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CONTENTS

Pistachio Export Development Strategies in Kerman Province, Iran

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Abstract: The historical study of pistachio exports shows that they constitute a major share of non-oil exports. In addition, pistachios exports need to recognize the internal and external factors that affect the trend of pistachio exports, and, furthermore, strategic planning is required. In this study, by using a measuring method, the strengths, weaknesses, opportunities and threats were identified in the form of a SWOT Table. To design the pistachio export development strategies for Kerman the presented strategies and the result of quantitative strategic planning matrix were considered, and market development strategies were introduced as the main and most beneficial strategies for pistachio. In addition, Kerman pistachio exports, for achieving success in global markets, should follow conservative strategies. Most of the conservative strategies including market development were determined by the position assessment matrix and strategic action (SPACE).
[Hamid Taboli, Mehdi Yadollahi. **Pistachio Export Development Strategies in Kerman Province, Iran** Life Science Journal,. 2011; 8(4): 304-313] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key word: SWOT analysis, pistachio exports, strategy implementation, Kerman pistachio

Introduction:

Pistachio is one of the agricultural products that are synonymous with the country of Iran. Furthermore, its production has a long historical background in the country, having been cultivated and exploited for millennia. Self-grown and wild forests of pistachio in the north-eastern region of Iran and adjacent areas of Turkmenistan and Afghanistan have a tremendous history and it is supposed that the pistachio tree has been planted and domesticated in Iran for about 3000 – 4000 years (Panahi, 2002).

Agricultural products comprise the major share of Iran's non-oil exports. Among the agricultural products, pistachio is very important, being the second major export good after hand-made carpets. Some studies have been carried out concerning the export of pistachio and pistachio export development strategies. These include:

Sedaghat (2008) conducted a study about the growth of pistachio and its effective factors on the competitive capability of Iranian pistachio in the global market. In this study, the exponential trend models, market stable share and time-series data related to the years of 1991-2003 were used. The results indicate that the growth rate of the area under cultivation is meaningful and is equal to 5/2%. While the growth rate of production, value of export was not significant, market distribution and the competitive effect played an important role in changing the rate of Iran's pistachio export between 1996 and 1999. However, the effect of product composition and market distribution were the most important factors in changing the rate of pistachio exports between 2000 and 2003 (Sedaghat, 2008).

Sedaghat (2008) analysed the export pattern and competitiveness of Iran's pistachio in the global market. The results of his study show that the exporting to Europe is the most efficient marketing path for Iran's pistachio, and exporting to central Asia and Arab countries has a higher efficiency in comparison with the other markets. Between 1996 and 2003, the United Arab Emirates was the most stable export market for Iran, with Russia and Germany following. In the years of 1996 to 1999, the changes in the export value were negative, whereas in 2000-2003 they were positive. The main reason for these changes was the effect of market distribution. His study also shows that the price of pistachio in the markets of Iran and the U.S.A. do not show an adequate correlation, therefore, it seems that Iran's Internal pricing system does not have adequate efficiency (Sedaghat, 2008).

Sedaghat and Surya Prakash(2006) studied the production and marketing constraints of Iran's pistachio. The results showed that from the viewpoint of producers, agricultural water shortage is in first place, inappropriate market structure, price fluctuations and producer's low prices are commonly in second place and chemical fertilizer shortage is in third place. However, from the viewpoint of exporters and producers, the first problem relates to the existence of Aflatoxin, inappropriate and changing policies of the government, and inadequate supply of products to market (Sedaghat & Suryaprakash, 2006).

Sedaghat and Surya Prakash (2006b) in addition to the presentation of the most important problems in the available system studied the possibility of a reciprocal contractual management system for Iran's pistachio marketing and production. They

believe that the current system of production management and pistachio market has low efficiency. Using the reciprocal contractual system could lead to promoting the production efficiency and improving the quality of pistachio production. In addition, by applying the new system the farmers' income and producers received price would improve (Sedaghat & Surya, 2006).

In this study, by using the results of the presented studies, in addition to enumerating the strengths and weaknesses, and opportunities and effective threats on pistachio exports, as well as the presentation of pistachio export development strategies, we provide a quantitative strategic planning matrix.

Methodology:

In this study, the descriptive and measurement method was used. Initially, by studying the internal factors (strengths and weaknesses) and external factors (opportunities and threats), a list of strengths, weaknesses, opportunities and threats were determined, then, through questionnaires recording the opinions of the Kerman pistachio exporters, the ranking and weighting of these internal and external factors were studied.

Subsequently, by extracting the Tables of the internal factors analysis summary (IFAS) and external factors analysis summary (EFAS), the SWOT model was designed and the appropriate strategies determined.

Weakness point W	Strengths point S	
WO	SO	opportunities O
WT	ST	threats T

Source (Daivid, 2006)

For determining the sample size the Sharp-Cochran method was used, subsequently, by using a random sampling method, a questionnaire was completed from the statistical universe.

Sharp-Cochran formula:

$$n = \frac{Nt^2pq}{Nd^2 + t^2pq}$$

N= population size
n= sample size
t= 1.96
P= the possibility of property existence (95%)
q= the possibility of property non-existence
d=sampling error (0.05)

By considering the Sharp-Cochran formula, 31 exporters were chosen as a population size.

Findings:

Internal factors affecting on Kerman pistachio exports

By studying the internal factors the basic weaknesses and strengths of the firms' duty units can be determined, which provide the list of the most important and effective internal factors for the firms to be competitive. Thus, the table of internal factors analysis (IFAS) is provided (Daivid, 2006).

Table 1: The summary of internal factors analysis of pistachio export (IFAS)

index	Score	Rank	Weight	
1- The existence of comparative advantage in pistachio export	0.151	2.85	0.053	Strengths points
2- The existence of relative share of export	0.132	2.60	0.051	
3- The productive capacity of country (physical factors such as water, soil, …)	0.137	3.20	0.043	
4- Entrepreneurship	0.168	3.50	0.048	
5- High profitability	0.138	3.30	0.042	
6- Comparative quality with foreign productions	0.160	3.20	0.050	
7- High nutritive value	0.153	2.95	0.052	
8- A vast background of pistachio export	0.111	2.86	0.039	
9- Market's need for production	0.110	3.15	0.035	
10- Possibility of providing foods and new material from pistachio	0.105	3.10	0.034	
11- Low production costs of the competitors	0.117	3.80	0.031	
12- The existence of surplus capacity to the province's need	0.094	2.95	0.032	

1- Liquidity shortage	0.162	3.25	0.050	Weakness points
2- Export organizations' weaknesses	0.118	3.12	0.038	
3- Inappropriate packaging and lack of mechanized packing system	0.164	3.17	0.052	
4- Lack of strategic planning for cultivation and world trade	0.109	2.80	0.039	
5- Non-utilization of successful exporter countries experiences	0.108	3.50	0.031	
6- Processing industry's weaknesses	0.142	3.48	0.041	
7- Inefficiency of available infrastructure (storage, cold storage…)	0.159	3.80	0.042	
8- Lack of standardization in particular pistachio	0.116	2.90	0.040	
9- Contamination of products with Aflatoxin	0.154	3.60	0.043	
10- Traditional and non-scientific presence in target market	0.129	2.95	0.044	
11- Lack of variation in export products	0.146	3.85	0.038	
12- Weaknesses of suitable and efficient information system	0.117	3.67	0.032	
Total	3.20	----	1	

The importance (weight) of effective indexes on pistachio exports and exporters' reaction to the mentioned factors (rank) were studied by 31 Kerman exporters.
(Total weight equals 1 and the rank criterion is from 1-5).

Weights and ranks in a questionnaire with the ranges of, very low, low, average, high and very high. By considering the Table 1, the number of strengths of Iran's pistachio exports is 12 and the number of weaknesses of Iran's pistachio exports is also 12. Among the strengths, the highest score of importance is related to the "existence of relative advantages in pistachio production" with the weight of 0.053 and rank of 2.85, and the lowest score of importance is related to the "low production costs of the competitors" in which its weight is 0.031 and its rank is 3.80. Also, among the weaknesses, the highest importance score is related to "inappropriate packaging and lack of mechanized packaging system" with a weight of 0.052 and rank of 3.17. The lowest importance score is related to "non-utilization of successful exporting countries, experience" with the weight of 0.031 and rank of 3.50. By considering (3.20), generally, it can be said that the reaction of exporters to the weaknesses and strengths in pistachio export was higher than average (Table1).

Effective external factors on Kerman pistachio exports

The aim of this study is to provide a final list of opportunities that can be used and also a list of threats that can be avoided. Strategists can evaluate economic, social, cultural, and ecological factors, political, governmental, environmental, legal, technological factors and competitive information by using an external factors analysis summary matrix (EFAS) (Daivid, 2006).

Effective environmental factors on Kerman pistachio exports (opportunities and threats of external environment) along with the rate of significance (weight) and the reaction of exporters to the mentioned factor (rank) were determined by the statistical sample (Total weight equals 1 and rank scale equals 1 to 5). By considering the calculated mode, among these opportunities, the highest importance score is related to the "existence of universities and higher education centres relevant to the agricultural section" with the weight of 0.053 and rank of 3.12, however, the lowest importance score is related to "the settling of business's advisors in the target countries" in which its weight is 0.035 and its rank is 2.81. In addition, among the threats, the highest importance score is related to the "available bureaucracy in customs, airports,….." in which its weight is 0.055 and its rank is 3.80, and the lowest importance score is related to the" government's excessive office in economy" with a weight of 0.039 and rank of 2.5.

By considering the total score (2.89), generally, it can be said that the reaction of exporters to the environmental opportunities and threats of pistachio exports has been less than average (Table2).

Table 2: The Summary of External Factors Analysis of Pistachio Export (EFAS)

index	Score	Rank	weight	opportun…
1- Internal and external exhibition	0.112	2.81	0.040	
2- The governments' attention to the non-oil exports	0.153	3.4	0.045	
3- Develop ports for expanding trade relations and goods' transit	0.165	3.3	0.050	

4-Proximity to the markets of central Asia, the Middle East and the Persian Gulf	0.166	3.2	0.052	
5- Possibility of investment in the private sector	0.149	3.11	0.048	
6- Universities and higher educational centres relevant to the agricultural section	0.165	3.12	0.053	
7- Export incentives	0.133	3.5	0.038	
8- Setting business advisors in target countries	0.098	2.81	0.035	
9-Government supportive policies from internal productions	0.121	2.90	0.042	
1- Problems resulting from bank credit	0.119	2.66	0.045	
2- Bureaucracy in the customs, airport…	0.209	3.8	0.055	
3- Competitors from abroad	0.189	3.5	0.054	
4- Competitors' advertising about Iran's pistachio pests in and out of the country	0.153	3.4	0.045	
5- Multiplicity and contradiction in expert instructions	0.137	2.6	0.053	
6- Production's frostbite and drought, and severe shortage of water resources	0.148	3.3	0.045	
7- Government's excessive office in economy	0.097	2.5	0.039	**threats**
8- Competitiveness ability reduction in global markets due to real foreign exchange's rate reduction	0.105	2.4	0.044	
9- Unhealthy competition of exporters in target markets	0.132	2.88	0.046	
10- Weakness of banking system in developing the commercial activities of the province	0.095	2.5	0.038	
11- High interest of bank facilities	0.099	2.21	0.045	
12- High fluctuations of export goods	0.097	2.31	0.042	
13- Lack of capable and experienced national institution in export	0.147	3.2	0.046	
Total	2.98	--	1	

Pistachio export development strategies design by using SWOT model

In the SWOT Matrix the balance and equilibrium is established between the firm's main external and internal factors. Strategists can present four types of strategy by using this matrix: SO strategies, WO strategies, ST strategies and WT strategies. In implementing SO strategies, the organization can exploit the external opportunities by using the internal strengths points. The aim of WO strategies is that a firm using the opportunities in an external environment tries to improve the internal weakness points. By implementing the ST strategies firms try to reduce the consequent effects of the threats in the external environment. The firms that implement WT strategies show a defensive state and their aim is to reduce the internal weakness points and avoiding the threats of an external environment (Daivid, 2006).

Providing a quantitative strategic planning matrix for pistachio export

For implementing the strategies a quantitative strategic planning matrix, (QSPM), is used as an analytical framework. By using this method, the various strategies or the best strategies can be determined, objectively. The process of providing a quantitative strategic evaluating matrix includes six stages, as follows (Daivid, 2006).

First stage- Opportunities, threats, weaknesses and strengths are written in the right hand column of the quantitative strategic planning matrix.

Second stage- Coefficient and weight is given to each of the external and internal factors in which they have a major role in firm's success. These coefficients are from zero (insignificant) to 1 (very important). Coefficients show the relative importance of one factor (In terms of firm's success in a relevant industry)

Third stage- The strategies obtained from the SWOT matrix are written in the top row of the quantitative planning matrix. If it is possible, these strategies should be contradictory.

Fourth stage- The attractiveness scores are determined. These are the numerical values that show the attractiveness of each strategy in a set of strategies. For determining the attractiveness score, the internal and external factors should be studied and whether these factors play a role in the process of strategy selection or not? The attractiveness score is in this form: 1= without attraction, 2= somewhat attractive, 3= reasonable attraction, 4= very attractive. If none of these factors plays a role in the process of strategies selection, then the attractive score should not be awarded.

Fifth stage- Total attractive score is calculated.

Sixth stage- Total attractive score is calculated. For this purpose, the total attractive score is calculated from each column, with the high scores indicating the strategies' attraction, by considering all the internal and external factors that can affect the strategic decisions.

The quantitative strategic planning matrix of pistachio export is as follows:

Table 3: SWOT Matrix of Pistachio Export

Internal factors / **External factors**	**Strengths (S)** 1- The existence of comparative advantage in pistachio export 2- The existence of relative share of export 3- The productive capacity of country (physical factors such as water, soil, …) 4- Entrepreneurship 5- High profitability 6- Comparative quality with foreign productions 7- High nutritive value 8- A vast background of pistachio export 9- Markets need for production 10- Possibility of providing foods and new material from Pistachio 11- Low production costs to the competitors 12- The existence of surplus capacity to the province's need	**Weaknesses (W)** 1- Liquidity shortage 2- Export organizations' weaknesses 3- Inappropriate packaging and lack of mechanized packing system 4- Lack of strategic planning for cultivation and world trade 5- Non-utilization of successful exporter countries experiences 6- Processing industries' weaknesses 7- Inefficiency of available infrastructure (storage, cold storage…) 8- Lack of standardization in particular pistachio 9- Contamination of products with Aflatoxin 10- Traditional and non-scientific presence in target market 11- Lack of variation in export products. 12- Weaknesses of suitable and efficient information system.
Opportunities (O) 1- Internal and external exhibition 2- The governments' attention to non-oil exports 3- Develop ports for expanding trade relations and goods' transit 4- Proximity to the markets of central Asia, the middle east and Persian Gulf 5- Possibility of investment in the private sector 6- Universities and higher educational centres relevant to the agricultural section 7- Export incentives 8- Setting business advisors in target countries 9- Government supportive policies from internal productions	**SO strategies (offensive)** Increasing exports to the Persian Gulf and central Asia S11, S2, S1, S3, S4, S5 Encouraging the inventors of agricultural Technologies (Market development) S1, S3, S11, O1, O2, O4, Employing the exports for producing high quantity production. S1, S2, S6, S9, O2, O4, O5 Introducing products to the markets (penetrate the market). S1, S3, S8, O1, O3, O4, O5 Customs facilities for the exporters of packaged pistachio appropriate to the modern Technology (Market development). S1, S2, O1, O4, O7, O9	**WO strategies (cautiously)** Variation in the pistachio export productions (product development). W2, W3, W11, W12, O1, O4, O6 Increasing and improving the research and development (R&D) activities (Market development). W2,W4,O1,O2,O9 Implementing the pistachio strategic planning in the field of pistachio export and cultivation (Market development). W2, W4, O2, O4,O8 Developing the processing industries in Kerman (product development) W2, W3, W6, W11, O2, O4 Information technology and e-commerce development (Market development). W2, W3, W5, O3, O4, O8

Treatments (T) 1- Problems resulting from bank credit 2- Bureaucracy in the customs, airport… 3- Competitors from abroad 4- Competitors' advertising about Iran's pistachio pests in and out of the country 5- Multiplicity and contradiction in export instructions 6- Production's frostbite and drought, and severe shortage of water resources 7- Government's excessive office in economy 8- Competitiveness ability reduction in global markets due to real foreign exchange rate reduction 9- Unhealthy competition of exporters in target markets 10- Weakness of banking system in developing the commercial activities of province 11- High interest of bank facilities 12- High fluctuation of export goods 13- Lack of capable and experienced national institution in export	**ST strategies (competitive)** Increasing control over the distribution system (forward integration). S1, S2, S9, T2, T3, T6, T9 Creating and expanding the free trade zones (vertical integration to top). S1, S2, S3, S8, T2, T3 Establishing laboratories for determining the bacterial and fungal contamination. S1, S2, S10, T3, T4	**WT strategies (defensive)** Increasing and improving marketing activities (penetrate the market) W2, W3, W4, T3, T4 Promoting packaging Technology (penetrate development). W2, W3, W11, T1, T2, T9 Following the global industrial standards (penetrate development). W2, W4, W8, T3, T8, T9 Using export models of successful exporting countries (Market development). W2, W4, W5, T3, T6, T8, T13

Table 4: Quantitative strategic planning matrix (QSPM) of Kerman pistachio

Factor determining success	Coefficient	Strategies under study 1		2		3	
Opportunities		AS	TAS	AS	TAS	AS	TAS
Universities and higher educational centres relevant to the agricultural section	0.15	3	0.45	4	0.6	2	0.3
Proximity to the markets of central Asia, the Middle East and Persian Gulf	0.15	3	0.45	3	0.45	2	0.3
Develop ports for expanding trade relations	0.08	3	0.24	3	0.24	----	----
Possibility of investment in the private sector	0.08	----	----	----	----	1	0.08
The governments' attention to the non-oil export	0.1	2	0.2	3	0.3	----	----
Threats							
Bureaucracy in the customs, airport…..	0.13	4	0.52	4	0.52	2	0.26
Competitors from abroad	0.1	3	0.3	4	0.4	----	----

Multiplicity and contradiction in export institutions	0.08	1	0.08	2	0.16	3	0.24
Lack of capable and experienced national institution in export	0.07	----	----	----	----	1	0.07
Production's frostbite and drought, and severe shortage of water resources	0.06	2	0.12	1	0.06	1	0.06
Strengths							
The existence of comparative advantage in pistachio export	0.15	1	0.15	4	0.06	3	0.45
The existence of relative share of export	0.12	3	0.36	2	0.24	2	0.24
Comparative quality with foreign productions	0.08	2	0.16	1	0.08	1	0.08
Entrepreneurship	0.08	1	0.08	1	0.08	2	0.16
The productive capacity of country (physical factors such as water, soil,)	0.07	----	----	----	----	----	0.07
Weaknesses							
Inappropriate packaging and lack of mechanized packing system	0.15	2	0.3	3	0.45	2	0.3
Liquidity shortage	0.1	2	0.2	3	0.3	1	0.1
Traditional and non-scientific presence in target markets	0.1	----	----	----	----	1	0.1
Contamination of products with Aflatoxin.	0.08	1	0.08	2	0.16	----	----
Inefficiency of available infrastructure (storage, cold storage…)	0.07	1	0.07	1	0.07	2	0.14
Total attractive score	1		3.76		4.91		2.97

1 =Market penetration, 2= Market development, 3= Product development
AS= Attractive score, TAS= Total Attractive score

By considering the quantitative strategic planning Matrix (QSPM) for pistachio, a Market development strategy with attractive score 4.91, is known as a more attractive strategy.

Appropriate strategies implementation.

Developing non-oil exports causes economic growth in developing countries and developed countries, and in this way, the payment deficits in these countries will be compensated. Government efforts for developing the exports can include the normal responsibilities of business such as research about the export markets, sales opportunities holding international exhibitions, establishment of export development office in relevant foreign countries and traditional forms of export development including tax incentives, foreign exchange subsidies, export special credits, export insurance, and export awards (Akhavy, 1995).

After studying the strengths and weaknesses and determining the Environmental factors (opportunities and threats), in the SWOT model and using the quantitative strategic planning matrix (QSPM), the appropriate strategies for pistachio export developments are:

- Increasing exports to the Persian Gulf and central Asia (Market development).
- Encouraging the invention of agricultural technology (Market development).
- Customs facilities for the exporters of packaged pistachio appropriate to the modern Technology (Market development).
- Increasing and improving research and development (R&D) activities (Market development).
- Implementing strategic planning in the field of pistachio export and cultivation (Market development).

- Information technology and e-commerce development (Market development).
- Using export models of successful exporting countries (Market development).

Also, the other presented strategies in the SWOT model for pistachio export development are:

A) SO strategies (offensive)

- Employment the exports for producing high quality production (product development).
- Introducing products to the markets (penetrate the market).

B) ST strategies (competitive).

- Increasing control over the distribution system (forward integration).
- Creating and expanding the free trade zones (vertical integration to top).
- Establishing laboratories for determining the bacterial and fungal contamination.

C) WO strategies (cautiously).

- Variation in the pistachio export production (product development).
- Developing the processing industries in Kerman (product development).

D) WT strategies (defensive).

- Promoting packaging Technology (penetrate development).
- Following the global industrial standards (penetrate development).

Strategic action and position evaluation matrix (SPACE)

A) Financial security determinants (FS).

	factors	score
1	Capital output	3
2	Imposing pressure (financial	4
3	leverage).	6
4	Capability of changing to money	4
5	Working capital	3
6	Liquidity	2
7	Easily in out of system	3
8	Risk at work	2
	Inventory turnover	
	Score average	24/8=3

B) Environmental stability determinants (ES)

	factors	
1	Change in Technology	-2
2	Inflation rate	-2
3	Change in demands	-2
4	Competitive degree in product	-1
5	Available barriers to enter the	-3
6	organization	-2
7	Competitive push in organization	-2
	Demand elasticity against price	
	Score average	-14/7= -2

C) Factors determining Strength (IS)

	factors	score
1	Organization potential growth	3
2	Organization potential profit	3
3	Financial stability of organization	2
4	Necessary skill in technology	3
5	Use of recourse	4
6	Capital density	4
7	Ease of entry to the organization	2
8	Productivity & resource usage	3
9	Other (flexibility against the changes in the market)	3
	Score average	27/9=3

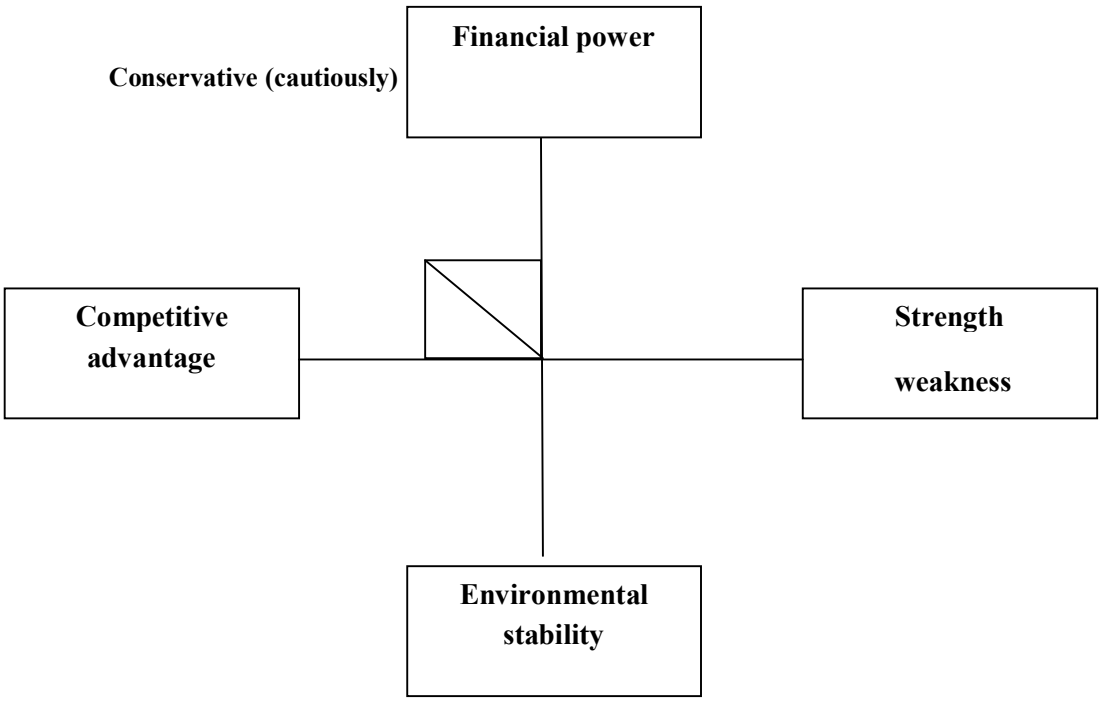
D) Factors determining Competitive advantage (CA)

	factors	score
1	The share of pistachio export from the	-3
2	country	-3
3	Service quality	-5
4	Educational courses' cycles	-5
5	Replacement cycle in pistachio export	-4
6	process	-4
7	Loyalty	-4
8	Using the competitive capacity	-4
	Technology special knowledge	
	Vertical integration	
	Score average	-32/8= -4

Results:

- The point is located on X axis.
  CA+IS= -4+3= -1
- The point is located on Y axis.
  ES+FS= -2+3= +1

Figure 1: evaluating position and pistachio strategies' action matrix



Pistachio exports in these conditions should perform a conservative strategy.

Organizations must keep their main competencies and not expose them self to the great risks. Most of the conservative strategies include penetrating the markets, market and product development, and homogenous diversities (David, 1999).

Conclusion and Suggestions

Pistachio as an agricultural strategic product can play an important role in export development and foreign exchange for the country. According to the presented analysis in the form of the SWOT model and studying and evaluating the internal factors (strengths, weaknesses), and external factors (opportunities and threats), the pistachio export strategies were presented. By considering the presented strategies and the results of the quantitative strategic planning matrix, market development strategy is presented as the most advantageous strategy for pistachio. In addition, for achieving success in global markets Kerman pistachio exports should follow the conservation strategies. Most of the conservative strategies include market development as determined by the strategic action (SPACE) and position evaluation matrix.

As can be seen, the results obtained from the quantitative strategic planning matrix suggest that the market development strategy matches the results of the strategic action and position evaluation's matrix, which suggests conservative strategies, and endorses the conservative strategies development and market development.

According to the results of this research, the following suggestions are presented for Kerman pistachio export development:

- Implementing the Kerman pistachio export strategic planning and presenting vision and mission.
- Providing special facilities to the agricultural graduates for their entrance to the manufacturing and pistachio export section.
- Preparing required facilities and equipment for developing the e-commerce and exchange facility.
- Establishing compulsory standards from the relevant organization for the standardization of production and packaging.
- Conducting research into the design of additional products and processing machines.
- Creating the required facilities for technology exporters.
- Reducing the in-bulk pistachio exports and moving towards packing based on international standards.

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Assessment of skin microcirculation and inflammatory markers of metabolic syndrome in a rat model

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Abstract: Analysis of the literature reveals that metabolic syndrome is invariably linked to microvascular disturbances, such as abnormalities in arteriolar reactivity, capillary recruitment, permeability, and hemorheology. The aim of this study was to assess skin microcirculation under baseline conditions and maximum skin hyperemia in response to heating (vasodilatory capacity) in control rats and in the rat model of metabolic syndrome. Twenty four young female rats were randomly assigned into control group (CG) fed on standard rat show & fructose induced insulin resistance group (FG) fed on fructose enriched show (60% of caloric intake) for 2 months. The skin microcirculation was assessed in the hairless ear of rat by Laser Doppler Flowmetry to measure skin blood flow, frequency of vasomotion waves, (frequency 1: 1-3 cycles/min (endothelial activity), frequency 2: 3-5 cycles/min (sympathetic activity), frequency 3: 5-20 cycles/min (vascular myogenic Activity)) & the Power of vasomotion (in perfusion units PU) in relation to the recorded frequencies . All the parameters were measured at 30^0 C and after local heating of the skin to 44^0C.The results demonstrated a significant increase in body mass index, serum glucose & insulin levels ($P<0.05$), systolic blood pressure, total cholesterol, low density lipoprotein cholesterol & triglycerides ($P<0.05$) in addition to a significant increase in nitric oxide, high sensitivity C reactive protein & tumor necrosis factor alpha ($P<0.05$), in FG compared to CG. So it can be claimed that use of fructose in diet for at least 2 months could be a model for experimentally studying the pathophysiological changes in the metabolic syndrome. Regarding parameters of microcirculation, there was a significant decrease in the % change in blood flow between blood flow at 30^0C and that after local heating of the skin to 44^0C ($P<0.05$) in FG compared to CG indicating impaired maximum skin hyperaemia induced by heating of the skin (vasodilatory capacity). Also FG showed a significant lower frequency values in the mid- range of frequency (frequency-2 i.e sympathetic dependent) at 30^0 C ($P<0.05$) and in the mid and high range frequencies (frequency-2 &frequency- 3 i.e sympathetic and myogenic dependent) at 44^0C ($P<0.05$) in addition to a significant decrease in the power of vasomotion (PU) at all frequency ranges (power-1, 2, and 3) after local heating of the skin to 44^0C in comparison to the CG ($P<0.05$). The microvascular dysfunction is a hallmark in our results that may be a potential factor explaining the clustering of several components of the metabolic syndrome & associated cardiovascular complications. Our results strongly suggest that targeting micro vascular and endothelial dysfunctions in patients with metabolic syndrome might help to prevent cardiovascular morbidity in those patients.

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Key words: Metabolic syndrome– microcirculation – nitric oxide- TNF-α – high sensitivity C reactive protein– rats.

1. Introduction

The metabolic syndrome is a multifaceted clinical entity resulting from the interaction of genetic, hormonal, and lifestyle factors. Over the past two decades, the number of people diagnosed with the syndrome has steadily increased and is associated with the global epidemic of obesity and diabetes[1].

National Cholesterol Education Program's Adult Treatment Panel III report (ATP III), [2] suggests a working definition of the metabolic syndrome that includes the presence of at least three of the following characteristics: abdominal obesity, hypertension, insulin resistance± glucose intolerance, dyslipidemia, pro-inflammatory and prothrombotic states. The pathophysiological basis of the metabolic syndrome is multiple and complex.

There is increasing evidence that microvascular dysfunction is a potential factor explaining the clustering of several components of the metabolic syndrome such as hypertension, obesity, and insulin resistance. Also, microvascular defects play an important role in the end-organ damage associated with the metabolic syndrome and may contribute to macrovascular dysfunction[3].

In a recognized experimental model of metabolic syndrome, the perfusion of multiple tissues has been shown to be compromised[4]. The direct mechanism of this decrease in perfusion, found in both humans and rats seems to be multi-faceted: a combination of altered responsiveness to vasodilator

and vasoconstrictor mechanisms, changes to the mechanical properties of the perfusing arteries, or a limit in the density/number of available microvessels to supply the tissue[5].

Thus, the microcirculation may present a promising future therapeutic and preventative target in the metabolic syndrome. Hence, clarification of pathophysiological pathways that contribute to microvascular dysfunction is essential.

Insulin resistance and endothelial dysfunction are characterized by elevated circulating markers of inflammation[6]. C-reactive protein (CRP), an inflammatory biomarker that has proven to be a strong, independent predictor of both incident diabetes and incident cardiovascular disease[7]. Tumour necrosis factor alpha (TNF-α) is another circulating marker of inflammation that has been associated with obesity. TNF-α was shown to be constitutively expressed by adipose tissue, to be hyperexpressed in obesity, and to mediate insulin resistance in the major animal models of obesity[8].

Aim of work:

The present study was designed to assess skin blood flow and capillary vasomotion by using Laser Doppler Monitor over hairless ear of the rat in addition to metabolic parameters and rat tail arterial blood pressure in fructose- induced insulin resistance rat as a model of metabolic syndrome.

2. Material & Methods

Experimental animals:

Twenty four female rats (150-250 grams) approximately 6 weeks old belonging to the local strain were used in this study. Veterinary care was provided by the laboratory animal house unit of Kasr Al-Aini Faculty of Medicine, Cairo University. Throughout the study period, the animals had free access to food and water all through the daytime with deprivation from food at night. Each rat was bred and housed individually in his own wire mesh cage at room temperature with normal light and dark cycle. Animals were allowed to acclimatize to their environment for 1 week before start of experiments. The animals were divided randomly according to the diet type into two groups of 12 rats each, control group (CG) fed on standard rat chow containing 75% of its caloric intake as carbohydrates & fructose induced insulin resistance group (FG) fed a fructose-rich diet contained 60% fructose, 21% proteins, 5% fat and 8% cellulose[9]for the entire study duration for 2 months**.**

Experimental procedures

Body mass index (BMI) measurement:

The animals were weighed in grams & the naso-anus length in cm was measured while the rats were anesthetized with ether, to make it easier and accurate. [10]

The obesity index was calculated according to an equation formulated by Dubuis *et al.*[11]:

BMI= cubic root of weight in grams x 1000/naso-anal length in cm

Systolic blood pressure (SBP) measurement:

Systolic blood pressure was measured by Harvard rat tail blood pressure monitor system in conscious animals. Four to six readings were averaged together to obtain a value for systolic blood pressure (MNL 490601 System)

Laser Doppler Flowmetry

Assessment of the skin capillary blood flow in anaesthetized rats was done using Laser Doppler Flowmetry (LDF) periflux 5000 satellite primed made in Sweden. The method involves conducting 2Mega Watt light from a laser system via a fiberoptic light guide to the skin surface using a probe held by a plastic adhesive tape. All measurements were performed in the morning in a quiet room at temperatures of approximately 28°C. The rats were placed on their side and the probe was fixed to the inner surface of the external ear. Local thermal hyperemia was induced using a heating disc surrounding the probe, connected to a heating unit. The probe was attached to the skin using a double-sided sticker. Recordings of the laser Doppler signal were made using PeriSoft for Windows. Baseline skin blood flow was recorded for 3 minutes with the local heating disc temperature set at 30°C[12]. This was immediately followed by rapid local heating to 44 °C which was maintained for 1 minute to obtain maximal vasodilatation[13]. After this, another 3 minutes of recording was then repeated at 30°C to study the microvascular reactivity to heat and maximum skin hyperemia. The data recorded are

- Basal skin blood flow at 30^0C in perfusion units (P.U), the percent change between blood flow at 30^0C and blood flow after local heating of the skin to 44^0C to study the maximum skin hyperaemia in response to heating & the slope of this change in milliseconds
- Frequency of vasomotion waves (cycles/minute) at 30^0C and after local heating of the skin to 44^0C. Three frequency ranges were recorded: -Frequency 1: 1-3 cycles/min (endothelial activity), frequency 2: 3-5 cycles/min (sympathetic activity) & frequency 3: 5-20 cycles/min (vascular myogenic Activity).
- Three Power of vasomotion (perfusion units PU) in relation to the recorded frequencies at 30^0 C and after local heating of the skin to 44^0C were

also recorded: Power-1: Increase in blood flow at frequency-1, Power-2: Increase in blood flow at frequency-2 & Power-3: Increase in blood flow at frequency-3

Biochemical analysis

After an over-night fast, blood samples were withdrawn through retro-orbital route and serum was separated and stored at -70°C until used except for the insulin & glucose; which were measured immediately after sampling.

Plasma glucose in blood samples was measured using oxidase- peroxidase method[14].

Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer's instructions[15].

Homeostasis model assessment of insulin resistance (HOMAIR)

HOMA is an indirect method for the assessment of insulin resistance. It depends on relationship between fasting plasma glucose and insulin based on a mathematical model:

HOMA-IR: [fasting plasma glucose (mmol/L) x fasting plasma insulin (uIU/ml)] /22.5[16].

HOMAIR values more than 4.0 are diagnostic of insulin resistance[17].

Measurement of lipid profile

Serum total cholesterol was assayed as described by Siedel *et al.* [18] while the protocols of Gordon and Gordon[19] and Jacobs and VanDenmark[20] were adopted for the determination of HDL-cholesterol and triglycerides (TG). LDL-cholesterol level was determined by calculation using the Friedwald formula[21] as follows:

$$LDL - C = Total\ cholesterol - \frac{TG}{5} - HDL - C$$

Measurement of NO:

Serum NO level was determined indirectly as its metabolic products (nitrate + nitrite ions) spectrophotometrically using a test kit (Boeringher, USA) in which all the nitrate ions in serum were first reduced to nitrite ions by nitrate reductase followed by the reaction between nitrite ions and the Greiss reagent (0.1% naphthylethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% H_3PO_4) to form a blue color solution[22]. Absorbance measurement was done at 540 nm against the reagent blank in which the serum sample was replaced with de-ionized water. The levels of nitric oxide in the experimental animals and control were determined by extrapolation from absorbance-concentration curve of the sodium nitrate standard solution (10–100 µM).

Measurement of hsCRP & TNF- α

Serum hsCRP levels were measured with a Rat C-Reactive Protein ELISA Kit (Alpha Diagnostic International, San Antonio, TX, USA).according to manufacturers instruction[23].

Serum TNF-α was measured by using ELISA (quantikine R & D system USA) according to the manufacturer`s instructions[24].

Statistical analysis:

Data was coded and entered using the statistical package SPSS (version 15). Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) and multiple comparisons (Post Hoc test) for quantitative variables while non parametrical (Kruskal-Wallis test) and (Mann-Whitney test) were used for quantitative variables not normally distributed. Correlations were done to test for linear correlations between quantitative variables. P-values < 0.05 were considered statistically significant

3. Results

As shown in table 1 & Fig-1A, BMI was significantly higher in FG than CG (298.3±9.7versus 279.04±2.7) (P<0.05).

These results demonstrated that high fructose diet significantly increased (P<0.05) the levels of serum glucose (mmol/L), serum insulin (uIU/ml) &HOMAIR compared to CG (5.89±.98), (15.32±1.8) and (4.02±.91) versus (3.20±.50),(10.20±.98) and (1.45±.31) respectively (Fig.1B).

Regarding serum lipids, there was significant elevation (P<0.05) in TC , LDL-C &TGs (mg/dl) in FG as compared to CG [(180.13±17.2), (124.7±10.07) & (105.6±8.9) vs (148.8±12.7), (87.7±12.4) & (75.7±7.4) respectively] while HDL-C level is significantly decreased (P<0.05) relative to the control (34.2±3.8)versus (45.5±4.9) (Fig. 1C).

Also, SBP increased significantly (P<0.05) from 115.8±4.1 in CG to 153.7±9.07 mmHg in FG (Fig.1D).

So rats fed on high fructose diet for 2 months showed the major components of metabolic syndrome, obesity, insulin resistance, high blood pressure & dyslipidemia

NO & inflammatory markers in metabolic syndrome:

Levels of nitric oxide (NO) µmol/l, the inflammatory markers; hs-CRP (mg/l) and TNF-α (ng/ml) were increased significantly (P<0.05) in FG as compared to CG [mean values (10.8±1.4),

(2.3±0.5) and (98.5±11.9) versus (2.4±0.6) , (0.42±0.35) and (61.2±7.9) respectively (Fig. 1D & E).

Table (1): The effect of high fructose diet on body mass index BMI, metabolic parameters , systolic blood pressure SBP, nitric oxide NO& inflammatory markers in young female rats (n=12)

Measured parameters	Control Group	Fructose induced insulin resistance group
BMI (%)	279.04±2.7	298.3±9.7*
Serum Glucose (mmol/L)	3.20±.50	5.89±.98*
Insulin (uIU/ml)	10.20±.98	15.32±1.8*
HOMA	1.45±.31	4.02±.91*
Total Cholesterol (mg/dl)	148.8±12.7	180.13±17.2*
HDL-C (mg/dl)	45.5±4.9	34.2±3.8*
LDL-C (mg/dl)	87.7±12.4	124.7±10.07*
Triglycerides (mg/dl)	75.7±7.4	105.6±8.9*
SBP (mmHg)	115.8±4.1	153.7±9.07*
hs-CRP (mg/l)	0.42±0.35	2.3±0.5*
TNF-α(ng/ml)	61.2±7.9	98.5±11.9*
NO(µmol/l)	2.4±0.6	10.8±1.4*

Results are mean±SD
HOMA:Homeostasis Model Assessment of insulin resistance .
HDL-C: high density lipoprotein .
LDL-C: low density lipoprotein .
hs-CRP :high sensitivity C-reactive protein
TNF-α :tumour necrosis factor -α .
*: significant P as compared to control group (P<0.05)

Parameters of microcirculation:

Table 2 showed that levels of % change in blood flow between blood flow at 30^{0}Cand 44^{0}C and Slope of change (m sec) were decreased significantly (P<0.05) in FG as compared to CG [mean values 29.09±4.3 and 0.49±0.19 versus 51.6±5.6 and 1.2±0.30 respectively] (Fig.2A).

At 30^{0}C the only frequency affected in FG was frequency 2(sympathetic activity) which decreased significantly (P<0.05) from 6.66±1.39 in CG to 5.082± 1.6. After local heating of the skin to 44^{0}C frequency 2 and 3 showed significant decrease (P<0.05) in FG as compared to CG [mean values (4.2±0.63), (44.6±4.0) versus (6.7±1.4), (52.48±8.43) respectively} while frequency 1, showed no significant difference (Fig. 2B).

Regarding vasomotion power, there was a significant decrease (P<0.05) in power 1,2 and 3 in FG in comparison to CG after heating to 44^{0}C [mean values (1.09±0.22), (0.97±0.11) and (0.53±0.05) versus (7.3±1.6), (4.9±0.83) and (4.3±0.62) PU, respectively] while no significant changes was observes at 30^{0}C (Fig. 2C).

Table (2): Mean ± SD of all parameters measured by Laser Doppler Flowmeter in control & fructose induced insulin resistance young female rats.

Measured Parameters	Control Group (n=12)	Fructose induced insulin resistance group (n=12)
Basal Bl Fl at 30^{0}C in P.U.	41.1±7.5	38.4±6.07
% change between Bl Fl at 30^{0}C and 44^{0}C	51.6±5.6	29.09±4.3*
Slope of change in Msec.	1.2±0.30	0.49±0.19*
Frequency. 1 at 30^{0}C in cycles/min	4.0±1.7	4.4±0.98
Power of vasomotion 1 at 30^{0}C in P.U.	0.97±0.42	0.79±0.13
Frequency 2 at 30^{0}C in cycles/min	6.66±1.39	5.08±1.6*
Power of vasomotion 2at 30^{0}C in P.U.	0.56±0.2	0.47±0.11
Frequency 3 at 30^{0}C in cycles/min	47.8±7.8	43.9±8.12
Power of vasomotion 3 at 30^{0}C in P.U.	0.51±0.16	0.41±0.06
Frequency 1 at 44^{0}C in cycles/min	4.5±1.6	4.25±0.7
Power 1at 44^{0}C in P.U.	7.3±1.6	1.09±0.22*
Frequency 2 at 44^{0}C in cycles/mi	6.7±1.4	4.2±0.63*
Power 2 at 44^{0}C in P.U.	4.9±0.83	0.97±0.11*
Frequency 3 at 44^{0}C in cycles/min	52.48±8.43	44.6±4.0*
Power 3 at 44^{0}C in P.U.	4.3±0.62	0.53±0.05*

n: number of rats
Bl.Fl : blood flow
PU: Perfusion unite
*: significant P as compared to control group (P<0.05)

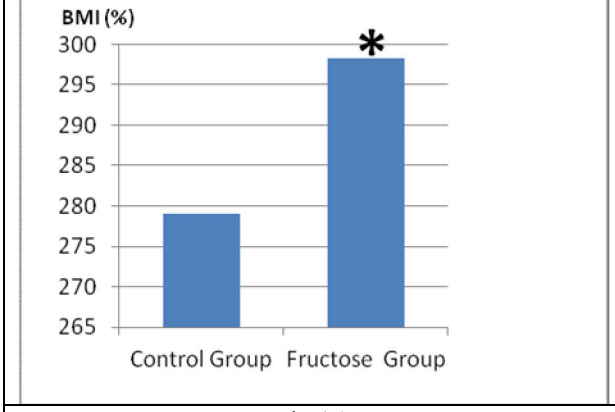
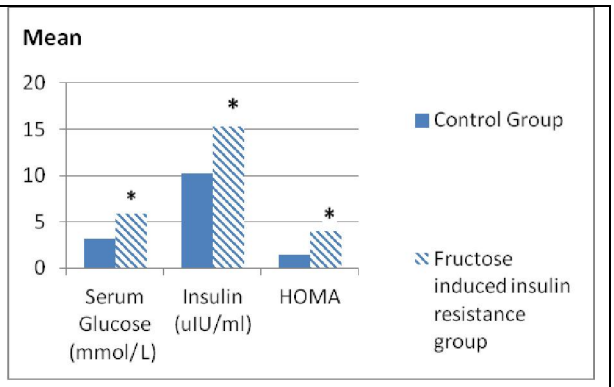


Fig 1A



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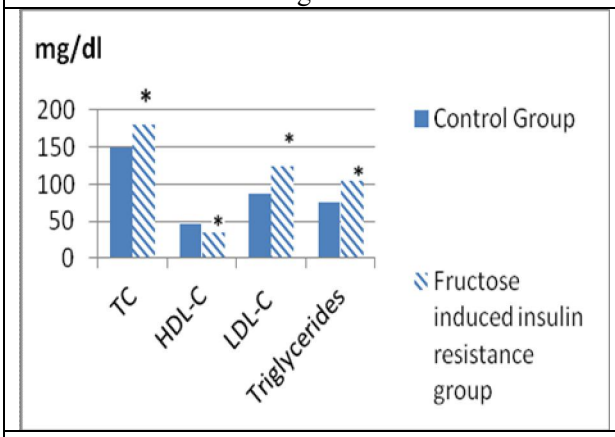

Fig 1C

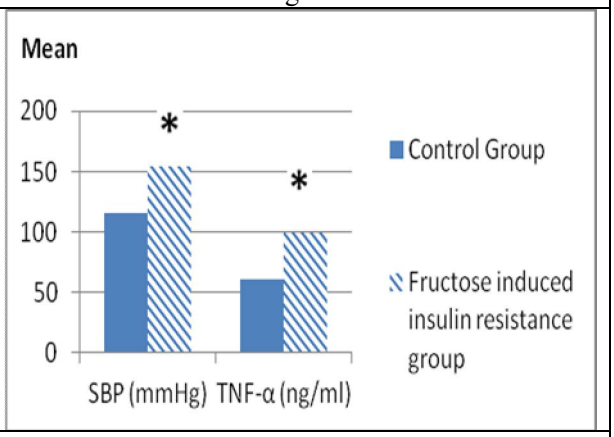

Fig 1D

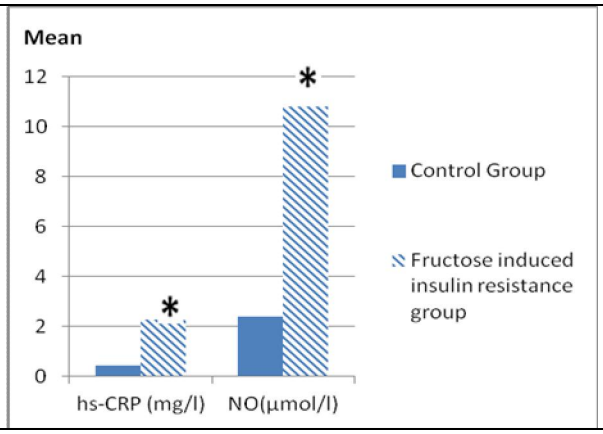

Fig. 1: The effect of high fructose diet for 2 months on body mass index BMI (Fig1A),serum glucose, insulin and HOMAIR (Fig1B), serum lipids (Fig 1C), tumor necrosis factor alpha TNF-α , systolic blood pressure SBP (Fig.1D) , high sensitivity C reactive protein, hsCRP & nitric oxide NO (Fig.1E) in young female rats
*: significant P as compared to control group (P<0.05)

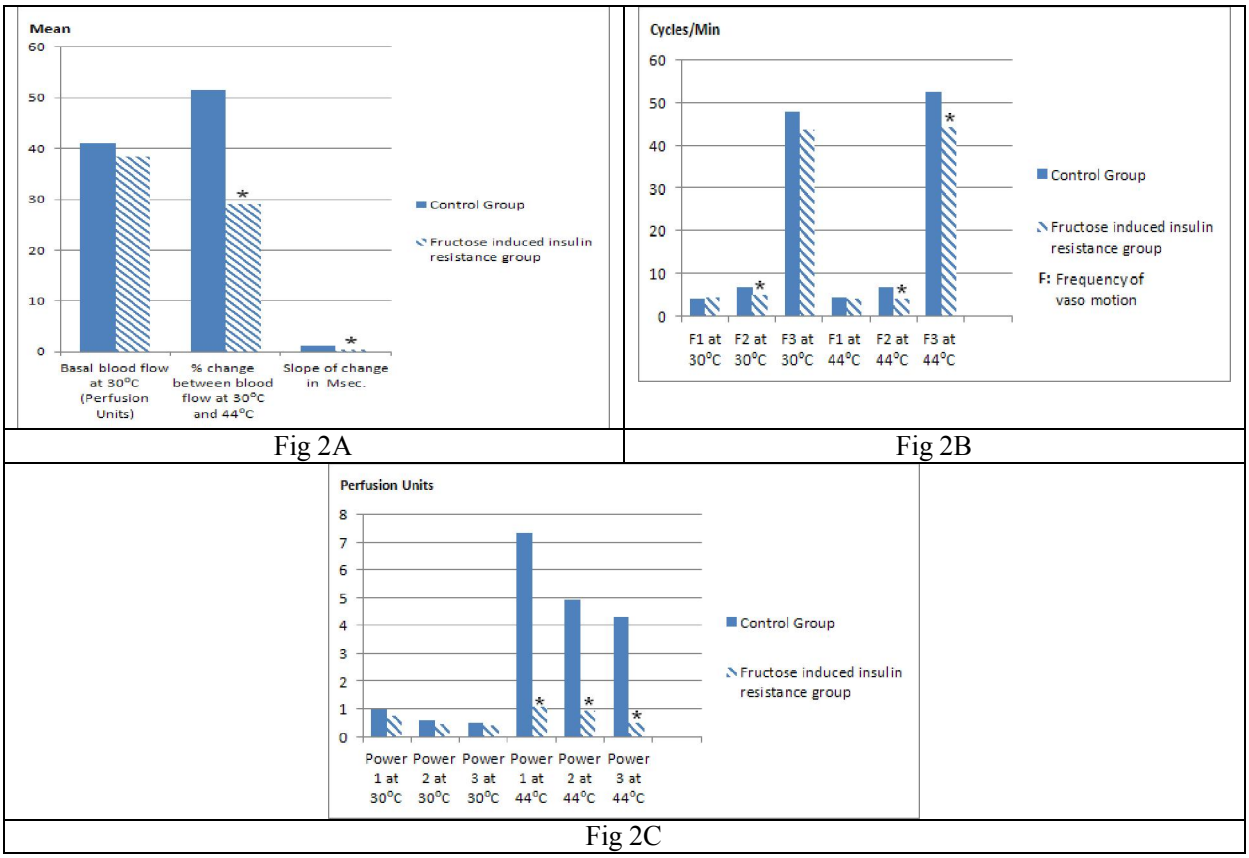

Fig 2A | Fig 2B

Fig 2C

Fig. 2: The effect of high fructose diet for 2 months on basal blood flow at 30C, % change of blood flow at 30^0 & 44^0 C & the slope of change (Fig.2A), frequency of vasomotion (Fig 2B) & power of vasomotion in relation to the recorded frequencies (Fig 2C) at 30^0C & after local heating of skin to 44^0 C in young female rates
*: significant P as compared to control group (P<0.05)

4. Discussion

The metabolic syndrome refers to the co-occurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia and hypertension. These conditions are interrelated and share underlying mediators, mechanisms and pathways[25].

In the present study, insulin resistance was clearly revealed in rats fed on high fructose diet (60% of caloric intake for 2 months) in relation to the rats fed on normal standard chow. Our results showed that rats on high fructose diet have significantly increased weight gain and BMI in comparison to the control rats.

Many results showed increased fasting plasma glucose and plasma insulin levels after high fructose consumption in rats[26&27].

On the other side, it was reported that short term intake of dietary fructose, is not a contributor to insulin resistance and hypersecretion in obese adolescents[28].

Increased insulin resistance on receiving fructose may be related to glucose transporter 5 (GLUT5), a fructose transporter that mediates the uptake of substantial quantities of dietary fructose, that was found to have significantly higher expression levels in young obese rats compared to lean controls[29]. Another theory explaining how chronic fructose over nutrition can lead to type 2 diabetes is the hexosamine hypothesis, where hexosamine flux is thought to regulate glucose and satiety-sensing pathways. With overexpression of glutamine, fructose-6-phosphate amidotransferase (the key regulatory enzyme in hexosaminesynthesis), the liver produces excess fatty acids, skeletal muscle becomes insulin resistant, and hyperinsulinemia results. This pathway of excess hexosamine flux leads to long-term storage of energy, and eventually obesity and type 2 diabetes[30]. Moreover, chronic fructose consumption has been reported to reduce adiponectin responses, contributing to insulin resistance[31].

The observation of increased body weight associated with fructose ingestion is of interest. One explanation for this observation could be that fructose ingestion did not increase the production of

the two hormones, insulin and leptin, that have key roles in the long-term regulation of food intake and energy expenditure[32].

Other features of the metabolic syndrome detected in rats fed high fructose diet included a significant elevation in the serum levels of total cholesterol, LDL-C, triglycerides and significant decrease in serum HDL-C.

Similar to our results, Taghibiglou *et al.*[33] concluded that fructose feeding in hamsters causes insulin resistance, hypertriglyceridemia, hepatic very-low-density lipoprotein over-production. In another study, consumption of moderate amounts of fructose significantly and dose dependently increased plasma triglyceride levels only in carbohydrate sensitive men[34].Moreover, Rader[35]reported that a low HDL cholesterol level is even more common in patients with insulin resistance than is hypertriglyceridemia.

In insulin-resistant states, two mechanisms lower HDL cholesterol: cholesterol ester transfer protein mediating the transfer of cholesterol from HDL to the apo-B- containing lipoproteins; and upregulation of enzymes, such as hepatic lipase and endothelial lipase, thus promoting hypercatabolism of HDL.

In contrast to our results Bantle *et al.*[36]demonstrated that fructose diet produced significantly higher fasting, postprandial, and daylong plasma triacylglycerol values in older men, although this effect of fructose was not seen in younger (< 40 y of age) men or in the older (≥ 40 y of age) women included in the study. The fructose diet had no significant effects on fasting plasma cholesterol, HDL cholesterol, or LDL cholesterol in either men or women

Rutledge and Adeli[37] suggested that dietary fructose has a direct impact on hepatic lipid metabolism by bypassing the enzyme phosphofructokinase, the regulatory step imposed on glucose. Allowing unregulated flow of fructose-derived carbons into lipogenesis . In addition to increased lipid production, fructose has been found to decrease lipid oxidation in humans[38].

In our study, rats fed high fructose diets showed highly significant elevation in their systolic blood pressure at the end of the study in comparison with the control rats. Even there was elevation reported in systolic blood pressure from the random samples taken after the first month.

Similar to insulin resistance and hyperlipidemia, many published experiments have shown that high-fructose diets induce hypertension in animals[39&40]. Our results clearly showed that SBP is highly correlated with insulin resistance (r; 0.652); this is in agreement with the fact that insulin resistance is one of the important mechanisms underling the development of the metabolic syndrome [41].

Hypertension in rats with the metabolic syndrome, due to chronic consumption of a high refined sugar has been reported to be associated with oxidative stress[42], the increase in sympathetic neural outflow and plasma catecholamine concentrations associated with increased plasma insulin concentrations[43], the anti-natriuretic effect of insulin to increase fluid reabsorption & lastly, the activated renin-angiotensin system found in obese individuals. [44]

Laboratory and experimental evidences indicate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process[45]. Based on these data, hs-CRP has been used as a marker of cardiovascular risk in the present study.

The present results showed a significant rise in the levels of inflammatory markers, hs-CRP and TNF-α in FG when compared with the CG. Moreover hs-CRP was positively correlated with systolic blood pressure (r = 0.733).

These results are in accordance with results of Women's Health Study where levels of hs-CRP were shown to correlate with the major components of the metabolic syndrome[46]. Numerous studies have revealed that persons who have the most or all features of the metabolic syndrome have increased levels of CRP[47&48].

Moreover, Andrea *et al.*[49] found increased TNF-α mRNA expression (5-fold), plasma concentration of TNF- α (8-fold), and protein expression of TNF- α (more than 3-fold in small coronary arteries) in Zucker obese fatty rats. Also Hotamisligil [50] found high TNF-α levels in metabolic syndrome patients.

The increases in proinflammatory cytokines including IL-6, TNF- α and CRP reflect overproduction by the expanded adipose tissue mass[51]. Studies done by Weisberg *et al.*[52], suggested that monocyte-derived macrophages reside in adipose tissue and may be at least in part the source of the generation of pro-inflammatory cytokines locally and in the plasma.

Our results showed a significant increase in the levels of nitric oxide (NO) in FG when compared with the CG. This is in agreement with results obtained by **Zahedi *et al.*** [53]**,** who found higher NO metabolites concentrations in subjects with metabolic syndrome and type 2 diabetes. Also an experiment done by Blouet *et al.* [54]on rats fed a high-sucrose diet for six weeks inducing insulin resistance,& showed that high-sucrose diet was accompanied with higher production of superoxide anion that account for the increase in NO scavenging and the resulting

production of peroxynitrite (a stable footprint of NO oxidation) which indicate a decrease in NO bioavailability in the studied rats.

These results and ours apparently contrasts with what had previously been reported under conditions of diet-induced oxidative stress in rodents associated with a reduction in NO production[55]. In rats fed a high-refined sugar and/or high-fat diet, an impairment of endothelial-dependant vasodilation was associated with decrease in endothelial nitric-oxide synthase eNOS expression, NO production and bioavailability, and reduced insulin-induced eNOS activation[56].

However, in the latter studies, these observations were made after more than 4 months of studying which is long duration in contrast to our study.

Another explanation for the unexpected increase in the levels of nitric oxide in our study could be the fact that inflammatory cytokines like TNF-α are known to trigger the transcription of inducible nitric-oxide synthase (iNOS), a proinflammatory mediator in chronic inflammatory states including obesity-linked diabetes[57].

Therefore, we suggested that a decrease in NO bioavailability is the first impairment that affects NO metabolism in the course of insulin resistance, and that subsequent impairment in NO metabolism lags behind.

One of the main goals of our study was to assess skin microcirculation under baseline conditions and maximum skin hyperaemia in response to heating (vasodilatory capacity) by Laser Doppler Flowmeter (LDF). Our results showed that the maximum skin hyperaemia induced by heating of the skin to ≥44°C (vasodilatory capacity) is impaired in FG. These results are in agreement with many of the intervention studies that investigated the effect of metabolic syndrome parameters; insulin resistance, hypertension, obesity and dyslipidemia on microcirculation.

It was revealed that the increases in blood flow, in response to body heating was markedly less in hypertensives than normal rats[58&59]. It was suggested that this difference indicate structural change in the skin vasculature in hypertension caused by rarefaction, vascular hypertrophy, or both[59].Moreover, there is evidence that experimental elevation of blood pressure causes an increase in generation of reactive oxygen species (ROS) in endothelial cells, which may trigger adverse functional and structural changes in microvessels [60].

Štulc *et al.*[61], noticed blunted skin vasodilator response to heating in hypercholesterolemic patients. Moreover in obese women, it was shown that postocclusive capillary recruitment , microvascular endothelium–dependent vasodilatation & insulin-induced increase of microvascular endothelium–dependent vasodilatation are decreased[62]. Studies done by Caballero *et al.* [66] revealed a significant inverse correlation between microvascular reactivity and systolic blood pressure, body mass index and index of insulin resistance HOMA. The previous mentioned studies are on line with our correlation studies as we found that % change between blood flow at 30^0Cand 44^0C after local heating of the skin was negatively correlated with body mass index(r = - 0.663), systolic blood pressure (r = - 0.807) and HOMA (r = - 0.589).

The pathophysiological mechanism behind the relationship between obesity and microvascular dysfunction is probably multi factorial. Adipose tissue secretes substances, such as FFAs, TNF-α, and adiponectin, that can influence microvascular function. An increase in FFAs impairs vascular function in resistance vessels in humans and in microvasculature in rats [63]. In addition, acute TNF-α elevation impairs insulin-induced capillary recruitment and glucose uptake in rats[64].Adiponectin levels are reduced in obesity and adiponectin has a vasoprotective effect, as demonstrated by associations between hypoadiponectinemia and impaired endothelial function in resistance vessels [65].

Regarding skin vasomotion, our results showed a significant lower frequency values in frequency-2 i.e sympathetic dependent) at 30^0 C and in the frequency-2 &frequency- 3 i.e sympathetic and myogenic dependent, at 44^0C & a significant decrease in power of vasomotion at all frequency ranges after local heating of the skin to 44^0C in FG as compared to the CG

Our results match those obtained by Rossi *et al.*[67] who reported that, the newly diagnosed essential hypertensive patients showed a reduced post-ischemic increase in sympathetic- and myogenic-dependent vasomotion, together with a normal post-ischemic response of the endothelial-dependent vasomotion. Moreover, De Jongh *et al.* [68] suggested that there is a decreased endothelial- and sympathetic-dependent skin vasomotion in obese women under basal conditions. More recently, John *et al.* [69]revealed that in acute insulin resistance induced by peripheral vasoconstrictor α-methyl serotonin (αMT), there is reduction in the myogenic component of vasomotion by 27% compared to baseline. They suggested that insulin directly interacts with insulin receptors on the vascular smooth muscle of the terminal arterioles that control capillary recruitment. The findings, however, do not rule out indirect effects of insulin for example via endothelial mechanisms to cause rhythmic contractions and relaxations of vascular smooth

muscle. The vasoconstrictor α MT that induces an acute state of insulin resistance blocks these vascular actions of insulin suggesting that vascular dysfunction of insulin resistance may involve a specific loss of effect of insulin on the vascular smooth muscle contribution to vasomotion in skeletal muscle.

In contrast to our results, Gryglewska *et al.* [70] found that in patients with masked hypertension the skin flow motion was characterized by higher power spectral density values of sympathetic and myogenic origin than in truly normotensive subjects.

Conclusion, the microvascular dysfunction is a hallmark in our results that may be a potential factor explaining the clustering of several components of the metabolic syndrome such as hypertension, obesity, and insulin resistance. Our results strongly suggest that targeting micro vascular and endothelial dysfunctions in patients with metabolic syndrome might help to prevent cardiovascular morbidity in those patients.

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Impact of water management transfer

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Abstract: Basically, water management is critical to the economic growth and environment protection. Its optimization is one of the general global strategies. In this regard, it is needed to develop local management and devolution of the operation and maintenance of water installation in the developing regions. This study surveys the effects of devolution of irrigation management to water users association to improvement of management on the water conveyance and water distribution in a case study, Qazvin plain, Iran. They investigated several eco-geographical variables including land and canal geographical location and beneficiary's habitats' area as travelling for water provision. The research was developed based on experimental investigation method to reduce the transportation of 3000 stockholder to headquarter office in the capital of the province. As a result, the function of water user assassinations was indicated travelling for water provision had reduced 75% because of shortening in distance in the every watering year. Overall, the feedback of users was investigated by comparing the users' satisfaction degree between before and after irrigation management transfer. The outputs showed that the travelling dimension for water provision is more important and significant. By the other means, users prefer to recourse to water local management bureaus as WUAs unions rather than long travelling to headquarter.
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Keywords: Water Users' Associations; Satisfaction; Water management transfer; Transportation.

1. Introduction

The world's climate is changing as a rate unprecedented in human history. It's risks are global, real and apparent in natural resources as components that constitute the livelihood (Nail, 2003). In addition, misuse of water has increased risk of human wellbeing, food security, and the ecosystems (UNESCO, 2003).In other words, it is needed to promote our societies' capacity on participation of environment protection and climate as the context of sustainable development (Sullivan, 2003; FAO, 2001). It is proven that the sustainable development's objectives and climate change are common and interlinked. Local participation yields high economic and environmental returns in implementing programs of management on water, sanitation, drainage, and etc. (World bank, 1992).

Optimization of water resources managemen t is one of the committals to achieve the environment protection as one of infrastructural goals on sustainable development (World bank, 1997) . By the same token, it is a global strategy as it starts by capacity building on human forces to the implementation of local water management. Moreover, management reform strategies vary dramatically hence accomplished various results (Vermllion, 1999).Overall, a participatory water management process is essential for both of the environment stability and economic development (World bank, 2003). It is run based on the enhancement devolution plan, operation and maintenance, O&M,on water installation to water users. As a result, natural resources are neededto bemanaged more effectively now and beyond the past (UN documents, 1992; Hamdy, 2007).

On one hand, Taleghan river basin extends almost 900 km2 which is in the north western of Tehran, Iran. It is computed the participation is 697.2 mmwhere is the climate relatively moisture in the Taleghanhighlands (Soltani, 2011). Therefore, different development which contains animals and plants and lots of spring, grass land, wildlife and some spring improve operational pasture and livestock productivity. It is the water resources zone at the upstream. On the other hand, Qazvin plain is a semi-arid zone which is the water consumption area as downstream. It is one of the developed zones in Iran. They were the Talegan reservoir dam whit 460 Million Cubic Meters, MCM, which has been

allocated annually for a part of Tehran drinking water (150 MCM), irrigating (287 MCM), artificial recharge (20 MCM) and traditional water rights and environmental requires at downstream (12MCM). The hug irrigation network (Length; 1200 KM, Capacity; 30 M3/S) and several hydro-mechanical equipment have implemented to water distribution for delivering to 30,000 farmers. The buildings and water utilities were built and operated since 1975 (Ghasemi, 1994).

Irrigation Management Transfer, IMT, plan includeddesign and implementation of the process of devolution of O&M to local communities institutions. Since it was formed, the Water Users Association, WUAs, was mandated by O&M. At present, 161 WUAs and 12 associations' unions and a Federation United (FUWUAs) play role of water management in Qazvin province. They are stated by capacity building of human resources of the 88 habitats in the suburbs. Formation had the biggest NGOs participation as the provincial federation in the agricultural section during three years, 2002 to 2004. The Qazvin Pilot Project, QPP, has been developed from bottom, land level to the provincial level (Ghasemi, 2005).

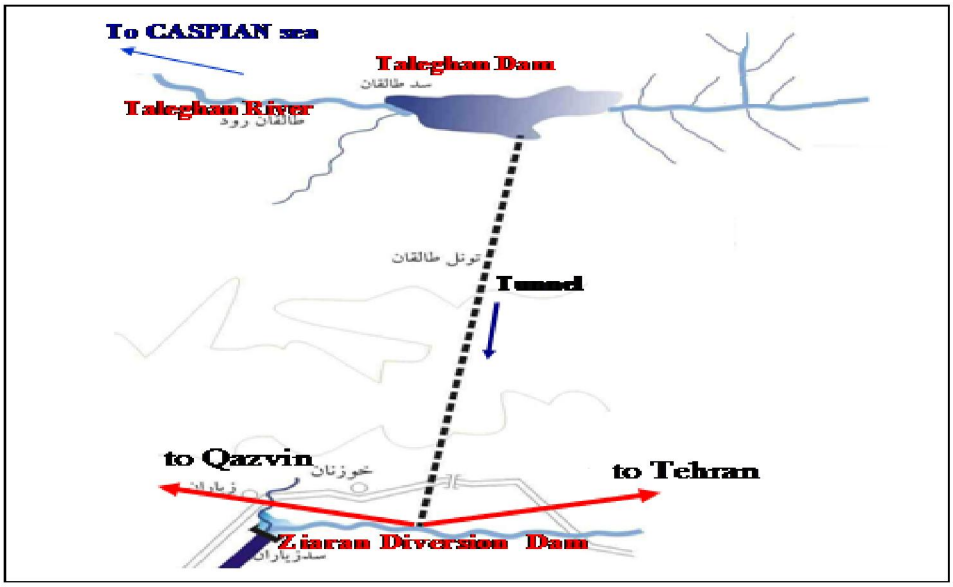

Fig1. Location of case; Taleghan river basin & Qazvin plain (Sumiani, 2008).

