fed 40 mg MT/kg, whereas it was insignificantly changed with other treatments. Albumin is synthesized by the liver using dietary protein. Its presence in the plasma creates an osmotic force that maintains fluid volume within the vascular space. Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins.

Most lipids are fatty acids or ester of fatty acid, act as energy storage, structure of cell membranes, thermal blanket and cushion, precursors of hormones (steroids and prostaglandins). Ahmad *et al*. (2002) reported that the *O. niloticus* showed the highest level in plasma total lipids at 40 mg MT/kg. Cholesterol is a chemical compound that is naturally produced by the body and is a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for hormones like estrogen and testosterone. About 80% of the cholesterol is produced by the liver.

The enzyme alanine aminotransferase (ALT) is widely reported in a variety of tissue sources. The major source of ALT is of hepatic origin and has led to the application of ALT determinations in the study of hepatic diseases. Ahmad *et al*. (2002) found that, the activity of ALT was the highest with

control *O. niloticus* fish and that fed low doses of 0.5-2.5 mg MT/kg, while the less one was obtained with 40 mg/kg. Plasma AST is one of several enzymes that catalyze the exchange of amino and oxo groups between alpha-amino acids and alpha-oxo acids. Ahmad *et al*. (2002) reported that, AST activity was significantly increased with high methyltestosterone doses of 20 and 40 mg/kg, while there was no significant change among other treatments.

Lactate dehydrogenase is an enzyme that helps produce energy. It is present in almost all of the tissues and becomes elevated in response to cell damage. Determination of creatine phosphokinase and lactate dehydrogenase isoenzymes provides a definitive diagnosis of acute myocardial infarction.

Molecular Biological analyses:-

Genomic approaches have shown that different classes of toxicants operating through different mode of actions (MOAs) can induce unique and diagnostic patterns of gene expression in fish. The use of molecular markers has provided important advances in the characterization and genetic variation in many species, including yeast and mammals, as well as fish (Horng *et al*., 2004 and Assem and El-Zaeem, 2005).

The genotoxic effects were indicated by appearance of some changes in polymorphism band patterns including lost of stable bands or occurrence of new bands. There also exists a distinct distance between the band patterns of exposed fish and protected or control fish samples. (Mahrous *et al*., 2006).

The present study aims to assess the effect of this hormone and its environmental impacts on sex reversal of fish species. Also its impacts on some biochemical parameters as well as histological examination of vital organs of fish especially the liver. On the other hand, to assess the effect of this hormone on liver. Furthermore, the present study aims to assess the effect of this hormone on alternation of DNA structure that can lead to abnormal changes of DNA fingerprints (discrimination as well as estimation of genetic variation).

2. Materials and Methods

The present work was carried out on water and Nile tilapia fish; *Oreochromis niloticus*. Samples were collected directly from two sampling sites were chosen.

There are two groups:-

First group (Untreated Control): samples of tilapia fish (growing in natural condition away from the hormonal effect) were taken from World Fish Center Farm (WFC) in El-Abbassa, El-Sharkeya governorate. This group was with range length between (10.80±0.26 cm : 22.77±0.49 cm) and range weight between (19.2± 1.1 g : 256.7 ± 12.9 g).

Second group (Treated group): Samples of tilapia fish were taken from El-Nubaria farm belongs to National Research Center (NRC) which is previously used the oral administration of the synthetic androgen (17 α-Methyl testosterone) hormone to produce all male tilapia at 60 mg/kg feed to newly hatched tilapia fry (9-11mm total length) for a period of 28 days which results in populations comprising 97 to 100% phenotype males (Popma and Green, 1990). This group was with range length between (11.53± 0.23 cm – 25.93 ± 0.78 cm) and range weight between was (25.4 ± 1.9 g – 287.4 ± 10.5 g). Samples of fish were taken through different months (April till November 2009) and also water samples will be taken to analyze all possible parameters to know the water quality using in the two sources and its contents.

Water samples were collected from the different studied sites through different months during a period from April to November (2009) and placed in clean sampling glass bottles according to Boyd (1990), then taken to analyze all possible parameters to know the water quality of the sources and its contents. .

Fish samples were collected from the different studied sites through different months during a period from April to November (2009). Fish were dissected to get liver which were kept frozen (-20 ^{o}C) for determination of residual testosterone in muscle and DNA analyses of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

The blood samples were taken from caudal vein of an anaesthetized fish by sterile syringe using EDTA solution as anticoagulant (Ahmad *et al*., 2002). The blood samples were examined immediately for the following: plasma total protein, Albumin, Globulin, A/G ratio, total lipids, cholesterol, aspartate amino transferase (AST) , alanine amino transferase (ALT) activities, Lactate dehydrogenase, Creatine phosphokinase of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites.

The method described for the plasma total protein determination is based on the report of Weichselbaum (1946) and Gomal *et al.* (1949).The violet color developed is proportional to the number of peptide bonds in the protein and is nearly independent of the relative concentration of albumin and globulin (Cannon, 1974). It is measured photometrically at wavelength 550 nm.

Albumin, in the presence of bromocresol green at a slightly acid pH, produces a color change of the indicator from yellow-green to green-blue. The intense of the color formed is proportional to the albumin concentration in the sample (Young, 2001),

and is measured photometrically at wavelength 630 nm.

Plasma globulin is calculated by subtraction of the plasma albumin value from the plasma total protein value.

Plasma total lipids were determined according to Boutwell (1972). The quantitative determination of the total lipid index in plasma is applied using the sulfo-phospho-ovanillin colorimetric method. In this method, lipids react with sulfuric acid to form carbonium ions which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically at wavelength (500-550 nm).

The enzymatic approach to cholesterol methodology was introduced by Flegg (1973) using cholesterol oxidase of bacterial origin following chemical saponification of the cholesterol esters. Roeschlau and Klin (1974) modified this technique and Allain (1974) published the first fully enzymatic assay, combining cholesterol oxidase and cholesterol esterase. The method presented is based on the Allain (1974) procedure and utilizes these enzymes in combination with the peroxidase/phenol-4-antipyrine reagent of Trinder (1969). The intensity of the final red color is proportional to total cholesterol concentration and is measured photometrically at wavelength (500 nm). Lipid Clearing Factor (LCF): a mixture of special additives developed by Stanbio is integrated into the cholesterol reagent to help minimize interference due to lipemia (Flegg, 1973 and Stein, 1986).

The enzyme reaction sequence employed in the Stanbio AST assay of aspartate aminotranseferase (AST) (Bergmeyer, 1978) is measured photometrically at wavelength 340 nm.

UV methods for ALT determination were first developed by Wroblewski and La Due in (1956). The method was based on the oxidation of NADH by lactate dehydrogenase (LDH). In 1980, the International Federation of Clinical Chemistry (IFCC) recommended a reference procedure for the measurements of ALT based on the Wroblewski and La Due (1956) procedures. The ALT reagent conforms to the formulation recommended by the International Federation of Clinical Chemistry (IFCC) (1980).

The procedure presented is essentially the Buhl and Jackson (1978) modification of Wacker (1956) which optimizes reaction conditions. LDH specially catalyzes the oxidation of lactate to pyruvate with the subsequent reduction of NAD to NADH. The rate at which NADH forms is proportional to LDH activity. The method described determined NADH absorbance increase per minute (Buhl and Jackson1978 and Wacker, 1956), and measuring was taken at wavelength 340 nm.

The kinetic procedure presented is a modification of Szasz (1975) of the Rosalki (1977) technique, which optimizes the reaction by reactivation of CPK activity with N-acetyl-Lcysteine (NAC). CPK specially catalyze the transphosphorylation of ADP to ATP, through a series of coupled enzymatic reactions, NADH is produced at a rate directly proportional to the CPK activity. The method determines NADH absorbance increase per minute at 340nm (Szasz, 1975 and Rosalki, 1977) and measuring was taken at wavelength 340 nm.

Molecular biological analyses:-

I- Quantitative analysis of DNA fragmentation

DNA (Deoxyribonucleic acid) fragmentation is an apoptosis marker. Hydrolysis of DNA leads to release of free deoxyribose that colorimetrically measured at (600 nm) after reaction with the diphenylamine reagent, as an indicator of cell death or apoptosis.

a- Diphenylamine reaction procedures:

The control and treated liver samples were collected immediately after sacrificing the Nile tilapia.

The proportion of fragmented DNA was measured by UV and calculated from absorbance reading at 600 nm using the formula:

$$\textbf{\%Fragmented DNA} = \frac{OD(S)}{OD(S) + OD(P)} \times \textbf{100}$$

OD(S) : Optical density of supernatants.

OD (P): Optical density of pellets.

b- DNA gel electrophoresis laddering assay :(Burton, 1956 and Lu et *al*., 2002)

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described by Lu *et al*., (2002).

c- Molecular Analysis :(Mahrous *et al*., 2006 and Khalil *et al*., 2007)

The genomic DNA was isolated using phenol/chloroform extraction and ethanol precipitation method with minor modifications (Sambrook *et al.*, 1989).

II-Random Amplification of Polymorphic DNA (RAPD-PCR) analysis: DNA amplification reactions were performed under conditions reported by **Williams *et al*. (1990) and Plotsky *et al*. (1995).**

Histopathological Examination:

Liver of Nile tilapia; *Oreochromis niloticus* , collected from different studied sites were fixed in neutralized formalin, dehydrated, embedded in paraffin wax and sectioned at 5 µm then stained

with Haematoxylin and Eosin according to Carleton *et al*. (1967).

Statistical analyses:

The results were statistically analyzed using Duncan's multiple range tests to determine difference in means Statistical Analyses System (SAS, 2000) and Software Program of Statistical Analysis (SPSS, 2008). One way ANOVA test (Analysis of variance) comparing the treated and untreated control groups in all months. Differences in all the studied parameters were assessed by one way ANOVA.

3. Results and Discussion

The efficacy of an androgen is affected by the mode of administration and by its source, whether synthetic or naturally occurring (White *et al*., 1973). Synthetic androgens such as ethlytestosterone and methyltestosterone are more effective when administered orally than naturally occurring androgens like testosterone, androstenedione which are most potent when injected intraperitoneally. Androgen treatments at both levels (30, 60 mg/ kg feed of MT and ET) for 35 and 59 days produced 100% male populations, ET- 60 and MT-60 at 25 days also produced 100% male population but ET- 30 and MT-30 at 25 days also produced 98.4 % and 99.2% male, respectively (Tayamen and Shelton, 1978) .

We have been select tilapia fish for our study because tilapia in general reproduces with great rapidity (DaSilva *et al.,* 1973), which makes them suitable for commercial production. However, their prolific rate of reproduction leads to overcrowding and hence stunting when mixed sexes are cultured in ponds (Guerrero and Abella, I976).Various practical measures have been employed to control. The males of Tilapia grow faster than the females (Van Someren and Whitehead, 1960 and Holden and Reed, 1972). Traditionally, tilapias are cultured in fresh water or cages in inland waters (Ishak, 1979).

In Egypt, there is a considerable interest in extending the culture of the Nile tilapia; *Oreochromis niloticus*, which gives a good quality fish with a high marketability and excellent growth rates (Kheir *et al*., 1998).

The use of 17α-methyltestosterone (MT) to produce a monosex male population in tilapia has been extensively reviewed by Hunter and Donaldson, (1983). Many factors are known to effect the success of the androgen treatment in masculinizing tilapia females such as species, age at which hormone is administered, duration of treatment, and type and level of hormone used. Doses of 30 to 60 mg/kg given for 15- 60 days have reported in this connection (Hunter and Donaldson, 1983). Feeding swim –up fry to 10 to 60 mg methyl testosterone /kg feed for 21 to 28 days results in populations with 95 to 100% male (Clemens and Inslee, 1968; Hanson *et al*., 1983; Pompa and Green, 1990 and Muhaya, 1985).

The present field investigations include the study of water quality of water samples collected directly from the studied sites (Table 1), which include also fish samples taken from El-Nubaria farm belongs to National Research Center which is previously used 17α-methyltestosteronehormone to produce all male tilapia, but the another source was from World Fish Center Farm in El-Abbassa, El-Sharkeya governorate. It also concerned with the study of some physiological and biochemical parameters of fish reared in the different studied sites.

Analyses of plasma constituents have proved to be useful in the detection and diagnosis of metabolic disturbance and disease (Aldrin *et al*., 1982). Many factors affect the biochemical composition of fish such as fishing area, type of food, water quality and pollution (Wassef and Shehata, 1991; El-Ebiary *et al*., 1997; El-Ebiary and Mourad, 1998; El-Naggar *et al*., 1998 and Shakweer *et al*., 1998).

The metabolic pathways of fish could be distinguished throughout assessing some physiological parameters. The present study showed insignificant changes in plasma total protein, albumin, globulin, A/G ratio, total lipids, cholesterol, AST, ALT, LDH, CPK activities in the untreated control and treated fish. However, these results reflect the healthy status of the cultured fish at this treatment.

Protein plays an important role in the metabolism and regulation of water balance (Heath, 1995). It is the basic building nutrient of any growing animal and also used as an indicator of their state of health (Alexander and Ingram, 1980 and Lea-Master *et al*., 1990)

Regarding the plasma total protein of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 2), is clear that there is no significant difference in the plasma total protein in the untreated control and treated fish collected from the different studied sites with the highest value in fish collected in the last month of the study for untreated control fish and treated fish.

Chan and O'Malley (1976) and O'Malley and Tsai (1992) reported that, the plasma total protein was significantly decreased at high MT doses, and this result may be due to the fact that androgens regulate protein synthesis by binding to cytosolic or nuclear receptors for steroids that than modulates transcription. Ahmad *et al.* (2002) reported significant reduction of plasma total protein in fish fed 40 mgMT/kg feed, whereas it was insignificantly changed with other treatments.

Table (1): Water Quality Criteria for Water Samples collected from each fish farm for untreated control and treated sampling sites with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* during April till November (2009).

Parameters	Water quality for untreated control Site (El-Abbassa)	Water quality for treated site (El-Nubaria)
Temperature (°C)	(19-24) 22.00 ± 0.68	(19-27) 23.3 ± 1.23
Dissolved Oxygen (mg/L)	(6.11-8.2) 6.86 ± 0.32	(5.8-7.3) 6.6 ± 0.24
pH	(6.82 – 7.8) 6.85 ± 0.26	(6.45-7.8) 7.3 ± 0.14
Ammonia (mg/L)	(1.1 – 1.4) 1.27 ± 0.05	(1.3- 2.4) 1.9 ± 0.14
Nitrate (mg/L)	(0.96 – 1.65) 1.26 ± 0.08	(1.43 – 1.94) 1.7 ± 0.08
Nitrite (mg/L)	(0.03 – 0.05) 0.04 ±0.002	(0.03 – 0.05) .045 ± 0.002
Total Hardness (mg/L as $CaCO_3$)	(111 – 132) 120.00 ± 3.24	(129 – 161) 147.2 ± 4.48
Total Alkalinity (mg/L as $CaCO_3$)	(186 -206) 189.33 ± 4.10	(196 – 228) 216.8 ± 4.53

- Data are represented as means of six samples ± SE.
- Student's t-Test between the two groups of the same parameter in the two studied sites for the whole studied period.

Table (2):Plasma total protein concentrations (g/dl) and Plasma albumin concentration (g/dl)for untreated control and treated Samples with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma total protein ntrol samples (El- Ab	Plasma total protein treated samples (El- Nuba		Plasma albumin in control samples (El- Abbassa)	Plasma albumin ated samples (El- Nu
April	5.81±0.13 [a]	5.97±0.14 [a]		1.50±0.03 [a]	1.45±0.05 [a]
May	5.65±0.18 [a]	6.09±0.11 [a]		1.53±0.04 [a]	1.58±0.03 [a]
June	5.52±0.18 [a]	6.07±0.12 [a]	*	1.59±0.04 [a]	1.57±0.06 [a]
July	5.61±0.23 [a]	6.43±0.29 [a]		1.51±0.03 [a]	1.59±0.07 [a]
August	5.51±0.25 [a]	6.20±0.33 [a]		1.51±0.09 [a]	1.58±0.04 [a]
September	5.33±0.18 [a]	6.26±0.15 [a]	**	1.54±0.07 [a]	1.54±0.05 [a]
October	5.58±0.23 [a]	6.25±0.21 [a]		1.55±0.09 [a]	1.61±0.05 [a]
November	5.62±0.23 [a]	6.43±0.20 [a]	*	1.53±0.08 [a]	1.60±0.04 [a]
F-Values	**0.412**	**0.593**		**0.157**	**0.937**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non-significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

Regarding plasma albumin and globulin of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 2&3), there is no significance difference in the untreated control and treated fish, while there is a slight increase in the albumin concentrations of treated fish during the study, which indicates that there is no effect of the used hormone during the growth of fish.

Concerning A/G ratio of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 3), there is no significance difference in the A/G ratio of the untreated control and treated fish. Despite there is a relative stability in the A/G ratio in treated fish and a slight increase in the A/G ratio of untreated control fish during this study. This indicates that, there is no effect of the used hormone during the growth of fish.

Lipids, as an important source of energy, play an important role in toelest fish (Shatunovsky, 1971; Harris, 1992; Haggag *et al*., 1993 and El-Ebiary *et al*., 1997). In contrast to mammals fish prefer to utilize lipids rather than carbohydrates as a main source of energy (Black and Skinner, 1986). Lipids affected by spawning cycle, food viability, seasonal variations and biochemical activity of fish (Bayomy *et al*., 1993). Lipids are important metabolites for locomotory and reproductory activities of fish.

Table (3): Plasma globulin concentrations (g/dl) and A/G ratio for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma globulin in control samples (El- Abbassa)	Plasma globulin in treated samples (El- Nubaria)		A/G ratio in control Samples (El- Abbassa)	A/G ratio in treated samples (El- Nubaria)
April	4.30 ±0.14 [a]	4.58 ±0.20 [a]		0.35±0.01 [a]	0.32±0.02 [a]
May	4.12 ±0.19 [a]	4.51 ±0.10 [a]		0.37±0.02 [a]	0.35±0.01 [a]
June	3.93 ±0.21 [a]	4.49 ±0.13 [a]		0.41±0.03 [a]	0.35±0.02 [a]
July	4.10 ±0.21 [a]	4.84 ±0.25 [a]		0.37±0.01 [a]	0.33±0.01 [a]
August	4.00 ±0.24 [a]	4.62 ±0.31 [a]		0.38±0.03 [a]	0.34±0.02 [a]
September	3.79 ±0.20 [a]	4.71 ±0.18 [a]	**	0.41±0.03 [a]	0.33±0.02 [a]
October	4.03 ±0.26 [a]	4.80 ±0.15 [a]	*	0.39±0.04 [a]	0.33±0.02 [a]
November	4.08 ±0.22 [a]	4.83 ±0.21 [a]	*	0.38±0.03 [a]	0.33±0.02 [a]
F-Values	**0.468**	**0.487**		**0.436**	**0.329**

Concerning the plasma total lipids and cholesterol of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 4), it is clear that, there was no significant changes in the plasma total lipids and cholesterol during the months of study in the untreated control fish samples and a gradual increase, relatively constant, in samples of treated fish. The present results of the plasma total lipids in the untreated control fish samples are similar to those reported by Ahmad *et al*. (2002) who reported no significant changes in plasma total lipids at moderate doses of MT (5, 10, 20 mg MT /kg feed) in Nile tilapia.

The decreasing in plasma total lipids reported by Dange and Masurekar (1984) in fish fed low levels of MT may be due to the increase of energy demand, which led to more consumption of protein and lipids. In contrast Ahmad *et al*. (2002) reported significant increase in plasma total lipids at 40 mg MT /kg feed in Nile tilapia.

Transamination represents one of the principal pathways for synthesis and deamination of amino acids, thereby allowing interplay between carbohydrates and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations. They also are considered to be important in the assessment of the state of the liver as well as some of the organs (Verma *et al.*, 1981). Therefore, attention has been focused on the changes in AST and ALT activities, which promote gluconeogensis from amino acids, as well as the effects of changes in aminotransferase activities on the liver condition (Hilmy *et al*., 1981 and Rashatwar and Ilyas, 1983).

Determination of transaminases, (AST, ALT) has proven useful in the diagnosis of liver disease in fish (Maita *et al*., 1984 and Sandnes *et al*., 1988). Cell injury of certain organs leads to the release of tissue-specific enzymes into the blood stream (Heath 1995and Burtis and Ashwood 1996).The animo transferases (AST &ALT) are considered a good sensitive tools for detection of any variations in the physiological process of living organisms as reported by Nevo *et al*. (1978) and Tolba *et al*. (1997)

Comparing the present results of plasma AST activity of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 5) , it is clear that, there is no significant difference in the untreated control fish all over the studied period. But there is a significant increase in the activities of AST for the treated fish during the studied period, with special significance increase in September. This is in agreement with Ahmad *et al*. (2002) who reported that, AST activities was significantly increased with high MT doses (20 and 40 mg MT /kg feed), while there was no significant changes among other treatment. These results are confirmed by the present histopathological examination of the liver which showed some hepatocytic vacuolations in the liver tissues.

Comparing the results of plasma ALT of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 5), it is clear that, there is no significant differences between the ALT activities in untreated control and treated fish. In contrast Ahmad *et al*. (2002) reported that, the activities of ALT was highest in untreated control Nile tilapia fish and that fed low doses of MT (0.5-2.5 mg MT/kg feed), while the less activities was obtained with 40 mg MT /kg feed.

The present results are confirmed by Bhasin *et al.* (1998) who reported that high dosages of exogenous male hormones,

including methyltestosterone, are known to cause side effects, especially liver damage, but lower levels actually produce various health benefits. Our results are confirmed by the present histolpathological examination, which showed diffuse vacuolar degeneration in the liver tissues.

Table (4): Plasma total lipids concentrations (g/l) and Plasma cholesterol concentration (mg/dl) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma total lipids in control samples (El- Abbassa)	Plasma total lipids in treated samples (El- Nubaria)	Plasma cholesterol control samples (El- Abbassa)	Plasma cholesterol in treated samples (El- Nubaria)
April	23.13±0.20^{a}	22.03±0.59^{b}	156.97±3.24^{b}	154.60±6.21^{b}
May	22.71±0.49^{a}	24.11±0.56^{ab}	156.58±2.23^{b}	160.85±3.89^{b}
June	22.61±0.65^{a}	24.70±0.65^{ab} *	151.87±2.58^{b}	174.25±5.84^{ab} **
July	23.08±0.97^{a}	24.93±0.74^{ab}	150.60±3.57^{b}	182.00±5.62^{ab} **
August	23.53±0.53^{a}	25.31±1.16^{a}	146.67±5.69^{b}	189.17±4.60^{ab} **
September	23.31±0.85^{a}	25.71±1.18^{a}	151.27±5.66^{b}	183.42±6.36^{ab} **
October	22.38±1.18^{a}	26.11±0.95^{a} *	151.53±5.80^{b}	205.28±5.92^{a} **
November	23.98±1.24^{a}	25.91±0.1.35^{a}	150.02±5.81^{b}	215.47±7.04^{ab} **
F-Values	**0.388**	**1.945**	**0.553**	**1.226**

Table (5): Plasma AST activities (U/l) and Plasma ALT (U/l) activities ffor untreated control and treated samples with 17 α methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Plasma AST in control samples (El- Abbassa)	Plasma AST in treated samples (El- Nubaria)	ALT in Untreated control Samples (El- Abbassa)	ALT in Treated Samples (El- Nubaria)
April	73.30±1.29^{a}	76.70±2.42^{b}	31.95±1.21^{a}	32.43±2.13^{a}
May	74.16±2.11^{a}	83.23±3.09^{ab} *	31.06±1.11^{a}	33.88±2.58^{a}
June	70.91±1.79^{a}	85.43±2.58^{ab} **	31.78±1.68^{a}	34.13±2.93^{a}
July	75.66±2.71^{a}	85.06±4.39^{ab}	32.90±1.67^{a}	32.93±2.56^{a}
August	76.16±2.39^{a}	85.15±5.00^{ab}	32.43±1.53^{a}	36.41±4.14^{a}
September	75.08±3.50^{a}	94.51±5.45^{a} **	32.05±1.87^{a}	37.53±2.90^{a}
October	74.83±3.41^{a}	86.11±6.54^{ab}	32.30±2.42^{a}	38.60±2.70^{a}
November	77.26±3.26^{a}	87.96±4.96^{ab}	31.28±2.15^{a}	37.30±2.89^{a}
F-Values	**0.528**	**1.192**	**0.116**	**0.645**

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.
-

Concerning plasma lactate dehydrogenase activities of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 6), it is clear that, there no significance difference in the (LDH) concentrations of the untreated control and treated fish.

Regarding plasma creatinine phosphokinase activities of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites (Table 6), there was no significant difference between the "CPK" activities in the untreated control fish, while there was a highly significant increase in the treated fish in October and November.

Molecular biological analyses:-

PCR-based techniques, such as RAPDs, have previously allowed the discrimination as well as estimation of genetic variation attributed to genotoxic elements. The exposure to genotoxic agents will give rise to alterations of DNA structure that can lead to abnormal changes of DNA fingerprints. Therefore, we have applied the random amplified polymorphism DNA (RAPD) method to evaluate the genotoxic effects.

The molecular biological results of the present study revealed that methyltestosterone was able to induce DNA fragmentation in liver (Table 7 and

Figs.1,2&3) of Nile tilapia; *Oreochromis niloticus*, in the first four studied months after MT treatment to induce sex reversal in farmed tilapias compared to the untreated control tilapia. In addition, the molecular genetic variability (using RAPD fingerprinting pattern) among the treated tilapia (in liver and testes tissues) was higher in the first four studied months after treatment than the untreated control tilapia.

Table (6): Lactate dehydrogenase activities (U/l) and Plasma creatinine phosphokinase activities (U/l) for untreated control and treated samples with 17 α- methyl testosterone hormone of the Nile tilapia fish; *Oreochromis niloticus,* collected from El- Abbassa and El- Nubaria fish farms during April till November (2009).

Parameter / Months	Lactate dehydrogenase in control samples (El-Abbassa)	Lactate dehydrogenase in treated samples (El-Nubaria)	Plasma creatinine phosphokinase in control samples (El-Abbassa)	Plasma creatinine phosphokinase in treated samples(El-Nubaria)
April	1445.63±21.11 [a]	1450.31± 6.79 [a]	10546.00 ±50.23 [a]	10587.50 ±209.53 [c]
May	1458.58±25.23 [a]	1457.63± 5.56 [a]	10546.83 ±50.72 [a]	10871.83 ±295.28 [bc]
June	1436.62±32.82 [a]	1465.66± 8.71 [a]	10538.50 ±88.08 [a]	10781.83 ±309.97 [c]
July	1456.55±39.32 [a]	1465.36±10.43 [a]	10526.50 ±57.68 [a]	10879.66 ±337.42 [bc]
August	1476.66±27.62 [a]	1467.73± 7.13 [a]	10552.16 ±56.27 [a]	10648.00 ±220.73 [c]
September	1474.60±49.24 [a]	1463.46± 11.60 [a]	10547.83 ±70.89 [a]	10879.00 ±370.69b [c]
October	1436.81±22.17 [a]	1476.40± 5.48 [a]	10574.33 ±56.01 [a]	11923.16 ±535.44 [ab] *
November	1457.11±41.10 [a]	1467.80± 7.85 [a]	10568.33 ±60.27 [a]	12360.33 ±435.98 [a] **
F-Values	**0.205**	**0.877**	**2.487***	**3.394****

Table (7): Effect of the 17 α methyl testosterone hormone on the DNA fragmentation ratio in liver tissues collected from Nile tilapia; *Oreochromis niloticus,* for several time intervals (April – November, 2009).

Parameter / Months	DNA fragmentation (%) liver tissues of untreated control sam (El- Abbassa)	DNA fragmentation (%) iver tissues of treated samples Nubaria)
April	10.66 ±0.33 [a]	13.33 ±0.33 [a] **
May	11.33 ±0.33 [a]	13.66 ±0.33 [a] **
June	10.66 ±0.33 [a]	13.66 ±0.33 [a] **
July	10.66 ±0.33 [a]	13.33 ±0.33 [a] **
August	11.33 ±0.33 [a]	12.33 ±0.33 [b]
September	11.66 ±0.33 [a]	12.66 ±0.33 [b]
October	11.66 ±0.33 [a]	11.66 ±0.33 [b]
November	10.33 ±0.33 [a]	11.66 ±0.33 [b] **
F-Values	**1.929**	**4.558 ** **

- Data are represented as means of six samples ± SE.
- Means with the same letter for each parameter in the same column between all months are non- significant different (P > 0.05); otherwise they do (SAS, 2000).
- Student's t-Test between the two groups in the same month for the whole studied period.
- One way ANOVA test (F-value) between all months in each group separately for the whole studied period.
- * Significant difference at P<0.05 ** Highly significant difference at P<0.01.

DNA gel electrophoresis laddering assay:-

Determination of the DNA fragmentation in liver tissues using DNA gel electrophoresis laddering assay in Nile tilapia; *Oreochromis niloticus* are summarized in figures (1 &2).

The results demonstrated that, the liver tissues collected from the Nile tilapia treated with testosterone showed DNA damage especially in the first four months after treatment (Fig. 1). In contrast, the liver tissues collected from untreated control Nile tilapia showed no changes in the genetic materials (Fig. 2). The DNA marker is in lane 1. Lane 2 to Lane 9 represent months of collection (April till November, respectively) of fish liver tissue samples treated with 17 α- methyltestosterone throughout the period of study.

For our knowledge, there are no data regarding the effect of methyltestosterone on the DNA damage in fish especially Nile tilapia. However, it could be postulated that the methyltestosterone residues were still existed in the fish tissues and/or in the fish

environment up to the first four months after treatment and then began to be disappeared, whereas the DNA fragmentation decreased after the first four studied months.

The action mechanism of testosterone treatment inducing genetic toxicity during the first months of age in tilapia (tilapia fry) is not investigated yet. In the present study, the negative effect of testosterone induced DNA damage may be attributed to the weakness in the immune system which may not be completed in growth yet. The main way in which steroid hormones interact with cells is by binding to proteins called steroid receptors. When steroids bind to these receptors, the proteins move into the cell nucleus and either alter the expression of genes (Lavery and McEwan, 2005) or activate processes that send signals to other parts of the cell (Cheskis, 2004) cause genetic toxicity. Beg *et al* (2008) reported that the possible genotoxicity of testosterone is depend on the metabolic activation. The first step of this mechanism may involve the aromatic hydroxylation catalyzed by cytochrome p450 as in the case of other steroids. Cytochrome p450 in liver fractions plays an important role in activating promutagens to proximate and/or ultimate mutagens.

The results of the present study revealed that the DNA damage attributed to methyltestosterone treatment was markedly disappeared after the first four studied months until it reached a relative stability rate similar to control untreated tilapia. It could be explained that the methyltestosterone residues in the fish tissues and/or in the fish environment were removed. Furthermore, disappearance of the DNA damage may be attributed to the increase of the immunity defense in the growing fish.

Traditionally, sex steroids are recognized as non-genotoxic carcinogens (Ho and Yu, 1993). To date, few studies have been reported on any aspect of DNA damage caused by testosterone treatment in organs of intact animals (Ho and Yu, 1993 and Ho and Roy, 1994). However, controversial results have been reported. Ho and Roy (1994) reported that testosterone combined with estrogen induced a dramatic increase in DNA strand breaks. In contrary, when female rats, converted to 'male type' by ovariectomy, treated with testosterone for one week, DNA damage caused by hepatocarcinogen (DL-ZAMI 1305) was completely abolished (Ragnotti *et al* ., 1987).

Marzin (1991) studied the mutagenicity of some synthetic androgen steroids, using number of genotoxicity tests *in vitro* and *in vivo* systems for gene mutations, chromosomal mutations and primary DNA damage demonstration. The results of this study showed no genotoxic activity attributed to these steroids. It is also found that 17α-alkylated steroids are directly toxic to hepatocytes, whereas the non-alkylated steroids show no effects at tested doses (Welder *et al*., 1995). Tsutsui *et al*. (1995) reported that testosterone did not induce gene mutations at the *hprt* or Na^+/K^+ ATPase locus. When testosterone was added at a final concentration of 2 mmol/l to DNA obtained from human surgical resections, rat liver, HepG2 cells, and calf thymus, did not form adducts with naked DNA. Furthermore, no adducts were observed in DNA isolated from HepG2 cells incubated with 10–100mol/l testosterone for 24 h (Seraj *et al*., 1996).

In agreement with our results, Hana *et al* (2008) reported that there were no significant differences in the frequency of total chromosomal aberrations between control and testosterone propionate-treated adult mice. In addition, they found that the molecular genetic variability using RAPD-PCR among the testosterone-treated adult mice was similar to control untreated mice. Whereas, all of the oligodecamers used revealed monomorphic bands in the control samples and those treated with testosterone propionate.

Additionally, Histopathological examination or biomarkers have been increasingly recognized as a valuable tool for field assessment of the impact of using 17 α-methyltestosterone hormone on fish organs (Heath, 1995; Schwaiger *et al*., 1996 and Teh *et al*., 1997). The investigated biochemical and physiological changes were confirmed by histopathological alterations of muscle, liver and testis of the Nile tilapia; *Oreochromis niloticus* collected from the two fish farms.

DNA gel electrophoresis laddering assay:-

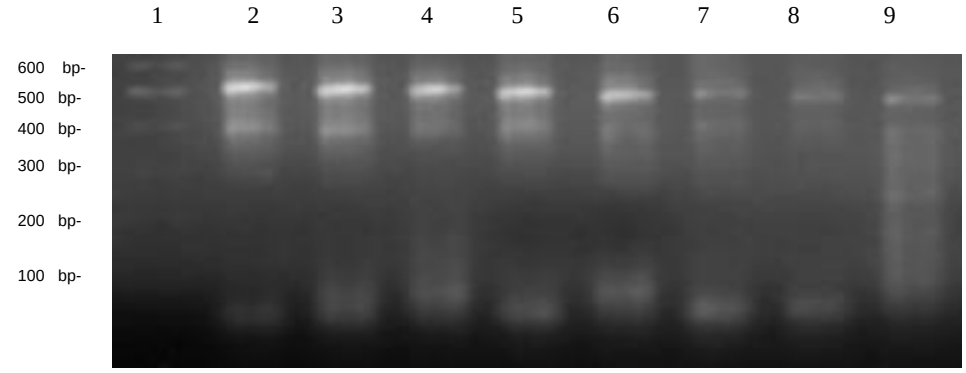


Figure (1): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from liver exposed to testosterone in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent liver tissues collected from April to November (2009).

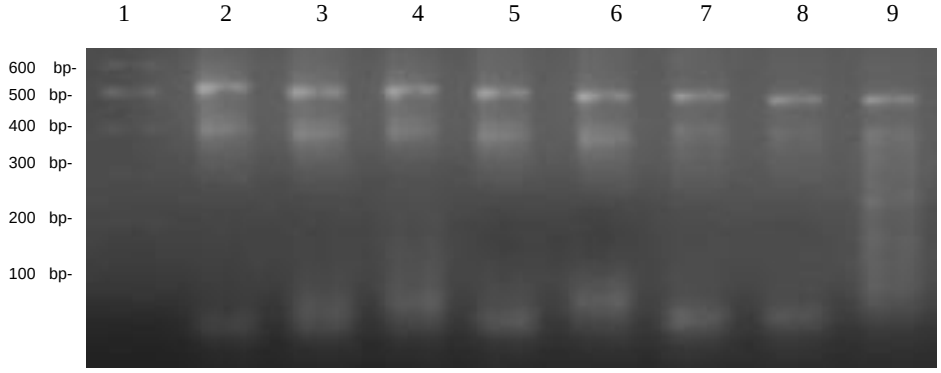
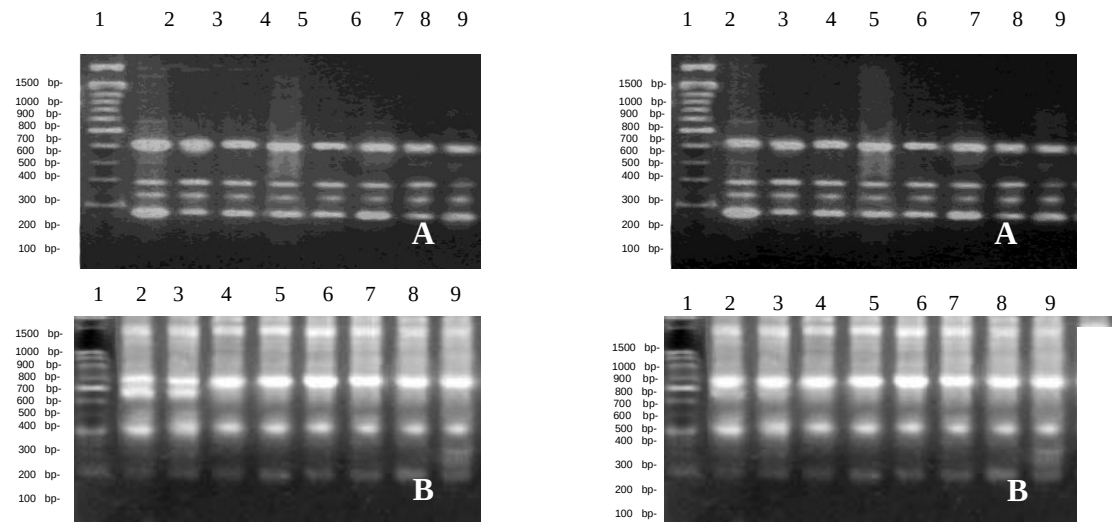


Figure (2): DNA fragmentation detected with agarose gel electrophoresis of tilapia DNA extracted from untreated liver in different time intervals analyzed by DNA gel electrophoresis laddering assay. Lane 1 represents DNA ladder. Lanes 2 to 9 represent liver tissues collected from April to November (2009).

Treated Liver **Untreated control Liver**



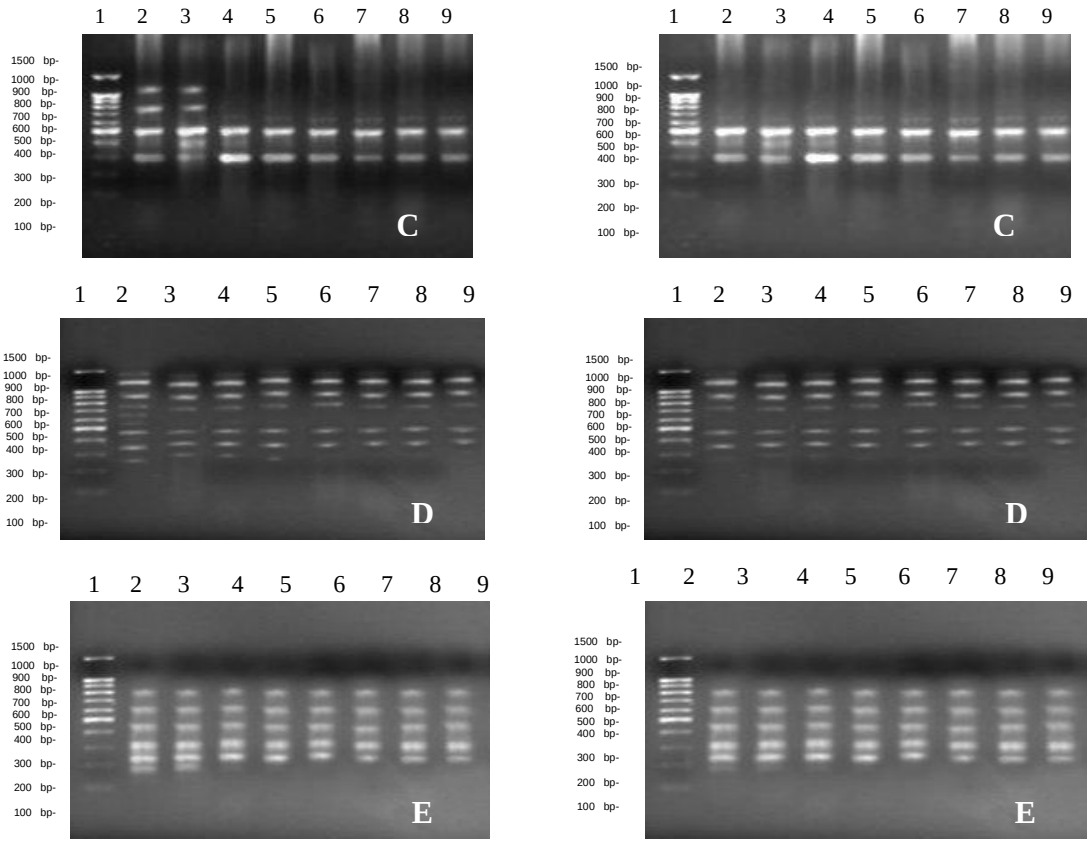


Figure (3)
Comparison of RAPD fingerprinting profiles of different tilapia genomic DNA: (A) Represents PCR products with primer A04, (B) Represents PCR products with primer A08, (C) Represents PCR products with primer A10,
(D) Represents PCR products with primer C09,(E) Represents PCR products with primer C12.

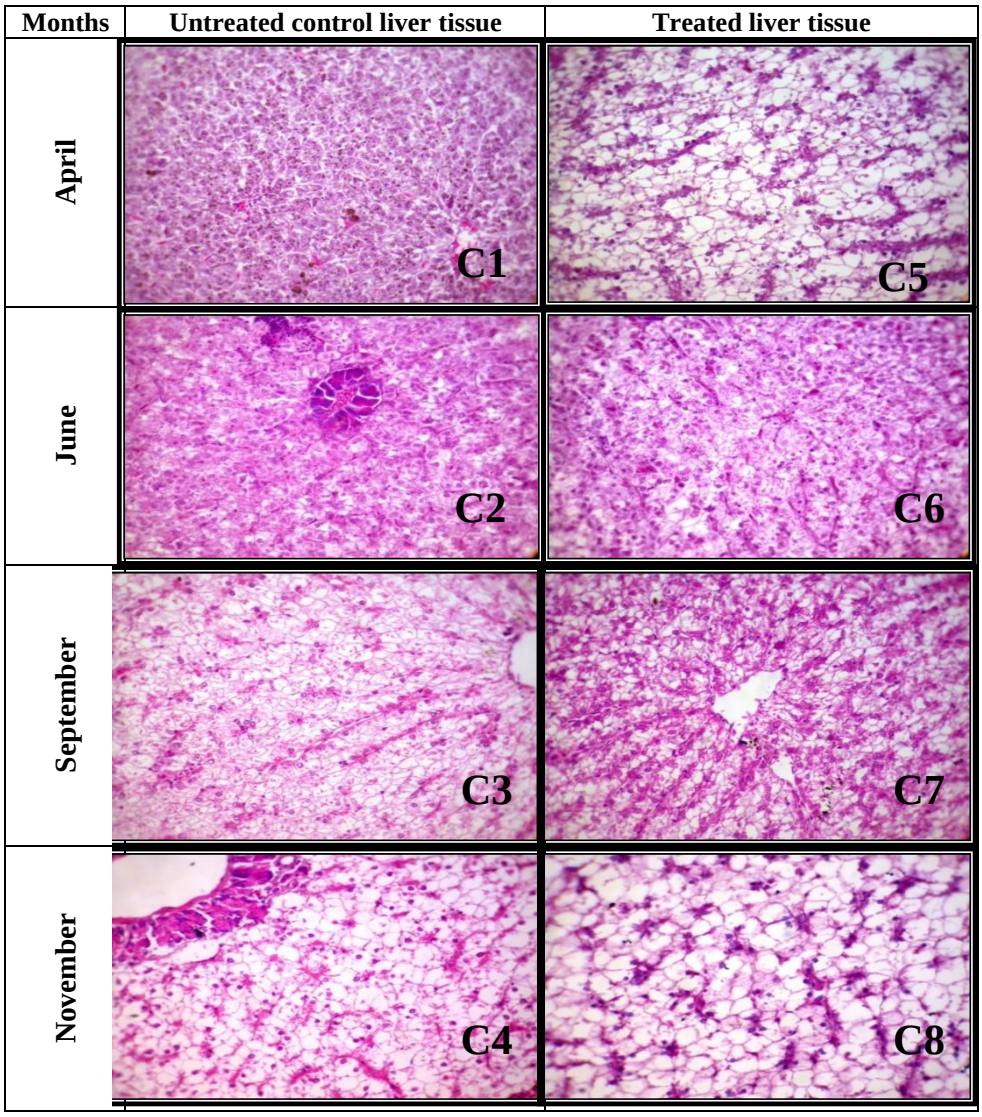
Liver:-

The liver section of the present studies (Photomicrograph 1) in the control group showed hepatic parenchymal arrangement consists of hepatocytes, which are rapidly arranged around central vein interconnecting laminae of two cells thickness, narrow straight sinusoid separating each lamina. This is in agreement with shown reported by Robert (2011).

Liver sections of untreated control fish (Sections, C1,C2,C3 &C4))showed the normal structure which followed by diffuse vacuolations at the end of the studied period, where diffuse severe hepatocytic vacuolations were appeared. On the other hand, liver sections of treated fish (Sections, C5,C6,C7 &C8)showed diffuse vacuolar degeneration followed by some hepatocytic vacuolations but at the end of the studied period severe hepatocytic vacuolations were appeared.

High dosages of exogenous male hormones, including methyltestosterone, are known to cause side effects, especially liver damage, but lower levels actually produce various health benefits, including reduced risks from cardio-vascular disease and cancer. Overall, it has been shown that the side effects of testosterone supplementation in humans are minimal when plasma testosterone levels are kept within the normal physiological range (Bhasin *et al.*, 1998).

Deborah (1990) studied the effect of the synthetic steroid 17α-methyltestosterone on the growth and organ morphology of channel catfish (*Ictalurus punctatus*) and found that there is no deviation from the normal morphology in livers taken from both treated and control specimens.



Photomicrograph (1):
Histological sections in Liver tissues of *Oreochromis niloticus* collected from the untreated control fish in El- Abbassa and treated fish in El-Nubaria fish farms from April till November (2009).

Untreated control
(C1) Liver showing normal structure (H&E 400X).
(C2) Liver showing normal structure (H&E 400X).
(C3) Liver showing diffuse severe hepatocytic vacuolations (H&E 400X).
(C4) Liver showing diffuse severe hepatocytic vacuolations (H&E 400X).

Treated
(C5) Liver showing diffuse vacuolar degeneration (H&E 400X).
(C6) Liver showing mild vacuolations (H&E 400X).
(C 7) Liver showing some hepatocytic vacuolations (H&E 400X).
(C8) Liver showing severe hepatocytic vacuolations (H&E 400X).

Also our results were in agreement with Khater (1998) who studied the effect of different doses (15, 30, 60, 90 mg) of 17α-methyltestosterone on the liver for 28 days and indicate that the hepatic parenchyma had diffused vacuolar degeneration. The central veins and hepatic sinusoids were congested.

Khater (1998) also reported that, liver tissue treated with 60 mg MT for 14 days, showed diffuse hydropic degeneration, the central vein was congested and hemorrhage was also seen in the hepatic parenchyma.

Finally, when 17 α-methyltestosterone is used for sex reversal treatment with dosage of 20 -40 mg/kg diet and the amount of 17 α-methyltestosterone ingested by tilapia is unlikely to exceed 10 µg/day. When tilapia are reared to a marketing weight of about 300 g, which under intensive culture conditions takes not less than 5 months (Melard and Philippart, 1981). Because the dose rates of 17 α-methyltestosterone used in human medicine ranged from 10 -50 mg daily (British Pharmacopoeia, 1980). Johanstone *et al*. (1983) concluded that, under normal circumstances, it would be unreasonable to suggest that hazardous levels of 17α-methyltestosterone might be ingested by consumption of adult fish treated as juveniles with this steroid.

Methyltestosterone treatment in tilapia farming is considered to be entirely safe provided the following recommended best practices are adopted by producers:

1. They restrict tilapia methyltestosterone treatment to the early fry stages, specifically to the first month from the time the fry are free-swimming/first-feeding.
2. They limit the dosage of methyltestosterone used to a maximum of 60 mg methyltestosterone /kg fry feed.
3. They rear methyltestosterone treated tilapia fry to adult size for at least five months after hormone treatment ends to ensure zero hormone residue remains in the fish.
4. As a precautionary measure, adopt safe handling protocols when preparing and administering methyltestosterone treated tilapia feed; use latex gloves and a protective face mask to avoid dermal contact or inhalation of methyltestosterone .
5. They keep a careful inventory of the amounts of methyltestosterone supplied to and used in each tilapia hatchery, and ensure that access of the hormone supply and record-keeping are controlled by the farm manager or hatchery supervisor.
6. They avoid direct release of hatchery water used for methyltestosterone treatment of tilapia fry into the environment. As a precautionary measure, tilapia hatcheries should utilize a gravel and sand filter, plus a shallow vegetated pond or an enclosed wetland, to receive and hold the hatchery wastewater for several days before discharge into the general environment.

Corresponding author
Hossam H. Abbas
National Research Center
*h3abbas@yahoo.com

References

Ahmad, M.H.; Shalaby, A.M.E.; Khattab, Y.A.E. and Abdel-Tawwab, M. (2002): Effects of 17 α–methyltestosterone on growth performance and some physiological changes of Nile tilapia fingerlings (*Oreochromis niloticus* L.). Egypt. J. Aquat. Biol. Fish, 4(4): 295-311.

Aldrin, J.f.; Messager, G.L. and Baudin Laurencin, F. (1982): La biochemie clinique en aquaculture. Interest et prespectives. Cnexo. Actes. Colloq., 14: 291-326.

Alexander, J. B. and Ingram, G.A. (1980): A comparison of five of the methods commonly used to measure protein concentration in fish sera. J. Fish Biol., 16: 115-125.

Allain,C.C.(1974):Quantitative-enzymatic-colorimetric determination of cholesterol in [lasma .Clin. Chem., 20:470 473.

American Public Health Association (APHA), American Water Works Association and Water Pollution Control Federation (1995): Standard Methods for the Examination of Water and Wastewater. 19th edition, New York, N.Y.

Assem, S.S. and El-Zaeem, S.Y. (2005): Application of biotechnology in fish breeding. II: production of highly immune genetically modified redbelly tilapia; *Tilapia zillii*. Afr. J. Biotechnol., 4: 449–459.

Bayomy, M. F.; Khallaf, E.A. and Gaber, N.(1993): Studies on the fat contents and their relation to the production of *Clarias lazera* (CUV and VAL) in Bahr Shebeen Nile Canal. J. Egypt. Ger. Soc. Zool., 10 (B): 165-182.

Beg, T.; Siddique, Y. H.; Ara, G. ; Afzal, M. and Gupta, J. (2008): Antioxidant Effect of ECG on Testosterone Propionate Induced Chromosome Damage. Human Genetics and Toxicology Laboratory, Department of Zoology, Aligarh Muslim University, Aligarh 202002 (UP), India.Interna.J.Phamacol. 4 (4): 258-263.

Bergmeyer, H.U. (1978): Quantitative determination of Aspartate Aminotransferase (AST) in plasma. Principles of Enzymatic Analysis. Verlag Chemie.

Bhasin, S.; Bagatell, C.J.; Bremner, W. J.; Plymate, S.R.; Tenover, J.L.; Korenman, S.G. and Nielschlag, E. (1998): Issues in testosterone replacement in old men. J. Clin. Endocrinol. Metabol., 83: 3435-3448.

Black, D. and Skinner, E. R. (1986): Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. Prog. Fish-Cult., 156(B): 497-502.

Boutwell, J.H.M. (1972): Quantitative colorimetric determination of plasma total lipids. USD. H.E.W.

Boyd, C.E. (1990): Water Quality in Ponds for Aquaculture. Birmingham Publishing Co. Birmingham, Alabama.

British Pharmacopoeia (1980): Vol .1. Her Majesty's Stationary Office, London, pp 291- 292.

Buhl, S.N. and Jackson, K.Y. (1978): Quantitative determination of Lactate Dehydrogenase in plasma. Clin. chem., 24: 828-830.

Burtis, C.A. and Ashwood, E.R. (1996): Tietz fundamentals of clinical chemistry. Philadelphia, PA: Saunders.

Burton, K. (1956): The study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62: 615–623.

Cannon, D.C. (1974): Quantitative colorimetric determination of plasma total protein. IN Clinical Chemistry- Principles and techniques, 2nd Ed. RJ Henry *et al*., Eds. Harper & Row, Hagerstown, MD, pp 411- 421.

Carleton, M.; Drary, Y.; Wallington, E.A. and Cammeron, H. (1967): Carleton's Histological Technique, 4 th ed., Oxford Univ. Press, New York, Toronto.

Chan, L. and O'Malley, B.W. (1976): Mechanism of action of the sex steroids. New Eng. J. Med. (June):1322-1328.

Chan,S.T.H. and Yeung, W.S.B. (1983): Sex control and sex reversal in fish under natural condition.In W.S. Hoar and D.J. Rondall and D.M. Donaldson (eds), Fish Physiology Vol.IX B, Academic Press, New York, pp. 171-213.

Cheskis, B. (2004): Regulation of cell signaling cascades by steroid hormones. J. Cellular Biochem., 93 (1): 20–7.

Clemens, H..P and Insleee, T. (1968): Production of unisexual broods by *Tilapia mossambica* sex-revered with methyl testosterone. Trans. Am. Fish. Soc., 97: 18-20.

Cruz, E. M. and Ridha, M. (1994): Overwintering tilapia; *Oreochromis spilurus* Gunther, fingerlings using warm underground sea water. Aquacul. Fish. Management, 25: 865- 871.

Dange, A.D. and Masurekar, V.B. (1984): Effect of naphthalene exposure on activity of some enzymes in Cichlid fish tilapia (*Sarotherodon mossambicus*) Peters. J. An. Morphol. Physiol., 31: 159-167.

Da Silva, A. B.; Sobrinho, A. C.; Fernandes, J. A. & Lovshin, L.L. (I973): Observation preliminaires sur l'obtention d'hydrides tous males des especes Tilapia hornorum et Tilapia nilotica.Notes et Documents sur la Peche et la Pisciculture n°7.

Deborah, A. S. (1990): The effects of the synthetic steroid 17-alpha-methyltestosterone on the growth and organ morphology of the channel catfish (*Ictalurus punctatus*), Aquacult., 84(1): 81-93.

EL-Ebiary, E.H. and Mourad, M.H. (1998): Response of florida red Tilapia (*Oreochromis urolepia hornorum* VS *Oreochromis hyprid*) fingerlings to different oil levels. Bull. Nat. Inst. of Oceanorg. And Fish. A.R.E., (24): 325-337.

El-Ebiary, E. H.; Zaki, M. A. and Mourad, M. H. (1997): Effect of salinity on growth, feed utilization and haematological parameters of Florida red Tilapia fingerlings. Bull. Nat. Inst. Oceanogr. Fish. A.R.E., (23): 203-216.

El-Naggar, G. O.; Zaghlol,K. H. ; Salah El-Deen, M.A. and Abo-Hegab, S.(1998): Studies on the effect of industrial water pollution along different sites of the River Nile on some physiological and biochemical parameters of the Nile tilapia, *Oreochromis niloticus*. 4th Vet. Med. Zag. Congress (26-28 August 1998 in Hurghada): 713-735.

Flegg, H.M. (1973): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. Ana. Clin. Biochem., 10:79- 81.

Gomal, A.C.G.; Bradawil, C.J. and David, M.M., (1949): Quantitative colorimetric determination of plasma total protein. J.Biol.Chem., 177:751-753.

Guerrero, R.D. and Abella,T.A.(1976): Induced sex reversal of Tilapia nilotica using methyltestosterone. Fish.Res.J.Phillipp., (1): 46-49.

Haggag, A.M.; Marie, M.A.S.; Zaghloul, K.H. and Eissa, S.M.(1993): Treatment of underground water for fish culture in Abbassa farm, Sharkia. III Biochemical and histological studies. Bull. Fac. Sci. Cairo Univ., 61: 43-69.

Hana, Y.H.; Khalil, W. K.B.; Elmakawy, A. I. and Elmegeed, G. A. (2008): Androgenic profile and genotoxicity evaluation of testosterone propionate and novel synthesized heterocyclic steroids. J. Steroid Biochem. Mol. Biol., 110: 284–294.

Hanson, R.; Sinith Evman, R.O.; Shelton,W.H. and Dunham, R.A.(1983): Growth comparison of monosex tilapia produced by separation of sexes hybridization and sex reversal, p. 570. 579. In L. Fishelson and Z. Yaron (compsi). Proceedings of International symposium on Tilapia in Aquaculture. 8-13 May 1983. Nazareth, Isreal. Aviv University, Israel

Harris, C. L. (1992): Materials and mechanisms of cells. In: Concepts of Zoology. Harper Collins Publ. Inc., New York, 19-50.

Heath, A.G. (1995): Water Pollution and Fish Physiology. CRC. Press. Inc. Boca Raton, Florida. 359pp.

Heberer, T. (2002): Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. *Toxicol. Lett.*, 131: 5–17.

Hilmy, A.M.; Shabana, M.B. and Said, M.M. (1981): The role of serum transaminases (SGOT and SGPT) and alkaline phosphatases in relation to inorganic phosphorus with respect to mercury poisoning in Aphanius dispar Rupp (Teleos) of the red sea. Comp. Biochem. Physiol., 68C: 69-74.

Ho, S.M. and Roy, D. (1994): Sex hormone-induced nuclear DNA damage and lipid peroxidation in the

dorsolateral prostates of Noble rats. Cancer Lett., 84 :155–162.

Ho, S.M. and Yu, M. (1993): Selective increase in type II estrogen binding sites in the dysplastic dorsolateral prostates of Noble rats. Cancer Res., 53: 528–532.

Holden, M. and Reed, W. (1972): West African Fresh Water Fishes.Longman Group Ltd., London 5I p.

Horng, Y.M.; Chen, Y.T.; Wu, C.P.; Jea, Y.S. and Huang, M.C. (2004): Cloning of Taiwan water buffalo male-specific DNA sequence for sexing. Theriogenol., 62: 1536–1543.

International Federation of Clinical Chemistry (IFCC) (1980): Quantitative determination of alanine aminotransferase (ALT) in plasma. *J. Clin. Chem.Clin.Biol.*,18:5231-5233.

Ishak, M.M. (1979): Development and progress of aquaculture in Egypt with special reference to cage and open cultures. International Workshop on open and Cage Culture of Fish. Tigbauan, Iioilo, Philippines; IDRC and SEAFDEC, 11-22 Feb., pp.31- 32.

Johnson, B. (2007): 17-Alpha Methyltestosterone Clinical Field Trials - INAD 9332. Annual Summary Report on the Use of 17-Alpha Methyltestosterone in Field Efficacy Trials, U.S. Fish and Wildlife Service Bozeman National INAD Office Bozeman, Montana. 15p.

Johnstone, R.; Macintosh, D.J. and Wright, R.S., (1983): Elimination of orally administered 17α-Methyltestosterone by *Oreochromis mossambicus* (Tilapia) and *Salmo gairdneri* (rainbow trout) juveniles. Aquacul., 35: 249-257.

Khalil, W.K.B.; Mahmoud, M.A.; Zahran, M.M. and Mahrous,K.F. (2007): : A sub-acute study of metronidazole toxicity assessed in Egyptian Tilapia ; *Tilapia zillii. J. Appl. Toxicol.*, 27: 380–390.

Khater, A.M.M. (1998): Sex reversal in *Tilapia nilotica*. Ph.D. Thesis. Zagazig University, Agricultural Science (Poultry Production- Aquaculture- Fish hatching and sex reversal), pp. 144.

Kheir, M.T.; Mechail, M.M. and Abo-Hegab, S. (1998): Growth of adult and newly hatched fry of *Oreochromis niloticus* reared at different saline concentrations. Egypt. J. Zool., (30):107- 115.

Lavery, D.N. and McEwan, I.J. (2005): Structure and function of steroid receptor AF1 transactivation domains: induction of active conformation. Biochemical J., 391 (Pt 3): 449–64.

Lea- Master, B.R; Brock, J.A.; Fujioka, R.S. and Akamura, R.M. (1990): Haematological and blood chemistry values for *Sarotherodon melanotheron* and a red hybrid Tilapia in freshwater and sea water. Comp. Biochem. Physiol., 97(A): 525-529.