2. Material and Methods

The study measured some geographical factors and it surveyed the impacts of implementation of WUAs on the users' satisfaction. It investigated the faction of participatory irrigation project on the water users travelling for water provision in that case. The beneficiaries'satisfaction was served into two sections before and after the implementation of IMT project and were compared. Some eco-geographical variables include canal and turn-out geographical location and water user's habitats were assessed by experimental design. Data collection which included, questionnaire and interviewer were discussed with theselected WUAs' representatives, WUAs' staffs, key stockholders, and members of the Qazvin Irrigation and drainagecompany. Sample size was computed for finite population on data collection. 161 WUAs were used for sampling size to be filled up by one of the representatives in the any WUAs. It had been confirmed by a typical formula which is verified by the reliability of the sampling method (Arkin, 1963). As a result, the statistical population contained 161 samples among 3000 water users' stockholders. The questionnaire format was organized in a bisectional format, which was divided depended on before and after the devolution of O & M services. The amount of shortening distance and satisfaction's levels due to saving time and cost were investigated. It was exercised by statistical socio-economic pattern for data analysis.

3. Results

The irrigation and drainage network were expended by 94 km as the main canal which includes suburbs as east and west zone and mainland as

nearby to headquarter in Qazvin city. The geographical location of canal users' residential area was computed for both times, before and after the IMT. Firstly, the total distance of 88 habitats to headquarter office in the capital of province was 102,106 kmwhich had been travelled annually by water users in the past. Overall, the water provision process was quite complicated before implementing theQPP and it wasted much travelling time and costs for water users. The beneficiaries faced were related to the time and effort each farmer had to spend in travelling to fulfil his demand once every ten days during the every watering period, and paying the charges and then again providing the proof of payment for water release. Finally, that issue was resolved completely (Ghasemi, 2005; UI-Hassan, 2007). At present, annual transportation of 3000 stockholder to the 12 WUAs' unions as the local management is 25,698 km. As a result, the function of NGOs was indicated travelling for water provision had reduced 75% because of shortening distance of average 25.5 km for each stockholder in every watering year. In other words, the QPP could be stopped at minimum of 76,400 km travelling as an environment protection goal. In addition, the socio-economic factors including WUAs trustworthiness and travelling for water provision which led to saving time and cost were measured. As seen in Table1, the statistical assessment showed there was a significant relationship between the above factors and satisfaction level with p-value < 0.001.

Table1. The influence of shortening distance on satisfaction due to saving time/cost indicator.

Variable. Before,After	Std. Deviation	Std. Error Mean	T	Df	Sig. (2-tailed)
WUAS Trustworthiness	0.835	0.066	- 19.258	160	0.000
Due to Saving Time & Cost	0.742	0.058	-24.975	160	0.000

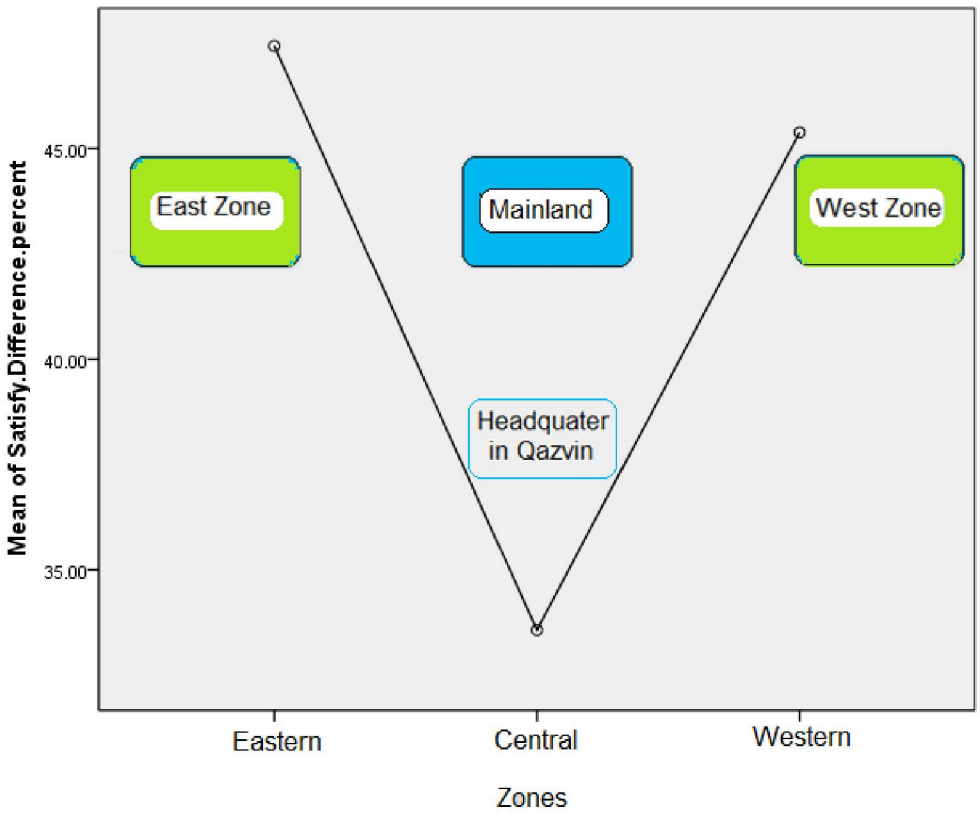

Fig2. The influence of distance to O&M bureaus on satisfaction level in Qazvin Plain, Iran.

Satisfaction level due to distances in bisectional times, before and after the IMT on lateral canals in the QPP was compared. In comparison, the impact of implementation of WUAs on satisfaction was relatively large in the suburbs area include eastern and western zones. It is less growth in the mainland includes laterals of headquarters' around. Overall, data analysis revealed that satisfaction due to shortening distance resulted in the most significant as key indicator for assessing beneficiaries' priorities. It showed that the satisfaction level was high in influential indicators, because of shortening distance by decreased of travelling for water provision to local bureaus rather than to the headquarter. It was divided into two zones; rural and mainland around of headquarter as determinant of distance of canals location to O&M offices. It was over 45% satisfaction level in the far canals against 33% in the mainland zone, Figure2.

4. Discussions

This paper investigated the implementation on participatory irrigation management in one of the famous plain in Iran. It was managed by the Qazvin Irrigation and Drainage Company since 2002.The QPP was made by empowerment of user'sto achieve higher participatory knowledge during three years in the IMT process as the establishment of 161 WUAs, 12 local water management around of irrigation network. It was stopped by the transportation to headquarter in Qazvin by the formation of the local bureaus through saving time and cost of water users. As a main result, the average of shortening distance for each of 3,000 stockholders is 25.5 km in the year which was fade save travelling almost 75%. It was decreased more than 76,400 km transportation for water users who have water right on Qazvin irrigation system. In addition, the feedbacks of respondents affirmed, the FUWUAs' function on O&M had improvement on O&M services.

Overall, the outputs showed that most significant was the transportation dimension for water provision. In other words, water users prefer to recourse to WUAs' local bureaus rather than long travelling to headquarter. Now, that issue isresolved completely by an intelligent strategy on environment protection goals. As a suggestion, the governance must support WUAs financially rather than government organisations to prevent their interference of tasks.

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Chemical, Biological and Biochemical Treatments to Improve the Nutritive Values of Sugarcane Bagasse (SCB): 2- *In Vivo* Studies to Evaluate the Nutritive Values of Untreated and Treated SCB

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Abstract: Twenty five Ossimi male growing lambs were randomly assigned into five treatments each of 5 animals to receive concentrate feed mixture plus one of the following roughages for 120 days feeding period: R_1, berseem hay; R_2, untreated sugarcane bagasse; R_3, 3% urea treated bagasse; R_4, fungi treated bagasse and R_5, bagasse treated with fungi + bacteria + 3% urea. Results obtained showed as a general evidence that, different treated bagasse rations indicated higher NH_3-N and TVFA's values compared with untreated bagasse ration. All estimated values of blood parameters in the present study were within the normal range. Animals given rations containing biological or biochemical treated bagasse showed higher DM intake, average daily weight gain and best feed conversion (Kg DMI or TDNI/ Kg gain) compared with those given rations containing untreated or urea treated bagasse. It could be concluded the possibility of replacing berseem hay (30% of the rations) by fungi treated bagasse in sheep ration without any adverse effect on lambs growth performance or feed utilization parameters.

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Key words: Sugarcane bagasse, chemical and biological treatments, lambs performance and economic efficiency.

1. Introduction

In Egypt, there is a wide gap between animal requirements and the available feedstuffs, although, there are about 26 million tons of agricultural plant by-products produced annually in Egypt **(Agriculture Economic and Statistics Institute, 2000)**. Approximately two thirds of the crop residues are burned or wasted, hence lead to environmental pollution and consequently health hazards. The poor quality roughages include; rice straw, wheat straw, bean straw, corn stalks and cobs, rice hulls, sugarcane bagasse and etc. Among these roughages, sugarcane bagasse represents about 4.13 million tons per year according to **Agriculture Economic and Statistics Institute (1995)**.

Sugarcane bagasses are secondary by-products derived during the industrial process of sugar production; its main components are cellulose, hemicellulose and lignin. As a result of its high lignin content, ruminal digestion is inhibited and thus, the nutritive value of bagasse and pith is low for ruminants.

The primary factors limiting the utilization of crop residues are its higher crude fiber and low protein contents, low digestibility and palatability. To improve the nutritive value of such agriculture residues, it is important to breakdown the linkages among cellulose, hemicellulose and lignin by mechanical, chemical or biological and /or biochemical treatments.

The possibility of biological treatment to deal with agricultural wastes has a great appeal as an alternative method to another expensive one; in terms of money and energy (chemicals) and to avoid the pollution hazards. Biological treatments are an alternative method to modify the fibrous materials by ruminants. The mode of fungal decay on roughages using the white rot fungi was shown to improve *in vitro* dry matter digestibility of the decayed substrate **(Dawson *et al.*, 1990)**. **Shoukry *et al.* (1985)** reported that CF content decreased while, CP content increased in sugarcane bagasse when treated with different fungi species, as a successive solution for the shortage in animal feeds.

Many scientists suggested the use of ammonia and urea to increase the crude protein contents of the poor quality roughages **(Fouad *et al.*, 1998)**. Biological treatments such as *Trichoderma viride* **(Khorshed, 2000, Villas-Boas *et al.*, 2002 and El-Ashry *et al.*, 2003)** were used to improve the nutritive value and digestibility of poor quality roughages. Increasing the digestibility of the diet by using exogenous feed enzymes obtained from fungal treatment would lead to beneficial effects on animal performance. Yeast treatment was also used to improve rumen digestibility of nutrients, especially crude fiber, elevation the ruminal fermentation and for more activation of the rumen microorganisms **(Dawson, 1992)**. The previous work **(Salman *et al.*, 2011)** suggested the possibility of improving the chemical composition and nutritive value of sugarcane bagasse by

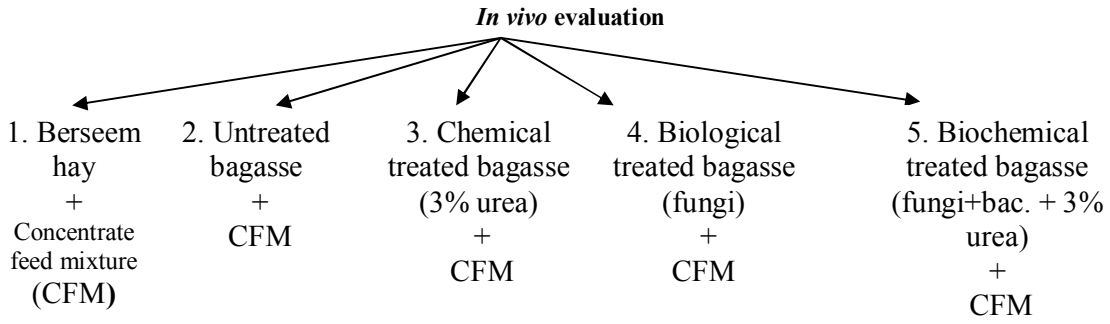
chemical, biological and biochemical treatments in ration for lambs.

Therefore, the main objective of the present study was to investigate the effect of replacing conventional roughages (berseem hay) by untreated or chemical, biological and biochemical treated sugarcane bagasse in rations of growing lambs, some ruminal and blood parameters along with growth performance were also investigated.

2. Materials and Methods

The present study was carried out at the Experimental Farm Station belongs to Faculty of Agriculture, Animal Production Department, Al-Azhar University, Nasr City, Cairo, Egypt and Laboratories of Animal Production Department, National Research Center, Dokki, Giza, Egypt, during the period from 2006 to 2009.

The scheme of the study is shown in the following diagrams:



This study was carried out to perform the best treatments detected in the previous work to be applied in feeding trials with growing lambs **(Salman *et al.*, 2011).** Rumen liquor and blood parameters were determined. Economic efficiency was also calculated. Sugarcane bagasse was purchased from Edfo Sugar Company, Aswan, Egypt. All sugarcane by-products were sun dried to 90% DM and bagasse was chopped to approximately 1-3cm.

Microorganisms:

a. White rot fungi (*Phanerochaete chrysosporium* NRRL-6361).

b. Bacteria (*Cellulomonas uda* NRRL-404).

The tested microorganisms were provided through the Genetic and Cytology Department, National Research Center, Dokki, Giza, Egypt.

Biological treatment:

Fungal propagation:

White rot fungi (*Phanerochaete chrysosporium* NRRL-6361) was maintained on potato dextrose agar medium (PDA), grown at 28°C for 72 hrs, then stored at 4°C and recultivated every two months.

Bacterial treatment:

Cellulomonas uda strain NRRL-404 was available from the same resource as mentioned before.

Procedure of bacteria inoculation:

The prepared inoculum was mixed well with the tested crop residues at the rate of 1:10 (v/w). The moisture content was adjusted to 65% then bagged and incubated at 30°C for the fermented period (up to 4 weeks).

Combined biological treatments:

The same procedure of fungal and bacteria treatments were used but the bags were inoculated with a mixture media containing 50% of fungi and 50% of bacterial media and continued to ferment till 10 days at 30± 2°C.

Chemical treatment (urea):

The required amount of urea (30g) was dissolved in 500 ml water and sprayed on 1kg bagasse. The treated bagasse was thoroughly mixed to be homogenous and the moisture content was adjusted to 65%, then ensiled (up to 3 weeks). At the end of incubation period, the chemical treated bagasse was taken out to be aerated for 2 days to get rid of the free ammonia and smell. Then samples were ground and stored in a glass bottles for later chemical analysis.

Biochemical treatment:

At the end of the fermented period of biological treated by-products, it was aerated overnight, mixed well with 3% urea then bagged, and stored up to 15 days.

***In vivo* evaluation:**

Animals and their managements:

Twenty five Ossimi male lambs with an average live body weight 32.7 kg and 180 days age were randomly assigned into five nutritional treatments (each of 5 animals) to receive one of the following roughages for 120 days feeding period: R_1: berseem hay; R_2: untreated bagasse; R_3: bagasse treated with 3% urea; R_4: bagasse treated with fungi and R_5: bagasse treated with fungi + bacteria + 3% urea. The composition of the five experimental rations are presented in Table (1).

Rations were offered to lambs *ad lib*, while, water and salt blocks were freely available all day time. Animals were fasted weighed at biweekly intervals, while changes in average live body weight, average daily gain, daily feed intake, feed conversion (kg DMI or TDNI/ kg gain), feed costs (LE/kg gain) and economic evaluation were estimated. Feeding costs of different experimental rations and net profit value were estimated according to current market price in 2007. Samples from experimental ingredients and rations were taken for chemical analysis according to **A.O.A.C. (1990)** and cell wall constituents according to **Goering and Van Soest (1970).**

Table (1): Composition of experimental rations

Item	Experimental rations, %					*Price L.E. /ton
	R_1	R_2	R_3	R_4	R_5	
- Berseem hay	30	-	-	-	-	750
- Untreated bagasse (Unt.B.)	-	30	-	-	-	200
- Bagasse treated with 3% urea (Urea Tr. B.)	-	-	30	-	-	300
- Bagasse treated with fungi (F Tr. B.)	-	-	-	30	-	300
-Bagasse treated with fungi + bacteria + 3% urea (F +B + urea Tr. B.)	-	-	-	-	30	300
- Ground corn grains	51	51	51	51	51	930
- Sunflower meal	7	7	7	7	7	1800
- Wheat bran	7	7	7	7	7	770
-Limestone, sodium chloride and minerals & vit. mix.	5	5	5	5	5	816
Total	100	100	100	100	100	-
*Current price of ration (L.E /ton, 2006-2007)	920	755	785	785	785	-

*Price of 1 ton bagasse = 200 L.E., besides 100 L.E./ ton of bagasse as cost for biological and chemical treatment + manufacture. Market price of 1 kg live body weight of sheep in (2007) = 17 L.E.

Rumen liquor measurements:

At the end of 2nd month from the feeding trial, 100 ml of rumen liquor were individually withdrawn by rubber stomach tube before feeding 0, 3 and 6 hrs after the morning meal, to determine pH, NH_3-N and TVFA's**.**

pH values of rumen liquor were immediately measured using Orion Research Digital pH meter, model 201. **Ammonia –nitrogen concentration,** was measured according to **Conway (1962),** while **total volatile fatty acids concentration**s were determined according to **Warner (1964).**

Blood parameters:

At the same time, blood samples were individually collected from the jugular vein in heparinized test tubes at 3hrs after feeding, to assess total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and creatinine contents. All the biochemical constituents of blood serum were measured colorimetrically using a specific kit by the Chemistry Auto-analyzer (Olympus AU 400). **Total protein,** was determined according to **Witt and Trendelenburg (1982); Albumin**, was determined according to **Tietz (1986),** while globulin and fibrinogen was calculated by subtracting the albumin value from the corresponding total protein value. **Albumin/ globulin ratio,** was calculated by dividing the albumin value on the corresponding globulin value. **Alanine aminotransferase (ALT)** and **aspartate aminotransferase (AST)** were measured according to **Reitman and Frankel (1957). Urea** was estimated according to **Coulombe and Favrean (1963),** while **creatinine** in blood serum was measured according to **Husdan (1968).**

Economic efficiency:

Based on free market prices of feed ingredients in (2006), while the cost of experimental ration was estimated as the total cost of concentrate feed mixture and berseem hay or sugarcane bagasse, in addition to the cost of chemical, biological and biochemical treatments of treated sugarcane bagasse (Table 1). Economic efficiency was calculated as the ratio between income (income from gain) and cost of feed consumed by experimental animals.

Statistical analysis:

Data were statistically analyzed according to **SAS (1998).** The significance among treatment means was tested by Duncan's Multiple Range Test **(Duncan, 1955)**. The statistical model used was:

$$Yij = u + Ti + eij$$

Where:
Yij: the observation.
u : the overall mean .
Ti: the treatment effect.
eij: the experimental error .

Chemical composition of the experimental ingredients:

Data presented in Table (2) showed the chemical composition of the experimental ingredients. It is obvious that the chemical composition of all ingredients was within the normal range.

3. Results and Discussion

Table (2): Chemical composition of the experimental ingredients (on DM basis, %)

Item *	DM	OM	CP	CF	EE	NFE	Ash
Berseem hay	87.10	82.20	12.41	39.00	2.68	28.11	17.80
Ground corn grain	90.72	97.94	10.81	7.82	4.55	74.76	2.06
Sunflower meal	92.35	94.42	28.73	31.72	3.72	30.25	5.58
Wheat bran	90.08	97.84	12.23	9.39	4.77	71.45	2.16
Unt. B.	91.90	94.70	1.80	49.50	1.16	42.24	5.30
Urea Tr. B.	96.20	92.30	5.20	43.70	1.30	42.10	7.70
F Tr.B.	93.80	90.65	4.60	43.20	1.26	41.59	9.35
F+B+urea Tr. B.	96.10	91.10	6.50	42.90	3.43	38.27	8.90

* Unt. B. = (untreated bagasse) * Urea Tr. B. = (3%urea treated bagasse) * F Tr. B. = (fungi treated bagasse)
* F + B + urea Tr. B. = (fungi + bacteria+urea treated bagasse).

As a general evidence, different treatments led to decrease OM and CF content but increased CP, EE and ash contents of sugarcane bagasse.

Data obtained in Table (3) showed the chemical composition of different treated and untreated rations. Data obtained indicated similar DM content of different experimental rations. Higher OM, CF and NFE contents were detected for different untreated and treated bagasse rations compared with the berseem hay ration (R_1). Lower CP content (9.75 to 10.32%) was recorded for different treated bagasse ration compared with the hay group (12.09%), but higher than that of the untreated bagasse ration (8.91%). Similar EE and NFE values for different rations, with lower ash content of untreated and treated bagasse rations than the hay ration (R1).

Table (3): Chemical composition of the experimental rations (on DM basis, %)

Item	Experimental rations				
	R_1	R_2	R_3	R_4	R_5
DM	91.79	91.48	91.18	90.70	90.73
OM	93.41	96.35	95.57	95.20	96.65
CP	12.09	8.91	9.93	9.75	10.32
CF	18.24	23.86	21.83	21.66	21.55
EE	3.69	3.23	3.27	3.27	3.91
NFE	59.39	60.35	60.54	60.52	60.87
Ash	6.59	3.65	4.43	4.80	3.35

Results of Table (4) illustrated cell wall constituents of different experimental rations. Data obtained indicated similar NDF values for different treated bagasse rations and hay ration (R_1). Lower NDF value was noticed with untreated bagasse ration (38.34%).

Table (4): Cell wall constituents of experimental rations (on DM basis, %)

Item	Experimental rations				
	R_1	R_2	R_3	R_4	R_5
NDF	42.76	38.34	41.65	42.93	40.22
ADF	19.14	16.85	25.49	25.50	26.30
ADL	5.99	2.68	7.76	8.76	9.12
Hemicellulose*	23.62	21.49	16.16	17.43	13.92
Cellulose**	13.15	14.17	17.73	16.74	17.18

* Hemicellulose = NDF – ADF ** Cellulose = ADF -ADL

Higher ADF, ADL and cellulose values were observed for different treated bagasse rations, but lower ones with both of $R_1$and R_2. In contrast, higher hemicellulose content was noticed with both

of R_1 and R_2, in compare with different treated bagasse rations.

Effect of different treatments on rumen liquor parameters of growing lambs:

Data presented in Table (5) indicated that pH values at 0 time was 6.22 and declined gradually 3hrs after feeding to 5.54 and tended to increase again to 6.76 and more than the corresponding value at 0 time (6.22). Lower ($P<0.05$) pH values were shown by lambs fed the biological and biochemical treated bagasse rations. Higher ($P<0.05$) pH values were shown by lambs fed R_1 (hay control) or R_2. These results agree with those obtained by **Abd El-Kareem (1990) and Tawila (1991)** who noticed that the ruminal pH values decreased gradually reaching the lowest values at 2-4 hrs after feeding and tended to increase again after 6 hrs.

Data presented in Table (5) indicated lower ($P<0.05$) NH_3-N values before feeding, increased to the maximum level 3hrs after feeding and tended to decline ($P<0.05$) again at 6 hrs after feeding. **Reddy *et al* (1989)** reported that the peak concentration of ammonia nitrogen being at 3hrs after feeding. Similar results were reported by **Williams and Newbold (1990)**, who concluded that the reduction of ammonia-N in the rumen liquor appear to be the result of increased incorporation of ammonia-N onto microbial protein and it was considered as a direct result to stimulated microbial activity. These results may be due to the change in fermentation rate with advancing time after feeding in which ruminal NH_3-N would satisfy microbial needs and hence maximize rate of fermentation in rumen.

As a general evidence, different treated bagasse rations indicated higher insignificant NH_3-N values, but without significant differences with the hay ration. Untreated bagasse ration led to lower ($P<0.05$) NH_3-N values at different time of sampling. Similar evidences were reported by **El-Ashry *et al.* (1997) and Khorshed (2000)** who noticed that NH_3-N concentration were increased in rumen of sheep and goats fed rations treated with white rot fungi or yeast culture. Moreover, ruminal ammonia-N concentration values were higher for lambs fed banana plant wastes silage treated by either urea or sodium hydroxide than the control or untreated **(Kropp *et al*., 1977 and Mohamed, 1998)**.

Data presented in Table (5) indicated lower ($P<0.05$) TVFA's concentration (meq/100ml) values before feeding and tended to increase ($P<0.05$) 3 hrs after feeding and declined at 6 hrs after feeding.

Table (5): Ruminal parameters of lambs fed rations containing untreated and treated sugarcane bagasse

Item	Sampling time (hours)	Experimental rations					Overall mean for time	±SE	Sig.
		R_1	R_2	R_3	R_4	R_5			
pH	0	6.93^{a}	6.40^{b}	6.27^{b}	6.03^{c}	6.17^{bc}	**6.22^{C}**	0.887	*
	3	5.73^{a}	5.67^{ab}	5.70^{ab}	5.27^{b}	5.50^{ab}	**5.54^{B}**	0.496	*
	6	6.17^{c}	7.03^{a}	6.63^{b}	6.53^{b}	6.83^{ab}	**6.76^{A}**	0.304	*
	Overall of mean	**6.28^{a}**	**6.37^{a}**	**6.20^{a}**	**5.94^{b}**	**6.17^{a}**	-	0.562	*
NH_3-N (mg/100ml)	0	16.74^{b}	12.11^{c}	19.01^{a}	19.78^{a}	20.31^{a}	**17.80^{C}**	1.004	*
	3	24.48^{a}	17.5^{b}	25.96^{a}	26.21^{a}	24.81^{a}	**23.62^{A}**	1.317	*
	6	19.24^{b}	14.3^{c}	20.76^{b}	20.28^{b}	22.67^{a}	**19.50^{B}**	0.835	*
	Overall of mean	**20.15^{a}**	**14.64^{b}**	**21.91^{a}**	**22.09^{a}**	**22.60^{a}**	-	1.052	*
TVFA's (meq/100 ml)	0	4.00^{ab}	4.17^{a}	3.67^{b}	3.57^{b}	3.57^{b}	**3.75^{C}**	0.125	*
	3	8.20^{a}	6.77^{c}	7.07^{c}	7.57^{b}	7.17^{bc}	**7.15^{A}**	0.143	*
	6	7.33^{a}	4.83^{e}	6.53^{c}	7.00^{b}	5.53^{d}	**5.97^{B}**	0.250	**
	Overall of mean	**6.51^{a}**	**5.26^{c}**	**5.76^{b}**	**6.05^{b}**	**5.42^{b}**	-	0.173	*

*: Significant differences at ($P<0.05$) **: Significant differences at ($P<0.01$)

a,b,c,d and e : Means at the same row and capital letters in the same column with different superscripts are different at ($P<0.05$)

As a general evidence and regardless of time of sampling, the hay group (R_1) recorded higher ($P<0.05$) TVFA's values in compare with different treated bagasse rations, which indicated insignificant differences between each other. While, the untreated bagasse ration, recorded the lower TVFA's value among different rations as an overall mean. **Fouad *et al*. (1998)** found that, the higher ruminal TVFA's concentration was associated with increased NFE intake. The TVFA's values were high in rations R_1, R_3, R_4 and R_5 which may be due to a higher microbial activity in the rumen of sheep fed these rations than those received the untreated (R_2). Similar results were obtained by **Singh and Gupta (1984)**; they found that the TVFA's production rate in rumen of lambs fed ammoniated roughage was higher than those fed untreated roughage. This improvement in TVFA's

concentration in R_4 and R_5 may be attributed to alteration in chemical composition of bagasse by the biological and biochemical treatments. Results of biological treatments might be related to the more efficient utilization of the dietary energy and the positive fermentation activity in the rumen. It was also worthy to note that, the lowest pH values were recorded after 3 hrs after feeding for the different rations. These results are in agreement with those obtained by **Reddy and Reddy (1986)** who reported that pH was inversely related to TVFA's concentration at different periods of incubation.

Effect of different treatments on blood parameters of growing lambs:

Data of hemato-biochemical parameters are presented in Table (6). Results obtained indicated that the biologically and biochemically treated bagasse (R_4 and R_5) led to increase ($P<0.05$) serum total protein and without significant difference with R_3 (ureated bagasse) compared with the other rations. However, albumin, globulin, urea and creatinine parameters indicated insignificant difference among different treatments. Such effects varied from being negative to positive one. These results agree with those obtained by **Khorshed (2000), Deraz and Ismail (2001) and Kholif *et al.* (2001)** who found that serum total protein and albumin concentration were higher with biological treatment than with the control groups. **Shehata *et al.* (2003)** reported that total protein concentration ranged from 6.30 to 7.53 g/l for growing lambs fed rations containing maize silage and CFM.

It is of interest to note that rumen liquor ammonia-nitrogen (Table 5) showed higher ($P<0.05$) NH_3 – N for such groups. Matching of both the two evidences led to suggest a positive correlationship between ruminal NH_3-N and serum total protein content of these groups. According to **Kumar *et al.* (1980)**, total protein reflects the nutritional status of the animal and it has a positive correlation with dietary protein.

Results of urea and creatinine as an indicator to protein metabolism, liver and kidney functions showed insignificant differences among different nutritional groups. However, it was of interest to note relatively higher insignificant values for R_3 (ureated bagasse ration); a result which may probably referred to urea treatment. In general, different treated bagasse rations did not indicate any significant differences in compare with the hay group as a standard conventional ration. Values of urea and creatinine however were within the normal ranges of the species and the performance of animals of different treated groups was the more pronounced truth.

Table (6): Blood parameters of lambs fed rations containing untreated and treated sugarcane bagasse

Item	Experimental rations					±SE	Sig.
	R_1	R_2	R_3	R_4	R_5		
Total protein (g/dl)	7.36^{b}	6.90^{c}	7.60^{ab}	7.81^{a}	7.85^{a}	0.101	*
Albumin (g/dl)	3.71	3.70	3.90	4.01	4.00	0.75	NS
Globulin (g/dl)	3.65	3.20	3.70	3.80	3.85	0.71	NS
A:G ratio	1.02	1.16	1.05	1.06	1.04	0.030	NS
Urea (mg /dl)	34.50	33.03	38.20	32.5	36.88	0.876	NS
Creatinine (mg /dl)	1.18	0.80	1.22	0.98	1.00	0.057	NS
ALT (U/L)	23.60^{c}	33.40^{b}	39.80^{a}	35.70^{ab}	34.87^{ab}	1.589	*
AST(U/L)	21.30^{c}	22.03^{c}	25.89^{ab}	23.40^{b}	27.30^{a}	0.726	*

NS: Non significant differences

*: Significant differences at ($P<0.05$)

a, b and c : Means in the same row with different superscripts are different at ($P<0.05$).

GOT (ALT) and GPT (AST) activity were found to be significantly increased due to chemically, biologically and biochemically treated bagasse compared with R_1 and R_2. Higher ($P<0.05$) ALT and AST values were detected with different treated bagasse rations, however R_3 (ureated bagasse rations) surpassed both of R_4 and R_5, while R_5 surpassed both of R_3 and R_4 for AST. Different treated bagasse rations, recorded higher estimates ($P<0.05$) than the hay group (standard conventional ration). Such results may suggest that, different chemical, biological and biochemical treatments led to exert to somehow, the liver functions. Although, all values obtained for blood biochemical parameters in the present study were within the normal ranges.

El-Ashry *et al.* (1997) stated that the serum GOT and GPT showed higher values with biological treatment than with untreated group. These results agree with **Khorshed (2000)** who reported that the use of biological treated sugar beet pulp in goat ration is useful and did not cause any abnormal condition on rumen activity, liver, kidney functions and animal performance.

Effect of different treatments on the performance of growing lambs:

Growth performance:

The average of body weight gain, feed intake and feed conversion are presented in Table (7).

Average daily weight gain was significantly ($P<0.05$) higher for sheep fed R_1 followed by R_4 and R_5 groups compared with R_2 group. However, no significant difference was detected between R_3 (urea treated bagasse) and the R_2 (untreated bagasse). **Deraz (1996) and Allam *et al.* (2006)** found that animals fed biologically treated roughages were the most efficient group followed by those fed chemically treated roughages. The average daily weight gain was considerably higher for lambs fed rations contained biological and biochemical treated sugarcane bagasse (R_4 and R_5, 193 and 183g, respectively), than those fed untreated bagasse (R_2, 151g). This is due to the higher nutritive value **(Salman *et al.*, 2011)** and intake of fermented sugarcane bagasse than untreated one. **Fazaeli *et al.* (2002)** showed that the improvements in the animal performance could reflect the use of more available nutrients due to the substitution of untreated wheat straw by the fungal treated wheat straw. Growth and feed conversion were all significantly affected by type of roughage and treatment besides intervals of the study period. Also, **Marghany *et al.* (2004) and Abdelhamid *et al.* (2007)** gave better daily body weight gain and **Mohamed (2005)** reported better feed conversion by feeding the biological fermented roughages.

No significant differences were detected in total dry matter intake (TDMI) and concentrate intake (g/h/d) due to different treatments, while a slight increased intake was noticed with R_1 (1330g/h/d) compared with R_2 (untreated bagasse, 1102g/h/d), followed by treatments R_5, R_4 and R_3 (1150, 1130 and 1113g/h/d, respectively). These results may be due to that (berseem hay) has more palatability than untreated bagasse ones. Roughages intake (g/h/d) was significantly differed ($p<0.05$) among R_1 (581g/h/d) and other treatments R_5, R_4, R_2 and R_3 (402, 395, 386 and 382g/h/d, respectively).

Table (7): Effect of chemical, biological and biochemical treatments on growth performance and feed conversion of growing lambs

Item	Experimental rations					±SE	Sig.
	R_1	R_2	R_3	R_4	R_5		
Days	120	120	120	120	120	-	-
No.of animals	5	5	5	5	5	-	-
Initial body wt.(kg)	32.7	32.6	32.8	32.7	32.8	0.405	NS
Final body wt.(kg)	58.65^{a}	50.7^{c}	51.92^{c}	55.8^{b}	54.76^{b}	0.642	**
Total body gain (kg)	25.95^{a}	18.1^{c}	19.12^{c}	23.1^{b}	21.96^{b}	0.609	**
Daily gain (g)	216^{a}	151^{c}	159^{c}	193^{b}	183^{b}	0.005	**
Metabolic body size($BW^{0.75}$)	11.58^{a}	8.78^{c}	9.15^{c}	10.54^{b}	10.14^{b}	0.283	**
Growth rate (%)* **	79.36	55.52	58.29	70.64	66.95	-	-
DM intake,g/h/d:							
Concentrate	749	716	731	735	748	0.026	NS
Roughage	581^{a}	386^{b}	382^{b}	395^{b}	402^{b}	0.023	*
Total	1330	1102	1113	1130	1150	0.043	NS
TDN,% ** **	81.00^{a}	68.99^{c}	72.16^{c}	76.52^{b}	76.29^{b}	0.823	*
DCP,% ** **	8.75^{a}	5.18^{c}	6.05^{b}	6.32^{b}	6.47^{b}	0.281	*
TDNI, g/h/d	1076.4^{a}	760.27^{c}	801.79^{c}	866.21^{b}	877.34^{b}	29.84	**
DCPI, g/h/d	116.38^{a}	57.17^{c}	67.22^{bc}	71.54^{bc}	74.41^{b}	5.666	**
DM intake, g/Kg $w^{0.75}$	114.85^{bc}	125.51^{a}	121.64^{ab}	107.21^{c}	113.41^{bc}	2.054	**
TDNI, g/Kg $w^{0.75}$	92.95	86.59	87.63	82.18	86.52	1.458	NS
DCPI, g/Kg $w^{0.75}$	10.05^{a}	6.51^{b}	7.35^{b}	6.79^{b}	7.34^{b}	0.428	*
Feed conversion ratio:							
kg DM intake/kg gain	6.16^{b}	7.30^{a}	7.00^{a}	5.85^{b}	6.28^{b}	0.165	**
TDNI/ kg gain(kg)	4.98^{a}	5.03^{a}	5.04^{a}	4.49^{b}	4.79^{ab}	0.075	*
DCPI/ kg gain(kg)	0.54	0.38	0.42	0.37	0.41	0.042	NS

NS: Non significant differences *: Significant differences at ($P<0.05$) ** Significant differences at ($P<0.01$)
a,b and c : Means at the same row with different superscripts are different at ($P<0.05$)
Total B .W. gain (Kg)/ Initial B. W. (Kg) x 100 * From **Salman *et al.* (2011)**

Biochemical and biological treatments increased total dry matter intake (g/h/d/). However, biochemical treatment showed the highest intake value followed by the biological treatment

compared with urea and untreated bagasse groups. These results were in harmony with those obtained by **Lewis *et al.* (1999)** who suggested an increase in dry matter intake with fungal or enzymatic treatment. **Kamra and Zadrazil (1988)** found that during microbial processes for conversion of lignocellulosic wastes into food, at least one of three objectives must be reached: an increase in the protein level, an increase in the digestibility of the lignocellulosic material and an improvement in the dry product palatability, although this last factor can be easily improved by ensiling or mixing the substrate with other more palatable food. **Bouattour (2004); Flores (2004); Titi (2004); Gonzalez (2004) and El-Kady *et al.* (2006)** reported that fungal or enzymatic treatments obtained from fungal were not altering dry matter intake. In contrast **Lewis *et al.* (1999) and McAllister *et al.* (1999)** reported that feed intake was increased by biological treatment. **Deraz (1996)** stated that chemical and biological treatments increased markedly voluntary DM intake of corn stalks of growing lambs by 63.3 and 33.8%, respectively, when compared with mechanically treated corn stalks.

Feed conversion:

Feed conversion in different terms i.e. Kg dry matter intake/ kg gain and as kg TDNI/ Kg gain indicated significant differences ($P<0.01$&$P<0.05$) among different nutritional groups, and the best values were recorded with R_1, R_4 and R_5, while no significant difference in feed conversion was shown between R_3 and R_2. These results were in harmony with those obtained by **Mahrous and Abou-Ammo (2005) and Bassuny *et al.* (2003 and 2005)**. **Mohamed *et al.* (1998)** indicated that the feed conversion of treated rice straw was better compared with untreated one. The improvement in term of Kg TDNI /Kg gain for rations contained biologically treated bagasse might be due to the improvement occurred in the chemical composition and the digestibility coefficients of such rations and consequently its nutritive values. Similar results were obtained by **Titi (2004); Plata *et al.* (2004); Haddad and Goussous (2005) and El-Kady *et al.* (2006)** who found that exogenous fibrolytic enzymes obtained from *Trichoderma viride* resulted in improved ($P<0.05$) feed conversion ratio and daily gain of fattened Awassi lambs and buffaloes, with no effect on feed intake. They added also that, fibrolytic enzymes could enhance the growth of fattened lambs and improve their conversion ratio, mainly through improving digestibility of rations nutrients. **El-Marakby (2003)** recorded better feed conversion values for lambs fed rations where 25 and 50% CFM proteins was replaced with biologically wheat straw.

Economical study:

Results of economical study (Table 8) showed that feed cost/ kg weight gain (L.E) of the R_1 ration showed the highest value while, the lowest cost was for that of R_4. The best relative economical efficiency was detected with (R_4). These results are in agreement with the result obtained by **Deraz (1996)** who indicated that the chemical and chemo-fungal treatment decreased the cost of feed used to produce one kg live body weight gain. In addition, **Abd El-Aziz (2002)** observed that replacing 40% of the CFM by biologically treated rice straw reduced the cost of feeding by 28.8%, while, **Allam *et al.* (2006)** reported that the biologically treated sugar beet pulp was to replace 0, 60, 75 or 100 % of corn grains for growing lamb groups, indicating that the feed cost /kg gain decreased with increasing replacing level.

Table (8): Effect of chemical, biological and biochemical treated sugarcane bagasse on economical efficiency of growing lambs

Item	Experimental rations					±SE	Sig.
	R_1	**R_2**	R_3	R_4	**R_5**		
Concentrate (DMI), g	749	716	731	735	748	0.026	NS
Roughage (DMI), g	581[a]	386[b]	382[b]	395[b]	402[b]	0.023	*
Total DM intake, g	1330	1102	1113	1130	1150	0.043	NS
Feed conversion Kg DM intake/ kg gain	6.16[b]	7.30[a]	7.00[a]	5.85[b]	6.28[b]	0.165	**
Av. daily gain (g/h/d)	216[a]	151[c]	159[c]	193[b]	183[b]	0.005	**
Av. feed cost /Kg weight gain (L.E.)	6.17	6.02	6.03	5.06	5.43	-	-
Net feed revenue (L.E.)	10.83	10.98	10.97	11.94	11.57	-	-
Economic feed efficiency (%)	175.5	182.4	181.9	236.0	213.1	-	-
Relative economic efficiency	100	103.9	103.6	134.5	121.4	-	-

NS: Non significant differences

*: Significant differences at ($P<0.05$) ** Significant differences at ($P<0.01$)

a,b and c : Means at the same row with different superscripts are different at ($P<0.05$).

Results of the present study indicated that, replacement of berseem hay by 30 % untreated sugarcane bagasse in lambs rations adversely affected both of growth rate and feed utilization i.e. 216g/h/day and 6.16 kg DMI /kg gain vs 151g/h/day and 7.30 kg DMI /kg gain, inspite of the

lower feed cost /kg gain, which may probably referred to the lower cost of untreated bagasse 200 L.E/ ton vs 750 L.E for berseem hay, respectively. However, replacement of treated sugarcane bagasse instead of berseem hay in sheep rations, particularly biologically and biochemically treated ones led to comparable values like that of hay ration, but at lower feed costs and more efficient utilization of feed (R_4 and R_5). The best treatment was that of R_4 (fungi treated-sugarcane bagasse) instead of berseem hay which significantly improved feed utilization parameters and also reduced feed cost/ kg diet and feed cost/ kg weight gain. The improvement in economic feed efficiency for groups fed bagasse treated with fungi compared with that of R_1 (hay + CFM) could be related to the low price of bagasse compared with the corresponding price of berseem hay, as well as the positive effect of biological treatment in improving the nutritive value and utilization of bagasse. Biological treatment reduced the feed cost by 16.82% **(Deraz, 1996)** to about 36% **(Belewu and Ademilola, 2002)**. However, biological treatments yielded the best economic efficiency **(Marghany *et al.*, 2004 and Hamza *et al.*, 2006)**.

Conclusion

According to the results obtained in the present study it could be concluded the possibility of replacing berseem hay (30% of the ration) by fungi-treated bagasse in sheep rations without any adverse effect on growth or feed utilization parameters, but relatively at lower feed costs.

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Preparation of autogenous bivalent vaccine for *M. bovis* and *M. bovigenitalium* in Egypt

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Abstract: In view of the decreasing effectiveness of antibiotics in controlling *Mycoplasma* infections and no vaccine is available against *Mycoplasma* in Egypt, the need for reliable vaccines has become even more urgent. The present study tried to prepare two bivalent autogenous vaccines (saponised and formalized vaccines) able to protect against *M. bovis* and *M. bovigenitalium*. The prepared vaccines were experimentally injected in groups of rabbits and challenged with virulent strain of *M. bovis* and *M. bovigenitalium*. Both saponised and formalized vaccines were able to protect rabbit against *M. bovis* and *M. bovigenitalium*. Meanwhile saponised vaccine was safe and more potent than formalized vaccine. Experimental work had shown that a vaccine inactivated with saponin can protect in the face of a large *Mycoplasma* challenge and was highly immunogenic.

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Key words: *M. bovis*, *M. bovigenitalium*, saponin, *Mycoplasma* vaccine, formalized vaccine.

1. Introduction:

Mycoplasma species are highly contagious pathogens cause a serious problem on dairy farms. *M. bovis* is a small, cell-wall less bacterium causing a number of diseases including bronchopneumonia, meningitis, otitis media, arthritis, mastitis, abscesses, keratoconjunctivitis and a variety of other diseases in cattle worldwide (Stipkovits *et al.,* 2005; Van der Merwe *et al.,* 2010; Maunsell *et al.*, 2011). *M. bovis* and *M. bovigenitalium* have the ability to colonize the reproductive tract and produce salpingo-oophoritis and reproductive failure in cattle (McEntee, 1990). Both mycoplasmas have been isolated from semen and are transmitted by natural breeding and by artificial insemination (Bielanski *et al.,* 2000). Recently Roy *et al.* (2008) recorded a first report of an intramammary infection caused by *Mycoplasma bovigenitalium* in a 7-weeks old Holstein calf.

Treatment of *Mycoplasma* diseases is difficult since *Mycoplasma* species lack a cell wall, which differentiates them from bacteria and is thus resistant to some commonly used antibiotics. Despite the seriousness of *Mycoplasma* diseases, there are few effective vaccines to combat them today. Indeed, those that are available are whole-cell vaccines, some of which are semi virulent, provide only transient or partial immunity and often induce unpleasant side effects. Furthermore, and alarmingly, attempts at vaccine improvement have often led to exacerbation of diseases, due to their immunopathological nature (Nicholas *et al.,* 2009). Saponins are natural glycosides of steroid or triterpene which exhibited many different biological and pharmacological actions such as immunomodulatory, antitumor, antiinflammatory, molluscicidal, antiviral, antifungal, hypoglycemic, hypocholesterolemicb (Lacaille-Dubois, 2005). The aim of the present study was to establish an early control method for bovine *Mycoplasma* diseases specially respiratory and genital form using saponised and formalized vaccines.

2. Materials and Methods

Identification of *Mycoplasma* isolates:

Two *M. bovis* and two *M. bovigenitalium* isolated from El-Kaliobia and Giza governorates were identified using the conventional methods, Immunoblotting (Towbin *et al.,* 1979) and polymerase chain reaction (Sambrook *et al.,* 1989) using the following Oligonucleotide primers used for detection of *M .bovis* and *M. bovigenitalium:*

Preparation of autogenous inactivated vaccines:

1. Preparation of *Mycoplasma* culture:

Local *M. bovis* and *M. bovigenitalium* isolates were inoculated into Modified Hay Flick's medium (Rosendal, 1994) for 48 hrs at 37º C, 5-10% CO_2. The broth cultures were grown on Modified Hay Flick's agar medium to check purity of *Mycoplasma* suspension. The suspension was centrifuged at 10000 rpm/min and washed 3 times with PBS.

2.Preparation of formalized inactivated *Mycoplasma* vaccine:

Mycoplasma suspensions were inactivated by 1 % formalin (38% analytical reagent grade) at 37º C for

24-48 hrs. After completion of activation, all isolates were mixed together.

Primer designation	Specificity	Length	Sequence (5̀-3̀)	Amplified Product size (bp)	Annealing temperature	Reference
MBsf-MN	*M .bovis*	19	CCA GCT CAC CCT TAT ACA T	442	52 ºC/1 minute	(Pinnow *et al*., 2001)
MBsr-MN		19	TGA ATC ACC ATT TAG ACC G			
MBsr-MN	*M. bovigenitalium*	18	ACC ATG GGA GCT GGT AAT	928	56ºC/1 minute	Gene Bank # AY 780797
MBmr-MN-927		18	TTC TTA CTT CTA AAG TAT			

3. Preparation of saponin inactivated *Mycoplasma* vaccine:

Mycoplasma suspensions were inactivated by saponin (Sapogenin glycosides, Sigma) at 2mg/ml overnight at 37º C. After completion of activation, all isolates were mixed together.

4. Preparation of emulsion for vaccines:

An oil emulsion vaccine with an aqueous phase was prepared. Mineral oil (Risella 17 oil) and SPAN 80 (Biobasic) were used as adjuvant (oily phase) while Tween 80 (HIMEDIA) and physiological saline were used as aqueous phase emulsifier.

Quality control of the prepared vaccines:

The prepared vaccines were tested for purity, sterility, completion of inactivation and safety test according to Standard International Protocols as described by the British Veterinary Codex (1970).

Challenge test (Nicholas, 2002):

Only 0.2 ml of *Mycoplasma* isolates suspension contain 1.2 x 10^5 cfu/ml were administrated by aerosol administration into vaccinated and unvaccinated rabbits at day 43 of designed experiment.

Experiment design:

Six groups of New Zealand rabbits (7-9 weeks old) weighting 1.5 kg were housed separately and vaccinated s/c. These groups represented as:

1- Group A (vaccinated / challenged): 3 rabbits were inoculated with formalized inactivated vaccine then boostering after 3 weeks and challenged 3 weeks later with aerosol administration of virulent mixture of *M. bovis* and *M. bovigenitalium* on consecutive days.
2- Group B (vaccinated / challenged): 3 rabbits were inoculated with saponin inactivated vaccine then boostering after 3 weeks and challenged 3 weeks later with aerosol administration of virulent mixture of *M. bovis* and *M. bovigenitalium* on consecutive days.
3- Group C (unvaccinated / challenged): 3 rabbits were challenged 3 weeks later with aerosol administration of virulent mixture of *M. bovis* and *M. bovigenitalium* on consecutive days.
4- Group D (vaccinated / not challenged): 3 rabbits were inoculated with formalized inactivated vaccine then boostering after 3 weeks and not challenged. These were monitored for adverse effects and antibody response.
5- Group E (vaccinated / not challenged): 3 rabbits were inoculated with saponin inactivated vaccine then boostering after 3 weeks and not challenged. These were monitored for adverse effects and antibody response.
6- Group F (unvaccinated / not challenged): 3 rabbits as control group.

Estimation of humoral immune response among the vaccinated group using:

1. Enzyme Linked Immunosorbent Assay ELISA (Maunsell *et al.,* 2009):

2. Micro-agglutination test according to Harry and Yoder (1982):

Estimation of anemia and carcinogenic effect of the prepared vaccines:

Blood and serum samples collected from vaccinated and unvaccinated groups were examined for detection of anemia tumor factor (CA125, CA19.5, CEA and AFP) using ELecsys 1010 (ROCHE) and IMMULITE (DPC) kits at the end of the experiment.

3. Results and Discussion

Mycoplasma species causes some of the most serious and economically most costly diseases of cattle. In Egypt *M. bovis* and *M. bovigenitalium* were isolated from bovine samples with percentage of 2.7 % and 1.7 % respectively (EL-Jakee *et al.,* 2008). Surprisingly, no vaccines are currently available in Egypt for protection against bovine mycoplasmae in the field. Therefore a critical need to develop

improved strategies for prevention of mycoplasmae associated disease. In the present investigation *M. bovis* and *M. bovigenitalium* isolates were identified according to Quinn *et al.* (2002) and confirmed to be *M. bovis* and *M. bovigenitalium* using PCR and immunoblotting as shown in Photos (1 and 2). Two autogenous bivalent vaccines (Formalized and saponin inactivated vaccines) were prepared from the collected *M. bovis* and *M. bovigenitalium* isolates. The bivalent vaccine would not only protect against the respiratory disease but might protect against other clinical manifestations, including otitis media (Friis *et al.,* 2002) and abortion (Shin *et al.,* 2003). The prepared vaccines were tested for purity, sterility and completion of inactivation according to Standard International Protocols as described by the British Veterinary Codex (1970). Also the prepared vaccines were assayed for side effects and safety by intraperitoneal administration of 1 ml of each vaccine to ten mice. None of the vaccinated mice died and the vaccines showed no reaction after vaccination.

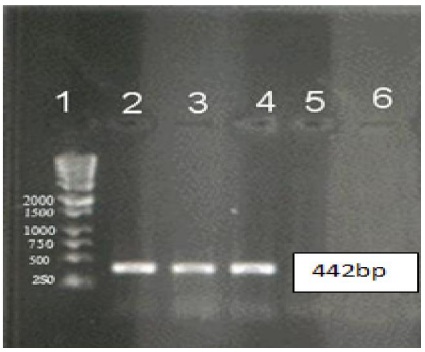
As shown in Figure (1), there was an increase in the body weight gain in groups B (vaccinated with saponin and challenged) and groups E (vaccinated with saponin and not challenged) in comparison with other groups. No local reaction was found in all rabbit injected with saponin compared with control group. The data illustrated in Figures (2-5) revealed that rabbits vaccinated with saponised vaccine had the highest antibody titers against *Mycoplasma bovis* and *Mycoplasma bovigenitalium* compared with other groups using ELISA and Microagglutination tests. Serological result of Delafe *et al.* (2007) indicated that saponin combined vaccines can produce a specific humoral immune response to *M. agalactiae* and *Mmm* LC over 6 months with antibody levels peaking at 45 days. The results from the work of Nicholas *et al.* (2002) reported that even a single dose of vaccine prepared from saponised *M. bovis* cells may provide effective control against *Mycoplasma* induced calf pneumonia.

No local reaction or clinical sign was observed among all rabbit injected with saponin in compared with control group, also no local reaction was found in all rabbits injected with formalized vaccines except one rabbit in group D (formalized vaccine and not challenged) had slightly local reaction. After challenge, *M. bovis* and *M. bovigenitalium* were isolated from nasal cavity, tracheal bifurcation, lung, vagina and joint fluid of rabbits in group C (unvaccinated and challenged). Pneumonia and swelling of joints were seen in the same group. Quillaja saponins have serious drawbacks such as high toxicity, undesirable hemolytic effect and instability in aqueous phase, which limits their use as adjuvant in human vaccination as recorded by Marciani *et al.* (2003). Meanwhile in our experiment no anemia or carcinogenic effect could be detected among the vaccinated and unvaccinated groups using ELecsys 1010 (ROCHE) and IMMULITE (DPC) kits.

The successful use of saponin in vaccines has already been demonstrated for other *Mycoplasma* infections such as CCPP and contagious agalactia. Its effectiveness must be associated with the fact that it apparently preserves the major antigens seen in untreated whole cells (Tola *et al.,* 1999). Previously, Kensil *et al.* (1991) speculated that the high level of protection seen with the use of saponins with vaccines in mice may be caused by the ability of saponins to induce an isotype profile similar to that seen in natural immunity to bacterial infections. This work highlights the effect of using saponin vaccine on protection against *Mycoplasma* associated respiratory disease.

Acknowledgement

To late Dr. El-Moustafa Barbar**,** Lecturer of Microbiology, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University.

A 

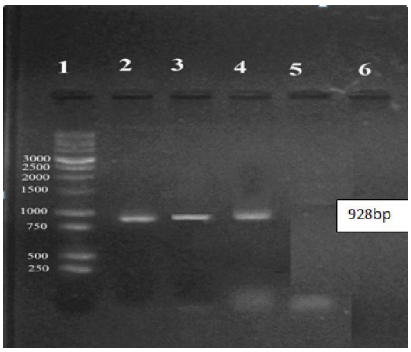
B 

Photo (1) Shows agarose gel electrophoresis showing amplification of 442 bp fragment of *M. bovis* (A) and 928 bp fragment of *M. bovigenitalium* (B).

(A)- Lane (1): DNA ladder (Sigma), Lane (2): *M. bovis* reference strain (PG45), Lane (3 & 4): *M. bovis* isolates and Lane (5): *M. bovigenitalium* reference strain (PG11). (B)- Lane (1): DNA ladder (Sigma), Lane (2): *M.*

bovigenitalium reference strain (PG11), Lane (3 & 4): *M. bovigenitalium* isolates and Lane (5): *M. bovis* reference strain (PG45).

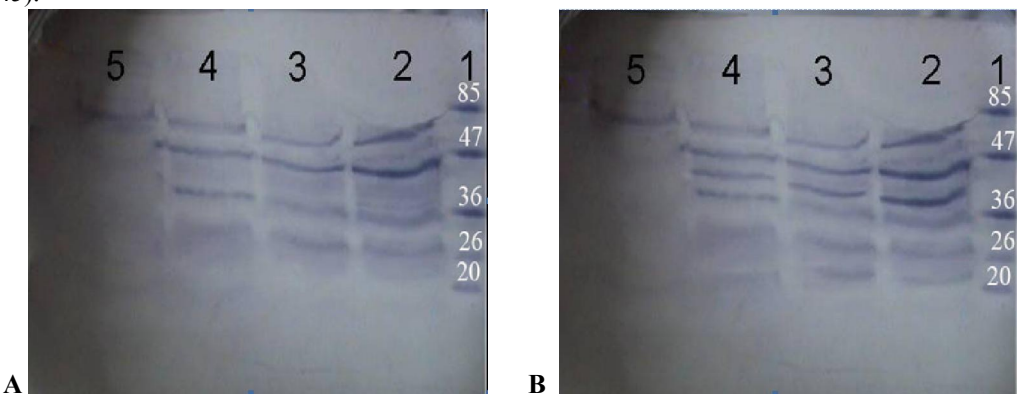


Photo (2) Shows immunoblotting against *M. bovis* (A) and *M. bovigenitalium* (B).
(A)-Lane (1): Protein marker (Ready to use) # 5M0441, Lane (2): *M. bovis* reference strain (PG45) Lane (3 & 4): *M. bovis* isolates and Lane (5): *M. bovigenitalium* reference strain (PG11). (B)- Lane (1): Protein marker (Ready to use) # 5M0441, Lane (2): *M. bovigenitalium* reference strain (PG11), Lane (3 & 4): *M. bovis* isolates and Lane (5): *M. bovis* reference strain (PG45).

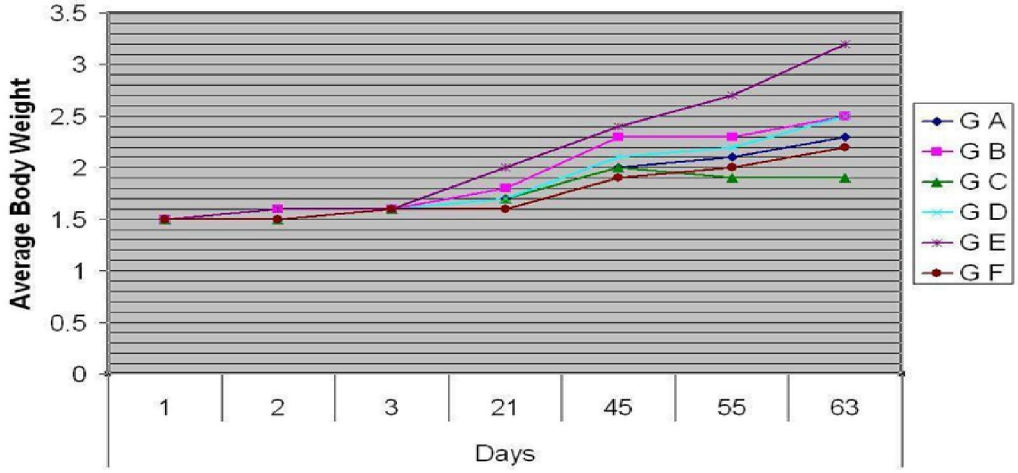


Figure (1) Body weight (kg) of vaccinated and unvaccinated rabbits.

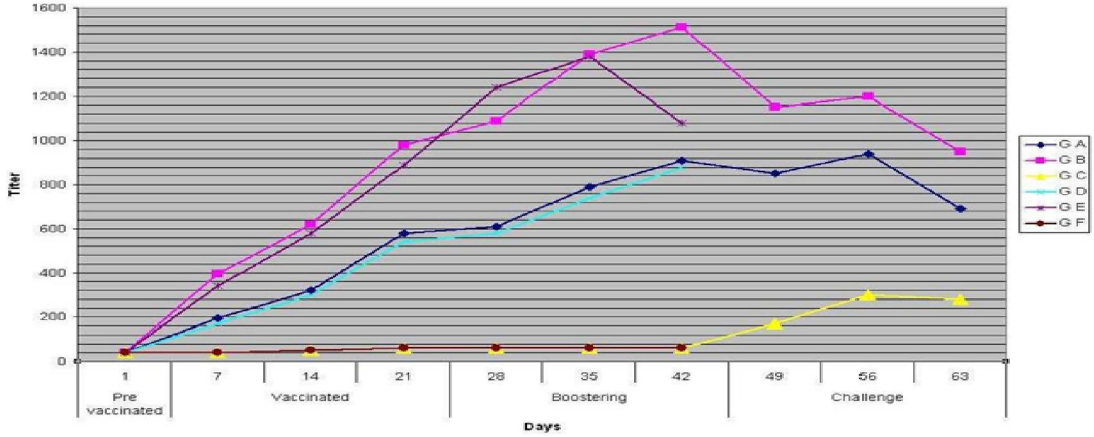


Figure (2) Antibody titers against *M. bovis* among the vaccinated and unvaccinated groups using ELISA test

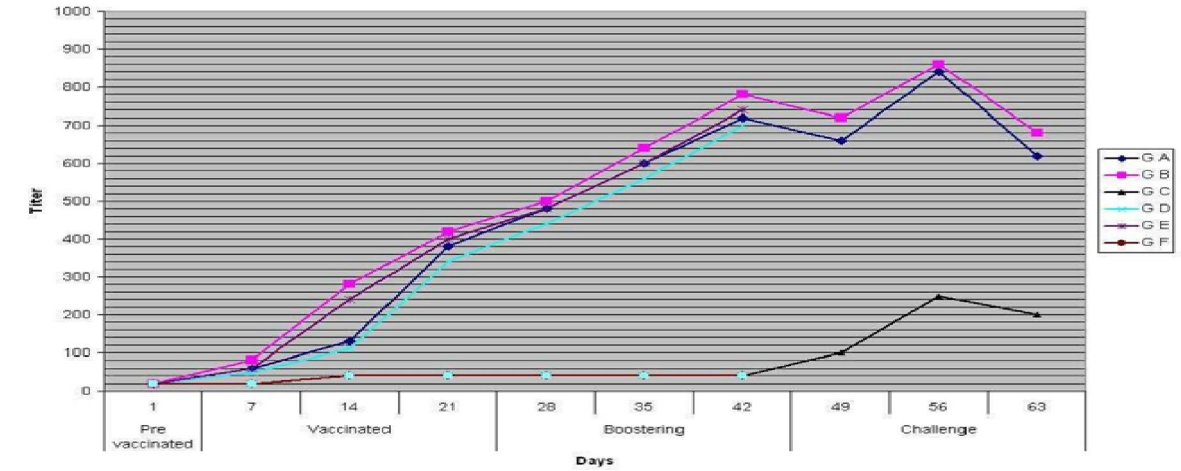


Figure (3) Antibody titers against *M. bovis* among the vaccinated and unvaccinated groups using Micro-agglutination test

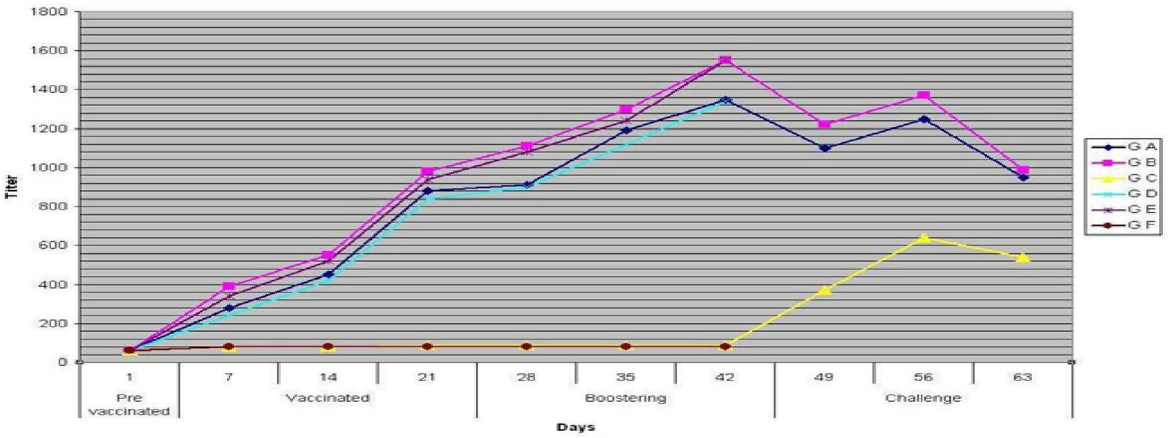


Figure (4) Antibody titers against *M. bovigenitalium* among the vaccinated and unvaccinated groups using ELISA test.

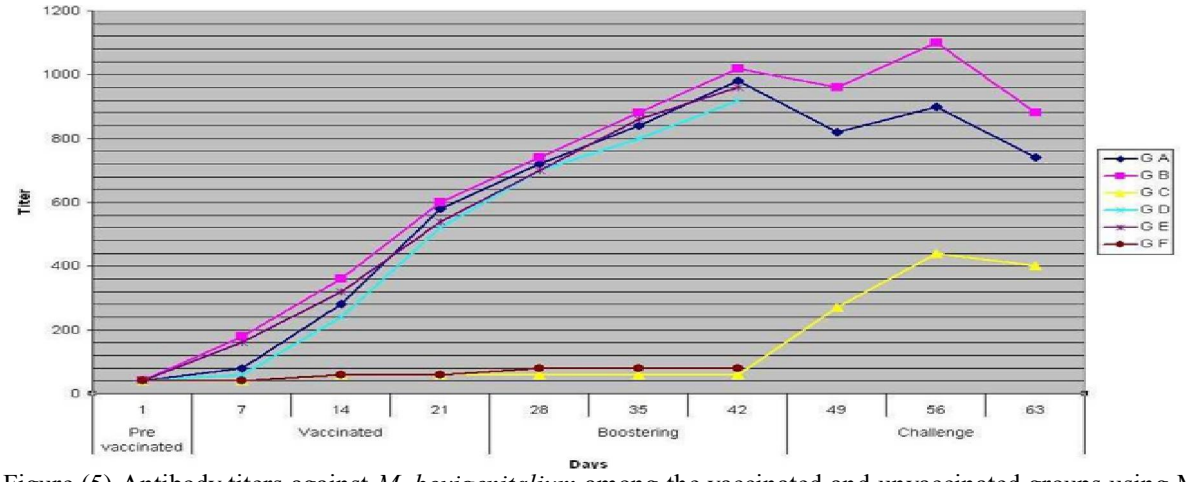


Figure (5) Antibody titers against *M. bovigenitalium* among the vaccinated and unvaccinated groups using Micro-agglutination test**.**

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Relationship between chronic HCV infection and diabetic microvascular complications in Egyptian patients

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Abstract: Background: Hepatitis C virus (HCV) infection and type 2 diabetes are two worldwide, major public health problems. Several studies demonstrated the link between HCV and microvascular complications of diabetes as regard progression and development while other studies fail to demonstrate that. The aim of the present study was to evaluate the effect of chronic HCV infection (Ch HCV) on the micro- vascular complications of type 2 diabetes mellitus (DM). Patients and methods: This study was conducted on 100 patients. They include 50 type 2 DM patients without chronic HCV infection (group I) and 50 type 2 DM patients with chronic HCV infection (group II) in addition to 20 healthy subjects as control group (group III). All patients were subjected to detailed history taking, clinical examination and laboratory investigations: including complete blood picture, fasting and post-prandial blood glucose and glycosylated hemoglobin (HbA1c), qualitative HCV RNA PCR test, liver profile (AST, ALT, serum bilirubin, serum albumin, INR), renal function (serum urea and creatinine, albumin/creatinine ratio (ACR). Ophthalmoscopic examinations for fundus and nerve conduction tests were done to prove retinopathy and peripheral nerve affection respectively. Results: Diabetic retinopathy was higher in diabetic patients without chronic HCV infection compared to diabetic patients with chronic HCV infection, while diabetic nephropathy and neuropathy were higher in diabetic patients with chronic HCV infection compared to diabetic patients without chronic HCV infection. Conclusions: Incidence of developing diabetic nephropathy and neuropathy was higher in diabetics with Ch HCV infection due to the double etiology, both diabetes and Ch HCV infection. On the other hand, incidence of diabetic retinopathy was lower in diabetic Ch HCV infected patients.