Luceri, C.; De Filippo C.; Caderni, G.; Gambacciani, L.; Salvadori, M.; Giannini, A. and Dolara, P. (2000): Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. *Carcinogenesis*, 21: 1753– 1756.

Mahrous, K.F.; Khalil, W.K.B. and Mohamoud, M.A. (2006): Assessment of toxicity and clastogenity of sterigmatocystin in Egyptian Nile tilapia. Afr. J.Biotechnol., 5(12): 1180- 1189.

Maita, M.; Shiomitsu, K. and Ikeda, Y. (1984): Health assessment by the climogram of hemochemical constituents in cultured yellow tail. Bull. Jap. Soc. Scient., (51): 205-211.

Marzin, D. (1991): Trenbolone application of the Ames test. Recent data, Ann. Rech. Vet. 22 (3): 257–262.

Melard, C.H. and Philippart, J.C.(1981): Pisciculture intensive du tilapia Sarotherodon niloticus dan les effleuents thermiques d'une central nucleaire en Belgique. K. Tiews (Editor), " Aquaculture in heated effluents and recirculation system." Heenemann Verlagsgesellschaft, Berlin (EIFAC/ 80/Symp/ Doc.E/11).

Muhaya, B.B.M. (1985): Growth comparisons of tilapia nilotica males produced through oral administarion of methyltestoserone at varing levels and durations, Auburn University, Auburn, Alabama, USA.28 P.M.S. thesis.

Nagy, A.; Becsenyi, M. and Csanyi, V.(1981): Sex reversal in crap
(*Cyprinus carpio*) by oral administration of methyletestosterone. Can. J. Fish Aquat. Sci., 38:725-728.

Nevo, E.; Shimony, T. and Libin, M.(1978): Pollution selection of allozymes polymorphisms in branchtes. Experentia, 36: 1562- 1564.

Plotsky, Y.; Kaiser, M.G. and Lamont, S.J. (1995): Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Animal Genetics, 26: 163-170.

Popma, T.J. and Green, B.W. (1990):. Sex reversal of tilapia in earthen ponds. International Center for Aquaculture and Aquatic Environments Research and Development Series No. 35, Auburn University, Alabama, pp: 15.

Ragnotti, G.; Presta, M.; Maier, J.A.M.; Rusnati, M.; Mazzoleni, G.; Legati, F.; Chiesa, R.; Braga, M. and Calovini, D. (1987): Critical role of gonadal hormones on the genotoxic activity of the hepatocarcinogen DL-ZAMI 1305. Cancer Lett., 36:253–261.

Rashatwar, S.S. and Ilyas, R. (1983): Effects of chronic herbicide intoxication on the *in vivo* activities of certain enzymes in the liver of fresh water fish Nemacheilus denisonii (Day). Toxicol. Let., 16: 249-252.

Robert, B.M. (2011): Biology of Fish. California Animal Health and Food Safety Laboratory System University of California.

Roeschlau, P. and Klin, Z. (1974): Quantitative-enzymatic-colorimetric determination of cholesterol in plasma .Chem. Clin. Biochem., 12: 226-228.

Rosalki, S.B. (1977): Quantitative determination of creatine phosphokinase (CPK) in Plasma. J. Lab. Clin. Chem., 23: 646-650.

Sambrook, L.; Fritsch, E.F. and Manitatis, T. (1989): Molecular cloning: A laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

Sandnes, K.; Lie, Q. and Waagbq, R. (1988): Normal ranges of some blood chemistry parameters in adult farmed Atlantic salmon; *Salmo salar*. J. Fish. Biol., (32): 129-136.

Schwaiger, J.; Fent, K.; Stecher, H.; Ferling, H. and Negele, R. D. (1996): Effect of sublethal concentrations of triphenyltriacetate on rainbow trout (*Oncorchynchus mykiss)*. Arch. Environ. Contam. Toxicol., (30): 327- 334.

Seraj, M.J.; Umemoto,A.; Tanaka,M.; Kajikawa, A.; Hamada K.and Monden,Y. (1996): DNA adduct formation by hormonal steroids *in vitro*. Mutat. Res., 370 (1): 49–59.

Shakweer, L.M; El-Ebiary, E.H. and Zaki, M.A. (1998): Comparative study on the major biochemical constituents in muscles of *Mugil cephalus* inhabiting the Mediterranean water, the north Delta lakes and fish farms of Egypt. Bull. Nat. Inst. Of Oceanongr. Fish. A.R.E., (24): 79-101.

Shatunovsky, M.I. (1971): Variations in different lipids and their relation to reproduction in the male fish *Clarias batrachus* (Linn). Indian J. Exp. Biol., (25): 639-640.

Software Program of Statistical Analysis, SPSS. (2008): Software Program of Statistical Analysis, Version 17.0 Edition for Windows. SPSS Inc., Chicago, IL, US.

Statistical Analyses System S.A.S. (2000): Statistical Analyses System, Version 6.2, SAS institution in corporation. Carry, NC27513 USA.

Stein, E.A. (1986): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. in Textbook of Clinical Chemistry, NW Tiez, Ed. W.B. Saunders, Philadelphia, p.p. 879-886, 1818, 1829.

Szasz, G. (1975): Proceedings of the second international Symposium on clinical Enzymology, Chicago, October 1975.

Tayamen, M.M. and Shelton,W.L. (1978): Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). Aquacul., 14:349-354.

Teh, S. J.; Adams, S. M. and Hinton, D. E. (1997): Histopathologic biomarker in feral freshwater fish populations exposed to different types of contaminant stress. Aquat. Toxicol., (37): 51- 70.

Tolba, M.R.; Mohammed, B. and Mohammed, A. (1997): Effect of some heavy metals on respiration, mean enzyme activity and total protein of the plumonate snails *Biomphalaria alexandria* and *Bulinus truncates*. J. Egypt.Ger.Soc.Zool., 24: 17-35.

Trinder, P. (1969): Quantitative-enzymatic-colorimetric determination of cholesterol in Plasma. Ann. Clin. Chem., 6: 24-26.

Tsutsui,T.; Konine,A.; Huff,J. and Barrett,J.C. (1995): Effects of testosterone, testosterone propionate, 17beta-trenbolone and progesterone on cell transformation and mutagenesis in Syrian hamster embryo cells, Carcinogenesis 16 :1329–1333.

Van Someren, V. D. and Whitehead, P. J. (1960): The culture of Tilapia nigra (Gunther) in ponds. The early growth of males and females at comparable stocking rates, and the length/weight relationship. East Africa. Argric. J., 25 (3): 69-I73.

Verma, S.R.; Rani, S. and Dalela, R.C. (1981): Isolated and combined effect of pesticides on serum transaminases in *Mystus vittatus*. Toxicol. Let., 9: 67-71.

Wacker,W.E.C.(1956): Quantitative determination of lactate dehydrogenase in plasma. New Engl. J. Med., 255:449-451.

Wassef, E. and Shehata, A.B. (1991): Biochemical composition of gilthead sea bream; *Sparus aurata* from Lake Bardawil. K.A.U. Mar. Sci., 2: 111-122.

Weichselbaum, T.E. (1946): Quantitative colorimetric determination of plasma total protein. Am. J. Clin. Patho., 7:40-42.

Welder, A.A.; Robertson, J.W. R. and Melchert, B. (1995): Toxic effects of anabolic–androgenic steroids in primary rat hepatic cell cultures, J. Pharmacol. Toxicol. Methods 33(4):187–195.

White, A.; Handler, P. and Smith, E.L. (1973): Principles of biochemistry, 5 edn. McGraw. Hill, New York, 1295pp.

Williams, J.K.G.; Kubelik, A.R.; Livak, K.J.; Rafalsky, J.A. and Tyngey, S.V. (1990): Molecular changes during gonad masculinization. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.

Wrobewski,F. and La Due, J.S.(1956): Quantitative determination of alanine aminotransferase (ALT) in plasma. Proc.Soc. Exper. Biol. Med., 91:569-572.

Young, D.S. (2001): Colorimetric Quantitative determination of albumin in plasma. Effect of disease on clinical lab. Tests, 4th Ed AACC.

Early Marker for Renal Impairment and Angiopathy in Diabetic Egyptian Children

Azza Abdel Shaheed Abdallah[1], Karima Abbas El-Shamy[2], William M. Morcos[1], Tahani Hanna Mikhail[2]and Nevein Naim Fadl[2]

Departments of [1]Child Health and[2]Physiology Department National Research Centre, Cairo, Egypt
*william_M_Morcos@hotmail.com

Abstract: Background: Since diabetes mellitus is a significant risk factor for early onset of many complications (atherosclerotic vascular disease, coronary heart disease, retinopathy, nephropathy, and neuropathy), there is therefore an essential need to know and understand more about the early detection of these complications in order to develop effective prevention strategies.**Objective**: The current work was carried out on type 1 diabetic children to investigate the level of retinol-binding-protein (RBP4) and endothelin-1 (ET-1) as an early marker for renal impairment and angiopathy. **Methods**: Forty-eight children (7-15 years) with type diabetes, 18 children with micro-albuminuria (MA^+ group) and 30 children were normoalbuminuria (MA^- group) and these two groups are compared with 24 apparently healthy non-diabetic children. A comparative study was performed for these groups as regarded to the levels of RBP_4, insulin, ET-1 and testosterone. **Results:** The high levels of serum and urine RBP4 in MA^- (normoalbuminuria) type 1 diabetic children group, indicates that RBP4 could be an early marker for renal impairment even in the absence of renal impairment (MA). The significantly higher level of plasma ET-1 in MA^- than in MA^+ diabetic group, may indicate that endothelial dysfunction, precedes the appearance of microabuminuria in type 1 diabetic patients, and could be used as an early marker for diabetic microangiopathy. Level of serum testosterone was significantly reduced in male diabetic children and showed direct correlation with age and insulin dose.**Conclusion:** (1) High levels of serum and urine RBP4 in MA^+ type 1 diabetic children groups, indicates that RBP4 (retinol binding protein) is an early marker for renal impairment even in the absence of MA; (2) The significantly higher level of plasma ET-1 (Endothelin-1) in MA^+ (normalalbuminurea) than in MA^+ (microalbuminurea) group, may indicate that endothelial dysfunction, precedes the appearance of microalbuminurea in type 1 diabetic patients, and could be used as an early marker for diabetic microangiopathy. In addition, no correlation was found between plasma ET-1 and both of serum insulin and insulin dose in the diabetic children; (3) Level of serum testosterone is reduced significantly in the diabetic children males and showed direct correlation with age and insulin dose.

[Azza Abdel Shaheed Abdallah, Karima Abbas El-Shamy, William M. Morcos, Tahani Hanna Mikhail and Nevein Naim Fadl. **Early Marker for Renal Impairment and Angiopathy in Diabetic Egyptian Children**]. Life Science Journal. 2011; 8(3):358-366] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: Diabetes children, angiopathy albuminuria

1. Introduction

Diabetes is one of the fastest growing disease in the world. The World Health Organization ***(WHO, 2008)*** estimates that more than 180 million people worldwide have diabetes. Type I diabetes mellitus is one of the most common chronic diseases in childhood, caused by insulin deficiency resulting from the destruction of insulin-producing pancreatic beta cells. Early functional and structural abnormalities may be present a few years after the onset of the disease in children and adolescents. Therefore, a regular screening for diabetic microvascular disease particularly retinopathy and nephropathy, early detection of diabetic microangiopathy and treatment of early signs of these complications have a vital role in prevention of blindness and end-stage renal failure in children and adolescents with diabetes ***(Chiarelli et al., 2002; Akbay et al.,2010).***

Children with diabetes mellitus type I (IDDM) that is not under strict control tend to develop hypercholesterolemia, micro and macroangiopathy and thus are at great risk of cardiovascular diseases.

Microalbuminurea (MA) is a predictor of future diabetic nephropathy and cardiovascular disease in both type 1 and type 2 diabetes. In type 1 diabetes urinary albumin excretion is an index of glomerular function whereas retinol binding protein "RBP" (RBP coded by the gene RBP_4) is an index of renal tubular function. The increased urinary excretion of RBP_4 in these patients suggests slightly impaired proximal tubular function in early stages of diabetic nephropathy.

A low level of testosterone might be related to the development of premature coronary artery disease in men. Increased testosterone levels in patients with diabetes could be related to increased risk for cardiovascular complication later in life ***(Meyer et al., 2000).***

Endothelin-1 (ET-1) has a role in type 1 and type 2 diabetes mellitus and the development of diabetic complications. As insulin stimulates endothelin-1 expression in a dose response relationship. Increased insulin exposure (hyperinsulinemia and hypertriglyceridemia in patients with diabetes may have long term effects on vascular wall structure through its stimulation of ET-1 expression ***(Sarafidis and Ruilope, 2006).***

Aim of the Work:

The present work was designed to evaluate and introduce more sensitive and specific biochemical early markers such as RBP4 (as an indicator of tubular disorder) and ET-1 (as a sensitive marker for angiopathy) in children with type 1 diabetes. In addition, testosterone was measured in these diabetic children to find out whether there is a correlation between daily insulin dose, duration of diabetes, metabolic control, age, and body mass index versus their levels.

2. Patients and Methods

Subjects:

Seventy-two children with age range of (7-17 years) were included in this study. They were 48 diabetic children with type 1 diabetes mellitus and 24 apparently healthy children as control (36 were males and 36 females). The duration of the illness varied from 1-10 years and their daily total insulin dose varied from 14-68 unit/day/ all patients were taken from those attending the diabetes program at the Diabetic Center, Kasr El-Eany Egypt. Medical history examination and measurement of weight and height for calculating the body mass index (BMI) were performed.

Under complete septic technique, 10 mL of blood were collected from each child and placed in three test tubes.

1st tube: contain EDTA for determination of glycohemoglobin (HbA_{IC}) in the whole blood at the same day ***(Abraham et al., 1978).***

2nd tube: contain EDTA and immediately immersed in ice then centrifuged within one hour in a cooling centrifuge at 0°C the plasma were stored at -20°C for later determination of endothelin-1 (ET-1) with ELISA Kits according to ***Tiyssen (1985).***

3rd tube: the collected blood allowed to clot naturally, then centrifuged at 4000 r.p.m. for 15 minutes to separate the serum. The fresh serum used for the estimation of the glucose concentrations, lipid profile (Total cholesterol, triglyceride and total lipid), kidney function (urea and creatinine), liver function (AST and ALT), insulin, testosterone, sex hormone binding globulin (SHBG) and retinol-binding protein (RBP4).

Methods:

Using laboratory and enzyme immunoassay ELISA Kits.

Part of the fresh serum was used for estimation of the glucose concentration the other part was stored at -20°C for further analysis of lipid profile total lipid, total cholesterol and triglyceride using the enzymatic colorimetric method described by ***Fossati and Prencipe (1982)*** kidney function (urea and creatinine) were performed according to ***Fabiny and Eringhausen (1971)***, liver function (AST and ALT) were determined according to the colorimetric method described by ***Reitman and Frankel (1957).***

Determination of total serum bilirubin by Randox Laboratory Kit

Insulin, testosterone, sex-hormone binding globulin (SHBG) and retinol binding protein (RBP4) by enzyme immunoassay (ELISA) Kits

In addition urine was collected from each child, part of it was stored at -20°C for later measurement of microalbuminurea (MA), urea and creatinine. The other part was adjusted to a pH (6-8) with NaOH then stored at -20°C for later urine RBP_4 measurement. According to the values of microalbuminurea, the diabetic children were classified into two groups.

Positive microalbuminurea children: MA^+ (+ve MA) group.

Negative microalbuminurea children: MA^- (-ve MA) group.

Statistical Analysis:

The data obtained in the present work were analyzed IBM compatible computer. Quantitatives variables were expressed by mean $\pm$ standard error (S.E.). In all tests, P.value was considered significant at level less than 0.05 ($P<0.05$).

3. Results

The current study was carried out on "48" randomized type 1 diabetic children which were divided into two groups:

Normoalbuminurea with negative microalbuminurea: MA^- group.

Microalbuminurea with positive microalbuminurea: MA^+ group.

Comparative data of the diabetics children with the data of apparently healthy children are give in (Tables 1-8), and correlation coefficient are shown in the graves.

Table (1): Comparison of the mean values of the parameters of children with type 1 diabetes (MA^- and MA^+) and control group

Groups / Parameters	Control children (n= 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n = 18)
Age (y)	**A** 12.21 ± 0.37	**A** 11.83 ± 0.30	**A** 12.22 ± 0.45
BMI (Kg/m^2)	**A** 20.96 ± 0.57	**B** 19.23 ± 0.66	**B** 18.94 ± 0.38

Table (2): Comparison of the mean values of duration of diabetes, insulin dose, serum glucose, and glycosylated hemoglobin (HbA_1c) in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children

Groups / Parameter	Control children (n= 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
Duration of diabetes (y)	–	**A** 3.20 ± 0.44	**A** 3.31 ± 0.57
Insulin dose (U/d)	–	**A** 38.40 ± 2.65	**A** 36.28 ± 3.29
Glucose (mg/dL)	**A** 87.67 ± 1.85	**B** 216.93 ± 16.43	**B** 179.50 ± 13.10
HbA_1c %	**A** 6.25 ± 0.26	**B** 8.03 ± 1.05	**B** 8.46 ± 0.74

Table (3): Comparison of the mean values of lipid profile in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children

Groups / Parameters	Control children (n=24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
Cholesterol (CH) (mg/dl)	**A** 171.00 ± 2.36	**B** 191.43 ± 6.57	**B** 188.50 ± 6.04
Triglycerides (T.G) (mg/dl)	**A** 53.33 ± 1.84	**B** 74.23 ± 4.30	**B** 67.56 ± 3.80
Total lipids (T.L) (mg/dl)	**A** 622.13 ± 10.85	**A** 632.80 ± 23.33	**A** 598.44 ± 20.77

n : number of children

All data are represented as mean ± S.E

Different letters at the same row mean significant difference ($p<0.05$) between groups at the level of (0.05), common letters mean non-significant difference ($p>0.05$).

Table (4): Comparison of the mean values of serum aspartate aminotransferase (s.AST) and serum alanine aminotransferase (s.ALT) in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children

Groups / Parameters	Control children (n = 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
s. AST (u/ml)	**A** 27.42 ± 0.83	**B** 35.70 ± 2.31	**B** 41.39 ± 3.07
s. ALT (u/ml)	**A** 8.46 ± 0.50	**B** 12.53 ± 1.08	**AB** 11.28 ± 1.02

Table (5): Comparison of the mean values of kidney function tests in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children.

Groups / Parameters	Control children (n= 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
S.urea (mg/dL)	**A** 16.58 ± 0.65	**B** 22.53 ± 1.13	**B** 22.11± 1.32
U.urea (g/L)	**A** 18.10 ± 0.73	**B** 13.11 ± 1.18	**B** 13.95 ± 1.04
S.creatinine (mg/dl)	**A** 0.33 ± 0.02	**B** 0.27 ± 0.02	**B** 0.24 ± 0.02
U. creatinine (g/L)	**A** 1.39 ± 0.08	**B** 0.84±0.05	**A** 1.27 ± 0.16
Microalbuminuria (µg/ml)	**A** 15.38 ± 0.39	**A** 13.93 ± 0.71	**B** 67.83 ± 4.73

Table (6): Comparison of the mean values of both serum and urine RBP4 in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children

Groups / Parameters	Control children (n= 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
S.RBP4 (mg/L)	**A** 13.67 ± 0.50	**B** 16.94 ± 0.60	**C** 18.94 ± 0.62
U.RBP4 (mg/L)	**A** 0.016 ± 0.00	**B** 0.020 ± 0.00	**C** 0.022 ± 0.00

n: number of children

All data are represented as mean ± S.E

Different letters at the same row mean significant difference ($p<0.05$) between groups at the level of (0.05), Common letters mean non-significant difference ($p>0.05$).

Table (7): Comparison of the mean values of serum insulin and plasma endothelin-1(ET-1) in children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children.

Groups / parameters	Control children (n= 24)	Diabetic children	
		MA^- (n= 30)	MA^+ (n= 18)
Insulin (µIU/ml)	**A** 44.50 ± 1.39	**B** 34.10 ± 2.57	**AB** 37.94 ± 2.934
ET-I (Fmol/ml)	**AB** 0.79 ± 0.04	**A** 0.99 ± 0.09	**B** 0.66 ± 0.11

Table (8): Comparison of the mean values of serum testosterone and sex hormone binding globulin (SHBG) in both males and females children with type 1 diabetes (MA^- and MA^+) and apparent healthy control children.

Groups / Parameters	Control children (n= 12)	Diabetic children	
		MA^- (n= 15)	MA^+ (n= 9)
Testosterone in males (ng/ml)	**A** 1.49 ± 0.06	**B** 0.26 ± 0.08	**B** 0.05 ± 0.01
Testosterone in females (ng/ml)	**A** 0.09 ± 0.02	**A** 0.15 ± 0.04	**A** 0.16 ± 0.04
SHBG in males (nmol/l)	**A** 27.67 ± 1.04	**B** 39.93 ± 3.13	**AB** 35.22 ± 1.42
SHBG in females (nmol/l)	**A** 22.92 ± 1.20	**B** 48.80 ± 2.25	**C** 32.33 ± 1.73

n : number of children

All data are represented as mean ± S.E

Different letters at the same row mean significant difference ($p<0.05$) between groups at the level of (0.05), common letters mean non-significant difference ($p>0.05$).

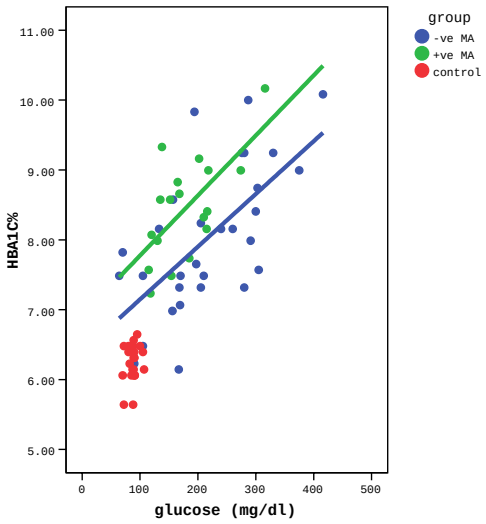


Figure (1-a): Direct correlation between HbA_1c value and serum glucose in MA^- group (r=0.61, P<0.01), and MA^+ group (r= 0.57, P<0.05).

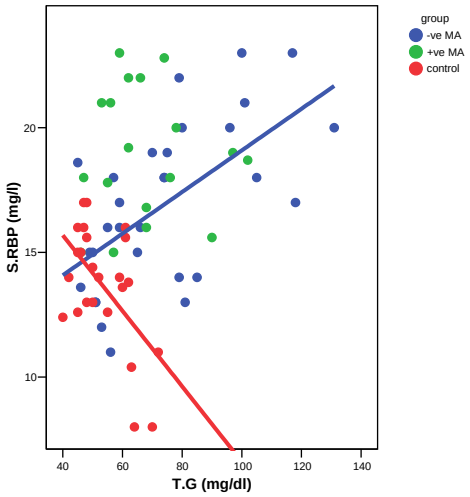


Figure (1-a): Direct correlation between HbA_1c value and ı glucose in MA^- group (r=0.61, P<0.01), and MA^+ group (r= 0.57, P<0.05).

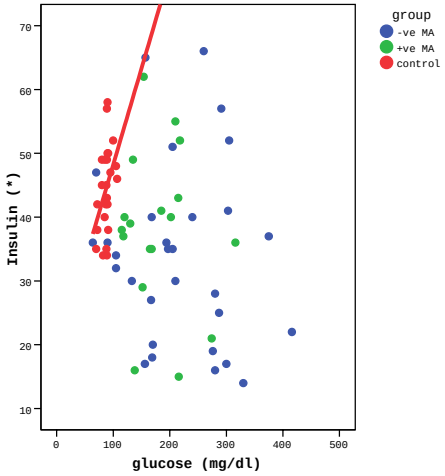


Figure (2-a): Direct correlation between serum insulin levels and serum glucose in healthy control group (r=0.45, P<0.05).

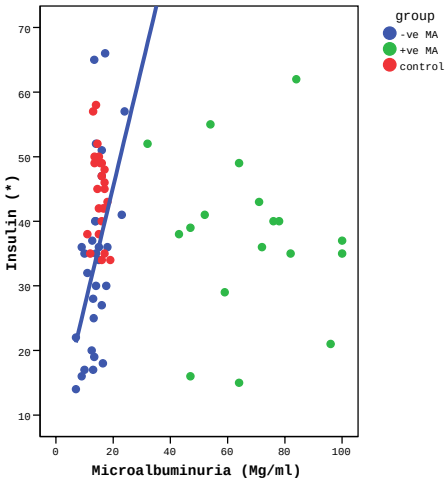


Figure (2-b): Direct correlation between serum insulin levels and microalbuminuria in MA^- diabetic group (r=0.53, P<0.01).

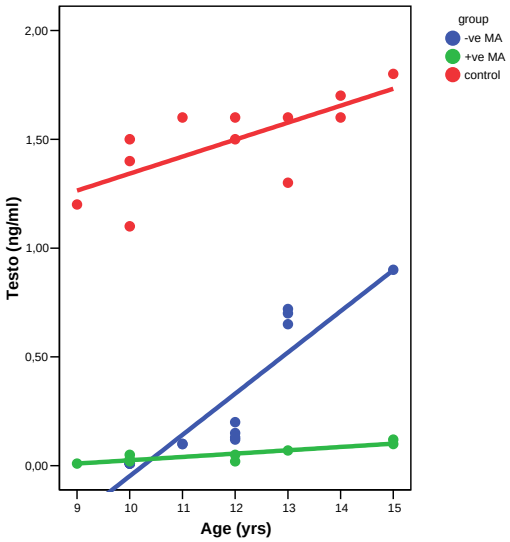


Figure (3-a): Direct correlation between serum testosterone (Testo.) and age in male control, MA^-, and MA^+ groups (r=0.73, P<0.01; r=0.91<0.01 and r= 0.89, P<0.01 respectively)

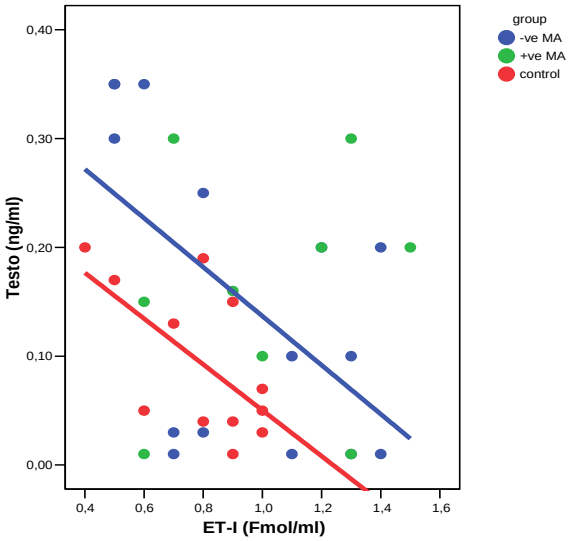


Figure (3-b): Inverse correlation between serum testosterone (Testo.) and plasma ET-1 in control and MA^- females groups (r= -0.62, P<0.05 and r=-0.56, P<0.05 respectively).

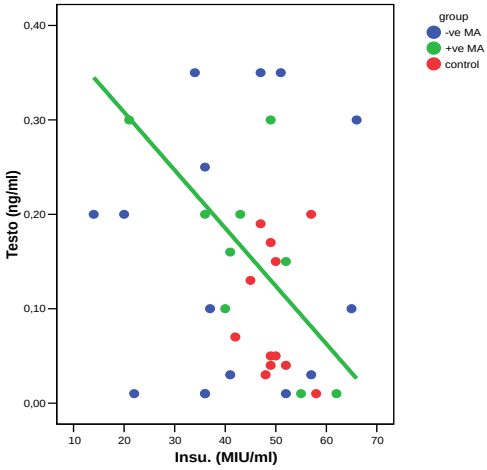


Figure (4-a): Inverse correlation between serum testosterone (Testo.) and serum insulin level in MA^+ diabetic female group (r=-0.69, P<0.05)

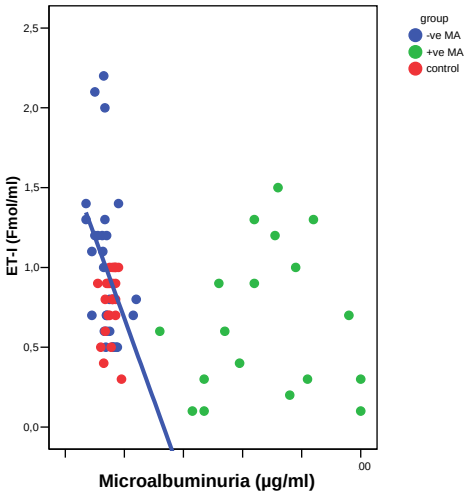


Figure (4-b): Inverse correlation between ET-1 and microalbuminuria in MA^- group (r=-0.41, P< 0.05).

4. Discussion

Diabetes is usually accompanied by an increased production of reactive oxygen species and free radicals, or by impaired antioxidant defenses *(Maritim et al., 2003),* which is widely accepted as important in the development and progression of diabetic complications. Research to help counteract these outcomes in diabetic patients has resulted in a number of new and advanced therapies, including novel antidiabetic medications, surgical interventions, islet cell transplantation, hematopoietic stem cell transplantation and gene therapy.

The purpose of this study has been to estimate the effect of diabetes on serum and urine RBP4 as a marker of nephropathy *(Pontuch et al., 1995; Turuner et al., 2011)* plasma ET-1 as a marker of angiopathy *(Schalkwijk and Stehouwer, 2005),* and to investigate the relationship between insulin dose and androgen in type 1 diabetic children. Results of the present work have shown a significant decrease in the body mass index (BMI) of children with diabetes whether MA^- or MA^+ compared to their control. A higher BMI was associated with a younger age (0-4.9 year) at diabetes onset and it gradually decreased with increasing age.

Chronic hyperglycemia during diabetes is known to cause glycation of body proteins that in turn leads to secondary complications affecting eyes, kidney, nerves, and arteries. Long-term hyperglycemia, as measured by HbA_1c is further related to cardiovascular mortality in men. Evidence from the current studies has indicated that in both group of type 1 diabetic children (MA^- and MA^+) there was a significant increase in the level of serum glucose and HbA_1c values compared to the control. A significant increase in HbA_{1C} concentration was detected in all diabetic patients, which may indicate an increased familial risk of diabetic microvascular disease.

In the present work HbA_1c value was slightly higher in MA^+ children diabetic group than in MA^- ones. Improvement of glycemic control declines the incidence of diabetic nephropathy *(Nordwall et al., 2004)* as it reduces hyperfiltration and diminishes albumin excretion rate.

A positive correlation between HbA_1c and serum glucose level has been detected in diabetic children (MA^- and MA^+). This finding is supported by many earlier studies.

The dyslipidemia of diabetes is associated with the increased cardiovascular disease found in type "1" diabetes. The similarity between the increased serum cholesterol and triglyceride levels in the current work is a proof that lipid abnormalities remain common in children and adolescents with type 1 diabetes. Abnormally high level of serum lipids is mainly due to the uninhibited action of lipolytic hormones on the fat depots, brought about by the action of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in the diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia, and insulin deficiency is also associated with hypercholesterolemia due to metabolic abnormalities.

Measurement of enzyme activity in serum is of important value because it helps to assess the state of the liver and other organs. Normally serum ALT and AST levels are low, but these enzymes are released into circulation after cellular damage and increased because they are cytoplasmic in location. The liver and heart release Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), and an elevation in their plasma concentrations are an indicator of liver and heart damage. Our results have shown significant increase in the activity of serum AST and ALT in the diabetic groups as compared to the control one.

Assessment of kidney functions in both children and adolescents with type "1" diabetes is necessary for the early detection of renal lesions and for the application of an adequate therapy. *Amann et al. (2006)* described the potential relation between change in kidney function and the potential consequences for the cardiovascular system as MA reflects impaired vascular function in general and is associated with a higher susceptibility to cardiovascular and renal events. Our results as well as those of former studies have shown that the level of MA was significantly higher in the diabetic children. The present study demonstrates that out of 48 type 1 diabetic children, 30 patients (~62%) had normoalbuminuria (MA^-) and 18 (~38%) had microalbuminuria (MA^+). Subjects diagnosed with type "1" diabetes before or during puberty are at a higher risk of nephropathy when compared with subjects diagnosed after puberty.

Usually the definition of renal impairment relies upon the serum creatinine and urea levels, or creatinine clearances, which, however, are not sufficient to demonstrate initial renal damage as they are insensitive, nonspecific, and change significantly only after significant kidney injury *(Vaidya et al., 2008).* For that, high and low-molecular weight urinary proteins are good indices of glomerular or tubular function. From these proteins, retinol-binding protein (RBP4), that is a good indicator of initial renal damage, even in the absence of alterations of the routine parameters.

The increase in the urinary excretion of RBP4 in children is highly specific for tubular disease, which occurs earlier than glomerular (albumin) affection, as urinary RBP4 excretion is increased in early diabetic nephropathy and might even be a marker of early renal damage preceding microalbuminuria *(Ziegelmeier et al., 2007; Axelsson et al., 2009).* The results of the present

study have shown a significant increase in the level of both serum and urine RBP4 in the diabetic children (MA^- and MA^+). Moreover a significant increase in its level was noticed in the MA^+ group compared to the MA^- one. These results are in line with many previous reports, which proved that increased concentrations of RBP4 both in serum and urine have been found in children in the absence of either MA or impaired renal function as assessed by serum creatinine.

Additionally, the absence of correlation between RBP4 and MA in our study may be explained on the basis that RBP4 is not usually correlated with the severity of the disease, but seems to be helpful in identifying a subset of patients with initial renal disorder *(Corso et al., 1999; Henze et al., 2010).* Supporting this concept, the present study indicated that serum RBP4 is the only reliable test for prediction of early renal impairment in the form of microalbuminuria. Moreover, the elevated concentrations of RBP4 in serum reflect that the kidney is the main site for RBP4 catabolism. Owing to its small size, RBP4 filtered at the glomeruli and both reabsorbed and metabolized in the proximal tubule.

As expected, our results showed that serum insulin level was lower in diabetic children as compared with control group. In the MA^+ diabetic group, serum insulin level was slightly higher, than in MA^- diabetic ones, and it correlates well with MA in the latter group (MA^-).

Children and adolescents with type 1 diabetes mellitus are prone to experience a delay in the onset of pubertal process *(Meyer et al., 2000).* In agreement with this concept, our study showed that serum testosterone level was significantly lower in male diabetic children as compared with the control. Among females, serum testosterone was slightly higher in those with diabetes than in nondiabetic siblings. This result is in line with *Meyer et al. (2000)* who reported that higher serum total and free testosterone were found in females with type I diabetes than in control.

According to the present study, SHBG was significantly higher in the diabetic children comparing with the control. This agrees with *Danielson et al. (2008)* who reported that type 1 diabetes is associated with elevated SHBG concentrations. Serum level of SHBG in the current study was significantly lower in MA^+ group as compared with MA^- diabetic females. This result is in accordance with *Rudberg and Persson (1995)* who reported that a hyperandrogenism and low SHBG level in female type 1 diabetic patients has been linked to MA risk.

Male serum testosterone in the current study showed a direct correlation with insulin dose in MA^- diabetic group and not in the MA^+ ones. Previously, *Meyer et al. (2000)* found no correlation between daily insulin requirements and serum androgen levels in adolescents with type 1 diabetes. Also male serum testosterone in the present study correlates directly with age in both diabetic and control groups. The present study showed that serum testosterone also correlates positively with BMI in the MA^+ diabetic male group.

Reports on plasma ET-1 (Endothelin-1) levels in patients with diabetes mellitus are conflicting because increased, unchanged or decreased *(Smulders et al., 1994)* levels have been reported previously.

In the present study, plasma ET-1 level was insignificantly higher in the diabetic children as compared with the control. Moreover, plasma ET-1 level was significantly higher in MA^- comparing to the MA^+. *Stehouwer et al. (1995) and Maier et al. (2009)* reported that endothelial dysfunction precedes the appearance of MA by about 3 years in IDDM patients. Therefore, circulating ET-1 levels could represent an indicator of early diabetes-related renal damage.

The present study recorded also a direct correlation between plasma ET-1 and triglycerides in the diabetic children with MA^+.

Insulin resistance has been widely accepted as risk factor for cardiovascular disease, where insulin could promote atherogenesis by direct action on the arterial wall and the enhanced formation of superoxide anion O_2 leading to an impaired endothelium dependent arterial relaxation *(Shinozaki et al., 2004).*

Consequently, the present study confirmed that the plasma ET-1 (Endothelin-1) might be considered as an early marker for angiopathy in diabetes (type 1).

Corresponding author
William M. Morcos
Departments of Child Health, National Research Centre, Cairo, Egypt
*william_M_Morcos@hotmail.com

References

Abraham EC, Huff TA, Cope ND, Wilson JB, Bransome ED, Huisman TH. Determination of the glycosylated hemoglobins with a new microcolumn procedure. Suitability of the technique for assessing the clinical management of diabetes mellitus. Diabetes. 1978; 27:931-937.

Akbay E, Muslu N, Nayir E, Ozhan O, Kiykim A: Serum retinol binding protein level is related with renal functions in diabetes. Journal of Endocrinological Investigation 2010; 33(10):7235-7239.

Amann K, Wanner C and Ritz E: Cross-talk between the kidney and the cardiovascular system. J Am Soc Nephrol. 2006; 17:2112-2119

Axelsson J, Obyrene SM, Blaner WS, Carrero JJ, Brunchfeld A, Heimburger O, Barany P, Lindholm B. Stenvin: Serum retinol-binding protein concentration and its association with chronic

kidney disease. American Journal of Nephrology 2009; 29(5):447-453.
Chiarelli F, Mohn A, Tumini S, Trotta D, Verrotti A: Screening for vascular complications in children and adolescents with type 1 diabetes mellitus. Horm Res. 2002; 53(1):113-116.
Corso A, Serricchio G, Zappasodi P, Klersy C, Bosoni T, Moratti C, Castagnola C, Lazzarino M, Pagnucco G, Nernasconi C: Assessment of renal function in patients with multiple myeloma; the of urinary protein. Hematol. 1999; 78:371-375.
Danielson KK, Drum ML, Lipton RB: Sex hormone binding globulin and testosterone in individuals with childhood diabetes. Diabetes Care. 2008; 31(6):1207-1213.
Fabiny DL and Eringhausen G: Calorimetric method for estimation of creatinine. Clin Chem. 1971; 17:696.
Fossati P and Prencipe L: Serum triglycerides determination colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem. 1982; 28:2077-2078.
Henze A, Frey SK, Raila H, Scholze A, Spranger J, Weickert MO, Tepel N, Zidek W, Schweigert GJ: Alternations of retinol-binding protein 4 species patients with different stages of chronic kidney disease. Biochemical and Biophysical Research Communications. 2010; 393(1):79-83.
Maier C, Clodi M, Neuhold S, Resl M, Elkenicky M, Prager R, Moerti D, Strunk G, Fuger A, Struck J, Packer P: Endothelial markers may link kidney function to cardiovascular events in diabetes. Diabetes Care. 2009; 32(10):1890-1995.
Maritim AC, Sanders RA, Watkins JB: Diabetes oxidative stress and antioxidants. J Biochem Mol Toxicol. 2003; 17:24-38.
Meyer K, Deutscher J, Anil M, Berthold A, Bartsch M, Kless W: Serum androgen levels in adolescents with type 1 diabetes: relationship to pubertal stage and metabolic control. J Endocrinol Invest. 2000; 23(6): 362-368.
Norwall M, Bojestig M, Arnqvist HJ, Ludvigsson J: Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population type 1 diabetes – the finkoping diabetes complications study. Diabetologia. 2004; 47:1266-1272.
Pontuch P, Toserova E, Vozar J, Bulas J, Kratoichvilova H: 24-h ambulatory blood pressure, daytime and midtime urinary albumin and retinol binding protein excretion in type 1 diabetic patients. J Diabetes Complications. 1995; 9(4):234-236.
Reitman S and Frankel S: A colorimetric method for the determination of serum GOT and GPT. Am J Clin Path.1957; 28:56-63.
Rudberg S and Person B: Indication of low sex hormone binding globulin (SHBG) in yong females with type 1 diabetes, and an independent association to microalbuminuria. Diabet Med. 1995; 12(9):812-822.
Sarafidis PA and Ruilope LM: Insulin resistance, hyperinsulinemia and renal injury: mechanisms and implications. Am J Nephrol. 2006; 26:232-244.
Schalkwijk CG and Stehouwer CD: Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin Sci (Lond). 2005; 109(2):143-159.
Shinozaki K, Ayajiki K, Kashiwagi A, Masada M, Okamura T: Malfunction of vascular control in lifestyle-related diseases: mechanisms underlying endothelial dysfunction in the insulin-resistant state. J Pharmacol Sci. 2004; 96:401-405.
Smulders RA, Stehouwer CD, Olthof CG, Van Kamp GJ, Teerlink T, De Vries PM, Donker AJ: Plasma endothelin levels and vascular effects of intravenous L-arginine infusion in subjects with uncomplicated insulin-dependent diabetes mellitus. Clin Sci (Colch). 1994; 87:37-43.
Soria B, Shoudy A, Martin F: From stem cells to beta cells: new strategies in cell therapy of diabetes mellitus. Diabetologia. 2001; 44(4):407-415.
Stehouwer CD, Fischer HR, van Kuijk AW, Polak BC, Donker AJ: Endothelial dysfunction precedes development of microalbuminuria in IDDM. Diabetes. 1995; 44(5):561-564.
Tiyssen P: Laboratory techniques, in biochemistry and molecular biology Practice and Theory of Enzyme Immunoassays. Vol. 15 Elsevier, Amsterdam. 1985; 221-241.
Toruner F, Altinova AE, Aktuk M, Arslan E, Bukan N, Kan E, Yetkin I, Arslan M: The relationship between adipocyte fatty acid binding protein-4, retinol binding protein-4 level in diabetes. Diabetes Research & Clinical Practice. 2011; 91(2):203-207.
Vaidya VS, Ferguson MA, Bonventre JV: Biomarkers of acute kidney injury. Annual Review of Pharmacology and Toxicology. 2008; 48:463-493.
World Health Organization. Fact sheet N°312, http:www.who.int/mediacentre/factsheets/ fs312/en/index.html. 2008.
Ziegelmeier M, Bachmann A, Seeger J, Lossner U, Kratzsch J, Blüher M, Stumvoll M, Fasshauer M: Serum levels of adipokine retinol-binding protein-4 in relation to renal function. Diabetes Care. 2007; 30(10):2588-2592.

7/28/2011

1-4 years old infant's acute diarrhea treatment with zinc sulfate and ORS solution: A case study at Eshkenan city, Fars province, Iran

Hakimeh S. Sajjadi [1], Ali Akbar Shaikhi Fini [2], Abdolvahab S. Samavi [2]

[1.] Hormozgan University of Medical Sciences, Ostandari Blvd, Bandar Abbas, Iran
[2.] University of Hormozgan, Minab Street, Bandar Abbas, Iran
hakimeh.sajjadi@yahoo.com

Abstract: An experiment was conducted to evaluate effects of treatment efficiency of zinc sulfate and ORS solution in combination or with ORS (only) on intensity and duration of diarrhea in 1-4 years old infants. A total of one hundred two of 1-4 years old cases were treated in two groups, control group (52 cases) and experimental group (50 cases). Treatment period were done at health-care center of Eshkenan city, Fars province, Iran. Obtained data were evaluated by t-test for detection of significant difference. Findings showed that zinc sulfate in combination with ORS had better treatment efficiency on shortening of acute diarrhea and lowering its intensity, in comparison with ORS, alone. From the results of this study, it is concluded that zinc sulfate is a suitable completive treatment accompanying with ORS in treating infant's diarrhea term. [Hakimeh S. Sajjadi, Ali Akbar Shaikhi Fini, Abdolvahab S. Samavi. **1-4 years old infant's acute diarrhea treatment with zinc sulfate and ORS solution: A case study at Eshkenan city, Fars province, Iran.** Life Science Journal. 2011; 8(3):367-369] (ISSN:1097-8135). http://www.lifesciencesite.com.

Key words: 1-4 years old infants; diarrhea; zinc sulfate; ORS solution

1. Introduction

Diarrhea, is a common disorder among infants world widely, and is a major reason of mortality in 1-4 years old in Iran that has a heavy economic costs on public health (King et al., 2003; Zhang and Junling Li, 2009). It is a main agent of grow delay and early mortality in developing countries (Bettger and Odell, 1981). In United States of America 2.1 to 3.7 million diarrhea cases were diagnosed annually and 300-400 cases of annual mortality were recorded because of acute diarrhea (Behrman et al., 2004). Patient with diarrhea causes high economic costs for developing countries, for example about 30 present of hospital beds in these countries were occupied by diarrhea suffered infants (Prasad, 1998). In Iran, diarrhea is a main reason of mortality for 1-4 years old group (Shams, 2001). In other word, about 12% 1-4 years old of infants in cities and 14% in villages were suffering from diarrhea (Iranian health ministry, 2002). Main reason of diarrhea related mortality is incidence of dehydration that commonly liquid intravenous injection was used for treatment (Arcasoy et al., 1990). In a research it is observed that treatment with only solution injection may cause lowering mortality incidence but can't decline duration of diarrhea period (Richard et al., 1993). Because of negative effect of acute diarrhea on body weight and immune system (Baqui et al., 1993), suggested treatment is including zinc sulfate syrup and ORS solution (Black et al., 1984). Efficiency of this kind of treatment was documented in researches (Reinhold and Charami, 1981; Al-Sonboli et al., 2003). Also, some studies decelerated that zinc supplementation can prevent respiratory disorders and can help for diarrhea period declining in acute or chronic diarrhea (Bhandari et al., 2002; Behrman et al., 2004; Raqib et al., 2004). With attention to effectiveness of ORS and zinc sulfate treatment, in present study, effect of both of treatments in 1-4 year old infants were compared.

2. Material and methods

This study conducted with clinical based diagnosis on patients (1-4 year old infants) at health-care center of Eshkenan city. The investigable patients have these parameters; 1-4 years old, suffering from diarrhea without hemorrhage and without antibiotic usage from began to end of treatment.

Patients with lower and higher ages (lower than one or higher than four), diarrhea with hemorrhage or without parents allowance were removed from our experimental groups.

Totally, 102 infant were divided in two experimental groups; 52 of them as control group and 50 of them as experimental or treatment group. In control group we had used only ORS and in experimental group, we had used ORS with zinc sulfate syrup according to hospital treatment protocol. Data were collected via communications with patient's parents, documents or disease history review and co-worker doctor's reports in same research project.

Data were analyzed by SPSS Ver. 16 software and t-test was done for comparison of two groups and detection of significant differences.

3. Results

Findings show 27.5 percent of diarrhea suffered infants were boy and 72.5 percent were girl. Age mean of infants was 2.41 years old and around 52.9 percent had lower than two years old. Demographic information of samples is presented as table 1. Diarrhea frequency and duration in control and experimental group are presented in tables 2 and 3. In both of parameters, superiority of experimental group was observed. Statistical analysis for diarrhea intensity show t-value: 11.45 with df: 100 and $p<0.001$. Also, Statistical analysis for diarrhea duration shows t-value: 7.17 with df: 100 and $p<0.001$. Comparative statistical description for treatments is presented in table 4.

According to tables 1-4, mean diarrhea frequency after zinc sulfate syrup and ORS was 2.24 time/ day that in comparison with control group (4.17 time/ day) had considerable declines. For treatment period duration, efficiency of treatment with both of zinc sulfate and ORS in comparison with only ORS, it was observed that mean healing period in experimental group was 2 day that was 3.21 day for control group.

Table 1. Demographic information of studied sample

Traits	Gender			Age (*months old)*				
	girl	boy	total	12	13-24	25-36	37-48	total
Number	74	28	102	24	30	30	18	102
Percent	72.5	27.5	100	23.5	29.4	29.4	17.6	100

Table 2. Diarrhea intensity (time/day) in control and experimental group

Group \ Time		2	3	4	6	≥ 6	Total
Control	No.	2	5	32	8	5	52
	%	3.8	9.6	61.5	15.4	9.6	100
Experimental	No.	38	6	6	0	0	50
	%	76	12	12	0	0	100

Table 3. Diarrhea duration (day) in control and experimental group

Group \ Day		1	2	3	≥ 3	Total
Control	No.	3	8	16	25	52
	%	5.8	15.4	30.8	48.1	100
Experimental	No.	12	29	6	3	50
	%	24	58	12	6	100

Table 4. Comparison of diarrhea duration and intensity of groups via *t*-test

Trait	group	mean	S.d	*t*-value	df	significance level
Intensity	control	4.17	0.87	11.45	100	p<0.001
	experimental	2.24	0.82			
Duration	control	3.21	0.91	7.17	100	p<0.001
	experimental	2.00	0.78			

4. Discussion

Findings of present study showed that synchronic application of ORS and zinc sulfate syrup in comparison with only ORS application is more efficient for both treatment parameters (declining of diarrhea frequency and shorting of healing period), qua in control group only 5.8% of infants in first 24 hours and 15.4% in 48 hours of treatment have healing signs, but in experimental group 24% of infants in first 24 hours and 58% of them in 48 hours had healing signs. About diarrhea intensity similar trend was observed; diarrhea frequency was 2 time/day in control group it was 3.8% and in experimental group it was 76%.

Obtained findings were according to past related studies (Sazawal et al., 1997; Dutta et al., 2000). In Sazawal et al. (1997) and Dutta et al. (2000), treatment with zinc sulfate and ORS solution could lower diarrhea intensity and duration and in overall it had healing effect on acute diarrhea in infants. It is concluded, zinc sulfate is a suitable completive treatment for ORS in term of infant's diarrhea treatment.

Acknowledgements:
Authors are grateful to Center of emergency care, Hormozgan University of Medical Sciences, Ostandari Blvd, Bandar Abbas, Iran for financial support to carry out this work.

Corresponding Author:
Dr. Hakimeh S. Sajjadi
Center of emergency care
Hormozgan University of Medical Sciences,
Ostandari Blvd, Bandar Abbas, Iran.
E-mail: hakimeh.sajjadi@yahoo.com

References

1. King CK, Glass R, Bresee JS, Duggan C. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. MMWR Recomm. Rep. 2003; 52(RR-16): 1-16.
2. Zhang Z, Junling Li WZ. Study of the risk factors of postoperative upper gastrointestinal bleeding of percutaneous coronary interventional therapy. Life Science Journal. 2009; 6(2): 63 – 64.
2. Bettger WJ, Odell BL. Acritical physiological role of zinc in the structure and function of biomembranes. Life Science 1981; 28(13): 1425-38.
3. Behrman RE, Kliegman RM, Jenson HB, Nelson OS. Textbook of pediatrics. 17th ed. Philadelphia: W.B. Saunders Company; 2004; 169-72.

4. Prasad AS. Zinc deficiency in humans: A neglected problem. Journal of American Collage of Nutrition 1998; 17(6):542-543.
5. Shams H. Educational demands of parents about acute diarrhea and application of ORS for fewer than five years old infants at gonabad city. 2001; OfogeDanesh Magazine, 2: 55. [In Persian]
6. Iranian health ministry. Health feature. Tabalvor publication, first edition, Tehran, 2002; p: 32.
7. Arcasoy A, Akar N, Ors U, Delibasi L, Karayalcin S. Ultra structual changes in the mucosa of the small intestine in patient with geophagic. Journal of pediatrics Gastroenterology and Nutrition 1990; 11:279-282.
8. Richard ZL, Claesonand M, Pierce NF. Manage met of acute diarrhea in children:lessons learned. Pediatric Infective Disease Journal 1993; 12(1): 5-9.
9. Baqui AH, Sack RB, Black RE, Chowdhury HR, Yunus M, Siddique AK. Cell- mediated immune deficiency and malnutrition are independent risk factors for persistent diarrhea in Bangladeshi children. American Journal of Clinical Nutrition 1993; 58(4): 543-548.
10. Black RE, Brown KH, Becker S. Malnutrition is a determining factor in diarrheal duration/not incidence among young children in a longitudinal study in rural Bangladesh. American Journal of Clinical Nutrition 1984; 39(1): 87-94.
11. Reinhold JG, Charami P. An attended study of the effect of Iranian village and urban flat Breads of the mineral balances of two men before and after supplementation with vitamin D. Ecology, Food and Nutrition 1981; 10: 169-77.
12. Al-Sonboli N, Gurgel RQ, Shenkin A, Hart CA Cuevas LE. Zinc supplementation in brazilian children with acute diarrhoea. Annals of Tropical Pediatrics 2003; 23(1): 3-8.
13. Raqib R, Roy SK, Rahman MJ, Azim T, Ameer SS, Chisti J. Effect of Zinc supplementation on immune and inflammatory responses in pediatric patients with shigellosis. American Journal of Clinical Nutrition 2004; 79(3): 444-450.
14. Bhandari N, Bahl R, Taneja S, Substantial TS. Reduction in severe diarrhea morbidity by daily Zinc supplementation in young north indian children. Pediatrics. 2002; 109(6): e 86.
15. Sazawal S, Black RE, Jalla S, Mazumdar S, Sinha A, Bhan MK. Effect of zinc supplementation on cell mediate dimmunted and lymphocyte subsets in preschool children. Indian. Pediatrics 1997; 34: 589-597.
16. Dutta P, Mitra U, Datta A, Niyogi SK, Dutta S, Manna B. Impact of zinc supplementation in malnourished children with acute watery diarrhea. Journal of Tropical Pediatrics 2000; 46(5): 259-263.

3/5/2011

Hepatotoxic Potential of Gibberellic Acid (GA_3) in Adult Male Albino Rats

Wafaa F. Hussein[1], Fatma Y. Farahat[1], Marwa A. Abass [1]& Azza S. Shehata[2]
Departments of [1]Forensic Medicine and Clinical Toxicology & [2]Histology and Cell Biology
Faculty of Medicine, Zagazig University

Abstract: Gibberellic acid (GA_3), a plant growth regulator, is widely used in agriculture of many countries including Egypt. However, its potential hazardous effects on human health were relatively unexplored. The purpose of this study was to investigate the effects of sub- chronic toxicity of GA_3 on hepatic function and structure in adult male albino rats and also to determine the effects of withdrawal of GA_3 on the affected parameters following 6 weeks of follow up. Forty adult male albino rats were equally divided into four groups; the first group was used as a negative control, while the second group (positive control group) received NaOH; the vehicle. Animals of the third group (GA3 group) received 75 ppm of GA3 daily in drinking water for six weeks. Animals of the last group (Recovery group) received the same treatment as the third group for six weeks then were left without any treatment for another 6 weeks. At the end of the experimental period, all rats were sacrificed for assessment of liver function tests; ALT, AST, GGT and ALP. Liver specimens were collected for histopathological examination and assessment of hepatic levels of SOD, CAT, GSHPx and MDA. The results revealed that GA_3 sub-chronic toxicity induced a significant increase in AST, ALT, GGT and ALP as compared to control group. There was also a significant increase in hepatic malondialdehyde level with a significant decrease in SOD, CAT, and GSHPx enzymes activity in comparison with control groups. Histopathological examination using light microscope showed; hepatocyte vacuolization and inflammatory cellular infiltration. Most of hepatocytes appeared shrunken with pyknotic nuclei. Moreover, Bcl-2 immunolocalization revealed over-expression of this protein in both hepatocytes and endothelial cells of hepatic sinusoids. Electron microscopic examination revealed most of hepatocytes were with shrinkage nuclei with condensation of its heterochromatin and cytoplasmic vacuolization. On the other hand, stoppage of GA3 administration for 6 weeks has resulted in some sort of regression of the previously mentioned hepatoxic effects. In conclusion: results of the current study suggested gibberellic acid was a potent pro-oxidant that induced a significant hepatotoxicity in adult male albino rats, while 6 weeks period of follow up was insufficient for complete recovery of these toxic effects.

[Wafaa F. Hussein, Fatma Y. Farahat, Marwa A. Abass & Azza S. Shehatan. **Hepatotoxic Potential of Ggibberellic Acid (GA_3) in Adult Male Albino Rats**. Life Science Journal, 2011; 8(3): 373-383] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Plant Growth Regulators; Gibberellic Acid; Hepatoxicity; Oxidative Stress; and Lipid Peroxidation.

1. Introduction

Many chemicals are currently used in agriculture nowadays. One of these chemicals is the plant growth regulators where its use began in the 1930s (Fishel, 2006). Plant growth regulators (PGRs) are also known as plant hormones or phytohormones. They are chemicals that regulate plant growth (Osborne and McManus, 2005).

Gibberellins are one of the six major classes of plant growth regulators according to the American Society of Agricultural Science (Fishel, 2006). Gibberellic acid (GA3) is one of the most active hormones of gibberellins. It affects many mechanisms of plant growth including stem elongation by stimulating cell division and elongation, flowering, fruit development and breaking dormancy (Neil and Reece, 2002).

Saber *et al.* (2003) mentioned that gibberellic acid (GA3) is used extensively in Egypt and other countries, to increase the growth of many fruits (such as strawberries and grapes) and vegetables (such as tomatoes, cabbages and cauliflower).

Gibberellic acid (GA3) is highly persistent and bioactive in soil for months. The Environmental Protection Agency has determined its use to be only allowed in low doses (Schwechheimer and Willige, 2009).

People may be exposed to residues of GA3 in diet derived from consumption of different types of fruits and vegetables treated with GA3. Exposure to residues may also be through drinking water (Tomlin, 2004).

Occupational exposure of the agricultural workers to **GA3** may occur through inhalation of powder and dermal contact with this compound at work places where **GA3** is produced or used giving the picture of acute toxicity (Arteca, 1996).

GA3-treated cells loose their ability to scavenge reactive oxygen species and this loss ultimately results in oxidative damage and cell death (Fath *et al.,* 2001).

A growing amount of evidence indicates that GA3 alters the antioxidative systems in the rat's tissues. Antioxidant enzyme activities were significantly decreased in the erythrocyte, liver, and brain tissues of rats treated with GA3 (Tuluce and Celik, 2006).

Three groups of enzymes play significant roles in protecting cells from oxidant stress: superoxide dismutase (SOD), catalase (CAT) glutathione peroxidases (GPx). These enzymes have been shown to be sensitive indicators of increased oxidative stress (Stadtman and Levine, 2000).

Furthermore, the lipid peroxidation end product malondialdhyde (MDA) was significantly increased in the erythrocyte, liver, brain and muscle of rats treated with GA3. MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation (Celik *et al.,* 2007).

The liver is the main target for the toxicity of several compounds. This is because 75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics in concentrated form (Lee and Senior, 2005). The pathophysiologic mechanisms of hepatotoxicity are still being explored and include both hepatocellular and extracellular mechanisms (Andrad *et al.,* 2005). There are reports in support of GA3 impairment effects on the hepatic function and structure of rats (Saber *et al.,* 2003).

Ozmen *et al.* (1995) found that, exposure to GA3 resulted in a significant reduction of the total protein amount of hepatic tissue when compared with the control mice. Moreover, Sakr *et al.* (2003) found that oral administration of GA3 induced different biochemical and histochemical changes in the liver of the treated rats. Biochemical changes were in the form of early increase followed by late decrease in liver enzymes ALT and AST. Histochemical observations revealed marked reduction in total carbohydrates and total protein contents in the hepatocytes.

The increasing use of this substance in agriculture making it as an interesting subject to investigate its possible adverse effects on the liver as one of the main target organs for different xenobiotics. So, the aim of this study was to evaluate hepatotoxic effects of GA3 in adult male albino rats for 6 weeks, and also to determine the effects of withdrawal of GA3 on the affected parameters following 6 weeks of follow up.

2. Material and Methods

Material:

1- Chemicals:

A-Gibberellic Acid (2,4a, 7-Trihydroxy-1-methyl-8-methylenegibb-3-ene-1, 10 - dicarboxylic acid 1,4a-lactone) in the form of white crystalline powder, was from Sigma -Aldrich chemical Co., Germany.

B- Sodium hydroxide: It was used to dissolve gibberellic acid. It was obtained from El- Nasr Co., Egypt.

2- Kits:

A. Eli tech-diagnostic kits: for estimation of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST).
B. Gamma – Glutamyl transferase (GGT) kit from Bio ADWIC, Egypt was used for this assay.
C. Alkaline phosphatase (ALP) kit from Bio-Systems Co., Spain.
D. Bio-diagnostic kits: for estimation of superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GSHpx) and malondialdehyde (MDA) levels. Kits were purchased from Diagnostic and Research Reagents (Giza, Egypt).

3- Experimental Animals:

In this experiment, 40 adult male albino rats weighting 150-200 g were obtained from Animal House in Zagazig Faculty of Veterinary Medicine. All animals were subjected to 14 days of passive preliminaries in order to adapt themselves to their new environment and to ascertain their physical wellbeing. They were housed in a separate well ventilated cages, under standard conditions, with free access to the standard diet and water *ad libitum*. The experiment was conducted at the Animal House of Faculty of Medicine Zagazig University. The experiment was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, 1996).

Methods:

1-Experimental design:

Grouping of animals:

The rats were divided into four groups, each group consisted of ten rats.

Group 1 (Negative control group): kept without treatment till end of experiment.

Group 2 (Positive control group): Sham received only the vehicle (each rat received 1ml of 1N NaOH added to 1000 ml of tap water and given orally for 6 weeks).

Group 3 (GA_3 group): 75 mg of GA3 were dissolved in 1ml of 1N NaOH and then were diluted with tab water until 1000ml to obtain a 75 ppm dose

(Tuluce and Celik, 2006) to be added to drinking water of these animals for 6 weeks**.**

Since all rats have the same physiologic characters, daily water consumption of all rats was approximately 30 ± 3ml during the tests. Consequently, the GA_3 intake amount of each rat was about 2.2± 0.3mg per day (Tuluce and Celik, 2006).

Group 4 (Recovery Group): Animals of this group received the same treatment as group 3 for 6 weeks, then they were left without any treatment for 6 weeks **(Saly, 1998)**.

At the end of the study, all rats were anaesthetized by ether and sacrificed for collection of blood samples for estimation of AST, ALT, GGT and ALP serum levels. Liver specimens were collected to be subjected for assessment of oxidative stress markers and histopathological examination using both light and electron microscopes.

2- Assessment of liver function tests (LFTs):

-Alanine aminotransferase (ALT) (IU/L): estimation of ALT has been carried out using kits of Eli Tech-diagnostic. It was done as described by **Reitman and Frankel (1957)** according to the pamphlet of Eli tech-diagnostic by method of enzymatic UV kinetic.
-Aspartate Aminotransferase (AST) (IU/L): estimation of AST has been carried out using kits of Eli Tech-diagnostic. It was done as described by **Reitman and Frankel (1957)** according to the pamphlet of Eli Tech-diagnostic by enzymatic UV kinetic**.**
-Gamma – Glutamyl Transferase (GGT) has been carried out using Biolabo- France kit. It was determining as described in **Tietz (1986).**
-Alkaline Phosphatase (ALP) enzyme assessment was done using bio- systems Co., kit, Spain.

1- Assessment of tissue oxidative stress markers:

Each liver specimen was divided into 2 parts, one part was wrapped with aluminum foil and embedded in liquid nitrogen for 1 hour then kept frozen in -80°C till used to asses SOD, CAT & GSHpx enzymes activity and MDA level in tissues. Assessment of enzyme activity of GSHpx & SOD & CAT was done according to Paglia and Valentine (1967) & Durak *et al.* (1996) and Soliman *et al.* (2010) respectively, while assessment of MDA level was done as described by Satoh (1978) according to the pamphlet of Bio diagnostic kits using calorimetric method. The 2nd parts were preserved for histopathological examination.

4- Histologic Study:

For light microscopic study, liver specimens were fixed in 10% formalin saline for histopathological examination using H&E stain by following the method described by Wilson and Gamble (2002).

For electron microscopic study, liver specimens were fixed in 2% glutaraldehyde and then post fixed in 1% osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections were cut and double stained with uranyle acetate and lead citrate and examined by transmission electron microscope at electron microscope laboratory in Histology Department, Faculty of Medicine, Zagazig University **(Glauret and Lewis, 1998)**.

5- Immunohistochemical study:

Immunostaining was performed using the avidin-biotin peroxidase technique for localization of Bcl-2. Paraffin sections mounted on coated slides were deparaffinized and treated with 0.01 M citrate buffer for 10 minutes to unmask antigens. Then sections were incubated in H_2O_2 for 10 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 2 hs at room temperature to inhibit the nonspecific immunoreactions. Primary monoclonal anti- Bcl-2 serum (Cell Marque Lot., 27068) were applied at 1:5000 dilutions. Sections were incubated with primary monoclonal antisera for 36 h at 4°C. after washing they were incubated with biotinylated secondary antibodies for 5 hrs, then followed by avidin-biotin peroxidase complex. Finally immune reaction was visualized with 0.05% diaminobenzidine. Then the slides were counter stained with Mayer's hematoxylin before mounting (Happerfield *et al.,* 1993).

6- Statistical analysis:

Data were represented as means ± SD. The differences were compared for statistical significance by ANOVA, LSD tests and student's t-test. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using Epi-Info version 6.1 (Dean *et al.,* 2000).

3. Results:

1- Biochemical results:

Statistical comparison between the negative and positive control groups regarding LFTs (AST, ALT, GGT and ALP) and hepatic oxidative stress markers (SOD, CAT, GSHPx and MDA) revealed no significant difference ($P>0.05$), so the negative control was used for comparison with other groups of the study **(Table 1).**

Table 1: Statistical analysis by student t-test of means values of LFTs and oxidative stress markers of negative control rats (groups 1) and positive control rats (groups 2) .

Parameter \ Groups		Group 1 (Negative Control)	Group 2 (Positive Control)
Liver Function Tests	AST (IU/L)	43.6 ± 1.9	44.9±1.5#
	ALT (IU/L)	27±1.82	26±1.63#
	GGT (IU/l)	2.73±0.462	2.88±0.502#
	ALP (IU/L)	45.0±1.72	45.8±1.63#
Oxidative Stress Markers	SOD (U/gm)	70.57±3.82	69.88±3.94#
	CAT (nmol/min/ml)	18.84±2.12	18.75±2.59#
	GSHPx (U/gm)	166.92±4.10	168.40±3.94#
	MDA (nmol/gm)	21.33±3.30	21.88±3.29#

Data are expressed as means ± SD with non significance (F) ≠ > 0.05,
n=10 in each group

1-1- Liver function tests (AST, ALT, GGT and ALP):

Processing the mean values of LFTs (AST, ALT, GGT and ALP) of the adult male albino rats of the negative control group, GA_3 treated group and the recovery group through (ANOVA) test revealed that there were statistically significant differences($p<0.001$) between them **(Table 2).**

There was a statistically significant increase in the mean values of LFTs of GA3 treated rats in comparison with those of the negative control group rats ($p<0.001$)**.** By the end of the follow up period there was an improvement in the mean values of LFTs of the recovery group. The results showed a statistically significant decrease in the mean values when compared with the mean values of LFTs of GA3 treated rats ($p<0.001$)**.** This improvement was partial because these values still higher ($p<0.001$)than those of the negative control group **(Table 2)** .

Table 2: Statistical analysis by ANOVA test and LSD of means values of LFTs of negative control rats (Group 1), GA3 treated rats (Group 3) and Recovery group (group 4) .

Parameters \ Groups		Group 1 (Negative Control group)	Group 3 (GA3 group)	Group 4 (Recovery group)	P
Liver Function Tests	AST (IU/L)	43.6 ± 1.9	82± 2.9*	57.4± 2.5*#	<0.0001†
	ALT (IU/L)	27± 1.82	66± 1.84*	42± 1.92*#	<0.0001†
	GGT (IU/l)	2.73± 0.462	12.55±0. 51*	7.42± 0.331	<0.0001†
	ALP (IU/L)	45.0± 1.72	79.82± 2.34*	51.55± 1.50*#	<0.0001†

†: significant difference ($p<0.05$) as compared by ANOVA test, * : Significant difference as compared to the negative control group ($P < 0.05$), #: significant difference as compared to GA3 treated group
n=10in all groups

1-2- Hepatic oxidative stress markers (SOD,CAT, GSHPx and MDA):

The mean values of the antioxidant enzymes (SOD, CAT, and GSHPx) and the lipid peroxidation end product MDA of the negative control group, GA_3 treated group and the recovery group through (ANOVA) test showed a highly($p<0.001$) statistically significant difference **(Table 3) .**

There was a statistically significant decrease in the mean values of the antioxidant enzymes activities of GA3 treated rats in comparison with those of the negative control group rats ($p<0.001$)**.** on the other hand GA3 treatment induced a significant increase in the hepatic level of MDA ($p<0.001$). the six weeks period of recovery didn't reveal non significant improvement in the hepatic MDA level, as statistical comparison of the mean values of MDA of the recovery group showed non significant difference when compared to those of GA3 treated group. In contrary there was a significant improvement in the mean values of antioxidant enzymes activities of the recovery group. The results showed a statistically significant increase in the mean values when compared with those of GA3 treated rats ($p<0.001$)**.** This improvement was partial because these values still higher ($p<0.001$)than those of the negative control group **(Table 3).**

Table 2: Statistical analysis by ANOVA test and LSD of means values of LFTs of negative control rats (Group 1), GA3 treated rats (Group 3) and Recovery group (group 4) .

Parameters \ Groups		Group 1 (Negative Control group)	Group 3 (GA3 group)	Group 4 (Recovery group)	P
Oxidative Stress Markers	SOD (U/gm)	70.57± 3.82	54.94± 3.31*	63.92± 4.05*#	<0.0001†
	CAT (nmol/ min/ml)	18.84± 2.12	15.61± 0.33*	17.04±1.5*#	<0.0001†
	GSHPx (U/gm)	166.92± 4.10	129.64±3 .86*	146.17±3.57 *#	<0.0001†
	MDA (nmol/ gm)	21.33± 3.30	144± 1.54*	142± 5.4*	<0.0001†

†: significant difference ($p<0.05$) as compared by ANOVA test, * : Significant difference as compared to the negative control group ($P < 0.05$), #: significant difference as compared to GA3 treated group
n=10in all groups

2- Histopathological Results:

2-1 Light microscopic examination:

2-1-1 H&E stain:

Examination of H&E stained liver sections of control groups showed the normal hexagonal or pentagonal lobules with central veins and peripheral hepatic triads (portal areas) contained branches of the portal vein, hepatic artery, and bile duct. Hepatocytes are arranged in trabeculae running radiantly from the central vein. They had stippled appearance of the acidophilic cytoplasm and contained large vesicular nuclei. The trabeculae were separated by sinusoidal spaces (Fig. 1).

Examination of H&E stained liver sections of GA3 treated group showed, mild piece meal necrosis of hepatocytes and inflammatory cellular infiltrates. Most of hepatocytes appeared apoptotic. They appeared shrunken with pyknotic nuclei. The hepatocytes also appeared vacuolated with few inflammatory cells in-between (Figs. 2,3).

Light microscopic examination of H&E stained liver sections of the recovery group showed partial recovery. Most of hepatocytes showed ground glass appearance. The cytoplasm appeared pale stained with apparently normal basophilic nuclei with appearance of some binucleated cells (Fig. 4).

2-1-2 Immunolocalization of Bcl-2:

Immunolocalization of Bcl-2 in all examined liver specimens of control groups revealed that hepatocytes were negative for Bcl-2 expression. On the other hand, 6 weeks of treatment with GA_3 resulted in increased overexpression of Bcl-2 in hepatocytes especially those surrounding the central vein and endothelial cells of blood sinusoids (Fig.5). Upon recovery both hepatocytes and endothelial cells sregain its negative expression of Bcl-2 (Fig. 6).

2-2 Electron microscopic examination:

Electron microscopic study of the control group showed the ultra structure of the liver. The hepatocytes appeared with euchromatic nuclei containing prominent nucleoli. The cytoplasm contained numerous mitochondria, smooth endoplasmic reticulum and rough endoplasmic reticulum. The hepatocytes were adjacent to each other (Fig. 7).

On the other hand, the electron microscopic study of GA3 treated group showed apoptosis of some hepatocytes with shrinkage of their nuclei and condensation of their heterochromatin. The cytoplasm appeared empty of cytoplasmic organelles (cytoplasmic rarification). Some apoptotic cells showed swelling of mitochondria and vacuolations (Figs. 8,9).

The electron microscopic study of the recovery group showed partial improvement of hepatocytes. There was reappearance of cell organelles (mitochondria & cisternae of ER). The nuclei appeared euchromatic with prominent nucleoli. The rest of cytoplasm was occupied by hypertrophied SER. There was some cytoplasmic vacuolization (Fig. 10).

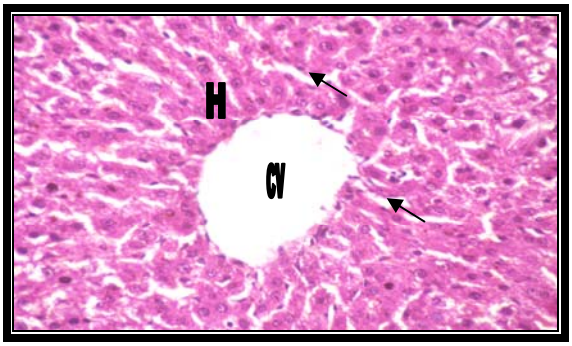


Fig. (1): A photomicrograph of a section in the liver of a negative control adult male albino rat showing part of hepatic lobule with central vein (CV) and sheets of hepatocytes (H) with sinusoidal spaces in between (arrows). (H&E X400)

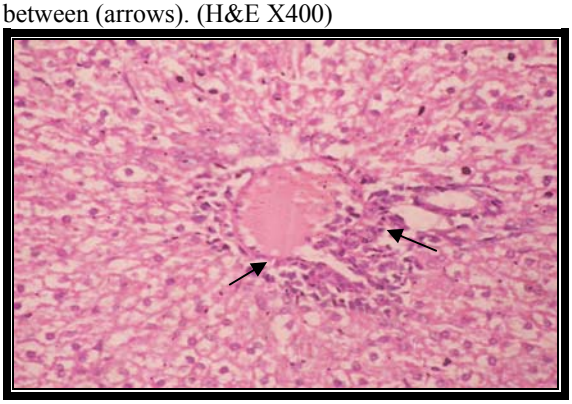


Fig. (2):A photomicrograph of a section of the liver obtained from an adult male albino rat from the 3rd group (GA_3 group) showing inflammatory cellular infiltrates (arrows). Most of hepatocytes showing cytoplasmic vacuolations. (H & E X400)

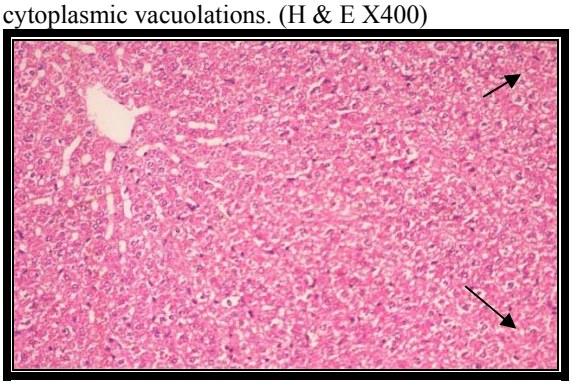


Fig. (3):A photomicrograph of a section of the liver obtained from an adult male albino rat from GA_3 group showing cytoplasmic vacuolations of hepatocytes and few inflammatory cells in between. Hepatocytes at the periphery of the lobules showed mild piece meal necrosis (arrows)(H &E X 200)

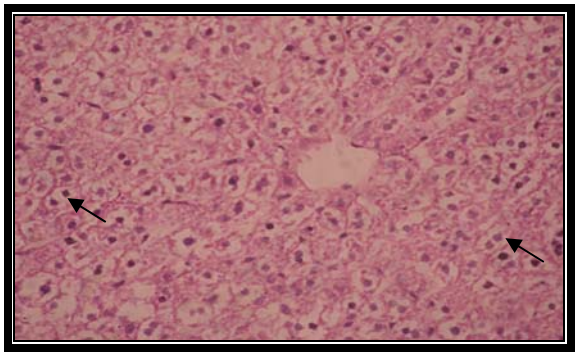


Fig. (4):A photomicrograph of a section of the liver obtained from an adult male albino rat from the 4th group (Recovery Group) showing ground glass appearance of hepatocytes. The cytoplasm appears pale stained. Apparently normal basophilic nuclei with appearance of some binucleated cells (arrows). (H&E X 200)

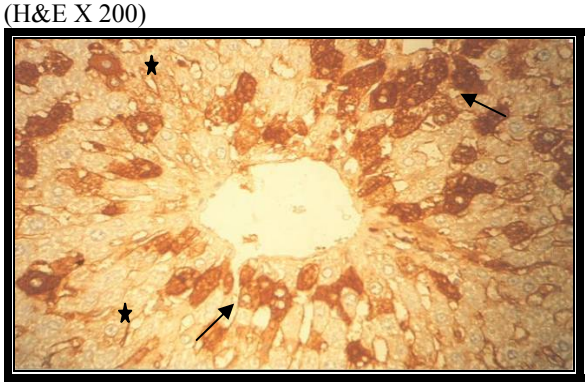


Fig. (5):A photomicrograph of a section of the liver obtained from an adult male albino rat from the 3rd group (GA3 Group) showing strong expression of Bcl-2 in hepatocytes surrounding the central vein (arrows) and endothelial cells of blood sinusoids (*). (avidin- biotin immunoperoxidase for Bcl-2 x 400)

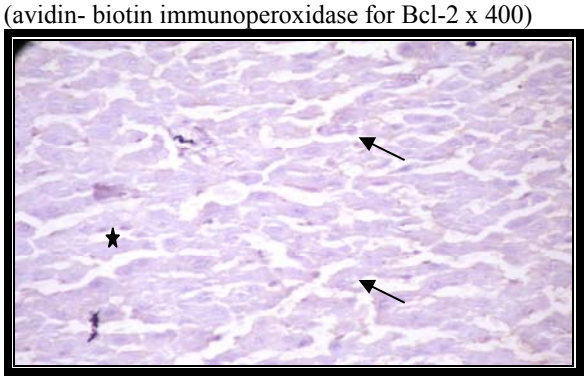


Fig. (6): A photomicrograph of a section of the liver obtained from an adult male albino rat from the recovery group showing negative expression of Bcl-2 in hepatocytes (arrows) and endothelial cells of the blood sinusoids (*). (avidin- biotin immunoperoxidase for Bcl-2 x 400)

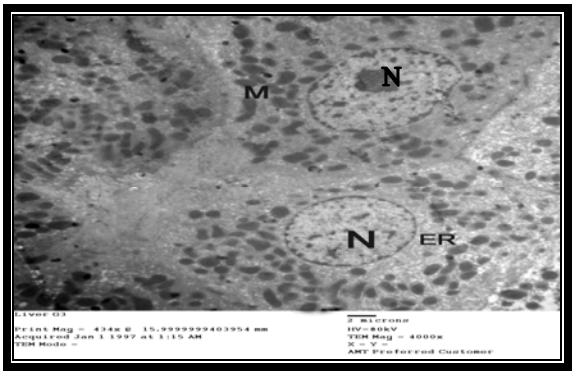


Fig. (7): An electron micrograph of a section in the liver of a negative control adult male albino rat showing adjacent hepatocytes with euchromatic nuclei (N) containing prominent nucleoli. The cytoplasm contains numerous mitochondria (M), smooth, and rough endoplasmic reticulum (ER). (X 4000)

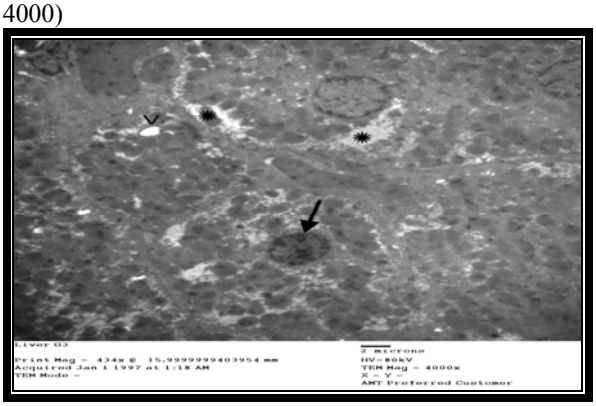


Fig. (8): An electron micrograph of a section of the liver obtained from an adult male albino rat from GA_3 group showing cytoplasmic rarification (*) and vacuolization (V) of hepatocytes with shrinkage of the nucleus and condensation of its heterochromatin (arrow). (X 4000)

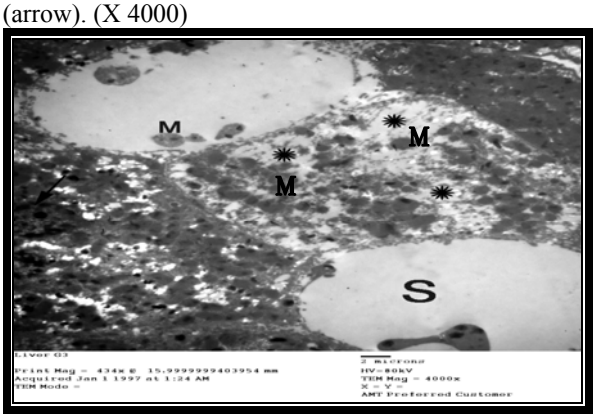


Fig. (9): An electron micrograph of a section of the liver obtained from an adult male albino rat from GA_3 group showing apoptotic nuclei (arrow), swelling of mitochondria (M) and cytoplasmic rarification (*). Notice part of sinusoid is seen (S)(X4000)

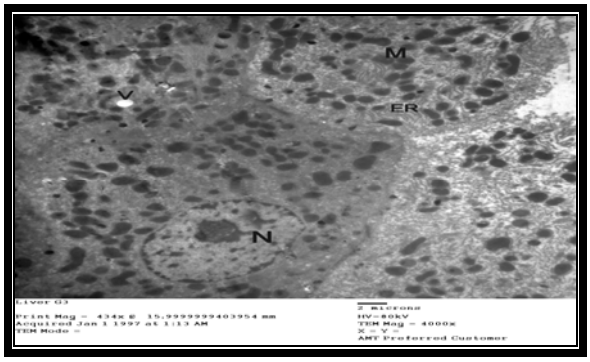


Fig. (10): An electron micrograph of a section of the liver obtained from an adult male albino rat from recovery group showing reappearance of cytoplasmic organelles (mitochondria (M) & cisternae of ER). The nucleus (N) appears euchromatic with prominent nucleolus. There is still some cytoplasmic vacuolization (V). (X4000)

4. Discussion

Gibberellic acid (GA3) is produced by a naturally-occurring fungus in large vats (Schwechheimer and Willige, 2009).

GA3 is used to increase fruit size, increase cluster size (in grapes), delay ripening of citrus fruits, speed up flowering of strawberries, and stimulate starch break down in barley (for beer making). Also, it is used to promote growth of male flowers on female plants and allows production of female-only seeds and seedless fruits (Cambell and Jane, 2002 & Seiler, 2005).

Although GA3 is extensively used in Egypt and other countries, little is known about its toxic effects in mammals as well as its potential hazardous effects on human health (Saber *et al.,* 2003 & Erin *et al.,* 2008).

The increasing use of this substance in agriculture making it as an interesting subject to investigate its possible adverse effects on the liver as one of the main target organs for different xenobiotics. So, the aim of this study was to evaluate hepatotoxic effects of GA3 in adult male albino rats for 6 weeks, and also to determine the effects of withdrawal of GA3 on the affected parameters following 6 weeks of follow up.

The present study revealed that, both control groups showed no abnormal findings as regards liver enzyme biomarkers: AST, ALT, GGT and ALP and also the antioxidant enzymes activities (SOD, CAT, GSHPx) and MDA level in liver. There was no significant difference between the negative and the positive control group as regard all these parameters. Also, there were no abnormal histopathological changes in the liver specimens of the adult male

albino rats of these groups all over the period of the study.

GA_3 treatment for 6 weeks induced a significant increase in the mean values of serum AST, ALT, GGT & ALP when compared with the negative control group. Upon recovery, there was an improvement in the mean values of liver function tests of the treated group as the recovery group showed a statistically significant decrease in LFTs when compared with those of GA3 treated group. This improvement was partial because these mean values still significantly higher than those of the negative control group.

These findings could be explained by Jaeschke *et al.* (2002) who found that leakage of the enzymes were produced within hepatocytes and small amounts constantly leak through the cell membrane which gave the normal serum enzymes level of these enzymes. Liver damage caused by liver cell injury (hepatocellular toxicity) made the membranes more permeable.

The results of the present work were parallel to Sakr *et al.* (2003). They observed a significant increase in serum ALT & AST after treatment with GA_3 which were considered to be a sensitive measure in evaluating hepatocellular damage.

In the present study the disturbance in LFTs was accompanied with disruption of the hepatic antioxidant enzymes activities with accumulation of MDA indicating GA_3 induced oxidative stress and lipid peroxidation in the treated animal livers. As there were statistically significant decreases in the mean values of SOD, CAT, GSHPx enzymes activities and significant increase in the mean values of MDA level in the hepatic tissues of the treated rats in comparison to that of the control group.

Upon recovery, the mean values of SOD, CAT and GSHPx enzymes showed a significant increase in comparison to GA_3 treated group. These mean values were still significantly lower than those of the negative control group. Also, the mean values of MDA level showed a non significant decrease in comparison to GA3 treated group.

The result of the present study was in agreement with Orrenius *et al.* (2003) who found that the plant growth regulators compounds including GA_3 can accelerate lipid peroxidation up to 65-fold, in different tissues and this was attributed to the formation of OH radicals that may react with the lipids, possibly by hydrogen abstraction leading to oxidative damage within the cell.

Moreover, Muthuraman and Srikumar (2009) investigated the effect of GA_3 on the antioxidant defense status and lipid peroxidation level in rats. They stated that sub-chronic treatment of rats with GA_3 caused enhancement of lipid peroxidation and reduction of antioxidant defense in treated animals when compared to the control rats.

Regoli and Principato (1995) mentioned that decreased CAT, GSHPx enzymes activities might have reflected a cellular oxidative stress due to GA_3 exposure. This decrease in antioxidant enzymes activities might be due to excessive consumption secondary to the flux of superoxide radicals or due to further decrease in the activities of these protective enzymes.

The flux of superoxide radicals can attack molecules in biological membranes, tissues, and mediate chain reactions which target lipids (Radi *et al.* 1991), polysaccharides, DNA and proteins, leading to various forms of cell injury (Stadtman and Levine, 2000).

Results of light and electron microscope examination of stained liver sections obtained in the present study have supported the above mentioned biochemical results.

Light-microscope examination of H&E stained liver sections of GA_3 treated rats after 6 weeks, showed mild piece meal necrosis, inflammatory cellular infiltrates and appearance of many apoptotic cells. These apoptotic cells appeared shrunken with pyknotic nuclei. The hepatocytes also appeared vacuolated with inflammatory cells in-between. Furthermore, 6 weeks of treatment with GA_3 resulted in increased intensity of staining of Bcl-2 especially in hepatocytes surrounding the central vein and endothelial cells of sinusoids.

Electron microscope examination of liver sections had clarified the above mentioned results. 6 weeks GA_3 treatment, resulted in apoptosis of some hepatocytes with shrinkage of the nuclei and condensation of their heterochromatin. The cytoplasm appeared empty of cytoplasmic organelles (cytoplasmic rarification). Some apoptotic cells showed swelling of mitochondria and vacuolations.

The histopathological findings of this study were coincided with Sakr *et al.* (2003) and Troudi *et al.* (2009) who stated that the liver sections of GA_3 treated rats revealed that hepatocytes were swollen and appeared with severe cytoplasmic vacuolization with degeneration of their nuclei. They also stated that such injuries were more obvious in the peripheral lobular zones than the pericentrally located ones. The intrahepatic blood vessels were congested. They also noticed massive cellular infiltrations with inflammatory cells in several areas of the lobules.

These results were in accordance with Abd El Maksoud *et al.* (1996) who recorded that the oral administration of GA_3 revealed significant histological changes in the liver cells in the form of appearance of large areas of rarified cytoplasm with disappearance of cellular organelles.

The biochemical interpretation of the cytoplasmic swelling and vacuolations had been subjected to wide speculations by many investigators. Sherlock and Dooly (2002) demonstrated that cytoplasmic swelling and vacuolization are one of the most important primary responses to all forms of cell injury. They occurred due to increased permeability of cell membranes leading to an increase of intracellular water. As water sufficiently accumulates within the cell, it produces cytoplasmic vacuolization.

Many earlier studies disclosed that mitochondrial dysfunction contributed to apoptosis via the production of reactive oxygen species (Mignotte and Vayssiere, 1998).

For interpretation of the mechanism of mitochondrial swelling reported in this study, Jaeschke *et al.* (2002) mentioned that oxidative stress had contributed to the opening of the mitochondrial permeability transition pore (PTP) which led to the formation of a high-conductance channel, in the inner mitochondrial membrane, and led to mitochondrial swelling and subsequent release of cytochrome c from the intermembrane space.

PTP opening appears to be associated with apoptosis or necrosis according to the presence or deficiency of ATP (Lemasters, 2002).

The histopathopathological changes in the present study as regard swelling of mitochondria were in accordance with the results of Pessayre *et al.* (2001) and Krahenbhul (2001) who reported that inhibition of mitochondrial function together with accumulation of reactive oxygen species and lipid peroxidation, all these factors led to cell death.

As regarding the follow up period in this study, examination of H&E stained liver sections showed partial improvement. Most of hepatocytes showed ground glass appearance with appearance of some binucleated cells. Immunolocalization of Bcl-2 revealed that most of hepatocytes and endothelial cells of sinusoids became negative for Bcl-2 expression.

The ground glass appearance of hepatocytes was clarified by electron microscopic picture which showed partial improvement in the form of reappearance of cell organelles (mitochondria & cisternae of RER). The nuclei appeared euchromatic with prominent nucleoli. The rest of cytoplasm was occupied by abundant SER. There still was some cytoplasmic vacuolization.

These results were coincided with Abd El Maksoud *et al.* (1996) who stated that following GA_3 withdrawal, a few cells became nearly similar to those of the control group, while the majority of cells remained affected.

The above mentioned results coincided with Saly (1998) who studied the chronic toxic effects of GA_3 on liver, heart and kidney and stated that 4 weeks of recovery from the destructive effects of GA3 was incomplete.

The ground glass appearance of hepatocytes detected in the recovery period of this study was coincided with Droge (2002) who mentioned that the glass appearance reflect adaptation of cells upon recovery.

These results were coincided with Kimball (2008) who stated that the ground glass appearance of liver cells was considered as adaptive mechanism and occurred as a result of increased synthesis of cellular organelles such as mitochondria and RER probably to increase function of individual hepatocytes.

Abundant SER in the recovery period could be also explained by Benedetti (2005) who stated that it takes part in the synthesis of phospholipids for building of cell membrane and membranes of cell organelles.

On the other hand the increased RER was explained by Kimball (2008) who mentioned that RER had a very important role in the synthesis and packaging of proteins. Some of these proteins might be used by the cell to synthesize membrane, other cell organelles, and the others were sent out. This was considered as a structural-functional response to enable cells to release the oxidative stress secondary to GA_3 toxicity.

Conclusion

From the previously mentioned results we can conclude that gibberellic acid was a potent pro-oxidant that induced a significant hepatotoxicity in adult male albino rats following 6 weeks of daily exposure. The hepatotoxic effects of GA_3 were manifested by impairment of the LFTs that associated with inhibition of the hepatic antioxidant enzymes with accumulation of MDA the end product for lipid peroxidation. GA3 induced lipid peroxidaton promote local apoptosis and upregualtion of Bcl-2 that end in hepatocyte apoptosis and cell death. On the other hand, 6 weeks period of follow up was in sufficient for complete recovery of these toxic effects.

RECOMMENDATIONS

1. Gibberellic acid (GA3) use should be under strict control.
2. Periodic monitoring of GA3 concentration in the soil and plants.
3. More studies are needed to explore other hazardous effects of GA3 on other body systems and organs.
4. Other studies with prolonged periods of administration of GA3 are recommended to learn more about its toxic effects.

Acknowledgement

Great thanks and gratefulness to research post graduate studies section of Zagazig University. As this work was funded by competitive research project fund – Zagazig University.

Corresponding author

Wafaa F. Hus

Departments of [1]Forensic Medicine and Clinical Toxicology

Faculty of Medicine, Zagazig University

References

1. Abd El Maksoud SA, Abd El Maksoud N and Abd El Hamid NA ,1996. Chronic toxic effect of plant growth promoting hormone, gibberellic acid, on the liver cells of adult male albino rat. Assut Med J., 20: 87-103
2. Andrade RJ, Lucena MI and Fernandez MC.,2005. Drug- induced liver injury: an analysis of 461 incidences submitted to Spanish registry over a 10- year period. Gastroent., 129: 512-521
3. Arteca R .,1996. Plant growth substances: principles and applications. New York: Chapman & Hall
4. Benedetti A.,2005. Endoplasmic reticulum: a metabolic compartment. FEBS Letters, 580:2160-2165
5. Cambell NA and Jane B.,2002. Biology, 6th ed. San Francisco., Benjamin Cumming., 145-155
6. Celik I, Tuluce Y and Isik I., 2007. Evalution of toxicity of abcisic acid and gibberellic acid in rats: 50 days drinking water study. J Enzyme Inhib Med Chem., 2: 219- 26.
7. Dean AG, Dean JA, Coulombier D *et al.*,2000. Epi-Info (6.1): A word processing data base and statistical program for epidemiology and micro-computer office, Center for disease control, Atlanta Georgia, USA.
8. Droge W.,2002. Free radical in the physiological control of cell function. Physiol Rev., 82:47-59
9. Durak I, Canbolat O, kavutch M *et al.*,1996. Activities of total, cytoplasmic and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. J Clin Lab Anal., 10:17-20.
10. Erin E, Afacan B, Ersony Y, Ercan F and Blaci MK., 2008. Gibberellic acid, a plant growth regulator increase mast cell requirement and alters substance P levels. Tox., 245: 75-81
11. Fath A, Bethke BC and Jones RI.,2001. Enzymes that metabolize reactive oxygen species are down regulated prior to GA-induced programmed cell death in barley aleurone. Plant Physiol., 126:156-166
12. Fishel FM., 2006. Gibberellins. Agronomy department, Florida cooperative extension service, Institute of food and agricultural sciences, University of Florida, USA. http://edis.ifas.ufl.edu.I
13. Glauret A and Lewis, 1998. Physiologic specimen preparation for transmission Electron Microscope1 ed., vol. 17., Portland Press, London.
14. Happerfield LC, Bobrow LG, Bains RM and Miller KD., 1993. Peroxidase labeling immunocytochemistry: a comparison of eleven commercially available avidin-biotin systems. J Histochem Cytochem., 41: 291-302.
15. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C, pp.21-55, http://www.nap.edu/openbook.php
16. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D and Lemasters JJ., 2002. Mechanism of hepatoxicity . Toxicol Sci., 56: 76-66
17. Kimball JW., 2008. Growth regulating hormones. http://users.rcn.com/jkimball.ultranet/biologypages
18. Krahenbhul S., 2001. Mitochondria: important target for drug toxicity. J Hepatol., 34:334-336
19. Lee WM and Senior JR., 2005. Recognizing drug-induced liver injury: current problems, possible solutions. Toxicol Pathol.,33:155-164
20. Lemasters JJ., 2002. Mechanisms of hepatotoxicity. Toxicol. Sci., 65: 166-167
21. Mignotte B and Vayssiere JL., 1998. Mitochondria and apoptosis. Eur J Biochem., 15:252:1-15
22. Muthuraman P and Srikumar K., 2009. A comparative study on the effect of homobrassinolide and gibberellic acid on lipid peroxidation and antioxidant status in rats. J Enz Med Chem., 24: 1122-1127.
23. Neil AC and Reece JB., 2002. Phytohormones (plant hormones) and other growth regulators: Gibberellinn. In: biology. 6th ed., San Fransisco, Benjamin Cummings.
24. Orrenius S, Zhivotovsky B and Nicotera P., 2003. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol., 4:552
25. Osborne DJ and McManus MT., 2005. Hormones, signals and target cells in plant development. 1st ed. Vol. 1. pp. 266, Cambridge University Press.

26. Ozmen M, Topcuoglus SF, Buzcuk S and Bozcuk NA., 1995. Gibberellic acid and abscisic on sexual differentiation and some physiological parameters of laboratory mice. Tuk. J. Biol., 19: 357-364
27. Paglia DE and Valentine WN., 1967. Studies on the quantitative and Qualitative characterizationof erythrocyte glutathione peroxidase. J Lab Clin Med., 70: 158- 169.
28. Pessayre D, Berson A, Fromrnty B and Mansouri A., 2001.Mitochondria in steatohepatitis. Semin Liver Dis., 21:57-69
29. Radi R, Beckman JS, Bush KM and Freeman BA., 1991. Peroxy nitrite- induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch Biochem Biophus., 288:481-487
30. Regoli F and Principato G., 1995. Glutathione, glutathione dependent antioxidant enzymes in Mussel. Mytilus galloprovincialis exposed to metals under laboratory conditions. Implication for uses of laboratory markers. Aquat Toxicol., 31:143-164
31. Reitman S and Frankle S 1957. Glutamic oxaloacetic transaminse colorimetric method. Am J Clin Pathol., 28: 56.
32. Sakr S A, Okdah Y A and F El- Abd S., 2003. Gibberellin A 3 induced histological and histochemical alterations in the liver of albino rats. Science Asia, 29: 327- 331
33. Saly YA., 1998. Chronic administration of plant growth hormones in rats; some histologic studies. Env Ass Un., 6:21-29
34. Satoh K., 1978.Serum lipid peroxide in cerebrospinal disorders determined by new colorimetric methods. Clin Chim Acta., 90:37-43.
35. Schwechheimer C and Willige B C., 2009. Shedding light on gibberellic acid signaling. Curr Opin Plant Biol., 12: 57-62
36. Seiler SE., 2005. Plant growth regulation. In: Forest Biology Textbook, chp 5., pp.150
37. Sherlock S and Dooly J., 2002. Diseases of the liver and biliary system. 9th ed., Blackwell Scientific Press.
38. Soliman H A E, MantawyM M and Hassan H M., 2010. Biochemical and molecular profiles of gibberellic acid exposed albino rats. Am J Sci., 6:18-23
39. Stadtman ER and Levine RL., 2000. Protein oxidation. Ann NY Acad Sci., 899:191-208
40. Teitz NW. 1986. Textbook Clinical Chemistry. Saunders Co., Philadelphia.
41. Tomlin CD., 2004. Gibberellic acid (77-06-5). In: The e-Pesticide Manual, 13th ed., vol 3. Surrey UK, British Crop. Protection council.
42. Troudi A, Mahjoubi SA and Zeghal N., 2009. Hepatotoxicity induced by gibberellic acid in adult rats and their progeny. Exp Toxicol Pathol., 18:127-133
43. Tuluce Y and Celik I.,2006. Influence of subacute and subchronic treatment of abcisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Pest. Biochem Physiol., 86:85-92.
44. Wilson I and Gamble M.,2002. The hematoxylins and eosins, In: Theory and practice of histological techniques, Bancroft JD and Gamble M (eds.), 5th ed., Churchill Livingston, Elservier Science Limited, London, UK, pp. 125-138.

6/20/2011

Laboratory diagnosis of FMD using real-time RT-PCR in Egypt

*Laila EL-Shehawy; Abu-Elnaga H.; Abdel Atty M.; Fawzy H.; Al-Watany H. and Azab A.

Department of Foot and Mouth Disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt
*drlaila15@yahoo.com; h.abu-elnaga@hotmail.com

Abstract: Definitive diagnosis of foot-and-mouth disease (FMD) requires the detection of virus antigen or genome in clinical material. The aim was performance of real-time RT-PCR (rRT-PCR) procedures for this purpose. Twenty nine cattle samples of vesicular epithelial and nasal swabs from four localities of Ismailia governorates were examined by ELISA, VN, RT-PCR and rRT-PCR. The results showed that 11 samples were positive by ELISA and virus isolation, 8 of serotype O and 3 for type A. Fourteen samples out of 29 were positive by RT-PCR and rRT-PCR. The features that influence sample quality appear to be less important for the rRT-PCR and RT-PCR as they can detect a small fragment of FMDV genomic RNA. Real-time RT-PCR provided an extremely sensitizer and rapid procedures that contributes to improved laboratory diagnosis of FMD.
[Laila EL-Shehawy; Abu-Elnaga H.; Abdel Atty M.; Fawzy H.; Al-Watany H. and Azab A**. Laboratory diagnosis of FMD using real-time RT-PCR in Egypt**] Life Science Journal. 2011;8(3):384-387] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: foot-and-mouth disease virus, rRT-PCR

Introduction

Control of outbreaks of foot-and-mouth disease (FMD) is dependant upon a system of monitoring and early detection, which requires basic familiarities with clinical signs and the ability to characterize the strains of virus responsible by laboratory tests. Definitive diagnosis of FMD requires the detection of virus, antigen or genome in clinical material. Ideally, the samples of choice should vesicular epithelium from clinically affected animals since, during the acute stage of the disease, it is rich in virus **(1-3)**. Consequently suspension of samples is propagated in sensitive cell culture **(4)** and the specificity of any isolated virus is confirmed by ELISA. Virus isolation methods are highly sensitive, they require four days before a negative result can be concluded. In emergence, speed of diagnosis (clinical and laboratory confirmation) is of paramount importance to control spread and eradicate the disease.

The development of real-time reverse transcription polymerase chain reaction (rRT-PCR) procedure has provided an additional tool, which can be used for FMD diagnosis **(3, 6-7)**. Real-time RT-PCR or quantitative PCR is a variation of the standard PCR technique used to quantify DNA or messenger RNA in sample using sequence specific primers, the relative number of copies of a particular DNA or RNA sequence can be determined. The quantification arises by measuring the amount of amplified product at each stage during the PCR cycles. Quantification of amplified product is obtained using fluorescent SYBR green. SYBR green is a dye bind to double stranded DNA. The intensity of fluorescent emissions increase as more double stranded amplicon is produced with the dye signal increase. The dye will bind to any double strand DNA molecule, while the 5' nuclease probe assay is specific to a pre-determined target.

2. Material and Methods

2.1. Samples

Twenty nine cattle samples, a vesicular epithelium (ep) and twenty nasal swabs (ns) were received from Fayed, Tal El-Keber, Kassasen and Kantara of Ismailia governorate during January, February, March and April 2011. Due to the contagious nature and economic importance of FMD, the laboratory diagnosis and serotype identification of the virus should be done in a facility that meets the requirements for containment group 4 pathogens with bio-safety and bio-security in the laboratory.

2.2. Virus isolation (VI) of the samples

The samples were filtered using 0.2µm filter, and then were inoculated onto monolayer BHK-21 cell line with three passages. The cultures were checked for specific cytopathic effect (CPE) every 24 hrs for 72 hrs. Cultures were stored at -70 ^{o}C until processing for ELISA **(8)**. Indirect sandwich ELISA was performed for the detection and identification of viral serotypes **(3)**.

2.3. RT-PCR

It was used to amplify genome fragment of FMDV in the samples. One-step RT-PCR was carried out as described by the manufacture's protocol to perform the reverse transcription and subsequent PCR by One-step RT-PCR (Qiagen, Germany) **(9-10)**. The

method amplifies a serotype specific segments of FMDV VP1 (1D) gene of type A and another for type O. The primer sequences were as listed in **Table 1**. Amplified products were analyzed on agarose gel. Negative control specimen and DNA ladder were involved in agarose gel electrophoresis.

Table 1. FMDV specific primer sequences

Primer	Orientation	Sequence (5' to 3')	Serotype Specificity	Genomic Location	bp
PH9	Forward	TAC CAA ATT ACA CAC GGG AA	A	1C	863-866
PH10	Reverse	GAC ATG TCC TCC TGC ATC TG	All serotypes	2B	863-866
PH2	Forward	GCT GCC TAC CTC CTT CAA	O	1D	402
PH1	Reverse	AGC TTG TAC CAG GGT TTG GC	All serotypes	2B	402

2.4. rRT-PCR

RNA extraction was carried out using the QIAamp® Viral RNA kit (Qiagen, Germany) according to the manufacturer's protocol. Primer pair (PoR/PoF) for real time RT-PCR was synthesized by BioBasic, Canada. PoF (5´- CCT ATG AGA ACA AGC GCA TC -3´) and PoR (5´- CAA CTT CTC CTG TAT GGT CC -3´) were derived from FMDV 3D polymerase **(11)**. RT-PCR was performed using QuantiTect® SYBR® Green RT-PCR Kit (Qiagen, Germany) as manufacturer's instructions. The cycling parameters were 50 ^{o}C for 30 min and 95 ^{o}C for 15 min; then 30 cycles consisting of 94 ^{o}C for 15 s, 55 ^{o}C for 30 s and 72 ^{o}C for 30 s. Negative control specimen was involved. Thermocycler Rotor-Gene Q (Qiagen, Germany) was used for real time detection of FMDV by RT-PCR.

3. Results and Discussion

The results achieved by ELISA, VI in cell culture, RT-PCR and real-time RT-PCR are summarized in **Table 2** for the comparison of the performance of four assays. FMDV was detected in 11 samples by VI and antigen ELISA, nine of tongue epithelial from Fayed, Tal El-Keber, Kassasen and Kantara while two nasal swabs from Kantara. These viruses represented 8 of serotype O and 3 of serotype A. There were broad agreement between RT-PCR and rRT-PCR, where 9 positive epithelial samples and 5 positive nasal swabs out of 20 samples from Fayed, Kassasen and Kantara were detected. All samples assigned negative by RT-PCR and rRT-PCR were also negative by virus isolation and ELISA **(Figs. 1 and 2)**

ELISA and VI have been the recommended laboratory procedures for FMD diagnosis, based on their suitability to detect the presence of FMDV antigen in tissue samples. If one considers that VI and ELISA procedures actually measure then it is evident that their effectiveness for diagnostic use is inherently compromised. Virus isolation is dependant upon the presence of infectious virus in sample submissions. While ELISA can detect both infectious and non-infectious FMD viral antigen, it is dependent upon the antigen being present in sufficient concentration to work **(1)**. RT-PCR can detect a small fragment of FMDV genome RNA, not just live virus. Real-time RT-PCR provides an extremely sensitive and rapid procedure that contributes to improve laboratory diagnosis of FMD **(1, 3)**. The aim of the study was to use real-time RT-PCR to detect FMDV in suspected samples. Five positive nasal swabs. Samples 1, 3 and 4 were identified by rRT-PCR, whereas only two of these samples were positive by VI and ELISA indicating comparable sensitivity between these diagnostic methods that was in agreement with previous authors **(2, 12-13)**. The negative results were likely to occur in samples in which cattle recovered from clinical lesions, since virus isolation was extremely reduced with more than 7-10 days after the appearance of gross lesions **(5)**.

In conclusion, the real-time RT-PCR method used in this study has proven to be highly sensitive and specific under laboratory condition.

Table 2 Positive samples from four localities of Ismailia governorate by VI and ELISA RT-PCR and rRT-PCR during 2011

Locality	Sample	No	VI	ELISA		RT-PCR		rRT-PCR
				A	O	A	O	
Fayed	ep	3	3	-	3	-	3	3
	ns	4	-	-	-	-	1	1
Tal El-Keber	ep	2	2	-	2	-	2	2
	ns	5	-	-	-	-	-	-
Kassasen	ep	2	2	2	-	2	-	2
	ns	6	-	-	-	-	1	1
Kantara	ep	2	2	1	1	1	1	2
	ns	5	2	-	2	-	3	3
Total		29	11	3	8	3	11	14

. ep= tongue epithelium, ns= nasal swab

Fig. 1 RT-PCR for detection of FMDV serotype A and O. M: 100 bp ladder, lanes 1-3: serotype A (863 bp), lanes 4-6: negative samples, lane 5: serotype O (402 bp)

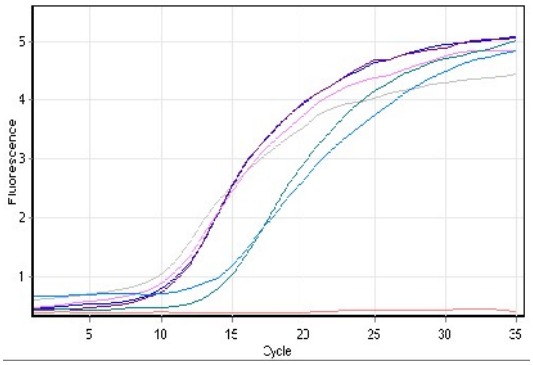


Fig. 2 Real Time RT-PCR result of FMDV isolates. Grey is positive control, red baseline is negative control, other curves are positive viral samples

Acknowledgement

This study is a part of the project entitled 'molecular characterization of FMDV Egyptian strains for improvement of local produced vaccine', ID number 1064. Science and Technology Development Fund (STDF), www.STDF.org.egy

Corresponding author
Laila EL-Shehawy
Department of Foot and Mouth Disease, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt
drlaila15@yahoo.com

References

1. Ferris N, Reid S, King D, Hutching G and Shaw (2004). Prospects for improved laboratory diagnosis of FMD using real time RT-PCR. European Commission for the Control of FMD 2004.
2. King DP, Ferris NP, Shaw AE, Reid SM, Hutchings GH, Giuffre AC, Robida JM, Callahan JD, Nelson WM and Beckham TR (2006). Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time reverse transcription-polymerase chain reaction assays. J Vet Diagn Invest., 18: 93-7.
3. OIE (2008). Foot and Mouth Disease. In: OIE Standards Commission (5th Eds.), Manual of Standards for Diagnostic Tests and Vaccines. Office International des Épizooties, Paris, France (Chap. 2.1.5).
4. Ferris N.P. and Dawson M. (1988). Routine application of enzyme- linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases. Vet. Microbiol., 16: 201–209.
5. Alexandersen S, Zhang Z, Donaldson AI and Garland AJ (2003). The pathogenesis and diagnosis of foot-and-mouth disease. J Comp Pathol., 29:1-36.
6. Reid SM, Grierson SS, Ferris NP, Hutchings GH and Alexandersen S (2003). Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. J Virol Methods, 107:129-39.

7. Alxandersen S, Klein J, Hussain M, PAton D and Afzal M (2006). Preliminary findings from a new project on the epidemiology of FMDV in Pakistan. European commission for the control of FMD 2006.
8. Macpherson I and Stoker M. (1962). Polyoma transformation of hamster cell clones-an investigation of genetic factors affecting cell competence. Virology, 16:147-51.
9. Knowles NJ, Samuel AR (1994). Polymerase chain reaction amplification and cycle sequencing of the 1D (VP1) gene of foot-and-mouth disease viruses. Paper presented at the session of the Research group of the standing Technical committee of European commission for the control of FMD, Vienna Austria, 19–22, September, 1994.
10. Laila EL-Shehawy, Abu-Elnaga H, Talat A, El-Garf E, Zakria A and Azab A (2011). A nucleotide sequencing of foot-and-mouth disease virus Egyptian strains. Journal of American Science (In press).
11. Shin JH, Sohn HJ, Choi KS, Kwon BJ, Choi CU, Kim JH, Hwang EK, Park J H, Kim JY, Choi SH and Kim OK (2003). Identification and isolation of foot-and-mouth disease virus from primary suspect cases in Korea in 2000. J Vet Med Sci., 65: 1-7.
12. Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL and Nelson WM (2002). Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc., 220:1636-42.
13. Paixão TA, Neta AV, Paiva NO, Reis JR, Barbosa MS, Serra CV, Silva RR, Beckham TR, Martin BM, Clarke NP, Adams LG and Santos RL (2008). Diagnosis of foot-and mouth disease by real time reverse transcription polymerase chain reaction under field conditions in Brazil. BMC Vet Res., 4:53.

6/20/2011

Comparison between Uses of Therapeutic Exercise and Heat Application on Relieve Pain, Stiffness and Improvement of Physical Function for Patient with Knee Osteoarthritis

Wafaa I. Shereif and Amira A. Hassanin

Department of Adult Care Nursing, Faculty of Nursing,Mansoura University, Egypt.

Abstract: Osteoarthritis is a common chronic disease and a major worldwide problem for medical, psychosocial, and economic reasons. Osteoarthritis leads to considerable morbidity in terms of pain, functional disability, lowered quality of life, and psychological problems and affected people are mainly cared for in the community. The aim of this study was to compare between uses of therapeutic exercise and heat application on relieve pain, stiffness and improvement of physical function for patient with knee osteoarthritis. This quasi-experimental study was conducted in the out patient clinic of orthopedic at Mansoura University main hospital. The data were collected from a convenience sample of 90 osteoarthritis patients of both sex and equally divided into 3 groups (G1) received training to used heat application with pharmacological treatment to decrease symptoms of osteoarthritis, (G2) received training of physical exercise with pharmacological treatment to decrease symptoms of osteoarthritis, (G3) received a combination training from physical exercise and heat application with pharmacological treatment prescribed by physician. The main inclusion criteria was diagnosis osteoarthritis of the knee based on attendant to out patient clinic at least twice / week. Results: knee stiffness improves from {mean= (G1, G2 & G3) 9.17, 22.08& 1.25 respectively to 66.25, 47.92& 85 respectively} after implementing education intervention. There were statistical significant difference between the three groups of studied sample pre – post teaching phase regarding experiencing total knee stiffness score ($P \leq 0{,}001$, 0,001& 0,001 respectively). Conclusion: the use of a combination of therapeutic exercise and heat application together for relieving pain, stiffness and improving physical function for patient with osteoarthritis was successful

[Wafaa I. Shereif and Amira A. Hassanin, Comparison between Uses of Therapeutic Exercise and Heat Application on Relieve Pain, Stiffness and Improvement of Physical Function for Patient with Knee Osteoarthritis**]** Life Science Journal,. 2011; 8(3): 388-396] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Knee osteoarthritis, Pain; Exercise; Heat application; Functional improvement.

Introduction

Osteoarthritis (OA) is one of the most frequent causes of physical disability among adults. More than 27 million people in the United States have the disease. By 2030, 20 percent of Americans - about 70 million people - will have passed their 65th birthday and will be at risk for osteoarthritis. Some younger people get osteoarthritis from joint injuries, but osteoarthritis most often occurs in older people. Both men and women have the disease. Before age 45, more men than women have osteoarthritis, whereas after age 45, it is more common in women (2,3).

The knee is one of the most commonly affected joints. OA is usually a slowly progressive degenerative disease in which the joint cartilage gradually wears away. It most often affects middle-aged and older people over 50 years of age, in particular in women.(4)

It can affect either one (unilateral) or both (bilateral) sides of the knee joint however it occurs more commonly on the inner (medial) aspect of the knee.

The bones of the knees joint (the backside of the kneecap, bottom of thighbone, and top of shinbone) are coated with smooth articular cartilage. When knee osteoarthritis develops, the cartilage undergoes gradual changes - loosing elasticity, hardening, and cracking, becoming more easily damaged and eroded by use or injury(5,3). Symptoms of knee osteoarthritis are stiffness (especially morning knee stiffness), knee pain that is aggravated by going up or down stairs, limitation in range of motion, a crunching feeling in the knee, and weakness of knee. The knee may be swollen but not red and hot (2,4,6) .

Osteoarthritis affects each person differently. In some people, it progresses quickly; in others, the symptoms are more serious. Scientists do not know yet what causes of the disease, but they suspect a combination of factors, including being overweight, the aging process, joint injury, and stresses on the joints from certain jobs, genetic susceptibility, lack of exercise / sedentary lifestyle, muscle Weakness - weak quadriceps (muscles of the thigh that attach to the knee), chronic overuse / overloading of knee joint (repetitive movements such as squatting/kneeling combined with heavy lifting or high impact activities such as running/jogging), skewed feet, and inappropriate footwear (high heels) (7,8) . The exact causes of osteoarthritis are unknown however there are a number of factors that are commonly associated with the onset of the disease (8).

Osteoarthritis pain can be effectively managed so that it does not significantly disrupt normal daily activities. Non-pharmacological management, combining several therapies may be required to adequately manage the symptoms of knee osteoarthritis as exercise, medications, knee taping,

un-loader knee braces in some cases, heat and cold, topical creams, and complementary supplements (9,10).

Exercise adherence improves long-term patient outcome for patients with knee osteoarthritis, because it strengthens leg muscles, supports the knee and absorbs shock before it gets to the knee. Exercising the quad muscles increase circulation in the knee joint and has been shown to stimulate beneficial biochemical changes in the joint fluid of the knee, improving its lubricating properties (11, 13). Exercise also improves the range of motion of the knee Exercise should be a core treatment for people with osteoarthritis, irrespective of age, co-morbidity, pain severity or disability. Exercise should include: Local muscle strengthening, and General aerobic fitness (12).

Also, heat application to the knee joint reduces stiffness and pain by increasing blood flow. The heat is also a comforting distraction from the knee pain. Do not apply heat to an inflamed joint. Usually, inflammation is not present in the early stages of knee osteoarthritis. Apply heat for 20 - 30 minutes at a time every day (13,15,31).

Nurses are responsible to empower the patient and family with teaching knowledge and skills necessary to reach optimal safe performance to manage pain and disability of osteoarthritis (14).

Aim of the study

The aim of this study was to compare between uses of therapeutic exercise and heat application on relieve pain, stiffness and improvement of physical function for patient with knee osteoarthritis.

2. Material and Methods

Research design:

A quasi experimental design used in this study

Setting:

This study conducted in the out patient clinic of Orthopedic and Rheumatology Clinic at El Mansoura University main hospital.

Subjects:

A convenient sample of 90 osteoarthritis patients from both sex this number all available patients came to out patient during 6 months and divided into three equal groups, (G1) received training to used heat application with pharmacological treatment to decrease symptoms of osteoarthritis, (G2) received training of physical exercise with pharmacological treatment to decrease symptoms of osteoarthritis , (G3) received a combination of training from physical exercise and heat application with pharmacological treatment prescribed by physician. The main inclusion criteria was diagnosis osteoarthritis of the knee without deformities based on attendant to out patient clinic at least twice / week and accept to participate in the study. Exclusion criteria: patients with chest pain, dyspnea, orthopnea, and sever inflammation in knee.

Tools:

Two tools were used in this study for data collection.

I-Patient' assessment sheet include the following:
Socio-demographic characteristics of patients such as sex, age, marital status, educational level, occupation, and part two included on medical profile such as, body weight, time of complain from osteoarthritis, which knee complain of osteoarthritis.

II- Knee injury and osteoarthritis outcome score (KOOS) this tool was developed by (31), The KOOS is a knee-specific instrument, developed to assess the patients' opinion about their knee and associated problems. The KOOS evaluates both short-term and long-term consequences of knee injury. It holds 42 items in 5 separately scored subscales; Pain, other Symptoms and stiffness, Function in daily living (ADL), Function in Sport and Recreation (Sport/Rec.), and knee-related Quality of Life (QOL). but the researchers of this study didn't used the final two items after carried out the pilot study on 10% of knee osteoarthritis patients before starting data collection and take the opinion of expert because this items away from the objective of this study.

Pain: It contains 9 items and used to assess amount of knee pain during the following activities (twisting/ pivoting on knee, straightening knee fully, bending knee fully, walking in flat surface, going up or down stairs, at night while in bed, sitting or lying and standing upright).

Symptoms it contains 7 items and used to assess amount of joint stiffness after first a wakening in the morning and after static poison and knee symptoms including ; grinding, hanging up, swelling, straightening, and bending knee fully.

Function, daily living: It contains 17 items and used to assess degree of difficulty during the following activities (descending and ascending stairs, rising from sitting, standing, bending to floor/ pick up an object, walking on flat surface, getting in/ out of care, going shopping, putting on socks, rising from bed, taking off socks, lying in bed (turning over, maintaining knee position) getting in / out of bath, sitting, getting on / off toilet, heavy domestic duties and light domestic duties.

Function, sports and recreational activities: It contains 5 items and used to assess degree of difficulty during the following activities (squatting, running, jumping, twisting, on the knee and kneeling).

Quality of life: It contains 4 items and used to assess knee related quality of life.