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Keywords: Hepatitis C virus, diabetes mellitus, nephropathy, neuropathy, retinopathy

1. Introduction

Hepatitis C virus (HCV) is a major cause of acute hepatitis and chronic liver disease, including cirrhosis and liver cancer. Globally, an estimated 170 million persons are chronically infected with HCV and 3 to 4 million persons are newly infected each year [(1)] .

Patients with chronic hepatitis C virus (Ch HCV)infection have significantly increased prevalence of type 2 diabetes mellitus (DM) compared to controls or HBV-infected patients, independent of the presence of cirrhosis [(2)] .

It was reported that several molecules, including tumor necrosis factor alpha, suppressor of cytokine signaling 1 and 3 proteins, insulin-receptor substrates 1 and 2, and other adipocytokines, potentially are involved in the development of insulin resistance in patients with chronic hepatitis C [(3)] .

Several studies demonstrated the link between HCV and microvascular complications of diabetes (diabetic retinopathy, nephropathy and neuropathy) as regard progression and development while other studies fail to demonstrate such a link [(1)] .

The aim of this work is to study the relation between microvascular complications of diabetes mellitus and chronic hepatitis C virus infection.

2. Patients

This study is a case-control comparative study which included 100 patients with type 2 DM and 20 controls selected from the outpatients of the Internal Medicine Department of Menoufiya University Hospital in the period from June 2010 to December 2010.

The patients were classified into three groups: **Group I included** 50 type 2 DM patients without HCV infection, **Group II included** 50 type 2 DM patients with chronic HCV infection and ***Group III*** included 20 normal subjects matched for the same age as control group. An informed consent was obtained from all subjects enrolled in the study.

Exclusion criteria:

In this study we excluded patients with advanced liver or renal failure and patients with history of interferon therapy.

All patients were subjected to detailed history taking including demographic data (age, sex, duration of diabetes, family history of diabetes), clinical examination and laboratory investigations: including complete blood picture, fasting and post-prandial blood glucose and glycosylated hemoglobin (HbA1c), Qualitative HCV RNA polymerase chain reaction PCR test using the Cobas Amplicor HCV version 2.0 (Roche Diagnostics Inc., Mannheim, Germany) assay,[(4)]

liver profile (AST, ALT, serum bilirubin), serum albumin, INR) were done on autoanalyzer SYNCHRON CX5 from Beckman, renal function (serum urea and creatinine, albumin/creatinine ratio (ACR) Albuminuria was detected by Cayman's HSA EIA Kit (competitive assay) Cayman Chemical Company, Ann Arbor, Michigan 48108 USA.[5] .Nephropathy was diagnosed if there was micro or macroalbuminuria (microalbuminuria if albumin level was between 30-300 mcg per mg creatinine and macroalbuminuria if it was more than 300 mcg per mg creatinine)[6]. Ophthalmoscopic examination for fundus was done by specialist of ophthamology to prove retinopathy and nerve conduction test was done by neurologist to prove peripheral nerve affection in Menoufiya University Hospital.

Statistical methodology

Data was analyzed using Statistical Package for Social Science (SPSS) software computer program version 15. Quantitative data were presented in mean and standard deviation (SD). Qualitative data were presented in frequency and percentage. To compare between groups we used: **t-test, Chi-square test**, **ANOVA test** (analysis of variance) and LSD (least significant difference). Significance level (P) value was $P \leq 0.05$.

3. Results

There were no significant differences between the three groups as regards age, sex and body mass index. There was no significant difference between groups I and II as regards the duration of DM.

The mean systolic and diastolic blood pressure were significantly higher in group I compared to groups II & III (Table I).

There was no significant difference between the three groups as regards RBC count, WBCs count and hemoglobin concentration. The mean platelet count was significantly lower in group II compared to groups I & III.

The mean fasting blood sugar, 2 hours post-prandial blood sugar and HbA1C were significantly higher in groups I and II compared to group III (Table III) .

The mean ALT, AST, serum bilirubin and INR were significantly higher in group II compared to groups I & III. The mean serum albumin level was significantly lower in group II compared to groups I & III.

The mean serum creatinine was significantly higher in groups I and II compared to group III. There was no significant difference between the three groups as regards mean blood urea.

Diabetic nephropathy was present in 12 (24%) patients of group I and 22 (44%) patients of group II, it was significantly higher in group II. Diabetic neuropathy was present in 14 (28%) patients of group I and 24 (48%) patients of group II, it was significantly higher in group II. Diabetic retinopathy was present in 18 (36%) patients of group I and 9 (18%) patients of group II, it was significantly lower in group II,

The mean age, mean duration of DM, HBA1C, systolic and diastolic blood pressure were significantly higher in nephropathy +ve patients compared to nephropathy –ve patients in both groups I & II.

In groups I & II, the mean age, mean of duration of DM and HBA1C were significantly higher in neuropathy +ve patients compared to neuropathy –ve patients.

In groups I & II, the mean age, mean of duration of DM and HBA1C were significantly higher in retinopathy +ve patients compared to retinopathy –ve patients.

Table I: Comparison between the three groups as regards blood pressure

	Group I (n= 50)	Group II (n= 50)	Group III (n= 20)	ANOVA test	LSD
Systolic blood pressure (mmHg)					
Mean ± SD	143.8± 22.57	122± 14.28	126 ± 17.88	P < 0.001*	GI vs GII & GIII
Diastolic blood pressure (mmHg)					
Mean ± SD	90.9 ± 9.51	70.0 ± 7.62	80.0 ± 9.98	P < 0.001*	GI vs GII & GIII

Table II: Comparison between the three groups as regards platelet count

	Group I (n= 50)	Group II (n= 50)	Group III (n= 20)	ANOVA test	LSD
Platelet count ($x10^3$/cc)					GI1vs GI & GIII
Mean ± SD	253.1±57.38	172.2 ± 52.6	254.1± 45.33	P< 0.001*	

Table III: Comparison between the 3 groups as regards blood glucose profile

	Group I (n= 50)	Group II (n= 50)	Group III (n= 20)	ANOVA test	LSD
FBS (mg/dl)					
Mean ± SD	146.04 ± 22.41	140.68 ± 32.88	81.35 ± 6.83	P < 0.001*	GI1vs GI & GIII
PPBS (mg/dl)					
Mean ± SD	211.90 ± 45.5	204.40 ± 42.33	101.4 ± 5.09	P < 0.001*	GI1vs GI & GIII
HBA1C (%)					
Mean ± SD	8.30 ± 1.27	8.37 ± 1.41	5.11 ± 0.39	P < 0.001*	GI1vs GI & GIII

Table IV: Comparison between group I and group II as regards incidence of micro-vascular complication

		Group I (n = 50)	Group II (n = 50)	X^2-test
Nephropathy	+ve	12 (24%)	22 (44%)	P = 0.035*
	-ve	38 (76%)	28 (56%)	
Neuropathy	+ve	14 (28%)	24 (48%)	P = 0.039*
	-ve	36 (72%)	26 (52%)	
Retinopathy	+ve	18 (36%)	9 (18%)	P = 0.042*
	-ve	32 (64%)	41 (82%)	

Table V: Comparison between nephropathy +ve and –ve patients in group I and group II as regard age, duration of DM, glycated hemoglobin level, blood pressure

Group I		**Nephropathy +ve (n = 12)**	**Nephropathy –ve (n = 38)**	**t-test**
Age (years)	Mean± SD	50.28 ± 8.9	43.11 ± 8.2	P = 0.009*
Duration of DM (years)	Mean± SD	16.71 ± 7.9	11.44 ± 7.3	P = 0.031*
HbA1C (%)	Mean± SD	9.04 ± 1.6	8.03 ± 1.0	P = 0.010*
Systolic BP (mmHg)	Mean± SD	159.29 ± 24.6	134.17 ± 21.7	P <0.001*
Diastolic BP (mmHg)	Mean± SD	92.68 ± 11.4	85.56 ± 10.6	P =0.037*
Group II		**Nephropathy +ve (n = 22)**	**Nephropathy –ve (n = 28)**	**t-test**
Age (years)	Mean± SD	51.00 ± 6.7	40.77 ± 6.8	P < 0.001*
Duration of DM (years)	Mean± SD	19.00 ± 6.0	8.25 ± 5.5	P < 0.001*
HbA1C (%)	Mean± SD	9.27 ± 1.7	7.86 ± 0.89	P < 0.001*
Systolic BP (mmHg)	Mean± SD	150.59 ± 17.8	121.5 ± 15.0	P<0.001*
Diastolic BP (mmHg)	Mean± SD	94.06 ± 8.7	76.97 ± 9.5	P <0.001*

Table VI: Comparison between neuoropathy +ve and –ve patient in group I and group II as regard age, duration of DM, glycated heamoglobin level, blood pressure

Group I		**Neuropathy +ve (n = 14)**	**Neuropathy –ve (n = 36)**	**t-test**
Age (years)	Mean± SD	56.0 ± 4.7	42.6 ± 7.4	P < 0.001*
Duration of DM (years)	Mean± SD	22.5 ± 4.2	10.57 ± 6.5	P < 0.001*
HBA1C (%)	Mean± SD	10.0 ± 1.4	7.88 ± 0.8	P < 0.001*
Systolic BP (mmHg)	Mean± SD	143.0 ± 31.3	133.85 ± 35.4	P =0.895
Diastolic BP (mmHg)	Mean± SD	89.0 ± 11.0	86.5 ± 10.3	P = 0.511
Group II		**Neuropathy +ve (n = 24)**	**Neuropathy –ve (n = 26)**	**t-test**
Age (years)	Mean± SD	54.00 ± 4.2	41.62 ± 6.05	P < 0.001*
Duration of DM (years)	Mean± SD	21.07 ± 4.5	8.91 ± 5.8	P < 0.001*
HBA1C (%)	Mean± SD	9.79 ± 1.8	7.87 ± 0.8	P < 0.001*
Systolic BP (mmHg)	Mean± SD	126.92 ± 20.2	132.97 ± 21.5	P =0.303
Diastolic BP (mmHg)	Mean± SD	77.69 ± 11.7	84.32 ± 11.9	P = 0.084

Table VII: Comparison between retinopathy +ve and –ve patient in group I and group II as regard age, sex duration of DM glycated heamoglobin level, blood pressure and duration of HCV infection

Group I		**Retinopathy +ve (n = 18)**	**Retinopathy –ve (n = 32)**	**t-test**
Age (years)	Mean± SD	50.7 ± 7.8	41.36 ± 6.9	P < 0.001*
Duration of DM (years)	Mean± SD	19.52 ± 5.6	9.57 ± 6.5	P < 0.001*
HBA1C (%)	Mean± SD	9.35 ± 1.6	7.74 ± 0.5	P < 0.001*
Systolic BP (mmHg)	Mean± SD	155.9 ± 25.3	133.6 ± 21.6	P =0.004*
Diastolic BP (mmHg)	Mean± SD	93.8 ± 11.7	84.7 ± 9.6	P =0.014*
Group II		**Retinopathy +ve (n = 9)**	**Retinopathy –ve (n = 41)**	**t-test**
Age (years)	Mean± SD	52.92 ± 4.9	41.66 ± 6.3	P < 0.001*
Duration of DM (years)	Mean± SD	20.07 ± 5.3	9.0 ± 6.1	P < 0.001*
HBA1C (%)	Mean± SD	9.87 ± 1.7	7.86 ± 0.8	P < 0.001*
Systolic BP (mmHg)	Mean± SD	147.86 ± 20.8	124.4 ± 17.8	P <0.001*
Diastolic BP (mmHg)	Mean± SD	89.3 ± 11.4	80.28 ± 11.1	P =0.014*

4. Discussion

Hepatitis C virus (HCV) infection and type 2 diabetes mellitus (DM) are two worldwide, major public health problems with increasing complications and mortality rates. Egypt contains the highest prevalence of hepatitis C in the world [7]

The classic view of metabolic and hemodynamic alterations as the main causes of microvascular injury in diabetes has been transformed significantly, with clear evidence indicating that these traditional factors are only a partial aspect of a much more complex picture. One of the most important changes is related to the participation of immune-mediated inflammatory processes in the pathophysiology of diabetes mellitus and its complications [7].

The current study found that nephropathy was higher in Ch HCV-DM patients (44%) compared to T2DM patients (24%). This agrees with **Sahar *et al.,*** [8] and **Soma *et al.,*** [9]. On contrary, **Kuriyama *et al.,*** [10] found that nephropathy was lower in Ch HCV-DM (10%) compared to T2DM (17%). In this study it was observed that the incidence of nephropathy was lower in Ch HCV-DM patients compared to our study which can be explained by better glycemic control observed in **Kuriyama *et al.,*** [10] study (HbA1C 6.8 %) compared to our study (8.3 %) also shorter duration of DM (8) years in T2DM patients and (5) years in Ch HCV-DM compared to (13.1 and 11.6) years in our study.

Hepatitis C virus (HCV) is known to have direct effects in the kidney, such as membranous nephropathy, cryoglobulinemia, and membrano-proliferative glomerulonephritis (MPGN). The presence of HCV worsens the progression of several renal diseases, and contributes to the excess of renal disease, and this effect may have a relatively greater impact among diabetic patients [11]. It was found that HCV is associated with increased risk for end stage renal disease (ESRD) among patients with DM. HCV has been reported to cause glomerular disease, increase the risk of albuminuria, and accelerate progression of diabetic nephropathy additionally [12,13] .

In study by **Errol *et al.,*** [14], HCV was a significant predictor of reaching ESRD independent of initial renal function, proteinuria, blood pressure, sex, race, presence of diabetic nephropathy, age, or duration of diabetes. Poorer renal survival in the HCV patients may be due to direct effects of HCV in the kidney. Baseline viral load is an independent positive predictor for chronic kidney disease [13] .

The mechanism of HCV-related renal disease is uncertain, research suggests that glomerular injury results from deposition of circulating immune complexes that contain hepatitis C antibodies, antigens, and complement [12]. Other study has shown that inflammatory cytokines, are determinant in the development of micro-vascular diabetic complications [15]. Diabetic patients with nephropathy have higher serum concentrations of tumor necrosis factor alpha (TNF-α) than non-diabetic subjects or diabetic patients without renal involvement, with a significant rise in serum TNF-α as diabetic nephropathy progresses [16,17]. TNF- is cytotoxic to glomerular, mesangial, and epithelial cells, and may induce direct renal damage. Moreover, TNF- has a direct effect on the protein permeability barrier of the glomerulus independent of alterations in hemodynamic factors or effects of recruited inflammatory cells [18].

Peripheral neuropathy (PN) in our study was found higher in Ch HCV-DM patients (48%) compared to T2DM patients (28%). **Sahar *et al.,*** [8] found that PN was higher in Ch HCV-DM (33.5%) compared to T2DM (24%). **Kuriyama *et al.,*** [10] found that PN was lower in Ch HCV-DM (20%) compared to T2DM (35%).

In study made by **Zaltron *et al.*,**[19] PN was found to be present in 23/68 patients with hepatitis C and detectable cryglobulins (CG).

PN is described in 9% of patients chronically infected by HCV and when cryoglobulinemia is present this number can rise to more than 30%.[20-22] Although PN in HCV has greater association with increased CG, several papers have described it in the absence of CG.[23-25] Recently, after finding the virus RNA in nerve biopsies, some authors suggested a direct viral aggression against the nerve[26] . However, PN seems to the result from immunomediated mechanisms determined by the HCV in the nerve rather than related to direct viral infection with consequent *in situ* lesion in the nervous tissue[27] .

PN associated with HCV is usually related to axonal damage, probably secondary to vasculitis, fascicular ischemia and subsequent axonal degeneration [28]. Usually the peripheral nervous system involvement is described as a sensory-motor, distal polyneuropathy often with cryoglobulinemia[29], but isolated mononeuropathy, such as carpal tunnel syndrome or multiple mononeuropathy seem common[30] .

PN is the most common complication of mixed cryoglobulinemia and result of axonal ischemic damage. Two main pathogenic mechanisms have been suggested, represented by deposits of CG in the vasanervrum microcirculation and vasculitis [31]. Recently a role of anti neuronal antibodies has been suggested[32].

Moreover, it was demonstrated that HCV core protein activates human glia and contributes to neurotoxicity. Direct exposure of HCV core protein to primary human neurons suppressed the neuronal autophagy, leading to neurite retraction. The change in neuronal membrane potential after exposure to HCV core protein indicated that core was biologically active at the cell membrane and was able to modulate ionic conductance in neurons. In addition to direct neurotoxicity, proinflammatory cytokines and other neurotoxins released from HCV core-activated microglia into supernatants were toxic to neurons[33] .

Retinopathy in our study was found higher in T2DM patients (36%) compared to Ch HCV-DM patients (18%).

The low prevalence of diabetic retinopathy in diabetic patients with hepatitis C chronic liver diseases may be related to liver disease induced abnormalities protecting the cardiovascular system from atherosclerosis (hypotension, coagulation defect and decreased platelet count)[34] .

In our study we observe that Ch HCV-DM patients had significantly lower mean systolic and diastolic blood pressure compared to T2DM. Also we observe significant decrease in platelet count and significant increase in INR in Ch HCV-DM compared to T2DM and control. These effects of Ch HCV infection may decrease diabetes induced hypercoagulation and premature atherosclerosis induced by factors including, increased Levels of platelet-derived micro particles (MPs) and monocyte-derived MPs[35] .

Increase mean platelet volume (a marker associated with platelet reactivity) has been demonstrated to be increased in diabetes particularly associated with diabetic retinopathy[35].

A lower activity of the system anti thrombin III-heparin, a higher activity of fibrinogen and activation of the fibrinolytic system were observed in diabetic patients with retinopathy (micro-angiopathy) compared to diabetics without retinopathy [36] . More than this chronic liver diseases (CLD) including Ch HCV infection have low serum level of lipids especially low serum lipoprotein LP(a) which competes with plasminogen for bending to fibrin impairing fibrinolysis. High LP (a) is associated with the development and progression of retinopathy in diabetic patients and there is correlation between the severity of diabetic retinopathy and LP(a)[37] .

Sahar *et al.*, [8] found that retinopathy was lower in Ch HCV-DM (29%) compared to T2DM (51%), **Kuriyama *et al.*,**[10] found that retinopathy was lower in Ch HCV-DM (20%) compared to T2DM (39%) and **Fujiwara *et al.*,** [38] found that retinopathy was higher in T2DM (53%) compared to LC-DM (16%), while, **Soma *et al.*,** [9] found that retinopathy was comparable in the two groups (47%) in Ch HCV-DM and (44.55) in T2DM.

Proliferative diabetic retinopathy is characterized by an early pathological micro-vascular obstruction and retinal ischemia. Platelet adhesion seems to be more important in pathogenesis of retinopathy (micro vascular occlusion, neovascularization and progression of retinopathy) than in other diabetic micro-vascular complications[39]. Unlike nephropathy and neuropathy, diabetic retinopathy is not known to be one of extrahepatic manifestations of HCV and this may give the chance for the protective effects of HCV CLD to take the upper hand making retinopathy lower in Ch HCV-DM compared to T2DM[37] .

This study clarified that several factors might affect the occurrence of diabetic micro vascular complications including longer duration of diabetes, high HbA1c, high blood pressure and longer age were observed in patients with diabetic triopathy as compared to those without triopathy.

Skyler *et al.*, [40] found that chronic hyperglycemia , duration of DM and hypertension are prominent risk factors for diabetic-angiopathy. **Rani *et al*.**, [41] found that the duration of diabetes, however, remained the strongest predictor for any diabetic retinopathy and its severity. Longer duration of diabetes, lean BMI, hyperglycemia coupled with other risk factors. Male gender was

observed to be associated with the presence of any diabetic retinopathy, but not its severity.

Conclusion

Incidence of developing diabetic nephropathy and neuropathy was higher in diabetics with Ch HCV infection due to the double etiology, both diabetes and Ch HCV infection . CH HCV can on its own produce nephropathy and peripheral neuropathy as extrahepatic manifestation. Incidence of diabetic retinopathy was lower in diabetic Ch HCV infected patients could be explained that retinopathy is not known to be one of extrahepatic manifestations of HCV. Platelet adhesion seems to be more important in pathogenesis of retinopathy (micro vascular occlusion, neovascularization and progression of retinopathy) than in other diabetic micro-vascular complications. This may give the chance for the protective effects of CLD (through Ch HCV induced thrombocytopenia, low coagulation functions) to take the upper hand making retinopathy lower in Ch HCV-DM compared to T2DM. Duration of diabetes, age, glycemic control and blood pressure are prominent risk factors for diabetic micro-angiopathy. Strict control of blood glucose, therefore, should be directed in patients with diabetic Ch HCV to help in preventing diabetic angiopathy in those patients. Further study is needed to find the relevance between diabetic angiopathy and insulin resistance in CLD patients.

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Chemical, Biological and Biochemical Treatments to Improve the Nutritive Values of Sugarcane Bagasse (SCB): 1- Chemical Composition, Scanning Electron Microscopy, *In Vitro* Evaluation, Nutrients Digestibility and Nitrogen Utilization of Untreated or Treated SCB

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Abstract: The present study aimed to evaluate the effect of chemical (3% urea), biological (fungi, yeast and bacteria and their combinations) and biochemical treatment (combined biological + urea) on the nutritive value of sugarcane bagasse. The effect of treatments on chemical composition, cell wall constituents, scanning electron microscopy, *in vitro* DM and OM disappearance and *in vivo* nutrients digestibility of bagasse was studied along with N- utilization with lambs. The results showed that different treatments increased DM, CP, EE and ash, while decreased OM, CF and NFE. The increments in CP content were 305, 188 and 156% due to biochemical, chemical and biological treatments, respectively. The chemical, biological and biochemical treatments decreased NDF, ADF, hemicellulose and cellulose, while increased ADL content. The obvious change in the structure of cell parenchyma was observed in chemical, biological and biochemical treated bagasse. Values of *in vitro* dry matter and organic matter disappearance were significantly higher (P<0.01) for biochemical, chemical and biological treated bagasse, respectively, than untreated bagasse. The nutritive values and N-utilization recorded with lambs fed rations containing biological and biochemical treated sugarcane bagasse were greater than those containing untreated or urea treated ones. It was concluded that, different treatments improved chemical composition, cell wall constituents, IVDMD and IVOMD disappearance, almost nutrients digestibility, TDN and DCP values of sugarcane bagasse with the superiority of fungi or fungi + bacteria + urea treatments.

[Fatma M. Salman; R. Salama; A. E. khattab[3]; S. M. Soliman and Y. A. El-Nomeary **Chemical, Biological and Biochemical Treatments to Improve the Nutritive Values of Sugarcane Bagasse (SCB): 1- Chemical Composition, Scanning Electron Microscopy, *In Vitro* Evaluation, Nutrients Digestibility and Nitrogen Utilization of Untreated or Treated SCB]** Life Science Journal,. 2011; 8(4):351-363] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: Sugarcane bagasse, chemical and biological treatments, chemical composition, *in vitro*, scanning electron microscopy, digestibility.

1. Introduction

Shortage in the animal feeds is a well-known problem; therefore several studies were carried out to improve the nutritive values of the poor quality roughages to find out an effective and practical solution to overcome the feed shortage problem in animal feeds and resources.

The estimated amount of poor quality roughages in Egypt includes rice straw, wheat straw, bean straw, corn stalks, corn cobs, rice hulls and sugarcane bagasse is about 19 million tons/ year (**El-Shinnawy and Shoukry, 2002**). Among these roughages, sugarcane bagasse represents about 4.13 million tons/ year according to **Agriculture Economic and Statistics Institute (1995)**. Under this circumstance, sugarcane becomes important as a potential feed resource given its high biomass yield and adaptation to the environment (**Conrad *et al*., 1990 and Molina, 1990**).

Sugarcane bagasse, filter cake and pith are secondary by-products in the process of sugar production. The main components of them are cellulose, hemicellulose and lignin. As a result of its high content of lignin, ruminal digestion is inhibited and thus, the nutritive value of bagasse and pith is low for ruminants.

To improve the nutritive value of these agriculture residues, it is important to breakdown the linkages among cellulose and lignin by mechanical, chemical or biological and combined biological plus chemical treatments. Many scientists suggested the use of ammonia and urea to increase the crude protein contents of the poor quality roughages (**Shoukry *et al.*, 1992 & Fouad *et al*., 1998**).

Physical and chemical pre-treatment such as ammonia explosion in combination with fungal may also upgrade the nutritional quality of marginal agricultural residues such as bagasse.

Biological treatment is an alternative method to modify the fibrous materials by ruminants. The mode of fungal decay on roughages using the white rot fungi was shown to improve *in vitro* dry matter digestibility of the decayed substrate (**Dawson *et al.*,1990).** Recently the production of microbial protein from agricultural crop residues received that attention of several workers (**El-Ashry *et al*., 1997 and 2002; Deraz and Ismail, 2001 and Kholif *et al*., 2001).**

The objective of this work was to study the possibility of improving utilization of sugarcane bagasse as feeds for ruminants using chemical, biological and biochemical treatments. The effect of different treatments on chemical composition, cell wall structure, IVDMD and IVOMD of sugarcane bagasse was studied. The effect of the best treatments detected on nutrients digestibility and N balance with lambs was also investigated.

2. Materials and Methods

This study was carried out at the Department of Animal Production, National Research Center, Dokki, Giza, Egypt and the Experimental Farm Station belongs to Faculty of Agriculture, Animal Production Department, Al-Azhar University, Nasr City, Cairo, Egypt.

Sugarcane by-product preparation:

Bagasse type of low quality roughages was obtained from Sugar Company in Edfo City, Aswan, Egypt, was sun dried to 90% DM and chopped to an approximate 1-3cm.

Chemical treatment:

The required amount of urea (30g) was dissolved in 500 ml water and sprayed on 1kg of chopped sugarcane bagasse. The treated bagasse were thoroughly to be homogenous then ensiled up to 3 weeks.

Biological treatment:

Fungi *(Phanerochaete chrysosporium* NRRL-6361), bacteria *(Cellulomonas uda* NRRL-404) and yeast (*Candida utilis* NRRL-1084) were obtained from the Genetic and Cytology Department, National Research Center, Dokki, Giza, Egypt.

The microorganisms were maintained on agar medium composed of (g/l) yeast extract, 3.0; malt extract, 30; peptone, 5.0; sucrose, 20 and agar, 20.

Biochemical treatment:

At the end of fermented period of biological sugarcane bagasse, it aerate overnight then mixed well with urea then bagged and stored up to 15 days.

This study included two parts of experiments:

The first part (laboratory trials):

The first part was laboratory trials which were carried out to study the effect of using chemical treatment (3% urea), biological treatment (fungi, yeast, bacteria and combined fungi plus yeast; fungi plus bacteria and bacteria plus yeast) and biochemical treatment (biological treatment followed by chemical treatment) on chemical composition, cell wall constituents and scanning electron microscopy of sugarcane bagasse.

Treatments were designed as follow:

T_1: Bagasse untreated (Unt.)
T_2: Bagasse treated with 3% urea (urea Tr.)
T_3: Bagasse treated with fungi (F Tr.)
T_4: Bagasse treated with bacteria (B Tr.)
T_5: Bagasse treated with yeast (Y Tr.)
T_6: Bagasse treated with fungi + yeast (F+Y Tr.)
T_7: Bagasse treated with fungi + bacteria (F+B Tr.)
T_8: Bagasse treated with bacteria + yeast (B + Y Tr.)
T_9: Bagasse treated with fungi + urea (F + urea Tr.)
T_{10}: Bagasse treated with bacteria + urea (B + urea Tr.)
T_{11}: Bagasse treated with yeast + urea (Y + urea Tr.)
T_{12}: Bagasse treated with fungi + yeast + urea (F+ Y +urea Tr.)
T_{13}: Bagasse treated with fungi + bacteria + urea (F+B+ urea Tr.)
T_{14}: Bagasse treated with bacteria + yeast + urea (B+ Y+ urea Tr.)

The second part (*in vitro* and *in vivo* trials):

The second part was carried out to evaluate *in vitro* dry matter and organic matter disappearance for bagasse with different chemical, biological and biochemical treatments under study. The treatments gave the best results were *in vivo* evaluated by digestibility and N-balance trials with lambs.

Digestibility and N-balance trials:

Twenty Ossimi male lambs with an average live body weight 32.7 kg and 180 days age were randomly assigned into five nutritional treatments (each of 4 animals) to receive one of the following roughages: R_1, berseem hay; R2, untreated sugarcane bagasse; R_3, bagasse treated with 3% urea; R_4, bagasse treated with fungi and R_5, bagasse treated with fungi + bacteria + 3% urea. The composition of the five experimental rations is presented in Table (1). Chemical composition and cell wall constituents of different experimental rations are presented in Table (2).

Rations were offered to lambs *ad lib*, while, water and salt blocks were freely available all day time.

Table (1): Composition of experimental rations

Item	Experimental rations, %				
	R_1	R_2	R_3	R_4	R_5

- Berseem hay	30	-	-	-	-
- Untreated bagasse (Unt.B.)	-	30	-	-	-
- Bagasse treated with 3% urea (Urea Tr. B.)	-	-	30	-	-
- Bagasse treated with fungi (F Tr. B.)	-	-	-	30	-
-Bagasse treated with fungi + bacteria + 3% urea (F +B +urea Tr. B.)	-	-	-	-	30
- Ground corn grain	51	51	51	51	51
- Sunflower meal	7	7	7	7	7
- Wheat bran	7	7	7	7	7
-Limestone, sodium chloride and minerals & vit. mix.	5	5	5	5	5
Total	100	100	100	100	100

Animals were individually confined to wooden metabolic crates. Digestibility and N-balance trials were carried out to determine nutrients digestibility, nutritive values and N-balance for the five experimental rations. Digestibility trials consisted of 21 days, where 14 days were considered as a preliminary period to allow animals a suitable adaptation followed by 7 days for total collection of feces and urine. Composite samples from collected feces and urine of each animal were taken for chemical analyses. Samples of rations offered and residuals if any, were weighed daily during the collection period for further chemical analysis.

Table (2): Chemical composition and cell wall constituents of the experimental rations (on DM basis, %)

Item	Experimental rations				
	R_1	R_2	R_3	R_4	R_5
DM	91.79	91.48	91.18	90.70	90.73
OM	93.41	96.35	95.57	95.20	96.65
CP	12.09	8.91	9.93	9.75	10.32
CF	18.24	23.86	21.83	21.66	21.55
EE	3.69	3.23	3.27	3.27	3.91
NFE	59.39	60.35	60.54	60.52	60.87
Ash	6.59	3.65	4.43	4.80	3.35
NDF	42.76	38.34	41.65	42.93	40.22
ADF	19.14	16.85	25.49	25.50	26.30
ADL	5.99	2.68	7.76	8.76	9.12
Hemicellulose*	23.62	21.49	16.16	17.43	13.92
Cellulose**	13.15	14.17	17.73	16.74	17.18

* Hemicellulose = NDF – ADF ** Cellulose = ADF -ADL

The Proximate chemical analysis of untreated and treated bagasse, berseem hay, concentrate feed mixture (CFM), feces and urine was determined according to **A.O.A.C. (1990).** Nitrogen free extract (NFE) was calculated by difference. Cell wall constituents were analyzed according to **Goering** and **Van Soest (1970),** to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose and cellulose were determined by difference. The electronic microscope scanning for untreated and treated bagasse was done according to **Baker *et al.* (1986**) using Electron Probe Microanalyzer.

***In vitro* evaluation:**

The *in vitro* dry matter and organic matter disappearance (IVDMD and IVOMD) were determined according to the method described by **Tilley and Terry (1963).** This was done on triplicate samples; rumen liquor was collected from cannulated Rahmani sheep using a stomach tube. The rams were maintained on all berseem hay diet for a period of at least 3 weeks before collecting the liquor. Hay was offered to the animals at a feeding level of 120% maintenance.

Statistical analysis:

Data concerning *in vitro* DM and OM disappearance and *in vivo* nutrients digestibility and N-balance trials were statistically analyzed according to **SAS (1998)**. A one-way classification analysis followed by Duncan's multiple-range test **(Duncan, 1955)** for testing the significance between means was used.

3. Results and Discussion

Chemical composition:

Results of chemical composition of untreated and treated bagasse with different treatments are presented in Table (3). The data obtained showed that DM content was increased after treatment in different

cases, the highest DM content was observed in bagasse treated with 3% urea (96.2%). On the contrary, the lowest value was recorded in untreated bagasse (91.9%). On the other hand, different biochemical treatments showed higher values in comparison with the biological treatments. Higher OM values were recorder with T_5 (96.6%). On the contrast the lowest value (82.4%) was recorded in T_{14} as a biochemical treatment, while untreated and treated bagasse with urea indicated intermediate values (94.7 and 92.3%). These results agree with those obtained by **Chandra *et al*. (1991)** who found that the decreased OM was a reflection to decrease in CF which was utilized by fungi, while the increase in total ash was inversely related to the OM content of treated paddy straw (untreated,*T. viride, A. niger* and mixed fungi treated paddy straw).

On the other hand, the increments in CP content (304, 188 and 156%) were due to biochemical, chemical and biological treatments, respectively. These results agree with those obtained by several authors **(Talha, 1990; Tabana, 1994; Mohamed, 1998 and Mousa *et al*., 1998)**. These effects were mainly due to nitrogen content of added urea in the chemical treatments, microbial protein from biological treatment due to growing fungi and bacteria and also to the biochemical treatments. **Chandra *et al*. (1991)** found that the increase in CP was reflected by a decrease in CF content, while, **Yang *et al.* (2001)** showed that solid state fermentation changed the composition of the straw. The protein content increased from 6.7% in unfermented straw to 14.7% in fermented straw.

Generally, biochemical treatment was the best treatment which led to decrease CF content followed by T_7 as a biological treatment and 3% urea as a chemical one. The decline in crude fiber content of the experimental rations could be resultant of the enzymes secreted by the biological treatment **(Gado *et al*., 2007)**. The decrease in CF content by urea treatment such as sodium hydroxide treatment may be due to the liberation of cellulose from its bonds with lignin (delignification) which increased the solubility **(Abd El-Ghani *et al.*, 1999)**.

As shown in Table (3) different biochemical treatments led to increase EE contents. These increase in EE due to synthesis of fatty acids through growth of bacteria **(Gado *et al*., 2007)**. **Zadrazil *et al*. (1995); Neclakantan and Singh (1998) and Rane and Singh (2001)** who found that the products of solid state fermentation gets in enriched fats, soluble sugars, vitamins and amino acids and can be used entirely as in animal feed.

There were decreases in the NFE contents of bagasse from 42.24% in untreated to 42.10, 38.21 and 37.56% in chemical, biological and biochemical treated bagasse, except T_5 and T_6 which were increased (44.7 and 43.78%), respectively, whereas the lowest value (32.57%) was recorded in T_{14}. On the other side, all biochemical treatments indicated lower values compared with the untreated bagasse.

Generally, all biochemical and chemical treatments increased ash content plus some biological treatments, compared with the untreated bagasse. These effects were due to added media of growing bacteria, fungi and yeast then degradation of DM to ash and OM. **Chandra *et al.* (1991)** found that the increased total ash on all treatments were a reflection to the decrease in CF and NFE contents. It could be concluded that all treatments had great effects on degradation of CF from 49.5 to 39.10% and increasing CP content from 1.8 to 8.8% of treated bagasse. The present finding is in agreement with **Bakshi and Langer (1991)**, who reported that CF decreased from 42.92 to 17.87% in the compost and spent in a treatment with cellulose enzymes. Supportive results were reported by **Streeter *et al.* (1982); Reader and Mc Queen (1983); Eduardo and Etienne (1985) and Lawrance and Abada (1987)**. **Abd El-Galil (2000)** who found that, when bagasse treated by *Cellulomonas sp.* bacteria CF was decreased from 44.9 to 30.21% and CP was increased from 1.75 to 15.9% with a reduction in DM content.

The present results confirmed the results obtained by **Shoukry *et al*. (1985) and Abdul-Aziz *et al.* (1997). Gado (1999)** fermented rice straw and bagasse with *Trichoderma ressei* and reported that CF, EE, NDF, ADF, cellulose and hemicellulose contents were lowered significantly ($P<0.05$) in both treated rice straw and bagasse. **Badr (2001)**found that biological treatment by *P. florida* decreased CF content than that in raw material, being 3.5% , while combined fungi and bacteria at level of 3.0% were more effective in decreasing CF content from 37.85% to 18.42% , followed by incubation of corn stalks by *P. florida* and *E. carotovora* at level 2% (being 47.37% CF for control).

Shoukry *et al.* (1985) found an increase in CP, EE and ash content when treated sugarcane bagasse with four different microorganisms. **Deraz and Ismail (2001) and El-Ashry *et al.* (2001)** reported that fungal treatment led to decrease OM and CF contents, while, CP and ash contents increased compared with the untreated roughages.

Table (3): Effect of chemical, biological and biochemical treatments on chemical composition of sugarcane bagasse (on DM basis,%)

Item	DM	OM	CP	CF	EE	NFE	Ash

T_1:Unt.	91.90	94.70	1.80	49.50	1.16	42.24	5.30
Chemical treatment : T_2:Urea Tr.	96.20	92.30	5.20	43.70	1.30	42.10	7.70
Biological treatments: T_3:F Tr.	94.10	90.20	4.80	45.80	1.58	38.02	9.80
T_4:B Tr.	94.10	87.90	4.30	45.60	1.44	36.56	12.10
T_5:Y Tr.	93.90	96.60	4.80	45.80	1.30	44.70	3.40
T_6:F + Y Tr.	93.95	95.40	4.50	46.70	0.42	43.78	4.60
T_7:F + B Tr.	93.80	90.65	4.60	43.20	1.26	41.59	9.35
T_8:B + Y Tr.	93.80	91.30	4.70	48.60	1.33	36.67	8.70
Overall mean of biological treatments	93.94	92.00	4.61	45.95	1.22	40.22	7.99
Biochemical treatments: T_9:F + urea Tr.	95.37	87.80	8.80	39.94	2.85	36.21	12.20
T_{10}:B + urea Tr.	94.60	94.10	8.20	42.30	1.96	41.64	5.90
T_{11}:Y + urea Tr.	93.90	87.60	6.20	40.80	2.55	38.05	12.40
T_{12}:F + Y + urea Tr.	95.92	88.10	7.20	39.10	3.16	38.64	11.90
T_{13}:F + B + urea Tr.	96.10	91.10	6.50	42.90	3.43	38.27	8.90
T_{14}:B + Y + urea Tr.	95.97	82.40	6.77	39.80	3.23	32.60	17.60
Overall mean of biochemical treatments	95.31	88.52	7.28	40.81	2.86	37.57	11.48

Effect of chemical, biological and biochemical treatments on cell wall constituents:

It is clear from Table (4) that chemical, biological and biochemical treatments had a significant effect on cell wall constituents (CWC) of bagasse, different treatments decreased NDF contents. The highest decrease in NDF content was recorded with bagasse treated by 3% urea. There were slight differences among treatments detected in ADF content. The highest decrease in ADF content was found in bacteria + yeast + urea treated bagasse. Hemicellulose content was decreased in different bagasse treatments except in T_{14} , while the highest decrease was recorded with urea treated bagasse (20.57%).

Table (4): Effect of chemical, biological and biochemical treatments on cell wall constituents of sugarcane bagasse (%)

Item	NDF	ADF	ADL	Hemicellulose.*	Cellulose**
T_1:Unt.	87.95	58.87	9.97	29.08	48.90
Chemical treatment : T_2:Urea Tr.	75.81	55.24	13.20	20.57	42.04
Biological treatments: T_3:F Tr.	79.19	57.06	9.53	22.13	47.53
T_4:B Tr.	81.20	53.50	9.84	27.70	43.66
T_5:Y Tr.	82.30	56.37	10.34	25.93	46.03
T_6:F + Y Tr.	80.79	52.70	10.09	28.09	42.61
T_7:F + B Tr.	83.13	54.13	10.15	29.00	43.98
T_8:B + Y Tr.	81.20	55.30	9.18	25.90	46.12
Overall mean of biological treatments	81.30	54.84	9.86	26.46	44.99
Biochemical treatments: T_9:F + urea Tr.	85.20	58.01	11.47	27.19	46.54
T_{10}:B + urea Tr.	83.50	58.40	10.33	25.10	48.07
T_{11}:Y + urea Tr.	80.13	58.20	11.22	21.93	46.98
T_{12}:F + Y + urea Tr.	79.90	53.09	9.15	26.81	43.94
T_{13}:F + B + urea Tr.	81.75	53.00	11.37	28.75	41.63
T_{14}:B + Y + urea Tr.	82.15	52.60	11.30	29.55	41.30
Overall mean of biochemical treatments	82.11	55.55	10.81	26.56	44.74

* Hemicellulose = NDF – ADF ** Cellulose = ADF -ADL

It was noticed that the lowest decrease in cellulose content was recorded with fungi + urea treatment, while the highest decrease was recorded with bacteria + yeast + urea as a biochemical treatment. In general, results indicated that treatments were significant effect on CWC of bagasse. These results agreed with those of **Van Soest *et al.* (1984)** and **Mann *et al.* (1988)**. **Hamissa *et al.* (1985)**

treated the fermented bagasse (*T. viride* fungal) with sodium hydroxide or sodium hypochlorite then it was sprayed with urea solution (4%), they also reported that all treatments significantly decreased CF, NDF, ADF, hemicellulose and NFE content of bagasse, while cellulose and ADF contents were nearly similar. **Abdel-Aziz** and **Ismail (2001)** found that NDF, ADF and cellulose contents of fungus treated rice straw decreased by 77.67, 48.81 and 39.73%, respectively. **Yang *et al*. (2001)** found that solid state fermentation changed the composition of the straw, hemicellulose and cellulose content decrease from 33.1 and 25.8% in unfermented straw to 26.1 and 16.0% in fermented one, respectively. **Deraz** and **Ismail (2001)** reported that CF, NDF, ADF, ADL, cellulose and hemicellulose of cotton stalks significantly decrease when fermented with *P. ostreotusor* white rot fungi. **El-Ashry *et al.* (1997, 2001** and **2002)** and **Abdul-Aziz *et al.* (1997)** obtained the similar trend.

Effect of chemical, biological and biochemical treatments on scanning electron microscopy of sugarcane bagasse:

The scanning electron micrograph (SEM) can provide information about the microbial community on the biofilter media. The biomass of individual particle can be mapped. From such precision, important factor such as filter media coverage, thickness and activity can be determined for modelling. The SEM after experiment has already been shown by some researchers **(Namkoong *et al.*, 2004** and **Chmiel *et al*., 2005**).

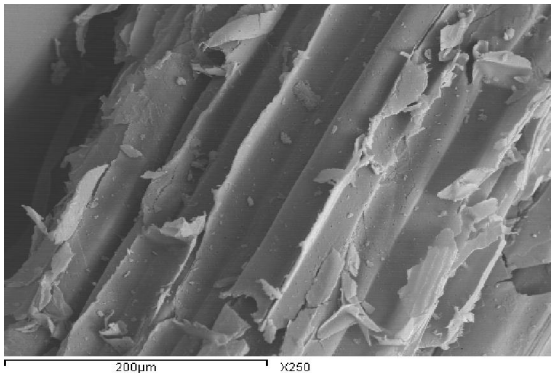


Fig.(1): Scanning electron micrographs (untreated bagasse)

A comparison of the scanning electron microscopic images of sugarcane bagasse before and after treatments is shown in Fig. (1, 2, 3 & 4). Compared to the initial sugarcane bagasse, a biofilm on the surface of the sugarcane bagasse was observed clearly after treatments. An even growth of microbial community on the surface of the pore of the bagasse is clearly visible. This occurred due to the attacks and decomposition of the microorganisms to cell wall constituents. Initially, the degree of acclimatized depends upon the adaptive capacity of the microorganism in the sugarcane bagasse, substrate concentration and its availability and on other necessary environmental conditions.

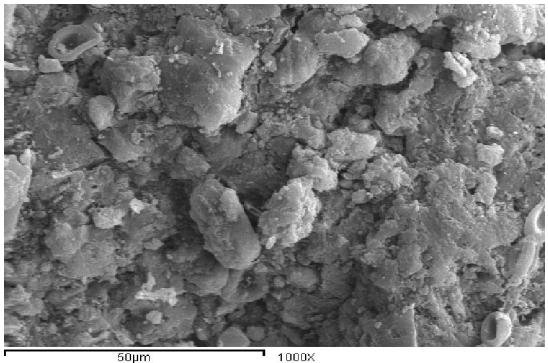


Fig. (2): Scanning electron micrographs (chemical treated bagasse)

The tissue of the untreated bagasse (Fig.1) did not reveal any degradation and shows a large amount of debris adhering to the surface of the fibre bundles, because they are coated with non-cellulosic material. Treatments with chemical, biological and biochemical treatments led to relatively cleaner surfaces (Fig. 2, 3 &4, respectively) which supports the removal of wax, pectin, lignin and hemicelluloses as supported by chemical analysis. Unfortunately, the resolution of the available SEM was inadequate to detect fine holes caused by fungal hyphae attacking fibre walls on treated fibre surfaces **(Daniel** and **Volc, 2004). Vazquez *et al.* (1992)** used NaOH, Ca $(OH)_2$, NH_4OH and H_2O_2 to treated bagasse pith, to investigate their action on single cell protein production using a mixed culture of *Cellulomonas flavigena* and *Xanthomonas sp*. SEM analysis showed that pith was mainly composed of microfibrils and parenchymatose cell wall. In addition, light microscopy illustreated swelling of vessels in pith pretreated with Ca $(OH)_2$ and NaOH, which was evident by the increased pore size of vessels in pith. **Kyong *et al.* (2009)** mentioned that the SEM of untreated and ammonia-pretreated rice straw showed that pretreatment induced physical changes in the biomass. **Bak *et al.* (2009)** indicated that pretreatment with aqueous-ammonia promoted the removal of external fibers. The removal of external fibers, in turn, led to increased surface area, which may have made cellulose more accessible to enzymes.

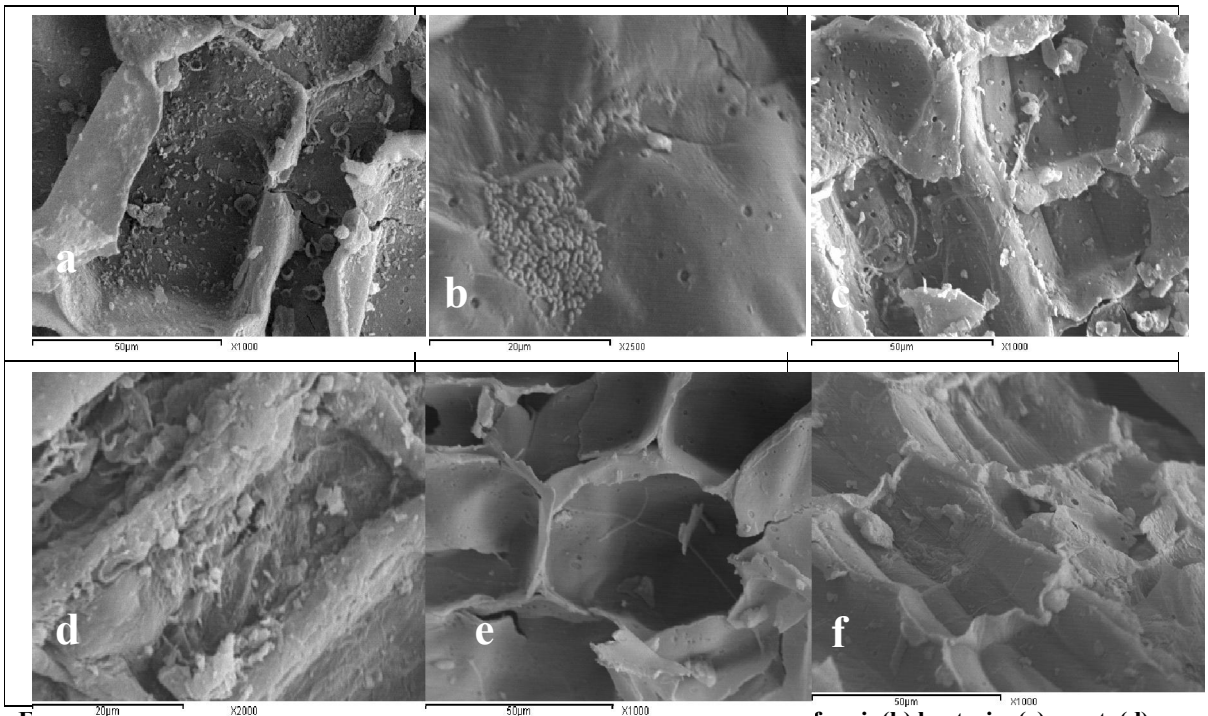
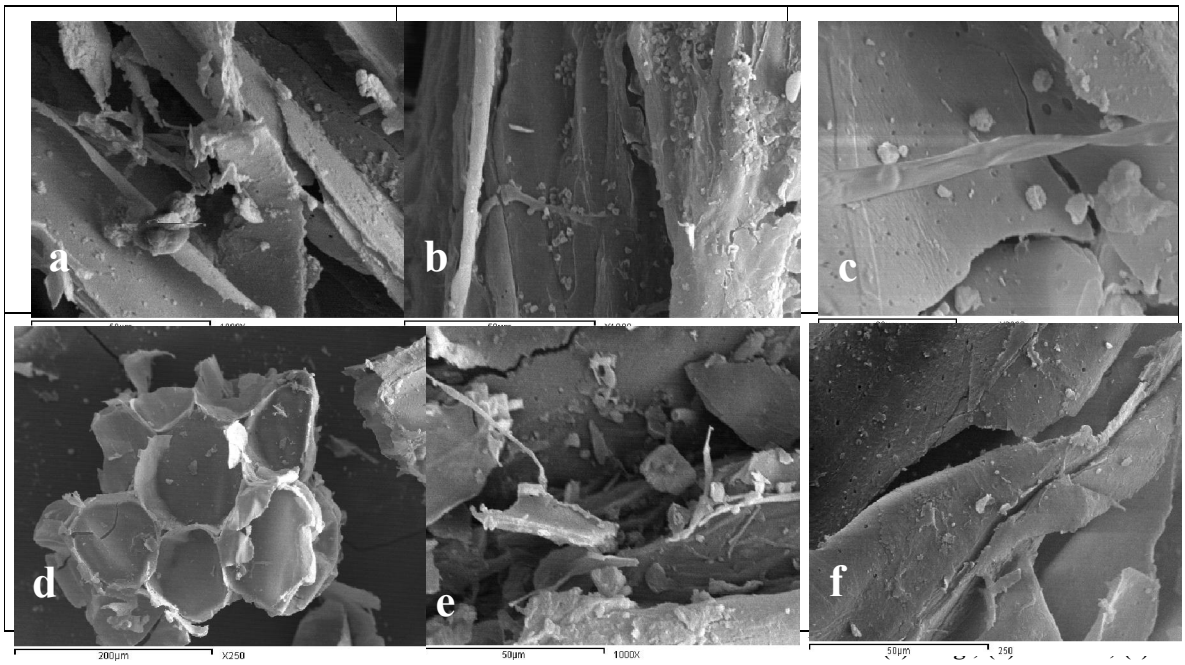


Fig. (5): Scanning electron micrographs for biological treated bagasse: (a) fungi, (b) bacteria, (c) yeast, (d) fungi + yeast, (e) fungi + bacteria & (f) bacteria + yeast



yeast, (d) fungi + yeast, (e) fungi + bacteria & (f) bacteria + yeast

Effect of chemical, biological and biochemical treatments on *in vitro* DM and OM disappearance:

The results from Table (5) showed that different treatments improved IVDMD and IVOMD than untreated, from 12.0 to 26.6% for IVDMD and from 18.0 to 33.23% for IVOMD for untreated and treated bagasse, respectively.

Generally, the best IVDMD value was obtained by T_{13} followed by T_{10} as a biochemical treatment and T_2 (3% urea) as a chemical treatment, respectively, the same results were obtained with IVOMD that different treatments increased IVOMD compared with untreated bagasse. The best IVOMD values were obtained by bagasse treated with fungi and treated with bacteria as a biological treatment

followed by 3% urea as a chemical treatment and treated with fungi + bacteria + urea and bacteria + urea as a biochemical treatment. These results agreed with those obtained by **Shoukry (1992)**, who found that IVDMD of corn cobs and sugarcane bagasse significantly (P<0.05) increased by 3% urea treatment. **Reis *et al*. (1995)** reported that treated rice straw with 5% urea increased IVDMD. **Swidan *et al*. (1996)** reported that IVDMD of corn cobs improved by 3 and 5% NH_3 treatment. **Surinder and Suman (1986)** reported that *Pleurotus ostreatus* and *S. pubverulentum* used as the biological treatment of paddy straw produce increases in IVDMD. **Shah and Rehman (1988)** noticed that IVDMD increased when cotton seed hulls fermented by *Bacillus polymexa* and *Trichoderma viride*. **Bassuny *et al*. (2003)** found that rice and bean straw treated with biological treatment significantly (P<0.05) improved IVDMD and IVOMD. Singh (2004) found that 4% urea and fungi when treated with wheat and rice straw, the values in IVDMD and IVOMD were significantly higher for urea treated straw (P<0.01) than untreated and fungi treated straw.

The results obtained of chemical composition, cell wall constituents, SEM and *in vitro* evaluation suggested that the best treatments detected were urea, fungi and fungi + bacteria + urea which were used in *in vivo* metabolism trials in comparison with untreated bagasse and hay rations.

Table (5): Effect of chemical, biological and biochemical treatments on *in vitro* DM and OM disappearance of sugarcane bagasse

Item	IVDMD, %	±SE	IVOMD, %	±SE
T_1: Unt.	**12.00^{g}**	**1.73**	**18.00^{h}**	**1.155**
Chemical treatment : T_2:Urea Tr.	**25.33^{ab}**	**1.45**	**32.0^{abc}**	**1.15**
Biological treatments: T_3:F Tr.	17.27^{ef}	0.696	33.23^{a}	0.710
T_4:B Tr.	14.26^{fg}	1.27	32.60^{ab}	1.50
T_5:Y Tr.	18.35^{e}	1.357	21.54^{gh}	0.889
T6:F + Y Tr.	17.06^{ef}	1.07	21.00^{gh}	1.155
T_7:F + B Tr.	22.70^{bc}	1.097	24.00^{fg}	1.155
T_8:B + Y Tr.	17.27^{ef}	0.696	27.87^{de}	1.657
Overall mean of biological treatments	**17.82**	**-**	**26.71**	**-**
Biochemical treatments: T_9:F + urea Tr.	22.40^{bcd}	0.231	25.40^{ef}	1.38
T_{10}:B + urea Tr.	25.40^{ab}	1.386	28.60^{cde}	1.15
T_{11}:Y + urea Tr.	20.10^{cde}	1.039	20.00^{h}	0.981
T_{12}:F + Y + urea Tr.	20.40^{cde}	0.693	26.20^{def}	1.154
T_{13}:F + B + urea Tr.	26.60^{a}	0.751	29.43^{bcd}	0.652
T_{14}:B + Y + urea Tr.	19.20^{de}	0.751	20.30^{h}	0.577
Overall mean of biochemical treatments	**22.35**	**-**	**24.99**	**-**

a, b,c,d,e,f,g and h : Means in the same column with different superscripts are different at (P<0.05).

Effect of different treatments on digestion coefficients and nutritive value of growing lambs:

Results of nutrients digestion coefficients and nutritive values of the experimental rations are shown in Table (6). Digestibility coefficients of the experimental rations indicated significant differences (P<0.05) for different nutrients, except DM and EE which showed insignificant differences. As for OM, R_4 (bagasse treated with fungi) indicated higher OM digestibility value compared with different experimental bagasse rations and without significant difference with R_5 (biochemical treated bagasse). However, different experimental bagasse rations indicated lower OM digestibility compared with the hay group.

The similar trend also observed with both of CP and CF, since R_4 indicated higher (P<0.05) digestibility values and without significant difference with R_5. The improvement in digestibility coefficient of CF, CP and DM may be due to the improvement in chemical composition, especially in CP and CF as a result of biological and biochemical treatments. These results are in a good agreement with those obtained by **Agosin *et al*. (1986)** who indicated that *Phanerochaete chrysosporium* increased DM digestibility.

The improvement in nutrients digestibility are associated with increasing the digestion of fibrous materials, particularly hemicellulose, in addition to the increased bacterial digestion of cell wall content **(Hassan *et al*., 2005)**.

Table (6): Nutrients digestibility and nutritive values of different experimental rations (%)

Item	Experimental rations					±SE	Sig.
	R_1	R_2	R_3	R_4	R_5		

Digestibility, %:							
DM	91.42	86.42	85.37	88.38	87.23	0.80	NS
OM	80.25 [a]	72.29[c]	70.33[c]	76.18[b]	73.81[bc]	1.35	*
CP	72.22[a]	58.18[c]	60.95[c]	64.81[b]	62.65[bc]	1.31	*
CF	77.77[a]	58.81[b]	63.73[b]	76.21[a]	73.57[a]	1.88	*
EE	85.60	86.57	87.56	83.34	86.25	0.987	NS
NFE	85.84[a]	72.09[c]	75.60[b]	78.64[b]	76.21[b]	2.28	*
NDF	62.92[a]	49.98[b]	62.62[a]	63.57[a]	62.63[a]	0.29	*
ADF	55.63[c]	31.78[e]	60.27[b]	71.32[a]	50.10[d]	3.01	*
Hemicellulose	75.74[b]	71.25[c]	66.54[d]	50.57[e]	83.69[a]	0.57	*
Cellulose	57.61[b]	36.35[c]	58.06[b]	71.69[a]	58.49[b]	2.63	*
Nutritive value, % on DM:							
TDN	81.00[a]	68.99[c]	72.16[c]	76.52[b]	76.29[b]	0.823	*
DCP	8.75[a]	5.18[c]	6.05[b]	6.32[b]	6.47[b]	0.281	*

NS: Non significant difference. *: Significant difference at (P<0.05).
a, b,c,d and e : Means in the same row with different superscripts are different at (P<0.05).

Consequently, it could be concluded that biological treatment with fungi and the mixture of fungi + bacteria + urea as a biochemical treatment could increase the digestibility coefficients of most nutrients compared with the untreated one. These results are in agreement with those reported by several researcher worked on stalks like **Azzam (1992) and Singh and Gupta (1990). Mahrous (2005)** who stated that the biological treatment could be used successfully to enrich poor quality roughages (cotton stalks) and improved the nutritive value (digestibility coefficients and feeding value).

Zewil (2005) showed that almost nutrient digestibilities were improved with *Trichoderma viride* (fungus) treatment in sheep rations. Similar results were obtained by **Deraz (1996), El-Kady *et al.* (2006) and Allam *et al.* (2006)** who found that animals fed biologically treated roughages were the most efficient groups followed by those fed chemically treated roughages.

Concerning the NDF, different treated bagasse rations indicated insignificant digestibility differences compared with the hay ration (R_1). And all were higher (P<0.05) than that of the untreated bagasse ration which recorded the lower (P<0.05) NDF digestibility.

Acid detergent fiber digestibility showed higher (P<0.05) value with fungus treated bagasse (R_4) compared with different treated bagasse rations and the hay ration. On the contrary, the untreated bagasse ration (R_2) recorded the lower (P<0.05) ADF digestibility value. The improvement in fiber fraction as a result of biological and biochemical treatments may be due to the effect of the cellulose enzyme of fungi, which may be responsible for the stepwise hydrolysis of cellulose to glucose. These results are in agreement with those obtained by **Abdel-Malik *et al.* (2003)** who reported that, digestibility coefficients of DM, OM, CP, CF , NFE , NDF, ADF, ADL, hemicellulose and cellulose were increased significantly for banana by-products treated with both chemical (urea or acid plus urea) and biological treatment (bacteria, fungi, or bacteria plus fungi) than those untreated. On the other hand, **Khorshed (2000)** reported that all biological treatments (*T.viride, S. cereviciae* or *T. viride* + *S. cereviciae*) significantly (P<0.05) increased apparent nutrients digestibility for DM, OM, CP, CF, NFE, NDF, ADF, ADL, hemicellulose and cellulose than their control. During alkali treatments of lignocellulosic materials the hemicelluloses are found to be partly solubilised and more accessible to microbial digestion by alkali swelling effect **(Thander and Aman, 1984)**. It is also indicated that, xylans are partly translocated during alkali treatment to a position in straw material where they are more available to rumen digestion **(Lindberg *et al.*, 1983)**. These highest digestibilities in NDF and ADF caused by fungi treatment are encouraging because they imply that the degradability and intake of sugarcane bagasse may be improved by chemical treatment.

As for hemicellulose, the biochemical treatment (R_5) indicated higher (P<0.05) digestibility value in comparison with chemical (R_3) and biological treated bagasse (R_4). The hay control ration and the untreated bagasse recorded higher (P<0.05) values in comparison with R_3 and R_4, but lower (P<0.05) than that of R_5. These results agree with those obtained by **Silva and Ørskov (1988)** who reported that treatment of straw with urea increased its nutritional value by making more digestible cellulose and hemicellulose available. This creates favorable conditions in the rumen for the developments of the cellulytic bacteria which best degrade the cell wall. Moreover, treatment with urea increased the degradable fractions of the straw, as well as the speed of degradation (**Nandra *et al.*, 1983 and Ibrahim *et al.*, 1989)**.

As for cellulose digestibility, R_4 (bagasse treated with fungi) recorded highest (P<0.05) value compared with different treated bagasse rations and the hay ration. The improved digestibility resulting from the treatments is due to two causes: (a) the solubilization of the hemicellulose (**Laurent *et al.*,**

1982 and Horton, 1983), and (b) the alteration of the crystalline structure of cellulose (favoring the action of microorganisms) owing to an indirect effect **(Han *et al.*, 1983)**.

As for the nutritive value (Table, 6), the hay ration recorded highest (P<0.05) nutritive values in terms of TDN and DCP compared with the different treated and untreated bagasse rations. These results may be due to the higher crude fiber content in untreated bagasse. On the other hand, these results indicated that the inclusion of treated bagasse at 30% in place of berseem hay did not improve the nutritive value of sheep rations .The highest TDN and DCP values were recorded with R_1 (berseem hay +CFM), while, the lowest values were recorded with R_2 (untreated bagasse).

Such results may suggest that different treatments led to improve the nutritive value of treated bagasse. However, both of the fungus treatment and the biochemical ones were more pronouncly effective in improving the nutritive value of bagasse rather than the chemical treatment in terms of TDN and DCP.

Such improvement was as high as 11% TDN and 25% DCP compared with the untreated bagasse (raw material). Results of the present study are in agreement with those obtained by **Abd El-Galil (2000)**,who found that the biological treatment of bagasse increased TDN value significantly (P<0.05) from 46.5 to 68.9 %. **El-Ashry *et al.* (1997)** found that TDN value increased from 63.93 and 63.35% in untreated rice straw and corn stalks to 72.31 and 72.88%in fungal treated ones, respectively. **Ward and Perry (1982)** compared ground corn cobs treated with cellulase from *T. viride* and untreated in diet of lambs. They found an increase in digestibility of DM (4.8%), NFE (9%) and TDN (18%) and a decrease in digestibility of CF (41%) and EE (8.8%) compared with untreated one.

Effect of different treatments on nitrogen balance of growing lambs:

Data presented in Table (7) illustrated dietary nitrogen utilization by sheep fed the different experimental rations.

Data obtained indicated significant differences among different experimental groups in different N terms. As shown highest (P<0.05) total nitrogen intake (TNI) was observed with hay group (R_1), but without significant difference with different treated bagasse rations. The lowest (P<0.05) TNI was observed with untreated bagasse ration (R2).

Nitrogen balance showed that all rations achieved positive N balance. However, the hay control group (R_1) retained more (P<0.05) N, but without significant difference with R4. Both the two groups indicated higher (P<0.05) N balance in compare with different treated and untreated bagasse rations. The variability in dietary nitrogen retained might be due to its escape from ruminal fermentation or may probably due to an increased utilization of ammonia in the rumen **(Holzer *et al.*, 1986).**

Table (7): Nitrogen balance of lambs fed rations containing untreated and treated sugarcane bagasse

Item	Experimental rations					±SE	Sig.
	R_1	R_2	R_3	R_4	R_5		
N. intake g/h/d	27.12^{a}	18.11^{b}	24.24^{a}	24.89^{a}	23.86^{a}	0.887	*
Fecal N g/h/d	12.86^{ab}	9.09^{c}	14.26^{a}	12.09^{b}	12.32^{b}	0.496	*
Urinary N g/h/d	7.37^{ab}	6.67^{ab}	6.14^{b}	8.47^{a}	7.48^{ab}	0.304	*
Total excreted N g/h/d	20.23^{a}	15.76^{b}	20.40^{a}	20.56^{a}	19.80^{a}	0.588	*
N digested g/h/d	14.26^{a}	9.02^{c}	9.98^{bc}	12.80^{ab}	11.54^{abc}	0.633	*
N balance g/h/d	6.89^{a}	2.35^{b}	3.84^{b}	4.33^{ab}	4.06^{b}	0.505	*
N balance % intake	25.41^{a}	12.98^{b}	15.84^{ab}	17.40^{ab}	17.02^{ab}	1.718	*
N balance % digested	48.32^{a}	26.05^{b}	38.48^{ab}	33.83^{ab}	35.18^{ab}	2.908	*

*: Significant difference at (P<0.05).

a, b and c: Means in the same row with different superscripts are different at (P<0.05).

As a general conclusion, dietary nitrogen utilization favored R_4 (biological treated bagasse ration) followed by R_5 (biochemical Tr. bagasse ration) as the most efficient groups in utilizing the dietary N of the treated bagasse rations. Such results indicated the effective role of both the fungus and biochemical treatments in improving dietary N utilization by experimental animals. And although, the biological treatment ranked first in compare with different applicable treatments in improving N utilization by experimental animals; however, different treated bagasse rations ranked in second category to that of the hay ration (R_1) i.e. conventional roughage. These results were in harmony with those obtained by **Langer *et al.* (1982) and Ahuja *et al.* (1986)** who found that ration containing biologically treated crop-residues showed positive nitrogen balance. According to **Mohamed (2005) and Allam *et al.* (2006)** with sugar beet pulp and **Deraz and Ismail (2001)** with biologically treated cotton stalks, diets contained 30% biologically treated sugar beet pulp showed higher (P<0.05) N balance than the control.

Conclusion

The present study suggested that the possibility of improving the chemical composition, nutritive value and nitrogen utilization of sugarcane bagasse

by different chemical, biological and biochemical treatments in rations for lambs. The superiority effect of fungi or fungi + bacteria +urea treatments was observed compared with urea treatment.

Further work is needed to study the effect of using untreated or treated sugarcane bagasse by different treatments in place of berseem hay in rations for growing lambs on their performance.