Standardized answer for knee injury and osteoarthritis out come score (KOOS). Each questions gets score from 0 to 4. A normalized score (100 indicated no symptoms and 0 indicated extreme symptoms) is calculated for each subscale. Traditionally in orthopedics, 100 indicates no problem and 0 indicates extreme problems. The normalized score is transformed to meet this standard by using formulas provided for each subscale:

1- Pain 100 – total score (p1- P9) x 100 = 100- -----=

36

2- Symptoms and stiffness 100 – total score (S1- S7) x 100 = 100- ----=

28

28

3- Function, ADL 100 – total score (p1- P9) x 100 = 100- -----= 68

68

Total score for each subscale will categorized into
< 100 – 66 Mild
< 66- 33 Moderate
< 33- 0 Extreme

Methods:

An official letterers were issued from the faculty of Nursing. Mansoura University to the Director of El-Mansoura University Hospital to obtain his approval in order to collect the necessary data from the out patient clinic of orthopedic and rheumatology clinic at El Mansoura university main hospital

Tools of data collection were designed by the researcher after reviewing the recent related literature.

The tools were tested for validity and reliability by five experts in the field of nursing staff.

A pilot study will be carried out on 10% of the subjects in order to assess the clarity, feasibility and applicability of the tool. Any necessary modifications will be done accordingly.

According to the schedule of work in the out patient clinic of orthopedic, two visits were done weekly on Sunday and Tuesday from 8AM to 2PM afternoon.

Each patient was interviewed individually in the out patient clinic of orthopedic and in the rheumatology clinic and obtained verbally consent to participate in the study.

Group 1 trained to compliance heat application on affected knee for 20-30 minutes at least from 2 to 3 time /day in the morning and before sleep along four weeks beside pharmacological treatment prescribed by physician. After that data were collected post educational intervention to know the level of complains of patient from pain and stiffness.

Group 2 trained to commit physical exercise activity especially the side of affected knee, the exercise made for five to ten minutes from 3 to 4 time /day along four weeks beside pharmacological treatment prescribed by physician. After that data were collected after educational intervention to measure the level of patient's complains from pain and stiffness and improvement of physical function occurred or not. The information and training for exercise contained stretching exercises such as (standing calf stretch, prone quadriceps stretch, and range of motion exercises) strengthening exercises such as (quadriceps strengthening exercise, and standing terminal knee extension).

Group 3 apply heat application and physical exercise activity together beside pharmacological treatment prescribed by physician. After four weeks, data were collected after educational intervention to measure the level of pain, stiffness and physical function according to KOOS scale.

Compare between three group's finding to evaluate the better methods used for improvement health status of patient with osteoarthritis.

The time taken to instruct and training the patient about uses of heat application or training for made physical exercise was 20 to 30 minutes.

Data collection covered a period of 6 months starting from August 2010 to June 2011.

Statistical analysis

Data was analyzed using SPSS (Statistical Package for Social Sciences) version 15. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-Square test. Data was presented as mean ± SD. Student t-test was used for comparison between groups. Wilcoxon Signed Ranks test used for comparison within group. $P < 0.05$ was considered to be statistically significant.

3. Results

Table (1) reveals that about two third in groups (G1, G2,and G3) 76.7% , 63.3% and70% respectively were female. Regarding age, the majority of G1, G2 (60%, 60%) were within 40- 50 years, respectively, while in G3 only 36.7% were at the same age. Concerning level of education nearly half of three groups (G1, G2, G3) of patients were university education (36.7%, 40% and 50% respectively). In relation to patients" occupation ,36.7%, 20% and 26.7% not works, respectively. The majority of three groups were married (90%, 80% and 86.7%) respectively. About two third patients of each groups were over weight (63.3%, 63.3% , 76.7) respectively. Regarding when patient experiencing osteoarthritic pain, above half patients of three groups were complain within year (76.7%, 60%, 80%) respectively. Mostly of the studied

sample (G1, G2, G3) were complains in right knee (46.7%, 43.3%, 76.7%) respectively.

Table 2 clarify that, with respect to symptoms, on pre educational intervention above half of G 1 (whom applied heat application) and G2 (whom applied exercise application) 60% and 70%, respectively complains *often* from symptoms, while only 40% and 26.7% of G1 and G2 complains *sometimes* and this percent increased after educational intervention to 93.3% and 40% respectively. Also a positive influence occurred to G3, whom applied both methods, as 90% complained *often* from symptoms during pre educational phase changed to 70% complained *sometimes* and only 30% *rarely* on the post educational phase. No statistical significant differences were found between three groups

Table (1): Distribution of the studied patients according to their socio-demographic characteristics and health status

	Heat applications (n = 30)		Exercise (n = 30)		Heat and Exercise (n = 30)		*P value*
	No	%	No	%	No	%	
Sex							
♦ Male	7	23.3	11	36.7	9	30	0.530
♦ Female	23	76.7	19	63.3	21	70	
Age							
♦ 20 – 30y	0	0	1	3.3	0	0	0.014*
♦ 30 – 40y	4	13.3	11	36.7	12	40	
♦ 40 – 50y	18	60	18	60	11	36.7	
♦ 50 – 60y	8	26.7	0	0	7	23.3	
Education							
♦ Illiterate	10	33.3	6	20	4	13.3	0.417
♦ Secondary	9	30	12	40	11	36.7	
♦ University	11	36.7	12	40	15	50	
Occupation							
♦ Manual	5	16.7	9	30	0	0	0.014*
♦ Employed	14	46.7	15	50	22	73.3	
♦ Not work	11	36.7	6	20	8	26.7	
Marital status							
♦ Single	0	0	1	3.3	2	6.7	0.206
♦ Married	27	90	24	80	26	86.7	
♦ Widow	3	10	2	6.7	2	6.7	
♦ Divorced	0	0	3	10	0	0	
Weight							
♦ Normal	8	26.7	10	33.3	5	16.7	0.529
♦ Under	3	10	1	3.3	2	6.7	
♦ Over	19	63.3	19	63.3	23	76.7	
When							
♦ 6 – 12 m	23	76.7	18	60	24	80	0.180
♦ < 12 m	7	23.3	12	40	6	20	
Which							
♦ Right	14	46.7	13	43.3	23	76.7	0.028*
♦ Left	4	13.3	8	26.7	4	13.3	
♦ Both	12	40	9	30	3	10	

In addition, with respect to pain the table 2, shows that G1 and G2 patient's pain relieved and improved from complains often (50%, 33.3%), respectively to complain sometimes (70%, 76.7 %,respectively), while G3 recovered from 50% complain sometimes and 50% complain almost to 93.3% complain rarely.

Moreover the data illustrate that G1 and G2 (80% and 40 %, respectively) were complained *Always* from stiffness on the pre educational phase,

improved post educational phase to 0.0% and 6.7% respectively.

On the other hand G3 whom complained from stiffness *Always* relived from 83% on pre educational phase to 0.0% on post educational phase.

Finally, with referred to the disability of functional activity, table 2 described that 63.3% , 76.7% and 90% from G1 , G2 and G3 respectively were have *Always* disability of functional activity before educational phase. Post educational phase the percent of those had *Always* disability of functional activity improved to 3.0%, 6.7% and 0.0% on the G3.

There are statistical significant differences in all variables between pre and post educational phase, in three groups(P= 0,001). Also the table illustrate that no statistical significant differences pre educational phase between three groups in mostly variables , but post educational phase highly statistical significant differences between three groups (P=0,001) .

Table (2) Number and Percent distribution of the studied sample according to factors of patients" complains

Items	Heat applications				Exercise				Heat and Exercise				P value	
	Pre		post		Pre		Post		Pre		post			
	No	%	No	%	No	%	No	%	No	%	No	%	Pre	Post
Symptoms														
- Never	0	0	0	0	0	0	0	0	0	0	0	0		
- Rarely	0	0	0	0	0	0	0	0	0	0	9	30		
- Sometimes	12	40	28	93.3	8	26.7	12	40	3	10	21	70	0.056	<0.001
- Often	18	60	2	6.7	21	70	18	60	27	90	0	0		
- Always	0	0	0	0	1	3.3	0	0	0	0	0	0		
P value (Pre versus Post)	<0.001				<0.001				<0.001					
Pain														
- Never	0	0	0	0	0	0	0	0	0	0	1	3.3		
- Rarely	0	0	9	30	0	0	0	0	0	0	28	93.3		
- Sometimes	1	3.3	21	70	0	0	23	76.7	0	0	1	3.3	0.341	<0.001
- Often	15	50	0	0	10	33.3	5	16.7	15	50	0	0		
- Always	14	46.7	0	0	20	66.7	2	6.7	15	50	0	0		
P value (Pre versus Post)	<0.001				<0.001				<0.001					
Stiffness														
- Never	0	0	0	0	0	0	0	0	0	0	1	3.3		
- Rarely	0	0	15	50	0	0	4	13.3	0	0	28	93.3		
- Sometimes	0	0	15	50	5	16.7	17	56.7	2	7	1	3.3	<0.001	<0.001
- Often	6	20	0	0	13	43.3	7	23.3	3	10	0	0		
- Always	24	80	0	0	12	40	2	6.7	25	83	0	0		
P value (Pre versus Post)	<0.001				<0.001				<0.001					
Disability of functional activity														
- Never	0	0	0	0	0	0	0	0	0	0	2	6.7		
- Rarely	0	0	8	26.7	0	0	0	0	0	0	27	90	0.081	<0.001
- Sometimes	0	0	22	73.3	1	3.3	22	73.3	0	0	1	3.3		
- Often	11	36.7	0		6	20	6	20	3	10	0	0		
- Always	19	63.3	0		23	76.7	2	6.7	27	90	0	0		
P value (Pre versus Post)	<0.001				<0.001				<0.001					

Regarding symptoms which patients complains it, table (3) reflects that in pre educational intervention mean of G1,G2&G3 improve from 45.17, 41.67& 40.0, respectively to 59.83, 45.5& 66, respectively. Also there is statistical significant difference between the three groups of studied sample post teaching phase regarding experiencing symptoms score ($P \leq 0.001$). Moreover, when compare between teaching phase pre - and post there were statistical significant difference to all groups ($P \leq$ 0.001, 0.026& 0,001 respectively).

-Also the table demonstrated that regarding knee stiffness the mean among G1, G2 & G3 improved from 9.17, 22.08& 1.25 respectively to 66.25, 47.92& 85 respectively after implementing education intervention. There were statistical significant difference between the three groups of studied sample pre – post teaching phase regarding experiencing total knee stiffness score ($P \leq$ 0,001, 0,001& 0,001 respectively). Also the table shows that statistical significant difference in compare with each others ($P \leq$ 0.001& 0.001).

Table 4 demonstrates that the studied sample whom experiencing pain have improved from pre to post therapeutic education for three groups (23.8, 21.11&25.74 to 69.81, 53.89& 92.5, respectively. In comparison of complain from knee pain among three groups there were statistical significant

difference ($P < 0.001$). No statistical significant pre educational phase between three groups

In relation to the function status it have the ability to make normal function after educational intervention especially in G3 than G1 and G2 in which clear by mean (G1,G2 & G3) pre educational intervention(18.07, 21.15& 16.98 respectively) and improved post educational intervention to 70.94, 54.11&92.6, respetively).

Also, table 5 illustrates that relation between studied groups pre and post compliance to therapeutic non pharmacological treatment and comparison with function of knee there were statistical significant difference ($P < 0.001$, < 0.001& < 0.001 respectively). There were statistical significant difference ($P < 0.001$) in compared the phase of pre of three groups.

Table (3) comparison between pre and post symptoms and knee stiffness of three groups (Presented by Mean ± Standered Deviation) for KOOS scale total score.

Symptoms		Heat applications (n = 30)	Exercise (n = 30)	Exercise & compresses (n = 30)	P value
Pre	Mean	45.17	41.67	40	0.076
	SD	9.05	11.09	5.57	
Post	Mean	59.83	45.5	66	< 0.001*
	SD	7.25	9.59	4.98	
P value		< 0.001*	0.026*	< 0.001*	
Stiffness					
Pre	Mean	9.17	22.08	1.25	< 0.001*
	SD	10.85	18.18	3.81	
Post	Mean	66.25	47.92	85	< 0.001*
	SD	10.96	19.72	6.89	
P value		< 0.001*	< 0.001*	< 0.001*	

Table (4) Comparison between pre and post teaching intervention regarding pain for three groups (Presented by Mean ± Standard Deviation) for KOOS scale total score.

Pain		Heat applications (n = 30)	Exercise (n = 30)	Exercise & compresses (n = 30)	P value
Pre	Mean	23.8	21.11	25.74	0.220
	SD	12.11	11.36	6.31	
	Min	0	0	16.67	
	Max	50	47.22	38.89	
Post	Mean	69.81	53.89	92.5	< 0.001*
	SD	6.43	14.66	6.11	
	Min	52.78	11.11	69.44	
	Max	80.56	72. 22	100	
P value		< 0.001*	< 0.001*	< 0.001*	

Table (5) Comparison between three groups of studied sample pre and post teaching intervention according to function status (Presented by Mean ± Standered Deviation) for KOOS scale total score

Function		Compresses (n = 30)	Exercise (n = 30)	Exercise & compresses (n = 30)	P value
Pre	Mean	18.07	21.15	16.98	0.271
	SD	12.33	10.71	7.08	
	Min	0	4.69	1.56	
	Max	37.5	53.13	35.94	
Post	Mean	70.94	54.11	92.6	< 0.001*
	SD	4.92	14.98	6.6	
	Min	57.81	14.06	71.88	
	Max	79.69	71.88	100	
P value		< 0.001*	< 0.001*	< 0.001*	

4. Discussion

Osteoarthritis (OA) is one of the oldest and most common forms of arthritis. Known as the "wear-and-tear" kind of arthritis, OA is a chronic condition characterized by the breakdown of the joint's cartilage (16) .Cartilage is the part of the joint that cushions the ends of the bones and allows easy movement of joints (17,18). The breakdown of cartilage causes the knee bones to rub against each other, causing stiffness, pain and loss of movement.

The treatment depends on the joint but often includes medication and exercise. There is no special osteoarthritis diet, but heat application and weight loss may improve symptoms of osteoarthritis (19,20) . Although the design was quasi-experimental and three study groups were comparable in terms of marital status, job status and body weight, with some differences in age, and educational level. These factors are important since they could play the role of confounders in the causation of knee osteoarthritis.

The aim of this study was to: Compare between uses of therapeutic exercise and heat application on improvement of functional status for patient with knee osteoarthritis.

According to the present study, the results reveals that about two third of groups G1, G2, and G3 were female (76.7%, 63.3% and70%, respectively). Regarding age, the majority of G1, G2 (60%, 60 %, respectively) were within 40- 50 years, while in G3 only 40% were between 30 to 40 years old. Also the present result come on accordance with Lawrence *et al.* (24) and Moss *et al.* (21) who supported this study by found that, osteoarthritis usually occurs in any age and age exemplifies one of the most important risk factors for osteoarthritis. Evidence of joint changes occurs at approximately age 40 and changes continue to occur to the extent that the majority of elderly persons reveal joint changes indicative of osteoarthritis and occurs more often in women than in men, (although more men are affected when osteoarthritis occurs in people younger than age 45) (22).

In the current study, the results showed that, about two third patients of all groups were overweight (63.3%, 63.3%, 76.7, respectively). This finding goes in line with Christensen (26) and Sharma (23). Excess weight places increased stress on the joints of the body; hence, many people suffering from osteoarthritis are overweight. And found correlations between osteoarthritis and being overweight: Osteoarthritis affects women more often than men and women have a greater tendency to be overweight. Excess weight stresses the joints, which intensifies pain and interferes with exercise which may deter overweight individuals with osteoarthritis from exercising, thus making it more difficult to lose weight. Also, this finding agree with Messier *et al.*(25). Who found the strongest link between overweight or obesity and knee OA and found a 9- to 13-percent increased risk for the development of the disease with each kilogram increase in body weight. That means that gaining 10 pounds would result in an increased risk of 40 to 59 percent. Being overweight during the early adult years can also increase the risk of developing the disease later on.

In relation to present study, Table 2 clarify that, with respect to symptoms, on pre educational intervention above half of G 1 (whom applied heat application) and G2 (whom applied exercise application) 60% and 70% respectively complains *often* from symptoms, while only 40% and 26.7% of G1 and G2 complains sometimes and this percent increased after educational intervention to 93.3% and 40%, respectively. Also a positive influence occurred to G3, whom applied both methods, as 90% complained *often* from symptoms during pre educational intervention changed to 70% complained *sometimes* and only 30% *rarely* on the post educational intervention. Regarding symptoms, table 3, showed total score of KOOS scale, found statistical significant difference($p< 0.001$) between pre and post-educational intervention in relation to symptoms. These findings come in consistent with The Centers for Disease Control and Prevention, (CDC 2010)(27) finding the onset of osteoarthritis symptoms develop slowly and may be stable for many years. Osteoarthritis symptoms may occur in any joint; however, the joints most often affected are the knees, hips, hands, and spine. And joints need motion to stay healthy. Long periods of inactivity cause the arthritic joint to stiffen and the adjoining tissue to atrophy (waste away). A moderate exercise program that includes low-impact aerobics, power and strength training has benefits for patients with osteoarthritis. And when combination between physical therapy and uses of heat application they can relieve osteoarthritis symptom for long period.

Concerning pain, table 2 showed that G1 and G2 patient's pain relieved and improved from complains *often* (50% & 33.3 %, respectively), to complain *sometimes* (70%, 76.7%, respectively), while G3 recovered from 50% complain *sometimes* and 50% complain *almost* to 93.3% complain *rarely*. In comparison of complain from knee pain among three groups regarding pain in table 4 there were statistical significant difference all of them ($P< 0.001$). These finding are agreement with Sharma (23) and Nefyn *et al.(*29) who revealed that the uses of heat application is time honored technique for relieving pain, stiffness and associated swelling that can result from osteoarthritis. This finding in the present study also come in accordance with Nefyn *et al.*(29) who concluded that, exercise helps patients with mild to moderate osteoarthritis in the hip or in the knee, and many patients who embark on an aerobic or resistance exercise program report less disability and pain and are better able to perform daily chores and remain independent than their inactive peers.

National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) Information (28) stressed that there is no cure for osteoarthritis, the therapeutic exercise can help to reduce the joint pain and improve flexibility and mobility of joints and most successful treatment programs involve a combination of treatments

tailored to the patient's needs, and health. Most programs include ways to manage pain and improve function. These can involve exercise, pain relief techniques, medications and heat application.

In relation of the function of knee it have the ability to make normal function after educational intervention especially in G3 than G1 and G2 in which clear by mean (G1, G2 & G3) pre educational intervention (18.07, 21.15& 16.98, respectively) and improved post educational intervention (70.94, 54.11& 92.6, respectively). This finding come in line with Martijn *et al*. (30). Who showed individuals with OA of the hip or knee experience pain, reduced muscle strength, decreased range of joint motion, and joint instability? According to the World Health Organization (WHO) OA is one of the ten most disabling diseases in developed countries. Further WHO estimates that 80% of those with OA have limitations in movement, and 25% cannot perform major daily life activities. Often OA patients are referred to physical therapy in order to reduce impairments and improve overall physical function to meet demands of daily living.

Conclusion and Recommendations

Based on the main study findings, it could be concluded that, the implementation of methods (heat application , therapeutic exercise, and) uses for relieve pain, pain and improvement of physical function had positive effect on relieve pain , stiffness and improvement physical function on two groups (G1, G2) of the studied sample but when combination between therapeutic exercises and heat application on group 3 of studied sample had a strong positive effect on relieve pain , stiffness and improvement in physical function so found in relation to the disability of functional activity, about 63.3% , 76.7% and 90% from G1 , G2 and G3 respectively were have *always* disability of functional activity before educational training and Post educational training the percent of those had always disability of functional activity improved to 3.0% , 6.7% and 0.0% on the G3. Therefore, it is recommended the following:

1- Use a combination of therapeutic exercise and heat application together as a methods of treatment to relieve pain, stiffness and improvement of physical function for patient with osteoarthritis when medications was contraindicated for those patients.
2- A simple manual of guidelines of uses heat application and therapeutic exercises for patients with osteoarthritis available in all out patient orthopedic clinic and rheumatology clinic to be provided to newly admitted patients.
3- The effect of uses heat applications and therapeutic exercises need to be explored more fully with a larger sample with osteoarthritis.

Corresponding author
Amira A. Hassanin
Department of Adult Care Nursing, Faculty of Nursing,Mansoura University, Egypt.

References:

1. Zhang, W., Moskowitz, R.W., Nuki, G., Abramson, S., Altman, R.D., Arden, N., Bierma-Zeinstra, S., Brandt, K.D., Croft, P., Doherty, M., Dougados, M., Hochberg, M., Hunter, D.J., Kwoh, K., Lohmander, L.S., Tugwell, P. (2008): OARSI recommendations for the management of hip and knee osteoarthritis, Part II: OARSI evidence-based, expert consensus guidelines. Osteoarthritis & Cartilage; 16:137-162.
2. Rattanachaiyanont, M., & Kuptniratsaikul, V.(2008): No additional benefit of shortwave diathermy over exercise program for knee osteoarthritis in peri-/post-menopausal women: an equivalence trial. Pub-med journal 16: 823-828.
3. Laufer, Y., Zilberman, R., Porat, R., Nahir, A.M.(2005) Effect of pulsed short-wave diathermy on pain and function of subjects with osteoarthritis of the knee: a placebo-controlled double-blind clinical trial. Clinical Rehabilitation; 19: 255-263.
4. Welch, V., Brosseau, L., Peterson, J., Shea, B., Tugwell, P., Wells, G.(2001): Therapeutic ultrasound for osteoarthritis of the knee. Cochrane Database Systematic Reviews; 3:132.
5. Brosseau, L., Yonge, K.A., Robinson, V., Marchand, S., Judd, M., Wells, G., Tugwell, P.(2003): Theramotherapy for treatment of Osteoarthritis. Cochrane Database of Systematic Reviews, 4: 522.
6. Silva, A.L.P., Imoto, D.M., Croci, A.T.(2007): Comparison of cryotherapy, exercise and short waves in knee osteoarthritis treatment. Acta Ortopedica Brasileria; 15: 204-209.
7. Durmus, D., Alayh, G.,(2007): Canturk, F. Effects of quadriceps stimulation program on clinical parameters in the patients with knee osteoarthritis. Clinical Rheumatology; 26: 674-678.
8. Gaines, J.M., Metter, E.J., Talbot, L.A.(2004): The effect of neuromuscular electrical stimulation on arthritis knee pain in older adults with osteoarthritis of the knee. Applied Nursing Research; 17: 201-206.
9. Fary R.E., Carroll, G.J., Briffa, T.G., Gupta, R., Briffa, N.K.(2008): The effectiveness of pulsed electrical stimulation in the management of osteoarthritis of the knee: a protocol for a randomised controlled trial. BMC Musculoskeletal Disorders; 9: 18.
10. American Physical Therapy Association. Guide to Physical Therapist Practice. (2001): 2nd Edition. Physical Therapy; 81: 9-744.

11. Kisner, C. Colby, L.(2002): Therapeutic Exercise: Foundations & Techniques. 4th Edition. Philadelphia: 644.
12. Wrightson, J.D., & Malanga, G.A. (2001): Strengthening and other therapeutic exercises in the treatment of osteoarthritis. Physical Medicine & Rehabilitation: State of Art Reviews; 15: 43-56.
13. Bashaw, R.T., & Teingstad, E.M.(2005): Rehabilitation of the osteoarthritic patient: focus on the knee. Clinical Sports Medicine; 24: 101-131.
14. Simkin, P.A., de Lateur, B.J., Alquist, A.D., Questad, K.A., Beardsley, R.M.,(2002): Esselman, P.C. Continuous passive motion of osteoarthritis of the hip: a pilot study. Journal of Rheumatology; 26: 1487-1491.
15. Oddis, C.V.(1996): New perspectives on Osteoarthritis. American Journal of Medicine; 100: 10S-15S.
16. Baker, K., McAlindon, T.(2003): Exercise for knee osteoarthritis. Current Opinion in Rheumatology; 12: 456-463.
17. Leroux, M.A., Cheung H.S., Bau, J.L., Wang, J.Y., Howell, D.S., Setton, L.A.(2004): Altered mechanics and histomorphometry of canine tibial cartilage followinf joint immobilization. Osteoarthritis & Cartilage; 9: 633-640.
18. Fontaine, K.R., Heo, M., Bathon, J.(2004): Are US adults with arthritis meeting public health recommendations for physical activity? Arthritis & Rheumatism; 50: 624-628.
19. Stuart, M.J., Meglan, D.A., Lutz, G.E., Growney, E.S., An, K.N. (2005):Comparison of intersegmenal tibiofemoral joint forces and muscle activity during various closed kinetic exercises. American Journal of Sports Medicine; 24: 792-799.
20. Suraj & Sudhir Kumar.(2006): Effect of knee complex mobilization on pain and active range of motion arc in osteoarthritis knee joint. Physical Therapy in Sport; 7: 176.
21. Moss, P., Sluka, K., Wright, A.(2007): The initial effects of knee mobilization on osteoarthritic hyperalgesia. Manual Therapy; 12: 109-118.
22. Fransen, M., Nairn, L., Winstanley, J., Lam, P., Edmonds, J. (2007): Physical activity for osteoarthritis management: A randomized controlled clinical trial evaluating hydrotherapy and tai chi classes. Arthritis & Rheumatism; 57: 407-414.
23. Sharma L (2003). Examination of exercise effects on knee osteoarthritis outcomes: Why should the local mechanical environment is considered? Arthritis and Rheumatism, 49: 255–260.
24. Lawrence, R. C., Helmick, C. G., Arnett, F. C., *et al.* (2006). Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis and Rheumatism, 65: 60-66 .
25. Messier SP, Loeser RF, Miller GD, Morgan TM, Rejeski WJ, Sevick MA, Ettinger WH, Paho M, Williamson JD. (2008): Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. Arthritis Rheum. ;50:1501-1510.
26. Christensen R, Astrup A, Bliddal H. (2005): Weight loss: the treatment of choice for knee osteoarthritis? A randomized trial. Osteoarthritis Cartilage. ;13:20-27.
27. The Centers for Disease Control and Prevention, (2010): "Osteoarthritis and you http://www.cdc.gov/Features/OsteoarthritisPlan/ . Accessed February
28. National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), (2010): Information Clearinghouse National Institutes of Health, Publication Date: July.
29. Nefyn H Williams, Elvis Amoakwa, Kim Burton, Maggie Hendry, John Belcher, Ruth Lewis, Kerenza Hood, *et al.*(2009):Activity Increase Despite Arthritis (AÏDA): design of a Phase II randomised controlled trial evaluating an active management booklet for hip and knee osteoarthritis, September 4. doi: 10.1186/1471-2296-10-62.
30. Martijn F Pisters, Cindy Veenhof, François G Schellevis, Jos WR Twisk, Joost Dekker, Dinny H De Bakker (2010): Exercise adherence improves long-term patient outcome in patients with osteoarthritis of the hip and/or knee. *Arthritis Care & Research*; volume 3: page,324-332 DOI: 10.1002/.20182.
31. Eaa,M. Roos and Stefan, Lohmander (2003):The knee injury and osteoarthritis outcome scor (KOOS): from joint injury healthqual life outcome journal. Volume.1 page. 64

8/12/2011

Seasonal Variations and Prevalence of Some External Parasites Affecting Freshwater Fishes Reared at Upper Egypt

Mahmoud A. El-Seify[1], Mona S. Zaki[*2], Abdel Razek Y. Desouky[1], Hossam H. Abbas[2], Osman K. Abdel Hady[2] and Attia A. Abou Zaid[3]

[1]Fac. of Vet. Medicine Kafrelsheikh University, Kafrelsheikh, Egypt
[2] Hydrobiology Department, National Research Center, Cairo, Egypt
[3]Animal Health Research Institute Kafrelsheikh, Kafrelsheikh, Egypt
[*]dr_mona_zaki@yahoo.co.uk

ABSTRACT: This study was carried out to detect prevalence and seasonal variation of external parasites affecting freshwater fishes. 330 *Oreochromis niloticus* and 140 *Clarias gariepinus* were collected from three different ecosystems at Kafrelsheikh province. Obtained results revealed that, the highest infection rate was recorded among *O.niloticus* followed by *C. gariepinus.* Also, seasonal dynamics among the examined *O.niloticus* were recorded.The isolated ectoparasites among examined fishes were *Cichlidogyrus tilapiae, Cichlidogyrus aegypticus, Cichlidogyrus cirratus, Quadricanthus aegypticus, Macrogyrodactylus clarii, Trichodina centrostrigeata, Trichodina rectinucinata, Chillodinella hexastica, Ichthyophthirius multifillis, Henneuguya branchialis, Lamproglena monody, Ergasilus sarsi and Copepodit stage (2nd stage) of Lernea cyprinacea.*

[Mahmoud A. El-Seify, Mona S. Zaki, Abdel Razek Y. Desouky, Hosam H. Abbas, Osman K. Abdel Hady and Attia A. Abou Zaid, **Seasonal Variations and Prevalence of Some External Parasites Affecting Freshwater Fishes Reared at Upper Egypt**] [Life Science Journal; 2011;8(3):397-400](ISSN:10978135). http://www.lifesciencesite.com.

Key words: External parasites, monogenetic trematodes, external protozoa, crustaceans, *O.niloticus, C. gareipinus.*

Introduction

Fish is one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shell fish

By the increasing intensification of fish production and lack of health management measures have lead to many disease problems of bacterial, viral, fungal and parasitic origin. About 80% of fish diseases are parasitic especially in warm water fish (Eissa, 2002). Ecto-parasites are the most dangerous group that causes severe mortalities (Shalaby and Ibrahim, 1988). In Egypt there are a long periods of optimum warm weather that enable external parasites for more production and cause bad effects on fish. The majority of the monogenitic trematodes of fishes are ectoparasites, Monogeneans (flatworms) are among the most host-specific of parasites in general and may be the most host-specific of all fish parasites. Monogenitic trematodes usually don't cause any problems in the natural environment unless the host is continually reinvested so that massive numbers of worms build up on the fish (Woo, 1995).

The most identified protozoa are belonging to ciliates. They can easily spread among most of the fish hosts. Uncontrollable or recurrent infection with ciliated protozoans is indicative of unhygienic husbandry problems (Al-Rasheid *et al.*, 2000).

Parasitic crustaceans are increasingly serious problem in cultured fish. Most Parasitic crustacean of freshwater fish can be seen by the naked eyes as they attach to the gills, body and fins of the host and it spent a large part of their life on fish, possessing an adhesive organs and mouth parts adapted for piercing and sucking fish blood (El Moghazy, 2008)

2. Materials and Methods

Fish samples:

A total number of 470 (330 *Oreochromus niloticus* and 140 *Clarias gariepinus*) freshwater fish were collected alive from three different ecosystem in Kafr El-Shiekh governorate River Nile Branch (Bahr Nashart), Drainage canal (Damroo Drainage canal) and Fish farm supplied water from damroo Drainage canal by the aid of fisher man and then transported alive to the laboratory of parasitology department-Faculty of Vetrinary Medicine-Kafrelsheikh university where they examined immediately (Table, 1)

Parasitological examination:

Parasitological examination was carried out for the detection and identification of the external parasites on the skin, gills and the accessory respiratory organs of the samples.

Collection and preparation of the detected ecto-parasites:

Monogenea: Monogenea were collected under binocular dissecting microscopic by means of small pipette in small Petri-dish and cleared several times with water to remove the attached mucous and debris.

The worms were then left in refrigerator at 4C till complete relaxation. Then, they were fixed in 5% formalin for permanent preparation, worms were washed carefully in water to get red of formalin traces and stained with Semichon's acetocarmine stain for about 5-10 minutes till reaching staining, the specimens were passed through ascending grades of ethyl alcohol (30, 50, 70, 90% and absolute) for dehydration. Then, cleared in clove oil, xylene and mounted in canda balsam (Pritchard and kruse, 1982), while the unstained Monogeneas were mounted in glycerin jelly (Abdel-Hady, 1998).

Protozoa:

Some of the positive slides were stained according to Klein's dry silver impregnation method in which the slides were air –dried, covered with 2% aqueous solution of silver nitrate ($AgNO_3$) for 8 minutes, rinse thoroughly in distilled water and exposed to UV light for 20-30 minutes or to direct sun light for 1-2 hr. The slides were allowed to dry and mount with neutral Canada balsam. This method is indispensable technique for staining *Trichodina* (Ali, 1992).

Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain for 20-30 minutes to detect the other protozoa. (Ali, 1992).

Crustacea:

The detected crustacean parasites were carefully collected by a fine brush and special needle, and transferred into Petri-dish for cleaning by using preserved and cleared in lacto phenol then mounting with polyvenylalcohol (Raef *et al.,* 2000).

3. Results

As shown in (Table, 2); from 330 examined *O. niloticus* taken from different three localities, the total infected number was 226 (68.5%), While the rates of infection in the River Nile branch, the drainage canal and the fish farm were 71.8% (84/117), 69% (69/100) and 64.6% (73/113) respectively. In addition; the total infection rate among *Clarias gariepinus* was 58.6% (82/140). While the rates of infection in the River Nile branch and the drainage canal was 53.7% (43/80) and 65% (39/60) respectively.

As described in (Table, 3); in *O. nilotica* the percentage of infection by monogenetic trematodes was higher in drainage canal than that of River Nile branch and fish farm, in case of infection by protozoa; it was higher in River Nile branch than that of drainage canal and fish farm, while the percentage of infection by crustacea was higher in drainage canal than that of fish farm and River Nile branch.

In case of *Cl. Gariepinus*, the percentage of infection by monogenetic trematodes was higher in drainage canal than that of River Nile branch and the infection was not detected in fish farm branch, protozoal infection among *Cl. Gariepinus* was higher in River Nile than that of drainage canal and not detected in fish farm locality. Parasitic crustacean was not detected among *Cl. Gariepinus* in all localities

Concerning the seasonal dynamics in the examined *O. niloticus* Table (4) revealed that the highest seasonal prevalence of ecto-parasites in examined *O. niloticus* was recorded in spring followed by summer then autumn and finally in winter. In The River Nile branch the highest prevalence of ecto-parasites was recorded in spring then winter followed by summer and autumn. But the highest prevalence of ecto-parasites in the drainage canal was recorded in summer followed by autumn then spring and winter, while in the fish farm the highest prevalence of ecto-parasites was recorded in spring then summer followed by winter finally in autumn.

Table (5) showed the peak of seasonal dynamic of Monogenea in total examined *O. niloticus* was during autumn followed by summer then winter and spring. while parasitic Protozoans recorded highest infection during spring followed by summer then winter and autumn. The highest seasonal prevalence of Crustaceans among total examined *O. niloticus* was recorded during summer then spring followed by autumn and finally in winter.

Table (1): Number of fish species examined from different localities:

Locality / Fish spp.	Examined number			Total Fish spp.
	River Nile Branch	Drainage canal	Fish farm	
Oreochromus niloticus	117	100	113	330
Clarias gariepinus	80	60	----	140
Total	197	160	113	470

Table (2): Prevalence of ecto-parasites in examined fish spp. In different localities

locality / Fish spp.	River Nile Branch			Drainage canal			Fish farm			total		
	No Ex.	No Inf.	% of Inf.	No Ex.	No Inf.	% of Inf.	No Ex.	No Inf.	% of Inf.	No Ex.	No Inf.	% of Inf.
O. niloticus	117	84	71.8	100	69	69	113	73	64.6	330	226	68.5
Clarias gariepienus	80	43	53.7	60	39	65	----	----	----	140	82	58.6

Table (3): Prevalence of different ecto-parasites in examined fish species in different localities.

Locality / parasites	River Nile Branch				Drainage canal				Fish farm				total			
	O. Niloticus no=117		*C. gariepinus* no=80		*O. Niloticus* no=1oo		*C. gariepinus* no=60		*O. niloticus* no=113		*C. gariepinus* no=0		*O. niloticus* no=330		*C. gariepinus* no=140	
	No Inf.	%	No Inf.	%	No Inf.	%	No Inf.	%	No Inf.	%	No Inf.	%	No Inf.	%	No Inf.	%
Monogenea	27	23	23	28.7	41	41	36	60	43	38	-----	----	111	33.6	59	42
Protozoa	76	65	29	36.3	48	48	12	20	53	46.9	-----	-----	177	53.6	41	29.3
Crustacean parasites	16	13.7	---	---	36	36	---	---	39	34.5	----	----	91	27.6	----	----

Table (4): Seasonal prevalence of ecto-parasites in examined *O. niloticus* in different localities:

Locality / season	River Nile Branch			Drainage canal			Fish farm			Total		
	No. Ex.	No. Inf	%	No. Ex.	No. Inf.	%	No. Ex	No. Inf	%	No. Ex.	No. Inf	%
Autumn	25	16	64	25	19	76	23	14	60.9	73	49	67
Winter	25	17	68	30	17	56.6	37	23	62	92	57	62
Spring	31	27	87	20	13	65	26	19	73	77	59	76.9
Summer	36	24	66.6	25	20	80	27	17	63	88	61	69

Table (5): Seasonal dynamics of different ectoparasites among examined *O.niloticus*:-

Parasites / Season	Monogenea		Protozoa		Crustacea	
	No. infected	%	No. infected	%	No. infected	%
Autumn N= 73	28	38.4	32	43.8	19	26
Winter N= 92	29	31.5	46	50	21	22.8
Spring N=77	22	28.6	51	66	22	28.6
Summer N=88	32	36.4	48	54.7	29	33

N= Number examined

4. Discussion

The present investigation revealed that *Monogenetic trematodes* recorded an incidence of (33.6%) which is nearly similar to those obtained byAbd El-Maged (2009) among examined *O. niloticus* was infected on the other hand higher value (80.76) was recorded by Abd El-Gawad (2004) which may be due to different of sample collection and changes in water quality in different localities.

In total examined *Clarias gariepinus*, our study revealed (42%) prevalence of *Monogenetic trematodes* which is considered higher than obtained by Ramadan (2000) 36.28%. and lower than recorded by Abd El-Maged (2009) (51.7%)

Parasitic protozoa recorded an incidence of (55.5%) among total examined *O. niloticus*. This result is found higher than that recorded by Abd El-Maged (2009) who recorded an infection rate of (6.3%).The prevalence of parasitic protozoa among

total examined *Clarias gariepinus* reached (29%). This result was in contrary with Abd El-hady (1998) who did not detect parasitic protozoans among *Clarias gariepinus* in River Nile and other water branches. This result may be related to different localities of sample collection.

The prevalence of Parasitic crustaceans in this study was (27%) in total examined *O. niloticus.* This result is higher than obtained by Abd El-Khalek (1998) who recorded that the prevalence was (24.73%), while being lower than that recorded by El-Moghazy (2008) who mentioned that the prevalence was (80%) While parasitic crustaceans not recorded is among *Clarias gariepinus*, being coincided with Abd El-Hady (1998). This is may be due to differences in localities and water quality in these localities.

With regard to the effect of the seasonal variation on the prevalence of *Monogenetic trematodes* in the present study, the highest rate of infection was during autumn. This result agreed with Ramadan (2000) and Abd El-Gawad (2004) Mean while, this result was in contrary with Abd El-Maged (2009) who recorded the lowest infection rate was obtained during autumn.

Regarding the seasonal dynamics of external protozoa, the highest infection rate was in spring. This result was in agreement with El-Sayed (1993) stated that the seasonal incidence of protozoal infection was high in spring.

Concerning the seasonal dynamics of crustacean's infection the maximum rate of infection was during summer. This result agreed El-Moghazy (2008) mentioned that the highest incidence was recorded during summer. But this result did not agree with Hassan (1992) who detected the crustacean during winter.

These differences in the rates and seasonal dynamics of infection between the different localities may be attributed to the differences in environmental conditions, fish species, and the differences in the degree of water pollution as well as number of examined samples.

Corresponding author

Mona S. Zaki
Hydrobiology Department, National Research Center, Cairo, Egypt
dr_mona_zaki@yahoo.co.uk

References

Abd EL- Hady. O. K. (1998): Comparative studies on some parasitic infection of fishes in fresh and polluted water sources. Ph.D. Thesis (parasitalogy), Fac. Vet. Med., Cairo Univ.

Abd El-Gawad, R.A. (2004): Studies on ectoparasites of freshwater fish. M.V.Sc. Thesis. Fac. Vet. Med., Zagazig. Univ.

Abd El-Khalek, H.M. (1998): Studies on the ectoparasites of some freshwater fishes in Beni-Suef Governorate. M.V.Sc. Thesis. Fac. Vet. Med., Beni-Suef. Univ.

Abd El-Maged, R.R. (2009): Studies on ecto-parasites of freshwater fishes in Dakahlia governorate. Ph.D. Thesis, Fac. Vet. Med., Kafrelsheikh Univ.

Ali, M.A. (1992): Biological and ecological studies on protozoan parasites infecting cultured *Tilapia* in Serow fish farm. M. Sc. Thesis, Fac. Science, Cairo Univ., Egypt.

Al-Rasheid, K.A.; Slim, A.; Sakran, T.; Abdel Bakr, A.A.and Abdel Ginaffer, F.A. (2000):"Trichodinid ectoparasites (Ciliophorea: Paritrichida) of some River Nile fish,Egypt." Parasitol. Int. Aug., 49(2): 131-137.

Eissa, I.A.M, (2002): Parasitic fish diseases in Egypt, Ist edition, pp: 52-53. Dar El-Nahdda El-Arabia publishing.

El-Moghazy, D.F. (2008): Studies on some parasitic diseases caused by harmful crastaceans in fish. Ph.D.Thesis, Fac. Vet.Med. Suez Canal University.

El-Sayed, E. (1993): Some studies on protozoal infection among cultured fish in Sharkia. M.V.Sc. Thesis, Zagazig University.

Hassan; M. A. (1992): Studies on some parasitic affections in fresh water fishes in Beni Suef governorate. Ph. D. Thesis, Fac. Vet. Med., Beni-Suef. Cairo Univ.

Pritchard, M.H. and Kruse, G.O.W. (1982): The collection and preservation of animal parasites. Univ. Nebraska, Lincolin and London, 141pp.

Raef, A.M.; El-Ashram, A.M. and El-Sayed, N.M. (2000): Crustacean parasites of some cultured freshwater fish and their control in Sharkia. Egypt. Vet. J., 28(2): 180-191.

Ramadan, R. A. M. (2000): Morphological and immunological studies on certain ectoparasites of some freshwater fishes. Ph.D. Thesis, Fac. Vet. Med., Suez Canal Univ.

Shalaby, S.I. And Ibrahim, M.M. (1988): The relationship between the monogenetic trematodes *cichlidogyrus tubicirrus magnus* first record in Egypt and morphological lesions of gills among tilapia nilotica.Egyption J. of comparative pat and Clin. Path., 1(9):116-126.

Woo, P.T.K. (1995): "Fish diseases and disorders." Vol. 1. Protozoon and metazoan infections phylum Arthropoda.

Study on Clinopathological and Biochemical Changes in Some Freshwater Fishes Infected With External Parasites and Subjected to Heavy Metals Pollution in Egypt

Mahmoud A. El-Seify[1], Mona S. Zaki [*2], Abdel Razek Y. Desouky[1], Hossam H. Abbas[2], Osman K. Abdel Hady[2] and Attia A. Abou Zaid[3]

[1]Fac. of Vet. Medicine Kafrelsheikh University, Kafrelsheikh, Egypt
[2] Hydrobiology Department, National Research Center, Cairo, Egypt
[3]Animal Health Research Institute Kafrelsheikh, Kafrelsheikh, Egypt
[*]dr_mona_zaki@yahoo.co.uk

Abstract: The present investigation was carried out to study the impact of external parasites and heavy metals pollution on some liver function tests of some freshwater fishes. 470 Fish species (330 *Oreochromis niloticus* and 140 *Clarias gariepinus)* were collected alive from three different ecosystems in Kafr-Elshiekh province, Egypt. The obtained results revealed that aspartate aminotransferase (AST), alanine aminotransferase (ALT) enzymes activities as well as creatinine and urea values were elevated in the external parasites infected fish as well as in the fish exposed pollutants. While fishes exposed to both external parasites infection and heavy metal pollution led to more drastic increase in serum AST and ALT enzymes activities as well as creatinine and urea values. In addition; heavy metals pollution increased the susceptibility of fish to protozoa infection while decrease prevalence of monogenea and crustacean infection. On conclusion; infection with external parasites in fishes exposed to heavy metals had the highest effect on liver and kidney functions in the studied fishes.

[Mahmoud A. El-Seify, Mona S. Zaki, Abdel Razek Y. Desouky, Hosam H. Abbas, Osman K. Abdel Hady and Attia A. Abou Zaid, **Some Study on Clinopathological and Biochemical Changes in of Some Freshwater Fishes Infected With External Parasites and Subjected to Heavy Metals Pollution in Egypt**] [Life Science Journal, 2011; 8(3): 401-405] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: External parasites, Heavy metals, *Oreochromus niloticus*, *Clarias gariepinus*, AST, ALT.

Introduction

Most of fish diseases might be occurred as a result of parasitic infection or environmental pollution (Hussain *et al.,* 2003) Knowledge of fish parasites is of particular interest in relation not only to fish health but also to understand ecological problems (Mahfous, 1997).

Aquatic pollution is still a problem in many freshwater and marine environments; it causes negative effects for the health of the respective organisms (Fent, 2007). The number of studies investigating effects of pollutants and concurrently occurring parasites is still relatively low (Sures, 2007). However the effect of environmental pollutants on fish parasites varies depending on the particular parasite and pollutant that interact (Lafferty and Kuris, *1999*). Pollutants may affect the immune system of the fish either directly or by change water quality; that in turn may reduce the fish immunity to parasites (Poulin, 1992) also, water pollution may accelerate the life cycle of the external parasites and promote their spread (Noor El-Din, 1997).

It is well known that certain blood parameters serve as reliable indicators of fish health as many parasites can live in a host, sometimes causing damage to it (Bond, 1979). Therefore, the changes associated with hematological parameters due to various parasites establish a database, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures. These measures are essential in fish farming and fish industry (Roberts, 1981).

Analysis of blood constituents is considered physiological indicators of the whole body and therefore they are important in diagnosis the structural and functional status of fish exposed to pollutants (Adhikari and Betal, 2004). In this respect; Ranzani-Paiva *et al*., (2000) demonstrated alterations in blood composition related to parasitism in fish from the Parana River, indicating that, determination of blood parameters of fishes is of great importance in evaluation of disturbance that caused by parasitism. Therefore, this study was aimed to investigate the impact of external parasites on some physiological parameters related to both liver and kidney functions of some freshwater fish *(Oreochromis niloticus* and *Clarias gariepinus)*, as well as to determine the relation between heavy metal pollution and the infection with external parasites.

2. Materials and Methods

Fish samples:

A total number of 470 (330 *Tilapia species* and 140 *Clarias gariepinus*) freshwater fish were collected a live from three different ecosystems at Kafr El-Shiekh governorate, North Egypt as follow. (River Nile Branch, Drainage canal and Fish farm) by the aid of fisherman and then transported a live to the laboratory where they examined immediately.

Parasitological examination

Parasitological examination was carried out for the detection and identification of the external parasites on the skin, gills and the accessory respiratory organs of the samples.

Collection and preparation of the detected ectoparasites

Monogenea:

Monogenea were collected under binocular dissecting microscopic by means of small pipette in small Petri-dish and cleared several times with water to remove the attached mucous and debris. The worms were then left in refrigerator at 4^{o}C till complete relaxation. Then, they were fixed in 5% formalin for permanent preparation, worms were washed carefully in water to get red of formalin traces and stained with Semichon's acetocarmine stain for about 5-10 minutes till reaching staining, the specimens were passed through ascending grades of ethyl alcohol (30, 50, 70, 90% and absolute) for dehydration. Then, cleared in clove oil, xylene and mounted in Canada balsam (Pritchard and kruse, 1982), while the unstained Monogeneas were mounted in glycerin jelly (Abdel-Hady, 1998).

Protozoa:

Some of the positive slides were stained according to Klein's dry silver impregnation method in which the slides were air –dried, covered with 2% aqueous solution of silver nitrate (Ag NO_3) for 8 minutes, rinse thoroughly in distilled water and exposed to UV light for 20-30 minutes or to direct sun light for 1-2 hr. The slides were allowed to dry and mounted with neutral Canada balsam. This method is indispensable technique for staining *Trichodina*. Other positive slides were also air-dried, fixed with absolute methanol and stained with 10% Giemsa stain for 20-30 minutes to detect the other protozoa (Ali, 1992).

Crustacea:

The detected crustacean parasites were carefully collected by a fine brush and special needle, and transferred into Petri-dish for cleaning by using preserved and cleared in lacto phenol then mounting with polyvol (Raef *et al.*, 2000).

Heavy metals detection:

Three samples of water from the same sources of fish collection were taken in the fore mentioned flask one liter volume capacity after its rinsing several times with distilled water and sterilized in hot air oven at 180 ^{o}C/hour. The collected water sample bottles were labeled with the locality, date, and time of collection. Chemical examinations of these samples were done to estimate some heavy metals including (copper, zinc, iron, lead, cadmium, selenium, mercury, manganese and nickel) according to Chapman and pratt, (1978).

Blood samples

Fresh blood samples were collected without anticoagulant from the caudal artery. The needle is run, quite deep, as much as possible through a middle line just behind the anal fin in a dorso-cranial direction till striking the vertebrate. By drawing the needle gently backward, blood is usually sucked into the syringe.

The collected blood was centrifuged post collection at 3000 rpm for 10 minutes to separate serum for biochemical analysis.

Biochemical analysis

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in serum were determined according to Reitman and Frankel, (1975). Creatinine value was determined according to Rock *et al*., (1987). Urea concentration was measured according to Pathson and Nauch, (1977).

All these biochemical analyses were measured calorimetrically using spectrophotometer and purchased kits.

3. Results

The effect of heavy metals pollution on the prevalence of ectoparasites on examined fish spp. are shown in Table (1) which indicate that the percentage of ectoparsites infection was 71.8% from the examined *Tilapia spp* in the River Nile branch where the pollution of water with copper (Cu), nickel (Ni), cadmium (Cd), selenium (Se) and mercury (Hg) were higher than the other localities where the degree of pollutants were 2.190, 0.102, 0.260, 3.630 and 1.90 respectively, In this degree of pollution, the present study revealed that ectoprotozoa infection of *Tilapia* spp. were the highest percentage of infection where 65% of examined *Tilapia* fish were infected and it was followed by Monogenea and Crustaceans where they reached 23% and 13.7% respectively.

In Drainage canal, where the heavy metals pollution were lower than that in river Nile branch, 69% of examined *Tilapia* were infected with ectoparasites, where the protozoa parasite decreased than in river Nile branch 48%. While monogenea and Crustaceans increased (41%, 38% respectively).

In Fish farms; the percentage of infection reached 64.9% from the examined fishes. Parasitic protozoa decreased than in River Nile branch 46.9%, while monogenea and Crustaceans increased (38.0%, 34.5% respectively).

In addition; the effect of polluted water on the ectoparasitic infection of *Clarias gariepinus*, it was recorded that, in river Nile branch the fish infected with monogenea (28.7%) and protozoa (36.3%), while in Drainage canal (less polluted with heavy metals) monogenea increased 60% while parasitic protozoa decreased 20% than in River Nile branch. With no infection obtained with Crustacean parasites in the examined areas.

Table (1): Effect of heavy metals on parasitic infection among examined Fish spp. in different localities.

Locality	Parasitic infection	% of infection		Heavy metal pollutants in ppm								
				Zinc *1	Lead * _	Manganes *1.5	Copper *1	Nicke *_	Cadmium *0.01	Selenium *_	Mercury *_	Iron *1
		Tilapia spp	*C. gariepinus*									
Locality 1 (River Nile Branch)	Monogenea	23	28.7	0.2	0	0.08	2.19	0.102	0.26	3.63	1.9	0.12
	Protozoa	65	36.3									
	Crustacea	13.7	0									
	Total	71.8	53.7									
Locality 2 (Drainage canal)	Monogenea	41	60	0.06	0.5	0.12	0.07	0.102	0.04	0.26	0.25	0.06
	Protozoa	48	20									
	Crustacea	36	0									
	Total	69	65									
Locality 3 (Fish farm)	Monogenea	38	Not examined	0.07	0.5	0.42	0.11	0.068	0.07	0.52	0.15	0.81
	Protozoa	46.9										
	Crustacea	34.5										
	Total	64.6										

- * Permissible limits of trace elements detected in ppm. According to Egyptian Law
- No available guide line

As shown in Table (2); Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were higher in infected *O. niloticus* taken from River Nile branch than infected *O. niloticus* taken from other localities as they were (78.6 U/l), (115 U/l), (52 mg/dl) and (1.9 mg/dl) respectively while in non infected *O. niloticus* were (69 U/l), (101.5 U/l), (41 mg/dl) and (1.54 mg/d/) respectively. In Drainage canal, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (61.3 U/l), (96.7U/l), (37.3 mg/dl) and (1.4 mg/dl) respectively in the infected *O. niloticus* while in non infected were (51.5 U/l), (85U/l), (26.5 mg/dl) and (1.18 mg/dl) respectively. In Fish farm, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (67 U/l), (97.3 U /l), (36.3 mg/dl) and (1.6 mg/dl) respectively in the infected *O. niloticus* while in non-infected were (55 U/l), (81 U/l), (31.5 mg/dl) and (1.3 mg/dl) respectively.

Table (3) showed that in River Nile Branch, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were higher in infected *Clarias garipinus* (101.7 u/l), (177.7 u/l), (69 mg/dl) and (2.17 mg/dl) respectively . Than non infected *Clarias garipinus* as they were (85 u/l), (130 u/l), (50mg/dl) and (1.6 mg/d/) respectively. While in Drainage canal, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea and Creatinene were (73.3 u/l), (94u/l), (38.7 mg/dl) and (1.63 mg/dl) respectively in the infected *Clarias garipinus* while in non-infected were (54 u/l), (76u/l), (28.5 mg/dl) and (1. mg/dl) respectively.

Table (2): Liver and Kidney function tests of *O. niloticus* infected with ecto-parasites in different localities.

locality / Parameter	River Nile Branch		Drainage canal		Fish farm	
	Non-infected	infected	Non-infected	infected	Non-infected	infected
ALT (U/L)	69	78.6	51.5	61.3	55	67
AST (U/L)	101.5	115	85	96.7	81	97.3
Urea (Mg/dl)	41	52	26.5	37.3	31.5	36.3
Creatinene (Mg/dl)	1.54	1.9	1.18	1.4	1.3	1.6

Table (3): Mean liver and kidney function tests of *Clarias gariepinus* infected with ecto-parasites in different localities.

Locality / Parameter	River Nile Branch		Drainage canal	
	Non.infected	infected	Non.infected	infected
ALT (U/L)	85	101.7	54	73.3
AST (U/L)	13O	177.7	76	94
Urea (Mg/dl)	50	69	28.5	38.7
Creatinene (Mg/dl)	1.6	2.17	1.1	1.63

Table (4): Mean liver and kidney function tests of fish spp. infected with ectoparasites in different localities

Locality / Parameter	River Nile Branch				Drainage canal				Fish farm	
	O.niloticus		*C. gariepinus*		*O. niloticus*		*C. gariepinus*		*O. niloticus*	
	Non-infected	Infected	Non-infected	Infected	Non-infected	Infected	Non infected	Infected	Non infected	Infected
ALT (U/L)	69	78.6	85	101.7	51.5	61.3	54	73.3	55	67
AST (U/L)	101.5	115	13O	177.7	85	96.7	76	94	81	97.3
Urea (Mg/dl)	41	52	50	69	26.5	37.3	28.5	38.7	31.5	36.3
Creatinene (Mg/dl)	1.54	1.9	1.6	2.17	1.18	1.4	1.1	1.63	1.3	1.6

4. Discussion

A negative relationship was detected between heavy metals pollution and prevalence of monogenic infection in River Nile drainage canal branch as well as fish farm during this study. This result agreed with Blanar *et al.*, (2009) which may be attributed to the toxic effect of the heavy metal on the parasite itself Gheorghiu *et al.*, (2006).

The present study denoted that the incidence of external protozoa among examined fish was higher percentage in River Nile Branch which it is more polluted with heavy metals than other localities this may be attributed to that the heavy metals decrease the immune system of the exposed fish which become more susceptible to protozoa infection Khan and Thulin, (1991). The lowest rate of infection with parasitic crustacean was recorded in River Nile Branch, where the heavy metals pollution increased, this result agree with Galli *et al.*, (2001) who recorded that the distribution of *Lamproglena pulchella* was limited to the unpolluted and slightly polluted river sectors. This negative relation may be attributed to the toxic effect of the heavy metals on the crustaceans which may cut its life cycle Ruben *et al., (*2006).

The blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were elevated in the infected fish species *(Oreochromus niloticus* and *Clarias gariepinus*) with external parasites than the non infected fishes in different localities, this indicate that the external parasites stimulated the activities of ALT and AST enzymes as well as both Urea and Creatinine. This result was agreed with Younis, (1999) recorded that aspartate aminotransferase (AST), alanine aminotransferase (ALT) and urea showed significant increase in *O. niloticus* infected with external protozoa and monogenetic trematodes. Osman *et al.,* (2009) reported that blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were increased in Trichodina infected *Clarias gariepinus*.

Concerning the effect of parasitic infection on biochemical parameters of examined fish in the presence of heavy metal pollution, the present study indicated that the blood serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) enzymes activities, Creatinine and Urea values were more higher in both *Oreochromis niloticus* and *Clarias gariepinus* that examined from river Nile branch (more polluted locality with heavy metals) than infected fish spp. taken from drainage canal and fish farm (less polluted with heavy metals). This indicated that exposure of fish to parasitic infection in the presence of heavy metals is more powerful in stimulating the activities of ALT and AST enzymes (Adams, 2002). This may be due to hepatic cells injury or increased synthesis of the enzymes by the liver (Yang and Chen, 2003). The elevation in the urea level in the infected fish may be due to gill dysfunctions as the urea excreted mainly through the gills (Murray *et al.*, 1990). Also these findings may be attributed to the inflammatory reactions and intoxications produced by the parasite in the affected fish.

Corresponding author

Mona S. Zaki
Hydrobiology Department,National Research Center, Cairo, Egypt
dr_mona_zaki@yahoo.co.uk

References

Abd EL- Hady. O. K (1998): Comparative studies on some parasitic infection of fishes in fresh and polluted water sources. Ph.D. Thesis (parasitalogy), Fac. Vet. Med., Cairo Univ.

Adham, K. G. (2002): Sub lethal effects of aquatic pollution in Lake Maryût on the African

sharptooth catfish, *Clarias gariepinus* (Burchell, 1822). Journal of Applied Ichthyology, 18:87-94.

Adhikari, S. and Betal, S. (2004): Effects of cypermehrin and carbofuran on certain haematological parameters and prediction of their recovery in fresh water teleost, *Labeo rohita* (Ham). Ecotoxicol. Environ. Safety58: 220-226.

Ali, M.A. (1992): Biological and ecological studies on protozoan parasites infecting cultured *Tilapia* in Serow fish farm. M. Sc. Thesis, Fac. Science, Cairo Univ., Egypt.

Blanar, A.C.; Munkittrick, R.K.; Houlahan, G.; Maclatchy, L.D. and

Bond, C.E. (1979): Biology of fishes. Saunders college publishing, Philadelphia, Pennsylvania, USA.

Chapman, H.D. and Pratt, P.F. (1978): Methods of Analysis for Soils, Plants and Waters. Univ. California Div. Agric. Sci. Priced. 4034.

Fent, K. (2007): Ökotoxikologie. Georg Thieme Verlag, Stuttgart, 2007.

Galli, P.G.; Crosa, G.; Mariniello. L.M.; Ortis, M. and D'Amelio, S. (2001): Water quality as a determinant of the composition of fish parasite communities. Journal of Hydrobiologia, 452:173-179.

Gheorghiu, C.; Cable, J.; Marcogliese, D.J and Scott, M.E. (2006): Effects of waterborne zinc on reproduction, survival and morphometrics of *Gyrodactylus turnbulli* (Monogenea) on guppies (*Poecilia reticulate)*. Int. J. Parasitology, 37:375-381.

Hussain, S.; Hassan, M.Z.; Mukhtar, Y. and Saddiqui, B.N. (2003): Impact of environmental pollution in human behaviour and up-left of awareness level through mass media among the people of Faisalabad city. Int.J.Agric.Biol., 5: 660-661.

Khan R.A., Thulin J. (1991): Influence of pollution on parasites of aquatic animals. Advances in Parasitology, 30:201–238.

Lafferty, K.D. and Kuris, A.M. (1999): How environmental stress affects the impacts of parasites. Limnol. Oceanogr., 44:925-931.

Mahfouz, N.B.M. (1997). Effect of parasitism on immunity of cultured freshwater fish. Ph D. Thesis, faculty of veterinary medicine, Tanta University, Egypt.

Murray, R.K.D.K.; Rranne, P.A.M. and Rodwell, V.W. (1990): Harper's Biochemistry Publisher, Norwalk, Connecticut/ Los Altos, California.

Noor El-Din, A.N. (1997): studies on the effect of water pollution along different sites of the River Nile on the survival and production of some freshwater fishes. Ph. D. Thesis, Zoology Dep, Fac. Science, Cairo Univ., Egypt.

Osman H. A. M.; Ismaiel, M.M.; Abbas, T.W and Ibrahim, T.B. (2009): An approach to the interaction between Trichodiniasis and pollution with Benzo-a-pyrene in catfish (*Clarias gariepinus*). World Journal of Fish and Marine Sciences 1(4): 283-289.

Pathson, C.J. and Nauch, S. R. (1977): Determination of serum urea. Anal. Chem., 49: 464-469.

Poulin, R. (1992): Toxic pollution and parasitism of freshwater fish.

Pritchard, M.H. and Kruse, G.O.W. (1982): The collection and preservation of animal parasites. Univ. Nebraska, Lincoln, London, 141pp.

Raef, A.M.; El-Ashram, A.M. and El-Sayed, N.M. (2000): Crustacean parasites of some cultured freshwater fish and their control in Sharkia. Egypt. Vet. J., 28(2): 180-191.

Ranzani-paviva, M.J.T.; Silva-souza, A.T.; Pavanelli, G.C. and Takemoto, R.M. (2000): Hematological characteristics and relative condition factor (Kn) associated with parasitism in *Schizodon borellii* (Osteichthyes, Anostomidae) and *Prochilodus lineatus* (Osteichthyes, Prochilodontidae) of the Paraná River, Porto Rico region, Paraná, Brazil. Acta Scientiarum, Maringá, 22(2):515-521.

Reitman, S. and Frankel, S. (1957): Colorimetric Composition of glutamic oxaloacetic and glutamic pyruvic transaminases, Am. J. Clin. Pathol., 28: 53-56.

Roberts, R.S.(1981) Patologia de los peces. Madrid, Mundi-prensa, pp:336.

Rock, R.C.; Walker, W.G. and Jennings, C.D. (1987): Nitrogen metabolites and renal function. In: Tietz, N.W., ed. Fundamentals of clinical chemistry, 3rd Ed Philadelphia: W.B. Saunders, pp: 669-704

Ruben, A.P.; Asbjorn, L.V.; Lars, E.W.F. and Antonio, B.S.P (2006): Effects of aqueous aluminum on four fish ectoparasites. Biology Journal of the Linnean Society 90(3):525-538

SURES, B. (2007): Host-parasite interactions from an ecotoxicological perspective. *Parassitologia*, *49*, 173-176.

Yang, J. and Chen, H. (2003): Serum metabolic enzyme activities and hepatocyte ultra structure of common carp after gallium exposure. Zoological studies, 42(3): 455-461.

Younis, A. A. E. (1999): Effect of some ectoparasites on the blood and serum constituents of *Oreochromis niloticus* fish with referring to treatment. Beni Suif. Vet. Med. J., 9(3): 341- 351.

Efficacy of Myrrh Extract "Mirazid®" to Reduce Lead Acetate Toxicity in Albino Rats with Special Reference to Cerebellum and Testes

Nahla AG Ahmed Refat [*1] and Marwa A Abass[2]

Department of Pathology, Faculty of Veterinary Medicine[*1] and Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine[2], Zagazig University, Egypt
[*]Nahla_kashmery@hotmail.com

Abstract: Lead is a ubiquitous environmental and industrial pollutant with a worldwide health problems. A total fifty adult male albino rats were equally divided into five groups to evaluate the toxic effects of lead acetate on liver, kidneys, brain and testes of albino rats, besides assessing the efficacy of myrrh extract in reducing these toxic changes. Group (1) was left without treatment. Each rats in group (2) was orally given 2 ml distilled water. Each rats in group (3) was given 20 mg lead acetate / kg B.wt. Each rats in group (4) was given 500 mg myrrh-extract / kg B.wt. Each rats in group (5) was simultaneously given similar doses of lead acetate and myrrh-extract as those given to groups (3&4). Treatments of groups (1-5) were administered daily by stomach tube for 3 months. At the end of the experimental period, all rats were sacrificed, necropsied and the gross lesions were recorded. Moreover, specimens were collected from the liver, kidneys, ,brain and testes of groups(1-5) and prepared for histopathological examination and morphometrical analysis of cerebellum and testes.. Histopathological examination of group (3) showed degenerative and necrotic changes in the hepatic and renal cells. Interstitial and perivascular aggregations of lymphocytes and hyperplasia of epithelial lining of some bile ducts with newly formed bile ductules were detected . Some hepatocytes and renal cells showed karyomegaly and cytomegaly with presence of eosinophilic intranuclear inclusion bodies. The brain revealed congestion, degenerated neurons, satellitosis, neuronophagia, encephalomalacia and coagulative necrosis of Purkinje cell. The testes showed interstitial edema among degenerated seminiferous tubules. The previous changes in cerebellum and testes were supported by the morphometric results which revealed a significant reduction of the mean thickness of the Purkinje cell layer when compared with the control rats. A significant decrease ($p<0.001$) of the width of germinal epithelial layer of the affected seminiferous tubules and number of Sertoli cells, spermatogonia and primary spermatocytes was detected. Group (5) showed marked amelioration of the encountered lesions in group (3). It could be concluded that, the adverse effects, induced by lead acetate, were markedly ameliorated by co-treatment with myrrh extract.
[Nahla AG Ahmed Refat and Marwa A Abass, **Efficacy of Myrrh Extract "Mirazid®" to Reduce Lead Acetate Toxicity in Albino Rats with Special Reference to Cerebellum and Testes]** Life Science Journal,. 2011; 8(4): 406-414] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Lead-acetate, Mirazid (Myrrh -extract), pathology, morphometrical analysis

1. Introduction

The environmental contamination by heavy metals has increased drastically along with the rapid development of modern industry. Among these metals is lead , of which its levels have increased substantially during the last few years (**Bilandz˘ic et al. 2009**). It is still mined and added to many commercial products including paints, eye cosmetics, gasoline , enamels and water pipes (**Fischbein et al. 1992, Sajitha et al. 2010 and Sansar et al. 2011)** .

Exposure to low levels of lead has been associated with functional and structural impairments in both human and experimental animals **(Reza et al. 2008**). The main targets of lead are the hematopoietic, nervous and renal tissues. Moreover, it hinders the efficacy of the hepatic, reproductive and immune function **(Teijón et al. 2006 and Durgut et al. 2008).** Many studies have shown that reproductive toxicosis is an important feature of lead toxicity. Lead toxicity induces apoptosis of the germinal epithelium with disruption of spermatogenesis (**Adhikari et al. 2001 and Batra et al. 2001)** . Furthermore, it has been shown that chronic lead exposure produces central nervous system impairments (**Antonio et al. 2003 and Sansar et al. 2010)**.

Calcium Disodium EDTA is a traditional synthetic lead expellant but it is toxic to the liver and kidneys. Therefore recently there have been many studies on the use of natural products such as vitamins and herbal drugs to expel lead **(Xu et al. 2005)**. There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical- induced tissue injury**(Siddique et al. 2000 and Koleva et al. 2002)**. Numerous plant products have antioxidant activity as they scavenger free radicals and inhibit of lipid peroxidation **(Scartezzini and Speroni , 2000 and Tapiero et al. 2002)**.

Mirazid is a new herbal anthelimentic drug. It is composed of purified myrrh extract. Myrrh is an oleo-gum resin extract from the stem of Commiphora

molmol plant **(Hassan et al. 2003)**.The oleo- gum resin of myrrh contains 2– 8% volatile oils and contains terpenes, sesquiterpenes, and cuminic aldehyde **(Chevallier,1986).** The various pharmacological activities of myrrh have been reported by many authors **(Sheir et al. 1986, Massoud et al. 2001 and Al-Rowais, 2002).** Myrrh extract (Mirazid) has been produced and marketed as an antischistosomal drug since 2001 **(Barakat et al. 2005).** Commiphora molmol was reported to have anti- inflammatory activity and antineoplastic activity equivalent to the standard cytotoxic drug cyclophosphamide **(Qureshi et al. 1993)**. Moreover, it was reported to have a protective effect on gastric ulcer that was attributed to its free radical- scavenging and prostaglandin- inducing properties **(Al-Harbi et al. 1997)**. However, up to our best knowledge, there is no previous studies that investigated the effects of myrrh extract upon the pathological changes induced by lead acetate, so the aim of this study was to evaluate the efficacy of myrrh extract in reducing the toxic effects of lead acetate in albino rats.

2. Material and Methods

Material:

1-Chemical:

A-Lead acetate ($_{C4H6o4}Pb3_{H2}O$) in the form of white crystals, was purchased from ADWIC (A governmental Pharmaceuticals Company), Egypt.

B- Myrrh -extract was obtained from Pharco Pharmaceuticals Company, Egypt, in the form of soft gelatin capsules commercially named "Mirazid".

2- Experimental Animals:

Fifty adult male Wistar albino rats, weighting 200±20 gm, were obtained from the Unit of Laboratory Animal, Faculty of Veterinary Medicine, Zagazig University. Animal care was performed according to the guidelines of the National Research Council and the American Association of Accreditation for Lab Animal Care **(NRC, 1994).** All animals were allowed 7 days for acclimatization to their new environment and to ascertain their physical well being. They were housed in separate well ventilated cages, under standard conditions, with free access to the standard diet and water ad libitum.

Methods:

1-Experimental design:

After 7 days adaptation period, rats were allocated randomly to five equal groups.

Group (1) was left without treatment. Each rats in group (2) was orally given 2 ml distilled water. Each rats in group (3) was given 20 mg lead acetate / kg B.wt. **(Nehru and Kanwar, 2004)**. Each rats in group (4) was given 500mg myrrh-extract / kg B.wt. Each rats in group (5) was simultaneously given similar doses of lead acetate and myrrh-extract as those given to groups (3&4).

Treatments of groups (1-5) were administered daily by stomach tube for 3 months. Myrrh extract solution(500mg /kg B.wt.) was given 1 hour before treatment with lead .The timing and dose of myrrh extract pretreatment have been selected on the basis of previous reports (**Badria et al. 2000 and Farid and Attia , 2007**) to build antioxidant pool in animal body before heavy metal exposure. At the end of the study, all the rats were sacrificed and necropsied to elucidate the lesions.

2- Pathological examination:

All animals were necropsied and the gross lesions were recorded. Moreover, specimens were collected from the liver, kidneys, ,brain and testes of groups(1-5) and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, stained with HE and examined microscopically **(Bancroft and Stevens, 2002)**.The H&E stained sections of cerebellum and testes were subjected for morphometric analysis.

3- Morphometrical analysis of the cerebellum and testes:

The image analyzer computer system (Leica Qwin 500) was used for the morphological analysis of the cerebellar and testicular sections in the Histology Department, Faculty of Medicine, Cairo University**.** The hematoxylin and eosin stained sections were examined using light microscope equipped by eyepiece graticule for measuring the structural changes. For cerebellar sections the mean thickness of the Purkinje cell layer in each group was measured **(Hoffer et al. 1987).**

The number of germ cells per seminiferous tubules, spermatogonia, primary spermatocyts, Sertoli cells (**Fawcett and Raviola, 1994)** and the seminiferous tubules diameter **(Batra et al. 2004)** were recorded for testicular sections. The examination was done at 1200 X magnification in ten randomly chosen non-overlapping fields, from five animals for each group.

4- Statistical analysis:

Data were presented as means ± SD. The differences were compared for statistical significance by student's t test. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using Epi-Info version 6.1 (**Dean et al. 2000).**

3. Results

1-Pathological findings:

The liver, kidneys, brain and testes of groups (1,2&4) showed neither gross nor microscopical lesions, meanwhile group (3), revealed enlarged and

congested liver with small grayish-white foci. Microscopically, the liver was congested .The portal areas showed fibroblastic proliferation infiltrated with leukocytes and bile ducts with hyperplastic epithelial lining with newly formed bile ductules (fig.1). Diffuse vacuolation of hepatocytes (fig.2) and coagulative necrosis of hepatocytes, evidenced by pyknosis and karyolysis (fig. 3) were found. Individualization and disorganization of the hepatic cords were encountered. Focal replacement of the hepatic parenchyma by round cells , mainly lymphocytes was detected (fig.4). Some hepatocytes showed karyomegaly and cytomegaly with presence of eosinophilic intranuclear inclusion bodies .

The kidneys were macroscopically enlarged. Microscopically, congestion and focal replacement of the renal parenchyma with mononuclears were noticed (fig.5). Cloudy swelling and vacuolation of the renal epithelium were seen in the convoluted tubules. Cystic dilation of some renal tubules with flattened epithelium was seen (fig.6). Some epithelial cells of renal tubules showed karyomegaly and cytomegaly (Fig .7) with presence of eosinophilic intranuclear inclusion bodies (fig.8).Other renal epithelium showed coagulative necroses, characterized by karyolysis . Focal replacement of renal parenchyma with mononuclears in presence of hyaline casts, besides necrotic renal epithelium and cystic renal tubules (fig.9).

The brain revealed congested and edematous meninges (fg.10). Brain edema was evidenced by dilated Virchow-Robin spaces (fig.11). Focal gliosis was seen partially replacing the midbrain parenchyma (fig.12.). Satellitosis and neuronophagia were seen in the gray matter of cerebrum. The cerebellar medulla exhibited encephalomalacia and the Purkinje cell layer was partially necrotic (fig.13). The necrotic Purkinje cells were surrounded with clear spaces, suggesting edema, and presented karyolysis (figs. 14). The third ventricle showed atrophic and necrotic epithelial covering of the choroid plexus (figs.15&16).Cerebral encephalomalacia was detected.

The testes revealed interstitial edema and atrophy of the leydig cells (fig.17). Aspermatogenesis was associated with absence of spermatids and spermatozoa (fig.18), besides presence of necrotic debris in lumina of seminiferous tubules (fig.19). Necrotic debris were seen in lumens of some seminiferous tubules with palisading Sertoli cells (fig.20).

Group (5) showed marked amelioration of the encountered lesions in group (3), where no gross abnormalities were detected in the collected organs. Microscopically, the liver was almost normal and revealed mild congestion (fig.21),besides hydropic degeneration and few round cell infiltration in portal areas . The kidneys showed mild congestion and hemorrhage. Small eosinophilic intranuclear inclusion bodies were rarely found together with a slight karyomegaly and cytomegaly. Few interstitial aggregations of round cells were observed. Rregenerative changes were detected in the epithelial lining of some renal tubules evidenced by hyperchromatic nuclei (fig.22). The brain showed mild encephalomalacia of the white matter of cerebellum with almost normal Purkinje cell layer (fig.23).The meningeal blood vessels were moderately congested. The normal structure of the majority of the seminiferous tubules was restored and spermatogenesis was evident (fig.24). Mild testicular congestion and interstitial edema were still encountered.

2-Morphometric study:

2-1- Morphometric cerebellar changes:

A significant decrease($p<0.05$) in the mean thickness (16.22±2.60µm) of the Purkinje cell layer of group (3) was observed ,while the mean thickness of Purkinje cell layer of group (1) was (19.61±3.16 µm) . There was a significant improvement in this measurement (18.72±3.09 µm) in group (5) compared with the control value (Table : 1).

2-2- Morphometric testicular changes:

The mean diameter of the seminiferous tubules was decreased significantly in group (3) (83.2±18.1 µm) when compared with group (1) (114.7±20.7 µm) ($p<0.05$). A significant decrease ($p<0.05$) in the mean number of Sertoli cells, spermatogonia and primary spermatocytes (8.9±4.1, 14.4±9.3, and 7.7±12.6 respectively) was observed in group (3) when compared with group (1) (15.2±4.3, 31.4±9.9, and 42.8±13.3 respectively). There was a significant improvement in these measurements (99.9±22.3, 11.9±3.0, 27.5±10.0, and 33.0±9.3 for mean diameter of seminiferous tubule, number of Sertoli cells, spermatogonia and spermatocytes respectively) in group(5) that was still significantly lower than the control (Table :2).

Table 1: Means and standard deviations (mean ± SD) of the thickness of Purkinje cell layer in the studied groups.

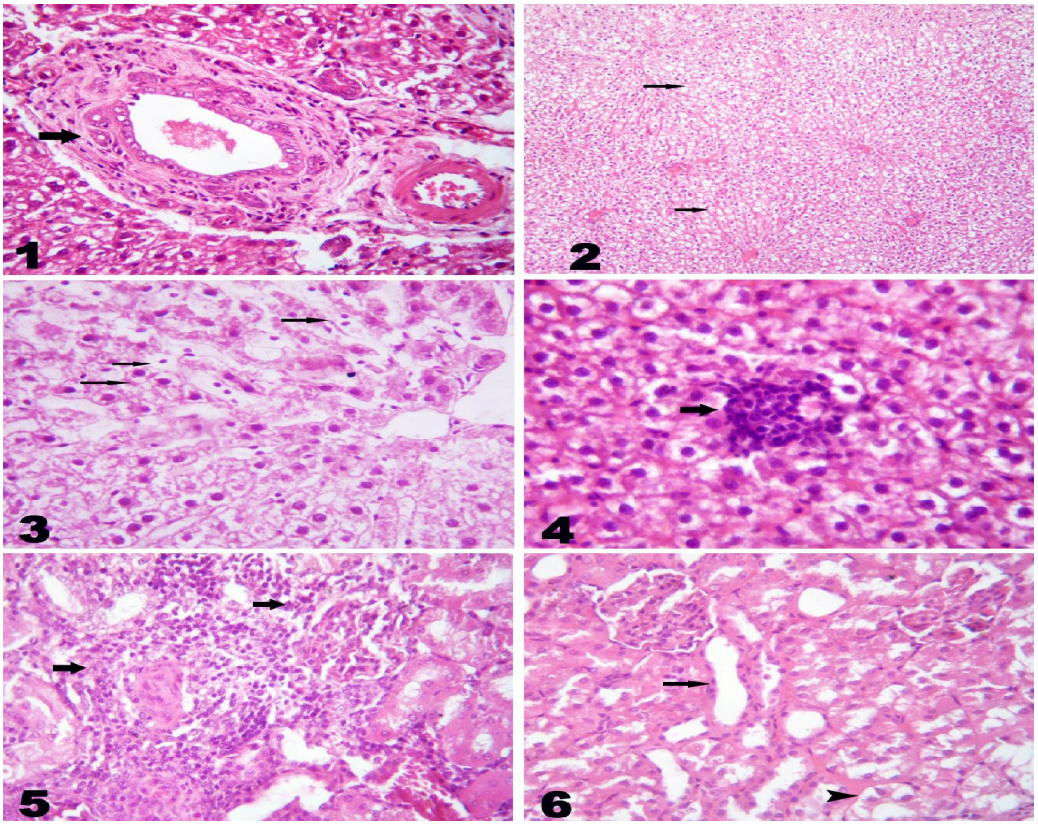
	Groups				
	1 mean ± SD	2 mean ± SD	3 mean ± SD	4 mean ± SD	5 mean ± SD
Purkinje cell layer thickness (µm)	19.61 ± 3.16	19.15 ± 3.40	16.22 ± 2. 60	19.58 ± 3.08	18.72 ± 3.09
P-value (compared with Group I)	-	0.5758	0.00019*	0.97004	0.27239

*Very highly significant ($P < 0.001$).
n=10 in all groups

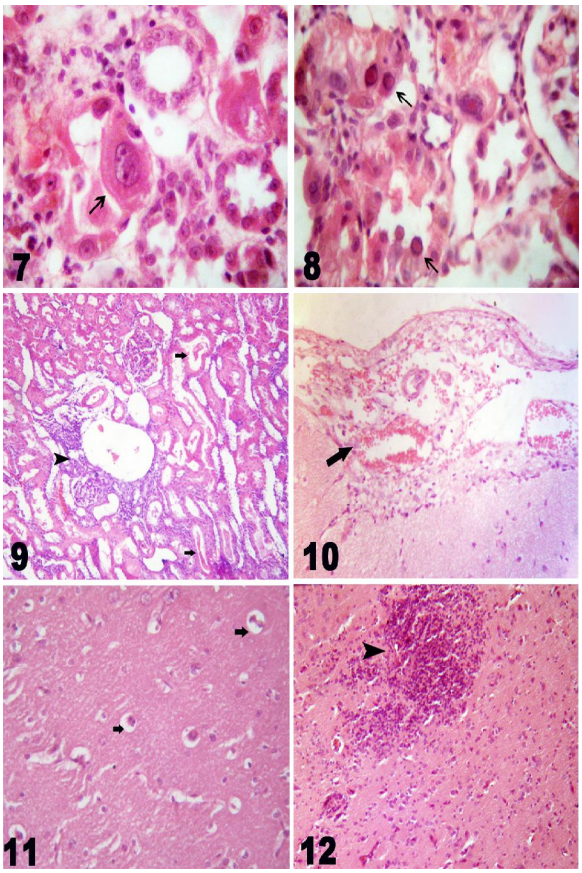
Table (2): Means and standard deviations (mean ± SD) of Morphometric parameters of testes in the studied groups .

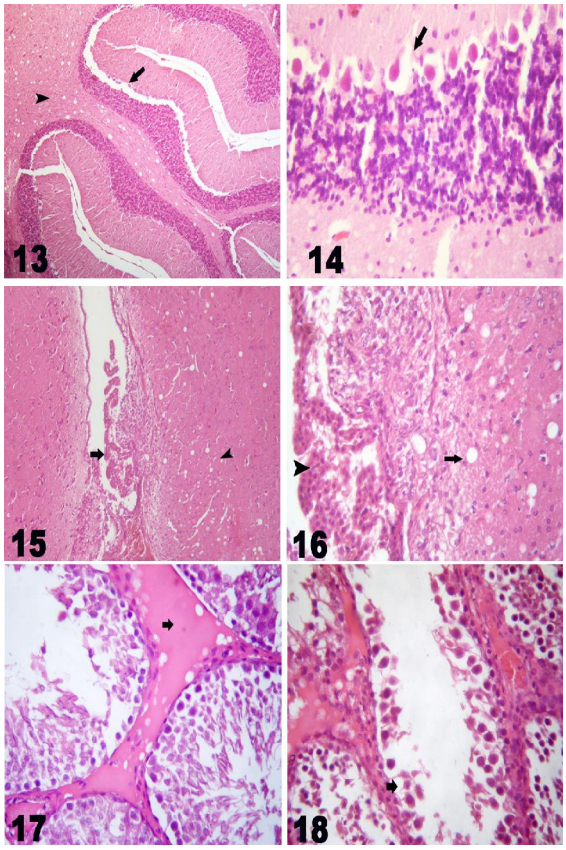
Groups	No.	Semineferous tubule diameter (µm) mean ± SD	Sertoli cells number mean ± SD	Spermatogonia Number mean ± SD	primary spermatocytes number mean ± SD
1	10	114.7±20.7	15.2±4.3	31.4±9.95	42.8±13.3
2	10	112.4±21.6	14.2±3.8	32.1±10.15	40.9±11.4
3	10	83.2±18.1 *◊≠	8.9±4.1 *◊≠	14.4±9.36 *◊≠	7.7±12.6 *◊≠
4	10	113.8±23.3	13.0±4.7	33.2±9.67	43.4±10.5
5	10	99.9±22.3 *◊≠†	11.9±3.0 *◊≠	27.5±10.00 *◊≠	33.0±9.3 *◊≠

*= Significant compared with control group($p<0.05$), ◊= significant compared with group(2)($p<0.05$), ≠ = significant compared with group (4)($p<0.05$),
†= significant compared with group (5)($p<0.05$).



Group (3): Figs. (1-4) Liver, Figs. (5&6) Kidneys. HE.
Fig.(1)- Hyperplastic epithelial lining of bile ducts with newly formed bile-ductules (arrow). x1200.
(2)- Diffuse vacuolation of hepatocytes (arrow). x 300 .
(3)-Coagulative necrosis of hepatocytes evidenced by pyknosis and karyolysis (arrow). x 1200.
(4)-Focal replacement of hepatic parenchyma with round cells (arrow). x 1200.
(5)- Focal replacement of renal parenchyma with mononuclears (arrow). x 1200.
(6)-Cystic dilation of some renal tubules (arrow) with necrotic renal epithelium (arrowhead). x1200.





Group (3): Figs. (7-9) Kidneys, Figs. (10-12) Brain. HE.

Fig. (7)- Karyomegaly and cytomegaly in some renal epithelium (arrow). x 1200.

(8)- Eosinophilic intranuclear inclusion bodies in renal epithelium (arrow) x 1200.

(9)-Focal replacement of renal parenchyma with mononuclears (arrowhead) besides hyaline casts (arrow) and cystic renal tubules. x 300 .

(10)-Congested (arrow) and edematous meninges . x 1200.

(11)- Edema evidenced by dilated Virchow-Robin spaces (arrow). x 1200.

(12)-Midbrain gliosis (arrowhead). x 1200.

Group (3): Figs. (13-16) Brain, Figs. (17&18) Testes . HE

Fig. (13)-Cerebellar medullary encephalomalacia (arrowhead) and necrotic Purkinje cells (arrow).x150.

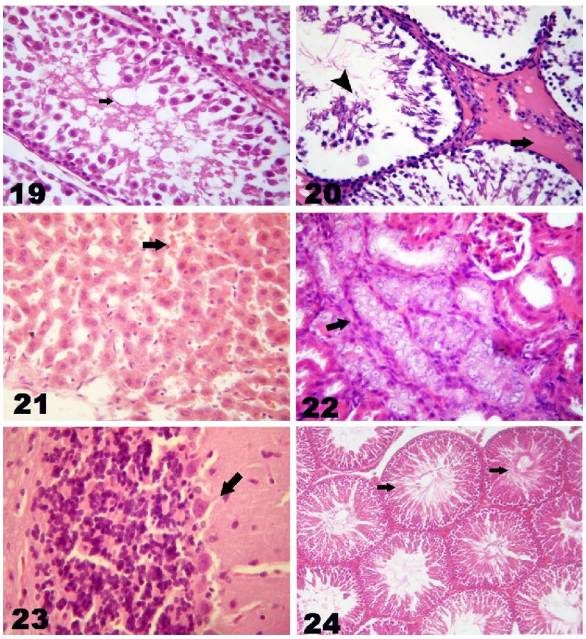
(14)-Magnification of fig. (13) to show necrotic and edematous Purkinje cell layer(arrow).x1200.

(15)-Midbrain displaying atrophic and necrotic choroids plexus (arrow) and vacuolated pons.x300.

(16)- Magnification of fig. (15) to show the atrophic and necrotic choroids plexus (arrowhead) and vacuolated pons. x1200.

(17)- Interstitial edema with atrophic leydig cells . x 1200.

(18)- Aspermatogenesis with absence of spermatids and spermatozoa .x 1200.



Group (3): Figs. (19&20) Testes. HE.
Fig. (19)- Aspermatogenesis with necrotic debris in lumen of seminiferous tubules (arrow).x1200.
(20)-Aspermatogenesis with desquamated germinal epithelium (arrowhead) and palisading Sertoli cells.x1200.
Group (5): Figs. (21-24). HE.
(21)- Liver: Almost normal liver with moderate congestion (arrow).x 1200.
(22)- Kidney: Regenerating renal cortical tubular epithelium evidenced by hyperchromatic nuclei (arrow).x 1200.
(23)- Brain: Almost normal Purkinje cell layer (arrow).x1200.
(24)- Testis: Most of seminiferous tubules displaying active spermatogenesis (arrow).x300.

4. Discussion

Both occupational and environmental exposure to xenobiotics such as lead, remains a serious problem in many developing and industrialized countries (**Yücebilgiç et al. 2003**).Our study was designed to evaluate the toxic effects induced by lead acetate treatment on liver, kidneys, brain and testes of adult male albino rats and to investigate the protective effect of myrrh extract against these toxic effects.

The results of the current study clearly demonstrated that, lead acetate treatment at a concentration of 20 mg/kg B.wt. induced degenerative and necrotic changes in the hepatic and renal cells, besides interstitial and perivascular aggregation of mononuclears. Hyperplasia of epithelial lining of some bile ducts with newly formed bile ductules was detected . Intranuclear inclusion bodies in the renal and hepatic cells is considered diagnostic and pathognomonic for lead toxicosis (**Weide et al. 2003).**These inclusions were represented by dense eosinophilic homogenous cores surrounded by a membrane,chemically considered as protein containing lead (**Jones et al. 1997).** The hepatic and renal cells, containing these inclusions showed karyomegaly, and cytomegaly. **Abdel-kalek et al. (2000), Ghorbe et al. (2001) and El-nattat et al. (2003)** reported that oral dosing of experimental animals with lead induced necrosis, hemorrhage and degenerative changes which are in general agreement with the current study. Congestion, degenerated neurons, satellitosis, neuronophagia, encephalomalacia, coagulative necrosis of Purkinje cell of cerebellar white matter were observed. These changes were supported by the morphometric results which revealed a significant reduction in the mean thickness of the Purkije cell layer as compared to control rats. Similar results were previously described by **Blood and Hinchcliff (2000), Sidhu and Nehru (2004) and Mc Gavin and Zachary (2007)** who reported that lead is a neurotoxin that damages the white and gray matter of both the central and peripheral nervous system. Furthermore, **Adonaylo and Oteiza (1999)** recorded that lead treatment for 2 months disrupted the normal arrangement of the cellular layers of the cerebellum with large spaces between the Purkinje cell layer and the granular layer.

Regarding lead induced testicular toxicity, the present study showed obvious testicular degenerations and necrosis, where most seminiferous tubules were lined with few germ cells, composed mainly of spermatogonia, besides interstitial edema. The previous results were correlated with **Hamir and Sullivan (2008), Al-Mansour (2009) and El-Sayed and El-Neweshy (2009). Koizumi and Li (1992)** attributed the lead induced testicular damage to its higher unsaturated fatty acids content which renders it more vulnerability to oxidative stress. An increased level of reactive oxygen species production in the male reproductive organs in rats was observed after lead exposure (**Acharya et al. 2003 and Marchlewicz et al. 2007)** and in human (**Naha and Manna , 2007 and Kasperczyk et al. 2008)**.The lead induced cellular damage, in the current work, may be attributed to its ability to generate reactive oxygen species that damages tissues by enhancing lipid peroxidation as reported by **El-Missiry (2000**). The lipid peroxidation inactivates the cell constituents by oxidation and ultimately loss of its membrane integrity (**Abdel-Wahhab, 2005 and El-Nekeety, 2009**). Moreover,

lead depletes the antioxidant reserves as superoxide dismutase, catalase and glutathione peroxidase (**Newairy and Abdou , 2009 and Ashry et al. 2010).**

On the other hand , myrrh extract treated rats did not revealed any pathological or morphometric deviations from the control rats. The current findings are in agreement with several experimental and clinical studies on myrrh extract which proved to be safe (**Al-Ashmawy et al. 2006 and Abdul-Ghani et al. 2009)**.The concurrent administration of myrrh extract with lead acetate ameliorated the lead acetate-induced lesions. Similar findings were described by **Farid and Attia (2007)** who found that myrrh extract at a dose of 500 mg/kg B.wt. significantly reduced the malondialdehyde and hepatic fibrosis induced by carbon tetrachloride toxicity. Interestingly, **Al-Ashmawy et al (2006)** reported that supplement of lead acetate with myrrh extract alleviated the lead induced lipid peroxidation in liver homogenate and genotoxicity in mice. The previous results may be attributed to the potent cytoprotective properties and antioxidant activity of Myrrh extract. The latter inhibits lipid peroxidation by increasing the activity of the anti-oxidant enzymes such as glutathione peroxidase and superoxide dismutase (**Qureshi et al. 1993 and Al-Harbi et al. 1994)**.

Conversely, the ameliorating effect of myrrh extract against lead –induced toxicosis could be attributed to the various antioxidant compounds present in the extract. Previous reports revealed that myrrh has immunostimulatory, anti- inflammatory and antioxidant potential(**Qureshi et al. 1993).** Myrrh causes a significant reduction in production of Interleukin-1β, IL-6, IL-8 (**Tipton et al. 2003)**. IL-1β is involved in lipid peroxidation induced by certain pathological conditions as cadmium toxicity and brain aging process(**Murray et al. 1999**).

It could be concluded that, lead acetate, at a dose of 20 mg/kg B.wt. induced lesions in the liver, kidneys, brain and testes of adult albino rats, meanwhile the concurrent administration of myrrh extract ameliorated and prevented the lead acetate -induced lesions.

Corresponding author
Nahla AG Ahmed Refat
Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt
Nahla_kashmery@hotmail.com

References

Abdel-Kalek MM , Hosny GA and Abdel-Rahman M (2000):Effect of chronic lead poisoning in broilers.J.Comp. Path., And Clinic.Path.13(2):66-83.

Abdel-Wahhab MA, Saeed A and Hufner A (2005): NMR and radical scavenging activities of patuletin from Urtica urens L. against aflatoxin B1. Pharm Biol.,43:515-525.

Abdul-Ghani RA, Loutfy N and Hassan A (2009): Myrrh and trematodoses in Egypt: an overview of safety, efficacy and effectiveness profiles. Parasitol Int.,58(3):210-214.

Acharya U R, Rathore R M and Mishra M (2003): Role of vitamin C on lead acetate spermatogenesis in swiss mice. Environ Toxicol Pharmacol.,13 (1): 9-14.

Adhikari N, Sinha N, Narayan R and Saxena D K (2001): lead-induced cell death in testes of young rats. J. Applied Toxicol., 21(4):275:227.

Adonaylo V N and Oteiza P I (1999): Lead intoxication: antioxidant defenses and oxidative damage in rat brain. Toxicology., 135(2– 3): 77–85.

Al-Ashmawy I M, Ashry K M, El-Nahas A F and Salama O M (2006): Protection by turmeric and myrrh against liver oxidative damage and genotoxicity induced by lead acetate in mice. Basic clin pharm toxicol .,98(1):32-37.

Al-Harbi M M, Qureshi S, Ahmed M M, Raza M, Giangreco A B, and Shah A H(1994): Anticarcinogenic effect of Commiphora molmol on solid tumors induced by Ehrlich carcinoma cells in mice. Chemotherapy., 40(5): 337– 347.

Al-Harbi M M, Qureshi S, Raza M, Ahmed M M, Afzal M and Shah A H (1997): Gastric antiulcer and cytoprotective effect of Commiphora molmol in rats. J. Ethnopharmacol., 55(2):141– 150.

Al-Mansour M I (2009): Histological alterations induced by lead in the testes of the quail Coturnix coturnix. Res J Environ Toxicol.,3(1):24–30.

Al-Rowais N A(2002): Herbal medicine in the treatment of diabetes mellitus. Saudi Med J.,23(11): 1327–1331.

Antonio MT, Corredor L and Leret ML(2003): Study of the activity of several brain enzymes like markers of the neurotoxicity induced by perinatal exposure to lead and/or cadmium. Toxicol Lett.,43:331–340.

Ashry K M, El-Sayed Y S, Khamiss R M and El-Ashmawy I M (2010): Oxidative stress and immunotoxic effects of lead and their amelioration with myrrh(Commiphora molmol) emulsion. Food Chem Toxicol.,48(1):236–241.

Badria F, Abou-Mohamed G, El-Mowafy A, Massoud A and Salama O (2000):

Mirazid: a newschistsomicidal drug. Pharmaceut. Boil., 93:127-131.

Bancroft JD and Gamble M (2002): Theory and Practice of Histological Techniques. 5th ed., Churchill Livingstone. New York, London, Philadelphia : 125-138.

Barakat R, Elmorshedy H and Fenwick A (2005): Efficacy of myrrh in the treatment of human schistosomiasis mansoni .Am. J. Trop. Med. Hyg., 73(2): 365–367.

Batra N, Nehru B and Bansal M P (2001): Influence of lead and zinc on rat male reproduction at biochemical and histopathological level. J Applied Toxicol.,21(6):507-512.

Batra N, Nehru B and Bansal MP (2004): Reproductive potential of male Portan rats exposed to various levels of lead with regard to zinc status. Br. J. Nutr., 91: 1887-1891.

Bilandz˘ic´ N, Sedak M, Vrataric´ D, Peric´ T, and S˘ imic´ B(2009):Lead and cadmium in red deer and wild boar from different hunting grounds in Croatia. Sci. Total Environ., 407: 4243–4247.

Blood D C and Hinchcliff K W (2000): Veterinary medicine: a Textbook of Sheep, Pig, Goats and Horses. 9th ed:.530–531.

Chevallier A (1986): The Encyclopedia of Medicinal Plants. Dorling Kindersley Inc., London, New York: 84– 90.

Dean AG, Dean JA, Coulombier D and Brendel K A (2000): Epi-Info (6.1): A word processing data base and statistical program for epidemiology and micro-computer office, Center for disease control, Atlanta Georgia, USA.

Durgut R, Koc A, Gonenci R, Bal R, Celik S, Guzel M, Altug M, and Atesoglu E (2008): Effects of high dose lead toxication on liver, kidneys, heart, brain and blood in rabbits: an experimental study. J Appl Biol Sci., 2: 11- 18.

El-Missiry MA (2000): Prophylactic effect of melatonin on lead induced inhibition of heme biosynthesis and deterioration of antioxidant system in male rats. J Biochem Mol Toxicol., 14:57-62.

El-nattat WS, Ali GA and El-Khadrawy HH(2003):The effect of lead acetate and vitamin C (antioxidant) on some enzyme activities in semen and serum of male rabbits.Egypt.J.basic and Appl.Physiol.,2(1):107-119.

El-Nekeety AA, El-Kady AA, Soliman MS, Hassan NS and Abdel-Wahhab MA(2009):Protective effect of Aquilegia vulgaris (L.) against lead acetate-induced oxidative stress in rats. Food Chem Toxicol., 47:2209-2215.

El-Sayed Y S and El-Neweshy M S (2009): Impact of lead toxicity on male rat reproduction at ‘‘hormonal and histopathological levels’’. Toxicol Lett., 189(.1): 219–220.

Farid A B and Attia H A (2007): Effects of selective natural products thioproline and pegasys ® on hepatic platelet activation factor (PAF) in CCL4 induced hepatic fibrosis in rats. Saudi Pharm J.,15(2) :96-104.

Fawcett DW and Raviola E (1994): Bloom and Fawcett, a Textbook of Histology. 12th Edn., New York, Champan and Hall.

Fischbein A, Wallace J, Sassa S, Kappas A, Butts G, Rohl A and Kaul B (1992): Lead poisoning from art restoration and pottery work: unusual exposure source and household risk. J Environ Pathol Toxicol Oncol.,11:7–11.

Ghorbe E, Boujelbene M, Makni F, Guremazi F, Murat J and El-Feki A (2001):Effect of chronic exposure on kidney function in male and female rats: determination of a lead exposure biomarker. Arch.Physiol.Biochem.,109(5):457-463.

Hamir AN and Sullivan N D (2008): Extra-neural lesions in experimental lead toxicosis of dogs. J Small Animal Pract.,24 (7):437–44.

Hassan M, El-Motaiem M, Afify H, Abaza B, El-Shafei M and Massoud A M (2003): In vitro effect of Mirazid on Schistosoma mansoni worms. J. Egypt. Soc Parasitol., 33 (3): 999-1008.

Hoffer B J, Lars O and Palmer M R (1987): Toxic effect of Lead in the developing nervous system: in oculo experimental models. Environ H Pers., 74: 169-175.

Jones TC, Hunt RD and King NW (1997):Veterinary Pathology.6th ED.,Williams and Wlikins A Waverly Company. Baltimore, Philadelphia, London.

Kasperczyk A, Kasperczyk S, Horak S, Ostalowska A, Grucka-Mamczar E, Romuk E, Olejek A and Birker E (2008): Assessment of semen function and lipid peroxidation among lead exposed men. Toxicol Appl Pharmacol., 228:378-384.

Koizumi T and Li Z G (1992): Role of oxidative stress in single dose, cadmium-induced testicular cancer. J Toxicol Environ Health.,37(1):25–36.

Koleva I I, Van Bleek T A, Linssen J P, De-Groot A and Evstatieva L N (2002): Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal., 13(1): 8- 17.

Marchlewicz M, Wiszniewska B, Gonet B, Baranowska-Bosiacka I and Safranow K (2007): Increased lipid peroxidation and ascorbic acid utilization in tesis and epididymis of rats chronically exposed to lead. Biol., 20: 9-13.

Massoud A M, El-Sisi S, Salama O M and Massoud A (2001): Preliminary study on therapeutic efficacy of a new fasciolicidal drug derived from Commiphora molmol (Myrrh). Amer J Trop Med Hyg., 65(2): 96–99.

Mc Gavin M D and Zachary J F (2007): In: Pathologic Basis of Veterinary Disease, 4th ed. 11830 West line Industrial Drive, St.louis, Missouri 63146: Mosby: 654-655.

Murray CA, Clements MP and Lynch MA (1999): Interleukin-1 induces lipid peroxidation and

membrane changes in rat hippocampus: An age-related study. Geront.,45(3):136-142.

Naha N and Manna B (2007): Mechanism of lead induced effects on human spermatozoa after occupational exposure. Kathmandu Univ Med J.,5(1):85-94.

Nehru B and Kanwar SS (2004): N-acetylcysteine exposure on lead-induced lipid peroxidative damage and oxidative defense system in brain regions of rats Biol Trace Elem Res., 101(3):257-264.

Newairy AS, Abdou HM (2009): Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem Toxicol., 47:813-818.

NRC (1994): Nutrition requirement of rat and mice.9th Revised Edition, National Academy press, Washington, DC.USA..

Qureshi S, AL- Harbi M M, Ahmed M M, Raza M, Giangreco A B and Shah A H (1993): Evaluation of the genotoxic cytotoxic, and antitumor properties Commiphora molmol using normal and Ehrlich ascites carcinoma cell- bearing Swiss albino mice. Cancer Chemoth Pharmacol., 33(2): 130– 138.

Reza B, Ali N, Azhdar H, Alireza A, Ali K (2008): Effects of low-level lead exposure on blood pressure and function of the rat isolated heart. Indian J Pharmacol.,40(2):69-72.

Sajitha GR, Jose R, Andrews A, Ajantha KG, Augustine P and Augusti KT(2010): Garlic Oil and Vitamin E Prevent the Adverse Effects of Lead Acetate and Ethanol Separately as well as in Combination in the Drinking Water of Rats. Ind J Clin Biochem ., 25(3):280–288.

Sansar W, Ahboucha S and Gamrani H (2010): Chronic lead intoxication affects glial and neural systems and induces hypoactivity in adult rat. Acta Histochem., doi:10.1016/j.acthis.2010.06.005.

Sansar W, Bouyata M, Ahboucha S and Gamrani H (2011):Effects of chronic lead intoxication on rat serotoninergic system and anxiety behavior. Acta Histochemica, doi:10.1016/j.acthis.2011.02.003.

Scartezzini P and Speroni E (2000): Review on some plants of Indian traditional medicine with antioxidant activity. J Ethnophamacol.,71 (1-2): 23-43.

Sheir Z, Nasr A A, Massoud A M, Salama O M, Badra G A, El- Shennawy H, Tariq M, Aqeel M A, Al-Yahya J, Mossa S, Al Said M S and Parmar N S (1986): Anti- inflammatory activity of Commiphora molmol. Agents Actions 17 (3-4): 381– 382.

Siddique M S, Eddeb F, Mantle D and Mendelow A D (2000): Extracts of Ginkgo biloba and Panax ginseng protect brain proteins from free radical induced oxidative damage in vitro. Acta Neurochir. Suppl., 76: 87-90.

Sidhu P and Nehru B (2004): Lead intoxication: Histological and oxidative damage in rat cerebrum and cerebellum. J Trace Elem Exp Med .,17:45–53.

Tapiero H, Tew KD and Ba GN (2002): Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. Biomed Pharmacother., 56(2): 200- 207.

Teijón C, Olmo R, Blanco D, Romer A, and Teijón J M (2006): Low doses of lead: effect on reproduction and development in rats. Biol Trace Elem Res.,111: 51- 165.

Tipton D A, Lyle B, Babich H and Dabbous M K (2003): In vitro cytotoxic and anti-inflammatory effects of myrrh oil on human gingival fibroblasts and epithelial cells. Toxicology in Vitro., 17(3): 301-310.

Weide R , Engelhart S , Farber H , Kaufmann F, Heymanns J and Koppler H (2003): Severe lead poisoning due to Ayurvedic Indian plant medicine.Dtsch.Med.Wochenschr.,128(46):2418-2420.

Xu Y, Li G, Han C, Sun L, Zhao R and Cui S (2005): Protective effects of Hippophae rhamnoides L. juice on lead- induced neurotoxicity in mice. Biol Pharm Bull., 28(3): 490-494.

Yücebilgiç G, Bilgin R, Tamer L and Tükel S (2003): Effects of lead on Na(+)-K(+)ATPase and Ca(+2) ATPase activities and lipid peroxidation in blood of workers. Int J Toxicol., 22:95-97.

7/6/2011

Some studies on fish deformity in freshwater fish in Egypt

Shawer.. R[1]., Safinaz Gomaa[2], Saleh. W[3]., Soliman. M. K[4]., Khalil R[5] and Mona S. Zaki[6]

[1]General Organization of Veterinary Services, Egypt
[2]National Inst. of Oceanography and Fisheries, Alex . Branch, Egypt
[3]Dept. of Microbiology, Fac. of Agriculture, Cairo Univ., Egypt
[4]Dept.of Poultry and Fish Diseases, Fac. of Vet. Med., Damanhour Univ., Egypt
[5]Dept. poultry and Fish Diseases, Fac. of Vet. Med, Alex. Univ., Egypt
[6] Hydrobiology Dept. ,Veterinary Research Division, National Research Center, Cairo Egypt.
elzahra2002@hotmail.com

Abstract: Fish anomalies are defined as presence of defects in particular parts of the body like vertebral column, mouth and caudal peduncle regions. This study was carried out on 400 fishes showed signs of anomalies (250 cultured, and 150 wild) collected from Alexandria, Kafr El-Sheikh and El-Behera Governorate in the period from June 2006 to May 2008. The clinical signs were in the form of, deformity of vertebral column, mouth and caudal peduncle. Also most fish were emaciated with dark discoloration of the external body. Internally, congestion of some internal organs (spleen, kidney and gills) with enlargement and paleness of liver, watery fluid in abdominal cavity were the main observed signs. Ration analysis from affected farms was carried out to detect calcium deficiency effect on fish deformity which revealed 17 samples had calcium deficiency from total examined 250 by a ratio of 6.8%. Deformed fish were examined for cytogenetic effect which revealed 6 samples have cytogenetic anomaly. Infection with ***Ichthyophonus hoferi*** was 68 samples from total number of 250 cultured fish by a ratio of 27.2% and 30 samples from total number of 150 wild fish by a ratio of 20% . Infestation with ***Myxosoma cerebralis*** was 68 from total examined 250 cultured fish by aratio of 27.2% and 14 samples from total examined 150 wild fish by a ratio of 9.3%. The prevalence of infection with ***Ichthyophonus hoferi*** and ***Myxosoma cerebralis*** were higher in Kafr El-Sheikh governorate followed by El- Behera and Alexandria. The prevalence of infection site with ***Ichthyophonus hoferi*** and ***Myxosoma cerebralis*** were higher in liver followed by kidneys, spleen and intestine respectively Histopathological changes of natural infected fish revealed changes of most affected organs as will as presence of cyst of ***Myxosoma cerebralis*** and spores of ***Ichthyophonus hoferi*** in many organs. Through this study we found that fish anomaliesproved to be affect fish economically either by low production or marketabilityAlso infectious causes of anomalies were of high percentage, so more studies and researches are of important in this situation to make planning for control.
[Shawer.. R., Safinaz Gomaa, Saleh. W., Soliman. M.K., Khalil R and Mona S. Zaki, **Some studies on fish deformity in freshwater fish in Egypt]** Life Science Journal,. 2011; 8(3):415-422] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keyword: fish deformity freshwater .

Introduction

The aquaculture industry has been considered as one of the fastest growing agribusinesses over the past two decades (***USDA2000)***.

Fish anomalies occur in both freshwater and marine fish. They have bad economical effect, as they affect marketability and during processing the fillets might be very soft slimy and strong with some times off odors ***(Reichenbach-klinke, 1965 and Amany 2010) .***

Infectious fish diseases considered as the main cause of reduction of fish farms production and its profitability ***Woo (2004) and Ramaiah (2006) .***

Fish anomalies can be attributed to genetic, pathogenic, environmental and / or nutritional may be involved ***(Noga, 1996 and Easa,1997).***

These anomalies may be genetic, resulting from mutation or recombination either epigenetic, acquired during embryonic development or post embryonic acquired during larval or post larval development***.(Noga, 1996).***

Skeletal anomalies ranging from modification in gill arch structures, fin rays to extreme vertebral deformation have been noted in fish farms polluted habitats ***(Sloof, 1982).***

The type of skeletal deformities differed according to the species of fish and causes ***(Easa, 1997).***

In Egyption study, the prevalence of infection with ***Ichthyophoniosis*** and ***mycobacteriosis*** was 32%. Prevalence was higher in cultured (40%) and female fish (44.7%) than for wild (24%) and males (22.6%). ***(Nadia Abdelghany et al. 2008).***

This study was aimed to throw the light on the causes of fish deformity among wild and cultured fish in Egypt.

2. Materials and Methods

Naturally deformed fish :

A total number of 400 fish (250 cultured *Oreochromis niloticus* and 150 wild fish including 2 *Mugil capito,* 2 *Mugil cephalus,*1 *Bighead carp,* 1 *Gold fish and* 144 *Oreochromis niloticus*) were

collected from 30 farms from different localities at Alexandria, Kafr El-Sheik and El-Behera Governorates (7 farms from Alexandria , 13 farms from Kafr El-Sheik and 10 farms from El-Behera) Ration samples were obtained from each farm for analysis .

The fish samples were collected during the period from June 2006 to May 2008. The body weight of the obtained fish was ranged from 40-150 g.

A total number of 12 fish were obtained a live from farms in El-Behera Governorate for studying the cytogenetic effect.

Clinical examination:

Clinical and postmortem examination of the collected fish were done according to the methods described by *Amlacher (1970) and McVicar*, *(1982)* to detect any clinical abnormalities like (Scoliosis, lordiosis , mouth deformity and loss of tail or fins) and any internal lesions.

Bacteriological and mycological examination:

Samples from affected organs (spleen, liver, kidneys) were used for cultivation of Mycobacterium species on Trypticase soya agar at 32 C for 48 hrs. The suspected colonies was transported to Dorset egg media then incubated at 25C for 2 weeks

Mycological examination was done according to *McVicar (1982)* and Amany (2010) for the fish showing any deformity . Samples were taken by using sterile dissecting needle from the internal organs (liver, kidney, spleen and intestine) and inoculated onto the MEM- 10 and on Sabouraud's dextrose agar with l% bovine serum. The inoculated plates and tubes were incubated at room temperature for 15 day.

Identification of the isolates was done according to the morphological characters including the hyphal growth and multinucleated spores through the microscopical examination of wet mount and stained preparation *McVicar (1982)* . From the nodules appeared in affected organs of naturally infected cases, squash preparations were prepared.

Calcium analysis:

Ration samples were obtained from every farm where fish samples were collected for calcium analysis. The calcium was analyzed according to the method described by *Khoof (1991)* by analytical chemical method and the obtained results were judged according to *N.R.C (1987)*

Diagnosis of *Myxosoma cerebralis*

A fresh fish sample was put between two sterile slides and compressed then examined under light microscope (high power) for refractile bodies (Myxobolus cyst) according to *(Wolf & Markiw 1984)* .

Cytogenetic analysis in deformed fish:

The effect of deformity on the somatic chromosomes of *Oreochromus niloticus* was investigated using micronucleus test (MN) as described by *(Hayashi et al., 1998)*.

X-rays examination X-ray technique was carried out for 10 samples of deformed fish.

Histopathological studies

Fresh specimens were collected from liver, spleen, gills, muscles and vertebral column for histopathological examinations Sections were stained by hematoxyline and eosin (H,E) according to the method described by *Culling (1983)*.

3. Results

Isolation and identification of *Ichthyophonus hoferi*:

The young culture of *Ichthyophonus hoferi* on SDA + 1% bovine serum showed rupture of multinucleated bodies and release of spores through extra material discharge after 9 days while the, culture of *Ichthyophonus hoferi* on MEM-10 PH 7.0 showed hyphae with different sizes and formation of multinucleated bodies after 8 days of incubation. Localization and fixation of multinucleated bodies (ameaboblast) at the end of each hyphae with rupture of some ameoboblasts were noticed .

At pH 3.5 showed starting of hyphal growth after 24 hours post incubation. The hyphae produced many branches, extending of the hyphae to grow and increased in length, migration of cytoplasm to the apex of hypae after 3 days was noticed .

Rounding up of the apices of the hyphae after 7 days was also observed , finally all the hyphae rounding up to form spherical hyphae terminal bodies after 10 days .

In old culture chlamydo-spores formation around the multinucleated bodies extend to the test of stacked hyphae at 3 weeks were seen.

Culture of *Ichthyophonus hoferi* showing foamy white color of hyphal growth on M E M- 10 (Fig 4)

Isolation of *Ichthyophonus hoferi* were from 98 fishes (1 gold fish , 1 big head carp and 96 from *Oreochromus niloticus*)

Calcium analysis :

Analysis of rations obtained from farms in which the fish showed deformities for calcium examination revealed calcium deficiency in 17 samples. The ratio was less than the reported ratio by FAO . According to *N. R . C (1987)* , the ratio was less than 3.8 mg calcium/kg feed considered to be Calcium deficiency

Identification *of Myxosoma cerebralis* :

The examination of gills and vertebral column of affected fish revealed the presence of refractile bodies indicates *Myxosoma cerebralis* spores at different stages in 68 samples . The spores were ovoid in shape contain two polar capsules with sporoplsm of different sizes (Fig5, 6, 7, 8, 9).

Cytogenetic analysis:

The genotoxic examination of collected fish revealed that , six samples monosex *Oreochromus niloticus* gave genotoxic effect for deformity (Fig. 10, 11)

Clinical signs and postmortem lesions of naturally deformed fish due to:-

Ichthyophonus hoferi* infection*:

Clinical signs of the deformed fish were in the form of excessive mucous on the skin, deformity of the vertebral column and congestion of some internal organs with paleness and enlargement of liver in some cases (Fig.2,3,4). There were 68 fish of *Oreochromus niloticus* from the total 250 by ratio of 27.2 % in cultured fish, and 30 fishes from the total 150 with a ratio of 20% in wild fish.

Calcium deficiency:

Emaciation and dwarfism, head size was comparatively larger than head region. The rays spin of fins were soft and easily turned down. Internally paleness of most viscera and watery fluid in the abdominal cavity were the main observed signs (Fig. 5,6) in case of calcium deficiency samples. There were 17 samples of calcium deficiency from the total 250 with a ratio of 6.8 % .

Myxosoma cerebralis* infestation*:

Emaciation, dark discoloration of external body, deformed mouth and body and internally congested liver, spleen, kidney and gills (Fig7, 8,9) were observed in case of deformed fish associated with *Myxosoma cerebralis* infestation . There were 68 samples from the total 250 with artio of 27.2 % in cultured fish and 14 samples from the total 150 with a ratio of 9.3 % in wild fish (Table 4) .

Cytogenetic deformity*:*

The results of signs which recognized in case of fish with genetic deformity were absence of tail , parrot and bull dog mouth , deformed body and internally congestion of internal organs in some cases . (Fig.10, 11)

Fish had mixed infection by both of *Myxosoma cerebralis* and *Ichthyophonus hoferi* found to be deformed in body , mouth or tail .

There were 15 wild fish samples found to be deformed in a ratio of 10 % from wild examined fish and 3.75 % from the total samples .

X-ray examinations

The X-ray of naturally infested fish by *Myxosoma cerebralis* and by genetically cause showed deformity of vertebral column (Fig 12).

Prevalence of deformed fish in different localities:

Mycological, parasitological examination, nutritional analysis and cytogenetic study of the collected fish revealed the prevalence of infection with *Myxosoma cerebralis*, *Ichthyophonus hoferi*, calcium deficiency and genetic defects among the examined fish. The data revealed that prevalence of infection in cultured fish was higher than in wild fish. With respect to the localities, the fish collected from Kafr- El-Sheikh showed higher infection rate than that obtained from Alexandria and El- Behera Governorates.

Seasonal prevalence of deformed fish:

Regarding to the seasonal prevalence of *Ichthyophonus hoferi*, *Myxosoma cerebralis* and calcium deficiency in the examined fish, in the three Governorates the infection recorded in a higher prevalence during Autumn followed by winter and in a lower prevalence during summer in the examined fish.

Twenty random samples were tested for Cytogenetic effect as a cause of deformity and the results revealed six samples were positive for cytogenetic effect as shown in .

Bacteriological examination:

The result of bacteriological examination revealed no bacterial growth on the dorset egg media .

Histopathological altration :

Result of naturally collected samples, proved the presence of resting degenerated spore of *Ichthyphonus hoferi*, in hepatic tissue with severe eosinophilic granular cells (EGCs) infiltration as a tissue reaction against infection. Perivascular severe lymphocytic infiltration with severe hepatic hydropic vacuolation, and diffuse filamentous necrosis were seen. Degenerated spores between muscle bundles with minimal tissue reaction appeared as infiltration of few melanophores adjacent to spores, enlargement and hyperactivation of melanomacrophage centers., multinucleated resting spores surrounded with fibrosis layers of chronic inflammatory cells as chronic tissue reaction were recorded.

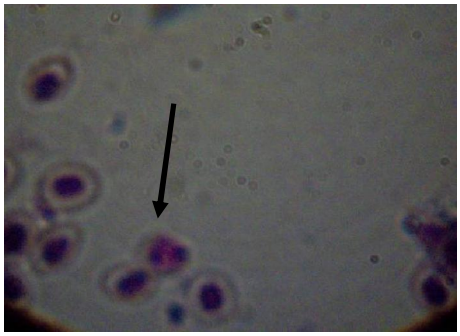


Fig 1 : Peripheral blood erythrocytes of ***Oreochromus. niloticus*** Arrow : Clear cytoplasm without micronucleus.Microscopic magnification X 1000

Fig 2 : ***Oreochromus niloticus*** , showing anomalies due to natural infection with ***Ichthyophonus hoferi***

Fig 3 : ***Oreochromus niloticus*** , showing anomalies due to natural infection with ***Ichthyophonus hoferi***

Fig 4 : ***Big head carp*** showing anomalies due to natural infection with ***Ichthyophonus hoferi***

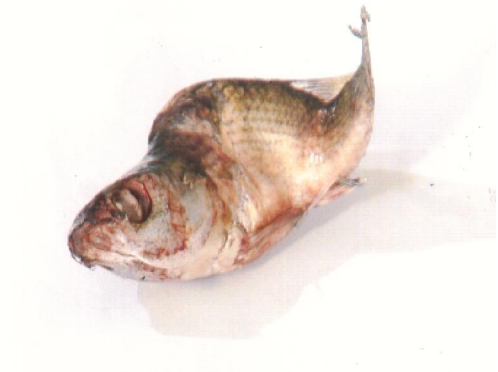


Fig 5 :***Mmugil capito*** showing anomalies due to calcium deficiency

Fig 6: ***Oreochromus niloticus***, showing anomalies due to calcium deficiency

Fig 7 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

Fig 8 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

Fig 9 : ***Oreochromus niloticus*** showing anomalies due to natural infection with ***Myxosoma cerebralis***

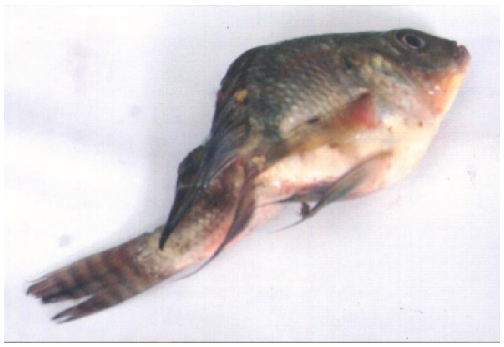


Fig 10 : ***Oreochromus niloticus*** showing anomalies due to cytogenetic effect in the form of mouth deformity .