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Impact of Portal Vein Thrombosis on Adult to Adult LDLT: 8 Years Experience

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Abstract: Background and Objective: Living donor liver transplantation (LDLT) for patients with portal vein thrombosis (PVT) involves technical difficulty. Its presence has frequently been presented as a relative or absolute contraindication in LDLT by numerous groups. The aim of this study is to demonstrate our experience in dealing with patients with preexisting PVT and its effect on the outcome of transplantation. Methods: From October 2001 to September 2009, 210 LDLT were performed by our team. Thirty one patients with intraoperatively confirmed nontumoral PVT formed the study group. The thrombus was removed by a simple technique of eversion thrombectomy. Anticoagulation was started after surgery to be stopped 6 months after confirmation of absence of PVT. A comparative analysis with intraoperative and postoperative variables was performed with 179 patients without PVT transplanted in the same period. Results: PVT was diagnosed preoperatively in 15 (48%) patients. The commonest type was grade II, occurring in 13(41.9%) patients. Total thrombectomy was successful in 29 cases and partial in two cases, but with adequate portal flow.The overall complications, Infections and portal vein rethrombosis were higher in patients with preexisting PVT but this was not associated with increase in ICU or hospital stay. PVT did not affect patient survival (70.6% and 60.2%, one and three year survival rate in patients with PVT vs. 81% and 62% in patients without PVT). Conclusion: PVT increases surgical difficulties and postoperative morbidity (PV rethrombosis, infections) but does not have an influence on patients survival.

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Key words: Impact of Portal Vein Thrombos, Adult , Adult LDLT ,Years Experience.

1. Introduction

Orthotopic liver transplantation (OLT) is now an accepted and efficient therapy for end-stage liver diseases. Portal vein thrombosis (PVT) is a complication of chronic liver diseases that occurs in approximately 5–15% of these patients[(1)].

At the beginning of OLT history, PVT was considered an absolute contraindication, till 1985, when the first report of a successful transplantationin two patients with PVT was published[(2)]. Nowadays, the presence of PVT is no longer considered a contraindication for LT, as many improvements have been made in the field of perioperative management and surgical technique, including vein grafts to overcome this problem[(3)].

Living donor liver transplantation (LDLT) has emerged as one of a variety of approaches to overcome the current lack of organ donation in the face of growing waiting lists and as the only modality in the absence of the cadaveric programs in some countries.

In LDLT, there are technical difficulties due to the need of distal dissection of vascular pedicle of the hilum and restricted availability of a vein graft. The presence of PVT in the recipient has frequently been presented as a relative or absolute contraindication in LDLT by numerous groups[(4, 5)].

To address this issue, an international survey[(6)] was performed to examine the attitude of transplant teams relative to LDLT in the setting of preexisting PVT in the potential recipient. They found that, 5 centers considered it to be an absolute contraindication (10.7%), 24 centers a relative contraindication (51%), and 18 as not being a contraindication (38.3%).

Aim of the study is to review our experience of performing LDLT in patients with PVT, in order to evaluate the feasibility of thrombectomy, and its influence on post-operative outcome.

2. Patients and method

From October 2001 to September 2009, 210 LDLT were performed at Wady El Nile and Ain Shams Specialised University Hospital including 31 patients with intraoperatively confirmed nontumoral PVT formed the study group. Patients with hepatocellular carcinoma (HCC) were excluded.

Preoperative assessment of liver transplant candidates for portal vein patency included both Doppler ultrasonography and abdominal computed tomography (CT) scan with CT portography (Figs. 1-4). Ultrasonography (US) is usually the investigation of choice, with a sensitivity and specificity ranging between 60% and 100% [(7)] ; it can

reveal the presence of solid, hyperechoic material into a distended portal vein or its tributaries, the presence of collateral vessels or a cavernoma. Doppler imaging can confirm the absence of flow in part or all the vassal lumen, and if present, a cavernomatous transformation[7]. Incidentally, US is less reliable in determining the extension of the thrombus to the mesenteric circulation. Instead, CT scanning can easily obtain this information, and, can estimate the impairment of the bowel and other adjacent organs. CT scanning is able to demonstrate hyperattenuating material in the portal vein lumen and the absence of enhancement after contrast injection. In addition, in hypoperfused areas, hepatic enhancement appears increased during the arterial phase and decreased during the portal phase[7].

The median delay between the last Doppler ultrasound examination and the transplantation was one week. Patients with confirmed PVT were classified into four grades according to the extent of thrombosis assessed intraoperatively, as described by Yerdel *et al.*[8]. (Figure 5): grade 1 :< 50% PVT without obstruction of the superior mesenteric vein (SMV); grade 2: grade 1 but >50% PVT; grade 3: complete PV and proximal SMV thrombosis; grade 4: complete PV and entire SMV thrombosis.

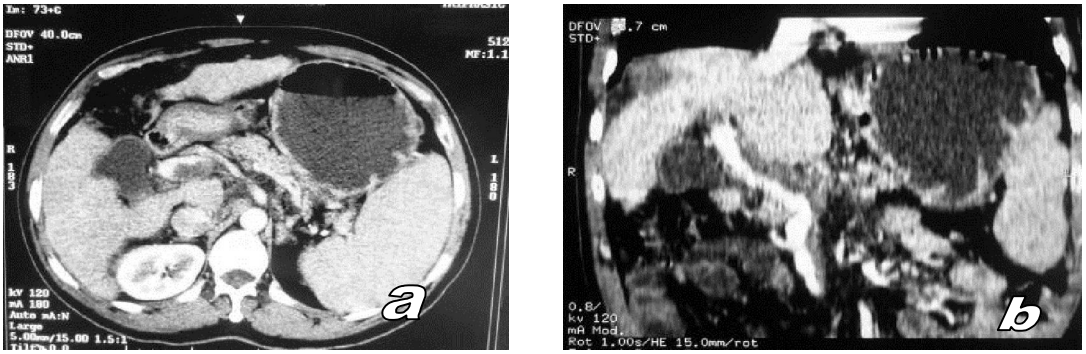


Figure (1): Male patient 55 years old liver cirrhosis and right hepatic lobe HCC. **(a)** Axial CT cuts in porto-venous phase showing partial eccentric main PV thrombus. (b) Coronal reconstruction in portovenous phase showing partial eccentric PV thrombosis which extend to involve the superior mesenteric vein causing eccentric thrombosis as well.

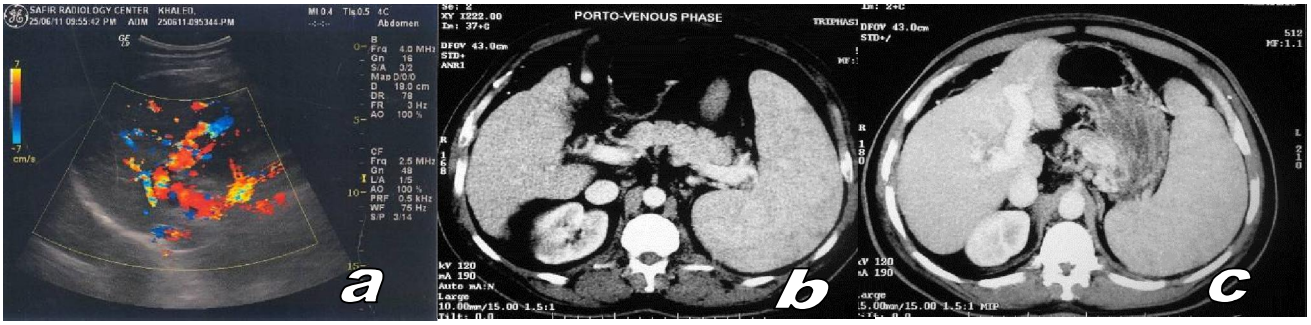
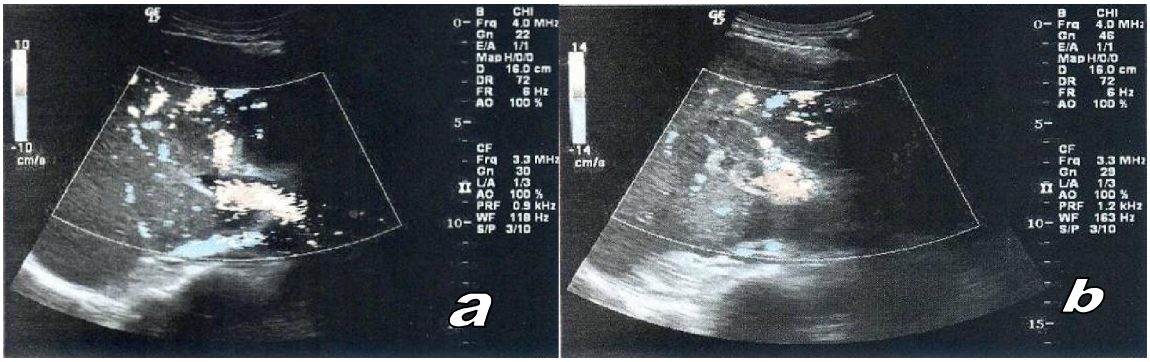


Figure (2): male patient 50 years old, liver cirrhosis **(a)** color Doppler examination showing patent main PV and left PV branch showing normal color flow pattern yet the right PV branch shows no color flow denoting its thrombosis. **(b)** Axial CT portography at extrahepatic level showing patent contrast enhanced opacified main portal vein with no evidence of thrombosis. **(c)** Axial CT portography at intrahepatic level showing patent contrast enhanced opacified left portal vein yet the right portal branch was not opacified and showed no contrast enhancement demoting it thrombosis.



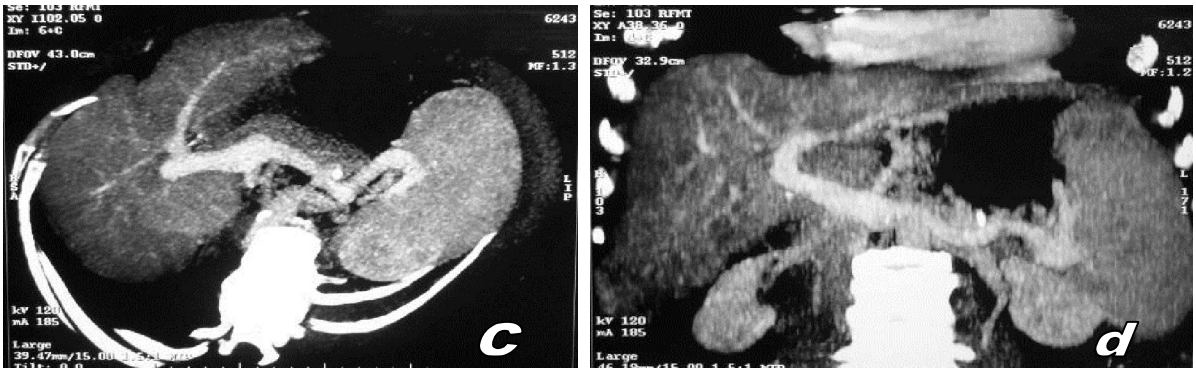


Figure (3): Female patient 56 years old, with liver cirrhosis. **(a)** Color Doppler examination showing patent main PV with normal color flow pattern with no evidence of thrombosis. **(b)** Color Doppler examination showing echogenic thrombus seen in right portal branch and no color flow denoting its thrombosis. **(c)** CT portography, axial cut showing patent opacified main portal and left portal vein branch, yet, non-opacified right portal vein branch denoting its thrombosis. **(d)** CT portography coronal reconstruction showing patent opacified main portal and left portal vein branch, yet non-opacified right portal vein branch.

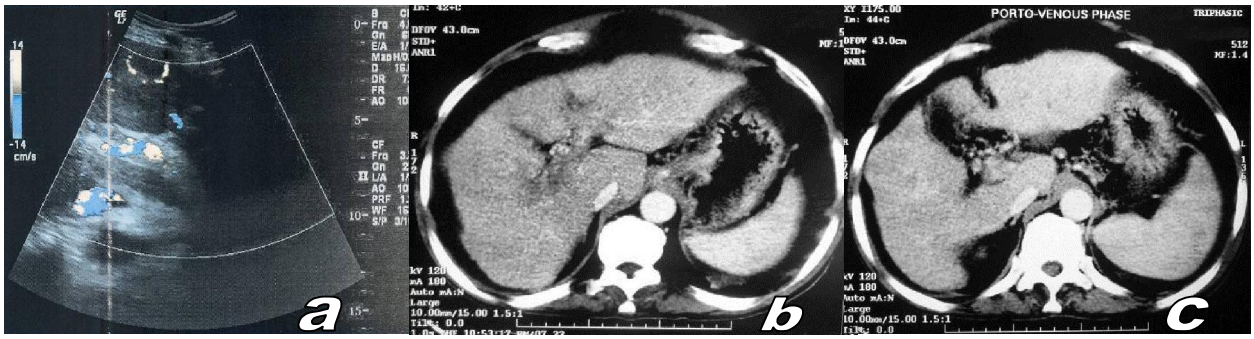


Figure (4): Male patient 58 years old, with liver cirrhosis. (a) Color Doppler examination showing patent hepatic artery with normal color flow pattern, yet the main portal vein showing intraluminal echogenic thrombus with no color flow signal denoting its thrombosis. (b) CT portography, axial cut showing non-opacified left and right portal vein branches denoting their thrombosis. (c) CT portography, axial cut showing non-opacified main portal vein denoting its thrombosis.

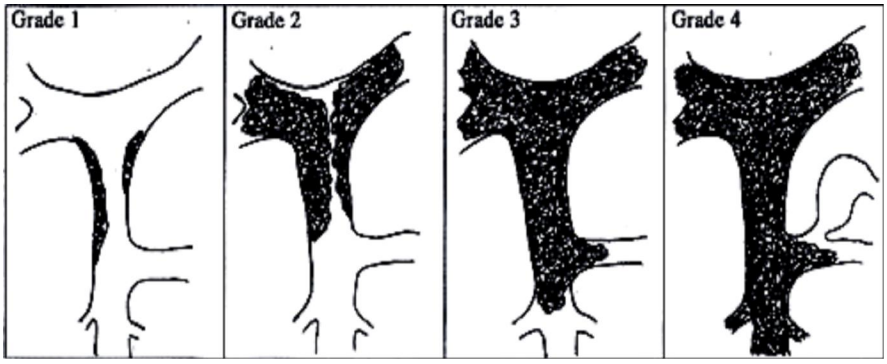


Figure (5): PVT grades according to Yerdel *et al.*[8].

Surgical technique:

Partial or complete PVT may be detected before or during surgery. In either case, our technique is the same. The hilum is dissected first, with isolation of the right and left hepatic arteries which aretied and transected as long as possible, followed by transection of the bile duct. This allows for an intimate exposure and dissectionof the portal vein in its entirety. The portal vein is manually and visually examined to determine the extent ofinvolvement by the thrombus. A portal clamp is applied at its lower part followed by transection of its right and left branches. The vein is maintained open by three tonsil clamps applied to its edge,and then the cleavage

plane between the thrombus and the intima was found. The clot was progressively and circumferentially freed (Figure 6), with the aid of a tonsilclamp by everting the venous wall, by clamping the free edge of the clot with a tonsil. This maneuver was extended to the splenic and/or superior mesenteric veins if necessary. After the clot had been pulled out, portal patency was assessed by introduction of the surgeon's index finger or a Hegar dilator. Usually, this technique allowed the entire clot material to be removed. Before completing the anastomosis, the blood flow in the recipientportal vein was verified by removing the clamp. The portal vein wasflushed with blood in order to eliminate residual or newly formed clots.Subsequently, portal flow was restored by end-to-end portal anastomosis and its patency was checked at the end of the operation by intra-operative Doppler ultrasound.

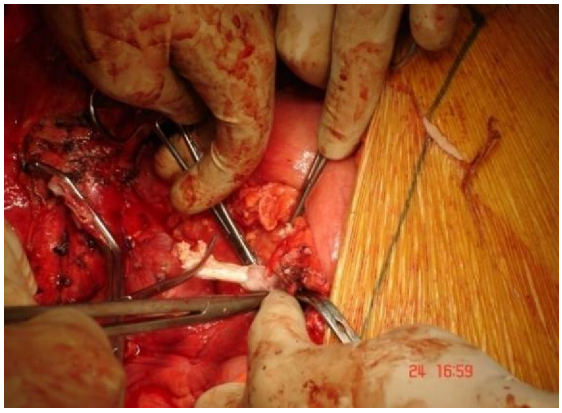


Figure (6): Holding the edge of portal vein opened by three tonsil clamps

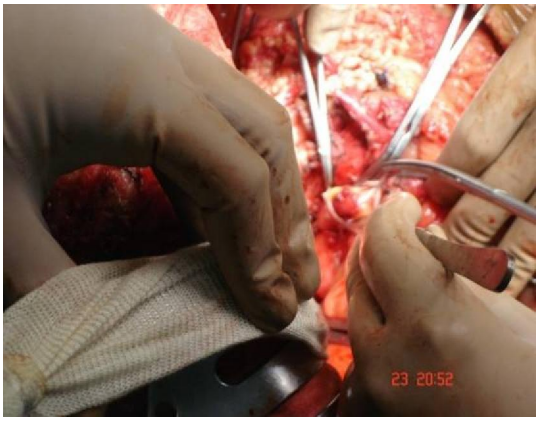


Figure (7): Dissection between the intima and the thrombus.

Hepatectomy was performed in all cases using the piggy-back technique.The choice between right or left lobe grafts depends on the graft recipient weight ratio (GRWR) and residual liver volume (RLV). A minimal 0.8 GRWR and 30% RLV criteria was applied.

Immunosuppression consisted of tacrolimus or cyclosporine and low-dose steroids. Anticoagulation was started postoperatively with enoxaparin1 mg/kg every 12 hours when INR>1.5 and platelet >20.000 with no evidence of bleeding tendency. Warfarin sodium was begun 1 week before discharge adjusting the INR between 2 and 3, to be stopped 6 months after confirmation of absence of PVT.

Post-operative arterial and venous patency was evaluated routinely using Doppler ultrasound at 24 h, daily for one week, weekly for one month, monthly for three months, and then as necessary. In case of any doubt of PVT, CT portography was done.

Intraoperative and postoperative variables analysed were: grade of PVT, thrombectomy whether partial or complete, type of graft, GRWR, blood requirements, cold ischemia time, operative time, recurrence of PVT, postoperative complications, early and late postoperative deaths, and survival. A comparative analysis was performed with 179 patients without PVT transplanted in the sameperiod.

Statistical analysis was performed using the analysis of variance or chi-squared test. The actuarial survival rate was calculated with the nonparametric Kaplan-Meier method and was compared with the Wilcoxon test throughout the study. Quantitative data are presented as median (range) and were analyzed using Kruskal-Wallis test. *P* values of less than 0.05 were considered significant.

3. Results

The pre-operative characteristics of the whole study group are shown in (Table 1).The incidence of PVT at the time of LT was 15%.Compared to patients without PVT, there were no differences in age, sex and Model for end stage liver disease (MELD) score, whereas the indication for LT was less frequently none viral hepatitis in PVT group.

PVT was diagnosed preoperatively in 15 (48%) patients; while in 18 (58%) was accidently discovered intra operatively. The commonest type was grade II, occurring in 13(41.9%) patients (Table 2). Total thrombectomy was successful in 29 cases. In two cases, (type III and IV) the remnant of the thrombus inside the SMV could not be extracted completely and a small residual thrombus was left but with adequate portal flow and reassessed at the end of the operation by intraoperative Doppler U/S. One patient needed ligation of the collaterals in gastro splenic ligament to increase the portal flow.None of them developed portal vein

rethrombosis.

On comparing the intra operative data between non PVT and PVT groups (Table 3), ischemia time was the only significant factor which was more prolonged in the PVT group (p=0.014). However blood transfusion requirements and operative time were nearly similar in both groups.

Table 1. Patient characteristics

	PVT (N=31)	Non-PVT (N=179)	p-value
Age (yr)	50 (28-64)	50(18-64)	0.36
Gender (M/F), n (%)	27(87)/4(13)	153(85.4)/26(14.6)	0.17
MELD	18(11-44)	16(7-29)	0.06
Etiology, n (%)			
HCV	29(93.5)	151(84.3)	0.11
HBV	1(3.2)	3(1.6)	
HBV+HCV		3(1.6)	
Other	1(3.2)	22(12.3)	
Graft			
Right lobe, n (%)	28 (90.3)	177(98.8)	0.09
Left lobe, n (%)	3(9.7)	2(1.1)	
GRWR	1.2(0.6-1.8)	1.2(0.7-1.9)	0.15

Continuous variables are reported in median and range.
PVT, portal vein thrombosis; MELD, Model for end stage liver disease ;HCV, hepatitis C virus; HBV, hepatitis B virus;; GRWG, graft recipient weight ratio.

Table 2. Degree and management of portal vein thrombosis

Grade	**n-value**	**Complete thrombectomy**
I, n (%)	**7(22.5%)**	**7**
II, n (%)	**13(41.9%)**	**13**
III, n (%)	**9(29%)**	**8**
IV, n (%)	**2(6.4%)**	**1**

Table 3. Intraoperative data of both groups

	PVT	Non PVT	p- value
RBC transfusion(U)	6 (0-28)	5 (0-40)	0.1
Ischemia time (min)	(35-175)	76 (50-214)	0.014
Operative time(min)	660(360-780)	570(320-930)	0.3

Variables are reported in median and range
PVT, portal vein thrombosis; RBC, red blood cells.

Influence on morbidity and mortality

The median follow up period is 14 months (1-79). Three patients 3/31(9.6%) developed re thrombosis in the early (within 2 weeks) postoperative period. Two of them, developed partial PVT on Day 2, 15 which was confirmed by duplex and elevation of the liver enzymes. They were successfully treated by medical treatment in the form of full hepiraniztion. Re-canalization occurred after 2 weeks, 2 months respectively with normalization of the graft function.One patient developed complete re-thrombosis on the 2nd day postoperative, confirmed by CT portography. This patient was urgently explored followed by transient clamping of the 3 venous outflow (right hepatic vein, vein of segment 5 and right posterior inferior hepatic vein), and hepatic artery. Thrombectomy followed by re anastomosis of PV and ligation of collaterals. The patient died on day 10 with renal and hepatic failure due to thrombosis of the segment 5 vein with development of the small for size syndrome (SSS).

On comparing the postoperative morbidity in patients with or without PVT (Table 4),the incidence of re thrombosis was significantly higher(p< 0.003) in the PVT group(9.6%versus 0.55%) .Two patients in the none PVT group developed portal vein stenosis postoperatively on day 31 and 45 with successful treatment by ultrasound guided dilation

with stent insertion. The incidence of infection was significantly higher (p<0.001) in the PVT group (n=19/31, 61%) than none PVT group46/179(31%). No difference was found between the two groups as regard the incidence of reoperation, postoperative bleeding ICU and hospital stay.

The overall morbidity in PVT group was significantly higher (p0.001) compared to none PVT group(23/31, 74.1%; versus 60/179, 33.5%)

Mortality occurred in 11 (35%) patients in the PVT group with only one patient developed portal vein re-thrombosis. The major cause of death was sepsis (n=6, 46.1%). Other causes included SSS (n=2, 6.4%), Bleeding (n=1, 3.2%), Hepatic artery thrombosis (n=1, 3.2%), and Cerebrovascular stroke (n=1, 3.2%).

The survival analysis (Figure 8.) showed that the 1 and 3years survival rates were 70.6% and 60.2%, respectively in PVT group compared to 81% and 62% in none PVT group however there was no statistical difference (p> 0.3) between the two groups.

Table 4. Percentages of postoperative complications with presence or absence of PVT

	PVT	Non PVT	p-value
Reoperation, n (%)	5/31(16%)	18/179(10%)	0.5
Bleeding, n (%)	1(3.2%)	9(5%)	0.3
PV complications, n (%)			
Re thrombosis	3/31(9.6%)	1(0.55%)	0.003
Stenosis	0	2(1.1%)	
Renal dialysis, n (%)	4(13%)	17/179(9.4%)	0.6
Infections, n (%)	19/31(61%)	46/179(31%)	0.001
Overall complications	23/31(74.1%)	60/179(33.5%)	0.001
ICU stay (days)	8(0-48)	7(0-56)	0.7
Hospital stay (days)	24(0-60)	28(2-61)	0.8

Continuous variables are reported in median and range
PVT, portal vein thrombosis; PV, portal vein

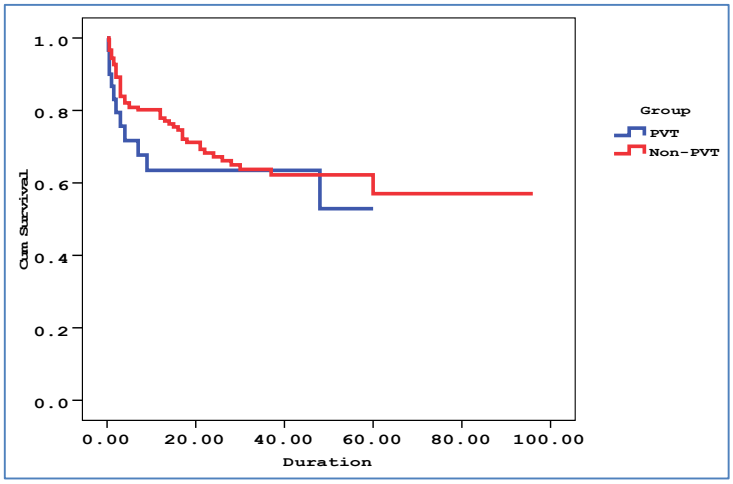


Figure (8): Overall patient survival rates in patients with and without

4. Discussion

Greater experience with LT and the description of several technical options have led PVT to beconsidered as a surgical challenge, rather than a contraindication for LT[9].

Theincidence of PVT among patients undergoing LT ranges from 2% to 26%, dependingon the reported series[10,11]. These differences are due to different diagnostic criteria andthe different study periods. There is a tendency in recent years towards an increasedincidence of PVT in patients undergoing LT[12]. In our series, it was 15%.

Imaging the portal vein is an important goal of pre-OLT patient evaluation and is usually based on Doppler ultrasonography, which is easily available, inexpensive and non-invasive but, its accuracy in detecting PVT ranges from 26% to 87%. This is explained by a high incidence of false negatives due

to the extension of PVT, the identification of portal collaterals as the PV, and the post-US thrombosis of the PV while patients are awaiting transplantation[13]. In our patients, although we use routinely both Doppler and CT portography, our detection rate of PVT preoperatively was 42%. This is similar to the results obtained by Dumortier *et al*[13], who had the rate of pre-OLT diagnosis of PVT of 44.7%.

An adequate portal inflow to the graft is essential for good liver function. Different approaches have been proposed to restore PV patency at the time of OLT, such as thrombectomy, the use of venous interposition grafts, the use of PV collaterals, and cavoportalhemitransposition[14]. In absence of cadaveric programs, eversion thrombectomy, represents the simplest way to restore portal flow. In our experience, thrombectomy with good portal flow restoration wasapplicable in most cases with the exception of grade 4 in which partial thrombectomy was done in one case and complete thrombectomy in the other.

When the portal flow is adequate in the setting of a small residual thrombus in the SMV and/or splenic vein after removal of the main thrombus, it is controversial whether or not to secure a perfect, but dangerous, further thrombectomy or to leave an incomplete, but safe thrombectomy. The two patients in our series with partial thrombectomy did not show portal rethrombosis in the follow up period.

The greater technical difficulty in a patient with preexisting PVT may be associated with longer operation time, anhepatic phase, and higher transfusion requirements[15]. In our patients,transfusion requirements and operative time were similar in both groups but longer Ischemia time in patients with preexisting PVT. The difficult hilar dissection in these patients may push us to rapidly control the pedicle which may have a reflection on minimizing operative time and blood loss.

It has been reported in the literature that OLT in patients with PVT is associated with a higher rate of complications, such as hepatic artery thrombosis, relaparotomy, pancreatitis, sepsis, and renal failure[8]. In our patients, the overallcomplications, Infections and portal vein rethrombosis were higher in patients with preexisting PVT but this was not associated with increase in ICU or hospital stay.

Although the incidence of portal vein rethrombosis was higher in patients with preexisting PVT (3/31, 9.6% vs. 1/179, 0.55%) this was not associated with direct effect on the outcome of these patients as two of them had partial thrombosis and was successfully treated with anticoagulant therapy and the third one died from SSS.

We observed that PVT did not affect patient survival (70.6% and 60.2%, one and three year survival rate in patients with PVT vs. 81% and 62% in patients without PVT), confirming most of the reports in the literature[13,16].

In conclusion, PVT increases surgical difficulties and postoperative morbidity (PV rethrombosis, infections) but does not have an influence on patients` survival. Grade IV had poor out come and may need venous Jump grafts or cadaveric OLT.

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Numerical simulation of turbulent characteristics (structures and statistics) in channel with periodic two-dimensional ribs

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Abstract: Numerical simulation of turbulent channel flow with periodic two– dimensional ribs has been performed in order to investigate the turbulent characteristic behind the ribs. The Reynolds numbers based on the friction velocity and the channel half width are 10- 1500. In the wake region, the mean flow becomes asymmetric with respect to the centerline of the geometry through the Coanda effect. Large – scale vortices are generated at the height of the ribs edges. The small – scale vortices are convicted toward the channel center. The budgets of the Reynolds stresses have been computed. The significant differences are found between the budgets in this study and those in backward – facing step turbulence. The positive Reynolds shear stress $\overline{u'v'}$ is observed owing to the flow contraction just behind the ribbed.
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Key words: Turbulent characteristic, wake region, rib, turbulent channel, vortices

1. Introduction

The ribs are often used to control the flow rate and to enhance the mixing in practical mechanical equipments. In the numerical studies of separated flows, the flow over a backward – facing step [4] , the ribbed channel [18] and the roughened channel flow [16],[14] are frequently employed. In particular, the flow through a two – dimensional backward – facing step is the most popular. The effect of step height was studied by [5]. The Kelvin-Helmholtz (K-H) vortices and the longitudinal vortices were observed behind the step [17]. Recently, the simulations for more practical configurations have been performed, for example, the flow over riblets or with one wavy wall [7].

In present work, the separation, the reattachment and also the contraction occur near and behind the ribbed therefore; the study of flow field with ribbed is useful to investigate the effects of the flow acceleration / contraction in the separated flow. In this study, the flow acceleration is defined as the positive streamwise mean velocity gradient ($\partial U/\partial x > 0$) and the flow contraction $\partial V/\partial x \neq 0$ and / or $\partial V/\partial y \neq 0$, where U is the streamwise mean velocity and V the wall – normal mean velocity.

In the present work, numerical simulation of a turbulent channel flow with periodic two – dimensional ribbed has been carried out for $Re_{\tau 0}$=10-1500, where is the friction velocity defined later, the channel half width and the kinematic viscosity. The purposes of this study are to obtain the turbulent statistics for the development in turbulence modeling and to examine the relationship between the turbulent structures and turbulent statistics behind the ribs.

2. Numerical model and validation

2.1 Governing equations

For steady incompressible turbulent flow, the Reynolds averaged equations for conservation of mass and momentum may be written as follows:

Continuity: $$\frac{\partial u}{\partial x} = 0 \quad (1)$$

Momentum:
$$\frac{\partial}{\partial x}\left[uu - (v + v_t)\frac{\partial u}{\partial x}\right] = -\frac{1}{\rho}\frac{\partial P}{\partial x} + \frac{\partial}{\partial x}\left[v_t \frac{\partial u}{\partial x}\right] \quad (2)$$

where u is the velocity component x-direction, P is pressure, v is kinematic viscosity and v_t is the eddy viscosity obtained from the turbulence model.

Among several variations of widely used two-equation turbulence models, the low Reynolds-number (near-wall) properly resolve the complex flow behind around the ribs, following the work of [19]. This particular model is chosen because of its proven robustness and unambiguous near-wall treatment, two essential attributes in numerical modeling of separated flow about distributed ribs. The eddy viscosity is determined from two transport equations:

Turbulence kinetic energy (k):
$$(3)\quad \frac{\partial}{\partial x}\left[uk - \left(v + \frac{v_t}{2}\right)\frac{\partial k}{\partial x}\right] = P_k - \beta^* k\omega$$

$$Re_\tau = \frac{k}{\omega v}, \beta^* = \frac{5}{100}\cdot\frac{4/15+(Re_\tau/R_\beta)^4}{1+Re_\tau/R_\beta}, R_\beta = 8 \quad (4)$$

Specific dissipation rate(ω):
$$(5)\quad \frac{\partial}{\partial x}\left[u\omega - \left(v + \frac{v_t}{2}\right)\frac{\partial \omega}{\partial x}\right] = \alpha\frac{\omega}{k}P_k - \beta\omega^2$$

$$\alpha^* = \frac{3/125+Re_\tau/R_k}{1+Re_\tau/R_k} \quad , \quad R_k = 6$$

$$\alpha = \frac{13}{25}\frac{1/9+Re_\tau/R_\omega}{1+Re_\tau/R_\omega}\frac{1}{\alpha^*} \quad , \quad R_\omega = 2.95 \quad , \quad \beta = \frac{9}{125}$$

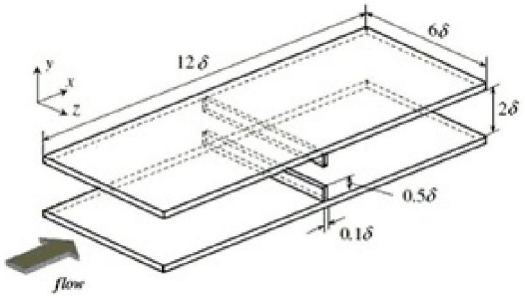
$$P_k = \nu_t\left(\frac{\partial u_i}{\partial x_j}+\frac{\partial u_j}{\partial x_i}\right)\frac{\partial u_i}{\partial x_j}$$

Where P_k the production of turbulence kinetic energy and the eddy viscosity is is related to k and ω as:

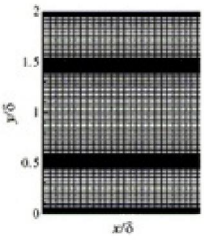
$$\nu_t = \alpha^*\frac{k}{\omega} \quad (6)$$

2.2. Computational domain and boundary conditions

The configuration of the computational domain is shown in fig. 1a. A periodically repeating spatial unit with two-dimensional ribs is simulated. The periodic boundary conditions are employed in the streamwise (x) and spanwise (z) directions. The no-slip boundary conditions are used on all the walls. To ensure the grid resolution even close to the ribbed wall, a large mesh number is employed in the streamwise direction (see table 1). In the wall–normal direction, the density of the computational mesh is high at the height of the ribbed edges and near the walls (sees fig. 1b). The specific dissipation rate, ω is specified at the first grid off the solid surface and given a value $6\nu/(9\Delta n^2/125)$ where Δn denotes the normal distance from the wall [21].



a



b

Figure.1.(a) configuration of the computational domain (b) computational mesh.

The ratio of ribbed height to channel height β is set to be 0.5, i.e., the distance between the wall and ribbed edge h is 0.5δ in this study. The non – dimensionalized local friction velocity $u_\tau^*(=u_\tau/u_{\tau 0})$ for is shown in fig. 2. The streamwise –averaged friction velocity $\langle u_\tau\rangle_{ave}/u_{\tau 0}$ is 0.26 in this case.

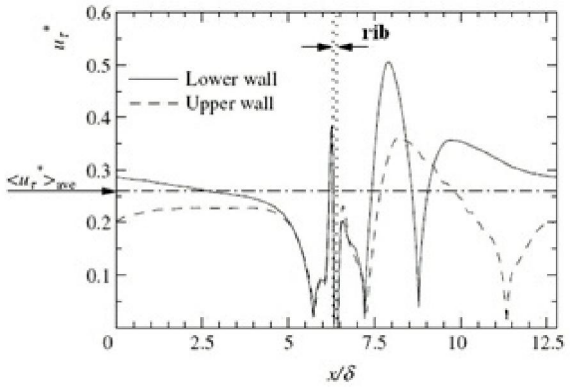


Figure.2. The non-dimentionalized actual friction velocity profiles for Reτ0=600.

2.3 Numerical solution procedure

The above equations are solved by a finite-volume method in an orthogonal body-fitted grid.

Second-order accuracy is assured by adopting the central differencing scheme throughout except for the convective derivatives that are discretized by the QUICK scheme. The continuity and the momentum equations, and the model equations for k and ω, are solved iteratively until convergence. The convergence criterion imposed in the calculation is that the sum of the residuals of mass source be less than 10^{-10}.

2.4. Validation tests

The major difficulty of model validation in the present case is the lack of adequately detailed experimental data for various rib shapes and block arrangements. In view of this, model validation is focused on two important features of the analysis: computation of separated flow and implementation of an orthogonal grid system.

The numerical model outlined above is validated against two test cases, namely, the backward facing step flow, and the square-ribbed channel flow, for which previous calculations provide a basis for comparison. Other researchers have also used these cases to validate their solution procedures and turbulence models. Therefore, it suffices to provide a very brief description of the results.

Fig.3. Backward facing step flow for $Re_h = 2800$ and $\delta_b/h = 1.1$ at $x/h = -3.8$: (a) streamlines and pressure contours; (b) c_f distribution along the channel.

, For the backward facing step flow, calculations were performed in a solution domain $-3.8 < x/h < 80$ with

a grid 180×80×240 , in which 30 of the 80 grid points are distributed in the expanded region, for a Reynolds number (Re_h), based on step height h and the mean inlet velocity, of 2800. The inlet velocity profile is constructed to match the turbulent boundary layer of the experiment, i.e.$\delta/h = 1.1$. Fig. 3 shows the overall flow field and the friction coefficient along the wall downstream of the step. There is good agreement with the measurements of Vogel and Eaton (1985) .The computed reattachment length of 6.67h. These results provide a degree of validation of the numerical method and turbulence model.

Finally , the flow over the regularly distributed square ribs with $h/D_e = 0.1$ and $w/h = 7.2$ where D_e is the hydraulic diameter, is examined for $Re_{De} = 7,200$ .The mean velocity profiles at various cross-sections plotted in Fig.4 are seen to be in good agreement with the measured data of [9].

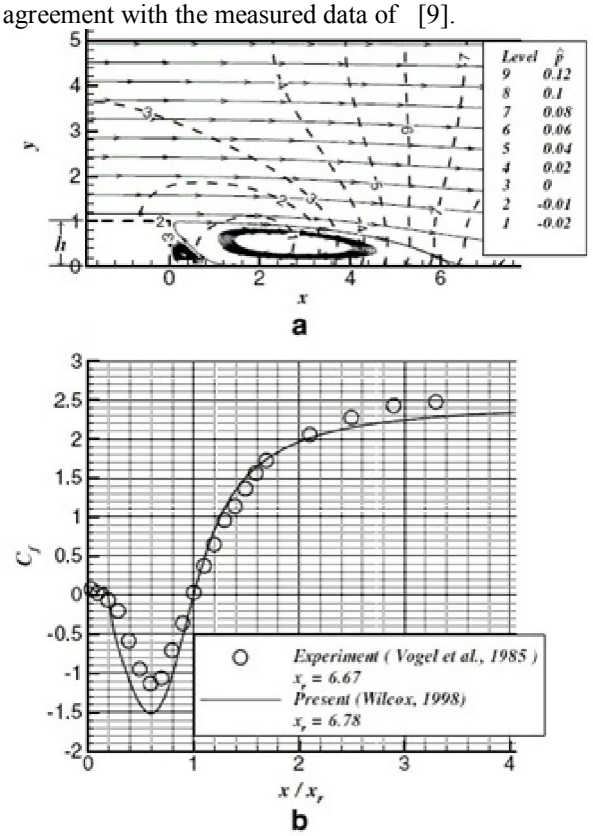


Figure.3. Backward facing step flow for Re_h=2800, (a)streamlines and pressure contours, (b)C_f distribution along the channel.

The discrepancy observed near the top surface of the rib, where the mean velocity attains a local maximum, was first suspected to be due to the insufficient grid resolution$(100 \times 80 \times 256)$. An additional calculation with much finer grid$(160 \times 150 \times 256)$ confirms that the solution is indeed grid-independent. The two-layer model of Chen and Patel (1988) may be the only exception that qualitatively shows the local maximum in the mean velocity in that region. The results of the standard $k-\omega$ model with the wall function are shown in the Fig.4.for reference.

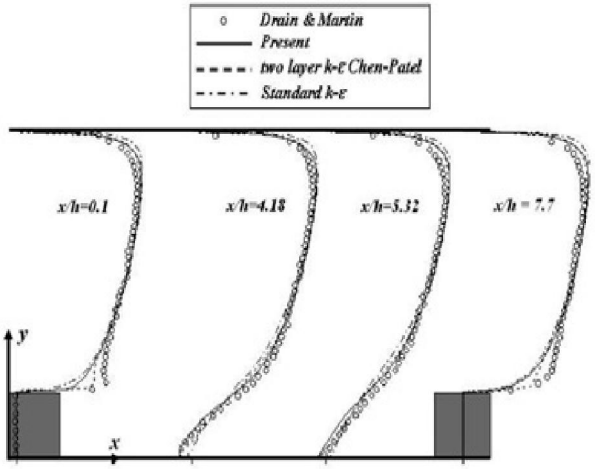


Figure.4. the mean velocity profiles at various cross-sections.

3. Discussion about results

3.1. Asymmetry phenomena and mean reattachment length

The streamlines of the mean flow for $Re_{\tau 0}$=20, 50, 300, 600, and 1500 are given in fig.5. The mean flow is asymmetry behind the ribbed in the cases of higher Reynolds numbers. These phenomena are referred to as the Coanda effect. The asymmetric direction depends on the initial flow field. We obtained the flow attachment to the upper and lower walls nearly with the same probability. In this study, the mean symmetric flow can be observed in the cases of $Re_\tau \leq 20$, i.e., $Re_b \leq 110$, and the mean flow becomes asymmetric in the cases of $Re_{\tau 0} \geq 30$ up to $Re_{\tau 0}$=1500, i.e., Re_b =7800.

In addition, we tested also an inlet- outlet boundary condition case (non- periodic) with a driver section which is a computational sub part to generate a fully developed turbulent channel flow for the inlet boundary condition [13]. The convective boundary condition is applied at the outlet boundary of the main part. Size of the used domain is 12δ ×2δ×6δ and the grid number is 256×128×256 for the driver section. For the main section, the domain is 16δ×2δ×6.4δ and the grid is the 512×128×256. The ribbed is stationed at 4δ in the streamwise direction from the connecting plane between the sub and main parts. The time–averaged streamlines in the case of the inlet- outlet boundary condition are shown in (Fig.6).The mean flow becomes asymmetric as seen in the periodicity adds less effect on the asymmetry phenomena.

A large primary bubble is observed from the back-end of the rib to 9δ in the x-direction at the lower wall (Lr_1in Fig.5). In addition, a secondary bubble (B in Fig.5) generates to approximately 7.3δ in the x-direction

(see Lr_2 in Fig.5), i.e., 1.8h from the rib for $Re_{\tau 0}$=600. Le et la. (1997) reported that the length of the secondary bubble in the x-direction Lr_2 is 1.76h for backward-facing step flow. Therefore, the length of the secondary bubble is almost same in both the cases.

The reattachment length Lr_1 and the secondary bubble length Lr_2 are shown in (Fig.7). as a function of the bulk Reynolds number. In this study, the reattachment location is determined by the location of $\partial U/\partial y = 0$ at the wall.

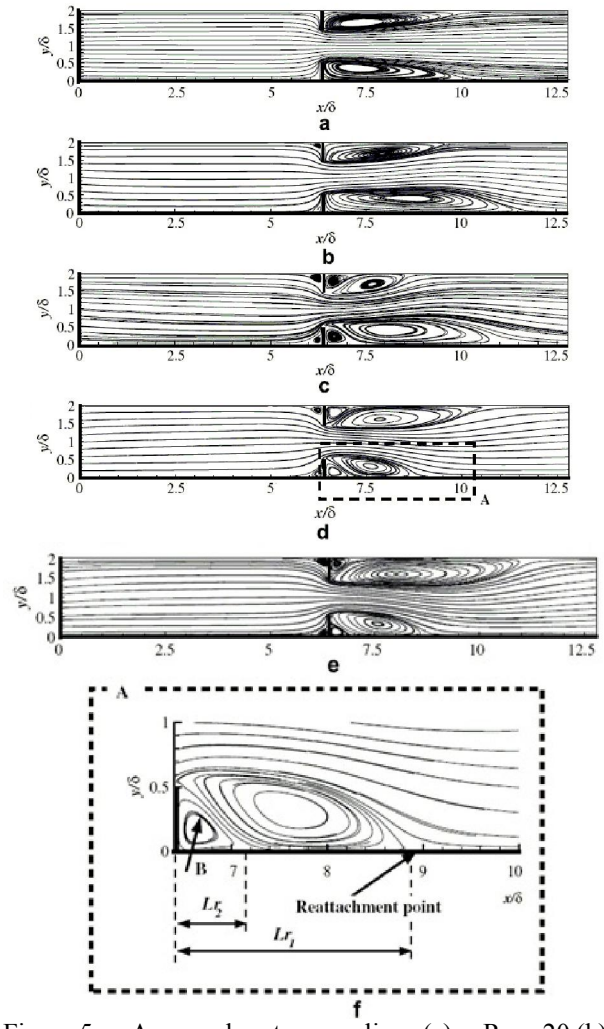


Figure.5. Averaged stream lines;(a) $Re_{\tau 0}$=20,(b) $Re_{\tau 0}$=50,(c) $Re_{\tau 0}$=300,(d) $Re_{\tau 0}$=600,(e) $Re_{\tau 0}$=1500,(f) enlarged view of the rectangular A in(d).

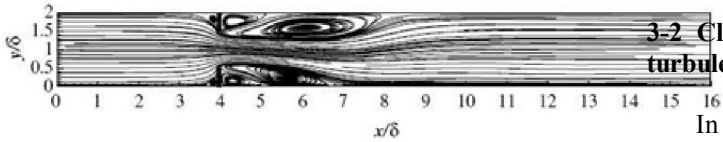
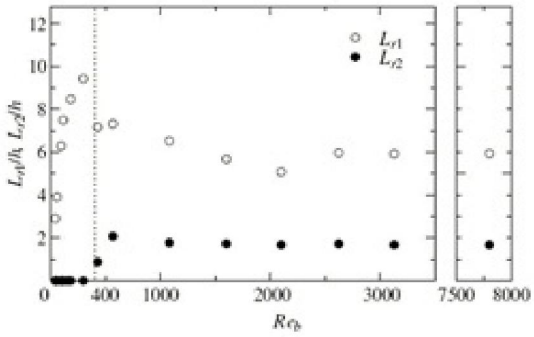


Figure.6. Averaged stream lines in the case of the inlet-outlet boundary condition.

The length Lr_1 is defined as the distance from the back-end of the ribbed to the reattachment location. Because the mean flow is asymmetric, the Lr_1 and the Lr_2 are obtained as an average of those on the upper and lower computational domain, i.e. the averaged reattachment point is calculated from (7).

$$1/2\left(\partial U/\partial y_{|lower} + \partial U/\partial y_{|upper}\right) = 0 \qquad (7)$$

In the range of Re_b<400 range, the Lr_1 increases with increasing the Reynolds number. On the other hand, in Re_b>400 range, the reattachment length $Lr_1$is characterized first by a sharp decrease and subsequently by a gradual one. These results are similar to those described in the case of the backward- facing step experiment [2]. One can clearly identify the laminar (Re_b<400), the transitional and the turbulent (400<Re_b<7800) ranges as implied by the shape of this profile.



(Fig.5). Reynolds number dependence of rectangular length Lr_1/h and secondary bubble length Lr_2/h from the back-end of the orifice.

The secondary bubble cannot be observed in the laminar region (Re_b<400). In the range of (400<Re_b<7800) ranges as implied by the shape of this profile.

The secondary bubble cannot be observed in the laminar region (Re_b <400). In the range of 400< Re_b <550, the secondary bubble length Lr_2 increases with increasing the Reynolds number and it becomes almost constant in Re_b>550 range. Therefore, the secondary bubble is generated in the transitional and the turbulent regime. In addition, the reattachment length Lr_1 at a higher Reynolds number (Re_b> 2000) is approximately 6h (=3δ) in this study. This corresponds roughly to the well- known length of 5-8h in the case of the turbulent flow over a backward–facing step [15].

3-2 Classification of wake region based upon the turbulent structures and statistics.

In this section, the relationship between the turbulent structures and the turbulent statistics in the case of $Re_{\tau 0} = 600$ are reported. In Fig.8 the wake region is classified based upon the budgets of the Reynolds stresses and the turbulent structures.

The region 1 is the channel center near the rib. The Reynolds stresses and the turbulent energy in this region are much smaller than in other regions. The minimum value of the $\overline{u'u'}$ near the channel center is about 4.3 of the maximum value in the shear layer at $x_r/\delta = 1.0$.

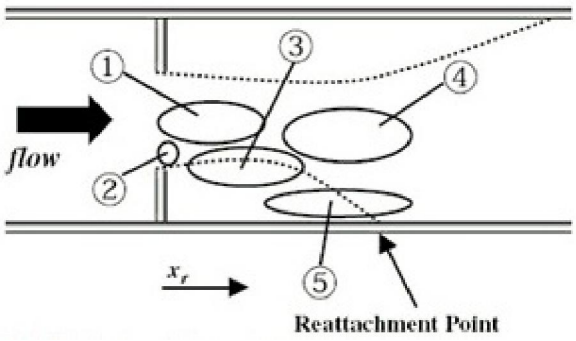
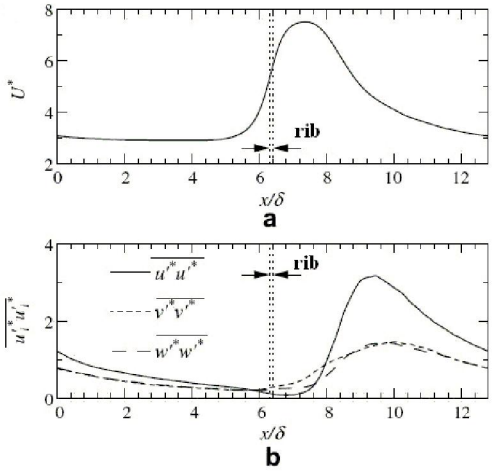


Figure.8. classification of the wake region.

Fig.9a and b show the streamwise evolutions of the streamwise mean velocity and the Reynolds stresses, respectively, where U^+ the non-dimensionalized is streamwise mean velocity at the channel center and $\overline{u_i'u_i'}$ indicates the Reynolds stresses on the centerline. The mean velocity increases in the streamwise direction in the range of $5.0 < x/\delta < 7.5$ by the flow acceleration through the ribbed.

The region 2 for region $(y/\delta > 0.6)$, Reynolds stress $\overline{u'u'}$ exhibits the significant value and the mean velocity gradient $\partial U/\partial x$ is positive. This positive $\partial U/\partial x$ is caused by the flow acceleration through the ribbed. Therefore, the negative production of $\overline{u'u'}$ is due to the flow acceleration/contraction through the rib.



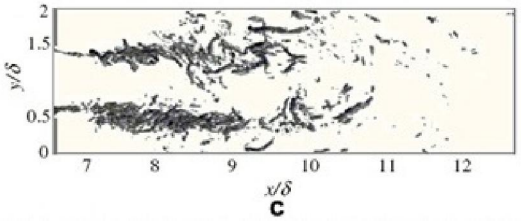


Figure.9.(a) Streamwise evolution of the streamwise mean velocity on y/δ =1line;(b) Streamwise evolution of the Reynolds stresses on y/δ =1line;(c) vortices behind the rib for $Re_{\tau 0}$=600 .

The region 3 indicates the shear layer in the recirculation region. These profiles are characterized by the sharp peaks of the production and the large negative pressure strain for $0.5 < y/\delta < 0.75$ on the lower side. The peak production is caused by the large positive mean velocity gradient $\partial U/\partial y$. In the budgets of $\overline{u'v'}$ the $\partial U/\partial y$ also mainly contributes to the negative peak of the production term. In the shear layer, the pressure strain terms of $\overline{v'v'}$ and $\overline{w'w'}$ exhibit large positive values. Hence, the redistribution from $\overline{u'u'}$ to $\overline{v'v'}$ and $\overline{w'w'}$ is the dominant process in this region.

In the region 4 ($x/\delta > 8$ and $0.5 < y/\delta < 1$ on the lower side of the channel), the turbulent diffusion term plays an important role. This term removes the turbulent energy from the shear layers and transports it to the channel center.

The contribution of the turbulent diffusion to the budgets becomes larger downstream in the center region of the flow (the center of the channel), i.e., $y/\delta = 1.0$, is not equal to the flow center because of the flow asymmetry. Thus, the turbulent diffusion contributes to the increase of the Reynolds stresses and the turbulent kinetic energy in the central region. Therefore, the turbulent diffusion adds more effects on the turbulent statistics at the channel center than in the case of the backward-facing step flow.

The region 5 is located near the reattachment point (cf. the time-averaged reattachment length $L_{r1}/\delta \simeq 2.6$ on the lower side for $Re_{\tau 0} = 600$).

4. Conclusion

In the present study, we performed numerical simulation of turbulent channel flow with the periodic two-dimensional rib in various Reynolds numbers for $10 < Re_{\tau 0} < 1500$ $(40 < Re_b < 7800)$ and investigated the asymmetry phenomena. Mean reattachment length, vortex structures and the relationship between turbulence statistics and structures. The mean flow becomes asymmetric behind the rib because of the Coanda effect. In this study, this asymmetry phenomena can be observed for

$30 < Re_{\tau 0} < 7800$, i.e., $180 < Re_b < 7800$.The Reynolds number dependence upon the reattachment length is similar to those described in the case of the backward-facing step experiment length is observed at $Re_b \simeq 400$ .This is the transition from laminar to turbulence. The secondary bubble can be seen in the turbulent regime. The length becomes constant in the higher Reynolds number range.

The budgets of the turbulent kinetic energy and Reynolds stresses were calculated. We classified the wake region into five regions based upon the budgets of the Reynolds stresses and the turbulent structures. The several differences are found between the Reynolds stresses budgets in the present case and those in backward–facing step turbulence. The potential region is generated in the channel center near the rib. In shear layers just behind the rib, the Reynolds shear stress $\overline{u'v'}$ becomes positive owing to the effect of the flow contraction. In addition, the production terms of $\overline{u'u'}$ exhib its negative value near the ribbed edge. This is because of the flow acceleration/ contraction effects. These behaviors cannot be observed in the backward-facing step turbulence. At the shear layer in the recirculation region, the redistribution from $\overline{u'u'}$ to $\overline{v'v'}$ and $\overline{w'w'}$ is the dominant process. In the channel center at the recirculation region, the turbulent diffusion term transfers the turbulent energy from the shear layer to the channel center. In the wall vicinity of the recirculation region, the redistribution from $\overline{v'v'}$ to $\overline{u'u'}$ is promoted by the large scale splitting effect.

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Endovascular Stenting with Drug-Eluting Stent for Symptomatic Ostial Vertebral Artery Stenosis

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Abstract: Objective: To evaluate the safety and efficacy of endovascular treatment with drug-eluting stent for symptomatic atherosclerotic ostial vertebral artery (VA) stenosis. Methods: Seventeen symptomatic patients (average age, 70.7±5.6 years) with 17 ostial vertebral artery lesions received 17 balloon-expandable drug-eluting stents. Follow-up angiography was performed when restenosis was suspected or during later catheterization for other indications. Restenosis was defined as 50% diameter narrowing. Results: The degree of stenosis ranged from 75% to 98% (mean 81 ± 5.2%). The technical success rate was 100%. Procedure-related complication rate, mortality rate, and permanent neurologic morbidity rate at 30-day follow-up were 0%. At 12-months follow-up, no patient was reported of having recurrent vertebrobasilar ischemic symptoms and all VA restenosis was ＜50%. Conclusion: This pilot study suggests that use of drug-eluting stents in angioplasty to treat symptomatic atherosclerotic ostial VA stenosis is feasible and promising in terms of potential safety and effectiveness on the prevention of recurrent ischemia and restenosis. These results could be helpful in the formulation of a larger prospective randomized controlled trial. [**Endovascular Stenting with Drug-Eluting Stent for Symptomatic Ostial Vertebral Artery Stenosis.** Life Science Journal. 2011;8(4):378-381] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: Vertebral Artery Stenosis; Drug-Eluting Stent; Stenting

1. Introduction

Approximately 20% to 25% of ischemic stroke are located in the posterior circulation involving the vertebrobasilar system (VBS) [1]. The prognosis for patients with atherosclerotic occlusion or thrombosis of the VBS is poor, with 80% to 100% mortality[2]. Medically refractory, symptomatic VBS disease carries a 5% to 11% incidence of stroke or death at 1 year[3]. Transient ischemic attacks (TIA) due to extracranial VBS disease are associated with a stroke rate of 30% at 5 years[4]. The Joint Study of Extracranial Arterial Occlusion examined 3,800 patients who presented for angiography due to symptomatic cerebrovascular disease and found a 40% incidence of veterbral artery (VA) stenosis and a 10% incidence of complete occlusion of either of the 1 vertebral artery[5]. The most frequently involved location of VA is ostium[6]. Endovascular intervention has been identified as an effective method for VB stenosis. Balloon angioplasty limited by elastic recoil and dissection, and restenosis rates reported in the literature was nearly 75%.[7] Stenting offers salvage following unsuccessful balloon angioplasty and primary stenting has been shown to be safe and effective[8]. However, the restenosis rate after stenting vary from 11.1% to 66.7%[9,10]. The major advantage of drug-eluting stent is to use the cytotoxic drug coatings on the stent to inhibit the occurrence of vascular restenosis due to intimal hyperplasia that is known to be associated with the use of stents. Drug-eluting stents are rationally superior to bare metal stents. The purpose of our study was to evaluate the feasibility and preliminary results of using paclitaxel-eluting stents for angioplasty and to treat symptomatic atherosclerotic ostial VA stenosis.

2. Methods

2.1 Study Design and Patient Population

With the approval of our institutional review board, a retrospective review of medical and radiological records was performed to identify patients referred to our tertiary center for endovascular treatment of symptomatic ostial VA stenosis refractory to adequate medication. The search identified 17 consecutive patients (11 men; mean age 70.7 years, range 54 to 88) who were treated with stent implantation for vertebral artery stenosis from March 2009 to October 2010. All patients presented with typical VBI symptoms (Table 1), such as severe dizziness, diplopia, vertigo, gait disturbance, or drop attacks, which was confirmed by a neurologist. Informed consent was obtained from every patient prior to the procedure, including follow-up angiography.

Table 1: Patient characteristics

	Male (n=11)	Female (n=6)
Age y	70±7	72±6
Hypertension	8	4
Diabetes	6	4
Hyperlipidemia	6	3
Smoking	7	1
Bilateral disease	2	1
Coronary artery disease	5	2
Symptoms		
Dizziness	7	3
Drop attack	2	0
Vertigo	2	1
Gait disturbance	3	1
Diplopia	5	2

2.2 Preprocedural Evaluation

The diagnosis of vertebral artery stenosis was made by cerebral angiography. Bilateral subclavian and nonselective vertebral artery angiograms were obtained to visualize the true vertebral ostium. The degree of stenosis in ostium was evaluated by using NASCET (North American Symptomatic Carotid Endarterectomy Trial) method (The diameter of a normal ipsilateral V2 segment was used as reference). The indications for stenting were: (1) diameter stenosis ＞ 75% in an artery with a reference diameter ＞3.5 mm or (2) diameter stenosis ＞50% in an artery with reference diameter ＞3 mm and contralateral occlusion. Lesions in an artery without continuation to the basilar artery or with severe stenosis beyond the V2 segment were excluded. Patients with severe stenosis in the basilar artery or its major branches were also excluded. Bilateral ostial vertebral artery stenting was allowed, as long as the stenting indications were fulfilled in individual lesions.

2.3 Stent Implantation

Procedural heparin was given at 70 U/Kg, and the activated coagulation time was kept at 250 to 300 seconds. A 6-F guiding catheter was placed via a femoral sheath into the proximal subclavian artery without engaging the vertebral ostium. A 0.014-inch coronary guidewire was carefully passed across the stenosis into the distal cervical vertebral artery, and no embolic protection devices were used in this series of patients. Predilation, if considered necessary by the operator, was done with an undersized coronary angioplasty balloon. A balloon-expandable drug-eluting coronary stent (Fire Bird Microport company, China) sized to match the reference diameter was positioned at the ostium and deployed at high pressure (9 to 12 atm). The goal was to achieve ＜ 20% residual stenosis, with complete lesion coverage. Aspirin (100 mg/d), combined with clopidogrel (75 mg/d), were started 5 days prior to stenting and was kept continued for 3 months.

2.4 Follow-up

All patients were followed for change in symptom and neurological status with monthly clinic visits. If new posterior stroke was suspected, CT or MRI was arranged for documentation. Recurrent VBI symptoms were carefully recorded and validated by a neurologist. Neck ultrasonography were done at 1 and 6 months post stenting to evaluate the stenosis of treated vessel by an independent neurologist. If restenosis was suspected, angiography was mandated to confirm the diagnosis. Vertebral angiography was also obtained in patients undergoing angiographic workup or intervention in other vascular territories, but no systematic angiographic follow-up was planned in the protocol. Angiographic restenosis was defined as diameter stenosis .50% at the stented site.

2.5 Statistical Analysis

The VA diameter, degree of stenosis, and length of the stenotic segment were measured, and the range, median, mean, and standard deviation were determined for each measurement. Since this was a single-arm study and the number of patients being small, statistical analysis was not performed.

3. Results

Technical success was 100%. The mean reference diameter was 4.0±0.6 mm and the pretreatment diameter stenosis was 92%±5% for the 17 target lesions. Predilation was done in only 22% (4/17) of the lesions before implantation of stents. No periprocedural death occurred. No patients suffered a periprocedural posterior stroke. One patient presented with nystagmus and severe vertigo just after stent implantation; of whom the MRI revealed multiple acute ischemic infarcts in the cerebellum, left thalamus, and bilateral occipital regions. The symptoms improved within 1 week, and no permanent neurological sequela remained thereafter. In the follow-up of angiogram 12 months later. One patient (5.9%) who had an episode of minor left hemispheric stroke 3 weeks prior to the procedure suffered from worsening right sided weakness on the day after the intervention. MRI revealed recent stroke in the left periventricular white matter, but no posterior infarction was found. The condition improved 5 days later, with complete neurological recovery. No patient had experienced recurrent vertebrobasilar stroke or transient ischemic attack at either the treatment-related vascular territory or the

treatment-unrelated vascular territory within 12 months follow-up after stenting. No patient had a VA restenosis of 50% or more. The degree of stenosis of the vessel lumen ranged from 0% to 45% (median, 25.5%; mean, 25.8% ±13.4). The management of stenting with drug-eluting stent is shown in Table 2. The degrees of VA stenosis measured before and immediately after stent placement and at 12-month follow-up of individual patients are shown in Table 3.

.

Table 2: Angiographic Measurements in 17 Ostial Vertebral Arteries

Predilation	4
Balloon diameter, mm	3.8 ± 0.5
Reference vessel diameter, mm	4.0 ± 0.6
Lesion length, mm	8.2 ± 2.5
Initial diameter stenosis, %	81 ± 5.2
Stent diameter, mm	4.0 ± 0.5
Stent length, mm	11.6 ± 3.8
Residual stenosis, %	25.8 ±13.4

Table 3: Proportional Distribution of Degree of VAS before and after Stent Placement

	Degree of VAS			
	⩽25%	25% ～50%	50%～＜70%	⩾70%
Before stent placement	0	0	5	12
Immediately after stent placement	12	5	0	0
At 12-month follow-up	9	8	0	0

4. Discussion

The present study demonstrates the feasibility and relative safety of primary stenting for ostial vertebral artery stenosis using balloon expandable drug-eluting coronary stents exclusively. Ostial VA stenoses are highly elastic lesions that require stents with high radial force. Accurate positioning is also necessary for complete lesion coverage and avoidance of stent protrusion into the subclavian artery. The shape-recovery capability of self-expanding stents is very important in the mobile cervical carotid artery to avoid the stent displacement that has been reported with balloon-expandable stents.

Current experiences with angioplasty and stent placement in the treatment of extracranial vertebral stenosis have almost exclusively been based on the use of balloon-mount coronary stents. The rate of recurrent ischemic attacks after stent placement varied from 3.8% to 28.5% of patients for a mean follow-up period of 11 months to 20.7 months [11,12]. Restenosis of the vessel in which the stent was placed at angiographic follow-up is usually defined as a degree of luminal narrowing of at least 50% [12,13]. In a retrospective study, Gupta et al.[14] reported on the off-label use of drug-eluting coronary stents in the endovascular treatment of VA. However, the exact location of the lesions in the extracranial VA was not specified.

Our early results showed that both the rate of recurrent ischemic symptoms and the rate of vascular restenosis ＞50% stenosis were 0% at 12-month follow-up. Drug-eluting stents were considered a major medical advance when they first appeared. The drugs released by these stents reduce the risk of restenosis by limiting macrophage accumulation and smooth muscle cell proliferation around the stent. However, such actions also inhibit re-endothelialization of the stent surface, and investigators became concerned that persistent exposure of the stent could increase the risk of delayed stent thrombosis due to localized hypersensitivity and that delayed endothelialization might occur and lead to vascular restenosis at longer-term follow-up.[15]

Our study had some limitations, namely, the small number of patients and the short follow-up period. To evaluate the clinical value of drug-eluting stents as opposed to bare metal stents or medical therapy in the treatment of VA stenosis would require a randomized controlled trial of a much larger scale.

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Cultural Transition, Social Change, Democratic and Islamic Citizen Approaches on Social Training

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Abstract: In this paper we discuss three approaches of cultural transition, democratic citizen, and social change in social training. Some of training implications of the approaches are also mentioned. Islamic approach on social training is described and some considerable verses of Quran are also stated.
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Keywords: Cultural transition, democratic citizen, social change, social training

1. Introduction

Social training is one of the most significant aspects of training which have specific place in learning and training processes. Social training is not only one of the objectives of teaching and training, but also is one of the dominant principals of teaching and training (Asqarian, 1989). In another respect, it's one of the main responsibilities of teaching and training (Shariatmadari, 1987).

In addition to the significant role of social training in learning and training, we can also consider its important function in cultural, social and economic development, and put emphasis on its relationship with development process in every society (Edalat Nejad, 1994).

Social training depends on a clear image of the concept of socialization; based on what entity or characteristics are defined for socialization, we will have special methods and principals for social training. In contrast, any principal or social training methods are dependent on certain presuppositions of nature of socialization (Bagheri, 1995).

Social science and education experts have different orientations about the whole concept of training and consequently on social training. John Lock, for instance, describes the responsibility of teaching and training as educating individuals according to their socialization methods (Naqib Zadeh, 1996), or Durkheim defines the function of social training as methodical socialization of young generation (Durkheim, 1997), in another word, they believe that teaching and training has conservative role and function in maintenance and strengthening of the current situation. This approach can also be called cultural transition and Functionalism. In contrast to the above mentioned theories, we can consider conflict theory or critical theory of Paulo Freire. These theories consider teaching and training as a tool to create fundamental changes in the society. In another word, in this approach teaching and training demonstrates its transformative power in society by training critics and reformers (Abd elazeem, 2011). The other approach is Democratic Citizen. Central issue in this approach is developing group work knowledge, to enable individual to participate in a democratic society (Miller, 1983).

In this paper, we will explain three approaches of social transition, democratic citizen, and social changes in social training context and illustrate some training implications of these approaches. Afterward we will discuss Islamic approach on social training.

2. Methods

2.1. Social transition approach

This approach is one of the primitive social approaches. Based on this approach, the school should lead the students toward appropriate social ways. Some of sociologists believe that school make the students familiar with social culture and the roles students will play in the society. Emile Durkheim describes teaching and training as the way of internalization of social facts or social being, not emphasize on individual growth, called individual being. Social life becomes a moral authority after confirming in the individuals (Durkheim, 1956).