Fig 11 : ***Oreochromus niloticus*** showing anomalies due to cytogenetic effect in the form of tail loss .

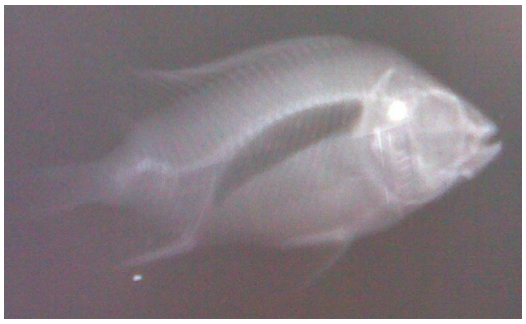


Fig 12 : X-ray film showing deformity of vertebral column of ***Oreochromis niloticus*** fish due to cytogenetic effect

4. Discussion

Lordiosis is one of the most severe deformities developing in reared fish and affect body shape, mostly the posterior abdominal region framed by the anterior and middle base of the pelvic fin. *Shimasaki et al. (2006)*.

The present study was carried out on cultured and wild fish from different species morphologically showed signs of anomalies to investigate the main causes of anomalies via cytogenitical, mycological, bacteriological and parasitological examinations in addition to ration analysis.

The results revealed that the fish anomalies due to either *Myxosoma cerebralis* or *Icthyophonus hoferi* were 136 cases from the total number of 250 cultured fish and 44 from the total number of 150 wild fish . These results proved that fish anomalies may be due to infectious or non infectious causes.

Noga (1996) and *Easa (1997)* mentioned that many factors, genetic, pathogenic, environmental and / or nutritional may be causes of fish anomalies.

The clinical signs of naturally infected fish revealed that the shape of anomalies among fish were varied from deformity of vertebral column to dwarfism, emaciation, deformed mouth or absence of tail. These signs were reported by *McCann and*

Jasper (1972). The differences in the shapes of anomalies may be related to fish species and causes of anomalies as well as severity of infection with the pathogenic agent *(Easa ,1997)* .

Ichthyophoniosis is considered as an important newly recorded disease among cultured tilapia species at different localities in Egypt *(Manal Easa 2002)*.

Spanggaard et al. (1996) and *Mellergaard and Spanggaard (1997)* have reported *Icthyophonus hoferi* as a cause of Ichthyophoniosis and deformity among fish .

During this study, *Icthyophonus hoferi* was isolated from different species of fish. The isolated fungus were submitted to complete morphological and cultural examinations

The young culture of *Icthyophonus hoferi* on SDA+1 %bovine serum showed rupture of multinucleated bodies and release of spores through extra material discharge after 9 days .

Culture on MEM at pH 7 showed hyphae with different sizes and formation of multinucleated bodies after 8 days .

At pH 3.5 showed starting of hyphal growth after 24 hrs post culturing which extend and produce many branches and form spherical hyphae terminal bodies after 10 days .

The growth characters of *Icthyophonus hoferi* were reported by *Ziedan (1999)*

Regarding to the clinical signs and post mortem lesions of *Icthyophonus hoferi* infection were mainly in form of hemorrhage, congestion of body surface with dark discoloration and emaciation. Internally enlargement of liver, congested kidney and gall bladder, heart with abdominal fluid

These may be attributed to the effect of quiescent cyst after settled in the different organs and made tissue damage (*Mcvicar and Mclay*, *1985).*

Deformity is the most important lesion occur due to *Icthyophonus hoferi* these may be due to migration of quiescent cyst (infective stage) to skeletal muscle around vertebral column. The fish try to localize the cyst which usually occurred by surrounding the cyst by connective tissue which replace the myofibers. The end result will be permanent extension of the muscles which lead to moving the vertebrae from its place and finally the deformity occur *(Chauvier and Mortier- Gabet ,1984 and* Amany 2010)

These signs mentioned by *Mcvicar* and *Mclay (1985)* who revealed that the most obvious lesions due to *Icthyophonus hoferi* infection occurred in the white muscle, heart, liver and kidney in herring . In cases of heavy infections normal organ tissue may be replaced by the cyst and C.T. Intern lead to impairing the organ function.

Also *Kocan et al . (2004)* reported that 20% of Yukon river purchased fish were discarded because of muscle tissue damage caused by *Icthyophonus hoferi* .

Myxosoma cerebralis is an important chronic parasitic disease of fish responsible for anomalies especially skeletal deformities *(Wolf et al . 1986)* .

In this study *Myxosoma cerebralis* spores revealed high incidence (21%) from examined (82) samples which found harboring *Myxosoma cerebralis* spores in organs.

This result can confirm the role played by *Myxosoma cerebralis* in fish deformity.

In the present study the signs of deformity resulting from *Myxosoma cerebralis* was mainly in vertebral column and mouth. That comes in accordance with the tropism of myxozoan spores to vertebral column multiplication between vertebrae causing deformity and to the upper and lower jaw resulting in deformity of mouth. In addition the emaciation and ascitis may be related to the chronicity of disease which make depletion of many elements of fish body especially protein which come in contact with that described by *(Wolf et al . 1986)* .

The deformity which occurred in case of *Myxosoma cerebralis* infestation may be attributed to myxozoan spores multiplication make destruction of the cartilaginous elements of the skeleton leading to the chronic phase of the disease characterized by skeletal deformities especially when the infestation occurred in young fish since the calcium precipitation not completed yet *EL Matbouli et al. (1995)*

Also Stoskoph(1993) and *Noga (1996)* reported that trout infected with *Myxosoma cerebralis* developed misshape of body that mainly in the form of deformed caudal area or curvature of the spine with permanently bent and opened mouth

In the present study the prevalence of *Myxosoma cerebralis* infestation was 68 samples from the total number of 250 by ratio of 27.2% in cultured deformed fish and 30 samples from the total number of 150 by a ratio of 9.6% in wild deformed fish. This may refer to the role of over-crowded and other aspects of culture systems which make the infestation more easy than wild fish infestation. Moreover, the chance for infestation is quite high.

Calcium deficiency is considered one of the main causes of fish deformity

During this study the ratio of deformed fish due to calcium deficiency was 6.8% which come in accordance with role of calcium deficiency in fish deformity. The main lesions were softness of fin rays and spins.

The results of ration analysis revealed that Calcium levels were less than 3.8 mg/kg diet . The *N. R. C (1987)* considered Calcium level less than 3.8 mg/kg as Calcium deficiency The negative results of bacteriological, parasitological and mycological examination of deformed fish plus low

level of calcium in ration (less than 3.8 mg / kg diet) gave us support to refer these deformities due to calcium deficiency .

Fish deformity due to calcium deficiency was more prevalent in young ages as This may be concerned to the nature of skeletal apparatus of young fish that mainly is gummy and weak so effect will be more and easy for deformity occurrence *Amlacher (1970).*

Also, in the young fish, the ossification center not closed yet and the precepitation of calcium is week.

Heupel et al . (1999) mentioned that skeletal anomalies are quite common in young fish and may be due to inadequate levels of vitamins, calcium or tryptophan.

Six cases from collected samples were due to genetic causes. These were supported by the genotoxic examination which gave positive results. In the same time the parasitological, bacteriological and mycological examinations were negative which highly supported the causes of genotoxic effect.

This conclusion was supported by *Heupel et al. (1999)* who found that genetic deformities in fish has a minor rank in the risk of aquaculture processes

Kumar and Thakar (2004) concluded that deformity caused alterations in the chromatin conformation of AR promoter and reduction in its accessibility to DN asel in the brain cortex of adult male mice.

Concerning to localities Kafr-elSheikh Governorate showed higher infection rate with *Myxosoma cerebralis*, *Icthyophonus hoferi* and cases of calcium deficiency than that collected from Alexandria and El-Behera. The possible explanation of higher prevalence in Kafr-elsheikh is that the high number of fish farms which present beside each other using the same water drained from the neighbor farms. Moreover they mainly use fish meal for rations and poultry manure which may contain infective stage of *Icthyophonus hoferi* (quiescent cyst) and infected fish used in feed manufacture *(Lauckner 1984)* .

Regarding to the seasonal prevalence of *Myxosoma cerebralis* and *Icthyophonus hoferi* infections in examined fish, the higher prevalence of infections was recorded during the autumn followed by winter, summer and spring seasons respectively.

The possible explanation may be due to the stress effects which caused by low temperature during the winter which interne facilitate the infection with *Icthyophonus hoferi* and infestation with *Myxosoma cerebralis*.

Amany Abdelwahab et al. (2003) reported that *Icthyophonus hoferi* proved to be higher during winter (68.1%) in *Oreochromis niloticus* .

In concerning to distribution of *Icthyophonus hoferi* and *Myxosoma cerebralis* in different organs of infected fish showed higher prevalence of infection in liver , kidney, spleen and intestine respectively

These can be explained that the infection occur mainly in the highly vascular organs with high blood supply. Also, these organs may be the right tropism for these causes .

Faisal et al. (1985) recorded a higher prevalence of infection in liver followed by kidney, spleen and intestine of examined *Claris lazera* infested with *Icthyophonus hoferi* in a rate of 42%, 36%, 14% and 4% respectively

Also *Noga (1996)* and *Ziedan (1999)* stated that the principle infected organs with *Icthyophonus hoferi* are that rich with blood (parenchymatous organs)

Histopathological changes were mainly in the form of viable multinucleated spores beside degenerated one in hepatic and pancreatic tissues with atrophy and necrotic focci , advanced acute cellular swelling of hepatic cells, perivascular severe lymphocytic infiltration with severe hepatic hydropic vacuolation this may be attributed to the effect of *Icthyophonus hoferi* infection .

Also severe eosinophilic granular cells infiltration of hepatopancreas , degenerated spores between muscle bundles with infiltration of melanophores adjacent to spores of *Myxosoma cerebralis* , degeneration of perichondrial tissue of cartilage these changes can be occurred due to *Myxosoma cerebralis* infestation.

These results refers mainly to the tissue reaction against both *Icthyophonus hoferi* and *Myxosoma cerebralis* spores beside the nature of multiplication through target organs lead to signs of inflammation. At the same time presence of the spores through infected tissues insure the infection

Rand (1991) detected the resting and germinating spores surrounded by chronic granulomatous reaction with pleocellular infiltration in the infected organs with *Icthyophonus hoferi* . Moreover the positive results of the roles played by *Icthyophonus hoferi* in deformity of vertebral column were proved by X- ray which done for the infected fish.

Corresponding author
Soliman. M. K
Dept.of Poultry and Fish Diseases, Fac. of Vet. Med., Damanhour Univ., Egypt
elzahra2002@hotmail.com

References

Abdelaziz, R.A. (2010): Some studies on Ichthyophonosis in cultured fresh water fish. M.V.Sc, Facu. of Veterinary Medicine Alexandria University .

Amany, A. A.; Shaheen, A. A.; Ahlam, A. and Manal, E. (2003): Studies on ***Icthyophonosis*** in Nile Tilapia (***Oreochromis niloticus***). 3rd Int. Sci. Conf. Mansoura . 1123 – 1140.

***Amany,M.* (2010):** Studies on some systemic mycotic affections in cultured freshwater fish . Ph.D.V.SC. Fac. of Vet. Med. Alex. Univ.

Amlacher, E. (1970): Textbook of fish diseases. P. 117 – 135. T.F.H. Publication, Neptune, U.S.A.

Cavas, R. C. and Ergene, G. ; Richards, R. S. and Bucke, D. (2005): Further consideration of the hematology of proliferative kidney disease (PKD) in ***Rainbow trout***, ***Salmogairdneri Richardson***. Journal of Fish Disease. 10, 435 – 444.

Chauvier, G. and Mortier, G. J. (1984): Premiers observations du pouvoir pathogene d' ***Ichthyophonus*** pour oiseaux. Annales Parasitologie Paris. 59, 427 – 431.

Culling, C. F. (1983): Handbook of histopathologic and histochemical staining . 3rd Ed . , Buterworth London.

Easa, A.I. (1997): Mycotic infection in cultured fish in Egypt . MVSC Suez Canal Univ.

El-Matbouli, M.; Hoffmann R. W.and Mandok, C. (1995): Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of ***Myxobolus cerebralis*** from epidermis into rainbow trout (***Oncorhynchus mykiss***) cartilage. J Fish Biol 46: 919-935.

Faisal, M.; Torky, H. and Richenbach-Klinike, H. H. (1985): A note on swinging disease among the labyrinth catfish (***clarias lazera***). J. Egy. Vet. Ass. 45, 1: 53 – 60.

Halos, D. S.; Alexandra, H. P.H. and Kocan, R. (2005): ***Ichthyophonus*** in Puget Sound Rockfish from the San Juan Islands Archipelago and Puget Sound, Washnigton, USA. Journal of Aquatic Animal Health. 17: 222 – 227.

Hayashi, R.; Wergeland, H. I.; Aasjord, P. M. and Endersen, C. (1998): Enhanced antibody response in Atlantic salmon ***(Salmo salar L.)*** to ***Aeromonas salmonicida*** cell wall antigens using a bacterin containing 1, 3.

Heupel, M. R.; Wooster, G. A.; Gethell, R. G. and Timmons, M. B. (1999): Mycobacteriosis infections of Tilapia (*Oreochromis niloticus*) in circulation production facility. J. of World Aquaculture Soc. 29, 335 – 339

Khoof, J. D. (1991): Zebrafish as a model host for ***Mycobacterial tuberculosis*** pathogenesis. Acta Trop. 91 (1): 53 – 68.

Kocan, R. and Hershberger, P. (2006): Differences in ***Ichthyophonus*** prevalence and infection severity between upper Yukon River and Tanana River ***Chinook salmon***, ***Oncorhynchus tsawytscha*** (Walbaum), stocks. Journal of Fish Diseases 29 (8): 497.

Kumar, J. D. and Thakur, T. (2004): Zebrafish as a model host for ***Streptococcal*** pathogenesis. Acta Trop. 91 (1): 53 – 68.

Lauckner, G. (1984): Agents: Fungi. In Diseases of Marine Animals. Vol. IV. Part I. Edited by O. Kinne. Biologische Anstalt Hegoland. Hamburg. PP. 89 – 113.

Leah, M. ; Lewis, M .and Santosh, P. L. (2007) : Effects of moderately oxidized dietary lipid and the role of vitamin E on the development of skeletal abnormalities in juvenile Atlantic halibut (***Hippoglossus hippoglossus***) . Aquaculture 262 (2007) 142-155

Manal, A. A. E. ; Nahla, R. H. El-K. and Easa, M. El-S. (1996): Some epizootiological aspects of ***Icthyophonosis*** in freshwater fish from Egypt. 7th Sci. Cong. Vet. Med. Asuit, Egypt., pp. 290 – 305.

McMann, H. and Jasper, R. (1972): Studies on the pathogenesis of bacterial infection in cultured yellow tails, ***Seriola Spp***: Effect of crude exotoxin fractions from cell-free culture on experimental ***Streptococcal*** infection. Journal of Fish Diseases. 5, 471 – 478.

Mcvicar, J. A. (1982) : Health maintainance and principle microbial diseases of cultured fishes, 1999 IOW a state University Press, Ames, Iwa-181 – 209.

McVicar, A. H. and McLay, H. A. (1985): Tissue response of plaice, haddock, and Rainbow trout to the systemic fungus ***Ichthyophonus***. In: Ellis, A. E. (ed.) Fish and Shellfish Pathology. Academic Press. London, pp. 329 – 346.

Mellergaard, S. and Spanggaard, B. (1997): An ***Ichthyophonus hoferi*** epizootic in herring in the North Sea, the Skagerrak, the Kattegat and the Baltic sea. Diseases of aquatic Organisms. Dis Aquat Org. 28: 191 – 199.

Nadia, A.; Abd-El-Ghany, M.S. and El-Ashram, M. M. (2008): Diagnosis of ***Icthyophoniasis*** in ***Oreochromis niloticus*** in Egypt by polymerase chain reaction (PCR). 8thInternational Symposium on Tilapia in Aquaculture. 1307 -1328.

Nadia, A.G. and Hoda, M. Abd-alla (2008): A trail for treatment of ***Icthyophonosis*** in cultured ***Oreochromis niloticus*** using focus and neem plants. 8th International Symposium on Tilapia in aquaculture pp: 1329-1349.

Noga, P. (1996): Response of eosinophilic granule cells of ***Gilthead seabream (Sparusaurata, Teleostei)*** to bacteria and bacterial products. Cell Tissue Res. Jan. 287 (1): 223 – 30.

N. R . C. (1987): Guidline of animal ration content and ration quality : Swiz No. 173 .

Ramaiah, A. (2006): ICES identification Leaflets for Diseases and Parasites of fish and shelfish. Bacteria of marine fish: Leaflet NO. 56.

Rand, T. G. (1991): Studies on the biology of ***Ichthyophonus hoferi*** (Plehn and Muslow, 1911) from Nova yellowtail flounder, ***Limandu ferruginea*** (Storer). Dis. Abst. Int. Pt. B. Sci. and Eng. Vol. 51, No. 11.

Shimasaki,Y. O.; Suguru, I.; Yoshiyuki, I. ; Joon, I .k. ; kei, N.; Hisaya, I.and Tsuneo, H. (2006) : Effect of tributyltin on reproduction in Japanese whiting , ***Sillago japonica*** . Marine Environmental Research 62 (2006) S 245 – S 248

Sloof , T. S. (1982) : bacterial isolation in marine fish . Japan conf. (7) : (3) : (105-108).

Stoskoph, T. A. (1993): Annu. Rev. Immunol. 2, 283-318.

USDA, (2000): Census of aquaculture. National Agricultural Statistics Services, Washington, DC.

Woo, P. T. K. (2004): Fish diseases and disorders. Vol. 3, Viral. Bacterial and Fungal Infections. CABI publishing, London, UK.

Wolf, K. and Markiw, M. E. (1984) : Biology contravenes taxonomy in the ***Myxosoma***: new discoveries show alternation of invertebrate hosts . Science , 225 : 1449 – 1452 .

Ziedan, A. H. H. (1999): Comparative study between hormonal effect and hybridization on the susceptibility of tilapia to infection with ***Ichthyophonus hoferi*** fungus. M. V. Sc. Thesis (Fish Medicine & management) Fac. of Vet. Med., Cairo University.

8/20/2011

Serum Tumor Necrosis Factor Alpha Receptor 2 in Pregnant Females Prior To Pre-Eclampsia

Khaled K. Aly[1], Mohammad AbdelHameed M. Nasr AdDeen[1], Gehane M. Hamed[2] and Nermeen G. Mohamed[1]

[1]Department of Obstetrics and Gynecology, [2] Department of Physiology,
Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT: This study aimed to measure the level of circulating soluble serum TNF-R2 to assess its accuracy as a predictor of pre-eclampsia during early pregnancy. Ninety pregnant women at 22-26 weeks of gestation having criteria making them liable to develop pre-eclampsia attending to the antenatal care and obstetric clinic department, at the faculty of Medicine, Ain Shams University, Maternity Hospital. They included 2 groups of women: Group I (n=45) including women who developed pre-eclampsia; and Group II (n=45) including women who remained normotensive and non-proteinuric till delivery. Both groups were subjected to careful history taking and physical examination, all the cases were subjected to serum collection within 22-26 wks of gestation, the blood was collected for determination of soluble tumor necrosis factor receptor-2 level using ELISA technique. The results of the present study showed that there were no significant differences between women of both groups concerning age and gestational age at recruitment. The mean value of serum level of TNF-R_2 was significantly higher in women who developed pre-eclampsia when compared to women of the control group. In addition, significant increase in mean serum TNF-R_2 was found in women who developed severe preeclampsia. Also, there was a significant positive correlation between serum TNF-R2 and each of systolic, diastolic and mean arterial blood pressure in preeclamptic women.

[Khaled K. Aly, Mohammad AbdelHameed M. Nasr AdDeen, Gehane M. Hamed and Nermeen G. Mohamed, **Serum Tumor Necrosis Factor Alpha Receptor 2 in Pregnant Females Prior To Pre-Eclampsia]** Life Science Journal. 2011; 8(3): 423-432] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keyword: Tumor Necrosis Factor Alpha Receptor 2, Pregnant Females, Pre-Eclampsia.

Introduction

Preeclampsia (PE) affects 5% -7% of healthy nulliparous women and is a major cause of maternal and fetal morbidity and mortality ***(Sibai et al., 2005).*** It is further subclassified into early onset and late onset PE, mild and severe PE, and into a maternal and fetal syndrome ***(Von Dodelszen et al, 2003).*** The syndrome is characterized by hypertension and proteinuria, and a common fetal feature is intrauterine growth restriction ***(ACOG, 2002).***

The pathophysiologic processes that underlie preeclampsia have been proposed by ***Roberts & Gamil (2005)*** to occur in two stages: stage 1; reduced placental perfusion, and stage 2; the maternal clinical syndrome**.** The authors added that placental ischemia/hypoxia causes release of a variety of placental factors that have profound effects on blood flow and arterial pressure regulation ***(Roberts & Gamil, 2005).*** Up till now, there is no effective prevention or treatment strategies for women with this disease , except for early delivery of fetus and placenta.

The initiating event in preeclampsia is thought to be inadequate trophoblastic invasion into the uterine spiral arteries early in gestation that leads to a reduction in uteroplacental perfusion with the potential for placental ischemia ***(Roberts & Gamil, 2005***). Placental ischemia is accompanied with a widespread dysfunction of the maternal vascular endothelium and the release of inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), Interleukin-1 (IL-1) and Interleukin-6 (IL-6); which have been shown to be elevated approximately two folds in women with preeclampsia as well as in placental explants from preeclamptic pregnancies compared to those from normal pregnant women cultured in hypoxic environment ***(Conrad & Benyo, 1997).***

TNF-α exerts its effect by interacting with two receptors, which have distinct biological effects, the 55 KDa TNF- receptor (TNF-R_1) that induces apoptosis and the 75- KDa (TNF-R_2) that induces proliferation through activation of the transcription factor ***(Bazzoni & Beutler, 1996).*** Shedding of the soluble receptors of TNF-α from the cell membranes plays a role in the regulation of TNF-α biological functions by decreasing its availability as a ligand ***(Aderka et al., 1998).***

TNF-R_1 is constitutively expressed in most tissues and seems to be the key mediator of TNF signalling. In contrast, TNF-R_2 is strongly regulated and predominantly found in immune cells indicating that this receptor plays a major role in the lymphoid system . The extracellular domains of both receptors can also be cleaved from the membrane resulting in

the production of soluble TNF (sTNF) receptors ***(Grell et al., 1995).***

Most, but not all studies reported elevated circulating maternal TNF-α ***(Page et al., 2000; Tosun et al., 2010),*** TNF-R_1, and TNF-R_2 concentrations during overt preeclampsia ***(Schipper et al., 2005).*** We could locate only 2 studies reporting elevations in sTNF-R_1 concentrations ***(Williams et al., 1999; Schipper et al., 2005)***, and one evaluating sTNF-R_2, prior to clinical manifestations of the disease ***(Sibai et al., 2009).*** Thus, this study aimed to measure the level of circulating serum sTNF-R_2 to assess its accuracy as a predictor to pre-eclampsia during early pregnancy in those at risk for development of preeclampsia.

2. Materials and Methods

This study was carried out on 600 pregnant women at 22-26 weeks of gestation attending to the antenatal care and obstetric clinic department, at the faculty of Medicine, Ain Shams University, Maternity Hospital during the period between January 2010 to October 2010.

Study size:

An assumption that incidence of preeclampsia is up to 10% in high risk group and to demonstrate a 30% difference in TNF-R2 between normal and preeclamptic pregnant women at a power of 80 and α-error of 0.05 in addition to an estimated drop at rate of 20% , it had mandated us to include 600 cases to be recruited originally to obtain a final number of 90 cases divided in to 2 equal .

Criteria of selected group:

Primigravida, subjected to detailed history with special reference to present, past, family and obstetric histories. Present history: Age, duration of marriage , occupation, residence, special habits; Past history: history of chronic illness especially hypertention, history of previous operations, blood transfusion, drug allergy; Family history: Consangunity, history of twin, history of congenital anomalies, history of preeclampsia, or hypertention with pregnancy; Obstetric history: Primigravid ; Menstrual history: Rhythm, rate, last menstrual period; Contraceptive history: Type of contraception , its duration, its side effects if found.

Complete physical examination:

- Blood pressure measured in a semi-recumbent position with a standard mercury sphygmomanometer with an appropriate sized cuff that will be placed at the level of the heart then two blood pressure recordings 6 hours apart will be obtained.
- Blood pressure is measured regularly every one month till 26 weeks of gestation then every two weeks till 36 weeks of gestation then every one week till delivery.

Methods of study

Serum was collected within 22-26 wks gestation, the blood was collected into plain tubes, centrifuged, and the serum fraction was aliquotted and was stored at -70° C for the future studies. After follow-up, cases developed pre-eclampsia were recorded, after exclusion of drop out cases, 45 cases were selected randomly to be enrolled in the analysis. On the other hand, 45 cases from the normotensive cases were selected randomly to be compared with the analysis.

Laboratory investigations:

Serum level of soluble tumor necrosis factor receptor-2 was measured using ELISA technique, as described by ***Tartaglia & Goeddel (1992).***

Statistical analysis:

The collected data were organized, tabulated and statistically analyzed by computer software using SPSS version 16 ***(Armitage & Berry, 1987)*** for quantitative data, the mean, standard deviation were calculated. Parametric data was compared using student T test, non parametric data was compared using chi square test. Correlation was analysed using Pearson correlation test. The diagnostic value of TNF-R2 was analyzed using sensitivity specifity positive & negative predictive values & accuracy. Area under the curve suggested the value that could be considered to be a cut of value for TNF-R_2. Significance was adopted at $P< 0.05$ for interpretation of the results of tests of significance.

3. Results

After exclusion of drop out cases, 52 cases had developed pre-eclampsia, from which 45 cases (Group I) were selected randomly to be enrolled in the analysis, and 45 women (Group II) including women who remained normotensive and non-proteinuric till delivery.

Both groups were matched concerning age and gestational age at recruitment ($P> 0.05$) (table-1). The mean gestational age at delivery was significantly lower in women who developed pre-eclampsia when compared to women of the normotensive group ($P< 0.001$) (table-1, figure-1).

The distribution of TNF-R_2 values in included cases and controls showed a non-normal (positively-skewed) distribution. Extreme values (3 in group I and 3 in group II) were, therefore, excluded from

analysis, in order to apply parametric tests on TNF-R_2 (Kolmogorov Smirnov test).

The mean serum level of TNF-R_2 was significantly higher in women who developed pre-eclampsia when compared to women of the control group (P< 0.001) (table-2, figure-2).

Receiver operator characteristics (ROC) curve was constructed for serum level of TNF-R_2 as predictor of development pre-eclampsia (figure-3). Area under the curve was 1.0 [95% CI (1.0 to 1.0), p<0.001] (table-3). The best cutoff point of serum TNF-R2 as predictor of developing preeclampsia was ≥ 2866 pg/ml [sensitivity 100%, specificity 100%, PPV 100%, NPV 100%, overall accuracy 100%, LR+ ∞, LR- 0] (table-4).

ROC curve was constructed for serum level of TNF-R_2 as predictor of development pre-eclampsia at gestational age < 34 weeks (figure-4). Area under the curve was 0.991 [95% CI (0.972 to 1.009), p<0.001] (table-5). The best cutoff point of serum TNF-R_2 as predictor of preeclampsia at gestational age < 34 weeks was ≥ 3586.5 pg/ml [sensitivity 100%, specificity 81.2%, PPV 84.1%, NPV 100%, overall accuracy 90.6%, LR+ 5.3, LR- 0] (table-6).

ROC curve was constructed for serum level of TNF-R_2 as predictor of delivery at gestational age < 34 weeks among pre-eclamptic women (figure-5). Area under the curve was 0.943 [95% CI (0.880 to 1.005), p<0.001] (table-7). The best cutoff point of serum TNF-R_2 as predictor of delivery at gestational age < 34 weeks was ≥ 3815 pg/ml [sensitivity 100%, specificity 89.5%, PPV 90%, NPV 100%, overall accuracy 94.4%, LR+ 9, LR- 0] (table-8).

There was a significant positive correlation between serum TNF-R_2 and each of systolic blood pressure (r_s=0.495, p=0.001), diastolic blood pressure (r_s=0.403, p=0.006) and mean arterial blood pressure (r_s=0.445, p=0.002) [table-9, figures 6-8].

The mean serum TNF-R_2 was significantly higher in women with severe pre-eclampsia when compared to women with mild pre-eclampsia (table-10, figure-9).

ROC curve was constructed for serum TNF-R_2 as predictor of severe pre-eclampsia. There was a significant association [AUC = 0.900, 95% CI (0.802 to 0.999), p<0.001] (figure-10, table-11). The best cutoff value of serum TNF-R_2 as predictor severe pre-eclampsia was ≥ 3801 pg/ml (sensitivity 87.5%, specificity 81.1%, PPV 50%, NPV 96.8%, overall accuracy 82.2%, LR+ 4.6, LR- 0.15) [table-12].

Table-1: Difference between the Study Groups concerning Initial Characteristics

	Group I [Pre-eclamptic Women] (n=45)	Group II [Control Women] (n=45)	*P**
Age (Years)			
Range:	17 – 35	17 – 39	>0.05
Mean ± SD:	23.62 ± 4.22	24.18 ± 5.4	NS
Gestational Age at Recruitment (Weeks)			
Range:	22 – 26	22 – 26	>0.05
Mean ± SD:	23.73 ± 1.42	24.24 ± 1.26	NS
Gestational Age at Delivery (Weeks)			
Range:	31 – 39	36 – 40	<0.001
Mean ± SD:	36.71 ± 1.69	38.78 ± 1.24	HS

* Analysis using Independent Student's t-Test NS: non-significant HS: highly significant

Table-2: Difference between the Study Groups concerning Serum Level of TNF-R_2

	Group I [Pre-eclamptic Women] (n=45)	Group II [Control Women] (n=45)	*P**
Serum TNF-R2 (pg/ml)			
Range:	3170 – 4250	1290 – 2562	<0.001
Mean ± SD	3681.19 ± 223.29	1597.69 ± 349.17	HS

* Analysis using Independent student's t-test HS: highly significant

Table-3: Area under the ROC Curve for Serum TNF-R_2 as Predictor of Pre-eclampsia

	AUC	*P*	95% CI
TNF-R2 as Predictor of Developing Pre-eclampsia	1.0	<0.001 HS	1.0 to 1.0

AUC area under the curve HS: highly significant 95% CI: 95% Confidence Interval

Table-4: Diagnostic Accuracy of Serum TNF-R_2 as Predictor of Pre-eclampsia

	Sensitivity	Specificity	PPV	NPV	Overall Accuracy	LR+	LR-
TNF-R2 as Predictor of Developing Pre-eclampsia ≥ 2866 pg/ml	100%	100%	100%	100%	100%	∞	0

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio

Table-5: Area under the ROC Curve for Serum TNF-R2 as Predictor of Developing Pre-eclampsia at Gestational Age < 34 weeks

	AUC	*P*	95% CI
TNF-R2 as Predictor of Developing Pre-eclampsia	0.991	<0.001 HS	0.972 to 1.009

AUC area under the curve HS: highly significant 95% CI: 95% Confidence Interval

Table-6: Diagnostic Accuracy of Serum TNF-R2 as Predictor of Developing Pre-eclampsia at Gestational Age < 34 weeks

	Sensitivity	Specificity	PPV	NPV	Overall Accuracy	LR+	LR-
TNF-R2 as Predictor of Developing Pre-eclampsia ≥ 3586.5 pg/ml	100%	81.2%	84.1%	100%	90.6%	5.3	0

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio

Table-7: Area under the ROC Curve for Serum TNF-R2 as Predictor of Delivery at Gestational Age < 34 weeks among Preeclamptic Group

	AUC	*P*	95% CI
TNF-R2 as Predictor of Delivery < 34 weeks among Pre-eclamptic Women	0.943	<0.001 HS	0.880 to 1.005

AUC area under the curve, HS: highly significant, 95% CI: 95% Confidence Interval

Table-8: Diagnostic Accuracy of Serum TNF-R2 as Predictor of Delivery at Gestational Age < 34 weeks among Preeclamptic Group

	Sensitivity	Specificity	PPV	NPV	Overall Accuracy	LR+	LR-
TNF-R2 as Predictor of Delivery < 34 weeks among Pre-eclamptic Women ≥ 3815 pg/ml	100%	89.5%	90%	100%	94.4%	9	0

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio

Table-9 Correlation between Serum TNF-R2 Level and Systolic Blood Pressure among Pre-eclamptic Group

		Systolic Blood Pressure	Diastolic Blood Pressure	Mean Arterial Blood Pressure
Serum TNF-R2	r_s	0.495	0.403	0.445
	P	0.001 S	0.006 S	0.002 S

r_s Spearman's rank correlation coefficient, S: significant

Table-10: Difference between the Mild and Severe Pre-eclamptic Women concerning Serum Level of TNF-R2

Pre-eclamptic Group	Mild Pre-eclampsia (n=37)	Severe Pre-eclampsia (n=8)	*P**
Serum TNF-R2 (pg/ml) **Range:** **Mean ± SD**	 3170 – 4250 3655.7 ± 223.32	 3710 – 3980 3869.8 ± 108.5	 0.043 S

* Analysis using Independent student's t-test, S: significant

Table-11: Area under the ROC Curve for Serum TNF-R2 as Predictor of Severity of Pre-eclampsia

	AUC	*P*	95% CI
TNF-R2 as Predictor of Severe Pre-eclampsia	0.900	<0.001 HS	0.802 to 0.999

AUC area under the curve, HS: highly significant, 95% CI: 95% Confidence Interval

Table-12: Diagnostic Accuracy of Serum TNF-R2 as Predictor of Severity of Pre-eclampsia

	Sensitivity	specificity	PPV	NPV	Overall Accuracy	LR+	LR-
TNF-R2 as Predictor of Severe Pre-eclampsia ≥ 3801 pg/ml	87.5%	81.1%	50%	96.8%	82.2%	4.6	0.15

PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio

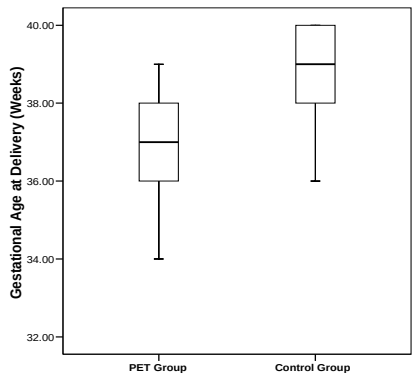


Figure-1: Box-Plot Chart showing Difference between the Study Groups concerning Gestational Age at Delivery.

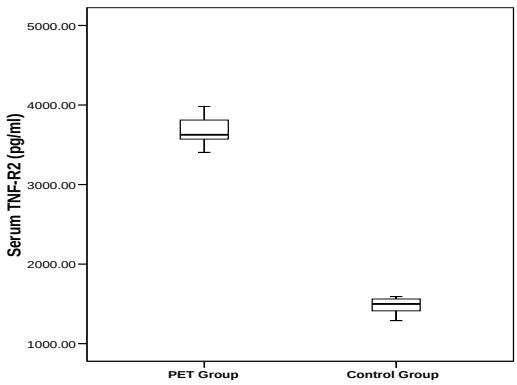


Figure-2: Box-Plot Chart showing Difference between the Study Groups concerning Serum Level of TNF-R2.

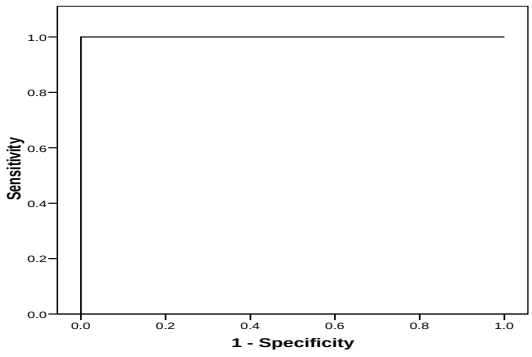


Figure-3: ROC Curve for Serum TNF-R2 as Predictor of Pre-eclampsia.

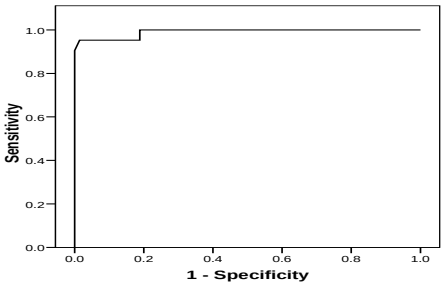


Figure-4: ROC Curve for Serum TNF-R2 as Predictor of Developing Pre-eclampsia at Gestational Age < 34 Weeks

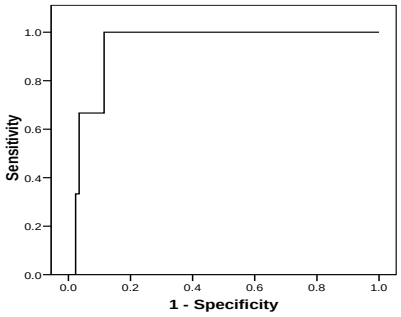
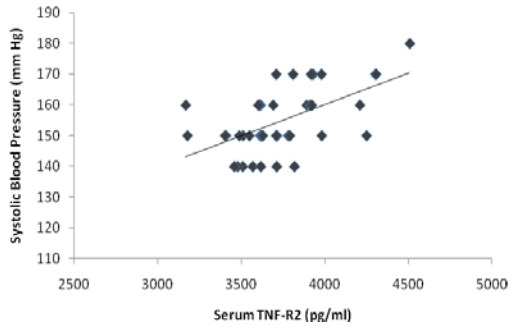
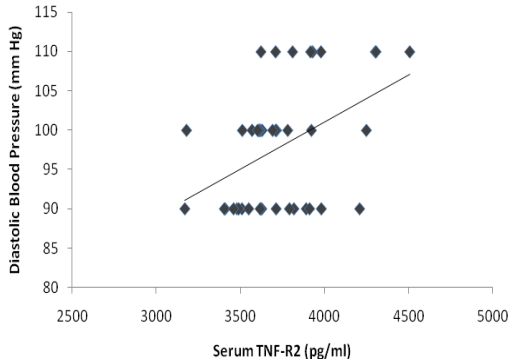


Figure-5: ROC Curve for Serum TNF-R2 as Predictor of Delivery at Gestational Age < 34 Weeks among Pre-eclamptic Women.



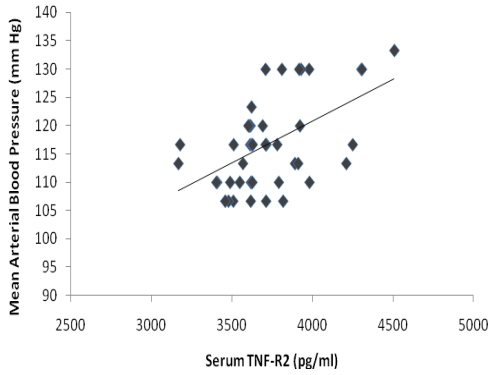
r_s=0.495, p=0.001

Figure-6 Scatter-Plot showing Correlation between Serum TNF-R2 Level and Systolic Blood Pressure among Pre-eclamptic women.



r_s=0.403, p=0.006

Figure-7 Scatter-Plot showing Correlation between Serum TNF-R2 Level and Diastolic Blood Pressure among Pre-eclamptic women.



r_s=0.445, p=0.002

Figure-8 Scatter-Plot showing Correlation between Serum TNF-R2 Level and Mean Arterial Blood Pressure among Pre-eclamptic women.

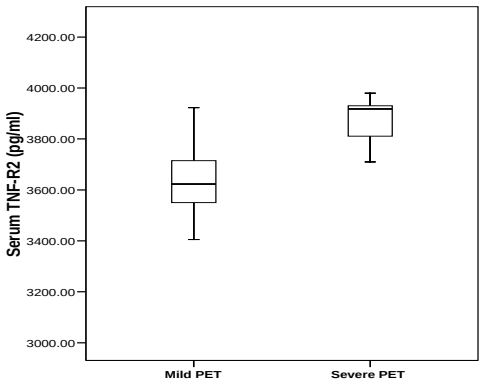


Figure-9: Box-Plot Chart showing Difference between the Mild and Severe Pre-eclamptic Women concerning Serum Level of TNF-R2

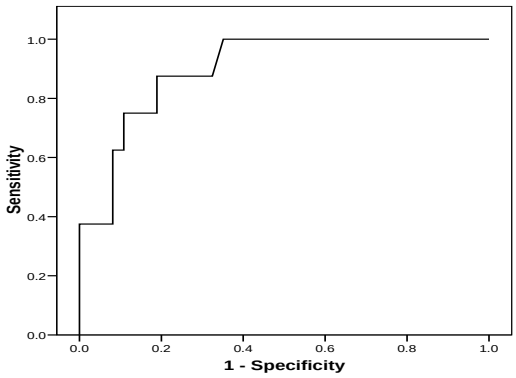


Figure-10: ROC Curve for Serum TNF-R2 as Predictor of Severity of Pre-eclampsia

4. Discussion

The present study revealed a significant elevation in the mean serum TNF-R_2, at 22-26 weeks' gestation in patients subsequently develop preeclampsia at less than 34 weeks gestation. Comparing women who develop severe preeclampsia with those who develop mild preeclampsia, a significant increase in mean serum TNF-R_2 was found in women who develop severe preeclampsia. In addition, there is a significant positive correlation

between serum TNF-R_2 and each of systolic, diastolic and mean arterial blood pressure. These results suggest the presence of an increased systemic inflammatory response early in pregnancy, reflected by increased serum TNF-R_2 concentrations, in patients destined to develop preeclampsia before 34 week's gestation.

Despite the indisputable role of TNF-*a* in the pathophysiology of preeclampsia, this protein is a bad biomarker in blood and its detection is not always reliable because of its high susceptibility to degradation. Thus, TNF-R_2 appears a better biomarker compared with TNF-*a*. It has already demonstrated that the expressions of TNF-R_2 and TNF-*a* were interdependent and follow the same pattern in placentas from women with and without preeclampsia ***(Kharfi et al., 2006).***

The elevated maternal concentrations of serum sTNF-R_2 in the present study, denotes elevation of TNF-alpha which may be a part of the pathogenesis of preeclampsia ***(Kocyigit et al., 2004).*** The strength of this study is that it provides information about sTNF-R_2 concentrations early in pregnancy in a considerable number of women considered at very high risk for development of preeclampsia. The best cutoff point of serum TNF-R_2 as predictor of preeclampsia at gestational age < 34 weeks was ≥ 3586.5 pg/ml [sensitivity 100%, specificity 81.2%, PPV 84.1%, NPV 100%, overall accuracy 90.6%, LR+ 5.3, LR- 0].

The results of the present study came in accordance with ***Sibai et al.*** results; which revealed an increase in serum TNF-R_2 in maternal blood before the clinical manifestation of preeclampsia. Using a cutoff value of the 75th percentile, the authors added that elevated concentrations of TNF-R_2 had poor sensitivity (27.3%) and a limited positive predictive value (23.2%) for subsequent diagnosis of preeclampsia, and so the authors suggested that measurement of s-TNF-R_2 early in pregnancy has limitations ***(Sibai et al., 2009).***

In parallel to the study of Sibai et al., it has been demonstrated increased levels of hydrogen peroxide (H_2O_2) early from the 10th week of gestation in maternal circulation of women with preeclampsia, as well as placentas from preeclamptic women exhibit more H_2O_2 than normotensive women. These results suggested that oxidative stress seen in preeclampsia affects both maternal circulation and the placenta. These findings also proved a potential link between H_2O_2 and TNF-R_2 in preeclampsia, providing two interdependent biomarkers ***(Aris et al., 2009).***

Under normal conditions, cells use their antioxidant defenses, which convert H_2O_2 to oxygen and water, thereby keeping the production of the reactive oxygen species (ROS) system under control. Increased H_2O_2 can result from overproduction of ROS and/or decreased antioxidant capacity. *In vitro* experiments showed that H_2O_2 induces increased release of sTNF-R_2 by cytotrophoblasts, confirming the hypothesis that H_2O_2 is an inductor of sTNF-R_2 synthesis and providing a convincing model of the induction of inflammation by oxidative stress, a phenomenon now called inflammatory stress. Excessive production of ROS and inflammatory factors may occur at certain windows in placental development and in pathologic pregnancies; such as those complicated by preeclampsia and/or intrauterine growth restriction, overpowering antioxidant defenses with deleterious outcome ***(Myatt & Cui, 2004).***

The etiology of preeclampsia is still open to debate, but oxidative stress and inflammation have been shown to be associated with shallow placentation in preeclamptic pregnancies. It is now postulated that these associations may result from a combination of immunologic, environmental, and genetic factors leading to the failure of normal trophoblastic invasion and remodeling of the uterine spiral arteries ***(Kharfi et al., 2003).*** These defects may cause underperfusion, ischemia, and hypoxia in placenta ***(Lyall & Myatt, 2002),*** which is then thought to release in maternal circulation a variety of mediators including proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-*a*), interleukins (IL-1 and IL-6), interferon gamma (INF-χ), and reactive oxygen species (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) ***(Kharfi et al., 2005).*** Such mediators are thought to cause endothelial dysfunction and permanent systemic vasoconstriction characterizing preeclampsia ***(Myatt & Webster, 2009).***

Several lines of evidence support the hypothesis that the ischemic placenta contributes to endothelial cell activation/dysfunction of the maternal circulation by enhancing the synthesis of cytokines such as TNF-α, which has been shown to induced structural as well as functional alterations in endothelial cells, as well as it enhances the formation of a number of endothelial cell substances such as endothelin and reduces acetylcholine-induced vasodilatation ***(Alexander et al., 2001).*** Some studies have found higher endothelin plasma concentrations of ≈2- to 3-fold in women with preeclampsia ***(Dekker et al., 1991).*** Typically, plasma levels of endothelin are highest during the latter stage of the disease, suggesting that endothelin may not be involved in the initiation of preeclampsia, but rather in the progression of disease into a malignant phase ***(Wang et al., 1994).***

It has also reported that the hypertension in response to chronic reductions in utero-placental perfusion pressure in the pregnant rat is associated with significant increases in renal expression of preproendothelin and serum levels of TNF-α ***(LaMarca et al., 2005).***

Moreover, there is considerable evidence linking angiotensin II to the regulation of TNF-α. TNF-α can be increased via angiotensin II–induced angiotensin type-1 receptor activation in endothelial cells (***Arenas et al., 2004)*** and can result in end-organ damage in both the heart ***(Kalra et al., 2002)*** and kidney ***(Ruiz-Ortega et al., 2002).*** In addition, apoptosis by TNF-α was found to require functional angiotensin type-1 receptor activation by angiotensin II in target cells ***(Wang et al., 1999 and Papp et al., 2002).*** Taken together, these and other reports suggest that angiotensin type-1 receptor signaling and the release of TNF-α are closely related. Therefore, in the setting of preeclampsia, excessive activation of the angiotensin type-1 receptor by the autoantibody may lead to deleterious increases in TNF-α, resulting in maternal symptoms ***(Irani et al., 2010).***

Considerable clinical evidence has accumulated that preeclampsia is strongly linked to an imbalance between proangiogenic and antiangiogenic factors in the maternal circulation. Also, plasma and amniotic fluid concentrations as well as placental soluble fms like tyrosine kinase-1 (sFlt-1) mRNA are increased in preeclamptic patients ***(Lam et al., 2005 and Lindheimer & Romero, 2007).*** Moreover, inhibition of vascular endothelial growth factor (VEGF-A) and placenta growth factor (PlGF) action through over-expression of soluble fms-like tyrosine kinase-1 (sFlt-1) causes a pre-eclampsia-like syndrome in pregnant rats was reported in the study of ***Maynard et al. (2003).*** Soluble fms-like tyrosine kinase-1 (sFlt-1) is formed by alternative splicing of the pre-mRNA encoding the full-length-signalling VEGF-R1 receptor, and lacks the cytoplasmic and transmembrane domains ***(Kendall & Thomas, 1993).*** Recently, Herse et al. in their study have reported that increased sFlt-1 may have a predictive value in diagnosing preeclampsia as concentrations seem to increase before manifestation of overt symptoms (e.g., hypertension, proteinuria) ***(Herse et al., 2009).*** These same clinical findings and imbalances in angiogenic factors were found to be reproducible in the rat model via lentiviral overexpression of sFlt-1 ***(Maynard et al., 2003; Gilbert et al., 2007).*** Following this discovery, other investigators revealed that infusion of a proangiogenic factor (e.g., vascular endothelial growth factor) into pregnant rats would attenuate blood pressure elevations and renal damage observed in pregnant rats overexpressing sFlt-1 ***(Li et al., 2007).***

In summary, our results reveal that among women at high risk for preeclampsia, the serum concentrations of TNF-R_2 at 22-26 weeks' gestation are higher in women later diagnosed with preeclampsia than in women not diagnosed with preeclampsia. This finding provides support that level of serum TNF- α receptor 2 should be taken into consideration as a predictor of preeclampsia and may act as a pathophysiological relevant factor in the development of preeclampsia.

In conclusion, the results of the present study should be verified in a prospectively designed study with serial measurements of sTNF-R_2 concentrations. Further studies however are to be recommended that will involve larger study population.

Corresponding author
Gehane M. Hamed
Department of Physiology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

References

ACOG Practice Bulletin No. 33 (2002): Diagnosis and management of preeclampsia. Obstet. Gynecol. 99:159-167.

Aderka D., Sorkine P., Abu-Abid S. (1998): Shedding kinetics of soluble tumor necrosis factor (TNF) receptors after systemic TNF leaking during isolated limb perfusion. Relevance to the pathophysiology of septic shock. J. Clin. Invest. 101: 650-9

Alexander B.T., Rinewalt A.N., Cockrell K.L., Bennett W.A., Granger J.P. (2001): Endothelin-A receptor blockade attenuates the hypertension in response to chronic reductions in uterine perfusion pressure. Hypertension. 37: 485-89.

Arenas I.A., Xu Y., Lopez-Jaramillo P., Davidge S.T. (2004): Angiotensin II-induced MMP-2 release from endothelial cells is mediated by TNF-α. Am. J. Physiol. Cell Physiol. 286:C779–C784.

Aris A., Benali S., Ouellet A., Moutquin J.M., Leblanc S. (2009): Potential biomarkers of preeclampsia: Inverse correlation between hydrogen peroxide and nitric oxide early in maternal circulation and at term in placenta of women with preeclampsia. Placenta 30:342–347.

Armitage P. and Berry G. (1987): Statistical Methods in Medical Reserve in left ventricular hypertrophy. Hypertension 5: 192-197.

Bazzoni F. and Beutler B. (1996): The tumor necrosis factor ligand and receptor families. N. Engl. J. Med. 334:1717-25.

Conrad K.P. and Benyo D.F. (1997): Placental cytokines and the pathogenesis of preeclampsia. Am. J. Reprod. Immunol. 37;240-249.

Conrad K.P., Miles T.M. and Benyo D.F. (1998): Circulating levels of immunoreactive cytokines in women with preeclampsia. Am. J. Reprod. Immunol. 10:102–11

Dekker G.A., Kraayenbrink A.A., Aeeman G.G. and van Kamp G.J. (1991): Increased plasma levels of the novel vasoconstrictor peptide endothelin in severe preeclampsia. Eur. J. Obstet. Gynecol. Reprod. Biol. 40: 215-220.

Gilbert J.S., Babcock S.A. and Granger J.P. (2007): Hypertension produced by reduced uterine perfusion in pregnant rats is associated with increased soluble fms-like tyrosine kinase-1 expression. Hypertension 50:1142–1147.

Grell M., Douni E., Wajant H., Lohden M., Clauss M., Maxeiner B., et al. (1995): The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. Cell 83:793–802.

Herse F., Verlohren S., Wenzel K., Pape J., Muller D.N., Modrow S., Wallukat G., Luft F.C., Redman C.W. and Dechend R. (2009): Prevalence of agonistic autoantibodies against the angiotensin II type 1 receptor and soluble fms-like tyrosine kinase 1 in a gestational age-matched case study. Hypertension 53:393–398.

Irani R.A., Zhang Y., Zhou C.C., Blackwell S.C., Hicks M.J., Ramin S.M., Kellems R.E. and Xia Y. (2010): Autoantibody-Mediated Angiotensin Receptor Activation Contributes to Preeclampsia Through Tumor Necrosis Factor-α Signaling. Hypertension 55: 1246-1253.

Kalra D., Sivasubramanian N. and Mann D.L. (2002): Angiotensin II induces tumor necrosis factor biosynthesis in the adult mammalian heart through a protein kinase C-dependent pathway. Circulation. 105:2198 –2205.

Kendall R.L. and Thomas K.A. (1993): Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc. Natl. Acad. Sci. USA 90: 10705–10709.

Kharfi A., Bureau M., Giguere Y., Moutquin J.M. and Forest J.C. (2006): Dissociation between increased apoptosis and expression of the tumor necrosis factor-alpha system in term placental villi with preeclampsia. Clin. Biochem. 39:646–651.

Kharfi A., Giguere Y., De Grandpre P., Moutquin J.M. and Forest J.C. (2005): Human chorionic gonadotropin (hCG) may be a marker of systemic oxidative stress in normotensive and preeclamptic term pregnancies. Clin. Biochem. 38:717–721.

Kharfi A., Giguere Y., Sapin V., et al. (2003): Trophoblastic remodeling in normal and preeclamptic pregnancies: Implication of cytokines. Clin. Biochem. 36:323–331.

Koçyigit Y., Atamer Y., Atamer A., Tuzcu A. and Akkus Z. (2004): "Changes in serum levels of leptin, cytokines and lipoprotein in pre-eclamptic and normotensive pregnant women," *Gynecological Endocrinology*, vol. 19, no. 5, pp. 267–273.

Lam C., Lim K.H. and Karumanchi S.A. (2005): Circulating angiogenic factors in the pathogenesis and prediction of preeclampsia. Hypertension 46:1077–1085.

LaMarca B.B., Benett W.A., Alexander B.T., Cockrell K.L. and Granger J.P. (2005): Hypertention produced by reductions in uterine perfusion in the pregnant rat role of tumor necrosis factor –α. Hypertention. 46;1022-1025.

Li Z., Zhang Y., Ying Ma J., Kapoun A.M., Shao Q., Kerr I., Lam A., O'Young G., Sannajust F., Stathis P., Schreiner G., Karumanchi S.A., Protter A.A. and Pollitt NS. (2007): Recombinant vascular endothelial growth factor 121 attenuates hypertension and improves kidney damage in a rat model of preeclampsia. Hypertension 50:686–692.

Lindheimer M.D. and Romero R. (2007): Emerging roles of antiangiogenic and angiogenic proteins in pathogenesis and prediction of preeclampsia. Hypertension 50:35–36.

Maynard S.E., Min J.Y., Merchan J., Lim K.H., Li J., Mondal S., Libermann T.A., Morgan J.P., Sellke F.W., Stillman I.E., Epstein F.H., Sukhatme V.P. and Karumanchi S.A. (2003): Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. J. Clin. Invest. 111:649–658.

Myatt L. and Cui X. (2004): Oxidative stress in the placenta. Histochem. Cell Biol. 122:369–382.

Myatt L. and Webster R.P. (2009): Vascular biology of preeclampsia. J. Thromb. Haemost. 7: 375–384.

Papp M., Li X., Zhuang J., Wang R. and Uhal B.D. (2002): Angiotensin receptor subtype AT(1) mediates alveolar epithelial cell apoptosis in response to ANG II. Am. J. Physiol. Lung Cell Mol. Physiol. 282:L713–L718.

Roberts J.M. and Gamil H.S. (2005): Preeclampsia: recent insights. Hypertention. 46:1243-1249.

Schipper E.J.I., Bolte A.C., Schalkwijk C.G., Van Geijn H.P. and Dekker G.A. (2005): TNF- receptor levels in preeclampsia result of a longitudinal study in high-risk women. J. Matern. Fetal Neonatal Med. 18:283-287.

Serin Y.S., Özçelik B., Bapbuõ M., Kýlýç H., Okur D. and Erez R. (2002): Predictive value of tumor necrosis factor alpha (TNF-α) in preeclampsia. Eur. J. Obstet. Gynecol. Reprod. Biol. 100: 143-145.

Sibai B., Dekker G. and Kupfermink M. (2005): Preeclampsia. Lancet February 26; 365(9461):785-99.

Sibai B., Romero R., Klebanoff M.A., et al. (2009): Maternal plasma concentrations of the soluble tumor necrosis factor receptor 2 are increased prior to the diagnosis of preeclampsia. Am. J. Obstet. Gynecol. 200:630.e631–e638.

Tartaglia L.A. and Goeddel D.V. (1992): Two TNF receptors. Immunology Today 13 (5): 151-153.

Tosun M., Celik H., Avci B., Yavuz E., Alper T., and Malatyalio˘glu E. (2010): "Maternal and umbilical serum levels of interleukin-6, interleukin-8, and tumor necrosis factor-α in normal pregnancies and in pregnancies complicated by preeclampsia," *Journal of Maternal-Fetal and Neonatal Medicine*, vol. 23, no. 8, pp. 880–886.

Von Dadelszen P., Magee L.A. and Roberts J.M. (2003): Subclassification of pereeclampsia. Hypertens. Pregnancy. 22:143-148

Wang M.X., Brown M.A., Brown M.L., Carlton M.A., Cario G.M. and Whitworth J.A. (1994): Endothelin excretion in hypertensive pregnancy: relationship to glomerular filtration rate, blood pressure and sodium excretion. Am. J. Hypertension 7: 308-313.

Wang R., Zagariya A., Ibarra-Sunga O., Gidea C., Ang E., Deshmukh S., Chaudhary G., Baraboutis J., Filippatos G. and Uhal B.D. (1999): Angiotensin II induces apoptosis in human and rat alveolar epithelial cells. Am. J. Physiol. 276:L885–L889.

8/23/2011

Gastroprotective Effect of *Cordia Myxa L.* Fruit Extract against Indomethacin-Induced Gastric Ulceration in Rats

Inas, Z.A. Abdallah[*a], Hala, A.H. Khattab[a] and Gehan, H. Heeba[b]
[a] Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt
[b] Pharmacology and Toxicology Department, Faculty of Pharmacy, Minia University, Egypt
[*]izaa2011@hotmail.com

Abstract: Gastric ulcer is one of the most serious diseases in the world. Although there are many drugs used for the treatment of gastric ulcer, most of these produce several adverse reactions. This study investigated the protective effects of Assyrian plum **(***Cordia myxa L.*) fruit extract (CME) against indomethacin-induced gastric ulcer in rats. Gastric ulceration was induced by a single intraperitoneal injection of indomethacin (30 mg/kg $^{-1}$ b.wt.). CME was administered orally at a dose of 125 mg/kg b.wt. and ranitidine (RAN), a reference drug, at a dose of 50 mg/kg b.wt. two weeks prior to indomethacin injection. Pretreatment with CME produced significant reduction in gastric mucosal lesions (U.I.), malondialdehyde (MDA), and serum tumor necrosis factor (TNFα) associated with significant increase in gastric juice mucin content and gastric mucosal catalase (CAT), nitric oxide (NO), and prostaglandin E_2 (PGE_2) levels. A similar increase in mucin content, NO and PGE_2 was not observed with RAN although it generated a preventive index of 75.9%. RAN significantly increased pH value and decreased pepcin activity, and gastric juice free and total acidity. Histological studies of stomach mucosa confirmed these results. Stomach of rats administrated with RAN showed leukocytic infiltration in submucosal layer. Meanwhile, stomach of rats administrated CME either alone or with RAN showed no histopathological changes. CME can protect indometacin-induced gastric ulceration due to its antioxidative and mucin enhancing properties. The protection afforded by co-administration of CME and RAN was found to be better than that of RAN alone. Results of the present study suggest that RAN should be used together with CME for better gastroprotective effect as well as to reduce H_2 antagonists drugs adverse effects.
[Inas, Z.A. Abdallah, Hala, A.H. Khattab and Gehan, H. Heeba. Gastroprotective Effect of *Cordia Myxa L.* Fruit Extract against Indomethacin-Induced Gastric Ulceration in Rats**]** Life Science Journal, 2011; 8(3): 433-445] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: *Cordia myxa* Extract, Gastroprotective, Indomethacin, Ranitidine.