Talcott Parsons also defines teaching and training as cultural transition; he believes schools have dual role, one is to socialize, which describes social roles of people and expectations of the others from a person, and the other one is human source distribution institute, which pays attention to training man-power (Parsons, 1964, Hsiangchi, 2011).

Other sociologists, who believe on functional learning and training school, represent analysis similar to Parson's. Cultural anthropologist, Udi Cohen, for example, illustrates the effect of school on students. Cohen makes difference between socialization and learning and training. Socialization is learning different behaviors by dealing with parents, relatives and members of society. On the

other hand, teaching and training can transfer the values and skills in more standard ways (Cohen, 1971).

Cohen states: "the country needs loyalty of citizens, and acquires this loyalty by pledging to a national world view. Flags, pictures, singing national anthem in schools is used to incorporate with this national loyalty. Since schools are entities under control of government, the government teaches its values, biases and honors to country like other things to students (Cohen, 1971, pp. 41-42).

Recently, the idea of socializing students through schools attracts the attention. Neil Postman, for instance, follows this theory in his book "Teaching as Conserving Activity". He states: "schools must consider manner education, which is standards of civilized interaction requires its students to follow motivations and interests."

Postman changes his idea in the book "Teaching as Subversive Activity" and believes schools have repressive manner and prevents individual development. This view support programs such as technical and professional teaching and behavioral plans such as cultural transition (Postman, 1997).

2.2. Democratic Citizen Approach

Central subject of this approach is to develop group work knowledge to enable individual to participate in a democratic society.

One of the main defenders of this approach is James Shaver, first working with Don Oliver and Freed Newman. Shaver, in association with William Strong authored a book named "Facing Value Decisions: Rationale Building for Teachers" and represented an approach toward social decisions based on democratic perception (Shaver, 1976).

Shaver believes democracy is composed of basic values such as individual dignity, self-representation, intelligence, pluralism and society. Self-representation means that within a society, democratic people have selection right especially is what effects their life. Relying on people's intelligence is also significant in democracy. Teaching and training should rely on deciding power by investigating the truth by taking various value statuses into consideration. The other value of democracy is pluralism; it means to value various values and life styles. Sometimes these values and methods conflict with each other, and people should make critical decisions. All of the above mentioned values evolve in certain values such as freedom of speech, freedom of assembling, and equality of opportunities. The role of teacher is to transfer democratic values in a way to improve intelligence of the students (Scheffler, 1960).

One aspect of democratic citizen process is to analyze conflict values within the situation. American creed is being taught to students to generate their mental concepts to democratic citizen concept, for example, to relate their own justice concept with the justice of equal behavior of law with people. This is called Label Generalization (Miller, 1983, p 61).

Teaching citizenship in a democratic situation mostly considers Analysis of value conflict. Harvard public issue, for example, considers this issue by proposing different case studies about moral, legal and social issues (Donald, 1974).

Jon Eisenberg et al, in Canada, focused on a case study method. Conceptual framework of Canadian is different from Shaver approach. Canadian project has two main objectives:

1- Developing social understanding of Canadian society about important issues and conflicts.

2- Developing discussion skills and making necessary decision to confront moral issues (Miller, 1983, 64).

Some of the programs relates to critical issues of Quebec, foreign ownership, cultural differences, urbanism issues, strikes, women right, etc. (Miller, 1983, 65)

2.3. Social Change approach

In this approach, the role of school is to change the society. The approach itself has three other approaches include: Social Reconstruction, Social literary action, and Social action.

a) **Social Reconstruction:** George Counts is one of the main speakers of this approach. He believes that teachers should actively engage in social changes and volunteer in political responsibilities. In his book "Dare the schools build a new social order?" he states: "teachers should cooperate with other groups in developing social changes." Today, his theories are followed by defenders of social reconstruction. Theodore Brameled, for instance, wants teachers to play more important role in social changes. He introduces Ralph Nader and Saul Alinsky as teachers who struggle social injustice and inequality: Social reconstruction theory requires teachers to pioneer in social changes (Miller, 1983, 65-66).

b) **Social Literacy Action:** Paulo Freire has a significant role in necessity of interference of schools in social change approach. His first book "The Pedagogy of the Oppressed" deals with describing his theories about literacy of Brazilian farmers. He codified the method to teach basic literacy skills of Brazilian students. His method is based on explaining cruelty. Cruelty or exploitation prevents people growth to reach

human perfection. Using sources such as marks, punishment, etc. to control students is also cruel.

Alfred Alschuler states: "the origin of discipline conflicts in schools is teachers who behave cruelly with their students and don't consider them as human.

Freire explains stages that people can pass to overcome the cruelty. People, in the first stage, named magical conforming, are passive and don't feel oppression. In second stage, Naïve Reforming, problems are considered as individual issues and there is no need to concentrate on social aspects. In third stage, critical transforming, people analyze their own culture and play an active role in changing their status (Miller, 1983, 66-71).

c) **Social Activity:** the other approach in social approaches is Fred Newman's social activity model. This model is much closed to social changes ideal, since the model encourages students to involve in social activities. This model not only support participating in social activities, but also tries to make changes by political and social activities.

Newman doesn't only focus on the activity itself to overcome student's passivity, but the main objective is environmental ability. Environmental ability emphasizes on the activity that brings special environmental outcomes. Newman believes citizenship training mostly focuses on self-oriented activities such as personal value clearness than development of abilities. He believes achieving environmental ability should be recognized as one of the important objectives of the school, but other abilities should not be ignored (Miller, 1983, 71-77).

Training implications of cultural transition, democratic citizen and social change approaches

In introduction, we stated that after explaining the approaches we will refer to some of training implications, here we will discuss it in the following aspects: *Training objectives, learning, training process, learner, learning environment, teacher's role and evaluation*.

- **Cultural transition approach**

1- **Training objectives:** value induction and significant cultural roles
2- **Learning:** process of self-adaptation with expectations of school and society, it means the students learn whatever needed to fulfill expectations of the society.
3- **Learner:** learner is a passive person, because information and values transfer to him.
4- **Training process:** teacher controls training process. Training methods include practicing and repeating other direct methods.
5- **Learning environment:** personal environment to have the control of the teacher.
6- **Teacher's role:** teacher is the pure dominant person and is responsible to transfer knowledge, value and roles to students
7- **Evaluation:** evaluation is to clarifying knowledge and value transferring to students (Miller, 1983, 77-78)

- **Democratic citizen approach**

1- **Training objectives:** valuing democratic values, analyzing skills, value conflict analysis skills of speech skills and adopting role and knowledge of democratic processes.
2- **Learning concept:** include interactive process among learner and environment. Learning is done by involving in general problems and conflicts.
3- **Learner:** learner is the one who can use his intelligence in participating in general affairs.
4- **Training process:** a) teacher proposes case studies or general affairs; b) value conflicts are specified and real information is collected; c) students involve in different issues; d) teacher uses Socratic conversation to discover student's position in the issue. Teacher uses comparison to encourage students to state their ideas and reform it; e) student uses Socratic conversation to investigate the issues and may adapt new theories.
5- **Learning environment:** materials such as case study, movie and papers which focus on general issues are used.
6- **Teacher's role:** teacher should be responsible to different roles; first he should make a sentimental and supporting environment to prepare students to participate, then he should use Socratic methods and parables to investigate student's position. Teacher should be able to interact and analyze different social issues.
7- **Evaluation:** focuses on general conflict analysis and participating in political discussion skills. Evaluation is done about students skills in presenting oral and written ideas (Miller, 1983, 78-79)

- **Social change approach**

1- **Training objectives:** student's participation in social issues and development of effective skills on social changes.
2- **Learning concept:** relates to direct contact with social issues. Learning is done through social problem, social changes, and environmental interaction and trying to affect the interaction.
3- **Learner:** learner is the one who can actively involve in social changes. Learner is considered as a change factor.

4- **Training process:** a) student clarifies the problem; b) student experiences different practical methods; c) investigates different roles related to moral, social and legal functions; d) he selects one solution; e) he practices the solution in school and society; f) student analyzes the issue by investigating effectiveness of the project and clarifying what he has learned.
5- **Teacher's role:** teacher tries to make the student's activity clear. Makes necessary sources available. Teacher should participate in social issues and form necessary relations between school and society.
6- **Evaluation:** it is done in different dimensions. Student's writing assesses the knowledge of a special case. It can be student's observation during his work (Miller, 1983, 79-80).

Comparing cultural transition, democratic citizen and social change approaches

When comparing these three approaches, we first discuss the main point of each approach. It is evident that democratic citizen and social change approaches are more conform to democratic society. Cultural transition has a passive approach toward students that is appropriate for absolute societies, but teaching some of the values and cultures is necessary even in free countries. Key point in orienting democratic citizen approach is freedom and selection right of students to analyze the issues.

Student's learning in democratic citizen and social change approach is different with each other. Democratic citizen focuses on cognitive skills and oral interactions. Social changes approach goes beyond oral interaction and focuses on student's participation in social and school issues. Although there is political analysis in class activities, the focus of main social change is on immediate environmental change of school (in social literary approach) or on society (social approach). Cultural transition and social change approaches are against each other, democratic citizen approach is in between of these approaches (Miller, 1983, 80-81).

Islamic approach on social training

At first it must be mentioned that in every training system, human is described as the base, since every element of training system including concepts, objectives, principals, levels and training methods observes human's status. In this regard Quran defines human as a creature with spirit, ego, nature, intelligence, will, authority, group identity and limitations and deficiencies. In the other word, analytic view of Quran about human is based on above mentioned anthropological resources, each of which should be analyzed separately (Baqeri, 1995). First, it must be mentioned that human is from God and return to God (Baqare, 156), it means, nature, entity, thoughts and deeds of individual in personal and social concept, should be analyzed in the context of origin and destination. Second, in a structural view by compiling above mentioned anthropological resources, it can be stated that human is described in Quran in a way with deep inside knowledge and appeal for God (mould), or having recognition (wisdom) and emotional (heart) factors, with owning power to determine its wills (authority and will), with group impressible and impressing the groups, with limitations and deficiencies from birth, with passion that will provoke in him. The significant aspect of human will appear in his endeavor and function (Bagheri, 1995). Third, the object of Islamic teaching and training, away from different objectives that are mentioned, is worship, the main aim of creation. Forth, human need to communicate with others because it's impossible to obviate needs alone. In one hand, tendency to obviate the needs and in the other hand tendency to eminence, are two strong motivations that make him to communicate in different levels. Furthermore, human has social responsibility along with personal responsibilities. In this regard, Quran states:

لكل امه اجل اذا جاء اجلهم فلا يستاخرون ساعه و لا يستقدمون (Yunus, 49).

For every nation there is an appointed time, when their time cometh, then they cannot put it off an hour, nor hasten (it).

Or it says: ان الله لا يغير مابقوم حق يغيروا ما بانفسهم (Al-Ra'd, 11).

Lo! Allah changed not the condition of a folk until they (first) change that which is on their hearts.

We can summarize that Quran believes human is a creatures in the path of origin and destination, have endeavor and justice, ultimate objectives like achieving God, wisdom, authority and social responsibilities. This believe about human observes an objective, conscious and practical training. In this regard we can say that Islamic training in general and in social and political concept should have religious and divine orientation, and should also be free, consciously and voluntary, since Quran disavows following those "whereof thou has not knowledge" (Al-Isra, 36) and focuses on authority of the people (Al-Kahf, 30). Also, the result of training should be represented in behavior of the people, as the consequent of all his endeavors (Marzoughi, 1377).

In this part we represent a group of training and prophecy principals of prophets related to social and individual issues. These principals constitute the base of prophet's invitation and their prophecy.

So these principals are scale for recognizing real prophecies and use them for recognition of bad and

good. They also present theoretical and practical basis of social training.

A- Establishing justice among people

* لقد ارسلنا رسلنا بالبينات، و انزلنا معهم الكتاب و الميزان، ليقوم الناس بالقسط (Al-Hadid, 25)

We verily sent our messengers with clear proofs, and revealed with them the Scripture and the Balance, than mankind may observe right measure

* يا ايهاالذين آمنوا كونوا قوامين بالقسط (Al-Nisa, 135)

O ye who believe! Be ye staunch in justice.

* قل امر ربى بالقسط (Al-A'raf, 29)

Says: My Lord enjoineth justice

* ... و اوفوالكيل و الميزان بالقسط (Al-An'am, 152)

Give full measure and full weight, in justice

* ... و ان حكمت فاحكم بينهم بالقسط، ان الله يحب المقسطين (Al-Ma'idah, 42)

But if thou judgest, judge between them with equality. Lo! Allah loveth the equitable. The most significant principals that messengers invite people to them and effort to strengthening its base are justice and just among people. In every issue of the life, what is clearly understood, according to Quran, is that justice and just among people are the main principal and the only objective of sending messengers and holy Books.

Therefore, a society cannot be called Islamic unless justice dominates all social issues and plans and its orientation (Hakimi, 1989).

B- Social justice

* ان الله يامر بالعدل و الاحسان ... (Al-Nahl, 90).

Lo! Allah enjoineth justice and kindness

* فلذلک فارع و استقم كما امرت و لا تتبع اهواء هم و قل: امنت بما انزل الله من كتاب، و امرت لا عدل بينكم ... (Ash-Shura, 15)

Unto this, then, summon and be thou upright as thou art commanded, and follow not their lusts, but say: I believe in whatever Scripture Allah hath sent down, and I am commanded to be just among you

* يا ايها الذين آمنوا كونوا قوامين الله، شهداء بالقسط، و لا يجرمنكم شنان قوم على ان لا تعدلوا، اعدلوا هو اقرب للتقوى. (Al-Ma'idah, 8).

O ye who believe! Be steadfast witness for Allah in equity, and let not hatred of any people seduce you that ye deal not justly. Deal justly, that is nearer to your duty.

* ان الله يامركم ان تودوا الامانات الى اهلها، و اذا حكمتم بين الناس ان تحكمو بالعدل ... (An-Nisa, 58).

Lo! Allah commandeth you that ye restore deposits to their owners, and, if ye judge between mankind, that ye judge justly…

* و ان خفتم ان لا تقسطوا فى اليتامى، فانكحوا ما طاب لكم من النساء مثنى و ثلاث و رباع، فان خفتم ان لا تعدلوا فواحده (An-Nisa, 3).

And if ye fear that ye will not deal fairly by the orphans, marry of the women, who seem good to you, two or three or four; and if ye fear that ye cannot do justice (to so many) then one.

Inviting to deal social justice is another important privilege of messenger's duty. Continuous effort to expand justice and make it practical among people and expanding social justice in human society, and to make people to accept justice is the most significant base that every creature depends on. Everyone who knows little about religious thought can recognize that Islam depends highly on social justice, and straight path is paced with it. Therefore, if there is no justice, there is no religion, and if social justice doesn't work and expanded, religious duty will not be satisfied (Hakimi, 1989).

C- Rescuing mankind

* الذين يتبعون الرسول النبى الامى الذى يجدونه مكتوبا عندهم فى التوراه و النجيل، يامرهم بالمعروف و ينهاهم عن المنكر و يحل لهم الطيبات، و يحرم عليهم الخبائث و يضع عنهم اصرهم و الاغلال التى كانت عليهم، فالذين آمنوا به عزروه و نصروه اتبعوا النور الذى انزل معه، اولئک هم المفلحون (Al-A'raf, 157).

Those who follow the messenger, the Prophet who can neither read nor write, whom they will find described in the Torah and the Gospel with them. He will enjoin on them that which is right and forbid them that which is wrong. He will make lawful for them all good things and prohibit for them only the foul; and he will relieve them of their burden and the fetters that they used to wear. Then those who believe in him, and honor him and help him, and follow the light which is sent down with him, they are the successful.

* و اذكروا اذا انتم قليل مستضعفون فى الارض، تخافون ان يتخطفكم الناس، فاواكم و ايدكم بنصره، و رزقكم من الطيبات، لعلكم تشكرون (Al-Anfal, 26).

And remember, when ye were few and reckoned feeble in the land, and were in fear lest men should extirpate you, how He gave you refuge, and strengthened you with His help, and made provision of good things for you, that haply ye might be thankful.

* قل يا اهل الكتاب تعالوا الى كلمه سواء بيننا و بينكم، الا نعبد الا الله و لا نشرک به شيئا، و لا يتخذ بعضنا بعضا اربابا من دون الله، فان تولوا فقولوا اشهدوا بانا مسلمون (Al-Imran, 64).

Say: O People of the Scripture. Come to an agreement between us and you: that we shall worship none but Allah, and that we shall ascribe no partners unto Him, and that none of us shall take others for lords beside Allah. And if they turn away, then say: Bear witness that we are they who have surrendered (unto Him).

*و ما لكم لا تقاتلون فى سبيل الله، و المستضعفين من الرجال و النساء و الولدان؟ الذين يقولون: ربنا اخرجنا من هذه القريه الظالم اهلها، و اجعل لنا من لدنک وليا و اجعل لنا من لدنک نصيرا (An-Nisa, 75).

Bring us forth out from this town of which the people are oppressors! Oh, give us from Thy presence some protecting friend! Oh, give us from Thy presence some defender!

*و لقد بعثنا فی کل امه رسولاً، ان اعبدالله و اجتنبوا الطاغوت، فمنهم من هدی الله و منهم من حقت علیه الضلاله، فسیروا فی الارض فانظروا کیف کان عاقبه المکذبین (An-Nahl, 36).

And verily we have raised in every nation a messenger, (proclaiming): Serve Allah and shun false gods. Then some of them whom Allah guided and some of them (there were) upon whom error had just held. Do but travel in the land and see the nature of the consequence for the deniers!

*ما کان لبشر ان یوتیه الله الکتاب و الحکم و النبوه ثم یقول للناس، کونوا عبادا لی من دون الله، و لکن کونوا ربانیین، بما کنتم تعلمون الکتاب و بما کنتم تدرسون. و لا یامرکم ان تتخذوا الملائکه و النبیین اربابا، ایامرکم بالکفر بعد اذ انتم مسملون (Al-Imran, 79-80)

It is not for any human being unto whom Allah had given the Scripture and wisdom and the Prophethood that he should afterwards have said unto mankind: Be slaves of me instead of Allah; but be ye faithful servants of the Lord by virtue of your constant teaching of the Scripture and of your constant study thereof. And he commanded you not that ye should take the angels and the Prophets for lords. Would he command you to disbelieve after ye had surrendered (to Allah).

*و تلک عاد جحدوا بآیات ربهم و عصوا رسله و اتبعوا کل جبار عنید. و اتبعوا فی هذا الدنیا لعنه و یوم القیامه، الا ان عادا کفروا ربهم، الا بعدا لعاد قوم هود (Hud, 59-60).

And such were Aad. They denied the revelations of their Lord and flouted His messengers and followed the command of every forward potentate. And a curse was made to follow them in the world and on the Day of Resurrection. Lo! Aad disbelieved in their Lord. A far removal for Aad, the folk of Hud!

* و تلک نعمه تمنها علی ان عبدت بنی اسرائیل (Ash-shu'ara, 22).

And this is the past favor wherewith thou reproachest me: that thou hast enslaved the Children of Israel.

* و لقد فتنا قبلهم قوم فرعوم و جائهم رسول کریم. ان ادوا الی عبادالله، انی لکم رسول امین (Ad-Dukhan, 17-18).

And verily We tried before them Pharaoh's folk, when there came unto them a noble messenger, saying: Give up to me the slaves of Allah. Lo! I am a faithful messenger unto you.

* اذهب انت و اخوک بآیاتی و لا تنیا فی ذکری، اذهبا الی فرعون انه طغی ... فایتاه فقولا، انا رسولا ربک، فارسل معنا بنی اسرائیل و لا تغذبهم ... (Ta-Ha, 42-43, 47).

Go, thou and thy brother, with My tokens, and be not faint in remembrance of Me. Go, both of you, unto Pharaoh. Lo! He hath transgressed… so go ye unto him and say: Lo! We are two messengers of thy Lord. So let the Children of Israel go with us, and torment them not.

Prophets employ their entire effort to do this, and make it their goal, because they are sent to free people from misery and calamity, and free them from slave of the others by worshiping God, to acquire their own freedom and not to obey any oppressors and know that he is mankind that has human munificence and freedom and is equal with other people (Hakimi, 1989).

D- Moral and living development

* ربنا و ابعث فیهم رسولا منهم، یتلوا علیهم آیاتک و یعلمهم الکتاب و الحکمه و یزکیهم، انک انت العزیز الحکیم (Al-Baqarah, 129).

Our Lord! and raise up in their midst a messenger from among them who shall recite unto them Thy revelations, and shall instruct them in the Scripture and in wisdom and shall make them grow. Lo! Thou, only Thou, art the Mighty, Wise.

* رسولا یتلوا علیکم آیات الله مبینات، لیخرج الذین آمنوا و عملوا الصالحات من الظلمات الی النور (At-Talaq, 11)

A messenger reciting unto you the revelations of Allah made plain, that He may bring forth those who believe and do good works from darkness unto light.

* و لقد آتینا موسی و هارون الفرقان و ضیاء و ذکر للمتقین (Al-Anbiya, 48).

And we verily gave Moses and Aaron the Criterion and light and a Reader for those who keep from evil

* هو الذی بعث فی الامیین رسولا منهم، یتلوا علیهم آیاته و یزکیهم و یعلمهم الکتاب و الحکمه و ان کانوا من قبل لفی ظلال مبین (Al-Jumu'ah, 2).

He it is who hath sent among the unlettered ones a messenger of their own, to recite unto them His revelations and to make them grow, and to teach them the Scripture and Wisdom, though heretofore they were indeed in error manifest.

* و لقد آتینا بنی اسرائیل الکتاب و الحکم و النبوه، و رزقناهم من الطیبات و فظلنا هم علی العالمین (Al-Jathiya, 16).

And verily we gave the Children of Israel the Scripture and the Command and the Prophethood, and provided them with good things and favored them above all people.

* فاذا قضیبت الصلاه، فانتشروا فی الارض و ابتغوا من فضل الله. (Al-Jumu'ah, 10).

And when the pray is ended, then disperse in the land and seek of Allah's bounty.

* و لقد بوانا بنی اسرائیل مبوا صدق، و رزقناهم من الطیبات (Yunus, 93).

And we verify did allot unto the Children of Israel a fixed abode, and did provide them with good things.

The domain of Prophet's activity is not limited only to spiritual and intellectual concepts, but also they consider to enhance life quality and at the same time spirit, because their trainings both included heartily believes and obvious truth. Prophecy school doesn't just pay attention to teach beliefs and wisdom to

people without trying to improve their life quality and strengthening their social relations, since this is incomplete and has no general effect on training. It's obvious that evolutionary movement of mankind and achieving God is done only by cooperation of body and mind, and this is achievable through good life and good social system, which is the aim of prophets.

E- Equality and brotherhood among people

* انما المومنون اخوه، فاصلحوا بين اخويكم، و اتقوا الله لعلكم ترحمون. (Al-Hujurat, 10).

The believers are naught else than brothers. Therefore make peace between your brethren and observe your duty to Allah that haply ye may obtain mercy.

* و اعتصموا بحبل الله جميعاً و لا تفرقوا، و اذكروا نعمه الله عليكم، اذ كنتم اعداء فالف بين قلوبكم، فاصبحتم بنعمته اخوانا (Al-Imran, 103).

And hold fast, all of you together, to the cable of Allah, and do not separate. And remember Allah's favor unto you: how ye were enemies and He made friendship between your hearts so that ye became as brothers by His grace

* و لا تكونوا كالذين تفرقوا و اختلفوا من بعد ما جاء هم البينات (Al-Imran, 105).

And be ye not as those who separated and disputed after the clear proofs had come unto them.

* و ان هذا صراطى مستقيماً فاتبعوه و لا تتبعوا السبل فتفرق بكم عن سبيله ذلكم و صاكم به لعلكم تتقون (Al-An'am, 153).

And this is my straight path, so follow it, follow no other ways, lest ye be parted from His way, this hath He ordained for you, that ye may ward off evil. One of the most important trainings of prophets is to create equality among people and inspiring brotherhood in society. They taught people that everybody is brother of the other people, and everyone is equal to others in their life rights, and every people from everywhere and every skin color are as a family member.

F- Grading improvement paths

* فقال الملاء الذين كفروا من قومه: ما نراک الا بشرا مثلنا، و ما نراک اتبعک الا الذين هم اراذلنا بادى الراى، و ما نراى لكم علينا من فضل بل نظنكم كاذبين. قال: يا قوم ارايتم ان كنت على بينه من ربى و انانى رحمه من عنده، فعميت عليكم، انلزمكموها و انتم لها كارهون؟ و يا قوم لا اسالكم عليه ما لا ان اجرى الا على الله، و ما انا بطارد الذين آمنوا، انهم ملاقوا ربهم و لكنى اراكم قوما تجهلون (Hud, 27-29).

The chieftains of his folk, who disbelieved, said we see thee but a mortal like us, and we see not that any follow thee save the most abject among us, without reflection. We behold in you no merit above us nay, we deem you liars. He said: O my people! Bethink you, if I rely on a clear proof from my Lord and there hath come unto me a mercy from His presence, and it hath been made obscure to you, can we compel you to accept it when ye are averse thereto? And O my people! I ask of you no wealth therefor. My reward is the concern only of Allah, and I am not going to thrust away those who believe. Lo! They have to meet their Lord but I see you a folk that are ignorant.

* فاما عاد فاستكبروا فى الارض بغير الحق و قالوا: من اشد منا قوه؟ اولم يروا ان الله الذى خلقهم هوا شد منم قوه؟ و كانوا باياتنا يجحدون (Fussilat, 15)

As for Aad, they were arrogant in the land without right, and they said: who is mightier than us in power? Could they not see that Allah who created the, He was mightier than them in power? And they denied Our revelations.

It's obvious that tyrants and arrogant didn't value any munificence and were considered as the main obstacle of law and accomplishment. They were oppressor, treacherous, and ravage people's wealth. They kill the boys and keep the girls alive and hang people on dates. Devilry, treachery, poverty, and ignorance are originated from them and return to them. Also it becomes clear that prophets are sent to reform human societies, remove destructions and free people from slave of cruel. Therefore, prophets were considered as the main danger for them, since the messengers were defending oppressed, standing against tyrants and aggressive, and grading the path to create social reformation and spread justice and establish human prestige (Hakimi, 1989).

According to above mentioned principles it is characterized that Quran has a deep and general view on human and society and avoids limiting to specific aspects.

3. Conclusion

In this paper we review four approaches on social training. In comparison with the other approaches, cultural transition approach prescribes submission. In another word, this approach considers unilateral communication of parents or preceptors with children. What happens to social training is providing solutions to form the children, in order to make them adaptable with social environment (Bagheri, 1995).

Contrary to this approach is social change approach which considers teaching and training as a mean to make fundamental social-political changes by informing deprived and oppressed people, and believes that teaching and training should be evolutionary power by growing up social critics. In another word, it signifies disharmony with current social order.

Democratic citizen approach, compare to two extravagant and wastage approaches, offers more balanced view and considers school as a place of

improving and training critical thinking, group work and other life-related skill in a democratic society.

The logic of Quran about social training is that prophets were instructors who had responsibilities. By expressing these responsibilities, we can perceive a Quran- based training system. The main concepts of this duty related to social and human issues include:

1) Establishing justice among people
2) Social justice
3) Making people free
4) Moral and living reformation
5) Equality and brotherhood among people
6) Grading improvement

In fact, what we mentioned here about Quran was a concise view on Quran; the other aspects of it can be characterized by deep thinking.

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Determining the Amount of Glucose, PTT, TT and Olfactory Nerve in 30-day-old Babies Rabbit of pregnant under Hypoxia

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Abstract: This research aimed at investigating the impact of hypoxia in embryonic period on the density of plasma glucose, PTT and TT and also the effect of disorder of olfactory nerve system on glucose, PTT and TT in baby rabbits. The research (which was carried out during 2009-2011) was based on an experimental design and used two groups: an experimental group and a control group including pregnant rabbits. The findings of the study indicated that embryonic period hypoxia and disorders of olfactory nerve system decreased the amount of glucose, PTT and TT and there was a meaningful difference between newborn babies in experimental and control groups. [Abdi, B., Aliyev, A., Qaziyev, A., Chekaniazar S. **Determining the Amount of Glucose, PTT, TT and Olfactory Nerve in 30-day-old Babies Rabbit of pregnant under Hypoxia.** Life Science Journal. 2011; 8(4):390-393] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: hypoxia, olfactory nerve, glucose, PTT, TT

1. Introduction

In their environment, living things are faced with great problems like hypoxia. Hypoxia causes disorders in structure and metabolism of normal cells. Breathing problems, cardiovascular and central nerve system disorders, bronchitis, asthma, jcerebralapoplexy, blood pressure are among the problems in initiated by hypoxia (king, 1985). As we know, embryo needs some special conditions in order to grow in mother's womb however; changes in the system of outer environments can result in fundamental problems in the growth of embryo in long term. It is also well known that long term hypoxia condition in pregnant animals can result in atrophy in the breathing system of babies given birth by these animals. External factors which result in hypoxia can create problems among babies in both birth and post-birth period (Giussani, 2007). Scientific findings show that embryos which have suffered from hypoxia for short or long time in their growth period in mothers, womb may also be affected by pathological conditions in their organs in post-birth growth period which can result in incurable diseases (30-40 percent mortality rate has been reported in this case) (Lig, 2003).

In order to prevent cellular hypoxia, the best way is to use O_3 in treatments. It is worth mentioning that the fact that we can breathe easily and relax after a rainy weather is related to the abundance of O_3 in the air which enters in O_3 form into the alveolus and in O_2 form into vascular system and cells. That is why O_3 therapy apparatuses are used in O_2-saturation of blood. O_3 derived from vascular system enters cells in O_2 form and at that time the person feels relaxed. Within 3 minutes hypoxia results in unrecoverable cardiovascular problems, headaches start, memory declines, sleep rhythm is disturbed and diabetic risk increases (kaur, 2006). Hypoxia usually initially appears in daytime activities and sleeping time. The halt of breathing results in the decline of blood oxygen which often happens in sleeping time. Due to the negligence of patient of their own problems and conditions, the doctors are not usually able to offer suitable treatments (Lyer, 1998).In this study, in addition to hypoxia in pregnant rabbits; in different stages olfactory nerve system of newborn baby rabbits has been investigated. The experiments have indicated that the cutting off of epithelium of newborn babies in experimental group results in the decrease of glucose, PTT and TT. In the process of research the newborn babies of the experimental group had been exposed to hypoxia. The experiment showed that there was a meaningful difference between the babies in control and experimental group regarding the decrease of the amount of glucose, PTT and TT. This decrease was more remarkable in experimental group. The findings of the present study are in line with the findings of other studies on olfactory nerve system. This can somehow be related to issues like air pollution and environmental control. Breathing the ingression of gases and materials from nostrils can result in the dangerous spread of these materials in sensory cells (Mucigant, 2006).

2. Material and methods

Healthy pregnant New Zealand rabbits were divided into four groups. Three female rabbit which was breathing natural air was used as the control, and the others-sample rabbits-were exposed to a daily 20-minute period of low oxygen: 10 days during 30 days of pregnancy for the first rabbit (day 1-10) 10 days during 30 days of pregnancy for the second rabbit (day 11-20) and 10 days during 30 days of pregnancy for the third rabbit (day 21-30).

- 7% O2 and 93% N2 compressed in a balloon were transferred into a non-toxic box.
- 7% oxygen was observed with oximeter pulse.

Within 10 days, sample pregnant rabbits breathed the rest of natural air after a 20-minute hypoxia, and new-born rabbits grew in natural air for 30 days. On the 31 day of their life, blood sampling was carried out and the amount of glucose, PTT and TT was determined (method of disabling Olfactory Nerves System (Epithelium): benumbing with deep lidocaine using Rhenoscope method). All samples were kept under supervision and care for four days due to the decision of the cultural committee. On the fifth day all blood samples (3 cc) from ear part were collected and then glucose from serum enzyme condition, PTT with German ORTO kit and TT with French STAGO kit were determined. All ethical issues were observed in this experiment.

2.1. Statistical –Analysis

One-way ANOVA analysis shows that there is a significant difference for glucose pretest-posttest, thrombin pretest-posttest and prothrombin pretest-posttest groups at a'=0.05 significance level.

2. Results

Also, Multiple Comparisons Dunnett (2-sided) shows that at a'=0.05 significance level there is a significant difference in glucose pretest and prothrombin pretest of first ten day group with control, second ten day group with control and third ten day group with control ($p<0.005$).

There is not a significance difference in glucose posttest of first ten day group with control at a'=0.05 significance level ($p=0.143$).but there is a significant difference in second ten day group with control and third ten day group with control at a'=0.05 significance level ($p<0.005$).

There is not significance difference in pretest thrombin of first ten day group with control at a'=0.05 significance level ($p=0.118$).but there is a significant difference in second ten day group with control and third ten day group with control at a'=0.05 significance level ($p<0.005$).

There is a significance difference in posttest thrombin of first ten day group with control and second ten day group with control at a'=0.05 significance level ($p<0.005$). But there is no significant difference in third ten day group a'=0.05 significance level ($p<0.005$).

There is no significant difference in posttest prothrombin of first ten day group with control at a'=0.05 significance level ($p=0.214$). but there is a significance difference in second ten day group with control and third ten day group with control at a'=0.05 significance level ($p<0.005$).

Table1. Descriptive Statistics for Glucose, PTT, TT

Traits \ Treatments		Control	1 – 10	11 – 20	21 -- 30
Glucose	Pretest	153 ± 8.767	133 ± 5.874	118 ± 5.874	132 ± 13.820
	Posttest	138.25 ± 14.584	125 ± 7.106	110 ± 7.071	105.5 ± 12.014
Prothrombin	Pretest	15.13 ± 1.126	12.6 ± 0.548	11.6 ± 0.548	11.5 ± 0.577
	Posttest	13.75 ± 1.035	12.4 ± 0.548	10.6 ± 0.548	10.25 ± 2.630
Thrombin	Pretest	30 ± 0.926	29 ± 0.707	27 ± 0.707	28 ± 0.816
	Posttest	1.488 ± 0.526	20 ± 1.581	18 ± 0.707	1.258 ± 0.629

3. Discussion

We carried out the research on the embryo in the sample rabbits' uterus under hypoxia in different time periods such as (1-10 days), (11-20 days) and (21-30 days). It was observed that newborn rabbits of these periods grew up 30 days at normal conditions. In comparison with newborn rabbits of control group that were not under hypoxia and had normal embryonic period and 30 days growth, we found some variations. For example, the rate of Glucose, PTT and TT in sample group's blood was less than that of control group. Experimental research on 30-day-old newborn rabbits among four samples and control group, after cutting the olfactory nerve, showed that Glucose, PTT and TT rate in sample rabbits was less than that of control ones. In fact, from physiological point of view, statistical analysis of experimental studies indicates that insufficiency of

hypoxia and olfactory nerve led to chronic and incurable diseases and also directly showed the secretion time of clotting on liver. Moreover the findings revealed that reduction of physical activities can have short time effect on thrombin in different tissues. Hilberg et al. (2003) found that maximal short term exercise will not activate blood clotting in healthy youth sample, Of course, there were intangible variations at normal rates. The current study suggests that PTT reduced instantly after taking exercise (Hilberg, 2005). However, contrasting results were obtained concerning the effect of exercise on PTT and TT. In general, most studies do not display noticeable effect on PTT, while some investigations were indicator of significant reduction on TT (Smith, 2003). Swimming activates clotting system through fiberinolystic activity (Lins, 2003) It is widely believed that long-term physical activates reduce TT compared to short-term activities. It is worth noting that the effect of muscular exercise on the clotting of blood can be regarded as diverse and wide-ranging areas of research on human being and laboratory animals. The results of such studies were relatively indicative of instant increasing of clotting after muscular activities. Some investigation also indicates the effect of heavy activities on blood clotting. Rebio et.al found that boring exercise in adults reduces relative time of activated TT (Riberiro, 2007). Furthermore, it is evident that heavy and boring activities resulted in variations of blood clotting and fiberinolysis (Ferguson, 1987). In this light, Hilberg et.al observed that maximal activities in 90 seconds were not able to simulate the clotting of blood. As a result, we conclude that hypoxia has a significant effect on PTT and TT rates in newborn rabbits. In addition, physical activities reduce TT in different tissues. Taking into account the previous studies in which rabbits' activities (aerobic) produced hypoxia in sample group and thus made noticeable variations in clotting times, I show that hypoxia causes meaningful variations in Glucose, PTT and TT rate. These findings are consistent with previous studies.

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Provably Secure Password-based Three-party Key Exchange Protocol with Computation Efficiency

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Abstract: Going along with the rapid development of web technologies, people can make a great quantity of service requests to service providers using mobile devices anytime and anywhere. However, the service requester and the service providers may not trust each other and they may locate at different domain. They require a communal trusted third party to help them establish a shared session key for secure communications. It is so-called three-party key exchange. Recently, many password-based three-party key exchange protocols were proposed against various well-known security threats. In those protocols, to prevent the password guessing attack, a widely used way is to employ public-key and/or symmetric-key cryptosystems to protect the exchanged messages. As we known, the encrypted and decrypted operations in a public-key cryptosystem are time-consuming. In this paper, we propose a password-based three-party key exchange protocol with the computation-efficiency without using public-key systems. Finally, we prove the security of the proposed protocol in the random oracle model.

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Keywords: cryptography; discrete logarithm problem; on-line undetectable password guessing attack; three-party key exchange.

1. Introduction

Today, people have many opportunities to obtain services or resources from application servers by using their mobile devices through the Internet. However, both of the clients and the servers may be distributed over different network domains and do not win the trust each other. A secure mechanism has to make sure that the identity of the clients and the server can be authenticated each other and the communications are secure against an unauthorized user from eavesdropping the delivery contents[1-2,5]. The client and the application server require a 3. communal trusted third party[3-4,17].

Password is widely employed to construct a secure key exchange protocol since password-based protocols are easily to be developed and to be maintained. However, users have to worry about whether their passwords (have low entropies) have been guessed or not. The password guessing attacks can be divided into three kinds[11-12]:

1. **On-line detectable guessing attack.** Attacker can enumerate all the candidature passwords and pick up one from the list. Then the attacker sends the chosen password to connect the server and verifies the server's response in on-line. Most password-based protocols can prevent this attack by the server limits the fail times.
2. **On-line undetectable guessing attack.** Attacker can enumerate all the candidature passwords and pick up one from the list. Then the attacker sends the chosen password to connect the server and verifies the server's response in on-line. Since the server cannot discriminate whether the request is malicious or honest, therefore the server always replies a honest response. The attacker can catch this chance to guess the password until the password is correctly obtained[23].

Off-line guessing attack. Since the communicated channel is open, any eavesdropper can collect all the communications. Then the attacker can enumerate all the candidature passwords to launch the attack off-line until a hit is obtained without the help of the server.

Many password-based three-party key exchange protocols were proposed and addressed to overcome the above guessing attacks by using the concept of public-key and symmetric-key techniques[10-11,19-20,26]. For enhancing the efficiency dramatically, in 2007, Lu and Cao proposed a simple three-party key exchange protocol[21] without using the server's public key. Unfortunately, Lu-Cao's key exchange protocol suffered from the unknown key sharing[1], the on-line undetectable guessing, and the impersonation

attacks[12,15,18,23]. For guaranteeing the quality of communication services, low communication and computation cost is required in a three-party key exchange protocol. In 2009, Huang[16] proposed an efficiency-enhanced password-based three-party key exchange protocol. Huang claimed that the proposed protocol is also more efficient than Lu-Cao's protocol and can be applied in practice. However, Huang's protocol is still not secure against the on-line undetectable guessing attack[25].

We propose a provably secure password-based three-party key exchange protocol to withstand various well-known security threats by using the random oracle model[3,11,22]. Compared with the related protocols [10-11,20], our proposed protocol is computation-efficient.

In the next section, we first give a notation of security. In Section 3, we propose a novel three-party key exchange protocol. In Section 4, we analyze the security of the proposed protocol. In Section 5, we analyze the efficiency among our proposed protocol and the related protocols.

1 An unknown key-sharing attack on a key Finally, we conclude this paper in Section 6.exchange protocol which provides the key confirmation property is an attack whereby an entity A believes that she shares a session key with the communicated entity B. Unfortunately, it is fact that if the entity B mistakenly believes that the session key is instead shared with another entity E, where $E \neq A$. A secure key exchange protocol should be against this threat[6,8].

2. Notations of Security

We first define some hard mathematical problems and security of a password-based three-party protocol.

2.1 Hard Problems

1. **Definition 1. Discrete Logarithm Problem (DLP).** Given two elements g and g^a, it is computationally infeasible to find a, where p is a large prime number, g is a generator with order q in 2. $GF(p)$ and $a \in Z_q^*$.

2. **Definition 2. Computational Diffie-Hellman Problem (CDHP).** Given three elements g, g^a, and g^b, it is computationally infeasible to calculate g^{ab}, where p is a large prime number, g is a generator 3. with order q in $GF(p)$ and both of a and $b \in Z_q^*$.

3. **Definition 3. Decisional Diffie-Hellman Problem (DDHP).** Given four elements g, g^a, g^b, and g^c, it is difficult to decide whether c mod q is equal 4. ab mod q, where p is a large prime number, g is a generator with order q in $GF(p)$ and all of a, b and $c \in Z_q^*$.

2.2 Security Definitions

The concrete security of a three party-based protocol is built up both the property of the session key indistinguishability and the protection of the password[7,22]. In a password-based protocol, an on-line detectable guessing attack[14] is inherent and is inevitable. However, this attack can be prevented by locking the account after some reasonable failed attempts in most password-based protocols. A more dangerous attack is the off-line guessing attack after an adversary copies a transcript of executions in a password-based protocol. The mission of a password-based protocol is to rule out the off-line guessing attack and to limit the adversary only to the on-line detectable guessing attack. For thwarting the online detectable guessing attack, the service requesters' requests are required to be authenticated for the operations of the trusted server from distinguishing malicious attempts from real requests. Also, for deterring the on-line undetectable and the off-line guessing attacks, the proposed protocol has to live up to the requirement of attackers that they may pick up the correct password but cannot verify their guessing from the eavesdropped messages.

We denote the proposed protocol, a service requester C_A and a service provider $C_B \in \hat{C} = \{C_1, ..., C_{NC}\}$ and a trusted server S. Each service requester C_A and a service provider $C_B \in \hat{C}$ hold memorial passwords pw_A and pw_B, and the server S maintains a password table $<P_1, ..., P_{NC}>$. We also assume that an adversary AD who controls all the communications that take place by C_A^i, C_B^j and S is a probabilistic machine, where we denote that C_A^i is the ith instance of the service requester C_A and C_B^j is the jth instance of the service provider C_B. AD can interact with all the participants (C_A, C_B, S) through the following oracle queries.

1. Execute(C_A^i, C_B^j), Execute(C_A^i, S), Execute(C_B^j, S): We use this query to model passive attacks where an attacker can eavesdrop all the communications between the instances (C_A^i, C_B^j) and between the instances (C_A^i, S), and (C_B^j, S) respectively.

2. SendClient(C_A^i, m): We use this query to model an active attack against that the attacker sends a message m to a participant C_A at the ith instance. Then query outputs the result of C_A from receiving the message m to generate.

3. SendServer(m): We use this query to model an active attack against that the attacker sends a message m to the server S. Then query outputs the result of S from receiving the message m to generate.

4. Reveal(C_A^i): We use this query to model an active attack against the known-key attack at the ith instance C_A. The query says that if the instance does not accept the session key, the output is $\perp$; otherwise, the output is the real session key.

5. Corrupt(C_A): We use this query to allow that an attacker AD can corrupt the complete internal state of an entity C_A.
6. Test(C_A^i): If an attacker AD queries this oracle and no session key for $C_A^i \in \hat{C}$ is accepted, this oracle outputs $\perp$; otherwise, the oracle flips a coin b. If $b = 1$, returns the real session key; if $b = 0$; returns a random key which has the same key with the real session key.

The security definition of the proposed protocol depends on the partnership and freshness of oracles, where the partnership of the oracles is defined using the session identifiers *sids* and the partnership is defined to restrict the adversary's Reveal and Corrupt queries. If the partnership is not accepted by the oracles, the adversary is trying to guess the session key.

1. Partnership: We say that two oracles C_A^i and C_B^j are partners, if and only if both of the oracles have accepted the same session key with the same session identifier and they have agreed on the same set of exchanging messages. Besides C_A^i and C_B^j, no other oracles have accepted with the same session identifier.
2. Freshness: We say that two oracles C_A^i and C_B^j are fresh if and only if the oracle C_A^i has accepted another partner oracle C_B^j, the oracle C_B^j has accepted another partner oracle C_A^i, and all the oracles C_A^i and C_B^j have not been sent a Reveal query a Corrupt query.
3. Session key security: We use the standard semantic security notation to model this property[22]. The security of session key is defined that the adversary who wants to discriminate a real key from a random one in the game G is indistinguishable, where the game played between the adversary AD and a collections of U_x^i oracles. The players $U_x \in \hat{C}$ and S and instances $i \in \{1, ..., N_I\}$. AD runs the game G with the following stages.

- Stage 1: AD is allowed to send the queries (Execute, SendClient, SendServer, Reveal and Corrupt) in the game.
- Stage 2: During the game G, at some point, AD can choose a fresh session and end a Test query to one of the fresh oracles C_A^i and C_B^j for the testing. Depending on the unbiased coin b, AD is given ether the actual session key K or a random one from the session key distribution.
- Stage 3: AD can continue to send the queries to the oracles Execute, SendClient, SndServer, Reveal and Corrupt for its choice. However, AD is restricted to send the Reveal and Corrupt queries to the oracles for its test session.
- Stage 4: Eventually, AD winds up the game simulation and decides to output its guess bit b'.

The success of AD from breaking the protocol in the game depends on passwords which are drawn from a dictionary D and is measured in terms of the advantage of AD from distinguishing whether the received value is the real key or a random one. Let $Adv_{P,D}^{G,AD}(k, q_{fake\text{-}C})$ be the advantage of AD and the advantage function be be defined as follows.

$$Adv_{P,D}^{G,AD}(k,q_{fake\text{-}C})=|Pr[b'\text{-}b]\text{-}q_{fake\text{-}C}/N\text{–}1/2*(N\text{-}q_{fake\text{-}C})| \quad (1)$$

where k is a security parameter, N denotes the size of the dictionary D and $q_{fake\text{-}C}$ denotes the number of attempts of the adversary from faking the client. After $q_{fake\text{-}C}$ times of faking the client, the intuition of the formulation is that the advantage of the adversary from finding the correct password and from faking the session key successfully should have the probability at most $q_{fake\text{-}C}/N$. The rest of non-successful faking cases could have the successful probability 1/2.

Password protection: An adversary may try to guess the password of a valid client and verify its guess through the interaction with the server or the client or from the intercepted messages. We require that the protocol has to provide the explicit authentication of a client's request for thwarting the online detectable guessing attack in which the server can do some actions such that the limitation of invalid request attempts cannot exceed the pre-defined threshold. Security against the adversary from launching the off-line guessing and the online undetectable guessing attacks, the protocol should not provide any advantageous information to outsiders or to a curious partner to verify its guess.

Definition 4. We say that a password-based three-party key exchange protocol is secure in our model when the following requirements are satisfied:

1. Validity: Among three oracles (C_A^i, C_B^j, S), the oracles (C_A^i, C_B^j) accept the same session key in the absence of an active adversary.
2. Session key indistinguishability: For all probabilistic, the advantage of the adversary AD is negligible within a polynomial time.
3. Explicit authentication: As the above mentioned, the protocol should make sure that the explicit authentication of two communicated parties is done for being against the online detectable guessing attacks.
4. Password protection: As the above mentioned, the protocol should not provide any advantageous information to outsiders or to a curious partner to verify its guess for being against the off-line guessing and the undetectable online guessing attacks.

3. Our Proposed Protocol

In our protocol, we define $h_1()$ and $h_2()$ are

secure cryptographic one-way hash functions and we will model the functions as random oracles in the security proof. The other parameters are introduced as follows:

A. The system selects a large prime number p, where $(p - 1)$ has a prime factor q.

B. Let g be a generator with order q in $GF(p)$.

C. TS denotes the trusted third party.

D. A and B denote two communicated parties.

E. pw_A and pw_B denote the passwords that A shared with TS and B shared with TS, respectively.

F. $\oplus$ denotes an exclusive OR operation.

G. For simplicity, all the exponentiation operations are under the modular p such as $g^x \bmod p \rightarrow g^x$.

1. Request that initiator A selects a random number x, calculates $R_A = g^x \oplus h_1(pw_A, A, B, sid)$, and sends (A, sid, R_A) to the responder B, where the sid denotes the session identity.

2. Upon receiving the request, B also selects a random number y, calculates $R_B = g^y \oplus h_1(pw_B, A, B, sid)$, and sends (B, R_B) with A's request to the trusted server TS.

3. (a) Upon receiving (A, B, sid, R_A, R_B), TS employs the passwords pw_A and pw_B to extract the exchanged information g^x and g^y, respectively. Then T selects three random numbers (z_1, z_2, z_3) and calculates (a, b, c, d), where $a = g^{xz_1}$, $b = g^{yz_1}$, $c = g^{z_2}$, and $d = g^{z_3}$.

(b) TS sends (A, sid, Z_{A1}, Z_{A2}) and (B, sid, Z_{B1}, Z_{B2}) to A and B in parallel, where $Z_{A1} = b \oplus h_1(pw_A+1, A, B, sid)$, $Z_{A2} = c \oplus h_1(pw_A+2, A, B, sid)$, $Z_{B1} = a \oplus h_1(pw_B+1, A, B, sid)$, and $Z_{B2} = d \oplus h_1(pw_B+2, A, B, sid)$.

4. Do in parallel

(a) Upon receiving (B, sid, Z_{B1}, Z_{B2}), B employs $h_1(pw_B+1, A, B, sid)$ and $h_1(pw_B+2, A, B, sid)$ to recover a and d. B then calculates the session key $K = h_2(A, B, sid, a^y)$, $S_{B1} = h_1(A, B, sid, K)$ and $S_{B2} = h_1(A, B, sid, d^y, a)$. B sends S_{B1} to A and S_{B2} to TS for identifying the validation of its identity and the session key.

(b) Upon receiving (A, sid, Z_{A1}, Z_{A2}), A employs $h_1(pw_A+1, A, B, sid)$ and $h_1(pw_A+2, A, B, sid)$ to recover b and c. A then calculates the session key $K = h_2(A, B, sid, b^x)$, $S_{A1} = h_1(A, B, sid, K+1)$ and $S_{A2} = h_1(A, B, sid, c^x, b)$. A sends S_{B1} to B and S_{A2} to TS for identifying the validation of its identity and the session key.

5. Do in parallel

(a) Both of A and B can authenticate each other by checking the validation of S_{B1} and S_{A1} and believe that the owned session key is fresh.

(b) Upon receiving A and B's responses, TS can check the validation of S_{B2} and S_{A2}. If any of the conditions does not hold, TS will return "*connection failure*" message to the corresponding parties and increase the fail times by one. We introduce the proposed protocol in Figure 1.

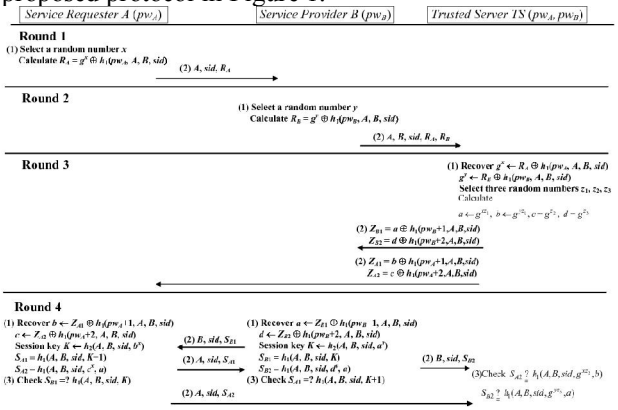

Figure 1. The proposed protocol

4. Security Analysis

In this section, we analyze that the proposed protocol is secure against some well-known attacks. Before our analysis, we first assume that the following mathematical problems are hard to be solved[9,13].

4.1 Analysis

Session Key Security.

(a) Even if $a = g^{xz_1}$ and $b = g^{yz_1}$ are known by an adversary, based on the difficulty of the CDHP, the adversary cannot derive the session key $K = g^{xyz_1}$ except the parties A and B.

(b) Based on the properties of one-way hash function and the exclusive-OR operator, the adversary is useless to derive (g^x, b, g^y, a) without the knowledge of A and B's passwords. The reason is that the extracted values cannot be verified. The adversary wants to discriminate (g^x, b, g^y, a) from $(R_A, R_B, Z_{A1}, Z_{B1})$, the probability of obtaining the session key K is equivalent to solve the CDHP on $(Z_{A1}, S_{A1}, Z_{B1}, S_{B1})$.

Replay Attack. An adversary who wants to imitate the requester A can resend the used messages $(R_A = g^x \oplus h_1(pw_A, A, B, sid))$ to B or to TS and expect to obtain some useful information from TS such as $(Z_{A1} = g^{yz_1} \oplus h_1(pw_A+1, A, B, sid), Z_{A2} = g^{z_2} \oplus h_1(pw_A+2, A, B, sid))$. Based on the CDHP assumption, the adversary not only cannot derive new session key $K = g^{xyz_1}$ without the knowledge of the ephemeral keys x, but also cannot win the trust of TS without the knowledge of the passwords pw_A since g^{z_2} is encrypted using the password pw_A.

Impersonation Attack. In Round 3 of our

proposed protocol, when someone sends the exchanged messages to *TS*, *TS* always returns the messages (Z_{A1}, Z_{A2}, Z_{B1}, Z_{B2}) back. The adversary can catch this chance to launch the attack. Note that *TS* waits the responses in Round 4. Since all the exchanged messages must be encrypted using the password independently, the adversary cannot know whether the guessed password is correct or not and also cannot judge whether the received message S_{B1} and the computed results (S_{A1}, S_{A2}) are correct or not. Based on the difficult of the CDHP, this way is blocked.

4. **Password Guessing Attack.**

(a) On-line detectable guessing attack. In current systems, there is a standard mechanism to defeat this attack. The solution is that the remote server logs and counts the number of trial failures. If the number is larger than the pre-defined threshold values, the server stops the connection. This concept can be applied to our protocol since *TS* verifies whether *A* and *B*'s responses (S_{A2}, S_{B2}) are correct or not in Round 4 and records the failure times.

(b) On-line undetectable guessing attack. To launch the attack successfully, the attacker has to get some useful information in advance for manipulating the data and verifying their guess on *TS*'s response (or *B*'s response). The attack cannot work on our protocol since all the requests have to be sent to *TS* and *TS* will wait the feedbacks from both of *A* and *B*. It implies that any trial process will be detected by *TS*. The attack fails.

(c) Off-line guessing attack. All the exchanged messages are encrypted using the passwords independently. The goal of the adversary is to guess the password and to verify the correctness on the intercepted messages. Based on the difficult of the CDHP, the adversary cannot employ the guessed password and derive messages to obtain any results on the messages (S_{A1}, S_{A2}, S_{B1}, S_{B2}) in Round 4.

5. **Forward/Backward Secrecy.**

(a) In each session, *A*, *B* and *TS* select their ephemeral keys (x, y, z_1, z_2) to construct ($R_A = g^x \oplus h_1(pwA, A, B, sid)$, $R_B = g^y \oplus h_1(pw_B, A, B, sid)$, $z_{A1} = b \oplus h_1(pwA+1, A, B, sid)$, $z_{B1} = a \oplus h_1(pw_B+1, A, B, sid)$). Based on the difficult of the CDHP, the adversary cannot calculate the session key $K = h_2(A, B, sid, g^{xyz_1})$ in all the sessions even if the passwords are guessed correctly. The property of the forward secrecy is provided.

(b) Even if one of the used session key $K = h_2(A, B, sid, g^{xyz_1})$ is compromised by the adversary, the adversary cannot obtain any useful information on the corresponding messages. For instance, the adversary may guess the password to get $g^{x'}$ and g^{yz_1}'. Based on the difficult of the CDHP, the adversary cannot verify the guessed password. As the above mentioned, without the knowledge of the password, the adversary cannot launch any attacks. Hence, the backward secrecy is also kept in our protocol.

Theorem 1. We claim that the proposed password-based three-party key exchange protocol is secure in the random oracle model if the CDHP is hard.

Proof. We then give the detailed proof in the appendix.

5. Efficiency Analysis

In this section, we analyze the computation cost of a service requester because the requester could use personal mobile devices to obtain the desirable services. Also, as introduced in[24], we can learn a relationship as follows: the time of one modular exponentiation is faster 5/3 times than the time of one public-key en/decryption operation, the time of one modular multiplication computation is faster 240 times than the time of one modular exponentiation operation, and the time of one one-way hash function operation is faster 600 times than the time of one modular exponentiation.

In Round 1, *A* calculates $R_A = g^x \oplus h_1(pwA, A, B, sid)$. The cost is one modular exponentiation plus one hash function operation. In Round 4, *A* recovers $b = Z_{A1} \oplus h\_1(pwA+1, A, B, sid)$ and $c = Z_{A2} \oplus h_1(pwA+2, A, B, sid)$. The cost is two hash function operations. Then *A* calculates the session key $K = h_2(A, B, sid, b^x)$, $S_{A1} = h_1(A, B, sid, K+1)$ and $S_{A2} = h_1(A, B, sid, c^x, a)$. The cost is two modular exponentiation plus 4 hash function operations. By the above, the computation cost of *A* is 3 modular exponentiations plus 6 hash function operations.

In the communication cost, we denote that:

1. Message Step denotes that one entity has sent data to the communicated party.
2. Communication Round means that if the sent data are independent between each message steps, one or more message steps can be integrated into the same communication round due to the sent data can be performed in parallel. The burden of the communication cost can be reduced.

We summarize the results in Table 1 and we can see that our protocol is more efficient than the related protocols[10-11,16,20-21].

5. Conclusions

In this paper, we have proposed a provably secure password-based three-party key exchange protocol to overcome some well known security

threats. Compared with the related protocols, the computation efficiency is still kept in our proposed protocol.

Table 1. Comparisons of the Computation Cost At Requester Side and the Communication Cost

	Our	Lu-Cao[21]*3	Huang[16]*4	Chien-Wu[11]	Chen *et al.*[10]*	Lo-Yeh[20]
T_{EXP}	3	4	2	2	3	3
T_{MUL}	0	2	0	0	0	0
T_H	7	3	4	4	4^{*1}	4^{*1}
T_{PKC}	0	0	0	1	1^{*2}	1^{*2}
T_{SYM}	0	0	0	0	1	1
Total(T_{MUL})	722.8	963.2	481.6	881.6	$1121.6+1T_{SYM}$	$1121.6+1T_{SYM}$
Rounds/Steps	4/8	5/5	5/5	4/4	5/5	4/6

T_{EXP} denotes the time of one modular exponentiation operation; T_{MUL} denotes the time of one modular multiplication computation; T_H denotes the time of one hash function operation; T_{PKC} denotes the time of one public-key en/decryption operation; T_{SYM} denotes the time of one symmetric-key en/decryption operation;
*: the protocol has been proven that the on-line undetectable guessing attack still exists[20].
*1: the computation cost of pseudo-random hash function is similar to the cost of one-way trapdoor hash function.
*2: the computation cost of one-way trapdoor function is similar to the cost of public key en/decryption.
*3: The protocol which is not secure against the unknown key sharing, the on-line undetectable guessing, and the impersonation attacks has been proven by[12,15,18,23].
*4: Wu had have shown that the protocol is not secure against the on-line undetectable guessing attack[25].

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Appendix

A. Security Proof

We prove that our protocol provides the session key indistinguishability property in the random oracle model under the CDHP assumption.

Proof. We use a contradiction way to prove it. We assume that an adversary AD can gain a non-negligible advantage to distinguish the test key in the game and AD can construct a breaker AD" to solve the CDHP problem, where the advantage of AD from differentiating the real session key from a random key as follows:

$\mathrm{Adv}_{P,D}^{G,\mathrm{AD}}(k,q_{fake\text{-}C}) = |\Pr[b'\text{-}b]\text{-}q_{fake\text{-}C}/N\text{–}1/2*(N\text{-}q_{fake\text{-}C})|$.

We suppose that an oracle C_A has accepted the session key of the form $K = h_2(A, B, sid, g^{xyz_1})$ with another fresh and partnership oracle C_B. We say that AD is successful if AD picks an oracle C_A or C_B to ask a Test query and can output the bit guess correctly. Thus, we have Pr[AD succeeds] = $q_{fake\text{-}C}/N + 1/2 * (N - q_{fake\text{-}C})/N + \eta(k)$, where $\eta(k)$ is non-negligible.

Let Q_h be the event that $h_1()$ or $h_2()$ has been queried on (A, B, sid, g^{xyz_1}) by AD or some oracles. Then Pr[AD succeeds] = $q_{fake\text{-}C}/N$ + Pr[AD succeeds | Q_h] * Pr[Q_h] + Pr[AD succeeds | $\overline{Q_h}$] * Pr[$\overline{Q_h}$]. Since $h_1()$ and $h_2()$ are random oracles and C_A and C_B are fresh oracles, it implies Pr[AD succeeds | $\overline{Q_h}$] = 1/2. Hence, $q_{fake\text{-}C}/N + 1/2 * (N - q_{fake\text{-}C})/N + \eta(k) \le q_{fake\text{-}C}/N + 1/2 * (N - q_{fake\text{-}C})/N + \Pr[Q_h]$. We then have $\Pr[Q_h] \ge \eta(k)$.

The adversary AD selects a fresh oracle C_A which has accepted a session key. Then the probability of $h_2()$ being queried on (A, B, sid, g^{xyz_1}) by AD or some oracles other than C_A and C_B is non-negligible. As mentioned before, we have assumed that AD constructs a breaker AD" which can solve the CDHP with non-negligible probability. The task of AD" is that: Given $X = g^x$ and $Y = g^y$, AD" outputs g^{xy}, where x and y are chosen randomly.

AD" executes the following process:

1. Randomly select C_A and C_B from $\hat{C} = \{C_1, C_2, ..., C_{NC}\}$ and instances u and i from $\{1, 2, ..., N_I\}$, where N_C and N_I denote the number of service requesters and service providers and the instances per entity. Note that all these parameters are polynomial on the security parameter.
2. Determine two oracles C_A^u and C_B^v who are partnership.
3. Guess that AD will choose one of C_A^u and C_B^v who have accepted the session to ask its Test query after AD decides to terminate the game.

Given the challenge $(X^* = g^x, Y^* = g^y)$ to AD", AD" sets the public parameters as (g, p). AD" also maintains the lists L_{h1} and L_{h2} for the random oracles $h_1()$ and $h_2()$ queries, L_{Send} for the communicated transcripts, and L_{Key} for the corresponding keys of each session. AD" selects the passwords pw for each C_A and $C_B \in \{C_1, C_2, ..., C_{NC}\}$ at random and lets $pw_{\hat{C}}$ be the password file of TS.

During the game, AD will ask some queries to AD". The answers are given as follows:

1. Hash query: AD" randomly responses $h_1()$ and $h_2()$ queries which are like real random oracles do, and records all the inputs and the corresponding outputs in L_{h1} and L_{h2}, respectively.
2. Corrupt(C) query: If C is one of C_A and C_B, AD" gives up; otherwise, AD" answers all the internal state of C to AD.
3. SendClient(C_X^i, m) query:

(a) If $(C_X = C_A)$ && $(i = u)$ && $(m = start)$, then AD" sets $N_X = X^*$ and responds the protocol says $\{CA, sid, N_X \oplus h_1(pw_{C_A}, C_A, C_B, sid)\}$. Finally, the oracle records the responsive transcript and the random exponent (?) in the L_{Send} list and $(h_1(pw_{C_A}, C_A, C_B, sid), (pw_{C_A}, C_A, C_B, sid))$ in the L_{h1} list, where ? denotes the corresponding exponent of X^* and is unknown.

(b) If $(C_X = C_B)$ && $(i = v)$ && (m has the form of $(C_A, sid, N_X \oplus h_1(pw_{C_A}, C_A, C_B, sid))$, then AD" sets $N_Y =$

Y^* and responds the protocol says $\{C_A, C_B, sid, N_X \oplus h_1(pw_{C_A}, C_A, C_B, sid), N_Y \oplus h_1(pw_{C_B}, C_A, C_B, sid)\}$. Finally, AD" records the responsive transcript and the random exponents (?) in the L_{Send} list and ($h_1(pw_{C_B}, C_A, C_B, sid)$, ($pw_{C_B}, C_A, C_B, sid$)) in the L_{h1} list, where ? denotes the corresponding exponent of Y^* and is unknown.

(c) If ($C_X \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of ("start", $C_Y \in \{C_1, C_2, ..., C_{NC}\}$ && $C_Y \neq C_X$)), then AD" selects an integer x' at random, calculates $X^* = g^{x'}$, and responds with the transcript $\{C_X, sid, X^* \oplus h_1(pw_{C_X}, C_X, C_Y, sid)\}$. Finally, AD" records the transcript and the randomly secret exponent x' in its L_{Send} and L_{h1} lists.

(d) If ($C_X \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of ($C_Y, sid, Y^* \oplus h_1(pw_{C_Y}, C_Y, C_X, sid)$), then AD" selects an integer x' at random, calculates $X^* = g^{x'}$, and responds with the transcript $\{C_Y, C_X, sid, Y^* \oplus h_1(pw_{C_Y}, C_Y, C_X, sid), X^* \oplus h_1(pw_{C_X}, C_X, C_Y, sid)\}$. Finally, AD" records the transcript and the randomly secret exponent x' in its L_{Send} list.

(e) If ($C_X = C_A \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of (C_X, sid, Z_{C_X1}, Z_{C_X2}) for $C_Y = C_B \in \{C_1, C_2, ..., C_{NC}\}$), then AD" consults its L_{Send} list by using sid to find a matched entry. If the matched entry can be found, AD" extracts the local value from L_{Send} to recover the received data and to calculate K, S_{C_X1} and S_{C_X2}. AD" responds with the transcript $\{C_X, sid, S_{C_X1}, S_{C_X2}\}$. Finally, AD" records corresponding data in its L_{Send}, L_{h1}, L_{h2} and L_{Key} lists respectively. Otherwise, AD" responses with error messages.

(f) If ($C_X \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of (C_X, sid, S_{C_X1})), then AD" consults its L_{Send} list by using sid to find a matched entry. If the matched entry can be found, AD" extracts the local values from L_{h1}, L_{h2} and L_{Key} lists and uses them to verify S_{C_X1}. If the verification does not hold, AD" gives up; AD" records corresponding data in its L_{Send} list.

(g) AD" responses with error messages for all the other cases.

4. SendServer(m) query:

(a) If (C_X and $C_Y \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of ("start", C_X, C_Y, sid, $X^* \oplus h_1(pw_{C_X}, C_X, C_Y, sid)$, $Y^* \oplus h_1(pw_{C_Y}, C_Y, C_X, sid)$)), then AD" uses pw_{C_X} and pw_{C_Y} to recover the received data. AD" selects three integers z_1, z_2 and z_3 at random and responds with the transcript $\{C_X, sid, Z_{C_X1}, Z_{C_X2}\}$ and $\{C_Y, sid, Z_{C_Y1}, Z_{C_Y2}\}$. Finally, AD records all the transcripts and the randomly secret exponents z_1, z_2 and z_3 in its L_{Send} list, L_{h1} list and L_{Key} list respectively.