1. Introduction:

Gasrtic ulcer is a major health hazard in terms of both morbidity and mortality (Chaturvedi et al., 2007). Untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding (Tortora and Grabowski, 2003). The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepcin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factors) (Repetto and Llesuy, 2002). According to Malyshenko et al. (2005) and Kim (2008), some other factors, such as inadequate dietary habits, cigarette smoking, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer. Several pharmaceutical products have been employed for the treatment of gastroduodenal ulcer and peptic diseases, resulting in decreasing mortality and morbidity rates, but they are not completely effective and produce many adverse effects (Rates, 2001).

Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H_2 receptor antagonists, antacids and to proton pump inhibitors (Wallace and Granger, 1996). A widely used drug associated with rare idiosyncratic hepatotoxicity is the histamine H_2 receptor antagonist ranitidine (RAN) (Bourdet et al., 2005). It is available over the counter for oral administration or by prescription for parenteral administration for treatment of gastric ulcers, hypersecretory diseases, and gastroesophageal reflux disease. Idiosyncratic RAN hepatotoxicity occurs in few people taking the drug (Fisher and Le Couteur, 2001). Most liver reactions are mild and reversible; however, extensive liver damage have occurred in individuals undergoing RAN therapy (Cherqui et al., 1989 and Ribeiro et al., 2000).

In recent years, there has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants (Rates, 2001 and Schmeda-Hirschmann and Yesilada, 2005). Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer (Alkofahi and Atta, 1999 and Schmeda-Hirschmann and Yesilada, 2005).

Cordia myxa fruit (family: *Boraginaceae*), is popularly used for the treatment of chest and urinary infections, and as an anthelminthic, diuretic, astringent, demulcent and expectorant agent (Alami and Macksad, 1974). Moreover, it has been reported that leaf extracts of certain species of *Cordia* such as *C. myxa*, *C. francisci,* and *C. serratifolia* have significant analgesic, anti-inflammatory, and antiarthritic activities in rats (Ficarra et al., 1995). The anti-inflammatory properties of the *C. myxa* fruit preparation in the treatment of experimental colitis have been demonstrated by Al Awadi et al. (2001). However, there are few data about its pharmacological effects on gastrointestinal system as

well as about its possible toxic properties and chemical composition. This promoted us to investigate the effect of *C. myxa* fruit extract (CME) on indomethacin induced gastric ulcer in rats as well as to evaluate its acute toxicity and qualitative phytochemical profile. RAN was taken as a reference drug with which the antiulcer potential of CME was compared, and the combined gastroprotective effect of CME with RAN was also evaluated**.**

2. Materials and Methods:

2.1. Drugs and chemicals:

Indomethacin was obtained from Sigma Chemical Co. (St. Louis, MO, USA), and was suspended in 1% aqueous solution of Tween 80. Ranitidine was kindly provided by GlaxoSmithKline, Egypt. Thiobarbituric acid,1,1,3,3-tetramethoxy-propane, trichloroacetic acid, ethanol absolute and diethyl ether were obtained from Sigma-Aldrich (USA). All drug solutions and suspensions were freshly prepared. Casein (>85% protein) was obtained from Misr Scientific Co. Dokki, Giza, Egypt. Cellulose and D-L methionine were purchased from Morgan Co. Cairo, Egypt. Minerals and vitamins constituent and sucrose were obtained from El-Gomhoriya Pharm. and Chem. Ind. Co. Cairo, Egypt. Corn oil was obtained from the local market. Corn starch was obtained from Starch and Glucose Co. Helwan, Cairo, Egypt.

2.2.Plant material:

Ripe Assyrian plum (*C. myxa L.*) fruits were collected from the farm of Medicinal and Aromatic Plants Research branch, Al Kanater Al Khayria, Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt under the supervision of **Prof. Dr. Said G. I. Soliman**, Professor of Medicinal and Aromatic Plants and branch manager.

2.3.Preparation of extract:

The *C. myxa* fruits were cleaned carefully and washed several times with running tap water. The ethanolic extract was prepared by soaking 500 g of *C. myxa* fruits in 1 liter of a solvent composed of 700 ml ethanol 95% and 300 ml distilled water, with daily shaking for 2 days and kept in a refrigerator. The infusion was filtered by a piece of double layer gauze and fresh solvent was then added to the plant materials. The combined filtrates were evaporated using a rotary evaporated apparatus (Swizerland) attached with vacuum pump then centrifuged at 3000 rpm for 10 min (Muralidharan and Srikanth, 2009). 100 gm of plant contain 43 gm husks and 40 gm seeds and 17 gm extract

2.4. Phytochemical screening:

The crude *C. myxa* fruit extract (CME) was analyzed for glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, tannins, phenolic acids, gums and mucilage using standard procedures of analysis (Evans, 2002 and Harborne, 2007).

2.5. Determination of acute toxicity of CME:

Acute toxicity of CME was performed as described by Souza Brito (1995). The male mice were divided into four groups of ten animals each. A group received saline (10 mL/kg) by gavage and kept as normal control. A single dose of CME was administered orally to group 2, 3 and 4 at doses of 50, 500, and 5000 mg/kg b.wt., respectively. The mortality, measured body weight and behavioral screening were recorded daily during 14 days after the extract administration.

2.6. Experimental Animals:

Forty male albino wistar rats weighing 190–200 g were obtained from the animal house of Faculty of Agriculture, Minia University, El-Minia, Egypt. They were used after acclimatization for a period of 1 week to animal house conditions and had free access to food and water. Basal diet was formulated to contain 14% casein, 10% sucrose, 5% corn oil, 5% fiber (cellulose), 3.5% mineral mixture, 1% vitamin mixture, 0.25% choline chloride, 0.3 % D-L methionine , and 60.95% corn starch (Reeves et al.,1993). Protocol was approved by the Local Animal Care Committee at Minia University (Egypt), and all the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals. The experiment was conducted at Faculty of Pharmacy, Minia University, Egypt.

2.7. Experimental design:

Rats were fasted for 24 h prior to the experiment in mesh-bottomed cages to minimize coprophagia but allowed free access to water except for the last hour before the experiment. All experiments were performed during the same time of the day to avoid diurnal variations of putative regulators of gastric functions. The animals were randomly classified into 5 groups (8 rats per each): (1) Control group; in which animals were left freely wandering in their cages for 3 h after receiving a single i.p. injection of 1% aqueous solution of Tween 80 (vehicle of indomethacin). (2) IND group; in which gastric ulceration was induced by i.p. injection of a single dose of 30 mg/ kg $^{-1}$ b. wt. indomethacin (IND). (3) IND + RAN group; in which animals pretreated with 50 mg/kg b. wt. RAN orally, two weeks before indomethacin administration. (4) IND + CME group; in which animals pretreated with 125 mg/kg b.wt. CME orally two weeks before indomethacin administration. (5) IND + CME + RAN group; in which animals concurrently pretreated with CME and RAN orally two weeks before indomethacin administration. The doses of CME and RAN used in this study were chosen according to Ficarra et al. (1995) and Prakash et al. (2007), respectively. Gastric ulceration was induced by intraperitoneal administration of indomethacin (30 mg/ kg $^{-1}$ b.wt., suspended in 1% aqueous solution of Tween 80) immediately after pyloric ligation (Khattab et al., 2001).

2.8. Pyloric ligation:

Pyloric ligation was carried out in each animal before indomethacin administration to collect gastric juice under light ether anesthesia, a mid-line abdominal incision was performed; the pyloric portion of the stomach was gently mobilized and carefully ligated with

a silk ligature around the pyloric sphincter taking care not to interfere with gastric blood supply. The abdominal incision was sutured and the animals were allowed to recover from anesthesia (Alumets et al., 1982).

2.9. Assessment of gastric mucosal lesions:

The animals were killed with an ether overdose three h after indomethacin administration. Each stomach was removed and opened along the greater curvature, and the gastric juice was collected. The stomachs were washed with ice-cold saline and examined for macroscopical mucosal lesions by an observer unaware of the treatment protocol. The gastric mucosal lesions were expressed in terms of ulcer index (U.I.) according to Peskar et al. (2002) which depends on the calculation of a lesion index by using of a 0-3 scoring system based on the severity of each lesion. The severity factor was defined according to the length of the lesions. Severity factor 0 = no lesions; 1 = lesions < 1 mm length ; 2 = lesions 2-4 mm length and 3 = lesions > 4 mm length. The lesions score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor. The U.I. for each group was taken as the mean lesion score of all the rats in that group. The preventive index (P.I.) of a given drug was calculated by the equation of Hano et al. (1976).

$$P.I. = \frac{\text{U.I. of IND group - U.I. of pretreated group}}{\text{U.I. of IND group}} \times 100$$

2.10. Analysis of gastric juice:

Gastric juice collected from each animal was centrifuged at 3000 rpm for 10 min to remove any solid debris and the volume of the supernatant was measured. The supernatant was then assayed for the pH (Moore, 1968), pepsin activity (Sanyal et al., 1971) and mucin concentration (Winzler, 1955). Free and total acid outputs were calculated by multiplying gastric juice volume by the measured free and total acid concentrations, respectively (Hara et al., 1991 and Feldman, 1998).

2.11. Biochemical analysis of gastric mucosa.

Gastric mucosal malondialdehyde (MDA) level was measured by the method of Mihara and Uchiyama (1978). Nitric oxide (NO) content was determined as total nitrites/nitrates, the stable degradation products of NO (Sastry et al., 2002). Catalase activity was estimated based on the method of Aebi (1984). Prostaglandin E_2 (PGE_2) assay was performed with PGE_2 enzyme immunoassay kit (R&D Systems, Inc., MN, USA) according to supplier's instructions. Serum tumor necrosis factor (TNF-α) was determined by enzyme-linked immunosorbent assay (ELISA) using rat TNF-α assay kit (Biosource, USA) as previously described by Su et al. (2002).

2.12. Histopathological studies:

The stomach from all groups were removed rapidly, opened along the greater curvature, and thoroughly rinsed with ice-cold saline. After recording the ulcers produced in the stomach, a longitudinal section of the gastric tissue was taken from the anterior part of the stomach and fixed in a 10% formalin solution. After 24 h of fixation followed by embedding in a paraffin block, it was cut into sections of 5 micron onto a glass slide and stained with hematoxylin-eosin for histological assessment of the gastric mucosa according to Bancroft et al. (1996).

2.13. Statistical analysis:

Data are expressed as Mean ± S.E.M., with a value of $p<0.05$ considered statistically significant. Statistical evaluation was performed by ANOVA followed by the Student's t-test. All analysis was made with the statistical software Microcal Origin (Version 6, Microcal Software Inc., Northampton, USA).

3. Results:

3.1. Phytochemical screening:

The Preliminary Phytochemical screening carried out on *C. myxa* fruit extract revealed the presence of phytoconstituents such as glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenolic acids, gums and mucilage (Table 1).

Table (1): Preliminary phytochemicals screening of *C. myxa* fruit extract .

Phytochemical Tests	Results
Test for glycosides	+
Test for flavonoids	+
Test for sterols	+
Test for saponins	+
Test for terpenoids	+
Test for alkaloids	+
Test for tannins	-
Test for phenolic acids	+
Test for gums and mucilage	+

+ : presence of the constituents.
- : Absence of the constituents.

3.2. Acute toxicity results:

The extract did not produce any toxic symptoms of mortality up to the dose level of 5000 mg/kg body weight in the treated animals, and hence it was considered safe for further pharmacological screening.

3.3. Effect of CME on indomethacin induced gastric lesions:

From Table (2), indomethacin administration caused a remarkably high ulcer index (21.6±2.01) when compared to control group. Pretreatment with RAN or CME offered significant protection against indomethacin-induced gastric ulcer in the experimental rats. CME reduced ulcer index to 7.5 ±0.44 showing 65.3% prevention whereas RAN reduced ulcer index to 5.2±0.31 showing 75.9% prevention. Pretreatment of rats with both RAN and CME produced higher gastroprotective effect as compared to RAN alone, they decreased the ulcer index to 2.1±0.15 providing 90.3 % prevention against gastric mucosal injury.

Table (2): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on ulcer index and preventive index in indomethacin (IND)-induced gastric ulcer in rats.

Groups	Ulcer index	Preventive index
Control	1.1 ± 0.01	-
IND	21.6 ± 2.01 [a]	-
IND + RAN	5.2 ± 0.31 [a,b]	75.9 %
IND + CME	7.5 ± 0.44 [a,b]	65.3 %
IND + CME + RAN	2.1 ± 0.15 [a,b,c]	90.3 %

Data represent the mean ± S.E.M. of observations from 8 rats.
[a] Significantly different from control group at P < 0.05.
[b] Significantly different from IND group at P < 0.05.
[c] Significantly different from IND+RAN group at P < 0.05.

3.4. Effect of CME on the gastric juice analysis:

Table (3) and (4) indicate the effect of CME on the gastric juice analysis. Table (3) shows that indomethacin administration caused significant decrease in pH value associated with significant increase in gastric juice free and total acidity. Pretreatment with CME produced insignificant changes in pH value and free and total acidity as compared to indomethacin group. Pretreatment with RAN either alone or with CME produced significant increase in pH value and significant decrease in free and total acidity when compared to indomethacin group. Co-administration of RAN and CME showed more potent efficacy in reduction of free and total acid output.

Table (4) shows that indomethacin administration caused significant increase in gastric juice pepcin activity associated with significant reduction in gastric juice mucin content. Pretreatment with CME produced insignificant change in pepcin activity and significant increase in mucin content as compared with indomethacin group. Pretreatment with RAN either alone or with CME significantly decreased the gastric juice pepcin activity as compared to indomethacin group. Meanwhile, pretreatment with RAN did not produce any significant change in the mucin content, co-administration of RAN and CME significantly increased mucin content as compared to indomethacin group and RAN pretreated group.

Table (3): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on pH, free and total acid output of the gastric juice in indomethacin (IND)-induced gastric ulcer in rats.

Groups	pH	Gastric juice	
		Free acid output (µEq/3hours)	**Total acid output** (µEq/3hours)
Control	2.88 ± 0.12	35.5 ± 3.1	49.3 ± 2.4
IND	1.62 ± 0.06 [a]	129.8 ± 12.1 [a]	152.6 ± 11.9 [a]
IND + RAN	2.67 ± 0.09 [b]	43.4 ± 4.2 [b]	70.5 ± 6.1 [a,b]
IND + CME	1.83 ± 0.15 [a,c]	110.5 ± 9.5 [a,c]	138.6 ± 10.2 [a,c]
IND + CME + RAN	2.71 ± 0.24 [b]	41.6 ± 3.8 [b]	61.3 ± 5.1 [b]

Data represent the mean ± S.E.M. of observations from 8 rats.
[a] Significantly different from control group at P < 0.05.
[b] Significantly different from IND group at P < 0.05.
[c] Significantly different from IND+RAN group at P < 0.05.

Table (4): Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on pepsin activity and mucin content of the gastric juice in indomethacin (IND)-induced gastric ulcer in rats.

Groups	Gastric juice	
	Pepsin activity (µg/ml tyrosine)	**Mucin content** (mg % hexose)
Control	189 ± 8.2	168.1 ± 11.4
IND	236 ± 18.9 [a]	93.7 ± 7.5 [a]
IND + RAN	184 ± 16.1 [b]	101.2 ± 9.3 [a]
IND + CME	219 ± 20.5 [a,c]	141.4 ± 13.2 [a,b,c]
IND + CME + RAN	180 ± 13.8 [b]	144.1 ± 12.6 [a,b,c]

Data represent the mean ± S.E.M. of observations from 8 rats.
[a] Significantly different from control group at P < 0.05.
[b] Significantly different from IND group at P < 0.05.
[c] Significantly different from IND+RAN group at P < 0.05.

3.5. Effect of CME on the gastric mucosal lipid peroxides (MDA):

As shown in Fig. (1), administration of indomethacin significantly elevated the gastric mucosal MDA concentration to about two folds the value observed for the control group, reaching 113.8 ± 11.4 nmol/g wet tissue as compared to 54.5 ± 3.12 nmol/g wet tissue for control group. Interestingly, all the pretreatments which used produced significant reduction in gastric mucosal MDA concentration as compared to indomethacin group. CME pretreatment reduced the gastric mucosal MDA concentration to 57.3 ± 4.05 nmol/g wet tissue. While, RAN pretreatment reduced the gastric mucosal MDA concentration to 65.4 ± 3.07 nmol/g wet tissue, co-administration of RAN and CME significantly augmented the decrease of gastric mucosal MDA concentration to 41.6 ± 3.18 nmol/g wet tissue.

3.6. Effect of CME on the gastric mucosal catalase (CAT) activity:

As shown in Fig.(2), administration of indomethacin significantly reduced the gastric mucosal catalase activity, reaching 3.46 ± 0.31 H_2O_2/g tissue/min. as compared to 5.65 ± 0.42 H_2O_2/g tissue/min. for control group. All the pretreatments which used induced significant increase in gastric mucosal catalase activity as compared to indomethacin group. CME pretreatment increased the gastric mucosal catalase activity to 7.51 ± 0.62 H_2O_2/g tissue/min. While, RAN pretreatment increased the gastric mucosal catalase activity to 6.21 ± 0.38 H_2O_2/g tissue/min., co-administration of RAN and CME significantly increased gastric mucosal catalase activity to 8.77 ± 0.55 H_2O_2/g tissue/min.

3.7. Effect of CME on the gastric mucosal nitrites/nitrates content:

In indomethacin group, gastric mucosal nitrites/nitrates content was significantly reduced from 325 ± 15.1 to 193 ± 11.5 nmol/g wet tissue. Pretreatment with RAN failed to alter significantly the gastric mucosal nitrites/nitrates content (207 ± 18.511.5) nmol/g wet tissue) when compared to indomethacin group. Meanwhile, pretreatment of CME significantly increased gastric mucosal nitrites/nitrates content to 298 ± 12.4 *nmol*/g wet tissue vs. indomethacin group. More increase in the gastric mucosal nitrites/nitrates content (334 ± 28.6 nmol/g wet tissue) was observed when CME co-administered with RAN (Fig. 3).

3.8. Effect of CME on the gastric mucosal prostaglandin E_2 (PGE_2) level:

The synthesis of mucosal PGE_2 was markedly suppressed by indomethacin compared to that in the normal rats (Fig. 4). However, the mucosal synthesis of PGE2 in the CME- pretreated rats increased significantly compared to that of indomethacion group. The effect of pretreatment with CME either alone or with RAN was significantly better than that of RAN alone, which increased the PGE_2 level marginally.

3.9. Effect of CME on serum level of proinflammatory cytokine (TNFα):

Serum level of pro-inflammatory cytokine (TNFα) in ulcerated rats upon administration of CME is presented in Fig.(5). Compared with control group, the serum level of TNF-α was significantly increased in indomethacin group. Interestingly, all the pretreatments used had a significant suppression effect on serum level of TNF-α when compared to indomethacin group. The effect of pretreatment with both RAN and CME was significantly better than that of RAN alone.

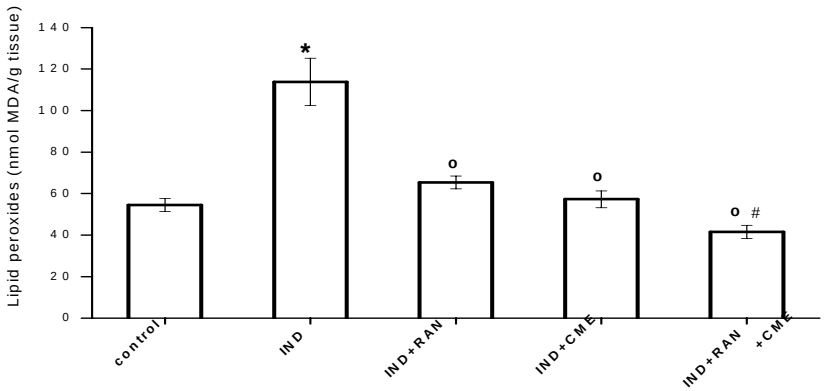


Figure 1: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal lipid peroxides in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean ± S.E.M. of observations from 8 rats.

* Significantly different from control group at $P < 0.05$.
° Significantly different from IND group at $P < 0.05$.
Significantly different from IND+RAN group at $P < 0.05$.

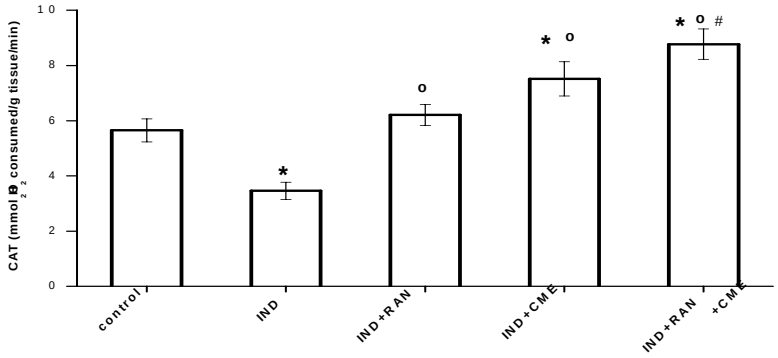


Figure 2: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal catalase activity in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean ± S.E.M. of observations from 8 rats.

* Significantly different from control group at P < 0.05.
° Significantly different from IND group at P < 0.05.
Significantly different from IND+RAN group at P < 0.05.

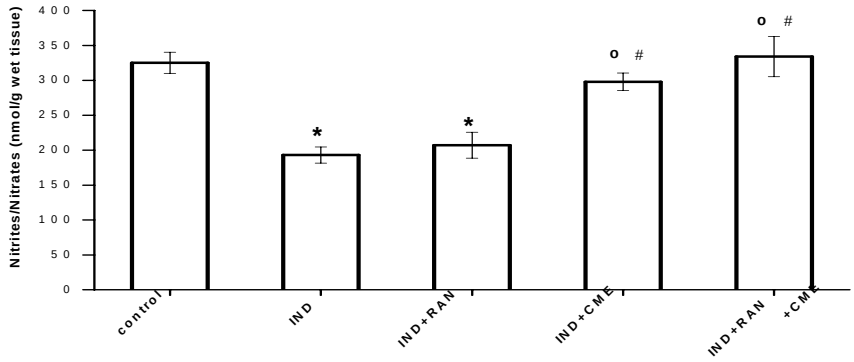


Figure 3: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal nitrites/nitrates in indomethacin(IND)-induced gastric ulcer in rats. Data represent the mean ± S.E.M. of observations from 8 rats.

* Significantly different from control group at P < 0.05.
° Significantly different from IND group at P < 0.05.
Significantly different from IND+RAN group at P < 0.05.

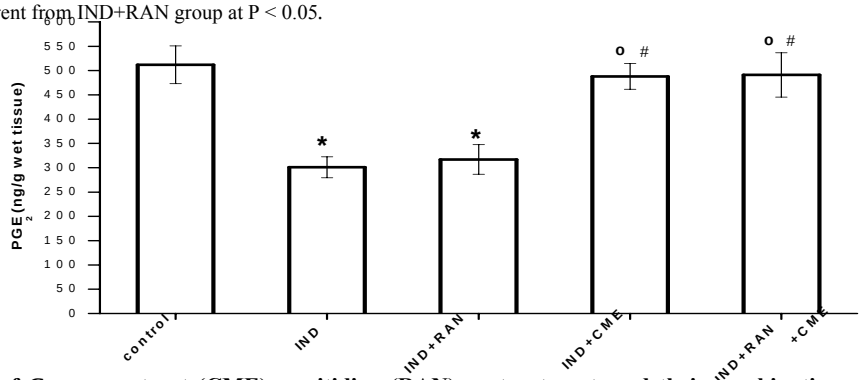


Figure 4: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on gastric mucosal prostaglandin E_2 in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean ± S.E.M. of observations from 8 rats.

* Significantly different from control group at P < 0.05.
° Significantly different from IND group at P < 0.05.
Significantly different from IND+RAN group at P < 0.05.

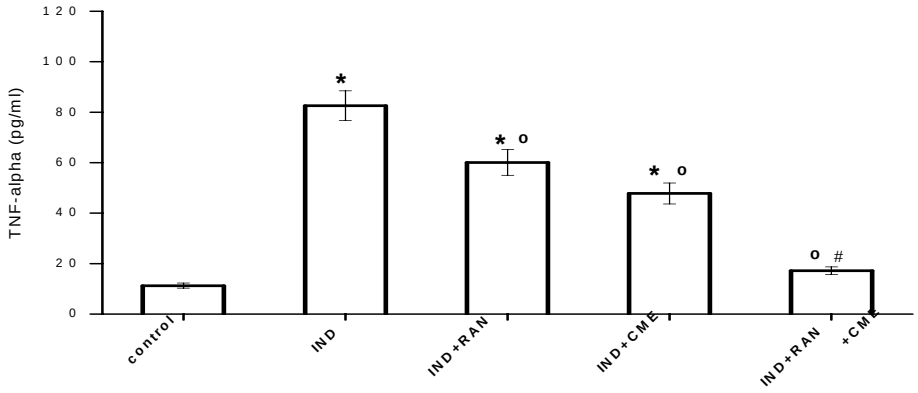


Figure 5: Effect of *C. myxa* extract (CME), ranitidine (RAN) pretreatment, and their combination on serum TNF-α in indomethacin (IND)-induced gastric ulcer in rats. Data represent the mean ± S.E.M. of observations from 8 rats.

* Significantly different from control group at P < 0.05.
° Significantly different from IND group at P < 0.05.
Significantly different from IND+RAN group at P < 0.05.

3.10. Histopathological results:

Figure (6) shows that stomach of rats from control group (A) revealed normal gastric mucosa. On the other hand, stomach of rats from IND group (B) showed necrosis of lamina epithelialis, exposed muscularis mucosa, congestion of blood vessels associated with massive leukocyte cells infiltration in lamina propria. However, examined stomach of rats concurrently pretreated with RAN + IND (C) showed leukocyte cells infiltration in submucosal layer. Meanwhile, stomach of rats concurrently pretreated with CME +IND (D) or CME+ RAN + IND (E) revealed no histological changes.

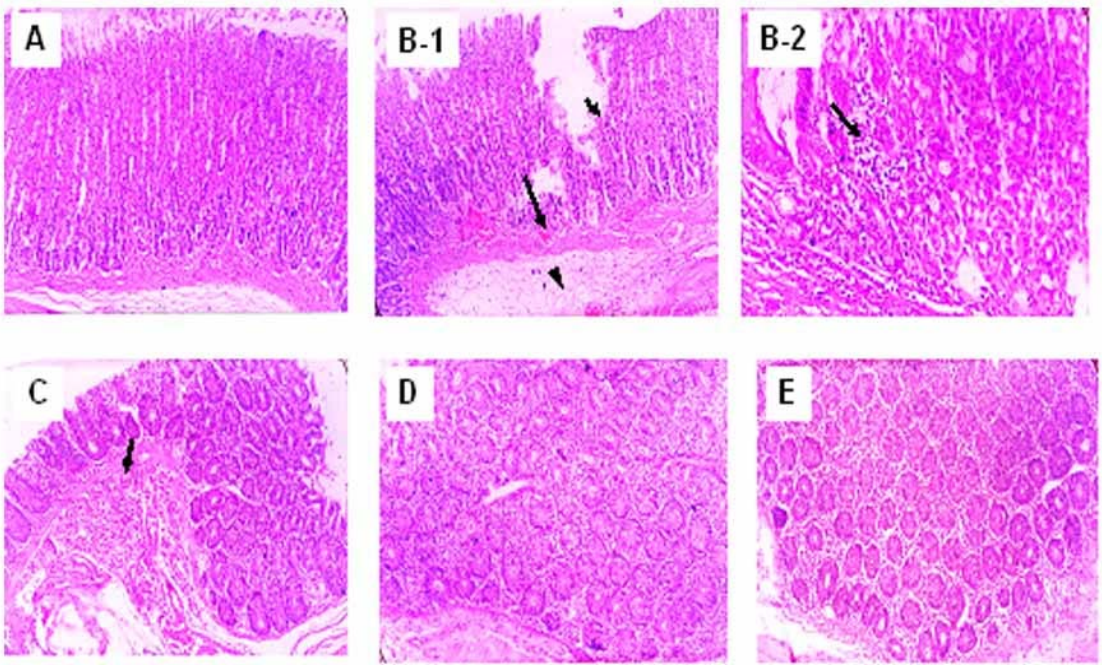


Figure 6: Light micrograph of rat stomach (A) control (B) treated with IND showing: (B-1) necrosis of lamina epithelialis (short arrow), exposed muscularies mucosa and congestion of blood vessels (long arrow), associated with submucosal edema (arrow head) (H&E X100), (B-2) massive leukocyte cells infiltration in lamina propria (H&E X200). (C) pretreated with RAN + IND showing leukocyte cells infiltration in submucosal layer (H&E X100). (D) pretreated with CME + IND and (E) pretreated with CME + RAN + IND showing no histopathological changes (H&E X100).

4. Discussion:

The use of nonsteroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater (Levi et al., 1990). There has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer (Pratt, 1992).

The phytochemical studies performed in the present study demonstrated that CME present carbohydrates, glycosides, flavonoids, sterols, saponins, terpenoids, alkaloids, phenols, gums and mucilage. Among these secondary compounds, saponins, terpenoids and flavonoids are referred as antiulcer compounds (Lewis and Hanson, 1991). This phytochemical composition of CME could explain the antiulcer activity produced by fruit extract which was detected in our study. Moreover, several plants containing high amounts of saponins have been shown to possess antiulcer activity in several experimental bioassays (Yesilada and Takaishi, 1999 and Morikawa et al., 2006) probably acting as an activator of mucus membrane protective factors (Saito et al.,1977). Triterpenoids are a widespread class of secondary compounds with several pharmacological activities, including anti-inflammatory effect in rat paw edema model (Jung et al., 2005) and antiulcer activities (Arrieta et al., 2003). Additionally, the gastroprotective effect of flavonoids has been previously reported (Reyes et al., 1996). Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borelli and Izzo, 2000).

The volume of acid present in gastric secretion which encompasses HCl, pepcinogen, mucus, biocarbonates, intrinsic factor and protein reflects acid volume. Exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration (Olsen, 1988). Another major aggressive factor responsible for ulcers is the content of acid present in gastric juice. Over secretion of histamine contributes to increased secretion of gastric juice (Grossman, 1978). When the concentration of hydrogen ions in gastric juice decreases, it is reflective of high pH. The genesis of ulcer and gastric damage is facilitated by hydrogen ions which serve as another aggressive factor (Lüllmann et al., 2000).

In the present study, indomethacin injection, a representative of NSAIDs family, caused a remarkably significant increase in ulcer index, gastric juice free and total acidity and pepcin activity. The ulceration induced by indomethacin is attributed mainly to various processes, including generation of reactive oxygen species, initiation of lipid peroxidation, infiltration of leukocytes, induction of apoptosis, and inhibition of prostaglandin synthesis (Bech et al., 2000). Decreased prostaglandin level impairs almost all aspects of gastroprotection and increases acid secretions which, in turn, aggravate the ulcer (Miller, 1983).

Oral administration of RAN significantly reduced ulcer index, gastric juice free and total acidity and pepcin activity. However, the drug has not produced any significant quantitative change in the mucin content. Gastric acid decimation by RAN is attributed to its ability to antagonize the binding of histamine to the H_2 receptor on the parietal cells (Banji et al., 2010). RAN can therefore counter the effect of indomethacin on acid secretion. Oral administration of CME produced significant decrease in ulcerative index, with insignificant change in free and total gastric acid values and pepcin activity. Therefore, this result reinforced the absence of antisecretory activity of CME and possible strengthening of gastroprotective factors such as antioxidant elements in this extract.

Gastric mucus (mucin) is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that cover the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto and Llesuy, 2002). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Penissi and Piezzi, 1999).

In this study, the decreased mucin secretion in the indomethacin-administered rats indicated reduced ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals (Naito et al., 1995). An increase in mucus production usually assists the healing process by protecting the ulcer crater against irritant stomach secretions (HCl and pepsin) thereby enhancing the rate of the local healing process. Treatment with CME protected the gastric mucosa from damage by increasing the mucin content significantly. Apparently, the free radicals scavenging property of CME might contribute in protecting the oxidative damage to gastric mucosa. In addition, the content of mucilage in CME might

carpet the gastroduodenal lining, thereby abrogating the impact of ulcerogens such as indomethacin.

Indomethacin is known to induce the reactive oxygen metabolites in animal models, which may contribute to mucosal injury (Chattopadhyay et al., 2006). These free radicals also damage the cellular antioxidant enzymes such as CAT, SOD and others, acting as the first line of cellular defense against oxidative injury. This might lead to aggravated tissue damage during stomach ulceration (El-Missiry et al., 2001). Our experimental results are in line with these previous data. Indomethacin- induced stomach ulceration was accompanied with a severe oxidative stress in gastric tissue causing damage to key biomolecules such as lipids. This was apparent from the stimulated lipid oxidation leading to increased accumulation of MDA as well as reduction in the gastric activity of CAT.

As shown in the present results, RAN treatment significantly reverted the indomethacin-induced changes in MDA and CAT. This significant reduction in MDA levels along with significant increase in CAT level suggest decreased lipid peroxidation and antioxidant activity of RAN. Ranitidine, an antisecretory drug, has often been reported to possess antioxidant and immunosuppressive actions, which may be responsible for its antiulcerogenic activity (Lapenna et al., 1994 and Ardestani et al., 2004). CME provided a marked suppression of oxidative damage due to its excellent radical scavenging capacity, It brought MDA level closer to normal levels, than observed in RAN with concomitant increase in CAT level.

Nitric oxide (NO) is an endogenous defensive factor for gastric cells and exhibits gastroprotective properties against different types of aggressive agents (Samini et al., 2002). It is involved in the maintenance of mucosal integrity through the regulation of mucus and alkaline secretion, gastric motility and microcirculation (Tsukimi and Okabe, 2001). NO is known to modulate acid levels, gastric mucus secretion, and blood flow in gastric tissues (Martín et al., 2001). NO has also been reported to prevent membrane lipid peroxidation (Hogg and Kalyanaraman, 1999), it may protect against NSAID damage by promotion of prostaglandin synthesis (Salvemini et al., 1993).

In the present study, indomethacin significantly reduced gastric mucosal NO level compared to control group. This finding was in accordance with Lanas et al. (2000) and Cadirci et al. (2007) who reported a decrease in NO level in stomach tissue damaged by indomethacin. Tripp and Tepperman (1995) also reported a decrease in NO biosynthesis, as a result of decreased nitric oxide synthase (NOS) activity that was associated with an increase in the extent of damage. Treatment with CME significantly increased mucosal NO level when compared to indomethacin treated rats. The possible mechanism of the increased NO action of CME may be due to its flavonoids content. Matsuda et al. (2003) reported that flavonoids are the major secondary metabolites class with several descriptions of antiulcer, antioxidant and gastroprotective properties.

Prostaglandin, a key molecule that stimulates the complex array of ulcer healing mechanism, gets synthesized in the mucosal cells by cyclooxygenase (COX) enzymes. It stimulates the secretion of biocarbonate and mucus, maintains mucosal blood flow and regulates mucosal turn over and repair (Hayllar and Bjarnason, 1995 and Hiruma-Lima et al., 2006). Suppression of prostaglandins synthesis by indomethacin results in increased susceptibility of stomach to mucosal injury and gastroduodenal ulceration. Indomethacin causes ulcer mostly on the glandular (mucosal) part of the stomach (Nwafor et al., 1996) by inhibiting prostaglandin synthesis through the inhibition of the cyclooxygenase enzymes (Rainsford, 1987). Prostaglandin and bicarbonate secretion and gastric blood flow have been shown to be reduced in animals by indomethain treatment (Selling et al., 1987). Our experimental results were in line with these previous data, indomethacin significantly reduced gastric mucosal prostaglandin E_2 (PGE_2) level compared to control. Treatment with CME significantly increased PGE_2 level when compared to indomethacin treated rats. This finding was explained by Borrelli and Izzo (2000) who reported that flavonoids may protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase.

Tumor necrosis factor (TNF-α) is a pro-inflammatory cytokine secreted by macrophages increasingly during ulcerative stress (Hamaguchi et al., 2001), it is a potent stimulator of neutrophil infiltration into gastric mucosa (Wei et al., 2003) and inducible nitric oxide expression (Calatayud et al., 2001). Overproduction of TNF-α increases the risk of gastric ulcer and cancer (Mitsushige et al., 2007). The inhibition of TNF-α and neutrophil infiltration will ultimately inhibit tissue destruction by reactive oxygen species (Kwiecien et al., 2002). In this study indomethacin significantly increased serum TNF-α as compared to control group. This finding was coincided with the finding of Appleyard et al. (1996) who reported that indomethacin up-regulated the synthesis of pro-inflammatory molecules like TNF-α contributing to mucosal injury. Moreover, Swarnakar et al. (2005) found that indomethacin-induced serum TNF-α and mucosal TBARS up-regulation at the

ulcer site seemed mostly to be responsible for ulcerogenesis.

Polymorphonuclear migration is an early and critical event in the pathogenesis of gastric mucosal injury caused by indomethacin. TNF-α was previously reported to be a proinflammatory cytokine that causes polymorphonuclear neutrophil migration, through up-regulating the expression of adhesion molecules in both neutrophil and endothelial cells (Santucci et al., 1995). Probably, prostaglandins inhibition by NSAIDs is responsible for the TNF-α rise, as these class of drugs markedly reduce prostaglandin synthesis, which were known to be potent inhibitors of TNF-α release from both macrophages (Kunkel et al., 1986) and mast cells (Hogaboam et al., 1993).

On the other hand, the up-regulatory action of indomethacin to serum TNF-α is possibly responsible for the decrease in mucosal NO. This action was in agreement with reported results of Bauer et al. (1997) who recorded that TNF-α is a potent inhibitor to constitutive NO, which mediated a protective effect in the stomach, mostly through modulation of cytokine production. CME produced significant suppression of TNF-α production and this may be attributed to the anti-inflammatory activity of CME. The anti-inflammatory effect of CME was previously proved by a reduction in myeloperoxidase activity in experimentally induced colitis in rats (Al-Awadi et al., 2001). It is interesting to find that in our study RAN showed a significant decrease in serum TNF-α. In accordance with our results, Odabasoglu et al. (2008) reported that ranitidine showed a decreasing effect on the myeloperoxidase activity. Van Zyl et al. (1993) reported also that ranitidine and other H2 antagonists in clinical use have potent inhibitory effect on myeloperoxidase catalyzed reactions.

Histopathological studies on the gastric mucosa revealed that indomethacin administration induced a mucosal ulceration, associated with significant increase in lipid peroxidation. This was manifested as lamina epithelial necrosis, blood vessels congestion, and leukocytic infiltration. This effect on mucosal oxidative stress and histological derangement was in accordance with the reports of (Valcheva-Kuzmanova et al. 2007 and El-Moselhy et al. 2009). CME had protective effect against indomethacin-induced inflammatory infiltration and congestion at the ulcer sites. It prevented gastric mucosal lesions through its flavonoid content. Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects (Alanko et al., 1999).

In conclusion, CME can protect indometacin-induced gastric ulceration due to its antioxidative, anti-inflammatory and mucin enhancing properties. The mechanism of its gastroprotective activity may be attributed to reduction in gastric mucosal lipid peroxidation (MDA), and serum TNFα along with elevation in gastric juice mucin content and gastric mucosal CAT, NO, and PGE_2 levels. The presence of phytoconstituents in this medicinal plant, particularly flavonoids and mucilage, might be responsible for these pharmacological actions. The protection afforded by co-treatment of CME and ranitidine was found to be better than that of CME alone and ranitidine alone. Consequently, CME could be used together with RAN for the treatment of gastric ulcer.

Acknowledgements

The authors would like to thank **Dr. Kawkab A. Ahmed**, Assistant Professor, Department of Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt, for her kind help in the histopathological study of this work.

Corresponding Author:
Dr. Inas Zeidan Abdou Abdallah, Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Egypt**.**
E. mail: izaa2011@hotmail.com

References:

Aebi, H. (1984). Catalase in vitro. Methods Enzymol. 105, 121-126.

Alami, R. and Macksad, A.(1974). Medicinal plants in Kuwait, Al-Assriya Press, Kuwait. p.6.

Alanko, J., Riutta, A., Holm, P., Mucha, I., Vapatalo, H. and Metsa-Ketela, T. (1999). Modulation of arachidonic acid metabolism by phenols: relation to their structure and antioxidant/prooxidant properties. Free radic. Biol. Med., 26 (Suppl 1-2):193-201.

Al-Awadi, F.M., Srikumar, T.S., Anim, J.T. and Khan, I. (2001). Antiinflammatory effects of *Cordia myxa* fruit on experimentally induced colitis in rats. Nutrition, 17(5):391-396.

Alkofahi, A. and Atta, A.H. (1999). Pharmacological screening of the anti-ulcerogenic effects of some Jordanian medicinal plants in rats. J. Ethnopharmacol., 67, 341-345.

Alumets, J., Ekelund, M., Hakanson, R., Hedenbro, J., Rehfeld, J.F., Sundler, F. and Vallgren, S. (1982). Gastric acid response to pylorus ligation in rats: is gastrin or histamine involved? J. Physiol., 323:145–156.

Appleyard, C.B., McCafferty, D.M., Tigley, A.W., Swain, M.G. and Wallace, J.L. (1996). Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leukocyte adherence. Am. J. Physiol. Gastrointest. Liver Physiol., 270:G42-G48

Ardestani, S.K., Janlow, M.M., Kariminia, A. and Tavakoli, Z. (2004). Effect of cimetidine and

ranitidine on lipid profile and lipid peroxidation in γ-irradiated mice. Acta Med. Iran., 42:198-204.

Arrieta, J., Benitez, J., Flores, E., Castillo, C. and Navarrete, A. (2003). Purification of gastroprotective triterpenoids from the stem bark of *Amphipterygium adstringens*, role of prostaglandins, sulfhydryls, nitric oxide and capsaicin-sensitive neurons. Planta Med., 69(10):905-909.

Bancroft, D., Stevens, A. and Turmer, R. (1996). Theory and practice of histological technique, 4th ed., Churchill Living Stone, Edinburgh, London, Melbourne. p. 47-67.

Banji, D., Singh, J. and Banji, O.J. (2010). Scrutinizing the aqueous extract of leaves of *pedalium murex* for the antiulcer activity in rats. Pak. J. Pharm. Sci., 23(3):295-299.

Bauer, H., Jung, T., Tsikas, D., Stichtenoth, D.O., Frolich, J.C. and Neumann, C. (1997). Nitric oxide inhibits the secretion of T-helper 1- and T-helper 2-associated cytokines in activated human T cells. Immunology, 90(2):205-211.

Bech, P.L., Xavier, R., Lu, N., Nanda, N.N., Dinauer, M., Podolsky, D.K. and Seed, B. (2000). Mechanisms of NSAID-induced gastrointestinal injury defined using mutant mice. Gastroenterology, 119(3):699-705.

Borrelli, F. and Izzo, A.A. (2000). The plant kingdom as source of anti ulcer remedies. Phytother. Res., 14(8):581-591.

Bourdet, D.L., Pritchard, J.B. and Thakker, D.R. (2005). Differential substrate and inhibitory activities of ranitidine and famotidine toward human organic cation transporter 1 (hOCT1;SLC22A1), hOCTt2 (SLC22A2), and hOCT3 (SLC22A3). J. Pharmacol. Exp. Ther., 315(3):1288-1297.

Cadirci, E., Suleyman, H., Aksoy, H., Halici, Z., Ozgen, U., Koc, A. and Ozturk, N. (2007). Effects of Onosma armeniacum root extract on ethanol-induced oxidative stress in stomach tissue of rats. Chem. Biol. Interact., 170(1):40-48.

Calatayud, S., Barrachina, D. and Esplugues, J.V. (2001). Nitric oxide: relation to integrity, injury and healing of the gastric mucosa. Microsc. Res. Tech., 53(5):325-335.

Chattopadhyay, I., Bandyopadhyay, U., Biswas, K., Maity, P. and Banerjee, R.K. (2006). Indomethacin inactivates gastric peroxidase to induce reactive-oxygen-mediated gastric mucosal injuty and curcumin protects it by preventing peroxidase inactivation and scavenging reactive oxygen. Free Radic. Biol. Med., 40(8):1397-1408.

Chaturvedi, A., Kumar, M.M., Bhawani, G., Chaturvedi, H., Kumar, M. and Goel, K.R. (**2007).** Effect of ethanolic extract of *Eugenia Jambolana* seeds on gastric ulceration and secretion in rats. Indian J. physiol. Pharmacol., 51(2): 131-140.

Cherqui, B., Desaint, B., Legendre, C. and Levy, V.G. (1989). Fatal hepatitis in a female patient treated with ranitidine. Gastroenterol. Clin. Biol., 13(11):952-953.

El-Missiry, M.A., El-Sayed, I.H. and Othman, A.I. (2001). Protection by metal complexes with SOD-mimetic activity against oxidative gastric injury induced by indomethacin and ethanol in rats. Ann Clin Biochem., 38:694-700.

EL-Moselhy, M.A., Abdel-Hamid, N.M. and Abdel-Raheim, S.R. (2009). Gastroprotective effect of nicorandil in indomethacin and alcohol-induced acute ulcers. Appl. Biochem. Biotechnol., 152(3):449-459.

Evans,W.C. (2002). Trease and Evan's Pharmacognosy. Haroourt Publishers, London, ISBN: 0702026174, p.336-337.

Feldman, M. (1998). Gastric secretion, normal and abnormal. In: Feldman M, Scharschmidt BF, Sleisenger MH Sleisenger & Fordtran's Gastrointestinal and Liver Disease: Pathophysiology/Diagnosis/Management. 6th edn. WB Saunders Co, Philadelphia, p.587-603.

Ficarra, R., Ficarra, P., Tommasini, S., Ragusa, S., Barbera, R. and Rapisarda, A. (1995). Leaf extracts of some *Cordia* species; analgesic and anti-inflammatory activities as well as their chromatographic analysis. Farmaco., 50(4):245-256.

Fisher, A.A. and Le Couteur, D.G. (2001). Nephrotoxicity and hepatotoxicity of histamine H2 receptor antagonists. Drug Saf., 24(1):39-57.

Grossman, M.I. (1978). Control of gastric secretion in gastrointestinal disease, Patho physiology- diagnosis and management. Sleisenzer, M.H, Fordtran, J.S., editors. 2nd ed. W B Saunders Co, Philadelphia, p.640-659.

Hamaguchi, M., Watanabe, T., Higuchi, K., Tominaga, K., Fujiwara, Y. and Arkawa, T. (2001). Mechanisms and roles of neutrophil infiltration in stress-induced gastric injury in rats. Dig. Dis. Sci., 46(12):2708-2715.

Hano, J., Bugajski, J. and Danek, L. (1976). Effect of adrenergic blockade on gastric secretion altered by catecholamines in rats. Arch. Immunol. Ther. Exp. (Warsz), 24(4):507- 524.

Hara, N., Hara, Y., Natsume, Y. and Goto, Y. (1991). Gastric hyperacidity and mucosal damage caused by hypothermia correlate with increase in GABA concentrations of the rat brain. Eur. J. Pharmacol., 194(1):77-81.

Harborne, J.B. (2007). Phytochemical methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London, ISBN: 81-8128-410-4, p.1-34.

Hayllar, J. and Bjarnason, I. (1995). NSAIDS, COX-2 inhibitor and the gut. Lancet, 346(8974):521-522.

Hiruma-Lima, C.A., Calvo, T.R., Rodriguez, C.M., Andrade, F.D.P., Vilegas, W. and Brito, A.R.M. (2006). Antiulcerogenic activity of *Alchornea castaneaefolia*: effects on somatostatin, gastrin and prostaglandin. J. Ethnopharmacol., 104(1-2):215-224.

Hogaboam, C.M., Bissonnete, E.Y., Chin, B.C., Befus, A.D. and Wallace, J.L. (1993). Prostaglandins inhibit inflammatory mediator release from rat mast cells. Gastroenterol, 104(1):122-129.

Hogg, N. and Kalyanaraman, B. (1999). Nitric oxide and lipid peroxidation. Biochim. Biophys. Acta, 1411(2-3):378-384.

Jung, H., Nam, J., Choi, J., Lee, K. and Park, H. (2005). 19α-Hydroxyursane-type triterpenoids: anti-nociceptive anti-inflammatory principles of the roots of *Rosa rugosa*. Biol. Pharm. Bull., 28(1):101-104.

Khattab, M.M., Gad, M.Z. and Abdallah, D. (2001). Protective role of nitric oxide in indomethacin-induced gastric ulceration by a mechanism independent of gastric acid secretion. Pharmacol . Res, 43(5):463-467.

Kim,J.W. (2008). NSAID-induced gastroenterpathy. Korean J. Gastroenterol., 52(3):134-141.

Kunkel, S.L., Wiggins, R.C., Chensue, S.W. and Larrick, J. (1986). Regulation of macrophage tumor necrosis factor production by prostaglandin E_2. Biochem. Biophys. Res.Commun., 137(1):404-410.

Kwiecien, S., Brzozowski, T. and Konturek, S.J. (2002). Effects of reactive oxygen species on gastric mucosa in various models of mucosal injury. J. Physiol. Pharmacol., 53(1):39-50.

Lanas, A., Bajador, E., Serrano, P., Fuentes, J., Carreňo, S., Guardia, J., Sanz, M., Montoro, M. and Sáinz, R. (2000). Nitrovasodilators, low-dose aspirin, other non-steroidal anti-inflammatory drugs, and the risk of upper gastrointestinal bleeding. N. Eng. J. Med. 343(12):834-839.

Lapenna, D., De Gioia, S., Mezzetti, A., Grossi, L., Festi, D., Marzio, L. and Cuccurullo, F. (1994). H2-receptor antagonists are scavengers of oxygen radicals. Eur. J. Clin. Invest. 24(7):476-481.

Levi, S., Goodlad, R.A. and Lee, C.Y. (1990). Inhibitory effect of NSAIDs on mucosal cell proliferation associated with gastric ulcer healing. Lancet 336(8719):840-843.

Lewis, D.A. and Hanson, P.J. (1991). Anti-ulcer drugs of plant origin. In: Ellis, G.P., West, G.B. (EDS.), Progress medicinal chemistry, vol.28. Elsevier Science Publishers, London, p.2001-2031.

Lüllmann, H., Mohr, K., Ziegler, A. and Bieger, D. (2000). Color Atlas of Pharmacology. 2nd ed. Thieme Stuttgart, New York, p.166.

Malyshenko,O.S.,Beloborodova, E.l., Vavilov,A. M. Lomivorotova,G.V. and Kasperskaia,V. I. (2005). Impact of age and type of behavior on the course of ulcer disease. Ter. Arkh., 77(2):28-31.

Martín, M.J., Jiménez, M.D. and Motilva, V. (2001). New issues about nitric oxide and its effects on the gastrointestinal tract. Curr. Pharm. Des., 7(10):881-908.

Matsuda, H., Morikawa, T., Ando, S., Toguchida, I. and Yoshikawa, M. (2003). Structural requirements of flavonoids for nitric oxide production inhibitory activity and mechanism of action. Bioorg. Med. Chem., 11(9):1995-2000.

Mihara, M. and Uchiyama, M. (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem., 86(1):271-278.

Miller, T.(1983). Protective effects of prostaglandins against gastric mucosal damage: current knowledge and proposed mechanisms. Am. J. Physiol., 235:G601-G623.

Mitsushige, S., Takahisa, F., Naohito, S., Akiko, N., Fang, X. and Masayoshi, K. (2007). Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcder and gastric cancer. J. Gastroenterol. Hepatol., 22(1):51-59.

Muralidharan,P. and Srikanth,J. (2009). Antiulcer activity of *Morinda Citrifolia linn* fruit extract. J. Sci. Res., 1 (2):345-352.

Moore, E.W. (1968). Determination of pH by the glass electrode: pH meter calibration for gastric analysis. Gastroenterology , 54(4):501-507.

Morikawa, T., Li, N., Nagatomo, A., Matsuda, H., Li, X. and Yoshikawa, M. (2006). Triterpene saponins with gastroprotective effects from tea seed (the seeds of Camellia sinensis). J. Nat. Prod., 69(2):185-190.

Naito, Y., Yoshikawa, T., Matsuyama, K., Yagi, N., Arai, M., Nakamura, Y., Nishimura, S., Yoshida, N. and Kondo, M. (1995). Effects of oxygen radical scavengers on the quality of gastric ulcer healing in rats. J. Clin. Gastroenterol., 21 Suppl 1:S82-S86.

Nwafor, P.A., Effraim, K.D. and Jacks, T.W. (1996). Gastroprotective effects of acqeous extracts of *Khaya senegalensis* bark on indomethacin-induced ulceration in rats. West Afr. J. Pharmacol. Drug Res., 12:46-50.

Odabasoglu, F., Halici, Z., Cakir, A., Halici, M., Aygun, H., Suleyman, H., Cadirci, E. and Atalay, F. (2008). Beneficial effects of vegetable oils (corn, olive and sunflower oils) and α- tocopherol on anti-inflammatory and gastrointestinal profiles of indomethacin in rats. Eur. J. Pharmacol., 591(1-3):300-306.

Olsen, C.E. (1988). Glutathione modulates toxic oxygen metabolite injury of canine chief cell monolayers in primary culture. Am. J. physiol., 254: G49-G56.

Penissi, A. and Piezzi, R. (1999). Effect of dehydroleucodine on mucus production: A quantitative study. Dig. Dis. Sci., 44 (4):708-712.

Peskar, B.M., Ehrlich, K. and Peskar, B.A.(2002). Role of ATP-sensitive potassium channels in prostaglandin-mediated gastroprotection in the rat. J. Pharmacol. Exp. Ther., 301(3):969-974.

Pratt, D.E. (1992). Natural antioxidants from plant material. In: Huang, I.M.T., Ho, C.T., Lee, C.Y., editors. Phenolic compounds in food and their effects on health. New York: American Chemical Society, p.54-72.

Rainsford, K.D.D. (1987). The effects of 5-lipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by

nonsteroidal anti-inflammatory drugs in mice. Agents Action, 21(3-4):316-319.

Rates, S.M.K. (2001). Plants as source of drugs. Toxicon, 39(5): 603-613.

Reeves, P.G., Nielsen, F.H. and Fahey, G.C.(1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123(11):1939-1951.

Repetto, M.G. and Llesuy, S.F. (2002). Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz. J. Med. Biol. Res., 35(5):523-534.

Reyes, M., Martin, C., Alarcon de la Lastra, C., Trujillo, J., Toro, M.V. and Ayuso, M.J. (1996). Antiulcerogenicity of the flavonoid fraction from Erica andevalensis Cabezudo-Rivera. Z Naturforsch C., 51(7-8):563-569.

Ribeiro, J.M., Lucas,M., Baptista, A. and Victorino, R.M. (2000). Fatal hepatitis associated with ranitidine. Am. J. Gastroenterol., 95(2):559-560.

Saito, H., Lee, Y.M., Takagi, K., Shoji, S. and Kondo, N. (1977). Pharmacological studies of Panacis Japonici Rhizoma. l. Chem. Pharm. Bull., 25(5):1017-1025.

Salvemini, D., Misko, T.P., Masferrer, J.L., Seibert, K., Currie, M.G. and Needleman, P. (1993). Nitric oxide activates cyclooxygenase enzymes. Proc. Natl. Acad. Sci. USA, 90(15):7240-7244.

Samini, M., Moezi, L., Jabarizadeh, N., Tavakolifar, B., Shafaroodi, H. and Dehpour, A. (2002). Evidences for involvement of nitric oxide in the gastroprotective effect of bromocriptine and cyclosporin A on water immersion stress-induced gastric lesions. Pharmacol. Res., 46(6):519-523.

Santucci, L., Fiorucci, S., Di Matteo, F.M. and Morelli, A. (1995). Role of tumor necrosis factor alpha release and leukocyte margination in indomethacin-induced gastric injury in rats. Gastroenterology, 108(2):393-401.

Sanyal, A.R., Denath, O.K., Bhattacharya, S.K. and Gode, K.D. (1971). The effect of cyproheptadine on gastric acidity. In: Pfeiffer CJ (ed). Peptic ulcer. Scandinavian University Books, Munksgaard, Copenhagen, p. 312–318.

Sastry, K.V., Moudgal, R.P., Mohan, J., Tyagi, J.S. and Rao, G.S. (2002). Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal. Biochem.,306 (1):79-82.

Schmeda-Hirschmann, G. and Yesilada, E. (2005). Traditional medicine and gastroprotective crude drugs. J. Ethnopharmacol., 100(1-2):61-66.

Selling, J.A., Hogan, D.L., Aly, A., Koss, M.A. and Insberg, J.I. (1987). Indomethacin inhibits duodenal mucosal bicarbonate secretion and endogenous prostaglandin E_2 output in human subjects. Ann. Intern. Med., 106(3):368-371.

Souza Brito, A.R.M. (1995). In. UNICAMP (Ed.), Manual de Ensaios Toxicol ´ogicos in vivo. Campinas, S˜ao Paulo, p.122.

Su, G.L., Goyert, S.M., Fan, M.H., Aminlari, A., Gong, K.Q., Myc, A., Alarcon, W.H., Steinstraesser, L., Remick, D.G. and Wang, S.C. (2002). Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. Am. J. Physiol. Gastrointest. Liver Physiol., 283(3):G640-G645.

Swarnakar, S., Ganguly, K., Kundu, P., Banerjee, A., Maity, P. and Sharma, A.V.(2005). Curcumin regulates expression and activity of matrix metalloproteinase 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. The J. Bio.l Chem., 280(10):9409-9415.

Tortora, G.J. and Grabowski, S.R. (2003). Principles of Anatomy and Physiology. 10th ed. John Wiley & Sons, Inc, NJ, p.889.

Tripp, M.A. and Tepperman, B.L. (1995). Effect of nitric oxide on integrity, blood flow and cyclic GMP levels in the rat gastric mucosa: modulation by sialoadenectomy. Br. J. Pharmacol., 115(2):344-348.

Tsukimi, Y. and Okabe, S. (2001). Recent advances in gastrointestinal pathophysiology: role of heat shock proteins in mucosal defense and ulcer healing. Biol. Pharm. Bull., 24(1):1-9.

Valcheva-Kuzmanova, S., Krasnaliev, I., Galunska, B. and Belcheva, A. (2007). Influence of DL-alpha-tocopherol acetate on indomethacin-induced gastric mucosal injury in rats. Auton. Autacoid Pharmacol., 27(3):131-136.

Van Zyl, J.M., Kriegler, A. and Van Der Walt, B.J. (1993). Antioxidant properties of H_2- receptor antagonists. Effects on myeloperoxidase-catalysed reactions and hydroxyl radical generation in a ferrous-hydrogen peroxide system. Biochem. Pharmacol., 45(12):2389-2397.

Wallace, J.L. and Granger, D.N. (1996). The cellular and molecular basis of gastric mucosal defense. FASEB J., 10(7):731-740.

Wei, X.M., Heywood, G.J., Di Girolamo, N. and Thomas, P.S.(2003). Nicorandil inhibits the release of TNF alpha from a lymphocyte cell line and peripheral blood lymphocytes.. Int. Immunopharmacol., 3(12):1581-1588.

Winzler, R.J. (1955). Determination of serum glycoproteins. Methods Biochem. Anal., 2: 279-311.

Yesilada, E. and Takaishi, Y. (1999). A saponin with anti-ulcerogenic effect from the flowers of *Spartium junceum*. Phytochemistry, 51(7):903-908.