(b) If ($C_X \in \{C_1, C_2, ..., C_{NC}\}$) && ($m$ has the form of (C_X, sid, S_{C_X2} for C_Y) $\in \{C_1, C_2, ..., C_{NC}\}$), then AD" consults its L_{Send} list by using sid to find a matched entry. If the matched entry can be found, AD" extracts the local values from L_{h1}, L_{h2} and L_{Key} lists and uses them to verify S_{C_X2}. If the verification does not hold, AD" responds an error message to C_Y and records corresponding data in its L_{Send} list.

(c) AD" responses with error messages for all the other cases.

Reveal(C_X^i) query: After receiving the query, AD" consults the records in the list of L_{Key} and reveals all the internal state and the session keys.

AD then answers its guess and requires AD" to searches its L_{h1} and L_{h2} list for the entry, where the entry has the input of the form (C_X, C_Y, sid, $(recovered\ data)^{secretexponent}$) for some K. Finally, AD" outputs K as the Diffie-Hellman key of C_X and C_Y. There are the two possible results for the above experiment:

1. AD" gives up if AD does not make its queries where C_A^u or C_B^v has accepted their session.
2. If AD does make its queries, then C_A^u or C_B^v will accept their session and hold the key formed $h_2(C_A, C_B, sid, (recovered\ data)^{secretexponent})$. It is the fact that the session key g^{xyz_1} is unknown to AD", AD" cannot calculate this key actually.

AD" will search its L_{h1} and L_{h2} lists for the entry and certainly wins its experiment if Case 2 does happen really. Hence, the probability of AD" outputting the correct value on $g^{xyz_1} \bmod p$ is: $\Pr[Q_h]/(N_C^2 N_I^2)\} \geq \eta(k)/(N_C^2 N_I^2)$, where the probability is non-negligible and the result contradicts our CDHP assumption. Hence, we can conclude that $\eta(k)$ must be negligible and is the advantage of $Adv_{P,D}^{G,AD}(k, q_{fake\text{-}C})$. The theorem is proven.

Effects and evaluation of creativity instructional methods on creativity of students

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Abstract: The purpose of this study to investigate and compare the efficacy of brain storming methods, group discussion and guided discovery of creative high school female students of Ahvaz was the third base. The study sample of 80 third grade high school students in Ahwaz city in the 89-90 school year as a cluster random sampling and then selected randomly in three experimental groups (brain storming, group discussion, guided discovery) and a control group (expository or traditional) were appointed to replace. The dependent variable to measure creativity, creativity test was used in Abedi. The research design and experimental test of the type pre and post test control group had. After random selection of experimental and control groups, first for all three groups, pre-test was performed, and then the pilot interventions in 10 sessions of 45-70minutes to the test groups were presented and after the training program after the test were. Analysis of covariance using a data track (ANOVAs) showed that techniques brain storming, group discussion and guided discovery than an expository of how creative the students had a positive impact. The results also showed that it isn't any of the methods in terms of impact than the other does not. .

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***Key words**: Creativity, Expository, Brain Storming, Group Discussion, Guided Discovery*

1. Introduction

We may consider the expansion of effective ways in teaching and learning as one of the psychologists' endless missions for helping the educational systems (Turnbull, Allison & Malcolm, 2010). Nowadays, Creativity and its operation and its effects on learning and teaching methods are psychologists and educational scholars' interest. In past few decades, many studies have been done relating to this subject in order to answer these two main questions: what is creativity? And is it teachable? (Teo2010).

Creative thinking is one of the most complex and outmost display of human thought. (Morris.W, 2006) According to Turnbull etal (2010) creativity is the ability to produce new thoughts and mix existing thoughts in a new form to find new solutions for the problem. While Robert Gangne (1984) considers creativity as special way of solving problem, Wool folk (1993) believes the core of all creative ideas and thinking is novelty concept. Torrance (1988), the scale of creativity inventor, with more than 40 researches regarding this matter believes creativity means to create, make and express exquisite ideas and it can be taught to others.

Psychologist and educational tutors believe there are two models for training creativity. One relates to person and other to the environment. In individual related way, child learns the ways and methods and uses them that lead to growth and prosperity of universal creativity. (Heausler1998). Four major methods used in this research can be the representative of these two above general practices.

In brain storming method, the main purpose is to separate the process of producing answer from the process of their evaluation, because evaluation mostly generates diverse answers and prevents its evaluation separately. In this method teacher gives examples to the students and asks them to express solutions that come to their mind for each issue. Before giving all answer no explanation or suggestion is not given by teacher or other students, this method is like the free association method use by psychologists, with this difference that free association is an individual process but brain storming is performed with other students participants (Mellew1996).

Of course we should mention that brain storming may be the way to evaluate input behaviors because the answer that are given by students at first are more dominated on them than others. Following the brain storming method will make students to think about more unlikely answers, also this method can teach students to remember concepts and basics required to solve an issue (kakia, & Madan dar Arani, 2010).

In traditional educational methods that are usually called expository method. Teacher in teaching materials gives students all concepts and basics for resolving an issue and then shows them the answer. But in Brain storming method, teacher doesn't give students concepts and basics rather the student is free

to find the solution for the problem any way he.she wants. Also in group discussion method that is a very common method in teaching, students unlike the traditional or explanatory methods participate actively in class. (Berg.R.2007) with this method we are able to create a better team work and make them involve in subject and finally help them to learn the material more stable than before(safavi, 2004).

Curran and his colleagues (2008) consider the approaches based on small group discussions effectiveness method in inter professional learning and believe that interaction factors in this method have important effect on interest, creativity, learning and satisfaction of students during learning process.

In Guided discovery method which is a combination of these two methods, student has an independent role in his work but still teacher supervises his activities and anytime he needs, teacher guides him through. For example, in guided discovery method teacher gives the concepts and basics to students and the student himself tries to solve the issue (kakia, & Madan dar Arani. 2010).

Results of Turnbul, Alison and Malcolm (2010) studies regarding education and group discussion designs and teaching methods effects on creation showed that this methods are effective in increasing the person's creativity and gives the ability to produce new ideas and mix existing ideas to new form in order to find a new solutions for problems.

Livingston (2010) in his research about creativity relation with academic education demonstrated that using new methods in academic education makes a creation of creativity in educational environments possible, he concluded that teaching in university creates a natural place for organizing educational structures and learning techniques, so this not only leads to creativity and individual training it also organizes people's relationships with each other, regulates and allocates time for finding the solution of one problem.

Sonz de Acedo Lizarraga, Oliver (2010) studied works of intellectual skills stimulation on intellectual skills, creativity, self organizing and educational success in 46 high school students between 16 to 18 years old. The results showed that stimulating intellectual skills with different methods of learning at first will lead to increasing the intellectual and creativity skills and then leads to higher self organization and educational improvement.

Sharifi and Davary (2010) execute a research with the purpose of studying and comparing the effects of 3 creativity training methods (brain storming, forced relationship and) in increasing the creativity of students in Shahrekord second grade of junior high school students. Their results showed a notable difference between pre-exam scores and post-exam scores in all groups except control group. (Brain storming group $P<0.05$, Sinectis $P<0.01$ and forced relation $P<0.05$) It also showed that none of the training creativity methods had superiority to others ($P<0.001$).

Dusold and Sadoski Study (2008) that also included the comparison of final exam result of two groups in form of group discussion following the individual study and lecture, showed no significant difference. In this study, base on researched performed by researchers, students in group discussions mentioned that they didn't need to study very much and most of the time they stick to those material discussed, while students in lecture group spent more time in preparing themselves for earning better scores.

Slavin etal (2008) in their study about the effects of four methods on creativity: a) traditional method b) combinational training methods using big and small groups and working or learning computer c) only teaching computer and d) processing programs that motivated teachers to create new methods in teaching, control groups were chosen accidentally and the result showed that partnership learning and combination methods have more effect on creativity and learning of audiences.

Feingold etal (2008) not only mentioned the difficulties of choosing a teaching method that can increase creativity and understanding level in students, but also offered a study named teaching by using small groups from university students in bachelor degree. Those who used interviews and observation as a collection tools for their data, reported that this method can significantly increase interaction between people, efforts and creativity to answer multidimensional questions.

Following this findings, Bourgeois JA and ET (2008) also by performing a study on PHD courses in California University, reported that courses based on small groups make a great impact on patient interaction, creative work in medical procedures, compliance with ethical issues and counseling approaches.

Also Kakia and Madan dar Arani (2009) in one research studied the female students' creativity according to evaluation and comparing effectiveness of brain storming and expository methods. The result showed teaching creativity by brain storming method in expansion level $P<0.001$ is approved but in other levels they didn't observe any significant difference between brain storming method and expository method.

Falavin(2006) in studying effectiveness of Brain storming, sinectis and forced relationship in Female and male students found out that the average of female creativity scores in brain storming method was higher than male scores in the same group although in

sinectis and forced relationship groups male had higher creativity levels(Sharifi and Davary,2010).

Asgary (2007) in his study about effectiveness of different teaching methods on creativity level in 574, 4th grade school girls in Hamadan province demonstrated that brain storming methods, research skills, creative study, active teaching (solving problem and group discussion), using self concept incremental approaches and available tools, familiarizing teachers with creativity and its value and importance, and using flexible curriculum in class have positive effect on students.

In another study, Marel (2003) by studying different creativity technique discovered that flexibility of training structure in class is effective in students' creativity. Morgan R.R and Et study(2000) on the role of intelligence and creativity methods in accordance with competitive environment showed that although intelligence has a important role in understanding human behavior but by using creativity, one can understand the ability of man to match himself with competitive environment and use appropriate and new methods.

Proctor (2002) in one experimental research, studied the effect of teachers' workshops on creativity, recognition, academic achievements of second grade gifted and non-gifted students. According to him factors related to school and teachers have the most important effect on student work in developing countries.

In his studies, Sternberg R.J (2001) showed that creativity is the ability to create new ideas in high level and it is teachable to students by using different techniques. This leads to a mixture of creativity power, flexibility, and sensitivity about existing beliefs in them. He believed that teaching creativity techniques to a person gives him the ability to think about other findings with reasonable thinking to have beneficial achievements for him and others.

Gardner and Jouler (2000) in their research regarding effectiveness of traditional and active learning methods declared that those students that use active learning not only have better learning but also enjoy the learning process. Because instead of being only a listener they participate in learning process actively and consider themselves as responsible for their learning (Yazdian poor, Yousefi and Haghani 2010).

However considering the previous researches, present study tries to evaluate the creativity power of third grade school girls in Ahvaz base on four teaching methods brain storming, group discussion, guided discovery and expository.

Therefore following hypothesizes were considered and studied in this research:

A. ***First hypothesis****: Teaching brain storming, group discussion, guided discovery methods is effective in third grade female students.*
B. ***Second hypothesis:*** *There is a significant difference between brain storming, group discussions, guided discovery methods in third grade female students' creativity levels.*

2. Methods

This study has been accomplished base on experimental method by using pre-exam and post-exam from control group. All of the third grade high school female students in Ahvaz during 1389-90 academic years were considered as statistical society of this paper. Statistical sample includes 80 people from this society that were chosen cluster randomly from high schools in Ahvaz's district 1. And base on simple sampling they were divided to 4 groups each twenty students (3 experimental groups and one control group). Some of the features of these sample groups are presented in Table 1.

As it is considered in Table 1, in Expository group 17 years old students had the most frequency with approximately 60% and 16 years old students had the lowest with 40 samples. Also in group discussion group, 17 years old students with the most frequency around 50% and 18 years old students had the lowest with 15 samples.

In guided discovery group, like two groups above, 17 years old students had the most frequency with 55% and 16 years old students had the lowest with 20 samples. In brain storming method, 17 years old students had the most frequency with 60% and 16 years old students had the lowest with 5 samples.

2.1. Research Tools

2.1.1. Creativity questionnaire

In order to measure the dependent variable – creativity- Jamal Abedi creativity evaluation test was used. This questionnaire includes 60 articles. Each article has 3 options a, b, c. that is scored respectively 1, 2, and 3. This questionnaire has 4 creativity, flowing, flexibility and expansion components that are respectively 16, 22, 11 and 11 questions. The final ratios reported for this questionnaire varies from 0.70 to 0.83.

In current research, final ratios of creativity questionnaire are calculated according to Tansif and Cronbach's alpha that were respectively 0.84 and 0.80. Those were the acceptable final results of this questionnaire.

Table 1: Distribution of subjects according to age group

Age (year)	Group									
	Expository Method		Group Discussion Method		Guided Discovery Method		Brain Storming Method		Total	
	frequency	*Percentage*	*Frequency*	*percentage*	*Frequency*	*percentage*	*Frequency*	*percentage*	*Frequency*	*percentage*
16	8	40.0	7	35.0	4	20.0	1	5.0	20	25.0
17	12	60.0	10	50.0	11	55.0	12	60.0	45	56.3
18	0	0	3	15.0	5	25.0	7	35.0	15	18.7
Total	20	100	20	100	20	100	20	100	80	100

.2.2. Research Findings

2.2.1. Descriptive findings

This research's descriptive findings include statistical indicators such as mean, standard deviation that are different for all variables. This point is mentioned in Table 2.

As it is mentioned in table 2, in pre-test period the average and deviation of creativity in each group respectively are as follow : control group (expository, "traditional and speech" method) 12.21 and 122.50, group discussion 14.59 and 120.35, guided discovery group 11.86 and 122.65, brain storming group 11.84 and 119.75 and in post exam period the average and deviation of each group are control group (expository, "traditional and speech" method) 14.02, 121.00, group discussion 14.77 and 128.00, guided discovery group 10.35 and 129.20 and brain storming method 11.23 and 125.40.

A- Findings regarding the research hypothesizes

First hypothesis: Teaching brain storming, group discussion, guided discovery methods is effective in third grade female students.

Table 2: Creativity Variable descriptive features in pre-test and post-test period
(S_d= standard deviation)

Variable	Group	Pre test		Post test	
		Average	S_d	Average	S_d
Creativity	Control (Expository)	122.50	12.21	121.00	14.02
	Group Discussion	120.35	14.59	128.00	14.77
	Guided Discovery	122.65	11.86	129.20	10.35
	Brain Storming	119.75	11.84	125.40	11.23

As it is shown in table 3, with controlling the creativity pre test a big difference was demonstrated among female students in control and experimental groups ($F=9.39$ and $P<0.001$)

In other word, There was a great creativity difference among students who experienced brain storming , group discussion and guided discovery (experimental groups) and those who didn't learn these methods and used the traditional way of expository (control group).

As it is shown in table 4, there is no significant difference between triple experimental group (group discussion, guided discovery, brain storming) by Pursuing Bonferoni test analysis. Therefore the second hypothesis is not confirmed. In other word, the effect of teaching in group discussion, guided discovery and brain storming to female students is almost the same.

Table 3: One track covariance study results (***ANOVAs***) a comparison of creativity average after exam Experimental and control groups before test

SOV	TS	df	MS	F	P
Pre-test	9691.52	1	9691.52	276.74	0.0001
group	987.22	3	329.07	9.39	0.0001
E	2626.47	75	35.02		

Second hypothesis: There is a significant difference between brain storming, group discussions, guided discovery methods in third grade female students' creativity levels.

Considering the fact that there is no significant difference among groups, using one track covariance analysis therefore, for this paper we used bonferoni analysis that its results are mentioned in Table 4.

Table 4: Pursuing bonferoni test Result among post creativity test average (*Av.*) scores. Experimental (*Exp.*) and control groups in monitored pretest

	Groupos	Av.	Exp.	Gr. dis	Gui. Dis	Br. S
1	*Control (Expository method)*	121.00		(0.0001)	(0.001)	(0.003)
2	*Group discussion method (Gr. dis)*	128.00			-	-
3	*Guided Discovery method (Gui. Dis)*	129.20				
4	*Brain Storming Method*	125.40				

3. Conclusion

As it was explained, this research was done with the purpose of studying and comparing the effectiveness of brain storming, group discussion and guided discovery teaching methods on third grade students' creativity level. The table 3 result showed that there is a significant difference among students who were taught according to brain storming, group discussion and guided discovery teaching method (experimental method) and those who didn't follow these methods and were taught in traditional way (control group). ($p<0.0001$) and this means that brain storming, group discussion and guided discovery teaching method is influential in third grade student creativity.

These findings are matched with researches done by Turnbul, Alison and Malcom (2010), Liunig Stone (2010) Sanz de asdo lizarga , oliver (2010), Sharifi and Davari (2010), Karan and et (2008) Dusold and Sadoski (2008) Slavin and et (2008) Fingled and Et (2008) Borgeuis and et (2008) Kakia and Madan Dar Arani (2009), Flavin (2006), Asgary (2007), Meril (2003), Peractor (2002), Steranberg (2001), Gardner and jouler (2000).

In order to explain these results, we can mention that new studies show, the concept of creativity can contain a vast range of social effects in person's individual growth especially in childhood. (kratzer, J.2008), these researches also confirm the positive effect of creativity on formation of" social wealth" in children and teenagers and provide an opportunity for most of people to use this wealth or source in their adult age. Therefore understanding the conditions of growth and expansion or teaching creativity can be considered as one of the knowledge areas that are not fully comprehensive for men. And this gives a good opportunity to psychologists and researchers to investigate and study.(Bandura A., 2001)

Although paying attention to creativity in psychology field was mostly started by Gilford's studies in 1950 AD and then followed by Turens researches in 1970s, but it entered schools as a basic field of study favorites by teachers and psychologists (Craft, A.). However recently many of Psychologists and teachers have tried to consider this issue more practically and expand its training in effective living areas such as educational system, especially in schools (Persaud, R.) Meanwhile teachers who consider creativity inflorescence and growth in students as the most important goal of education, tried to apply active learning method (like methods used in this research) and help students to use their creativity as far as it's possible. (Hosseini2002)

In this regard, Persaud (2008) believes that producing and creating creativity in schools requires deep attention toward the quality of education. Many scientific researches consider teaching and learning by brain storming method as an active teaching that after dividing evaluation process from answer production process plays an essential role in idea creation of students. (Abedi1993). This method increases the effort of students to give more direction to their answers hence lead to increase of answers produced by students (Persaud2008).

It must be acknowledged that brain storming can be a way to evaluate the input behaviors because the answers which students first give are probably those that they are more dominance.

Continuing the brain storming method can eventually make students to test their weaker answers. Also this method can make students to try to remember the concepts and basics required for solving the issue (Madan Dar Arani & Kakia, 2009).

Group discussion method, one of the methods apply in this research, is also introduced as active learning and increases creativity and innovational skills in students. As a matter of fact, applying this method can help create a better interaction among students and this way increase their involvement with the lesson subject and eventually create a more stable learning. Those teachers who believe in active participations of students in class and give them relative independence so they can feel proud and possession toward the class not only increase the students' inner motivation but also provide a ground for reinforcement and development of creativity(Am ably, 1996).

Group discussion method, regardless of educational aspects and enough opportunity to analyze the details being discussed, has undeniable effects on enhancing social culture and people communications. This method is valuable in

communication improvement, creating self confidence by discussing the topic, improving the ability of transferring and transparency of ideas, listening ability reinforcement, observing other people's reaction about what is being said, disagreeing without showing any resistance and entrenchment, free expression of ideas and asking mutual questions that in some points can be the starter and starting point of one research (Barras R, 2002).

Regarding the manner of guided discovery method effectiveness which is a combination of brain storming and traditional or expository method, we can say that student unlike expository or speech method uses teacher's instructions and supervision while keeping his independence. Although in this method students are completely free to find the solution but teachers also help them anyway they can. However this help is for preparing and assisting them to remember the knowledge or concepts or basics and skills required to solve an issue. This method can play an important role in students' growth and creativity because it teaches students to take responsibility for finding relations and organizing the information and also anytime needed teacher's support and guidance is provided (Seif2000).

Also considering the fact that second hypothesis about the lack of differences in brain storming, group discussion, guided discovery method effectiveness on female students in third grade of Ahvaz high school showed no real meaning. We can consider that according the results in table 3, this research like Sharifi and Davary's researches 2010, Asgary 2007, Neka 1984, Mirzaeian 2004 showed no superiority among creativity methods, although in this regard, in some researches (flavin 2006, Hasani 2000, Bahrami, Rashidi and Arizi 2000) one creativity method was considered better that the others.

Therefore considering the mixed scientific information about the differences of creativity training methods 'effectiveness, it seems that this research's limitations in addition to test inefficiency in considering the environmental, cultural, social and moral variables in students is due to inadequate number of teaching creativity sessions, insufficient volume content, lack of intelligence control (although in this research other variables such as sex, economical –social conditions was controlled) .

However, in order to enhance the quality of the future studies following points are recommended, it is better to increase the test time, the sample size of research and number of training sessions and diversification of creativity training content in addition to use other creativity training methods such as model making or combinational methods and comparing their results in different creativity levels.

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The prevalence of musculoskeletal disorders and its relationship to general health statement in hospital nurses

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Abstract: **Introduction:** The term musculoskeletal disorders (MSDs) covering over 200 conditions that affect the muscles, joints, tendons, ligaments, peripheral nerves and supporting blood vessels causing pain and functional impairment to sufferer. This study was directed as an epidemiologic survey with a view to "assessing the prevalence of MSDs among inpatient hospital nurses of Oil Company's grand hospital in Ahvaz-Iran over the Last 12 months and last week separately, measuring the general health state and its relationship to the prevalence of MSDs of specific sites of body". **Aims:** Estimation of the prevalence of MSDs among different groups of inpatient hospital nurses**,** Demonstration the relationship of MSDs and mental health state in hospital nurses**, and hypotheses:** Estimation the prevalence of MSDs in specific sites of the body, Categorize the severity of mental health state in hospital nurses, The relationship between MSDs in specific site of body and mental health state. **Materials and methods:** All of the nurses and nurse aides who are working in inpatient wards are included in this study but not outpatient and other clinical nurses. In this study the shift-workers are the nurses who are intermittently working in morning (6am -2pm), in afternoon (2pm – 10pm), and at night (10pm -6am). In the retuned-back questionnaires the questionnaires which contained at least one answer about MSDs YES or NO is recorded as a responder. From the 195 distributed questionnaires 161completed questionnaires were retuned back with the response rate of 82.5%. The SPSS software was used for processing and analysing the data. **Result:** From the 161 responders 10.5% of them had a $15<\text{score} \geq 20$ which suggests the evidence of distress, and 4.3% of them had a score more than 20 which suggests severe problem and psychological disorders. The minimum score of GHQ was zero which is the indicator of best general health state, while the maximum score was 34 which indicate the poor general health state. The mean of GHQ score was 10.8(table 5), the distribution of GHQ scores from zero to 34 is demonstrated. The correlation between poor general health state and all of the musculoskeletal disorders over 12months was strongly positive except for neck, elbow, hand and wrist disorders.The correlation between hand/wrist disorders in last 12 months and poor general health was weakly positive but there was not any relationship between elbow and neck disorders in last 12 months and poor general health state.

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Key words: prevalence of musculoskeletal disorders, general health,

1. Introduction

1.1. Definition and history

The term musculoskeletal disorders (MSDs) covering over 200 conditions that affect the muscles, joints, tendons, ligaments, peripheral nerves and supporting blood vessels causing pain and functional impairment to sufferer (1).

Investigations have demonstrated that MSDs are the leader causes of occupational injuries, disability, absenteeism and incapacity among workers in developed and developing countries.

1.2. Musculoskeletal disorders in nurses

1.2.1. The prevalence of MSDs among nurses

There are some investigations to support that MSDs are more common among nurses than other groups of work forces. For example in the study by Karahan (2) it is demonstrated that MSDs are common among Turkish hospital staff and in compare to other employees in hospital, the nurses have the highest prevalence. According to this study the prevalence of low back pain in hospital staff has been overall 65.8% while the highest prevalence (77.1%) was reported by the nurses. This study included only low back pain and not all of MSDs such as neck, shoulder, hip, knee, and ankle disorders. In another study among hospital nurses in Nigeria by Fabunmi (3) it is demonstrated that the 12 month prevalence of musculoskeletal disorders at any site of the body to be 90.7% and the low back pain is a commonly reported MSDs with the prevalence rate of 78%.

Studies of back-related worker compensation claims in USA reveals that nursing personnel have the highest claim rate of any occupation or industry, and 12 percent of nurses leave their profession

annually as a result of back injuries, and more than 52 percent of them complain of chronic back pain (4) (Nevada RN formation 2003) .

In the review of research of musculoskeletal disorders among Italian nursing personnel with a particular focus on studies that had examined individual, physical and psychosocial risk factors by Lorusso (5) (2008) it was shown that low back pain prevalence rate was varied widely among different investigations conducted in Italy, ranging from 33 % to 86 %. In the study by Mehrdad et al (6) (2010) the prevalence of MSDs among hospital nurses in Iran has been as follow: back pain 73.2%, knee pain 68.7%, shoulder pain, 48.6% and neck pain 46.3%. In the other study in Iran by Choobineh et al (7) (2006) it was demonstrated that 84.4% of the hospital nurses had experienced some symptoms of MSDs during a 12 months period.

1.3. MSDs in nurses, causes and risk factors

The causes of MSDs in nurses are widespread and patient care and treatment such as turning position and transferring, handling drugs, handling medical equipment and devices, work station design, physical environment, welfare and psychosocial factors are common causes. In the study among Japanese nurses by Smith and Derek (8) (2006) alcohol consumption, tobacco smoking, and having children have been shown to be significant risk factors, with adjusted odds ratio of 1.86, 2.45, and 2.53 respectively.

Also the prevalence of musculoskeletal disorders is increased by work volume; work hours per week and job experiences (3). It should be considered that there are some non occupational components such as leisure, play and daily physical activities and psychosocial disorders that contribute to occurrence of MSDs. According to a prospective cohort study among nurses by Nidd hammer (9) (1994) the risk factors associated with cervical, dorsal and lumbar pain are smoking, experience symptom of psychological disorders and physical work load. Psychological disorders are the risk factors for recurrent or chronic back pain. Age and history of previous MSDs are risk factors for cervical pain, and have children under 3 -year and tobacco consumption are risk factors for dorsal pain. In another study by Harcombe et al (10) (2010) in New Zealand nurses, job strain had the strongest association with neck (OR 3.46) and wrist/hand pain. It means that addressing job strain could provide significant benefit for those with neck and wrist/hand pain. In the study by Nick pour et al (11) (2009) on Iranian hospital nurses it was demonstrated that previous history of MSDs, increasing BMI and lifting patients are related to the prevalence of MSDs.

1.4. The psychosocial factors

Exposure to physical work factors and the development and prognosis of particular disorders may be modified by psychosocial factors. Understanding these associations and relating them to the causes of disease is critical for identifying exposures amenable to preventive and therapeutic interventions. For this reason the remediation strategies which focus only on manual handling task would probably are suboptimal in reducing MSDs among nurses (8) (Smith & Derek 2006).

For managing and reducing the risk of MSDs among high risk group of work forces such as nurses, estimation of MSDs prevalence, identification of risk factors and exposure assessment is mandatory.

This study was directed as an epidemiologic survey with a view to "assessing the prevalence of MSDs among inpatient hospital nurses of Oil Company's grand hospital in Ahvaz-Iran over the Last 12 months and last week separately, measuring the general health state and its relationship to the prevalence of MSDs of specific sites of body".

1.5. Aims, objectives and hypotheses

1.5.1. Aims

A. Estimation of the prevalence of MSDs among different groups of inpatient hospital nurses
B. Demonstration the relationship of MSDs and mental health state in hospital nurses

1.5.2. Objectives

[1] Estimation the prevalence of MSDs in specific sites of the body
[2] Categorize the severity of mental health state in hospital nurses
[3] The relationship between MSDs in specific site of body and mental health state

1.5.3. Hypothesis

I. There is a correlation between the prevalence of MSDs and mental health state
II. There is the correlation between MSDs in specific site of body and mental health state

1.6. Materials and methods

1.6.1. Sampling

All of the nurses and nurse aides who are working in inpatient wards are included in this study but not outpatient and other clinical nurses.

1.6.2. Questionnaires and Data Collection

All of the data which were needed in this study are based on self reporting via the

questionnaires provided. In this study the shift-workers are the nurses who are intermittently working in morning (6am -2pm), in afternoon (2pm – 10pm), and at night (10pm -6am). The nurses who are usually working at morning but sometimes working as over time in afternoon or night, or she/he has a history of shift working not included as shift workers. In this survey, a smoker is defined as a person who smokes at least one cigarette per day.

The MSDs' Nordic questionnaire which specifies the organ involvement of the persons who have or have had MSDs, in recent 7days or during the last 12 months according with the attached picture was used to demonstrate the pain or any discomfort from head, neck, shoulder, elbow, wrist and hand, upper back , lower back, hip and thigh, knee and ankles.

Among the general health questionnaires (GHQ) which demonstrate the recent weeks' mental health state of the responders and non-psychotic disorders, the GHQ-12 which is quick, reliable and sensitive was preferred to be used.

In the retuned-back questionnaires the questionnaires which contained at least one answer about MSDs YES or NO is recorded as a responder. From the 195 distributed questionnaires 161completed questionnaires were retuned back with the response rate of 82.5%. The SPSS software was used for processing and analysing the data.

GHQ score:

Scoring – L**ikert Scale 0, 1, 2, 3 from first** to **4th choice**.

12 items, 0 to 3 for each item

Score range is from 0 to 36.

Scores about 11-12 is typical, Score >15 suggest the evidence of distress and Score >20 suggests severe problems and psychological distress

1.6.3. Analysis

The answers to questions of MSDs in Nordic questionnaires including NO or YES were converted to 0 and 1. The minimum score of musculoskeletal disorders for each person was zero and maximum score was 20. And in the same way responses to smoking habits No or Yes were converted to 1 and 2. Also the age groups converted to 1, 2, 3, and 4.

For estimating the prevalence of MSDs as a whole, the person who had reported at least one disorder were considered as a case then the prevalence was calculated as follow: the number of cases/161 multiplied by 100 (161 is the total number of responses). In the same way the prevalence of who had at least two or more disorders were calculated. The prevalence of each disorder such as disorders of neck, shoulder, elbow, back, etc also calculated separately. For demonstrating the relationship between the numerical data such GHQ score and the prevalence of MSDs, the Pearson's correlation coefficient (r) estimation was used.

1.7. Results

1.7.1. Demographic data

According to demographic data 87% of valid cases were female, 3.1% of them were smoking and 76.4% were working as shift workers. 25.9% of responders were between 20-30 years old, 5o.6% were 30-40, and 21.5% were between 40-50 years old and only 1.4% of them were between 50-60 years. The range of experience was between 0-5 years to more than 25 years (table 1).

Table1. Demographic data of inpatient hospital nurses of National Iranian Oil Company (NIOC) grand hospital nurses – Ahvaz

Male	19 person	13%
Female	127 person	87%
Experience 0-5 years	39 person	24.7%
Experience 5-15 years	81 person	51.3%
Experience 15-25 years	37 person	23.4%
Experience > 25 years	1 person	0.6%
Age between 20-30 years	41 person	25.9%
Age between 30-40 years	80 person	50.6%
Age between 40-50 years	34 person	21.5%
Age between 50-60 years	3 person	1.9%
Non shift workers	37 person	23.6%
Shift workers	120 person	76.4%
Non smoking nurses	156 person	96.9 %
Smoking nurses	5 person	3.1%

1.7.2. Reliability of the questionnaires:

The reliability of Nordic questionnaire for MSDs and GHQ 12 questionnaire by Cronbach's Alpha test was 83.5% and 87.1% respectively.

1.7.3. Prevalence of musculoskeletal disorders:

The prevalence of MSDs during the last week and over last12 month are demonstrated in tables 2 & 3 respectively.

In overall 90.1 percent of the responders reported that they had at least one disorder of musculoskeletal system during last week while 81.4 percent of them had at least two disorders and 65.8 percent had more than two disorders (Fig1).

As is demonstrated in figure 1 only 9.9% of responders were free of symptoms during last week. 19.3% had 3 disorders and 1.9% of them had complained from all of the disorders.

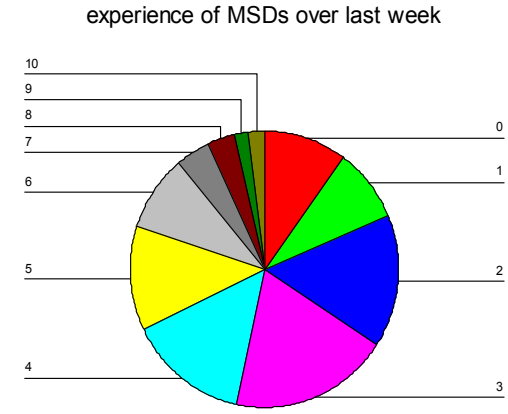

Fig1. Frequency of nurses who had experienced o, 1, 2, 3, ….of MSDs during last week in grand hospital of NIOC in Ahvaz

1.7.4. General Health statement

From the 161 responders 10.5% of them had a 15<score ≥ 20 which suggests the evidence of distress, and 4.3% of them had a score more than 20 which suggests severe problem and psychological disorders (Table 4).

The minimum score of GHQ was zero which is the indicator of best general health state, while the maximum score was 34 which indicate the poor general health state. The mean of GHQ score was 10.8(table 5), the distribution of GHQ scores from zero to 34 is demonstrated in figure 2.

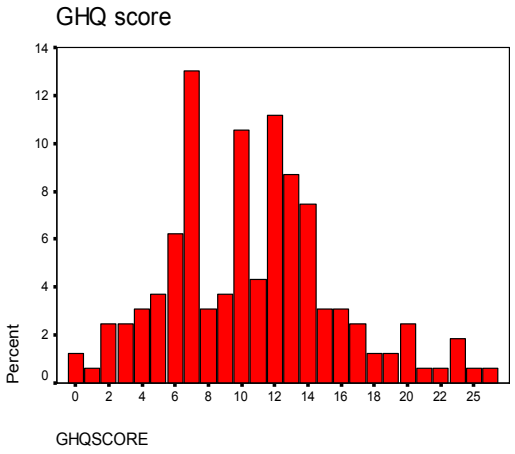

Fig2. Distribution of GHQ score among inpatient hospital nurses of NIOC's grand hospital in Ahvaz

1.7.5. Correlation analysis

Is there any correlation between GHQ score and MSDs?

By Pearson's correlation test between MSDs and general health state, there was a strong positive correlation between poor general health state (high GHQ score) and the prevalence of MSDs during last 12 month and last week separately and in combination, with the confidence of more than 99% (table 6).

The detailed correlation test between each site of body disorders during last week (table 7) and last 12 months (table 8) and general health score demonstrated that some disorders are more affected by general heath state than others. For example the correlation between poor general health state and musculoskeletal disorders of foot/ ankle, hip/ thigh, lower back and upper back are stronger than other disorders over last week while there was not any correlation between neck disorders in last week and general health state.

The correlation between poor general health state and all of the musculoskeletal disorders over 12months was strongly positive except for neck, elbow, hand and wrist disorders (table 8). The correlation between hand/wrist disorders in last 12 months and poor general health was weakly positive but there was not any relationship between elbow and neck disorders in last 12 months and poor general health state.

1.8. Discussion

The data collected in this study was based on questionnaires and self reporting, and like to any other type of questionnaires, it measures the attitude and individual perception of pain and discomfort, pleasure, happiness and so on. For this reason the prevalence of MSDs among special group of workers in different countries even with the same hazards may be different due to their different attitude and perception.

The strongly positive correlation between poor general health statement and short term and long term MSDs (over last week and last year) supports the ideas of who believe that general health and psychosocial statement have an important role in developing MSDs. Psychosocial risk factors as well as leading to stress, which is a hazard in its own right, can lead to musculoskeletal disorders. The stress-related changes in the body (such as increased muscle tension) can make people more susceptible to musculoskeletal problems (13) (HSE book, 2000).

Table 2. Prevalence of MSDs in specific sites of the body over last week among inpatient hospital nurses of NIOC's grand hospital in Ahvaz

head	Neck	shoulder	elbow	Hand/wrist	Upper back	Lower back	Hip/thigh	knee	Ankle/foot
53.7%	45.3%	39.8%	14.9%	42.9%	32.3%	37.9%	20.5%	46%	26.1%

Table 3. Prevalence of MSDs in specific sites of body over last 12 months among inpatient hospital nurses of NIOC's grand hospital in Ahvaz

head	Neck	shoulder	elbow	Hand/wrist	Upper back	Lower back	Hip/thigh	knee	Ankle/foot
62.1%	64%	44.1%	14.3	49.7%	41.6%	48.4%	25.5%	54.7%	37.9%

This relationship is also demonstrated in previous studies such as the study by Warming (14) (2009), which demonstrated that stress and transfer task are associated with low back pain. Another example is the study by Simon, et al (15) (2008), "back or neck- pain-related disability of nursing staff in seven European countries" which showed a pronounced association between psychosocial factors and back or neck-pain-related disability. In this survey as is demonstrated in tables 8 and 9 all of MSDs except neck, elbow and hand/wrist have a strong positive correlation with general health statement score.

As demonstrated in this survey general health score has a strong positive correlation to develop MSDs (nurses with poor general health more involved). It means that in any program for prevention of MSDs the general health state of employees should be considered.

Recommendations

There are some recommendations for reducing the prevalence of MSDs among nurses and its consequences, direct and indirect costs. These recommendations are to who have a role in prevention of MSDs such as policy makers, employers, clinicians and other stakeholders.

A. Identification of vulnerable group who suffering from some evidence or severe psychological problem and appropriate intervention to control it

B. Providing the psychosocial support and welfare facility to improve the motivation and mental health levels.

Table 4. Frequency of GHQ score among inpatient hospital nurses of NIOC's grand hospital in Ahvaz

GHQ Score	f_i	%
0	2	1.2
1	1	.6
2	4	2.5
3	4	2.5
4	5	3.1
5	6	3.7
6	10	6.2
7	21	13.0
8	5	3.1
9	6	3.7
10	17	10.6
11	7	4.3
12	18	11.2
13	14	8.7
14	12	7.5
15	5	3.1
16	5	3.1
17	4	2.5
18	2	1.2
19	2	1.2
20	4	2.5
21	1	.6
22	1	.6
23	3	1.9
25	1	.6
34	1	.6
Total	161	100.0

Table 5. Descriptive Statistics of GHQ score among inpatient hospital nurses of NIOC's grand hospital in Ahvaz

	Number	Minimum	Maximum	Mean	Std. Deviation
GQH SC.	161	0	34	10.76	5.306

Table 6. Correlations between job experience, MSDs and GHQ score among hospital nurses in NIOC's Grand hospital

Correlation analysis (Pearson Correlation)	MSDs over last12 months	MSDs over Last week& last 12months	Years of experience	GHQ score
MSDs over Last week	.715(**)	.917(**)	.143	.369(**)
MSDs over last12 months		.935(**)	.222(**)	.417(**)
MSDs over Last week& last12months			.200(*)	.426(**)
Years of experience				-.003

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Table 7. Correlation between GHQ score & MSDs in specific site of body over last week among hospital nurses in NIOC's Grand hospital of Ahvaz

MSDs in specific site of body →	ankle/ foot last week	Knee last week	hip/ thigh last week	lower back last week	upper back last week	hand/ wrist last week	elbow last week	shoulder last week	Neck last week	head last week
GHQ SCORE Pearson Correlation	.216 (**)	.190 (*)	.267 (**)	.224 (**)	.217 (**)	.188 (*)	.160 (*)	.185 (*)	. 038	.202 (*)

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

Table 8: Correlation between GHQ score & MSDs in specific site of body over last12 months among hospital nurses of NIOC's Grand hospital of Ahvaz

MSDs in specific site of body →	head 12m	neck 12m	shoulder 12m	Elbow 12m	hand/wrist 12m	upper back 12m	lower back 12m	hip/ thigh 12m	Knee 12m	ankle/ foot 12m
GHQ SCORE Pearson Correlation	.273 (**)	.118	.260 (**)	.129	.183 (*)	.266 (**)	.281 (**)	.228 (**)	.231 (**)	.333 (**)

** Correlation is significant at the 0.01 level (2-tailed), * Correlation is significant at the 0.05 level (2-tailed)

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Effect of Testosterone on Hind Limb Regeneration in Tadpoles of the Egyptian Toad, *Bufo Regularis* Reuss

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Abstract: The present study investigated the role of Testosterone on the regenerative capacity in two metamorphic stages of the tadpoles of the Egyptian toad, Bufo regularis Reuss, after amputation of the hind limb at the mid-shank level. It indicated an enhancing effect of Testosterone treatment on limb regeneration in the prometamorphic (stage 56), where 90% of the cases regenerated toes ranging from five to one compared with 77.3% in the control group, also the differential effect of testosterone on the number of toes was obvious in the treated animals, where 30% and 35% of the cases regenerated five and four toes respectively compared with 27.3% and 31.8% in the control group. In the metamorphic stage (stage58), the effect of testosterone was also obvious, where 38.6% of the treated cases restored toes compared with 13.3% of the cases in the control group. 45.5% of the treated cases restored part of the foot compared with 20 % of the cases in the control group. Histological observations of the treated limbs revealed that the formation of thick epithelial covering and complete skin is faster than that of the control animals. This may indicate that the enhancing effect of testosterone on limb regeneration, this may be due to the acceleration of wound healing either by its action upon the proliferative phase of healing which involves immune processes such as reepithelialization and angiogenesis or by the production of IGF-1 or by its stimulatory effect through Wnt/β-catenin signaling resulting in the initiation of the early phases of limb regeneration.
[Hamida Hamdi, Abdel-Wahab EL-Ghareeb, Alaa Shamakh and Sakina Saeed **Effect of Testosterone on Hind Limb Regeneration in Tadpoles of the Egyptian Toad, *Bufo Regularis* Reuss]** Life Science Journal,. 2011; 8(4): 416-429] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key Words: Limb regeneration, Amphibia, Testosterone.

List of Abbreviations:
As Astragalus **BL** Blastema **C** Cartilaginous collar **CD** Cellular debris **CF** Cartilage or procartilage formation **CP** Cartilaginous cap **E** Epiphysis **FC** Fibrocytes or fibrocellular accumulation **M** Muscle fibres or Muscle Group **MG** Multicellular glands **ML** Melanophores **MS** Mesenchymal cells **PH** Phalanges **SC** Scar of fibrocellular tissue **TF** Tibio-fibula **WE** Wound epithelium

Introduction

Limb regeneration is one of the best examples of organ/appendage regeneration in vertebrates and has been called 'epimorphosis' since it requires blastema formation and proliferation (**Brockes, 1997; Suzuki *et al*., 2006**) though there has been criticism of the classical definition of epimorphosis and morphallaxis (**Agata *et al*., 2007**). Among tetrapods, the cellular and molecular mechanisms involved in limb development are highly conserved, where fully developed limbs share a common skeletal pattern (**Muneoka and Sassoon, 1992**). On the other hand, the regenerative responses of limbs after amputation differ from animal to animal among tetrapods. Birds cannot regenerate limbs at any stage of development and, surprisingly, mammals have slightly better limb regenerative capacity than that of birds. Embryonic and neonatal mice can regenerate their digit tips if they are amputated through the distal phalanx (**Borgens 1982; Reginelli *et al*., 1995**), and similar digit tip regeneration occurs in humans (**Douglas, 1972; Illingworth, 1974**). After amputation at a more proximal level, a neonatal mouse cannot regenerate lost parts, and hypertrophy of amputated bones occurs (**Masaki and Ide, 2007**). In contrast, amphibians have exceptionally high regenerative capacity for limb regeneration. Urodele amphibians such as newts and salamanders can regenerate their limbs following amputation any time during their life cycles, although there is a non-regenerative mutant in axolotls (**Sato and Chernoff, 2007**). Anuran amphibians such as *Xenopus* are intermediate between urodele amphibians and other vertebrates in terms of their regenerative capacity, in that they can completely regenerate developing hind limb buds prior to the onset of metamorphosis, but regenerative capacity declines gradually as metamorphosis proceeds (**Dent, 1962; Muneoka *et al*., 1986; Suzuki *et al*., 2006**).

Many investigations have dealt with the factors affecting either retardation or enhancement of the regenerative capacity among urodeles and anurans, by using several experimental means such as mechanical, electrical, chemical, and hormonal means.

Hormones as well as hormone-like growth factors are well known to promote cellular differentiation and regeneration (**Leon *et al.*, 1998; De luca *et al.*, 1999**).

Dyson and Joseph (1968) concluded that the treatment of rabbit females with testosterone stimulates their regenerative growth in the ear.

Beran *et al.* (1982) concluded that testosterone and some of the synthetic analogs tested exert their hemopoietic effect, at least partly, by affecting the maintenance of erythroid and granulocytic stem cells, directly by increasing their survival or proliferation or indirectly by increasing the input from multipotent stem cell pool, or by both mechanisms.

Kinderman and Jones (1993) proved that testosterone propionate administration during facial nerve injury results in an increase in ribosomal levels in hamster facial motoneuron system (FMN).

Bardin, 1996; Katznelson *et al*., 1996; Bhasin *et al.*, 1997&2000; **Swerdloff and Wang, 2003** stated that treatment with testosterone improves muscle mass and strength, bone density, and reduces visceral fat in a variety of subjects.

Brown *et al*. (2001) suggested that testosterone enhances the rate of regeneration by increasing the neuronal cytoskeletal response after axonal injury. And suggested a common mechanism for gonadal steroid action on regenerating motoneurons across species.

Ustünel *et al.* (2003) determined that testosterone can induce protein synthesis in gastrocnemius muscle fibres, and induces changes in shape and size, and also can change the appearance and the number of fibres.

Sinha-Hikim *et al*. (2003) Concluded that testosterone -induced muscle fiber hypertrophy is associated with an increase in satellite cell number, a proportionate increase in myonuclear number, and changes in satellite cell ultrastructure.

Prokai-Tatrai *et al.*(2007) stated that testosterone can activate synthesis of bcl-2 protein, which prevents cell apoptosis in the injured regions.

Cayan *et al.* (2008) showed that testosterone has a significant role to increase bladder smooth muscle, leading to improvement in bladder functions in postmenopausal women with urogenital system dysfunction.

Little *et al*. (2009) suggested that testosterone has neuroprotective effects on morphology and function in both highly androgen-sensitive as well as more typical motoneuron populations, further supporting a role for testosterone as a neurotherapeutic agent in the injured nervous system.

Wilson *et al.*(2009) suggested that Testosterone has neuroprotective effects on morphology in both males and females.

Fu *et al.*(2011) suggested that testosterone promotes cell proliferation and differentiation via G protein-coupled receptors and different downstream pathways in the L6 cell line, although the related molecular mechanisms need to be elucidated in future studies.

The present study was deemed necessary in view of elucidating further the effect of testosterone on hind-limb regeneration in a prometamorphic stage (stage 56) and a metamorphic stage (stage 58) of the Egyptian toad, *Bufo regularis* Reuss after amputation at the mid-shank level.

2 Material and Methods:

Early tadpoles of *Bufo regularis* Reuss were collected from the ponds of Abou Rawash, Giza Governorate, Egypt. The tadpoles were reared in glass aquaria (60 x 30 x 30cm) in the laboratory at room temperature 27°C ± 2°C, in Department of Zoology, Faculty of Science, Cairo University.

Two stages were selected for this study. Staging of the individuals before the operation was carried out according to the normal table of **Sedra and Michael (1961)**. The selected stages were numbered 56 and 58. The most distinctive external criteria of these stages are as follows:

Stage 56 (A prometamorphic stage)

Age 28 days, length 25mm and tail is 14.5 mm long. Hind limb is about 1.9 mm in Length. Landmarks between thigh, shank and foot are more distinct. Melanophores are scattered on all toes and are especially dense on the last three toes.

Stage 58 (A metamorphic stage)

Age 39 days, length 30 mm and tail is 17 mm long. Elbow of right fore-limb is piercing or has already pierced the overlying skin. The left fore-limb has passed out through the wide, spout-like opening of the branchial chamber; thus this limb is now completely exposed. About 4-5 tubercles on the palm are prominent. The fourth toe is the longest. Web clearly developed between 2-3 and 3-4 toes.

Tadpoles of each stage were randomly divided into two groups: a control group and a treated one. Chloretone (Sigma) was used as an anaesthetic medium in tap water with the concentration 1:2000. Each operated case was then transferred to a Petri dish containing half concentration of the anaesthetic medium, then shortly to another Petri dish containing tap water in which the operated case recovered and became motile within few minutes.

For each group, amputation was carried out on the left hind-limb at the level of the mid-shank (Fig. 1). The right limb was kept intact. Amputation was carried out by using iridectomy scissors and fine watch-maker's forceps. Experimental tadpoles were injected intraperitoneally after amputation with Testosterone

propionate (Testone-E, Misr Co. for Pharm Ind., Cairo, Egypt) at a single dose of 5µl / individual.

To clarify the early post-operative histological changes within the stump tissues, individuals were selected and fixed at regular intervals, in a time series of 1, 3, 5, 7, 10 and 15 post-operative days, while the remaining tadpoles were left to reach two weeks after complete metamorphosis. A total of 250 cases were operated. Out of these, 80 cases were serially sectioned and studied microscopically.

The amputated limbs were then dehydrated through ascending grades of alcohol, cleared in cedar wood oil and finally embedded in parablast. Limbs were serially sectioned longitudinally at a thickness of 7 microns. The sections were stained with haematoxylin and eosin for general histological structures. A total of 160 cases (metamorphosed toadlets) were fixed, and morphologically examined.

To investigate the pattern of skeletal elements, transparencies of the operated limbs by using Victoria blue stain (**Bryant and Iten, 1974**) and Alizarin Red S stain (**Sedra, 1950**) were made.

Photomicrographs of representative sections and limbs of both control and treated animals were prepared at a known magnification.

3 Results

STAGE 56Group: 1/56 (Control group)A) Histogenesis of time-series•

By the first post-amputation day, the wound surface was covered with two or three layers of epithelial cells. Epithelial covering was thicker at the most distal tip of the regenerate. Nuclei of the epithelial cells were large and rounded. The basement membrane was indistinct. The activity of macrophages in removing cellular debris was noticed at the stump surface (Fig. 4).• By the third post-amputation day, the main bulk of the blastema was fibro-cellular in nature. Some blastemal cells began to redifferentiate into cartilage cells added to the stump skeleton .• By the fifth post-amputation day, the upper layer of the epidermis was cornified. Procartilaginous streaks were evident in the distal regions as an early indication of autopodial elements on the way to redifferentiate, with indentation at the most distal part of the regenerate as a first sign of toes formation. Muscles were surrounding the skeleton of the regenerate (Fig. 5).• By the seventh post-amputation day,the epidermis was stratified and the dermis was thin and multi-cellular glands were embedded within the dermis. The shank region was restored completely with its skeletal support. Distally, a chondrifying centre representing the foot skeleton was observed. Muscles were surrounding the skeleton of the regenerate. Lymph spaces were seen beneath the skin.• By the tenth post-amputation day, the shank was completely restored. Distally, long skeletal elements were redifferentiated representing the autopodial skeleton. Muscles were well-redifferentiated surrounding the skeletal elements. The skeletal elements were normally articulating with each other .• By the fifteenth post-amputation day, redifferentiation progressed distally resulting in the restoration of toes with their skeletal support (Fig. 6).

B) Final cases

i) General morphological characteristics (Table 1 & Fig.2):

- 44 cases were operated. Out of these:

• 12 cases had regenerated five toes**.** One of them, that was demonstrated with Victoria blue stain, weak chondrification of phalanges of the 1st toe and the terminal phalanges of 2nd toe was observed (Fig. 7).

• 14 cases had regenerated four toes each. One of them, showed that all the regenerated limb segments were normal while the fourth toe was short (Fig. 8).

• Four cases had regenerated three toes each. One of them, that was demonstrated with Alizarin red preparation, astragalus and calcaneum were short and completely fused (Fig. 9).

• Two cases had regenerated two toes each.

• Two cases had regenerated one toe each.

• Two cases had regenerated part of foot.

• Four cases had regenerated part of the shank region with a tapering end.

• Four cases had regenerated part of the shank region with a blunt end.

ii) Histological observations

Two cases were studied microscopically. Both of them showed advanced histogenesis, restored normal skeletal elements with normal configuration and articulation between phalanges. Muscles were well-restored surrounding the skeletal elements (Fig. 10).

Group: 2/56 (Testosterone -treated group):

A) Histogenesis of time-series:

• By the first post-treatment day, a thick epithelial covering closed the wound surface. Basement membrane was indistinct; the wound cover was dermis free. Activity of macrophages in removing cellular debris was noticed. Dedifferentiation of muscles began around the stump skeleton (Fig. 11).

• By the third post-treatment day, the epidermal covering was two or three cells thick. Basement membrane was discontinuous. Unicellular glands were observed. The dedifferentiated mesenchymal cells formed a blastema. The whole regenerate was in the form of a cone (Fig.12).

• By the fifth post-treatment day, melanophores and multicellular glands were spread beneath the epidermis. Mitotic activities of the blastemal cells resulted in more elongation of the regenerate with its pointed distal tip.

• By the seventh post-treatment day, the tibio-fibula was completely restored. Distally, a chondrifying centre representing the autopodial skeleton was observed. Muscles were surrounding the skeleton of the regenerate (Fig. 13).
• By the tenth post-treatment day, more redifferentiation was observed. redifferentiation of muscles around the skeletal elements was observed
• By the fifteenth post-treatment day, further redifferentiation progressed distally resulting in the restoration of toes with its skeletal support. Skeletal elements were normally articulating with each other (Fig. 14).

B) Final cases:

i) General morphological characteristics (Table 1 & Figs. 2):

- 40 cases were operated:
• 12 cases had regenerated five toes each. One of them, that was demonstrated with Alizarin red preparation, weak ossification of terminal phalanges of 1st , 2nd ,3rd and 5th toes were noticed (Fig. 15).
• 14 cases had regenerated four toes each. In one of them, that was demonstrated with Victoria blue stain, most skeletal elements were strongly chondrified (Fig. 16).
• Five cases had regenerated three toes each. One of them, that was demonstrated with Victoria blue stain, it showed partial fusion between the basal phalanges of the 1st and 3rd toes with skeletal elements of foot region was noticed (Fig. 17).
• Two cases had regenerated two toes each.
• Three cases had regenerated one toe. In one of them, that was demonstrated with Victoria blue stain, chondrifying phalanges supporting the toe were obvious (Fig. 18).
• Two cases had regenerated part of foot.
• Two cases had regenerated part of the shank region with a tapering end.

ii) Histological observations:

Two cases were studied microscopically. In the first case, the one that regenerated three toes, restoration of most skeletal elements and soft tissues was well observed. In the second case; that regenerated two toes, most of the skeletal elements of the foot and toes were restored (Fig.19).

STAGE 58

Group: 1/58 (Control group)

A) Histogenesis of time-series

• By the first post-amputation day, the wound surface was covered with stratified epithelium. The epithelial cells were having large and rounded nuclei. Some activity of macrophages in removing cellular debris was noticed.
• By the third post-amputation day, the epithelial covering was two or three cells thick. Basement membrane was seen discontinuous. Dermis was still hardly seen. Mitotic activities of the blastemal mesenchyme cells were noticed (Fig. 20).
• By the fifth post-amputation day, few melanophores and multicellular glands were noticed. Cartilage redifferentiation was noticed on both sides of stump skeleton. The distal part of the blastema was still fibrocellular in nature.
• By the seventh post-amputation day, the upper layer of the epidermis was cornified.. Cartilage redifferentiation progressed at both sides of the tibio-fibula shaft. Mesenchymal cells of the blastema were still noticed at the distal end of the regenerate (Fig. 21).
• By the tenth post-amputation day, the upper layer of the epidermis was cornified. Melanophores and multicellular glands were noticed. cartilage redifferentiation progressed to form a cap above the collar, while blastema cells were still fibrocellular in nature.
• By the fifteenth post-amputation day, melanophores and multi-cellular glands were highly spread beneath the skin. A cartilaginous collar was formed around the distal end of the shaft of the tibio-fibula. The collar was extending apically to form a cap. A fibrous scar was surrounding the distal part of the skeleton (Fig. 22).

B) Final casesi) General morphological characteristics (Table 1 & Fig. 3)

-30 cases were operated. Out of these:
• Four cases had restored one toe each. In one of them, the shank region was straight with a thin foot ending with a toe-like protrusion. Upon demonstration with Victoria blue preparation, chondrifying phalanges supporting the toe were obvious (Fig. 23).
• Six cases had restored part of the foot. In one of them, that was demonstrated with Victoria blue stain the regenerated part of foot was small,chondrification was obvious at the base of foot (Fig. 24).
• Eight cases had restored the shank region with a tapering end. One of them, that was demonstrated with Alizarin red preparation, there was incomplete restoration of skeletal elements (Fig. 25).
• Eight cases regenerated part of the shank region with a blunt end.
• Four cases were negative.

ii) Histological observations

The examined case regenerated nearly the whole shank with a blunt end; it had a toe-like protrusion laterally, in which, multicellular glands and melanophores were well-spread within the skin. A large cartilaginous condylar cap was formed around the stump skeleton. Muscle fibres were surrounding the

skeletal tissue, but merging distally into fibrous scar (Fig. 26).

Group: 2/58 (Testosterone -treated group):

A) Histogenesis of time-series:

• By the first post-treatment day, the wound surface was covered with a thin layer of epithelial cells that were condensed at the distal margin of the stump. These cells were cuboidal with rounded nuclei while the most outer cells were squamous with flattened nuclei-basement membrane and dermis was not seen. Activity of macrophages in removing cellular debris was noticed (Fig. 27).

• By the third post-treatment day, the epithelial covering was two or three cells thick. Basement membrane was discontinuous. Some blastema cells were redifferentiated into procartilage cells above the stump skeleton, while other blastema cells were still undifferentiated having fibro-cellular nature. The regenerate ended with ablunt end (Fig. 28).

• By the fifth post-treatment day, epidermal covering was formed of thick stratified squamous epithelium with underlying thin dermis. Melanophores and multicellular glands were observed. Most of the blastema cells were redifferentiated into cartilage cells and were added to the stump skeleton, while fibrocellular tissue was still observed beneath the skin. Some muscle fibres were redifferentiated at the stump edges, surrounding the skeletal elements (Fig. 29).

• By the seventh post-treatment day, the upper layer of the epidermis was cornified, melanophores and multicellular glands were seen. The addition of cartilage cells to the stump skeleton resulted in the formation of thick collar around the tibio-fibula shaft. mesenchymal cells of the blastema were intermingled with fibres above the stump skeleton (Fig. 30).

• By the tenth post-treatment day, melanophores and multicellular glands were noticed. A chondrifying centre was formed distally and articulating with the cartilaginous cap. Muscle fibres were redifferentiated around the skeletal tissues. Loose fibrocellular connective tissue was obviously seen beneath the skin. The distal end of the regenerate was protruding outwards (Fig. 31).

• By the fifteenth post-treatment day, epidermis was cornified. The cartilaginous collar formed around the distal part of the shaft of tibio-fibula was extending apically to form a cap. Fibrous bundles were forming a scar underneath the skin (Fig. 32).

B) Final cases:

i) General morphological characteristics (Table 1& Fig.3):

- 44 cases were operated:

•Two cases had restored two toes, One of them, that was demonstrated with Victoria blue stain, the shank region was short and the foot was considerably ending with toes appeared as two fused small protuberances (Fig.33).

• 15 cases had restored one toe, in one of them, that was demonstrated with Victoria blue preparations, the lateral toe -like protrusion was supported by chondrifying phalanges (Fig.34)

• 20 cases had restored part of the foot. In one of them, that was demonstrated with Alizarin red preparations, complete restoration of tibio- fibula and no skeletal support at the restored part of foot was shown (Fig. 35).

• Six cases had restored the part of the shank region with a tapering end.

• One case had restored part of the shank region with a blunt end.

ii) Histological observations:

The examined case had regenerated part of the foot. A cartilaginous collar was formed representing the distal epiphysis of tibio-fibula and extending distally into a cartilaginous element which is the skeletal support of the foot part (Fig. 36).

4. Discussion

The present study aimed to investigate the effect of testosterone on the restoration of the regenerative capacity in a prometamorphic stage (stage 56) and a metamorphic stage (stage 58) of the Egyptian toad, *Bufo regularis* Reuss, after amputation at the mid-shank level. Selection of the experimental stages was based on the following: Stage 56 represents the prometamorphic stage, during which the regenerative capacity starts to drop down. Stage 58 represents the metamorphic stage, where the regenerative capacity is reduced or completely lost.

Scadding (1979) stated that neither gonadectomy, nor injections of testosterone or 17-beta estradiol, had apparent effect on the rate of regeneration or histological appearance of limb regenerates in the newt *Notophthalmus viridescens*. Neither promotion nor inhibition of limb regeneration was observed.

Tarsoly *et al*., (1979) concluded that testosterone exerts a direct peripheral effect on the callus cells, presumably on their enzyme system.

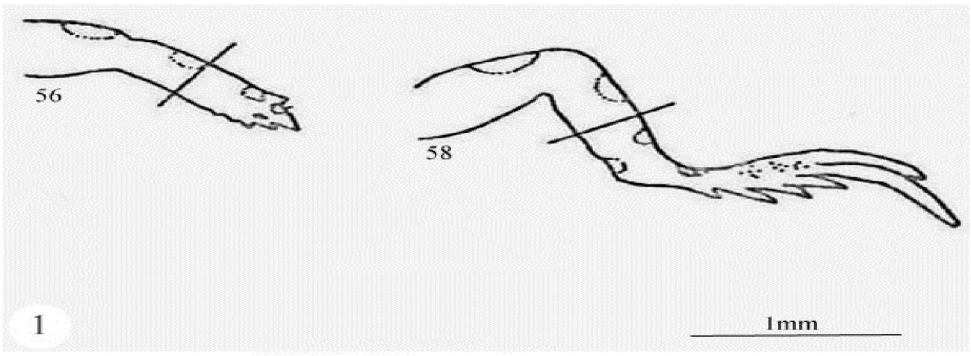
Vita *et al*.(1983) tested the effect of testosterone on the reinnervation of the anterior tibialis sciatic nerve following crush in rabbits. And showed that there is accelerative effect of Testosterone on the regeneration process.

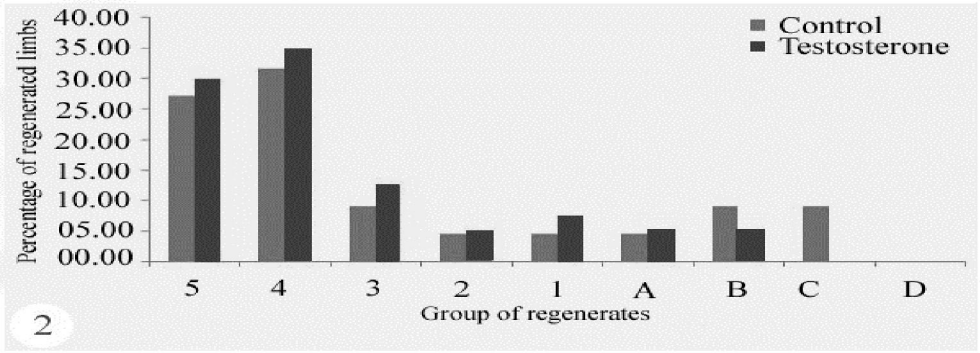
Sassoon *et al*. (1986) concluded that testosterone induces both chondrogenesis and myogenesis in juvenile larynx and that this process may contribute to the pronounced sexual dimorphism of the adult vocal organ.

Testosterone has a documented ability to modulate the activity of immune, fibroblast, and

myogenic precursor cells, which are all components of regeneration (**Grounds, 1987; Zhang *et al.*, 1998; Friedl *et al.*, 2000; Horiguchi *et al.*, 2002 and Schneider *et al.*, 2003**).

Jones *et al.*(2001) showed that exogenous administration of testosterone immediately after nerve injury impacts positively on the functional recovery through actions mediated by the androgen receptor. The mechanism by which steroidal enhancement of the regenerative properties of injured motoneurons occurs may involve pre-existing androgen receptors, heat shock proteins, and modulation of the cellular stress response.





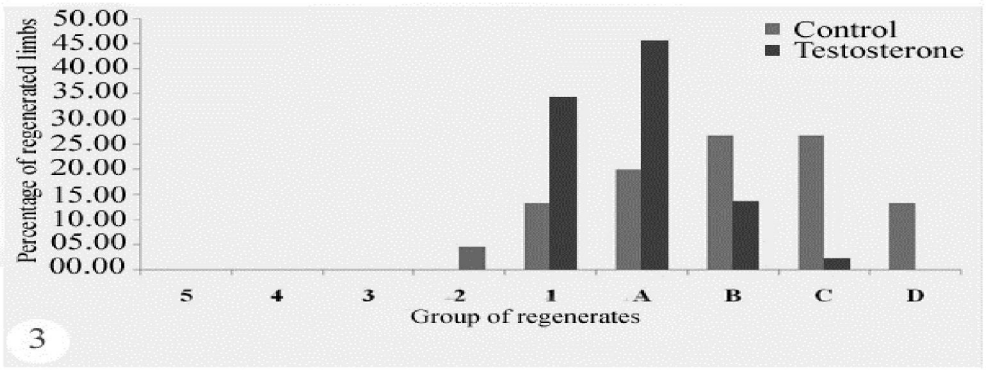


Fig. (1): Diagrammatic drawing of the left hind-limbs of stages 56 and 58 of the tadpoles of *Bufo regularis* Reuss, shown in antero-lateral view. The level of amputation is represented by a line transecting the mid-shank level.

Fig. (2): A histogram showing comparison between the regenerates of control and experimental tadpoles treated with Testosterone after amputation at the mid-shank of stage (56) of *Bufo regularis*.

Fig. (3): A histogram showing comparison between the regenerates of control and experimental tadpoles treated with Testosterone after amputation at the mid-shank of stage (58) of *Bufo regularis*.

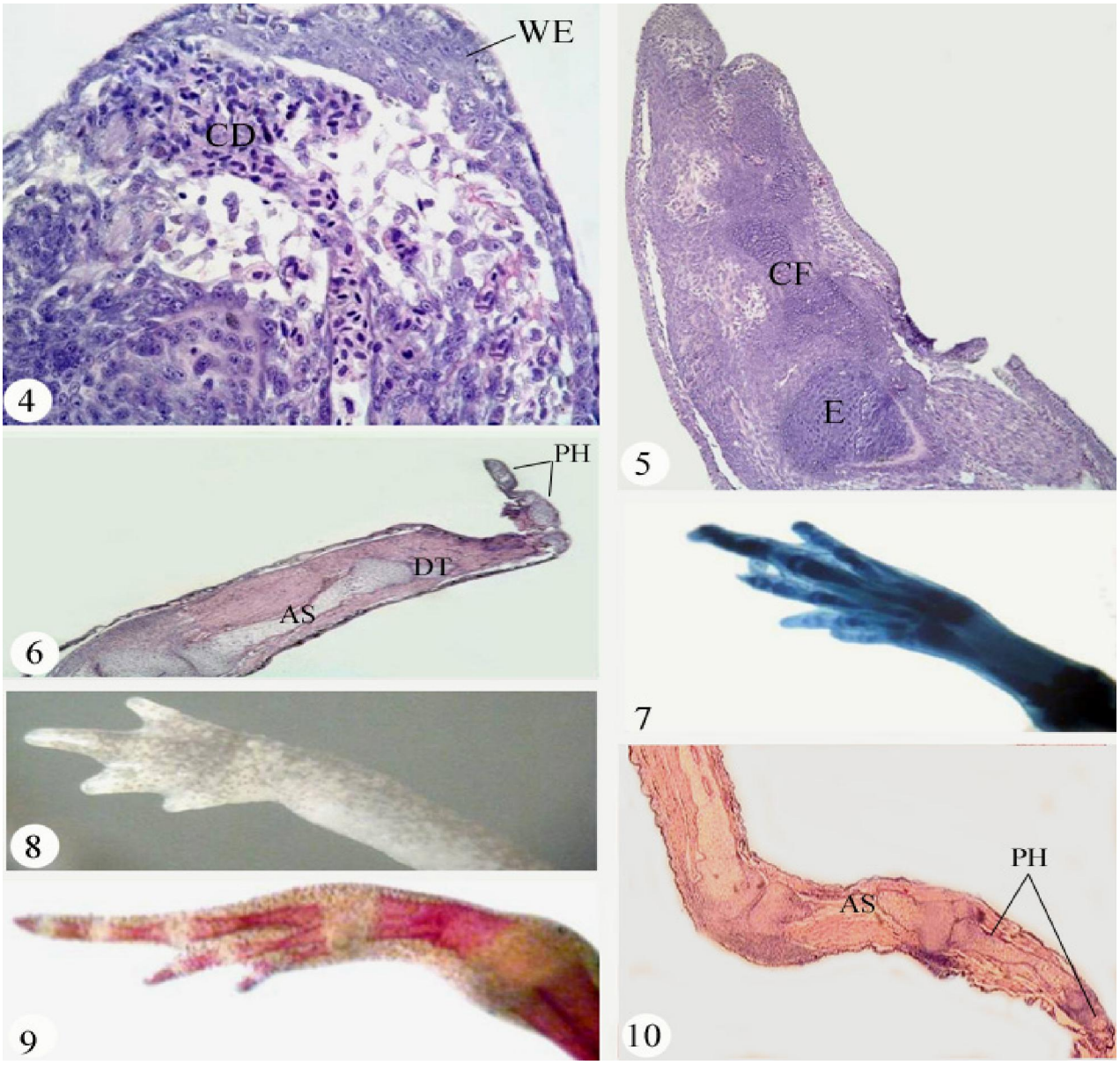


Fig. (4)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed one day after amputation. H&E stain (X 200).
Fig. (5)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed five days after amputation. H&E stain (X 100).
Fig. (6)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed fifteen days after amputation. H&E stain (X 100).
Fig. (7)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).
Fig. (8)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis(X 25).
Fig. (9)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Alizarin red stain (X 25).
Fig. (10)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed two weeks after metamorphosis. H&E stain (X 40).

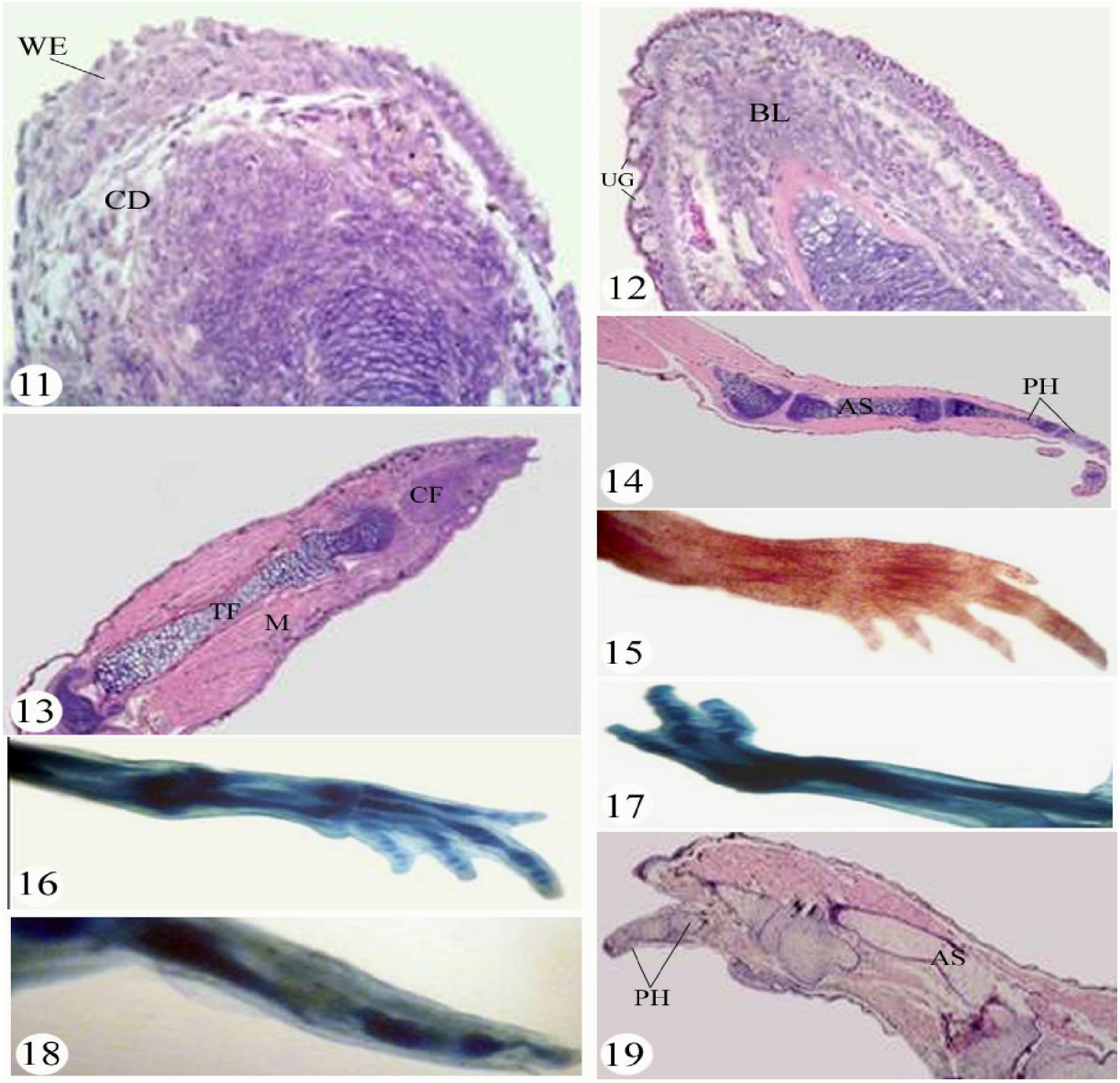


Fig. (11)**:** A photomicrograph of alongitudinal section of a regenerate of left hind limb, fixed one day after amputation. H&E stain (X 200).
Fig.(12)**:** A photomicrograph of alongitudinal section of a regenerate of left hind limb, fixed three days after amputation. H&E stain (X 200).
Fig.(13)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed seven days after amputation. H&E stain (X 40).
Fig. (14)**:** A photomicrograph of aLongitudinal section of a regenerate of left hind limb, fixed fifteen days after amputation. H&E stain (X 40).
Fig. (15)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Alizarin red stain (X 25).
Fig. (16)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).
Fig. (17)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).
Fig. (18)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).
Fig. (19)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed two weeks after metamorphosis. H&E stain (X 40).

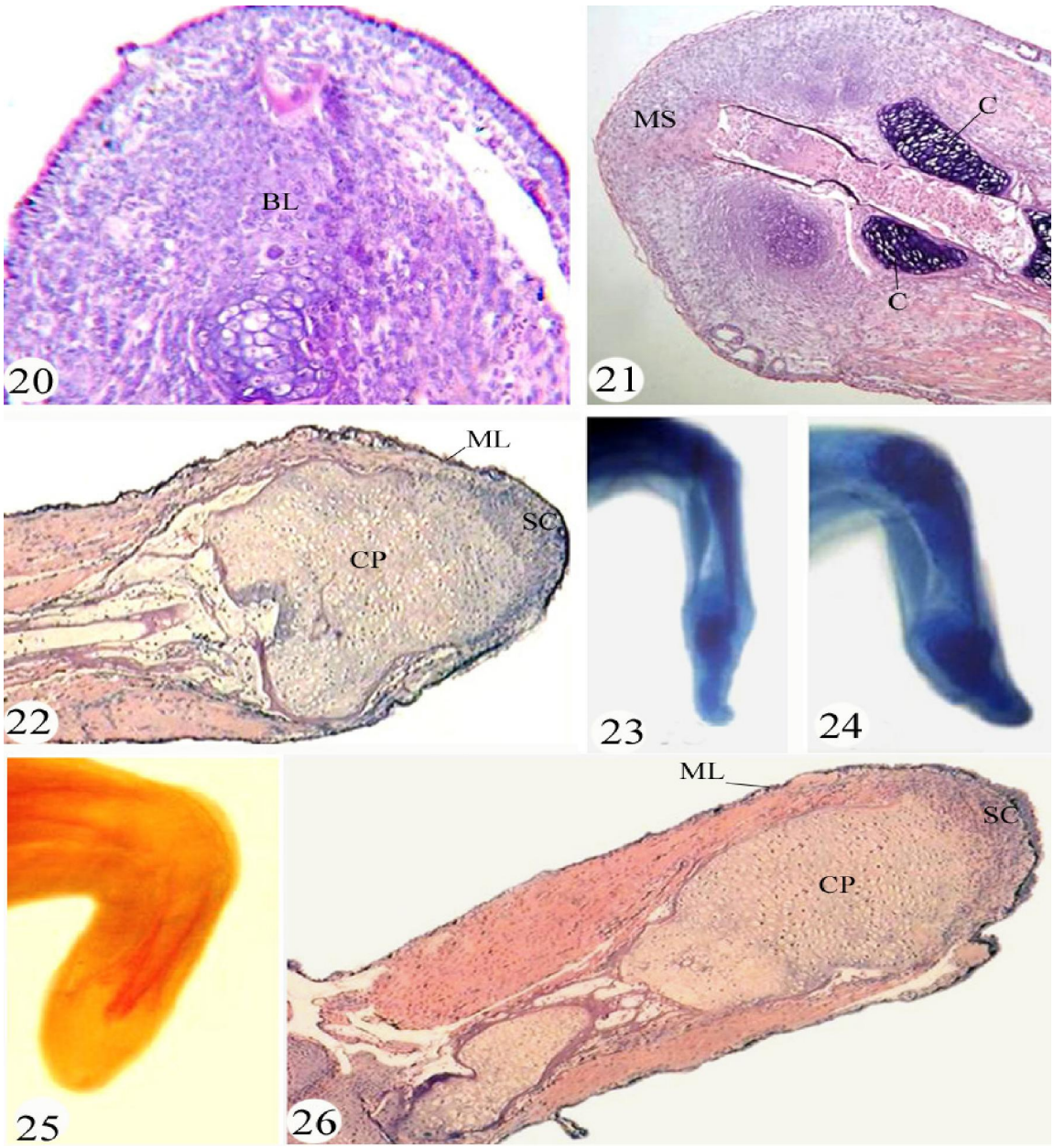


Fig. (20): A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed three days after amputation. H&E stain (X 100).

Fig. (21)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed seven days after amputation. H&E stain (X 40).

Fig. (22)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed fifteen days after amputation. H&E stain (X 100).

Fig. (23)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).

Fig. (24)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).

Fig. (25)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Alizarin red stain (X 25).

Fig. (26)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed two weeks after metamorphosis. H&E stain (X 40).

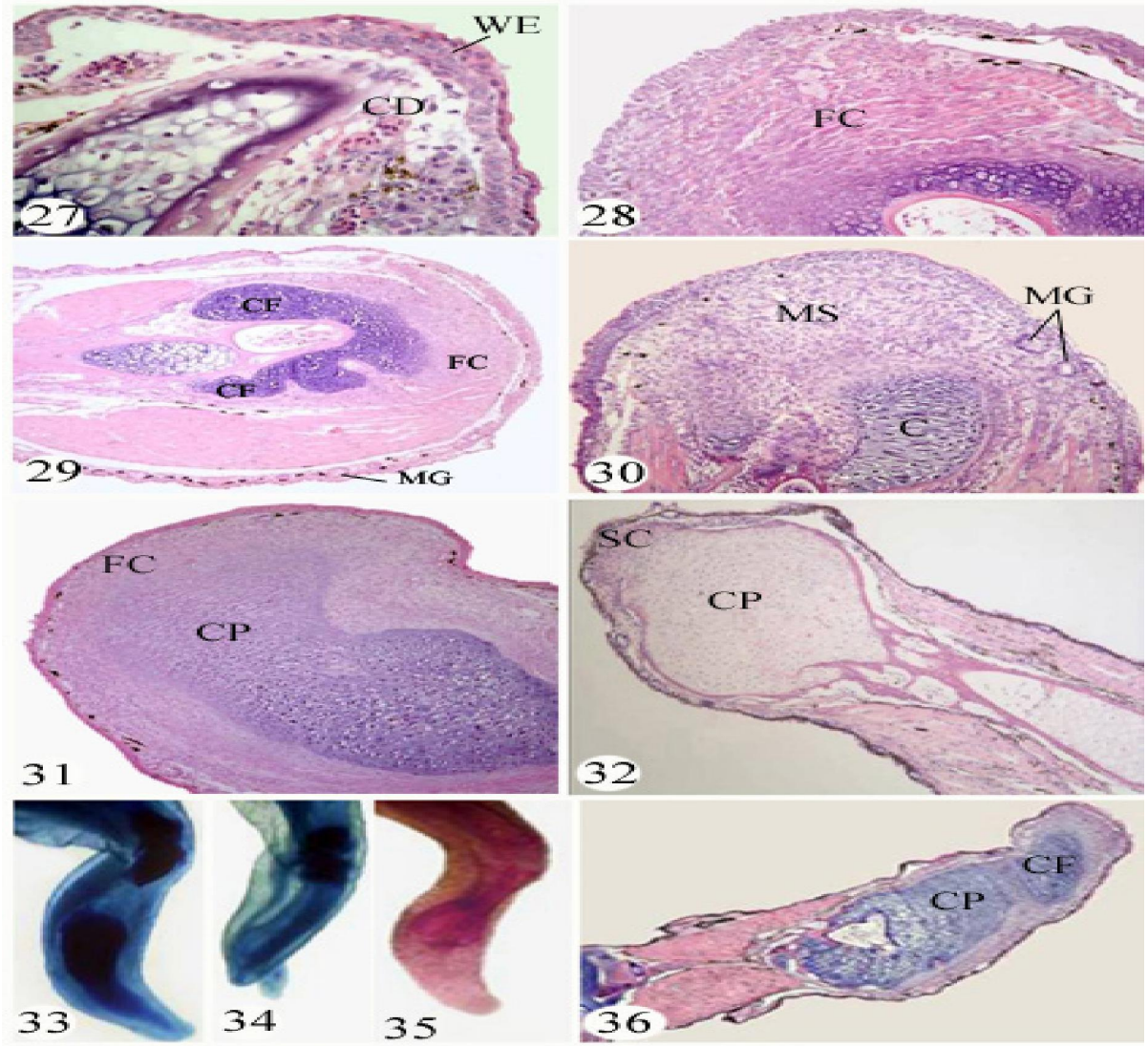


Fig. (27)**:** A photomicrograph of alongitudinal section of a regenerate of left hind limb, fixed one day after amputation. H&E stain (X 200).

Fig.(28)**:** A photomicrograph of alongitudinal section of a regenerate of left hind limb, fixed three days after amputation. H&E stain (X 200).

Fig.(29)**:** A photomicrograph of aLongitudinal section of a regenerate of left hind limb, fixed five days after amputation. H&E stain (X 100).

Fig.(30)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed seven days after amputation. H&E stain (X 100).

Fig.(31)**:** A photomicrograph of alongitudinal section of a regenerate of left hind limb, fixed ten days after amputation. H&E stain (X 100).

Fig.(32)**:** A photomicrograph of aLongitudinal section of a regenerate of left hind limb, fixed fifteen days after amputation. H&E stain (X 40).

Fig. (33)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).

Fig. (34)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Victoria blue stain (X 25).

Fig. (35)**:** A photomicrograph of a regenerate of left hind limb, fixed two weeks after metamorphosis and demonstrated with Alizarin red stain (X 25).

Fig. (36)**:** A photomicrograph of a longitudinal section of a regenerate of left hind limb, fixed two weeks after metamorphosis. H&E stain (X 40).

Testosterone generally has immunosuppressive and anti-inflammatory properties (**McCruden and Stimson, 1991; Giglio *et al.,* 1994; Wichmann *et al.,* 1997; Savita and Rai, 1998**), although there is evidence that Testosterone promotes inflammation in dermal wound healing (**Ashcroft and Mills, 2002; Ashcroft *et al*., 2003**).

Testosterone increases expression of the nerve growth factor (**Tirassa *et al.*, 1997**) and mediates promotion of neurite growth and interneural communication through branching and arborization (**Kujawa *et al.*, 1991**).

Phillip *et al.*(2001) conclude that testosterone has a direct, local, GH-independent effect on growth of the tibial epiphyseal growth plate and IGF-1 receptor abundance in hypophysectomized and castrated rats.

White *et al.* (2009) showed that Nandrolone decanoate (ND) (exogenous testosterone) administration can enhance castrated mouse muscle regeneration during the recovery from bupivacaine-induced injury. ND had a main effect for increasing muscle MyoD and cyclin D1 mRNA expression at 14 days.

The present results indicated an enhancing effect of testosterone treatment on limb regeneration in stage 56, where 90% of the cases regenerated toes ranging from five to one compared with 77.3% in the control group, also the differential effect of testosterone on the number of toes was obvious in the treated animals, where 30% and 35% of the cases regenerated five and four toes respectively compared with 27.3% and 31.8% in the control group.

In the metamorphic stage (stage58), the effect of testosterone was also obvious, where 38.6% of the treated cases restored toes compared with 13.3% of the cases in the control group. And 45.5% of the treated cases restored part of the foot compared with 20 % of the cases in the control group.

The present results agree with and support the results of (**Dyson and Joseph 1968; Vita *et al*., 1983;Grounds 1987; Zhang *et al.*, 1998; Friedl *et al.*, 2000; Horiguchi *et al.*, 2002 and Schneider *et al.*, 2003**). Who showed the accelerative effect of testosterone on the regeneration process.

Histological observations of the treated limbs revealed that the formation of thick epithelial covering and complete skin is faster than that of the control animals.

Demling (1999) found that anabolic agents, human growth hormone, HGH, and the testosterone analogue, oxandrolone, after severe burn injury, significantly decreased weight and nitrogen loss and increased healing with nearly identical benefits.

Testosterone is needed for the wound healing process since decreased levels impede healing (**Stanford *et al*., 1999; Demling, 2000; Demling and Orgill 2000**).

Karim *et al.* (1973); **Janssens and Vanderscheuren (2000)** demonstrated a significant increase in net protein synthesis, especially in muscle and skin, with high doses of Testosterone delivered parenterally.

Previous studies have shown the importance of testosterone on dermal wound healing (**Ashcroft and Mills, 2002; Ashcroft *et al.,* 2003**) and the modulatory effects of this hormone on immune responses (**Cutolo *et al.,* 2002; Palaszynski *et al.,* 2004**).

Robert and Demling (2005) showed that exogenous administration of anabolic agents ,human growth hormone, insulin-like growth factor-1, insulin, testosterone and its analogs maintained or increased lean body mass as well as directly stimulate the healing process through their anabolic and anticatabolic actions.

The anabolic properties of testosterone were defined in the 1930s. These include an increase in muscle size, synthesis, and strength. Increased skin thickness has also been noted with administration of testosterone to hypogonadal men. The importance of testosterone is evident by the complications seen with low Testosterone levels, which include sarcopenia or lost lean mass, increased rate of development of osteoporosis, anemia, thinning of skin , weakness, and impaired wound healing (**Carson-Jurica *et al.*, 1990; Kuhn, 2002 and Matsumoto, 2002**)

Engeland *et al.* (2009) suggested that human mucosal healing rates are modulated by testosterone levels. Based upon when between-group differences were observed, testosterone may impact upon the proliferative phase of healing which involves immune processes such as reepithelialization and angiogenesis.

Hobbs *et al.* (1993) indicated that 6 weeks treatment of normal men with testosterone leads to an increase in serum IGF-I levels.

IGF-1 is considered to be a wound healing stimulant, increasing cell proliferation and collagen synthesis **(Lieberman *et al.,* 1994; Lin *et al.,* 1998*;* Coerper *et al.,* 2001; Blumenfield *et al.,* 2002).**

From the conclusions of **Stanford *et al.* (1999); Demling (2000); Demling and Orgill (2000);Engeland *et al.* (2009)** it may be suggested that the enhancing effect of Testosterone on limb regeneration may be due to its acceleration of wound healing either by its action upon the proliferative phase of healing which involves immune processes such as reepithelialization and angiogenesis or by the production of IGF-1. **Bhasin *et al.* (2006)** proposed that testosterone could promote the differentiation of mesenchymal multipotent cells into the myogenic

lineage while inhibiting adipogenic differentiation by modulating nuclear translocation of β-catenin.

Singh *et al.*(2009) indicated that testosterone promotes the nuclear translocation of β-catenin through an AR-mediated mechanism in C3H 10T1/2 cells.

Hong *et al.*(2011) Concluded that testosterone regulates β-catenin protein level and proliferation rate in mesenchymal tumour(desmoid tumour).

Zhao *et al.*(2011) provided that Testosterone increases cellular β-catenin content which promotes the expression of β-catenin-targeted genes and myogenesis in the muscle-derived stem cells of cattle.

B-catenin is essential for adult skeletal muscle growth and regeneration *in vivo* (**Polesskaya *et al.*, 2003; Reya and Clevers, 2005; Armstrong *et al.*, 2006**)

Yokoyama *et al.* (2007) demonstrated that Wnt/β-catenin signaling plays an essential role during the early phases of limb regeneration and is important, but not absolutely required, during the subsequent phases of limb regeneration in *Xenopus*.

From the conclusions of **Bhasin *et al.* (2006) ; Yokoyama *et al.* (2007); Singh *et al.* (2009); Zhao *et al.* (2011)**. It is suggested that Testosterone may enhance the limb regeneration by its stimulatory effect through Wnt/β-catenin signaling resulting in the initiation of the early phases of limb regeneration.

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The Ogden Model for Coronary Artery Mechanical Behaviors

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Abstract: A nonlinear coronary artery Ogden model is proposed to describe the possible artery mechanical behavior during the stent expansion process. The artery model parameters were curve fitted from measured coronary artery circumferential stress-stretch curves. The proposed Ogden model parameters were compared with the data cited from other literature. The proposed Ogden artery model employed to simulate stent inflation and deflation during its expansion process. The numerical results reveal that the proposed nonlinear Ogden model feasibly simulates the stent expansion process.

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1. Introduction

Stent implantation has been applied to treat arterial stenosis in last few decades. The finite element method has been frequently used to investigate stretch behaviors of artery, plaque and metal stent during implantation. Proper artery and plaque mechanical models play an important role in the simulation. In many pioneering works, different stress-stretch relationships have been proposed to describe artery mechanical behavior. These proposed artery models have been extensively studied for many years with much data investigated for various biomedical applications. The inflation-extension tests were used to measure the mechanical properties of disease-free and diseased coronary arteries for surgical development [1-3]. The coronary artery stress-strain relationships yielded by inflation-extension tests were employed to compute the Green strain tensor components to establish a mathematical description of arterial behavior and determine the constitutive equation parameters for the different arterial wall layers [4-6]. Tensile tests were utilized to investigate the stress-stretch curves of different human iliac artery layers and plaque to establish a constitutive atherosclerotic artery model [7]. The mechanical properties of blood vessels of various sizes obtained using the tensile test were investigated. These blood vessels include human cerebral arteries and veins, and porcine abdominal aorta, vena cava, carotid arteries and iliac arteries [8-11]. The uniaixal and biaxial tension tests were used to measure arterial elastin mechanical properties for determining the strain energy function and to investigate the elastic behavior of porcine coronary artery tissue for stent design studies and stent implantation simulations [12-15].

The stent implantation has been applied to various arteries including intracranial arteries, coronary arteries, carotid arteries, iliac arteries and renal arteries [16-19]. The stent-graft insertion has been used to open abdominal aorta aneurysms, thoracic aorta aneurysms and aortic arch aneurysms [20-22]. Identifying the mechanical properties of arteries is necessary for stent implantation simulation research. Accordingly, the tensile stress-stretch curves of porcine coronary arteries were used to derive the artery model in this study. The nonlinear elastic Ogden strain energy function was employed to fit the measured data and applied to the finite element simulation of stent expansion in a coronary artery.

2. The Nonlinear Ogden Model of Coronary Artery

Based on the nonlinear solid mechanics, the principal stresses in an isotropic hyper-elastic material depend only upon principal stretches and can be expressed using the following relation [23],

$$\sigma_i = J^{-1}\lambda_i\left(\frac{\partial \Psi}{\partial \lambda_i}\right) \qquad for \quad i = 1,2,3 \tag{1}$$

where σ_i : is the principal stress.
λ_i : is the principal stretch.
Ψ : is the strain energy function.
J : is the product of $_1$, $_2$, and $_3$.

The strain energy function Ψ is a scalar-valued isotropic tensor function and can be expressed as bellows,

$$\Psi = \Psi(\mathbf{C}) = \Psi(\mathbf{B}) \tag{2}$$

where $\mathbf{C}$: is the right Cauchy-Green tensor.
$\mathbf{B}$: is the left Cauchy-Green tensor.

The right and left Cauchy-Green tensors can be

expressed using the deformation gradient **F** as the following equations, respectively.

$$\mathbf{C} = \mathbf{F}^{\mathrm{T}}\mathbf{F}, \quad \text{and} \quad \mathbf{B} = \mathbf{F}\mathbf{F}^{\mathrm{T}} \tag{3}$$

Because the biological soft tissue is an isotropic hyper-elastic material, the strain energy function may be expressed in terms of the independent strain invariants of symmetric Cauchy-Green tensors **C** and **B** as the following function

$$\Psi = \Psi\left[I_1(\mathbf{C}), I_2(\mathbf{C}), I_3(\mathbf{C})\right] = \Psi\left[I_1(\mathbf{B}), I_2(\mathbf{B}), I_3(\mathbf{B})\right], \tag{4}$$

Where the invariants I_i (i=1, 2, 3) can be expressed in terms of stretches as

$I_1 = \lambda_1 + \lambda_2 + \lambda_3$ (5a)

$I_2 = \lambda_1\lambda_2 + \lambda_2\lambda_3 + \lambda_3\lambda_1$ (5b)

$I_3 = \lambda_1\lambda_2\lambda_3$ (5c)

Therefore, the strain energy function can be expressed in terms of stretches as

$$= (\lambda_1, \lambda_2, \lambda_3) \tag{6}$$

A biological material can be regarded as incompressible with the constraint condition $I_3 = J = \lambda_1\lambda_2\lambda_3 = 1$. Based on the relation between the strain energy function and the stretches and the constraint condition of incompressibility, Ogden proposed the strain energy function as [23]

$$U = \sum_{n=1}^{N} \frac{\mu_n}{\alpha_n}\left(\lambda_1^{\alpha_n} + \lambda_2^{\alpha_n} + \lambda_3^{\alpha_n} - 3\right) \tag{7}$$

U denotes the strain energy function of the nonlinear elastic Ogden model. N is the item number. λ_1, λ_2, and λ_3 are principal stretches. μ_n and α_n are material constants used in the Ogden model.

The nonlinear elastic coronary artery Ogden model is curve fitted from the measured stress-stretch relationships of arteries in this study. To illustrate the feasibility of the proposed models, these curve fitted models were employed in the finite element analysis to simulate the inflation and deflation of a coronary stent during its expansion process.

Table 1. Measured data for different specimens in the CS test

specimen No.	measured dimensions			calculated values	loading rate (*mm/min.*)	strain rate (*1/sec.*)
	length (L_o) (*mm*)	width (W_o) (*mm*)	arterial wall thickness (t_o) (*mm*)	specimen volume (V_o) (mm^3)		
C1	13.3	4.39	0.725	79		0.023 %
C2	14.4	5.49	0.560	85		0.019 %
C3	14.0	5.73	0.570	88		0.019 %
C4	10.7	4.99	0.905	89	4	0.017 %
C5	12.4	5.71	0.900	119		0.016 %
C6	16.2	7.11	1.105	238		0.013 %
C7	15.9	7.40	0.780	175		0.015 %

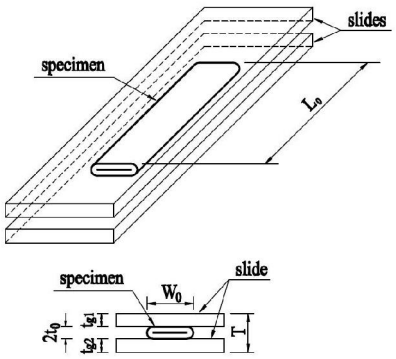


Figure 1. Dimension measurement for specimens used in the CS test

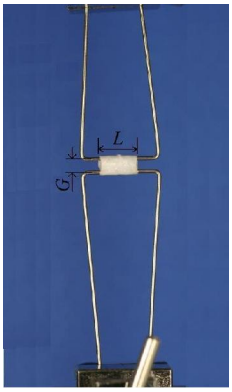


Figure 2. Measurement set up for the specimens in the CS test

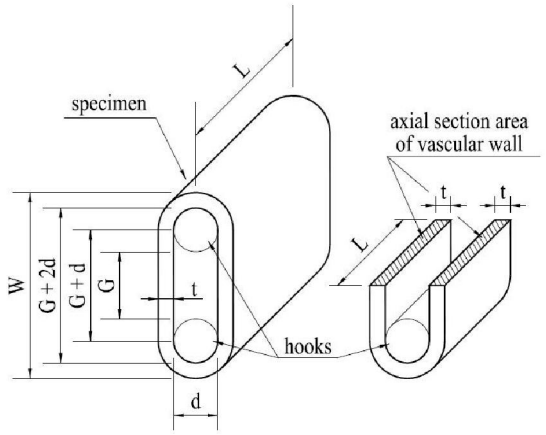


Figure 3. Sketch of artery specimen dimensions

3. Experimental Setup and Material Parameters in Ogden Model of Coronary Artery

3.1. Specimens for Tensile Tests

Porcine coronary arterial specimens were measured in this work. To maintain the artery mechanical properties from the live body to testing, all specimens were placed in phosphate buffered saline prior to testing. All tests were completed within 18 hours of slaughter [24]. The coronary artery specimens were from the anterior descending and circumflex coronary arteries. There were seven coronary specimens used for the arterial circumferential stretch (CS) test. The artery was placed between two thin slides as shown in Figure 1 to measure the specimen dimensions. The total thickness values (T), slide thicknesses (t_{g1} and t_{g2}), length L_o and width W_o were measured. The arterial wall thickness t_o was obtained by deducting t_{g1} and t_{g2} from T, as

$$t_o = \frac{T - \left(t_{g1} + t_{g2}\right)}{2} \qquad (8)$$

The coronary specimen shape was tubular. The specimen volume (V_o) was calculated from the measured length L_o, width W_o and arterial wall thickness t_o, as

$$V_o = L_o t_o \left[2\left(W_o - 2t_o\right) + \pi t_o\right]$$

$$\text{or} \quad = \frac{L_o \pi \left[D_o^2 - \left(D_o - 2t_o\right)^2\right]}{4} \qquad (9)$$

The outside diameter D_o of tubular specimen was estimated using Eq. (9) leading to

$$D_o = \frac{2}{\pi}\left[W_o + t_o\left(\pi - 2\right)\right] \qquad (10)$$

The measured data for all specimens in the corresponding CS tests are listed in Table 1.

The tensile tests were measured using a 500N SHIMADZU EZ-Test with the maximum stroke of 500 *mm* and measurement accuracy 0.01 *mm*. In the CS test the specimen was extended using two hooks as shown in Figure 2. The coronary arterial specimens were stretched and measured during the tensile test. A preload of 0.02 *N* was applied in these tests for the zero adjustment. The variation in tensile load and stroke were recorded continuously during the test. A high resolution digital camera was used to record the dimension variation. The correspondent stretch and stress variations were computed via the measured data. The loading speed in the CS test for coronary arteries was controlled at 4 *mm/min*.

3.2. Circumferential Stress-Stretch Curves in Arterial Circumferential Stretch (CS) Tests

During the arterial CS test, the specimen was stretched into an oval form as shown in Figure 3. The length L and the distance between hooks G were measured. To obtain the circumferential stress, the variation in sectional area of the specimen must be calculated during the test. Because the specimens were not deformed uniformly, an average sectional area was used in this work. Based on biological soft tissue being incompressible [7] assumption the specimen volume can be considered to be constant during loading. The variation in average cross section area of the measured specimen was estimated from the original volume and the measured specimen length L and distance between hooks G as shown in Figure 3. From the specimen shape, the average thickness t of the specimen during the test was derived using the following equation.

$$t = \frac{1}{\pi}\left[\sqrt{\left(G + d + \frac{\pi d}{2}\right)^2 + \frac{\pi V_o}{L}} - \left(G + d + \frac{\pi d}{2}\right)\right] \qquad (11)$$

where V_o : is the volume of specimen.

L : is the measured length of specimen during the tensile test.

d : is the hook diameter.

G : is the measured distance between hooks during the tensile test.

t : is the average thickness of arterial specimen during the tensile test.

The variation in sectional area A and the average circumferential true stress σ_c of the measured arterial specimen was computed as

$$\sigma_c = \frac{F_c}{A} = \frac{F_c}{2tL}, \qquad (12)$$

where F_c is the measured tensile load during the tensile test.

The corresponding circumferential stretch ratio value λ_c of the measured specimen was derived as

$$\lambda_c = \frac{C}{C_i} \qquad (13)$$

With

$$C = 2(G+d)+\pi(d+t), \qquad (14)$$

where $_C$: is the circumferential stretch of specimen during the tensile test.

C : is the mean circumference of specimen during the tensile test.

C_i : is the initial mean circumference of specimen with preload applied only.

4. Measured Results and Curve Fitted Ogden Model Parameters

A number of coronary artery specimens were measured in the CS tests. The measured load-elongation curves in the corresponding artery CS tests are plotted in Figure 4. The different sizes of the specimens resulted in different load-elongation curves. The corresponding variation in true stress and stretch curves are shown in Figure 5. The applied strain rates ranged from 0.013% to 0.023% per-second as listed in Table 1. The variation in measured data and the corresponding true stress of specimen C1 was tabulated and illustrated in Table 2.

The elastic modulus of a coronary artery can be approximated as the tangential slope of the measured stress-stretch curve. The measured stress-stretch curves indicate that the elastic modulus is sensitive to the load. Roughly, the modulus variation can be divided into four stages as shown in Figure 6. In the first stage, the elastic modulus value is low. When the stress curve rises over the heel over point (HOP) [11], the loaded coronary artery goes into the transitional portion, i.e. the second stage. Figure 5 presents the HOP of a coronary artery circumferential curve located in the stretch range between 1.1 and 1.3. In the second stage a positive stress-stretch curve slope is observed. In other words, the modulus increases gradually with the load in this stage. An almost constant modulus remains in the following third stage. Generally, an abrupt modulus drop is observed after passing the peak load. An undulating stress-stretch curve is presented in the final fourth stage. However, the measured stress-stretch curves of some specimens, i.e. specimen C2 and C3, have only stages 1, 2 and 3 as shown in Figure 5. Most coronary arteries have a stress reduction when the stretch reaches around 1.4. In arterial histology, the main arterial wall composition elements are elastin, collagen and smooth muscle [25]. Collagen strength is much greater than that of elastin and smooth muscle. Therefore, some softer tissues failed during the test and the remaining tougher tissues continued to bear the load. This may be the reason for the stress undulation in the fourth stage as shown in Figures 5 and 6.

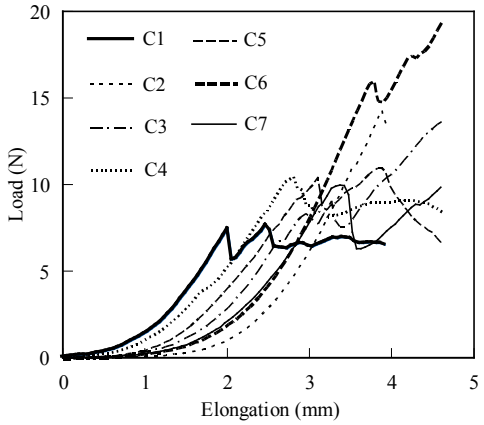


Figure 4. The variation in measured load-elongation curves for coronary artery specimens

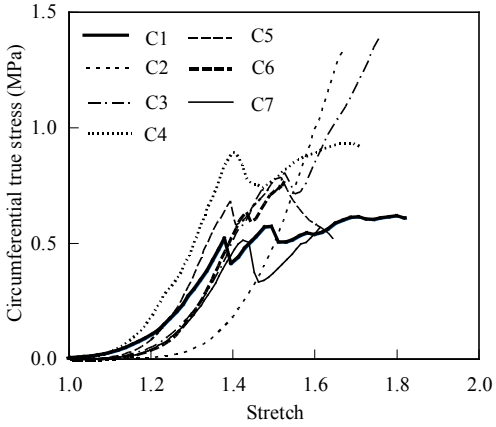


Figure 5. The variation in measured stress-stretch curves for coronary artery specimens

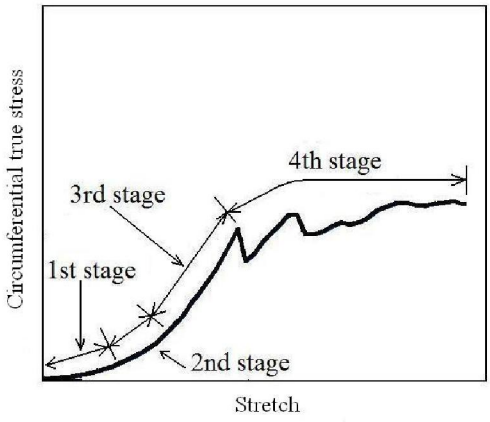


Figure 6. The four stages of modulus variation in measured stress-stretch curve

Table 2. Measured results for the coronary artery specimen C1 in the circumferential stretch test

measured data			calculated values				
length (L)	distance between two hooks (G)	load (f_C)	arterial wall thickness (t)	axial sectional area (A_A)	specimen's girth at half thickness (C)	circumferential true stress ($_C$)	cir. stretch ($_C$)
(mm)	*(mm)*	*(N)*	*(mm)*	*(mm^2)*	*(mm)*	*(N/mm^2)*	
10.99	0.13	0.000	0.89	19.36	8.17	0.00	1.00
10.99	0.14	0.025	0.88	19.25	8.19	0.00	1.00
10.99	0.17	0.054	0.87	19.14	8.22	0.00	1.01
10.97	0.28	0.119	0.86	18.77	8.38	0.01	1.03
10.93	0.52	0.268	0.82	17.95	8.76	0.01	1.07
10.92	0.69	0.454	0.80	17.44	9.02	0.03	1.10
10.90	0.78	0.592	0.79	17.15	9.17	0.03	1.12
10.89	0.96	1.000	0.76	16.63	9.46	0.06	1.16
10.87	1.11	1.391	0.75	16.21	9.70	0.09	1.19
10.84	1.28	1.963	0.73	15.77	9.98	0.12	1.22
10.76	1.41	2.638	0.72	15.41	10.21	0.17	1.25
10.70	1.56	3.534	0.70	15.03	10.47	0.24	1.28
10.70	1.69	4.413	0.69	14.73	10.68	0.30	1.31
10.70	1.84	5.435	0.67	14.39	10.93	0.38	1.34
10.65	2.05	7.208	0.65	13.93	11.30	0.52	1.38
10.65	2.22	5.943	0.64	13.59	11.57	0.44	1.42
10.65	2.37	6.735	0.62	13.29	11.83	0.51	1.45
10.57	2.51	7.378	0.62	13.02	12.09	0.57	1.48
10.55	2.67	6.382	0.60	12.72	12.37	0.50	1.51
10.55	2.83	6.367	0.59	12.44	12.64	0.51	1.55
10.51	2.99	6.585	0.58	12.17	12.93	0.54	1.58
10.44	3.15	6.482	0.57	11.89	13.23	0.55	1.62
10.37	3.30	6.788	0.56	11.65	13.51	0.58	1.65
10.29	3.46	6.935	0.55	11.40	13.80	0.61	1.69
10.19	3.63	6.704	0.55	11.15	14.11	0.60	1.73
10.11	3.78	6.624	0.54	10.92	14.40	0.61	1.76
10.05	3.87	6.607	0.54	10.80	14.57	0.61	1.78
9.99	3.95	6.576	0.53	10.69	14.72	0.62	1.80
9.91	4.04	6.399	0.53	10.56	14.90	0.61	1.82

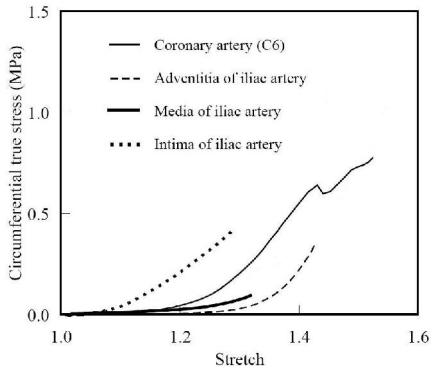


Figure 7. The measured stress-stretch data from specimen C6 and the published data

In anatomy, the artery includes three main layers i.e. adventitia, media and intima [25]. The measured stress-stretch curve of specimen C6 was compared with the published stress-stretch curves for an iliac artery [7], as shown in Figure 7. The results in Figure 7 indicate that the data measured from the proposed CS test agrees with the published data [7]. Within the stress range from 0.2 to 0.4 *MPa*, the adventitia and intima moduli vary within 2.0 and 4.8 *MPa*. This agrees with the aforementioned modulus range for coronary arteries.

For simplicity, the coronary artery is considered to be extended in the circumferential direction. The axial deformation effect is so small that it can be ignored. Therefore, the nonlinear strain energy

function, i.e. Equation (7), was introduced to derive the stress-stretch relationships of coronary artery and plaque in simulation. Based on the coronary artery incompressibility assumption, i.e. J = $\lambda_1 \lambda_2 \lambda_3$ = 1, three principal stretches can be approximated as $\lambda_2 = \lambda_3 = \lambda_1^{-1/2}$ in uniaxial tensile condition. The corresponding uniaxial true stress can then be expressed in terms of uniaxial stretch as

$$\sigma_1 = \sum_{n=1}^{N} \mu_n \left(\lambda_1^{\alpha_n} - \lambda_1^{-\frac{\alpha_n}{2}} \right). \qquad (15)$$

For getting a good curve fitting and also considering computational efficiency, the item number N=2 was used in this study. It leads to

$$\sigma_1 = \mu_1 \left(\lambda_1^{\alpha_1} - \lambda_1^{-\frac{\alpha_1}{2}} \right) + \mu_2 \left(\lambda_1^{\alpha_2} - \lambda_1^{-\frac{\alpha_2}{2}} \right) \qquad (16)$$

Table 3. Curve fitted Ogden model parameters for coronary artery and plaque

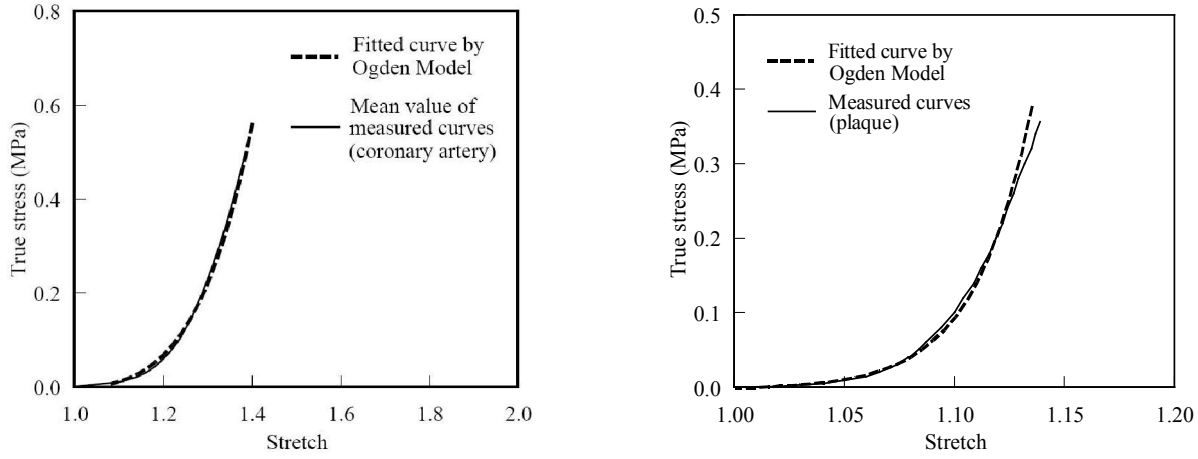
	(MPa)	(MPa)		
artery*	1.755×10^{-2}	-11.39	10.79	1.661×10^{-2}
plaque**	1.437×10^{-3}	4.366×10^{-9}	45.03	32.48

*: The Ogden model parameters fitted from the measured data proposed in this study.

**: The Ogden model parameters fitted from the published data [7].

The least-squares method was applied to curve fitted all the material parameters, and from the measured coronary artery circumferential stress-stretch curves. The mean stretch value of different curves, corresponding to same true stress, was computed first. The stress from 0 to 5.0 N/mm^2, 20 equally-spaced points were chosen for computing the mean value of measured curves as shown in Figure 8(a). The same method was applied to curve fit the measured plaque data which cited from the literature [7]. The extracted material constants are listed in Table 3. The difference between the measured and fitted plaque curves is shown in Figure 8(b).

The feasibility of the proposed Ogden mechanical coronary artery model is illustrated in this work using a stent implantation process simulated using the elastic-plastic finite element method. The traditional Palmaz type stent, the initially folded balloon and discontinuous distributed plaque were included in the simulation model. Figure 9 shows the *1/16* symmetrical three-dimensional model with a *1/8* circumferential and *1/2* axial symmetry component. The corresponding dimensions and material properties of the different parts in the finite element model are listed in Table 4. The simulated stent implantation under different pressures is shown in Figure 10. The stent diameter variation in the inflation and deflation process can be simulated using the proposed coronary artery material properties. The artery also causes an elastic recoil in the stent diameter during the deflation process. The simulated results indicate that the proposed Ogden coronary artery mechanical model is adequate to simulate the stent implantation process.



(a) Coronary artery (b) Plaque

Figure 8. Ogden model fitted stress-stretch curves for coronary artery and plague

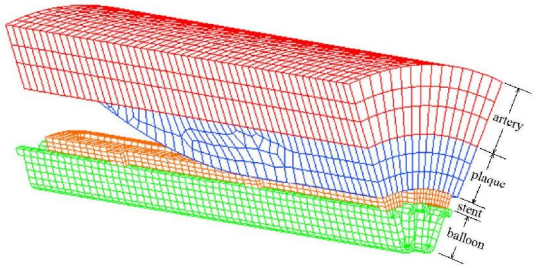


Figure 9. The 1/8 circumferential and 1/2 axial symmetry finite element model for stent implantation simulation

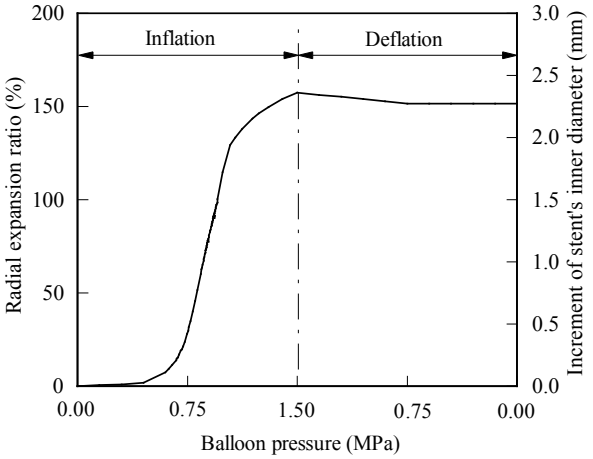


Figure 10. The simulated stent implantation under different pressures

Table 4. Component dimensions and material properties in the illustrated example

item	arterial wall	plaque	stent	balloon
outer diameter (*mm*)	3.5	2.5	1.7	nil
thickness (*mm*)	0.5	0.4	0.1	0.05
length (*mm*)	4.284	3.360	4.284	4.284
material model	nonlinear (Ogden)	nonlinear (Ogden)	elastoplastic	linear elastic
Young's modulus (*GPa*)	nil	nil	196	0.69
Poisson's ratio	nil	nil	0.3	0.3

5. Conclusions

This article proposed a nonlinear Ogden material model for the stent implantation process in a deformed coronary artery. The corresponding Ogden model parameters were derived by collecting the stress-stretch values from over seven coronary artery specimens. The data may be able to provide valuable samples for deriving the stress-stretch function of the coronary specimen when the applied load is not over the heel over point (HOP).

For validation, the proposed nonlinear Ogden material model for coronary artery was applied to a Palmaz type stent implantation process. The simulated stent deformation was found to be reasonable. It had a good correlation with the measured results. However, since the material parameters were derived only from coronary artery specimens, the use of the material parameters for different types of arteries should be done with caution.

Acknowledgments

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Analysis of Heavy Metals and Organic Pollutants of Ground Water Samples of South Saudi

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Abstract: The groundwater quality was determined in Jazan city southwest of Saudi Arabia, groundwater samples were selected during July 2010 (dry season). Selections of metals which may be toxic in excess when present in drinking water were further discussed (As, Cd, pb, Cu, Cr, Hg, Mn, Ni, Zn, Fe, and Se). Quantitative identification and determination of Total Petroleum Hydrocarbon (TPH), Hydrocarbon C_{10}-C_{40} (diesel hydrocarbon fraction), Volatile Organic Compounds (VOCs), Poly Aromatic Hydrocarbons (PAHs), Total Herbicides and Organochlorine Pesticides in samples based on applications of gas chromatograph (GC). The chemical analysis of groundwater samples show that all samples comply with WHO standards for the parameters measured. Overall the water quality is found to be suitable for drinking purposes without any prior treatment.

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Keywords: Groundwater; heavy metals; pesticides; WHO limits; Jazan.

1. Introduction

Water-related diseases are responsible for 80% of all illnesses/deaths in developing countries, and they kill more than 5 million people every year (**UNESCO, 2007**) Water, the precious gift of nature to human being is going to be polluted day by day with increasing urbanization. Although three-forth part of earth is being surrounded by water but a very small portion of it can be used for drinking purposes. Ground water is an important source of drinking water for humankind. It contains over 90% of the fresh water resources and it is an important reserve of good quality water. Ground water, like any other water resource, is not just of public health and economic value(**Armon *et al.,* 1994**). The water pollution by heavy metals has become a question of considerable public and scientific concern in the light of the evidence of their toxicity to human health and biological systems (**Anazawa *et al.,* 2004**). Heavy metals receive particular concern considering their strong toxicity even at low concentrations (**Marcovecchio *et al.,* 2007**). They exist in water in colloidal, particulate and dissolved phases (**Adepoju - Bello *et al.,* 2009**) with their occurrence in water bodies being either of natural origin (e.g. eroded minerals within sediments, leaching of ore deposits and volcanism extruded products) or of anthropogenic origin (i.e. solid waste disposal , industrial or domestic effluents) (**Marcovecchio *et al.,* 2007**). Some of the metals are essential to sustain life-calcium, magnesium, potassium and sodium must be present for normal body functions. Also, cobalt, copper, iron, manganese, molybdenum and zinc are needed at low levels as catalyst for enzyme activities (**Adepoju-Bello *et al.,* 2009**). Lead is a commutative poison and a possible human carcinogen (**Bakare - Odunola, 2005**), while for Mercury, toxicity results in mental disturbance and impairment of speech, hearing, vision and movement (**Hammer and Hammer, 2004**). But growth rate in population compared to olden days, industrialization and hence greater load of wastewater and use of numerous chemicals in industry and agriculture such as insecticides, pesticides, herbicides, hydrocarbons, fertilizers, petroleum products, plastics and polymers etc. have resulted in tremendous build up of organics in many forms in water. Many unexpected organics are reported to be found even in remote parts like hills of Himalayas, Alaska and North Pole due to man-made devastation of our environment. The environmental education has aroused much awareness about the toxicity of traces of environmental pollutants in general and organics in particular. The aim of this study was to investigate the quality of the ground water of the Jazan wells samples in the southwest of K.S.A.

2. Material and Methods:

Sample collection and analysis:

Samples of wells water were collected from two places in dry season (July 2010) from the study area. Before water sampling, all the glass bottles were cleaned and rinsed thoroughly with water to be analyzed.All reagents used were of analytical grade. Samples were unfiltered and the concentration of the different parameters could correspond to the total concentration of the ground water was used by the consumers for drinking. The ground water samples were stored at 1-4°C temperature prior to analysis in the laboratory. Extraction of water samples by liquid-

liquid extraction procedure according to EPA method no. 3510C. Clean-up of organic extract was carried out according to EPA method no. 3600C (**APHA, 2005**).

The apparatus used in the study:

Spectrophotometer Company Hach Lange/LPV 42299–00001. Gas Chromatograph (GC) HP 6890 equipped with ultra 1 HP column (25 m x 0.25 mm x 320 µm. Atomic absorption Spectra AAS Varian 220 equipped with several lamps.

3. Results and Discussions:

In Saudi Arabia, ground water is considered as the first water source for irrigation and other uses. Jazan, figure 1., is located in the southwest of K.S.A, along the red sea cost, the climate of the Jazan can be described as being hot, windy and arid with humidity due to the influence of red sea. The source of drinking water in Jazan city is only ground water. The aim of this study was to investigate the quality of the ground water. Samples were collected during July 2010 (dry season) from the Jazan wells.

Figure 1. Location map of the study area

The ground water from the study area had no color, odor and turbidity. The results of the chemical analysis of ground water from this area are presented in the following Table 1 . So, it is necessary to make a comparison of ground water quality of the study areas with drinking water standards. According to **lode, 1992**, a heavy metal is a chemical element with a specific gravity that at least, 5 times of the specific gravity of water , which is 1.0 at 4°C. However in medicine, heavy metals are loosely defined to include all toxic metals, irrespective of their atomic weight (**John, 2002**). In the light of these definitions, the metals determined in this project work, fall into the heavy or toxic metal category e.g. Iron has a specific gravity of 7.9, Lead = 11.34. In this reference, some of these metals, in tolerable concentrations, are very beneficial to consumers. Iron for example, is made as portion of some multivitamin drugs and products. On the other side, they become toxic when in excess, they are not metabolized by the body and they accumulate in the soft tissues. In cooperation with the U.S Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease registry (ATDR), has compiled a priority list called the Top 20 hazardous Substance. On this list, Lead (Pb) remains second to arsenic. Lead happens to be the only metal amongst the five, In addition, Lead and Mercury may cause the development of autoimmunity in which a person's immune system attacks its own cells. This can lead to joint diseases and ailment of the kidneys, circulatory system and neurons. At higher concentrations, Lead and Mercury can cause irreversible brain damage (**Lane *et al.*, 2000**). However, excess exposure to heavy metals can cause toxicity. Heavy metal can cause serious health effects with varied symptoms depending on the nature and quantity of the metal ingested (**Adepoju - Bello and Alabi, 2005**). They produce their toxicity by forming complexes with proteins, in which carboxylic acid (–COOH), amine ($–NH_2$), and thiol (–SH) groups are involved. These modified biological molecules lose their ability to function properly and result in the malfunction or death of the cells. When metals bind to these groups, they inactivate important enzyme systems or affect protein structure, which is linked to the catalytic properties of enzymes. This type of toxin may also cause the formation of radicals which are dangerous chemicals that cause the oxidation of biological molecules. The most common heavy metals that humans are exposed to are Aluminium, Arsenic, Cadmium, Lead and Mercury. Aluminium has been associated with Alzheimer's and Parkinson's disease, senility and presenile dementia. Arsenic exposure can cause among other illness or symptoms cancer, abdominal pain and skin lesions. Cadmium exposure produces kidney damage and hypertension.

The concentrations of all target elements are summarized in Table 1. The concentration level ranged from 0.0001mg/l to 0.01 mg/l. There was no detectable heavy metal in the wells water. For the protection of human health, guidelines for the presence of heavy metals in wells water have been set by different International Organizations such as USEPA, WHO, EPA, European Union Commission (**Marcovecchio *et al.*, 2007**), thus, heavy metals have maximum permissible level in water as specified by these organizations. Maximum contaminant level (MCL) is an enforceable standard set at a numerical value with an adequate margin of safety to ensure no adverse effect on human health. It is the highest level of a contaminant that is allowed in a water system.

(**WHO, 2000; Hammer and Hammer , 2004**).

Table 1 gives the summary of the results obtained in this study for concentrations of the major metals in the wells water.

Table 1: Major metal concentration in ground water samples

Elements	Unit	Mean of concentration
As	mg/l	<0.001
Cd	mg/l	<0.0001
Pb	mg/l	<0.0001
Cu	mg/l	<0.01
Cr	mg/l	<0.002
Hg	mg/l	<0.0005
Mn	mg/l	<0.01
Ni	mg/l	<0.001
Zn	mg/l	<0.005
Fe	mg/l	<0.01
Se	mg/l	<0.002

Pesticides consist of a large group of chemicals that are used in agriculture and residential settings to control plant and animal infestation. The use of synthetic organic pesticides has grown rapidly since the 1950s because, when used in conjunction with fertilizers, they increase crop yields. Pesticides are, however, a risk to human health. In the case of a pesticide spill or misapplication near a well, the levels of pesticides in drinking water may reach high enough levels to cause immediate health problems, such as the damage of the nervous system, some pesticides can cause cancer; some can also result in birth defects, Thyroid Changes, Nervous System Effects, Blood Changes, Kidney and Liver Changes. Maximum Contaminant Levels (MCLs) have been established by the USEPA and NJDEP for many pesticides. MCLs are set at levels well below those known to cause harmful health effects. MCLs are limits that public water systems are required to meet by law. For most pesticides, In addition, USEPA has issued Health Advisories for many pesticides.

Table 2. Show that by using standards USEPA and NJDEP all of the wells water samples within the optimum value. This would indicate that most wells are suitable to use as drinking water.

Table 2. Results of pesticides in wells water samples.

Parameter	Water sample
Monocrotopho	47.4 ng/l
Bioaltherin	286.2 ng/l
Primiphose	287.04 ng/l
Pyrozophose	666.2 ng/l
Fenthion	ND
Permethrine	ND
Total	1286.84

ND: less than detection limit.

One of the best known classes of ground water contaminants includes petroleum-based fuels such as gasoline and diesel. Nationally, the U.S. Environmental Protection Agency (EPA) has recorded that there have been over 400,000 confirmed releases of petroleum-based fuels from leaking underground storage tanks. Gasoline consists of a mixture of various hydrocarbon that dissolve to some extant in water, and often are toxic. Table 3 shows that Using standards USEPA all of the wells water samples within the acceptable limits. This would indicate that most wells are suitable to be used as drinking water.

Table 3. Organic results of wells water samples.

Parameters	Water sample
Total Petroleum Hydrocarbon (TPH)	230.41ug/l
Hydrocarbon C_{10}-C_{40} (diesel Hydrocarbon fraction)	144.59 ug/l
Poly aromatic Hydrocarbons (PAHs)	ND
Volatile Organic Compounds (VOCs)	ND
Total Herbicides	ND
Organochlorine Pesticides	ND

ND: less than detection limit

Conclusion

Drinking water can be obtained from a number of sources, the one used often depending on the relative availability of surface water (such as rivers, lakes, and reservoirs) and ground water aquifers). In Jazan southwest Saudi ground water from wells is an important source of drinking water. However, in some cases ground water may contain chemical constituents hazardous to the health. This research give the summary of the results obtained in this study for concentrations of heavy metals level ranged from 0.0001mg/l to 0.01 mg/l. There was no detectable of heavy metal in the wells water. Both Pesticides and organic compound in wells water were within the optimum value using standards USEPA. It means that water is not polluted. This would indicate that most wells are suitable to be used as drinking water source. However, regular monitoring should be ensured by the authorities concerned.

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Comparative morphometric study on eight seed bug tribes of subfamily Rhyparochrominae (Hemiptera-Lygaeoidea- Rhparochromidae)

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Abstract: The detailed morphological characters of *Remaudiereana annuilpes* (Baerensprung) (tribe: Myodochini) are given as a model of the subfamily Rhyparochrominae, also the scanning electron microphotographs were presented to the metathoracic scent gland, abdominal trichobothria and armature of fore leg to this species. The following species *Emblethis gracilicornis* Puton [Gonianotini], *Lethaeus lethierryi* (Puton) [Lethaeini], *Lamprodema maura* (Fabricius) [Megalonotini], *Remaudiereana annulipes* (Baerensprung) [Myodochini], *Marmottania simonis* Puton [Ozophorini], *Phasmosomus priesneri* (Wagner) [Phasmosomini], *Dieuches mucronatus* (Stal) [Rhyparochromini] and *Stygnocoris breviceps* Wagner [Stygnocorini] were investigated to clear the comparative morphometric study of this subfamily. The results are arranged in tables and clarifiedwith labelled drawings and colored pictures to facilitate the determination of the main taxonomic differences between the eight tribes through their representatives in Egypt.
[Rawda M.Badawy, Salwa K.Mohamed Hassan H.Fadle, Sohair M. Gadalla and Rabab F.Sawaby **Comparative morphometric study on eight seed bug tribes of subfamily Rhyparochrominae (Hemiptera- Lygaeoidea- Rhparochromidae)**. Life Science Journal, 2011; 8(4):442- 461] (ISSN: 1097-8135).
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Key words: Rhyparochromin tribes, scanning microphotographs, *Remaudiereana annulipes* (Baerensprung) [Myodochini], colored pictured.

Introduction

Subfamily Rhyparochrominae, the small dull seed bugs constitutes the largest numbers and the most diverse species of the family Rhyparochromidae, superfamily Lygaeoidea, order Hemiptera (Aukema & Rieger, 2001); (Dobbs & Brambila, 2004) and Brailovsky (2009). It is a fertile field for investigation owing to the host associations of most species, many interesting cases of mimicry, ecological differentiation, are unknown and the degree of host-specificity is poorly understood (Schuh and Slater, 1995); (Cassis & Gross, 2002); (Wheeler,2003); (Cervantes & O'donnell, 2009) & (Namyatova et al, 2011).

Rhyparochromins are characteristically ground bugs living in seed litter below plants, there is a variable degree of host specificity and certainly great differences in habitat, but all species in this subfamily are seed feeders, some climbing herbaceous vegetation when seeds are available. Many species, difficult to collect in the field, frequently come to light (Slater & Baranowski, 1990). This large family can reduce 60-90% of fig seed germination depending on the species and the host species (Cervantes & Carranza, 2008).

The systematic of this subfamily is complex, based on difficult characters such as the position of the abdominal spiracles and of the trichobothria [Slater and Baranowski, 1990]. So the comparative morphological works provide an impetus for clear identification and classification of this subfamily.

2. Material and Methods

Materials for the investigations were collected by light traps, aerial nets and pitfall traps from El Gabal El Asfar (Qalubyia); Abu Rawash & Kafr Hakim (Giza); Qena and Saint Katharine (Southern Sinai). Some specimens were pinned and labeled indicating the date and site of collection, other specimens were preserved in 70% glyceride – ethanol for dissecting purposes. In order to prepare the genitalia, genital capsule (pygophore) was carefully removed from the body, softened in hot water for 10 minutes, and then transferred to warm 10% KOH solution, (Ashlock, 1963 and Slater, 1963). Drawings were made from parts mounted on microscopic slides or preserved in alcohol and glycerin, using binocular microscope and light microscope. Scanning electron microphotographs were presented to reveal the metathoracic scent gland, abdominal trichobothria and armature of fore leg in *Remaudiereana annulipes* (Baerensprung) (tribe: Myodochini). These structures were dissected, dehydrated in ethanol. After drying the materials mounted on stubs, coated with gold and photographed with aid of JEOL JEM-100 scanning electron microscope operating at an accelerating voltage of 25 Kv. Scanning was carried out in the

Central Laboratory, Faculty of Science, Ain Shams University.

3. Results and Discussion

General morphological characters (Fig.1):

Body length 5.5-6 mm, subovoid in shape, brownish yellow in color and wholly punctured.

Head and head appendages (Figs. 2-6):

Head capsule of the opistognathous type, with **vertex** slightly concave; **clypeus** slightly raised apically at middle; **juga** triangular in shape and shorter than **clypeus**. **Compound eyes**: laterally bulging. **Ocelli** two in number and lie much closer to eyes. **Antennae:** four-segmented, antennal tubercle 0.25 x as long as compound eyes, 1st - 3rd segments with long sparse spines, the 1st segment the thickest and shortest one, 2nd segment the longest one and 4th segment fusiform with dense large spines. **Mouth parts:** piercing sucking with **labrum** flap like extension; **stylets** hollow and look like slender tubes; **labium** four segmented proboscis provided with a dorsal groove which harbors the **stylets** when not used.

Thorax (Figs. 7-20). Dorsal side (Figs. 7&8). Pronotum large, exceeding in size any other body segment(Fig.7). **Mesonotum** consists of a median marginal **phragma**,

A subrectangular **prescutum**, a narrow **scutum** and a triangular **scutellum**, which is surrounded by a narrow **postscutellum**. The **Anterior** and **Posterior notal processes** are situated on **scutum** and **scutellum** respectively. Only **prescutum** is provided with one **middle suture** and two lateral **convergent sutures** (Fig.8). **Ventral side (Fig.9, 11-16).** Ventral side in each of prothoracic, mesothoracic and metathoracic segments is differentiated into lateral pleura and middle sternum. **Sternum** consists of a middle **basisternum** ending in a **sternellum**. Metathoracic segment with two scent glands, which are represented by an external **ostiolar pertreme** or **auricle** and surrounded by an **evaporative area**.

Legs (Fig. 10, 17&18). clothed with bristles and spines; **Coxae** short or truncate, cone shaped; fore **coxa** skull-like, with a basal arm on each side; **trochante**r one segmented; fore **femora** swollen with 4 strong arms on ventrolateral side, middle and hind **femora** simple; **tibiae** cylindrical and apically toothed with strong setae; **tarsi** three segments, the 1st segment the longest one and the 3rd segment ends in two sclerotized sickle shaped **claws** and two distinct pulvilli.

Wings (Figs. 19&20) Fore wing (Fig.19) of the hemelytra type, divided into a triangular **corium**; a narrow **clavus** and a distal part or a **membrane**. Its veins: **costa**, **subcosta**, **radius**, **median**, **cubitus** and **vannal**. **Axillary sclerites** three in number. The first and second ones broad and nearly of equal size while the third sclerite narrow and forked. **Hind wing (Fig. 20)** membranous and characterized with an oblong **discal** cell. Its veins: **costa**, **subcosta**, **hamus**, **radius**, **median**, **intervannal**, **cubitus, vannal** and **juga**. **Axillary sclerites** are four in number. The first, second and third ones broad, second and third ones nearly of the same size while the fourth sclerite is narrow.

Abdomen (Figs. 21-33): Ten segmented, with the first seven segments differentiated into: **Terga (Figs. 21&22):** provided with rudiments of two median abdominal scent glands and limited laterally by **connexivum**; **Sterna (Figs 23-28):** abdominal sterna with incomplete suture between 4th & 5th segment and characterized by presence of large spiracle, with 28 abdominal **trichobothria** , each **trichobothria** consists of cuticular filiform hair surrounded by circular area of dense papilli-like structures.

Genital segments: the eighth and ninth segments don't show distinct terga and sterna and are considered as genital segments, the tenth segment reduced into a very small ring carrying the anus.

Male genital segments (Figs. 29-31): Consist of: **Pygophore** sclerotized capsule derived from the ninth segment and overlapped by the ring like eighth segment (Fig.29). **Parameres** two symmetrical sclerotized structures attached posteriorly to the **pygophore**. Each paramere consists of a basal **shank** and an apical **blade** (Fig.30). **Aedeagus** (Fig. 31) contained in the **pygophore** and differentiated into:

Basal plate sac like body, horse-shoe shaped (**Conjunctiva)** with a delicate membranous part limited posteriorly with the **ejaculatory reservoir**, (**vesica**) consists of a proximal ejaculatory reservoir and a distal tubular part (**vesical tube**).