Evaluation of safety and efficacy of intra-arterial thrombolysis for acute stroke in patients 80 and older − two center study

ZHAO Jie[1], XU Hao-wen[1],TAN Song[1], LI Ming-hua[2], FANG Chun[2], XU Yu-ming[1]

1. Department of Neurology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan 450052, China.
2. Department of Interventional Radiology, the Shanghai Sixth People's Hospital, Shanghai Jiao Tong University, Shanghai 200026, China.
Co-first author: XU Hao-wen, neuron@126.com; Corresponding author: XU Yu-ming, xuyuming@zzu.edu.cn

[Abstract] Object The purpose of this study was to evaluate the feasibility, safety and efficacy of very elderly patients (≧80 years) with acute ischemic stroke treated with intra-arterial thrombolytic therapy. **Method** The characteristics and clinical outcome of patients aged ≧ 80 years (n =21) were compared retrospectively contemporaneous patients aged < 80 years (n =65) from a registry of consecutive patients treated with intra-arterial thrombolysis and control group who aged ≧ 80 years and did not received thrombolytic therapy (n =50). **Results** There were no significant difference in favorite recanalization rate, short-term outcome and incidence of symptom intracranial hemorrhage between the very elderly and younger cohorts who received thrombolytic therapy (P=0.528, P=0.102, P=0.353). The incidence of symptom intracranial hemorrhage in the very elderly patient group was lower than that of normal age patient group (42.9% versus 50.8%, P=0.042), however, which is higher than that of the control group (42.9% versus 16%, P=0.017). The mortality of very elderly group who received thrombolytic therapy was similar to that of the control group (23.8% versus 28%, P=0.816), which was higher than that of younger cohort group (23.8% versus 10.8%, P=0.034). **Conclusions** There were relatively high feasibility, safety and efficacy in very elderly patients (≧80 years) with acute ischemic stroke treated with intra-arterial thrombolytic therapy. These findings demonstrate that the use of intra-arterial thrombolytic therapy in very elderly patients should not be avoided but pursued advisably.

[ZHAO Jie, XU Hao-wen,TAN Song, LI Ming-hua, FANG Chun, XU Yu-ming. **Evaluation of safety and efficacy of intra-arterial thrombolysis for acute stroke in patients 80 and older − two center study.** Life Science Journal. 2011;8(3):446-450] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: elderly; cerebral infarct; intra-arterial thrombolysis

Introduction

Age is the most important unmodified risk factor of actue ischemic stroke (AIS), and the mortality rapidly increased with age[1]. The treatment of very elderly patients with AIS presents physicians with a unique and increasingly important challenge. Brown et al reported that persons aged 85–94 years experience a 2-fold higher incidence of AIS compared with persons aged 65–74 years and a 26-fold higher incidence compared with persons aged 45–54 years[2]. The old person is rapidly growing worldwide. The person aged 65 years and older account for 8.87% of all population in china[3]. For AIS, thrombolysis remains the only proven effective therapy, despite hemorrhagic complications. Schwark et al found Among the patients with AIS received IV recombinant tissue plasminogen activator within 3 hours after symptoms onset, the incidence of symptomatic hemorrhage in patients ≧80 is notably higher than that of in patients <80[4]. However, Intra-arterial thrombolysis (IAT) has the merit of high rate of recanalization and low incidence of hemorrhage related with thrombolysis. It is unclear that if very elderly patients (≧80 years) is suitable for IA thrombolytic therapy. The aim of study was to compare the efficacy and safety of acute ischemic stroke patients aged ≧80 years treated by IAT with those younger than 80 years of age.

Methods and subjects

From May 2006 to May 2010, 91 consecutive patients (44 women and 56 men) with AIS were treated with IAT using urokinase or rt-PA in the first affiliated hospital of ZhengZhou university and the sixth hospital of ShangHai JiaoTong university. Five of these patients were lost in 9o-day follow-up. Therefore, a total of 86 patients (52 men and 34 women) were chosen in this study. Three groups was classified according age: very elderly patient group (≧80 years), younger cohort groups (<80) and control group. There were 21 and 65 patients in two groups respectively. 50 patients (≧80 years) with AIS who did not receive IAT because the admission time was from six hours to twenty four hours after symptom onset. The baseline characteristic of these patients were shown in table 1.

Inclusion criteria of IAT : (1) neurological signs indicate ischemic lesion in the anterior circulation; (2) 4≤baseline NIHSS score ≤ 24; (3) 3 hours≤ the

time window of IV/IAT or IAT ≤6 hours; (4) patients aged ＜ 80 years; (5) CT excluded intracranial hemorrhage or significant mass effect.

Exclusion criteria of IAT: (1) symptomatic ischemic stroke within 6 weeks, (2) head trauma within the previous 90 days, (3) surgery within 90 days, (4) biopsy involving a major organ within 2 weeks, (5) a history of intracranial hemorrhage, (6) a clinical presentation suggestive of subarachnoid hemorrhage or septic embolism, (7) gastro-intestinal or any serious hemorrhage within 3 weeks (8) Systolic BP＞185 mmHg or diastolic BP ＞110 mmHg despite the use of IV nitroprusside or labetalol, and (9) Laboratory exclusion criteris: Protime ＞15 seconds; platelets ＜ 100,000/mm3; Blood glucose＜2.8 or ＞22 mmol/L.

Bilateral internal carotid artery and the vertebral basilar system angiography were performed via femoral route to evaluate the site and extent of thrombus and collateral blood flow. Occlusion and recanalization were graded by according to Mori grade [70]. After identification of the occlusive artery, a 6F guide catheter was advanced into the lesion-related side common carotid artery or internal carotid artery. Heparin at a dose of 500 U/hour was continuously infused in the catheter until the end of the procedure. A microguidewire and microcatheter were used to traverse the occluded segments several times in an attempt to disrupt the clot, and then the microcatheter was embedded into the clot. UK (60,000-120,000 unit) or rt-PA (0.5mg/kg) was administered through microcatheter. Mechanical disruption of thrombus with the microguidewire and microcatheter was fitfully attempted during the procedure. Diagnostic angiography through the guide catheter or microcatheter was performed at 10-minute intervals. Termination of the IA treatment procedure occurred if there was: (1) achievement of thrombolysis in Mori grade 4; (2) beyond the 6 hours from stroke onset.

All patients underwent nonenhanced head CT scanning within 24 hours of thrombolysis and immediately upon neurologic deterioration. After thrombolytic therapy, all patients were admitted to neuro-intensive care units for close monitoring of vital signs. No antiplatelet agents and anticoagulation were administered within 24 hour after symptom onset. Heparin was reversed with protamine immediately if parenchymal hyperdensity shown on CT. 24 hours later, all patients without sICH were administered aspirin 100 mg orally daily for at least 6 months.

Neuroradiological and Outcome Evaluation

Severity of neurological deficit was assessed at admission and 7 days after onset by a neurologist using the NIHSS. Early neurologic improvement was defined as an improvement NIHSS score by four or more points on the NIHSSS between baseline and 7 days or a NIHSSS of 0 to 1 at 7 days, although this was not recognized as the end point of the study [15]. Arterial patency was rated by the thrombolysis in Mori grade in which 0 indicates no perfusion, 1 is minimal reperfusion, 2 is reperfusion of less than 50% of the territory of the occluded artery, 3 is reperfusion of more than 50% of the territory of the occluded artery, and 4 is complete reperfusion. We defined Mori grade 2-4 as recanalization, Mori grade 3-4 as good recanalization, and Mori grade 0-2 as poor recanalization. Symptomatic ICH was defined as neurological worsening ⩾ 4 points in NIHSS and attributable to cerebral hemorrhage shown on CT. Clinical outcome was assessed with mRS at 90 days which was grouped into two categories: good outcome (mRS score, 0–2) for those patients who were functionally independent, poor outcome those who were dependent (mRS score, 3–5), and death corresponds to mRs score of 6 [1]. The investigators were not blinded to the baseline NIHSS scores, angiographic results and clinical outcome.

Statistical Analysis

Descriptive and frequency statistical analysis were obtained and comparisons were made using the statistical software SPSS 15.0. For calculation of significant differences among the three groups were analyzed with variance analysis, the chi-square test respectively, the Mann-Whitney U test and logistic regression analyses. Ninety-five percent confidence intervals were calculated for odds ratios and for relative risk. The following variables were analyzed: age, sex, baseline NIHSS scores, early neurologic improvement, time from symptom onset to thrombolysis, mortality, incidence of sICH and good outcome. A probability value of $P<0.05$ was considered statistically significant. All values are presented as means ±SD or as medians

Results

General Characteristics

Among 86 patients who received IAT, 21 patients were older than 80 years (the mean age was 83.4±3.1, from 80 to 89 years), 65 patients were younger than 80 years (the mean age was 61.2±8.7 years, from 36 to 79 years). The mean age of 50 patients in control group was 82.3±3.7 years，from 80 to 92 years. There were significant difference in age and incidence of coronary disease among the three groups (p=0.000). Sex and risk factors including hypertension, diabetes, TIA and atrial fibrillation did not differ among these groups. Baseline patient characteristics for the 2 groups are presented in Table 1.

There were 7 patients (33.3%) whose NIHSS score dropped by 4 points in very elderly patients

group as compared with 35 patients (53.8%) in younger cohort group (p=0.528). Good recanalization rates (Mori 3-4) were 42.9% which is lower than that of younger cohort group (50.8%), but there were no significant difference between the two group (p=0.102). The incidence of sICH in very elderly patients group (19.0%) was notably higher than that of young cohort group (12.3%， p=0.353)and control group (6%， p=0.034). 42.9% of very elderly patients group had a favourite outcome (mRS score of 2 or less) at 90 days after stroke as compared to 50.8% of young cohort group ((p=0.042), which was higher than 16% of control group (p=0.017). Mortality was 23.8% in very elderly patients, which was higher than that of younger patients (p=0.034), however was lower than that of control groups (p=0.816). the result indicated that the clinical outcome of very elderly patients who received IAT was better in comparison with similar patients who did not receive IAT, nevertheless, which was worse than that of younger patient who received IAT (Table 2,3).

Table 1. Baseline Characteristics of three groups

	Intra-arterial thrombolysis		Congrol group
	≧ 80 years (n=21)	< 80 years (n=65)	≧ 80 years (n=50)
Age (year)*	83.4	61.2	82.3
Sex, male	61.9%	60.5%	64.0%
Hypertension	66.7%	50.8%	62.0%
Diabetes	61.9%	40.0%	56.0%
TIA	28.6%	12.3%	18.0%
Atrial fibrillation	28.6%	23.1%	26.0%
Coronary artery disease *	71.4%	26.2%	56.0%
Baseline NIHSS	15.6	14.5	17.3
Location of infarct			
Anterior circulation	85.7%	86.2%	92.0%
Posterior circulation	14.3%	13.8%	8.0%
Thrombolytic agent			
urokinase	15 (71.4%)	51(80.5%)	
r-tPA	6 (18.6%)	14(21.5%)	

There were no significant difference in baseline characteristics among the three groups except for age and coronary disease. (p>0.05)

*** there were** significant difference in age and coronary disease between the two groups which received IAT(p<0.05), however, no significant difference in all baseline characteristics between the two groups whose patients were older than 80 years(p>0.05).

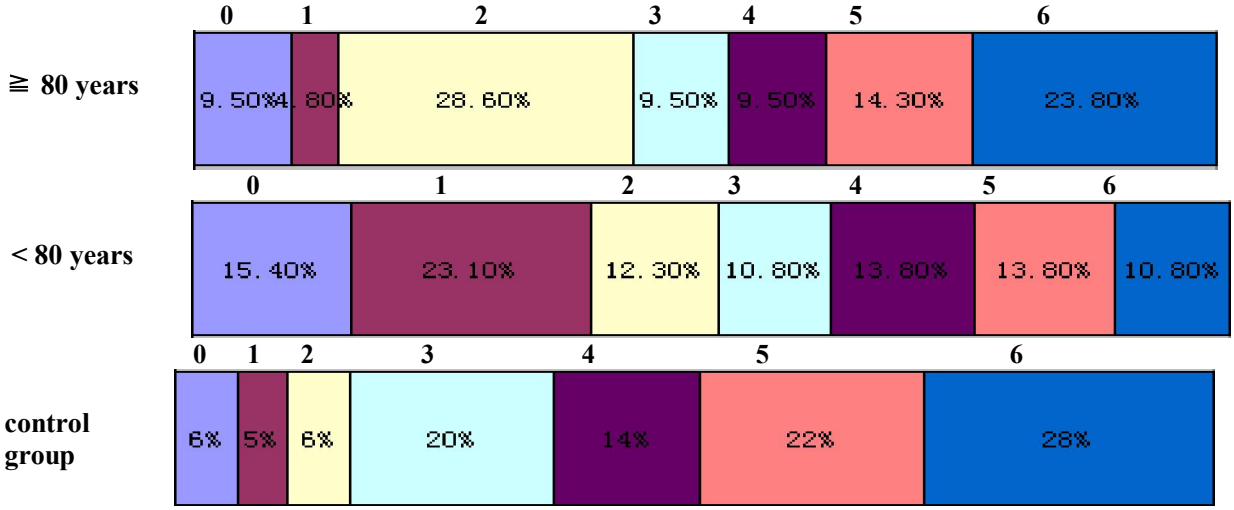
Table 2. The clinical outcome of the three groups

	Intra-arterial thrombolysis		Control group
	≧ 80 years (n=21)	< 80 years (n=65)	(n=50)
Early neurologic improvement (NIHSS improvement ≧4)	33.3%	53.8%	
Good recanalization after IAT （Mori grade 3-4）	42.9%	50.8%	
Symptomatic ICH	19.0%	12.3%	6.0%
good outcome（mRS 0-2）	42.9%	50.8%	16.0%
Mortality	23.8%	10.8%	28.0%
Complication of femoral artery puncture	1(4.8%)	4(6.2%)	

Table 3. Comparison of the clinical outcome among the three groups

	≧ 80 years and < 80 years		≧ 80years and control group	
	χ2 值	*P* 值	χ2 值	*P* 值
Good recanalization	0.398	0.528		
Early neurologic improvement	2.673	0.102		
Symptomatic ICH	0.863	0.353	4.491	0.034
good outcome	4.140	0.042	5.858	0.017
Mortality	4.472	0.034	0.085	0.816

Chart 1. Modified Rakin scale score at 90 days in different groups



Discussion

Stroke primarily affects an elderly population. Brown reported that more than half of the strokes in this population affected subjects aged ≧75 years and nearly one quarter affected subjects aged ≧85 years[2]. A Multicenter Cohort Study Across Canada found that 38% of hospitalized patients with an ischemic stroke occurred in individuals aged 80 or older[5].

In 2007, Kim et al firstly compared the safety and efficacy between very elderly patients (≧ 80 years) and younger patients (< 80 years) with AIS who all received IAT[6]. They found significant differences in recanalization rates and symptomatic ICH could not be detected between the very elderly and younger patients, the good outcome was worse in the former compared to the latter. However, one limitation of this study was no comparison of safety and efficacy between very elderly patients treated with IAT and without IAT. Our study not only compare the safety and efficacy of AIS patients (≧ 80 years) treated with IAT and that of younger patients, but also designed a control group of patients 80 years and older who did not receive IAT to provide relative estimates of treatment benefit.

This pilot study demonstrates the feasibility of treatment of very ischemic stroke patients (≧ 80 years) treated with IAT. Despite this success, clincial outcome of very elderly patients was not improved compared with that of younger patients, but inference is limited because of a small numbers of patients, an excess of adverse events unrelated to treatment assignment in the very elderly group. The safety of a two groups which received intra-arterial thrombolysis was acceptable in this small pilot study. The rate of symptomatic ICH and recanalization in younger group was 12.3% and 50.8%, which were similar to the rate of the PROACT II study and previous IA cohort series report[7, 8]. A higher rate of symptomatic ICH and lower proportion of recanaliation and good clinical outcome accomplished in very elderly patients in comparison with younger patients and Kim study. In a study involving 33 ischemic stroke patients who were 80 years or older, all patients were treated with intra-arterial thrombolytic therapy. Kim and coworkers emphasized the feasibility of IAT for very patients with AIS and demonstrated that the rate of recanalization, symptomatic ICH , good clinical outcome and mortality at 3 months were 79% were 79%,7% , 44% and 43% sequently. We think the differences may be related with discrepancy of race, baseline characteristics, time to treatment, thrombolytic drugs and dosage of drug.

Ever since thrombolysis was first administered to

stroke patients in the 1950s[9]. Symptomatic ICH associated with cerebral revascularization therapy has deterred its use. Kidwell thought the risk factors for symptomatic ICH were age, high NIHSS Score, long time to treatment, hypertension, diabetes, leukoaraiosis, severe ischemia and high dose thrombolytic therapy[10]. Several studies have identified age as an independent risk factor for intracerebral hemorrhage after intravenous fibrinolysis, administered for both myocardial and cerebral ischemia[11-13]. Nevertheless, a meta-analysis conducted by Mijderwijk and coworkers found age is not a risk factor for symptomatic intra-cerebral hemorrhage (sICH) for acute stroke patients after intra-arterial thrombolysis[14]. This finding contradicted our result. In our study, the rate of symptomatic ICH in patients aged ≧80 years were apparently higher than patients aged < 80 years. Trouillas reported that high incidence of symptomatic ICH in very elderly patient may be connected with cerebral amyloid angiopathy, fragile vasculature and delayed half-life period of thrombolytic drug[15]. We approve of Trouillas's view.

In our study, compared to patients aged < 80 years, the lower rate of good functional outcome and higher mortality rate observed in the patients aged≧80 years after IAT. We think a variety of factors probably contribute to reduced functional outcome, despite successful reperfusion in the elderly, (1) a higher frequency of prior stroke/TIA, higher frequency of prestrike comorbid conditions and poststroke medical complications. (2) impaired collateral circulation, Collateral circulation is believed to salvage parts of the ischemic territory and, thus, positively affect eventual functional outcome. In the elderly population, the ability to form collateral pathways may be acutely decreased. (3) reduction of multi-organ function. It's important to note that the good clinical outcome of very elderly patients who received IAT was better than people of the same age who did not receive IAT. This finding support the concept that the very elderly ischemic stroke patients are worth being treated with IAT, because it can remarkably improve the clinical outcome and reduce the mortality rate. With the introduction of combined intra-venous and intra-arterial thrombolytic therapy, mechanical recanalization and clot retrieval device such as Penumbra and Merci, the efficacy of interventional therapy will be improved.

Because of the small sample size, our results are preliminary and preclude us from reaching completely generalizable conclusions. Large-scale prospective randomized studies in future are required to confirm the results of this study.

References

1. Whisnant JP, Wiebers DO, O'Fallon WM, et al. A population-based model of risk factors for ischemic stroke: Rochester, Minnesota. Neurology. 1996;47(6):1420-1428.
2. Brown RD, Whisnant JP, Sicks JD, et al. Stroke incidence, prevalence and survival: secular trends in Rochester, Minnesota, through 1989. Stroke 1996; 27:373-380.
3. Zhao Y, Yao Z, D'Souza W, et al. An epidemiological survey of stroke in Lhasa, Tibet, China. sroke. 2010; 41(12):2739-43.
4. C Schwark, P D Schellinger. Is old age really a reason to withhold thrombolytic therapy? J Neurol Neurosurg Psychiatry 2006; 77:289-295.
5. Gustavo Saposnik, Robert Cote, Stephen Phillips, et al. Stroke Outcome in Those Over 80 A Multicenter Cohort Study Across Canada. Stroke. 2008; 39:1-7.
6. D Kim, GA Ford, CS Kidwell, et al. Intra-Arterial Thrombolysis for Acute Stroke in Patients 80 and Older: A Comparison of Results in Patients Younger than 80 Years. America Journal Neuroradiology. 2007; 28:159–163.
7. Anthony Furlan, Randall Higashida, Lawrence Wechsler, et al. Intra-arterial prouro- kinase for Acute Ischemic Stroke: The PROACT II Study: A Randomized Controlled Trial. Journal of American Medical Association 1999; 282:2003–2011.
8. Furlan A, Higashida R, Wechsler L, et al. Intra-arterial prourokinase for acute ischemic stroke. The PROACTII study: a randomized controlled trial. Prolyse in Acute Cerebral Thromboembolism. JAMA 1999; 282:2003–1.
9. Sussman BJ, Fitch TS. Thrombolysis with fibrinolysin in cerebral arterial occlusion. JAMA. 1958; 167:1705–1709.
10. Kidwell CS, Saver JL, Carneado J, et al. Predictors of hemorrhagic transformation in patients receiving intra-arterial thrombolysis. Stroke 2002; 33:717–724.
11. Tanne D, Kasner SE, Demchuk AM, et al. Markers of increased risk of intracerebral hemorrhage after intravenous recombinant tissue plasminogen activator therapy for acute ischemic stroke in clinical practice: the multicenter rT-PA stroke survey. Circulation 2002;105:1679–85
12. Larrue V, von Kummer RR, Muller A, et al. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). Stroke 2001;32:438–41
13. Gurwitz J, Gore J, Goldberg R, et al. Risk for intracranial hemorrhage after issue plasminogen activator treatment for acute myocardial infarction. Participants in the national registry of myocardial infarction. Ann Intern Med 1998;129:597–604
14. Mijderwijk , A.E. Bras, D.W.J. Dippel. Symptomatic Intra-Cerebral Hemorrhage in Ischemic Stroke Patients Treated with Intra-Arterial Thrombolysis. Erasmus Journal of Medicine. 2010; 5:19-21.
15. Trouillas P, Kummer R. Classification and pathogenesis of cerebral hemorrhages after thrombolysis in ischemic stroke. Stroke. 2006; 37:556-561.

7/7/2011

Developing methods for anatomical identification of the genus Zygophyllum L. (Zygophyllaceae) in Saudi Arabia. Original research

Nahed M. Waly*, Faraj A. Al-Ghamdi**, Rahma I. Al-Shamrani,**

*Faculty of Science- Botany department- Cairo University, Egypt
** Faculty of Science- Biology department- King Abdel Aziz University, Jeddah, Saudi Arabia
*Corresponding author, e-mail: nwaly89@hotmail.com

Abstract: Zygophyllum L., the largest genus of Zygophyllaceae comprises about 100 species known from the Mediterranean to Central Asia, more than eleven species growing in Saudi Arabia specially in desert and saline habitats. This study deals with taxonomy of Zygophyllum species growing in Saudi Arabia depending on anatomical characters of leaves and petioles to eleven investigated species. Anatomical features of leaves and petiole of the eleven investigated Zygophyllum species show characters of major importance such as stem outline, leaf outline, the arrangement of leaf vascular tissue, in addition to the number of vascular bundles in the inner leaf whorl, and characters of minor importance such as leaf mesophyll, and the branching of the main vascular bundle in the petiole Those characters enable us to separate Z. simplex from the other ten species by its cup shape transverse section in stem. Leaf vascular tissue is arranged either in straight line, or in two whorls . Petiole vascular bundles tissue arranged in two whorls the outer with number of vascular bundles, the inner whorl with two type: one main central vascular bundle and one main central vascular bundle associated with two peripheral vascular bundles. According to all those anatomical characters an artificial key explain the difference between the eleven investigated Zygophyllum species. Life Science Journal. 2011;8(3):451-459] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Zygophyllum species, anatomical features, leaf vascular tissue

Introduction

The genus Zygophyllum L., the largest genus of *Zygophyllaceae*, comprises about 100 species known from the Mediterranean to central Asia, South Africa and Australia. It includes perennial shrubs or under-shrubs, with succulent cylindrical rarely flattened leaves, simple or 1-2 foliate (Boulos 2000).El Hadidi (1977) described *Zygophyllum migahidii* and *Z.mandavillei* as new species to Saudi Arabia. Migahid (1978) recognized 7 species of *Zygophyllum* grow in Saudi Arabia: *Zygophyllum album, Z. coccineum, Z. decumbens, Z. gaetulum, Z. mandavillei, Z. migahidii* and *Z. simplex* . In her revision of the genus, Hosny (1978) added another 5 species of *Zygophyllum* viz. Z. *aegyptium, Z. berenicense, Z. propinquum. Z. qatarense* and *Z. hamiense*. Collonette (1999) added the following 2 species: *Z. boulosii* and *Z. fabago*. Ahmed (1989) carried out an anatomical study on *Zygophyllum album* in Egypt and reported the effect of the different ecological factors on the stem growth. These anatomical characters mentioned in her study highlighted the importance of investigated these features of the *Zygophyllum* species growing in Saudi Arabia which can't be done before.

Therefore the aim of this works: 1- Investigate the anatomical characters of each of the eleven *Zygophyllum* species growing in Saudi Arabia; 2-Based on these anatomical features recognized, an artificial key utilizing major and minor characters for the diagnosis of these species will be developed.

1. Materials and Methods:

2.1. Sample collection:

Fresh materials of 9 *Zygophyllum* species growing wild in sand plain region and salt marches were collected. Attention was paid for studying specimens who were collected from localities representing the geographical range of each species (Table 1). Two herbarium specimens were studied where the fresh materials was not available during this seasons. The collected materials were identified according to Migahid (1978) and Chaudhary (2001). Samples of the identified materials were kept at Botany Department Faculty of Science King Abdul-Aziz University (Girls section).

2.2 Sample preparation:

For anatomical investigations specimens were fixed in formalin- glacial acetic acid- ethyl alcohol .Petioles and leaves blade were microtome serially at 10-15 **µ** after being embedded in paraffin wax. Sections were stained in saffranin and light green dehydrated in alcohol-xylol series, cleared in clove oil and mounted in Canada balsam, photographed by Nikon Microscope.

3. Results:

3.1. Anatomical result:

In table (2&3) the 11 investigated *Zygophyllum* species were arranged according to systematic treatment followed by Van Huyssteen (1937). Two species viz. *Z. decumbens* and *Z. simplex* belong to section Bipartita of subgenus *Agrophyllum*, while *Z.album ; Z. boulosii ; Z. coccineum; Z. hamiense; Z. mandavillei; Z. migahidii; Z. propinquum. Z. qatarense* belong to section Mediterranea of the same subgenus. The last row comprise *Z. fabago* belong to section Fabago of subgenus *Zygophyllotypus*, the anatomical characters are arranged horizontally.

Table (1): The collected specimens of Saudi Arabia *Zygophyllum* species

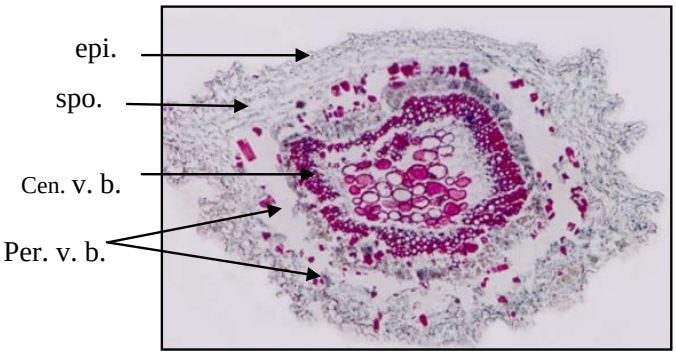
Species	***Locality***	***Date of collection***
1-Z. album	El-Sheaba 90 Km south Jeddah South-Obher 30Km south Jeddah	15/2/2003 20/2/2003
2-Z. boulosii	North –Obher 20 Km north Jeddah	12/7/2003
3-Z. coccinum	North–Obher 40Km north Jeddah South-Obher 30Km south Jeddah Jeddah-Makka road	7/3/2003 20/2/2003 25/3/2003
4-Z. mandavillei	East part Abou-Hedrea road	12/2/2003
5-Z. migahidii	EL-Raid 20Km south El-Mattar road	15/4/2003
6-Z. qatarense	East part Abou-Hedrea road	12/2/2003
Z. simplex 7-	El-Sheaba 90 Km south Jeddah Jeddah-Makka road	15/2/2003 25/3/2003
8-Z. propinquum	Al-Shaiba, 80 Km south of Jedda	9/4/1999 Herbarium specimens
9- Z. fabago	Al-Asawia,10Km before Tabarjal, S.Abedin,M.Al-Tahya, M.Al-Said,5-iv-88(19038-RIY)	Herbarium specimens
10-Z. hamiense	10 km north Dammam road	22/4/2003
11-Z. decumbens	20Km North west Jeddah	17/3/2003

Table (2): Petiole anatomical characters of the Zygophyllum species in Saudi Arabia

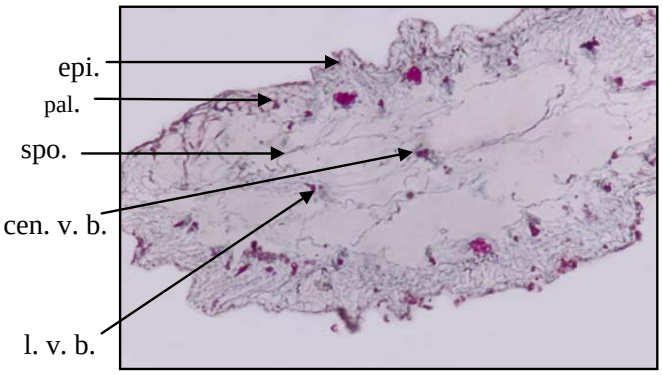
Characters	*Agrophyllum*										*Zygophllotypus*
	Bipartita		*Mediterranea*								*. fabago*
	Z. decumbens	*Z. simplex*	*Z. album*	*Z . boulosii*	*Z. coccineum*	*Z. hamiense*	*Z . mandavillei*	*Z . migahidii*	*Z. propinquum*	*Z. qatarense*	*Z. fabago*
Out line	Cylindrical wavy margin	--------	Cylindrical	linear	kidney	kidney	linear	Cylindrical	Cylindrical	Cylindrical	winged irregular
Mesophyll	unilateral	--------	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	unilateral
Main vascular tissue	central asymmetric vascular cylinder	--------	central cress shape V.B. accompanied by 2 lateral V.B.	central tribranched V.B. accompanied by 2 lateral V.B.	central cress shape V.B. accompanied by 2 lateral V.B.	only central V.B.	central cress shape V.B. accompanied by 2 lateral V.B.	only central cress shape V.B.	central cress shape V.B. accompanied by 2 lateral V.B	central cress shape V.B. accompanied by 2 lateral V.B	complete vascular cylinder accompanied by 2 lateral V.B

Table (3): Leaf blade anatomical characters of the *Zygophyllum* species in Saudi Arabia

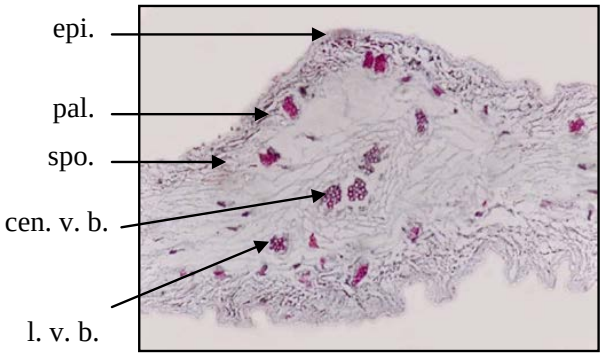
Characters	Agrophyllum										Zygophllotypus
	Bipartita		*Mediterranea*								*. fabago*
	Z. decumbens	*Z. simplex*	*Z. album*	*Z . boulosii*	*Z. coccineum*	*Z. hamiense*	*Z . mandavillei*	*Z . migahidii*	*Z. propinquum*	*Z. qatarense*	*Z. fabago*
Out line	winged linear	elliptical	elliptical	linear	kidney	kidney	linear	linear	elliptical	linear	winged linear
Mesophyll	unilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	bilateral	unilateral
Main vascular tissue	central V.B. accompanied by 6 lateral V.B. at same level	only central V.B.	central dichotomous V.B. accompanied by 2 lateral V.B.	central V.B. accompanied by 2 lateral V.B.	5-7 central separated V.B accompanied by 2 lateral V.B.	5-7 central separated V.B.	central V.B. accompanied by 2 lateral V.B.	central V.B. accompanied by 1 lateral V.B.	only central dichotomous V.B.	only central cress shape V.B.	complete vascular cylinder accompanied by 5 lateral V.B .in each wing
Crystals	----------	druses	----------	----------	druses	druses	----------	----------	druses	druses	----------



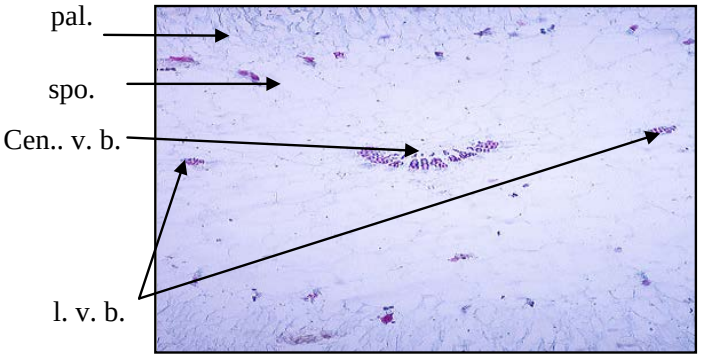
T.S. in *Z. decumbens petiole* x 50
Figure.2b



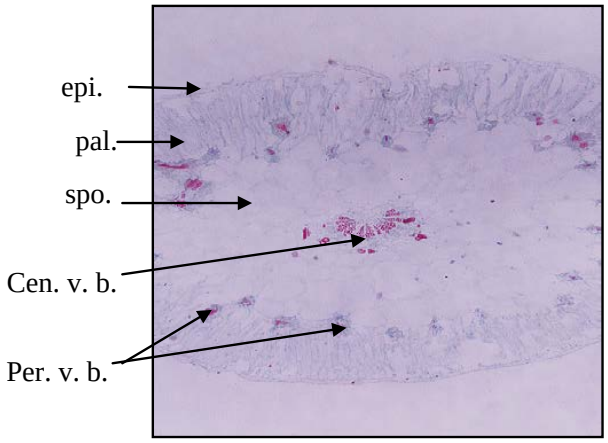
T.S. in *Z. mandavillei petiole* x 50
Figure.3b



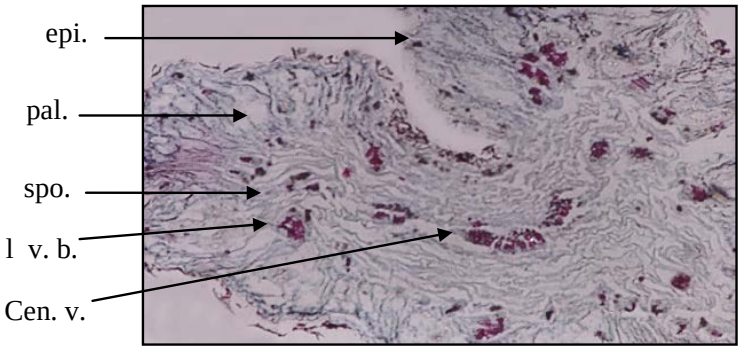
T.S. in *Z. boulosii petiole* X50
Figure.4b



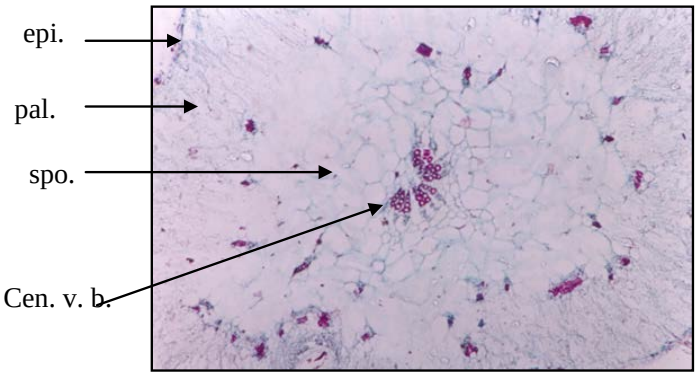
T.S. in *Z. album petiole* X 50 Figure.5b



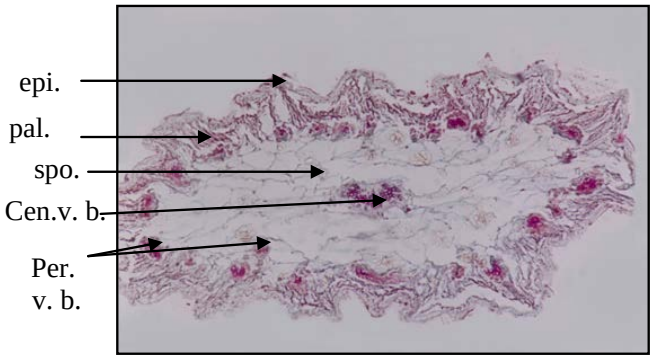
T.S. in *Z. migahidii petiole* x 50 Figure.6b



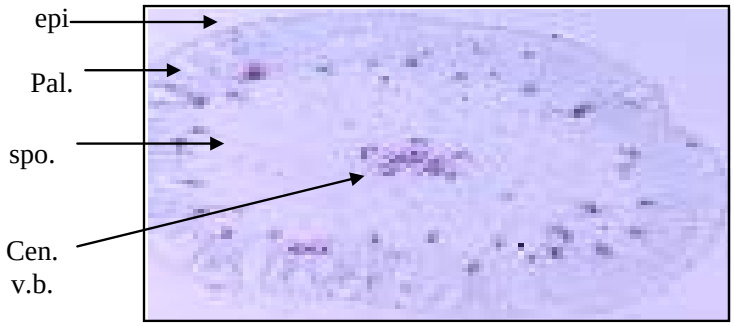
T.S.in *Z. propinquum petiole* x 50 Figure.7b



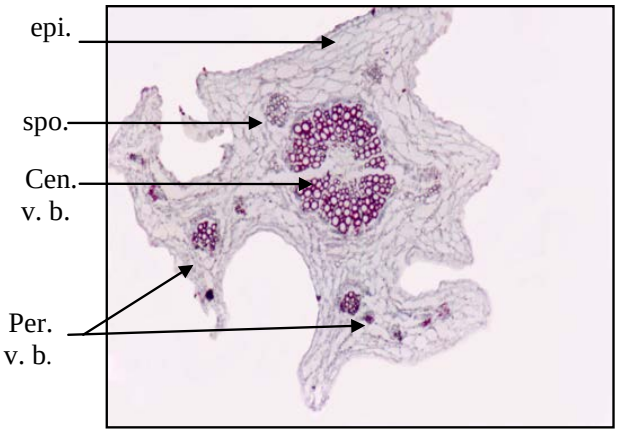
T.S. in *Z. coccineum petiole* X50
Figure.8b



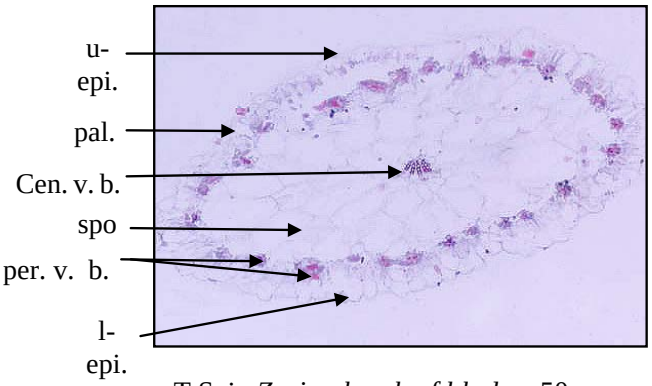
T.S. in *Z. hamiense petiole* x 50 Figure.9b



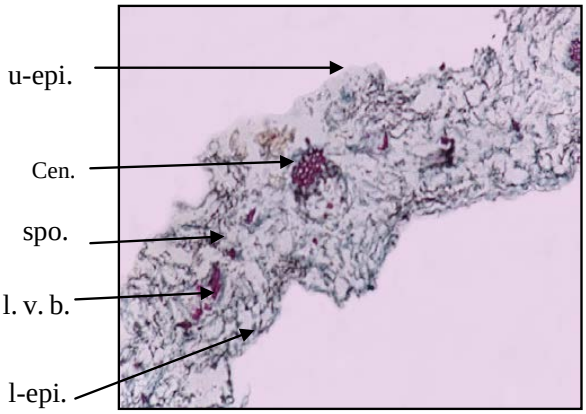
T.S. in *Z. qatarenes petiole* x 50 Figure.10b



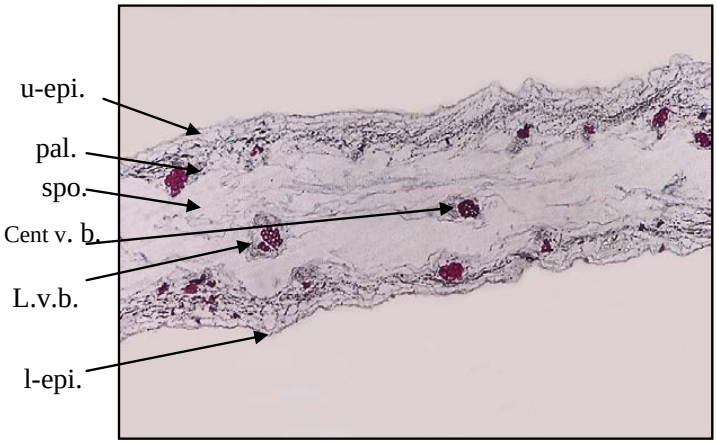
T.S. in *Z. fabago petiole* x 50 Figure.*11b*



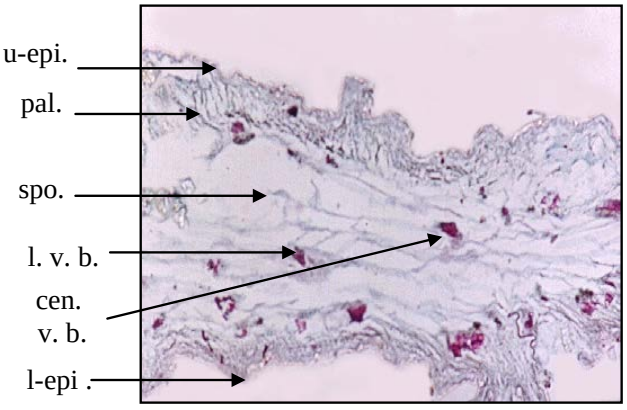
T.S. in *Z. simplex leaf blade* x 50
Figure.1



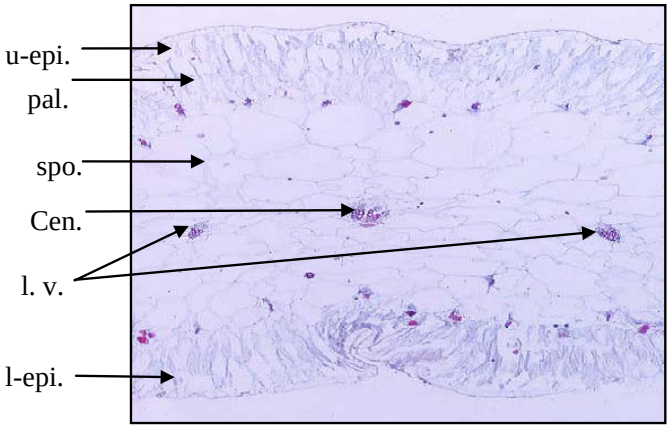
T.S. in *Z. decumbens leaf blade* x 50
Figure.2a



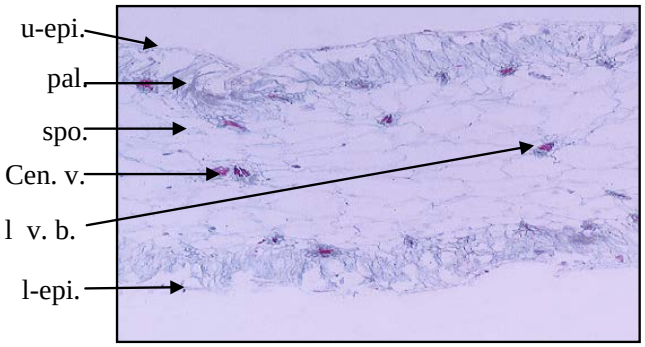
T.S. in Z. mandavillei leaf blade x 50 Figure.3a



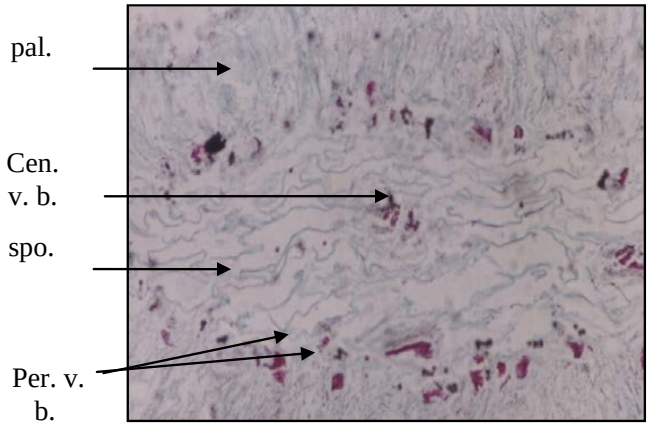
T.S.in *Z. boulosii leaf blade* X50 Figure.4a



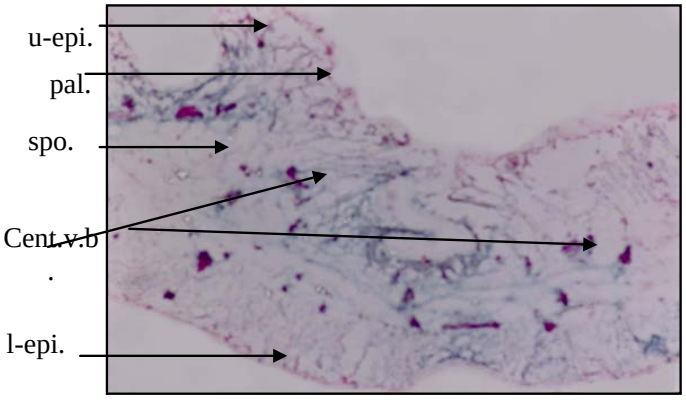
T.S. in *Z. album leaf blade* X 50 Figure.5a



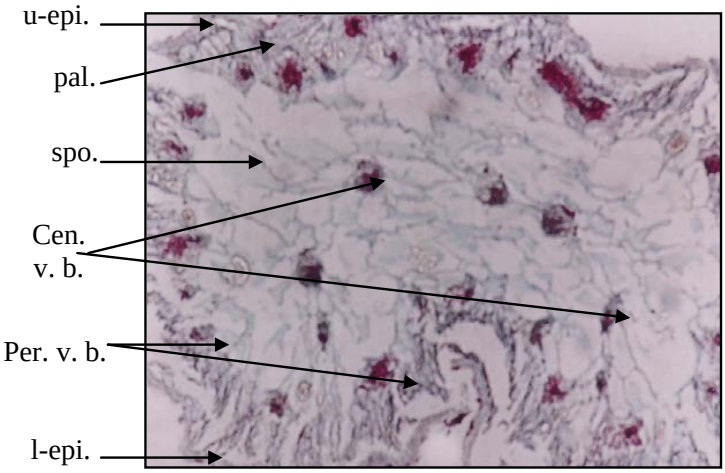
T.S. in *Z. migahidii leaf blade* x 50 Figure.6a



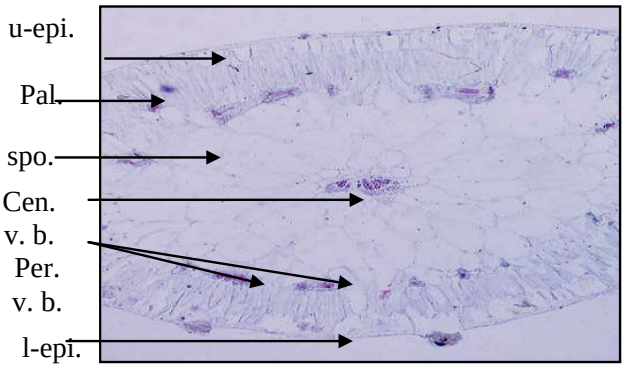
T.S. in *Z. propinquum leaf blade* x 50 Figure.7a



T.S.in *Z. coccineum leaf blade* X50 Figure.8a



Z. *hamiense leaf blade* x 50 T.S. in Figure.*9a*



T.S. in *Z. qatarenes leaf blade* x 50 Figure.10a

3.2. Statistical results:

In table (4 &5) and figure (12&13) the distribution of lateral vascular bundles and their position relative to the central vascular bundle varied from one species to another in leaf blade except in Z. *boulosii and Z. mandavillei* .were similar. Also the distribution of lateral vascular bundles and their position relative to the central vascular bundle varied from one species to another in leaf petiole except in *Z. album, Z.coccineum, Z. mandavillei, Z propinquum and Z.quatarense* which are similar

Table (4)Tally for Discrete Variables: VB in Leaf blade

VB type	Count	Percent
5-7centra VB +lateral VB	1	9.09
5-7central VB+2lateral VB	1	9.09
central cress VB	1	9.09
central di VB	1	9.09
central di VB +2 lateral VB	1	9.09
central VB	1	9.09
central VB+1lateral VB	1	9.09
central VB+2lateral VB	2	18.18
central VB+6lateral VB	1	9.09
complete VB + 5 lateral VB	1	9.09
N= 11		

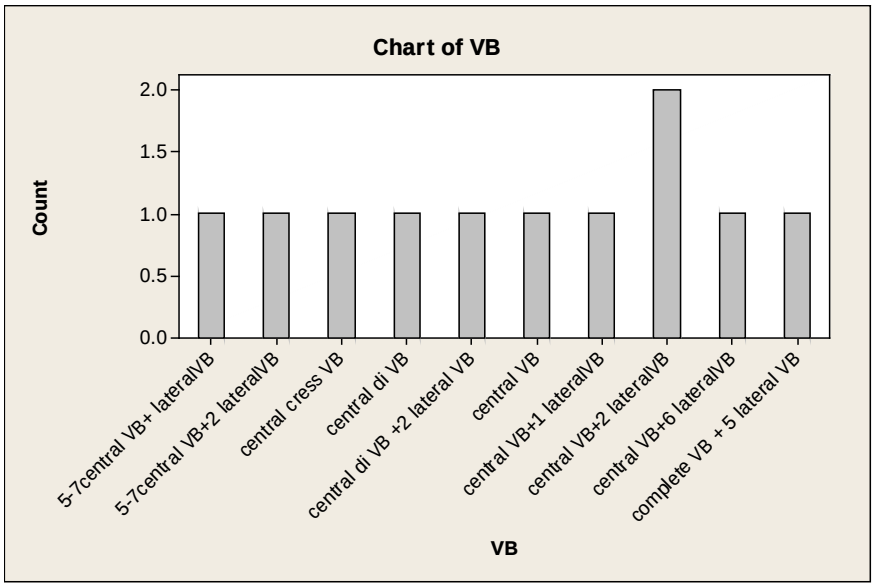
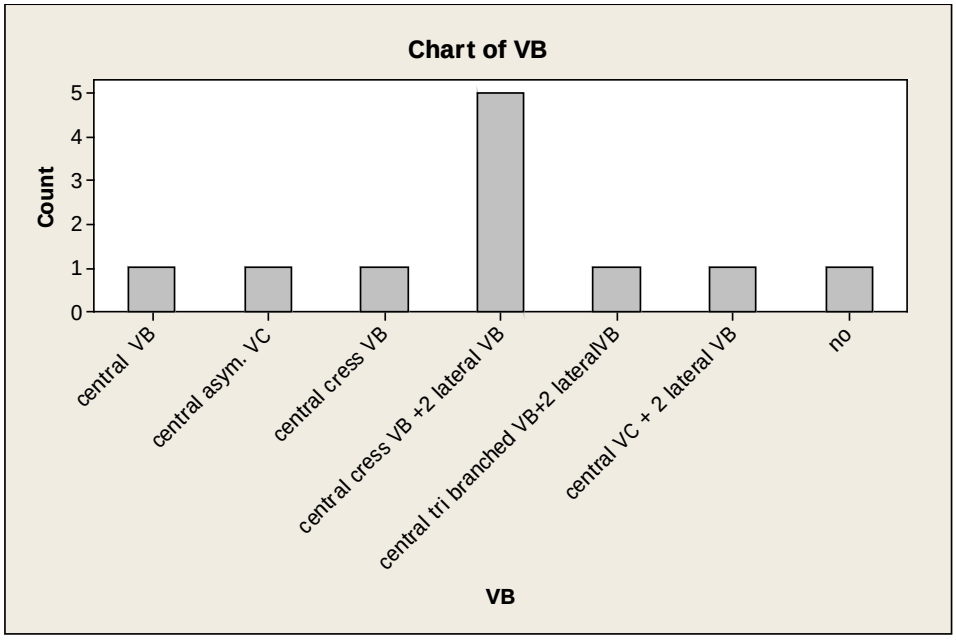


Figure (12) Position of lateral Vascular bundle in relation to central vascular bundle in leaf blade

Table (5) Tally for Discrete Variables: VB in petiole

VB type	Count	Percent
central VB	1	9.09
central asymmetric VB	1	9.09
central cress VB	1	9.09
central cress VB +2 lateral VB	5	45.45
central tri branched VB+2lateral VB	1	9.09
central VC + 2 lateral VB	1	9.09
no petiole	1	9.09
N= 11		



Figure(13) Position of lateral Vascular bundle in relation to central vascular bundle in leaf petiole

4. Discussion

Vascular tissue in both leaf blade and petiole are proved to be the most significant characters among the investigated taxa. It is to be noted that the vascular system is composed of two whorls the inner whorl with main or central vascular bundle or bundles accompanied with number of lateral vascular bundles and the outer one with number of peripheral vascular bundles representing the veinlets. Comparing the leaf blabe characters of both *Zygophyllum* species of section Bipartita: *Z. simplex* (Figure. 1) have only one central vascular bundle while *Z. decumbens* (Figure. 2a) have one central vascular bundle accompanied by 6 lateral vascular bundles 3 in each wing . In species of section Mediterranea vascular tissue show high variation: *Z. mandavillei* (Figure. 3a)and *Z. boulosii* has (Figure. 4a) one central vascular bundle accompanied by 2 lateral vascular bundles, while the central vascular bundle in *Z.album* (Figure. 5a), *Z. migahidii* (Figure. 6a) and *Z. propinquum* (Figure. 7a) is dichotomously branched. In *Z. coccineum* (Figure. 8a) and *Z. hamiense* (Figure. 9a) the central vascular tissue is subdivided into number of vascular bundles. *Z. qatarense* (Figure. 10a) which *belong* also to section *Mediterranea* take a cress shape central vascular bundle. *Z. fabago* (Figure. 11a) (section Fabago of subgenus *Zygophyllotypus*) shows a distinct complete vascular cylinder in both leaf blade and petiole

Vascular tissue in petiole of the *Zygophyllum* species show a less variation even *Z. simplex* is sessile all the other species has one central vascular bundle accompanied by 2 laterals. Shape of the central vascular tissue range between an asymmetric cylinder in *Z. decumbens*, (Figure. 2b) tribranched in *Z. boulosii* (Figure. 3b) and a cress shape in all other lifted species. Table (2)&(3) also show other characters viz. petiole and leaf blade out line, type of mesophyll and presence or absence of crystals, which are of less systematic value and can be used for the distinction of certain species. Leaf blade out line ranging between winged linear in *Z. decumbens* and *Z. fabago* and linear in, *Z. mandavillei; Z. migahidii* and *Z. qatarense*, while it is elliptical in *Z. simplex*, *Z.album* and *Z. propinquum* , it takes an irregular kidney shape in *Z. coccineum* and *Z. hamiense* .Petiole out line takes an irregular cylinder shape with wavy margin in all species except elliptical in *Z. mandavillei* (Figure.3b) and linear in *Z. boulosii*(Figure.4b) . Type of mesophyll in both leaf blade and petiole is identical, unilateral in *Z. decumbens* (Figure. 2b) and *Z. fabago* (Figure.11b) and bilateral in all other species. Druses crystals can be seen in *Z. simplex, Z. qatarense* (Figure. 10b) *Z. coccineum* (Figure 8b) , *Z. hamiense* and *Z. propinquum.* (Figure. 7b) Utilization of artificial key based on anatomical diagnosis is specially recommended in seasons where the plants are devoid of flowers

5. Conclusion

In view of the previous results differentiating the various anatomical characters on leaf blade and petiole the following key shows the efficiency of such characters between the 11 investigated species of *Zygophyllum.*

1.a. Leaf blade vascular tissue of one central vascular bundle----------2
b Leaf blade vascular tissue of separated vascular bundles ----------10
2. a.Central vascular bundle not accompanied with lateral V.B. ----------3
b. Central vascular bundle accompanied with lateral V.B. ----------5
3.a. Central vascular bundle dichotomous branching ---------- *Z. propinquum*
b. Central vascular bundle simple ----------4
4.a.. cress shape---------- *Z. qatarense*
b .Not as above ---------- *Z. simplex*
5.a. Central vascular bundle dichotomous branching----------6
b. . Central vascular bundle simple----------7
6.a. Central vascular bundle accompanied with 2 lateral V.B-------- *Z.album*
b. Central vascular bundle accompanied with 1 lateral V.B---- *Z. migahidii*
7 .a.Central vascular bundle accompanied with 2 lateral V.B----------8
b. Central vascular bundle accompanied with more than 2 lateral V.-------9
8.a. Central petiole vascular bundle tribranched---------- *Z. boulosii*
b. Central petiole vascular bundle not as above---------- *Z. mandavillei*
9.a.. Asymmetric vascular cylinder in petiole---------- *Z. decumbens*
b. Complete vascular cylinder in petiole---------- *Z. fabago*
10.a. Central vascular bundle accompanied with lateral V.B-- *Z. coccineum*
b Central vascular bundle not accompanied with lateral V.B- *Z. hamiense*

References:

1) Ahmed, K. A. Anatomical Studies On the Desert Plant *Zygophyllum album* L. *Desert Inst. Bull.,A.R.E*., (1989):39 (Suppl.), 433- 448p.
2) Boulos, L. Flora of Egypt. Alhadara publishing. Cairo. Egypt. 2000: 2
3) Chaudhary, S. A.. Flora of the Kingdom of Saudi Arabia Illustrated Ministry of Agriculture & Water, Kingdom of S. A. 2001:2 (1)
4) Collenette, S. Wild Flowers of Saudi Arabia. National Commission for Wildlife Conservation and Development (NCWCD) Kingdom of Saudi Arabia. 1999
5) El-Hadidi, M. N. Two new Zygophyllum Species from Arabia. *Cairo Univ. Haerb*. 1977: 327-329. (7&8)
6) Engler, A. & Prantle, K.).Zygophyllaceae-Engler, A. prantal.,K. (edts). Die naturlichen Planzenfamilien 1931:.2 (19) a *Leipzig: Engelmann*.
7) Hosny, A. I. . Genus Zygophyllum L. in Arabia. *Täeckholmia* 1988 *(* 11): 19-32.
8) Migahid, A. M. and Hammouda .). Flora of Saudi Arabia. King Saud Univ Libaries. Riyadh. 1978:2(1)
9) Van Huyssteen, D. C. Morphologish-systematische studien .Uber die Gattung *Zygophllum* mit besonderer berucksichtigung der africanischen Arten. Diss, *Math. Naturwiss. Fak. Friedrichs. Wilhelms Universitat* Berlin

3/11/2011

Life Science Journal

(Acta Zhengzhou University Overseas Edition)

Call for Papers

The academic journal "***Life Science Journal***" (ISSN: 1097-8135) is inviting you to publish your papers.

Life Science Journal, the Acta Zhengzhou University Oversea Version registered in the United States, is an international journal with the purpose to enhance our natural and scientific knowledge dissemination in the world under the free publication principle. The journal is calling for papers from all who are associated with Zhengzhou University-home and abroad. Any valuable papers or reports that are related to life science-in their broadest sense-are welcome. Other academic articles that are less relevant but are of high quality will also be considered and published. Papers submitted could be reviews, objective descriptions, research reports, opinions/debates, news, letters, and other types of writings. Let's work together to disseminate our research results and our opinions.

Please send your manuscript to editor@sciencepub.net.

Address:
Life Science Journal - Acta Zhengzhou University Overseas Edition
Marsland Press
PO Box 180432, Richmond Hill, New York 11418, USA
Telephone: (347) 321-7172
Emails: editor@sciencepub.net; sciencepub@gmail.com; lifesciencej@gmail.com;
Website: http://www.sciencepub.net; http://www.lifesciencesite.com

Volume 8, Number 3, (Cumulative No.26) Part 3 September 25, 2011 ISSN:1097-8135

Life Science Journal

Marsland Press

PO Box 180432, Richmond Hill, New York 11418, USA

Website:
http://www.sciencepub.net

Emails:
editor@sciencepub.net
sciencepub@gmail.com

Phone: (347) 321-7172

ISSN 1097-8135