Female genital segments (Figs. 32 & 33): represented by an elbow like **ovipositor** connected to a **spermatheca**. **Ovipositor:** composed of a pair of **ventral valves** and a pair of **dorsal valves (Fig. 32)**. **Ventral valves** arising from the eighth segment and consist of a triangular **first gonocoxa** attached posteriorly to a narrow **first gonapophysis**. The valve is supported anteriorly with a cylindrical **gonangulum** and laterally with the **first armus**. **Dorsal valves** arising from the ninth segment and consist of a crescent shaped **second gonocoxa** attached posteriorly to the **second gonapophysis** and supported laterally with the **second armus**. The attachment of the **dorsal** and **ventral valves** takes place by the inter locking of the first and second rami to each other. **Spermatheca:** composed of an apical **spermathecal bulb** connected posteriorly to a longitudinal **spermathecal duct** (Fig. 33).

Sexual dimorphism:

Male smaller than female with body 5.5mm in length, trichobothria arranged on the abdominal sternites as follows: 6 trichobothria in two anterolateral groups on 3rd and 4th sterna, 6 trichobothria in two lateral groups on 5th and 6th sterna and 4 trichobothria in two lateral groups on 7th sternum. Apex of abdomen with rounded pygophore. **Female** larger than male with body 6mm in length, trichobothria arranged on the abdominal sternites as follows: 8 trichobothria in two anterolateral groups on 3rd sternum, 6 trichobothria in two anterolateral groups on 4th sternum, 4 trichobothria in two lateral groups on 5th sternum, 6 trichobothria in two lateral groups on 6th sternum and 4 trichobothria in two lateral groups on 7th sternum. Apex of abdomen narrow with ovipositor bends on 7th sternum.

Comparative morphometric study of the eight tribes in Tables(I-VIII)& Figs(34-95).

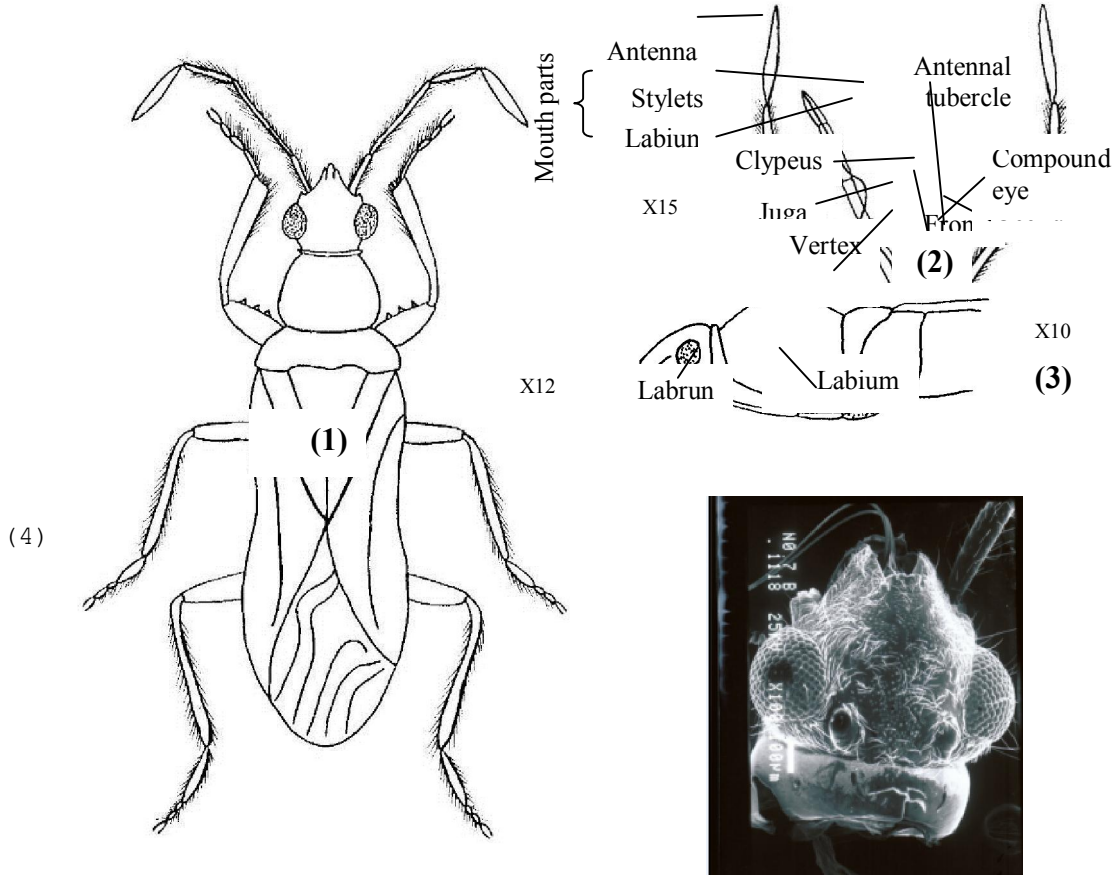
The comparative morphometric study of the 8 tribes of the subfamily Rhyparochrominae in Egypt are: Gonianotini, Lethaeini, Megalonotini, Myodochini, Ozophorini, Phasmosomini, Rhyparochromini and Stygnocorini were investigated through their representative bugs, ***Emblethis gracilicornis*** Puton, ***Lethaeus lethierryi*** (Puton), ***Lamprodema maura*** (Fabricius), ***Remaudiereana annulipes*** (Baerensprung), ***Marmottania simonis*** Puton, ***Phasmosomus priesneri*** (Wagner), ***Dieuches mucronatus*** (Stal) and ***Stygnocoris breviceps*** Wagner, respectively.

So the general morphological characters shared with the representative species of the 8 rhyparochromin tribes are: ocelli two in number; antennae 4- segmented; membrane of hemelytra with 4 or 5 veins; tarsi 3- segmented; ventral suture between 4th and 5th abdominal segments, not reaching to the connexival margin; abdomen with rudiments of 2 or 3 scent glands on tergum 3-5; abdominal trichobothria 28 in number in both sexes, arranged on the sternites 3-7 and female ovipositor, elbow-like. These results indicated that the mentioned characters are distinctive for the subfamily Rhyparochrominae as previously reported by Slater, 1964 and Schuh & Slater, 1995.

On the other hand, the differences in characters among the species represented 8 tribes, which facilitate the differentiation between them are labium slender, tapering, with 4th segments not extending beyond the hind coxae, except in the tribe Phasmosomini where labium with the 4th segment extending beyond hind coxae. Shape of pronotum varies in the 8 tribes and is characterized by, lamelliformed lateral margins in Gonianotini and Rhyparochromini;being broad and flat in Lethaeini, Megalonotini and Stygnocorini; strongly constructed behind middle in Myodochini; little sinuate at middle in Ozophorini and Phasmosomini. Abdominal spiracles vary in position in the different tribes. In Gonianotini the spiracles are dorsally placed only on segment 4; while in Lethaeini, Ozophorini, Phasmosomini and Stygnocorini, all spiracles are ventrally placed. On the other hand, the spiracles on segments 3 and 4are dorsally placed in Megalonotini, Myodochini and Rhyparochromini. Aedeagus, particularly basal plate and vesical parts have characteristic features at tribal level. Tribe Lethaeini characterized by free-floating wings of the ejaculatory reservoir, while in other tribes wings contiguous with body. Structure of ovipositor constant in the 8 tribes, but differs in size as it is distinctly, narrow and elongated in Megalonotini, Myodochini, Rhyparochromini Phasmosomini and Ozophorini; on the other hand it is wide and short in Gonianotini and Lethaeini. These findings supported the results reaches by those of Puton and Leithery, 1887;. Wagner (1958) & (1959); Scudder (1957); Sweet (1967); Harrington (1980); Linnavuori (1994); Schuh & Slater (1995); Cassis & cross (2002); Brailovsky (2009) and Cervantes & O'donnell (2009) . Spermatheca are known to show high degree of variation which is very important for differentiation at the specific level (Scudder, 1957 & 1959 and Sweet, 1967). In the present investigation it was found that, the shape of spermathecal bulb varies from triangular-shape in Gonianotini; to cap-like in Lethaeini; funnel-shaped in Megalonotini; kidney-shaped in Myodochini; spherical in Ozophorini and Stygnocorini; bladder-like in Phasmosomini and oblong heart-shaped in Rhyparochromini.

Accordingly, the relationships between the 8 tribes could be concluded as follows: Ozophorini and Phasmosomini show a degree of resemblance in shape of pronotum (little sinuate at middle), position of abdominal spiracles (all spiracles ventrally placed) and long ovipositor. Meanwhile the different structure in, inner laterotergites, abdominal scent glands, aedeagus of male and spermatheca of female can easily differentiated between the two tribes. Megalonotini and Myodochini also show a degree of similarity, especially in the position of abdominal spiracles (3 & 4 dorsally placed) and elongated ovipositor but differ in, the shape of pronotum, inner laterotergites which present in Megalonotini but absent in Myodochini and the shape of aedeagus, so that the two tribes are not related. Gonianotini, Lethaeini, Rhyparochromini and Stygnocorini are placed in unique position. These suggestions are confirmed by the opinions of Sweet (1967), Harrington (1980), Linnavuori (1994).

Moreover, scanning electron microscope examination of metathoracic scent gland and abdominal trichobothria of ***Remaudiereana annulipes*** (Baerensprung) reveled that, trichobothria consists of cuticular filiform mechanoreceptor hair, surrounded by circular area of dense papilli-like structures, that may be play a role in responding to air currents and sound as mentioned by Keil (1997) in his study on the functional morphology of insect mechanoreceptors. On the other hand evaporative area of metathoracic scent gland are found to be consists of two types of microsculptures, a limited area of rod-like microsculptures and a wide area of mushroom-like microsculptures. The main function of the adult scent glands appears to be defensive, although sexual alarm and aggregation functions may also exist (Remold, 1963). The variable types of microsculptures detected in the present investigation may be responsible for the performance of the function of the evaporative area suggested by Carayon (1971) and Carver (1990). They reported that evaporative area increase the effectiveness of the scent secretion in defense and also serve in restricting spread of the scent fluids to a circumscribed area of the body because the scent fluids are known to be toxic to the bugs that secrete them as well as to potential predators.



(Figs. 1-33): *Remaudiereana annuilpes* (Baerensprung(1): General characters of adults (male and female); (2): Dorsal side of head; (3): Lateral side of head and thorax; (4): Dorsal side of head (Scanning photographe)

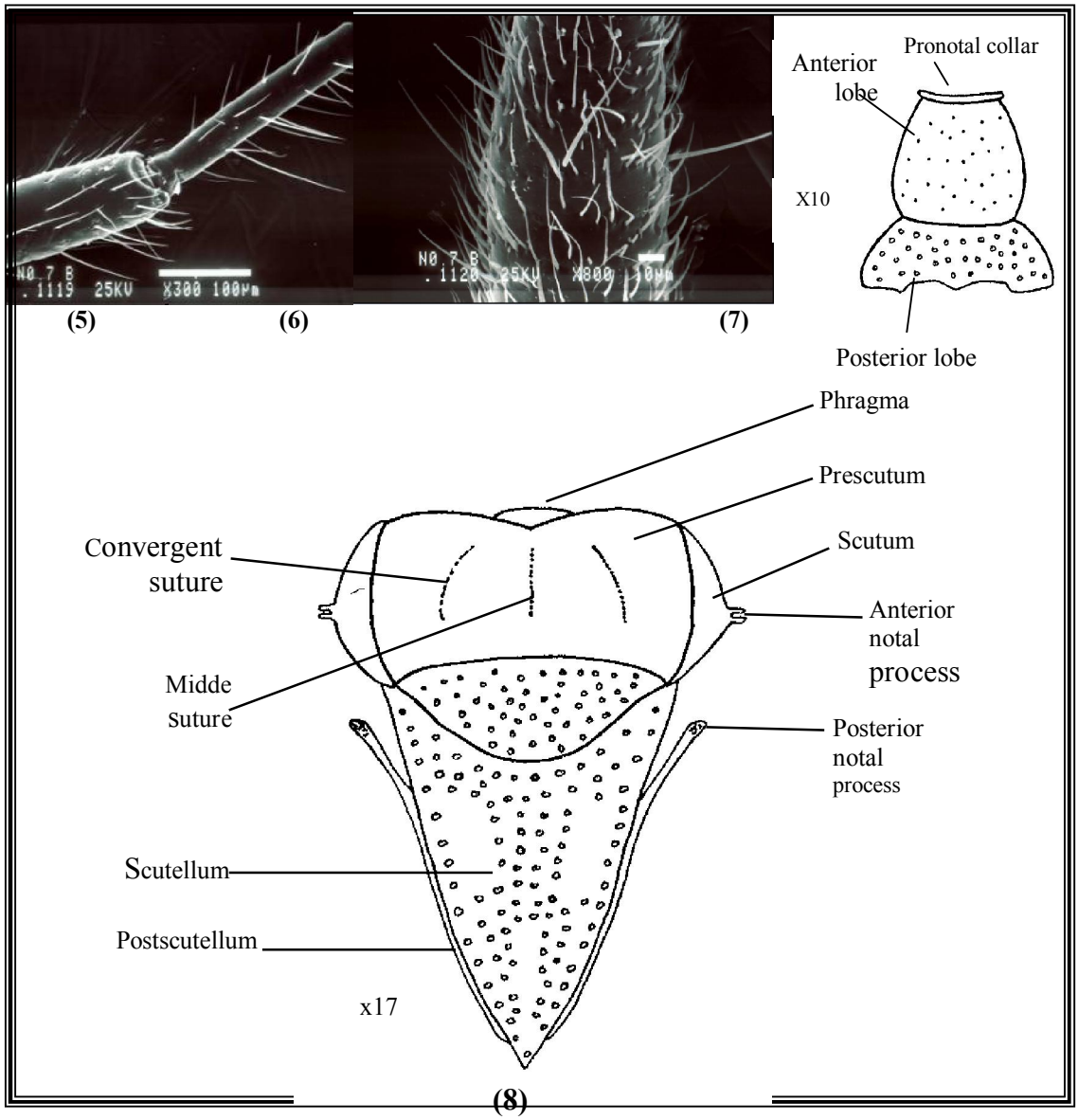
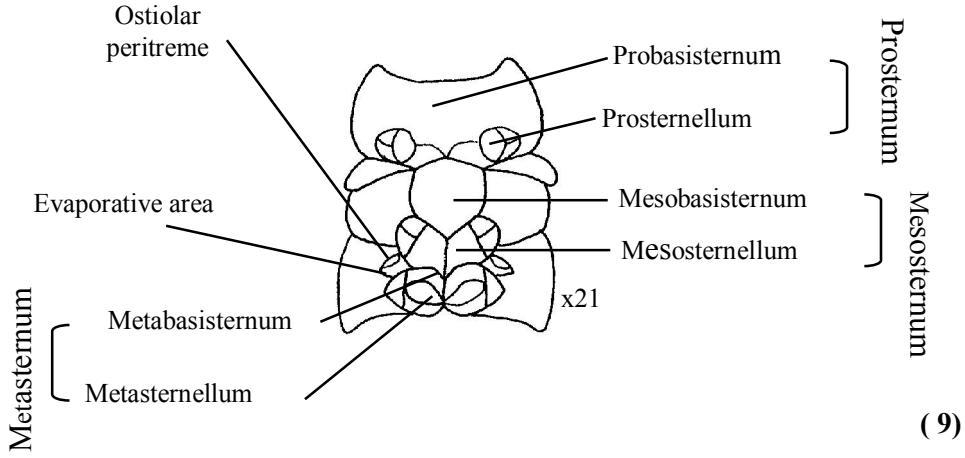
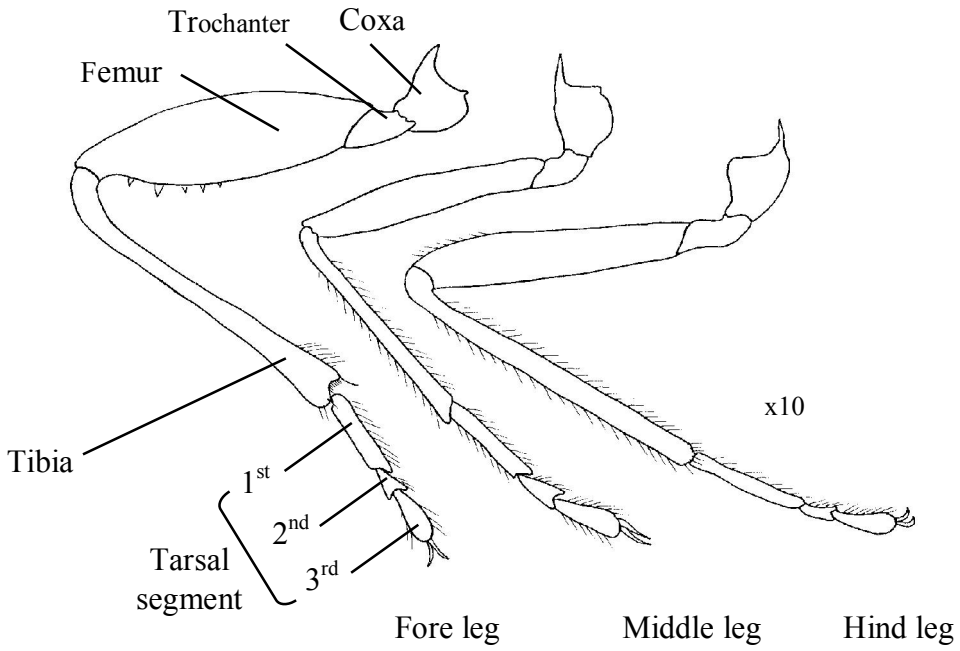


Fig. (5): 1st & 2nd antennal segments; (6): 4th antennal segment; (7): Pronotum; (8): Dorsal side of mesonotum.

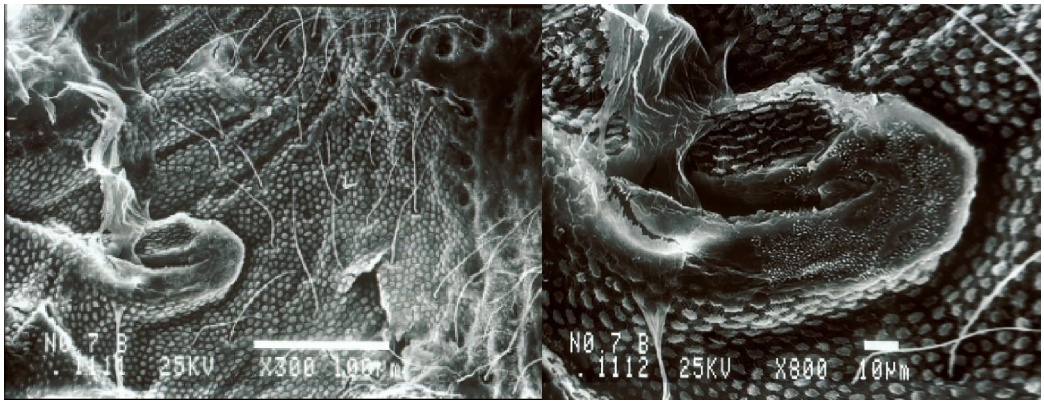


(9)

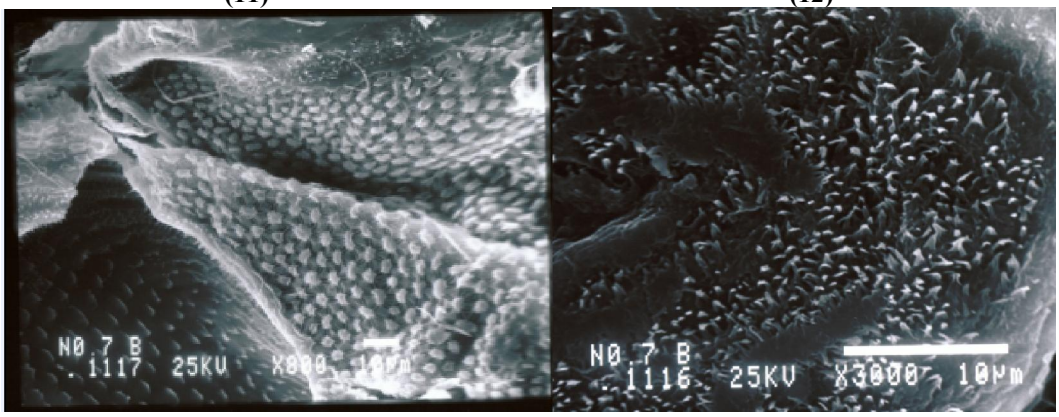


(10)

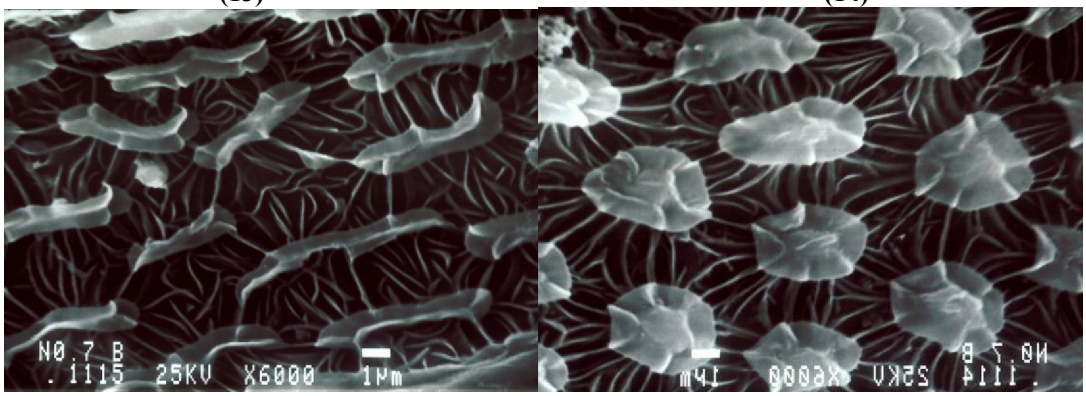
(Fig. (9): Ventral side of thorax; (10): Legs.



(11) (12)



(13) (14)

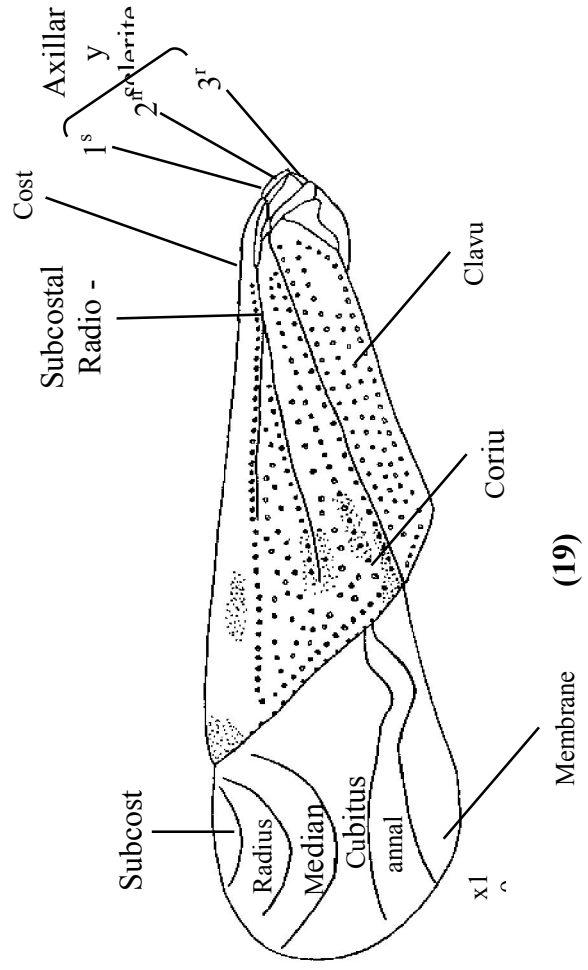


(15) (16)

Fig.(11): Metathoracic scent gland,X300; (12): Metathoracic scent gland,X800 (13): Canal of metathoracic scent gland; (14): Apex of auricle of metathoracic scent gland; (15) Rod-like microsculpture Evaporative area; (16): Mushroom-like microsculpture.

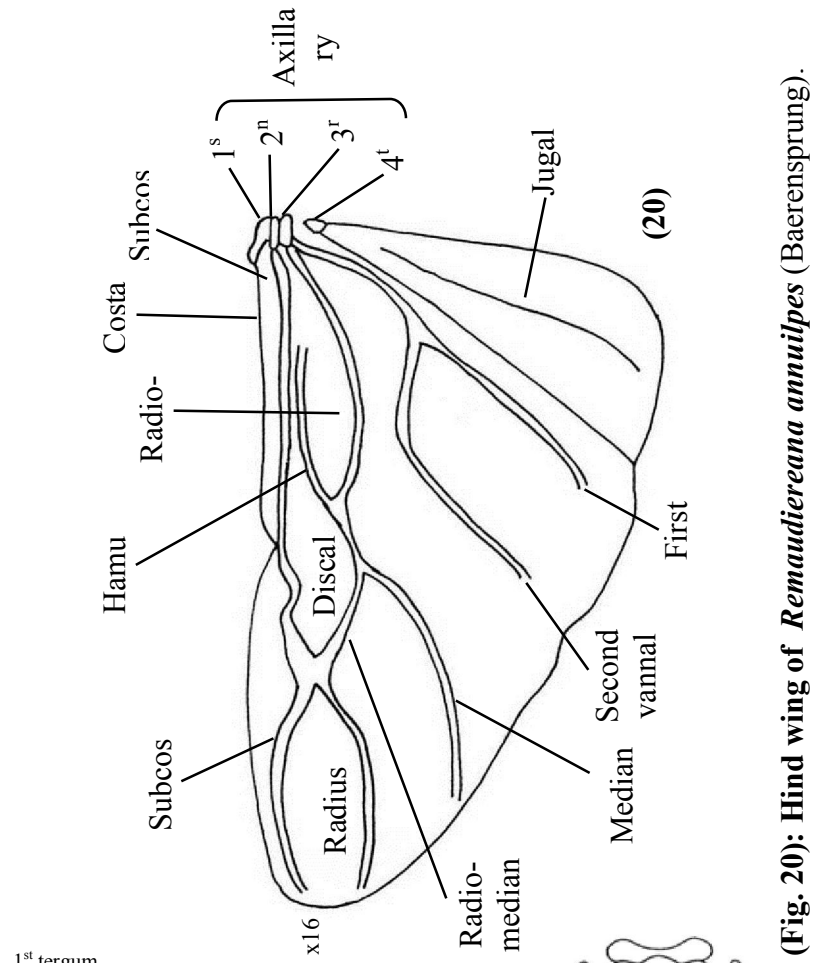
(17) For leg coxal arm

(18) For leg femoral arms.

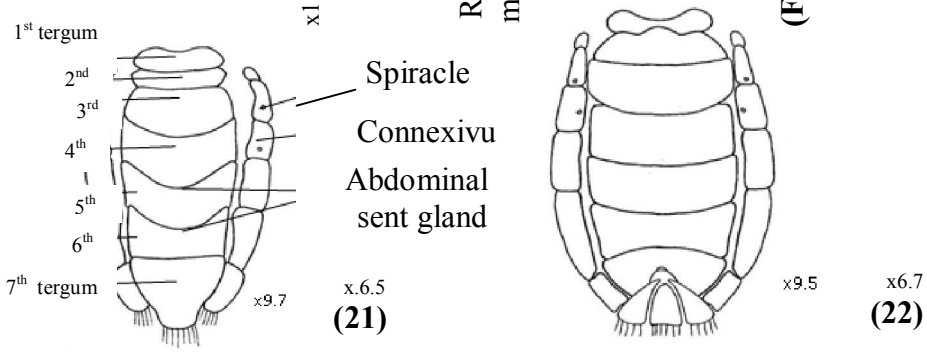


(19)

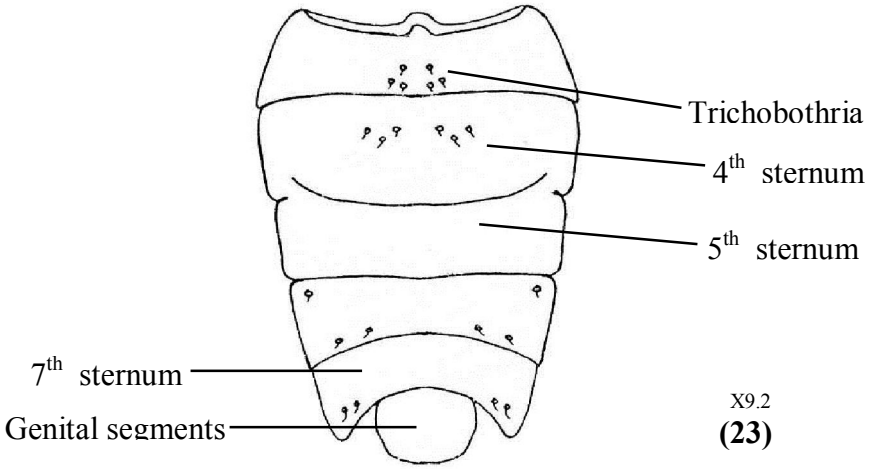
(Fig. 19): Fore wing of *Remaudiereana annuilpes* (Baerensprung).



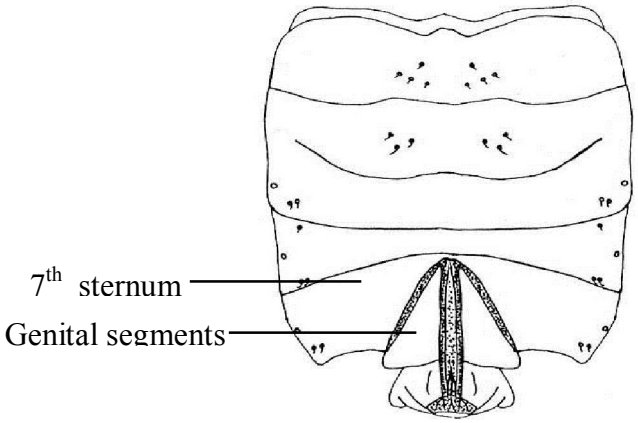
(Fig. 20): Hind wing of *Remaudiereana annuilpes* (Baerensprung).



Abdomen dorsally (21): Male; (22): Female.

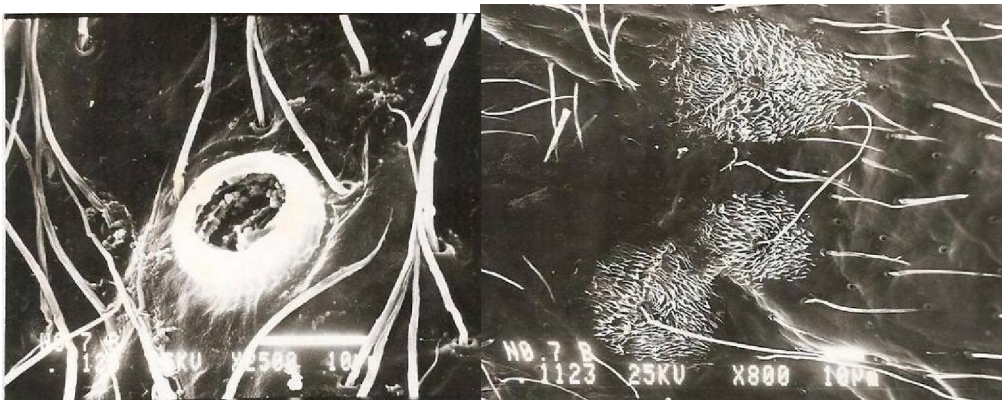


X9.2
(23)

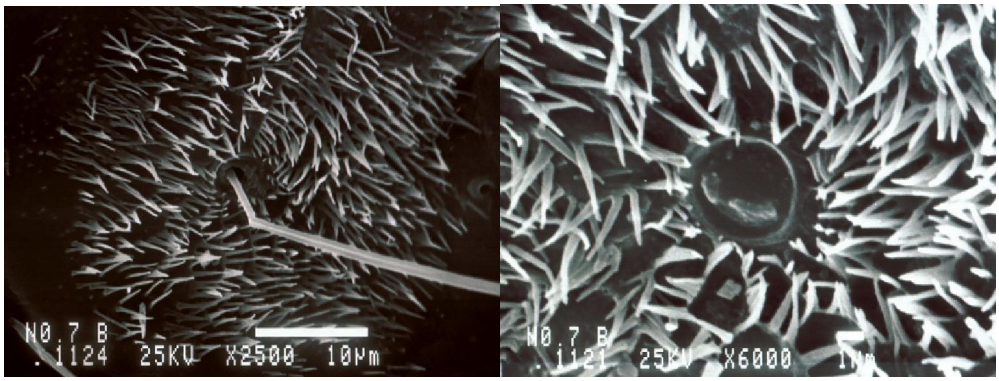


X8.9
(24)

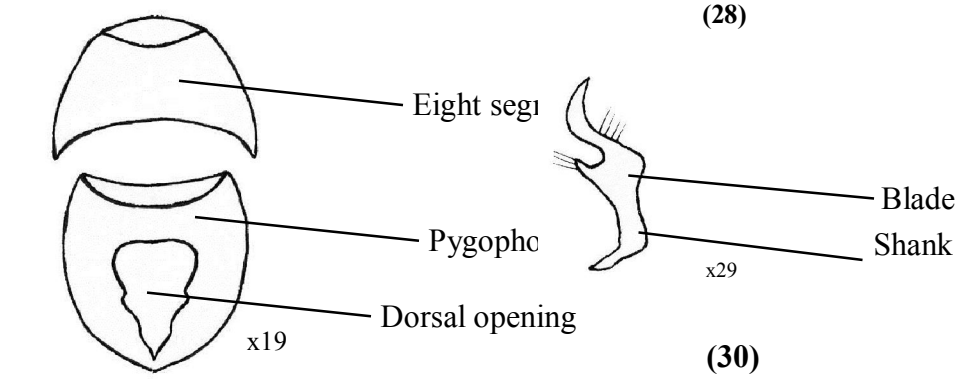
Abdomen ventrally, **(23): Male; (24): Female**.



(25): Spiracle of abdominal segment; (26). Abdominal trichobothria

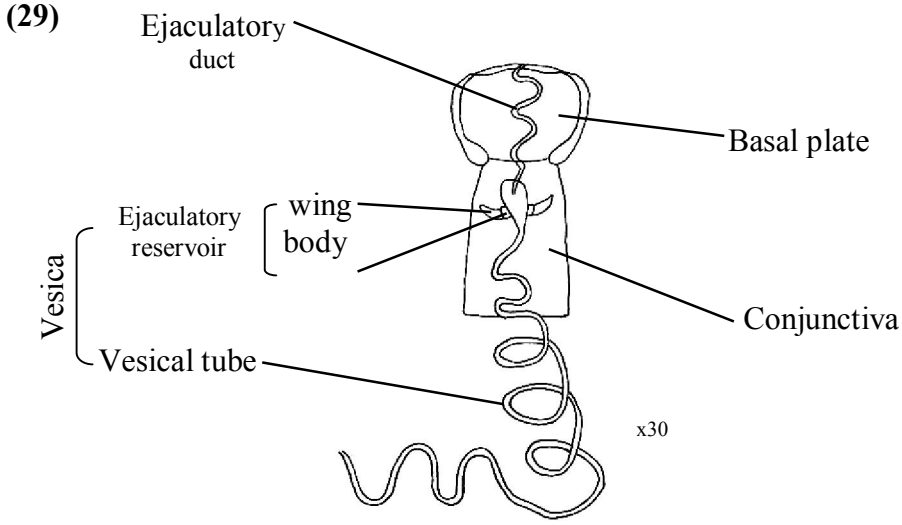


(28)



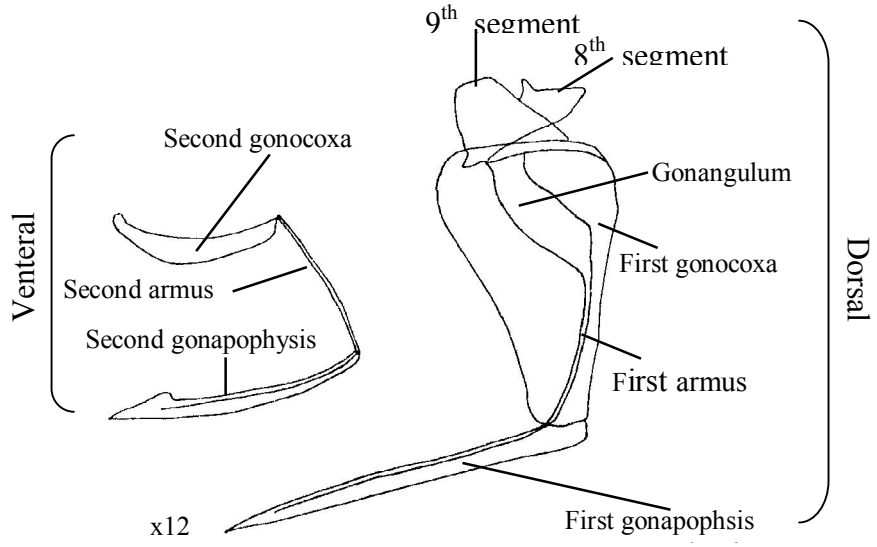
(29)

(30)

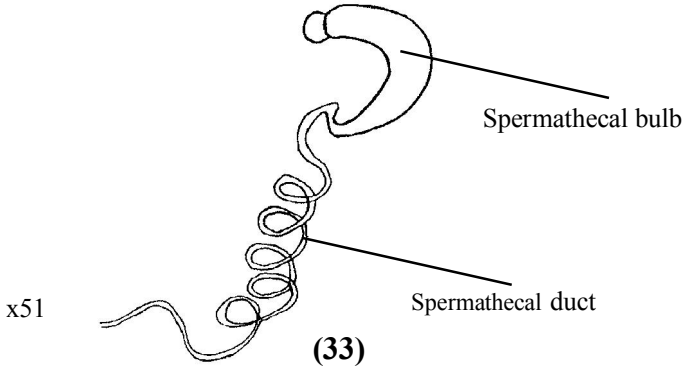


(31)

Figs(27),(28): Abdominal trichobothria; Figs (29-31): Male genitalia, (29): Pygophore; (30): Paramere; (31): Aedeagus.



(32)



(33)

(Figs. 32 & 33): Female genitalia, (32): Valves of ovipositor; (33): Spermatheca.

Table I: General characters of Adults (Males & Females)

Tribe	Gonianotini	Lethaeini	Megalonotini	Myodochini	Ozophorini	Phasmosomini	Rhyparochromini	Stygnocorini
Species	***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
Length	7.5-8 mm.	6.5-7 mm.	4-4.5 mm.	5.5-6 mm.	4.5-5.5 mm.	7-8 mm.	11-11.5 mm.	4-4.5 mm.
Width	2-2.5 mm.	2-2.5 mm.	1.3 mm	2 mm.	1-1.2 mm.	2mm.	3-3.5 mm.	1.5-2 mm.
Form	Slender.	Oblong ovate.	Acromion, weal like.	Subovoid.	Elongated, narrow, somewhat ant-like.	Elongated, glabrous.	Cylindrical, elongated.	Slender.
Color	Yellowish with brown punctures.	Brownish.	Bronze metallic.	Brownish yellow.	Brownish yellow, brilliant.	Yellow to dark brown.	Reddish brown marked with yellowish white.	Grayish brown.
Upper Surface	Wholly punctated.	With strong punctures.	With strong punctures.	Wholly punctated.	Wholly punctated.	Wholly punctated.	Wholly punctated.	Wholly punctated.

Table II: Lateral side of Head

Tribe		**Gonianotini**	**Lethaeini**	Megalonotini	**Myodochini**	**Ozophorini**	Phasmosomini	Rhyparochromini	**Stygnocorini**
Species		***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
Labrum		As long as 1st labial segment.	As long as 1st labial segment.	Shorter than 1st labial segment.	Shorter than 1st labial segment.	Nearly as long as 1st labial segment.	Shorter than 1st labial segment.	As long as 1st labial segment.	Shorter than 1st labial segment.
Labium	1st segment	Shorter than head.	Shorter than head.	As long as head.	Nearly as long as head.	Shorter than head.	Shorter than head.	Slightly longer than head.	Longer than head.
	2nd segment	Extending to fore coxae.	Extending between fore coxae.	Extending between fore coxae.	Extending before fore coxae.	Extending to mesosternum.	Extending to middle coxae.	Extending between fore coxae.	Extending between fore coxae.
	3rd segment	Extending to mesosternum.	Extending between middle coxae.	Extending to middle coxae.	Extending behind fore coxae.	Extending to middle coxae.	Extending to hind coxae.	Extending to mesosternum.	Extending between middle coxae.
	4th segment	Extending between middle coxae.	Extending to metasternum.	Extending to metasternum.	Extending to mesosternum.	Extending to hind coxae.	Extending behind hind coxae.	Extending between middle coxae.	Extending to metasternum.

Table III: Dorsal side of Thorax

Tribe		**Gonianotini**	**Lethaeini**	***Megalonotini***	**Myodochini**	**Ozophorini**	**Phasmosomini**	***Rhyparochromini***	**Stygnocorini**
Species		***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
Pronotum		Flat, lateral margin lamelliform.	Flat.	Broad, flat.	Strongly constricted behind middle.	Slightly constricted at middle.	Slightly constricted at middle.	Elongated, lateral margin lamelliform.	Broad, flat.
Scutellum		Shorter than pronotum with pointed apex.	Nearly as long as pronotum with pointed apex.	Longer than pronotum with pointed apex.	Longer than anterior lobe of pronotum with pointed apex.	Shorter than pronotum with pointed apex.	Shorter than pronotum with pointed apex.	Shorter than pronotum with pointed apex.	Nearly as long as pronotum with rounded apex.
Hemelytra	clavus	With 2 regular rows of punctures and another scattered punctures.	With 2 regular rows of punctures and another scattered punctures between them.	With 4 rows of regular punctures.	With 4 rows of regular punctures.	With 2 regular rows of punctures and another scattered punctures between them.	With 3 rows of punctures, median row irregular.	With 2 regular rows of punctures and another scattered punctures.	With 4 rows of regular punctures.
	membrane	With 5 veins.	With 4 veins.	With 4 bright veins.	With 5 veins.	With 4 veins.	With 4 veins.	With 5 veins.	With 4 veins.

Table IV: Dorsal side of Abdomen (Terga)

Tribe	**Gonianotini**	**Lethaeini**	Megalonotini	**Myodochini**	**Ozophorini**	**Phasmosomini**	Rhyparochromini	Stygnocorini
Species	***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
Position of spiracles	Abdominal spiracle 4 only located dorsally.	All abdominal spiracles located ventrally.	Abdominal spiracle 3&4 located dorsally.	Abdominal spiracle 3&4 located dorsally.	All abdominal spiracles located ventrally.	All abdominal spiracles located ventrally.	Abdominal spiracle 3&4 located dorsally.	All abdominal spiracles located ventrally.
Connexivum	5 segments.	5 segments.	6 segments.	6 segments.	6 segments.	6 segments.	6 segments.	6 segments.
Inner laterotergites	Present.	Present.	Present.	Absent.	Absent.	Present.	Present.	Present.
Scent gland scars	2	2	3	2	3	3	2	3

Table V: Male genital segments (Pygophore & Paramere)

Tribe		Gonianotini	Lethaeini	Megalonotini	Myodochini	Ozophorini	Phasmosomini	Rhyparochromini	Stygnocorini
Species		*Emblethis gracilicornis* Puton	*Lethaeus lethierryi* Puton	*Lamprodema maura* Fabricius	*Remaudiereana annuilpes* (Baerensprung)	*Marmottania simonies* Puton	*Phasmosomus priesneri* (Wagner)	*Dieuches mucronatus* (Stal)	*Stygnocoris breviceps* Wagner
Pygophore	shape	Rounded.	Quadrate.	Spherical.	Rounded.	Quadrate.	Spherical.	Rounded.	------
	Dorsal opening	Cylindrical.	Inverted bell-shaped.	Narrow with dented at lateral sides.	Inverted cup-shaped.	Diamond-shaped.	Rounded, wide.	Heart-shaped.	-------
Blade of paramere		Straight with rounded apex.	Straight with rounded apex.	Curved with rounded apex.	Curved with pointed apex.	Straight with rounded apex.	Curved with rounded apex.	Straight with rounded apex.	-------

Table VI: Male genital segments (Aedeagus)

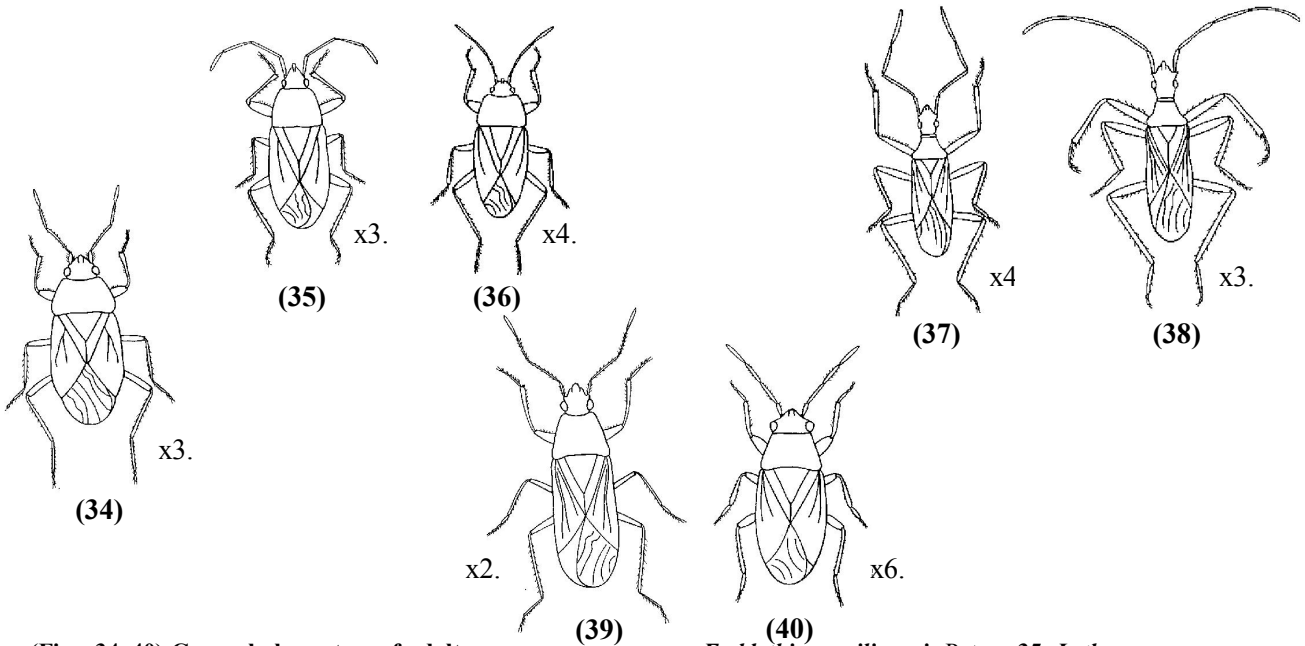
Tribe	Gonianotini	Lethaeini	Megalonotini	Myodochini	Ozophorini	Phasmosomini	Rhyparochromini	Stygnocorini
Species	***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
Shape of Basal plate	Rounded.	Subquadrate.	Triangular.	Rounded.	Quadrate.	Rounded.	Subquadrate.	Quadrate.
Body	Cup-shaped.	Kidney-shaped.	Small, rectangular.	Ball like.	Ball like.	Cylindrical.	Small, rectangular.	Rounded.
Wing	Spindle-shaped.	Triangular.	Ear-like.	Cup-shaped.	Cup-shaped.	Cup-shaped.	Triangular.	Cup-shaped.
Vesical tube	Long and coiled.	Short and coiled.	Short and uncoiled.	Long and coiled.	Long and coiled.	Long and coiled.	Long and coiled.	Long and coiled.

Table VII: Female genitalia (Valves of ovipositor)

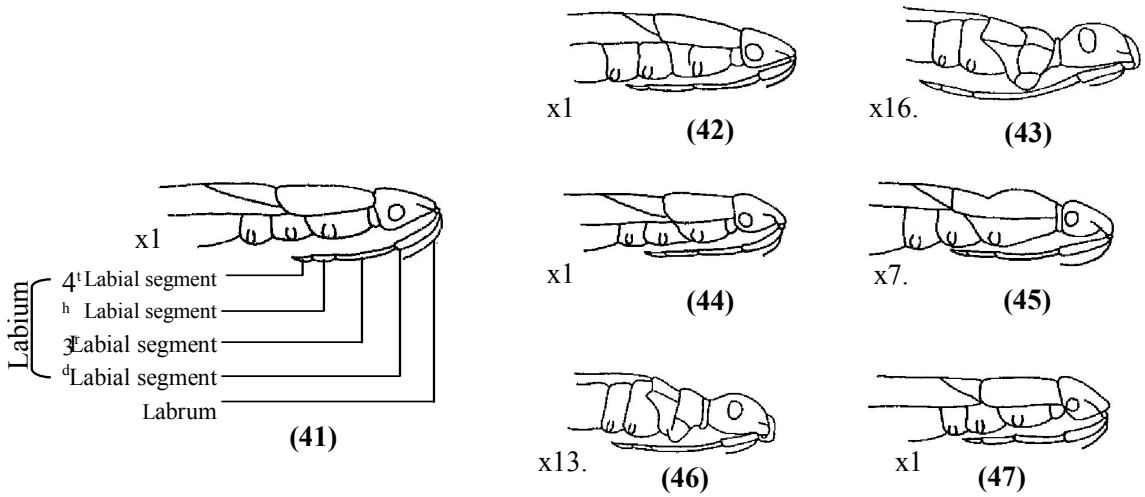
Tribe	Gonianotini	Lethaeini	Megalonotini	Myodochini	Ozophorini	Phasmosomini	Rhyparochromini	Stygnocorini
Species	***Emblethis gracilicornis*** Puton	***Lethaeus lethierryi*** Puton	***Lamprodema maura*** Fabricius	***Remaudiereana annuilpes*** (Baerensprung)	***Marmottania simonies*** Puton	***Phasmosomus priesneri*** (Wagner)	***Dieuches mucronatus*** (Stal)	***Stygnocoris breviceps*** Wagner
First gonocoxa	Flat and longer than 1st gonapophysis.	Flat and longer than 1st gonapophysis.	Narrow and shorter than 1st gonapophysis.	Flat and shorter than 1st gonapophysis.	Narrow and as long as 1st gonapophysis.	Narrow and shorter than 1st gonapophysis.	Narrow and shorter than 1st gonapophysis	--------
First gonapophysis	Narrow and insinuate.	Wide and sinuate.	Narrow and insinuate.	Narrow and insinuate.	Narrow and insinuate.	Narrow and insinuate.	Narrow and sinuate.	--------
Second gonapophysis	Wide with acute apex.	Wide with rounded apex.	Narrow with rounded apex.	Narrow with acute apex.	Narrow with rounded apex.	Narrow with acute apex.	Narrow with rounded apex.	--------

Table VIII: Female genitalia (Spermatheca)

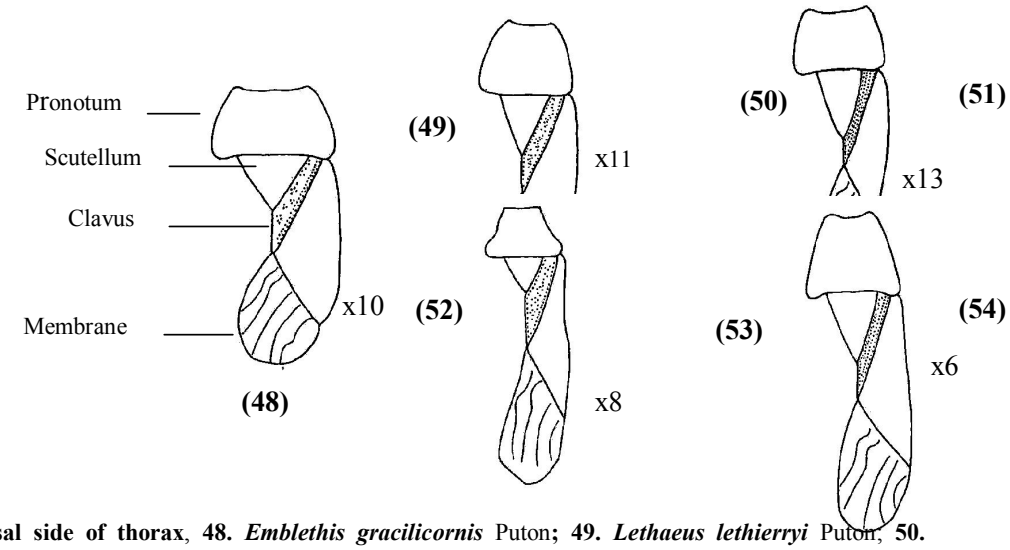
Tribe	Gonianotini	Lethaeini	Megalonotini	Myodochini	Ozophorini	Phasmosomini	Rhyparochromini	Stygnocorini
Species	*Emblethis gracilicornis* Puton	*Lethaeus lethierryi* Puton	*Lamprodema maura* Fabricius	*Remaudiereana annuilpes* (Baerensprung)	*Marmottania simonies* Puton	*Phasmosomus priesneri* (Wagner)	*Dieuches mucronatus* (Stal)	*Stygnocoris breviceps* Wagner
Spermathecal pulp	Triangular-shape.	Cap-like.	Funnel-shaped.	Kidney- shaped.	Spherical.	Bladder-like.	Oblong heart-shaped.	Spherical.
Spermathecal duct	Long and coiled.	Short and uncoiled.	Short and uncoiled.	Long and coiled.	Long and coiled.	Short and uncoiled.	Long and uncoiled.	Long and coiled.



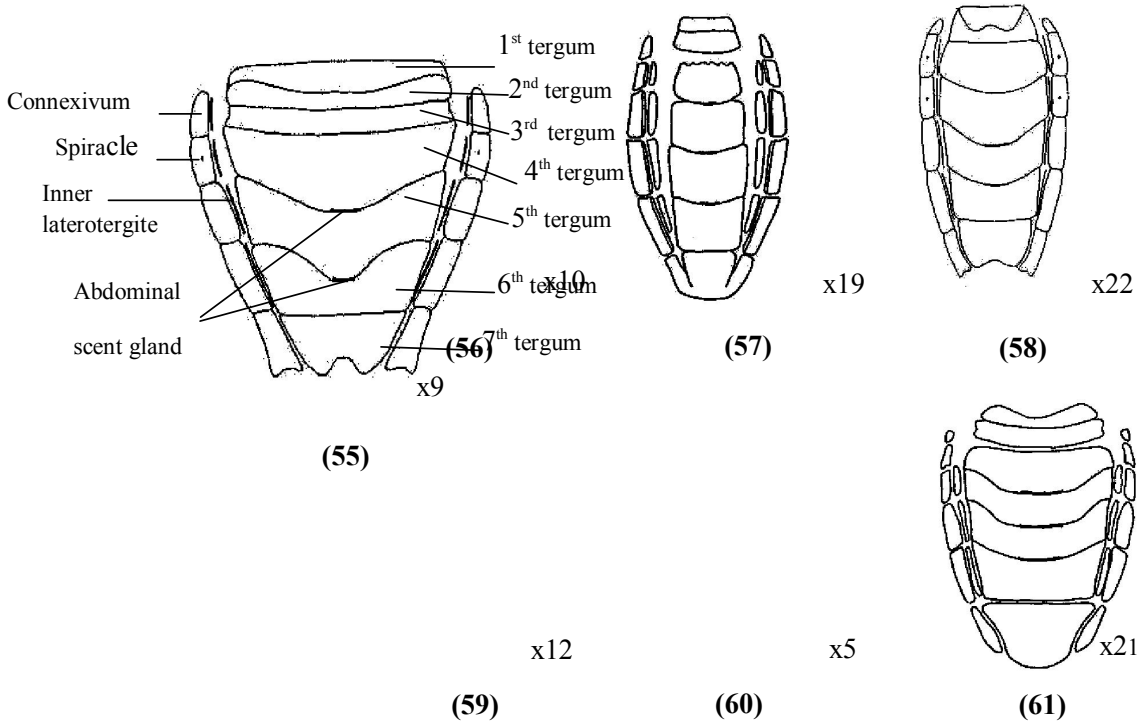
(Figs. 34–40) General characters of adults (Male& Female), 34. *Emblethis gracilicornis* Puton**; 35. *Lethaeus lethierryi*** Puton; **36. *Lamprodema maura*** Fabricius; **37. *Marmottania simonis*** Puton; **38. *Phasmosomus priesneri*** (Wagner); **39. *Dieuches mucronatus*** (Stal); **40. *Stygnocoris breviceps*** Wagner.



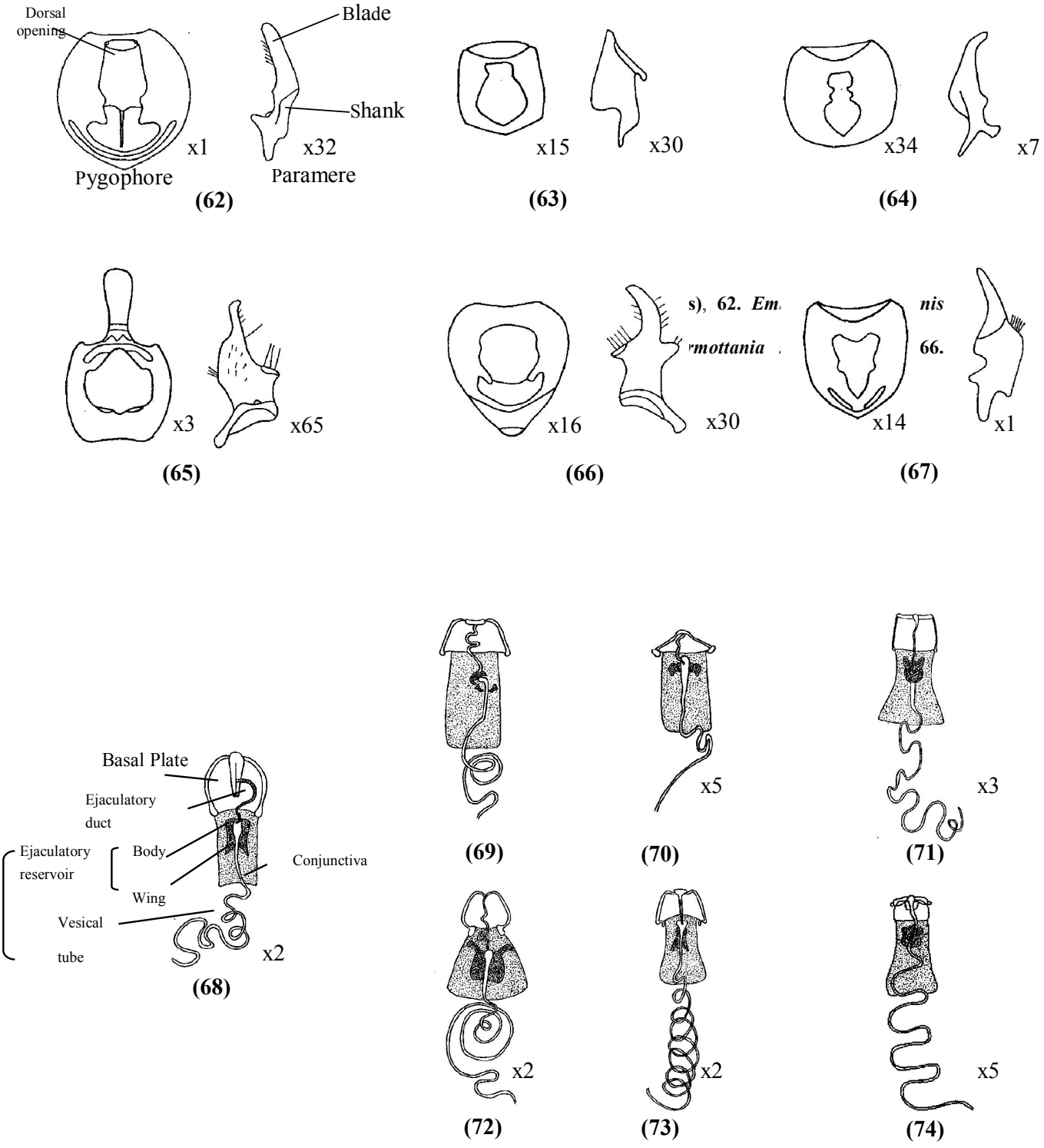
(Figs. 41-47) Lateral side of head and thorax, 41. *Emblethis gracilicornis* Puton**; 42. *Lethaeus lethierryi*** Puton; **43. *Lamprodema maura*** Fabricius; **44. *Marmottania simonis*** Puton; **45. *Phasmosomus priesneri*** (Wagner); **46. *Dieuches mucronatus*** (Stal); **47. *Stygnocoris breviceps*** Wagner.



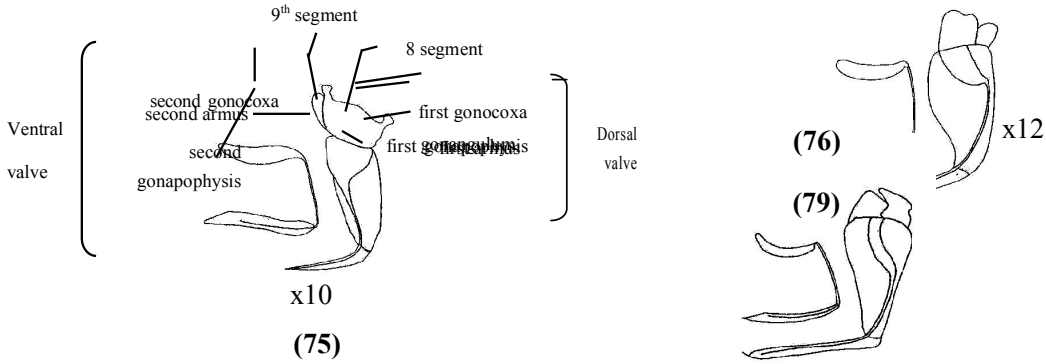
(Figs. 48-54) Dorsal side of thorax, **48.** ***Emblethis gracilicornis*** Puton**; 49.** ***Lethaeus lethierryi*** Puton; **50.** ***Lamprodema maura*** Fabricius; **51.** ***Marmottania simonis*** Puton; **52.** ***Phasmosomus priesneri*** (Wagner); **53.** ***Dieuches mucronatus*** (Stal); **54.** ***Stygnocoris breviceps*** Wagner.



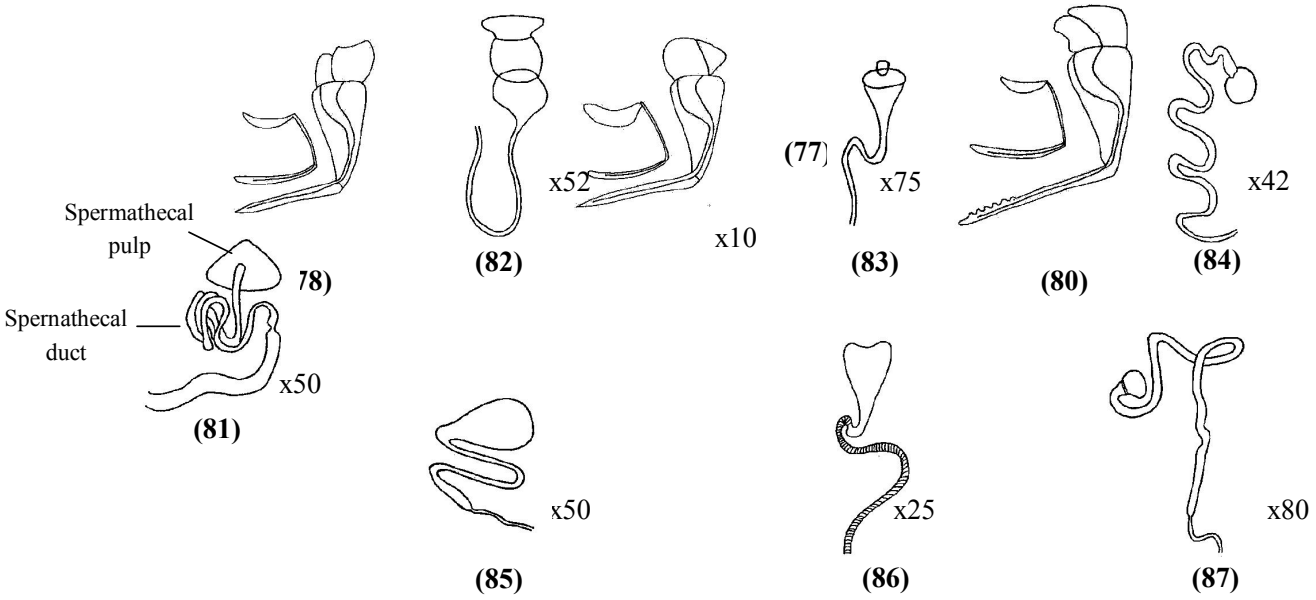
(Figs. 55–61) Dorsal side of abdomen (Terga), **55.** ***Emblethis gracilicornis*** Puton**; 56.** ***Lethaeus lethierryi*** Puton; **57.** ***Lamprodema maura*** Fabricius; **58.** ***Marmottania simonis*** Puton; **59.** ***Phasmosomus priesneri*** (Wagner); **60.** ***Dieuches mucronatus*** (Stal); **61.** ***Stygnocoris breviceps*** Wagner.



(Figs. 68–74) Male genital segments (Aedeagus), **68.** ***Emblethis gracilicornis*** Puton; **69.** ***Lethaeus lethierryi*** Puton; **70.** ***Lamprodema maura*** Fabricius; **71.** ***Marmottania simonis*** Puton; **72.** ***Phasmosomus priesneri*** (Wagner); **73.** ***Dieuches mucronatus*** (Stal); **74.** ***Stygnocoris breviceps*** Wagner (After Sweet, 1967).



(Figs. 75–80) Valves of ovipositor (female), **75.** ***Emblethis gracilicornis*** Puton**; 76.** ***Lethaeus lethierryi*** Puton; **77.** ***Lamprodema maura*** Fabricius; **78.** ***Marmottania simonis*** Puton; **79.** ***Phasmosomus priesneri*** (Wagner); **80.** ***Dieuches mucronatus*** (Stal).



(Figs. 81–87) Female spermatheca, **81.** ***Emblethis gracilicornis*** Puton**; 82.** ***Lethaeus lethierryi*** Puton; **83.** ***Lamprodema maura*** Fabricius; **84.** ***Marmottania simonis*** Puton; **85.** ***Phasmosomus priesneri*** (Wagner); **86.** ***Dieuches mucronatus*** (Stal); **87.** ***Stygnocoris breviceps*** Wagner (After Sweet, 1967).

Fig.(88): *Emblethis gracilicorius* Puton (Gonianotini); (89): *Lethaeus lethierryi* Puton (Leyhaeini); (90): *Lamprodema Maura* (Fabricius) (Megalonotini); (91): *Remaudiereana annulipes* (Baerensprung) (Myodochini).

Fig.(92): *Marmottania simonies* Puton (Ozophorini); (93): *Phasmosomus priesneri* (Wagner) (Phasmosomini); (94) *Dieuches mucronatus* (Stall) (Rhyparochromini); (95): *Stygnocoris breviceps* Wagner (Stygnocorini).

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