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Evaluation the Physical Properties between Flexible, Cold –Cured and Hard Heat-Cured Acrylic Resin (*In-Vitro* Study)

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Abstract: In this study compare between the flexible resin system (marketed in the U.S. by DENTSPLY Austenal), hard heat-cured acrylic resin and cold acrylic,(U.S. by DENTSPLY Austenal). Tensile strength and transverse strength tests were performed on 100 specimens from each material using computerized testing system . The results revealed statistically significant differences in ultimate tensile strength, yield strength, percentage elongation, and modulus of elasticity between the tested specimens. The flexible acrylic resin showed higher value than the other hard acrylic resin and cold acrylic resin.

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Keywords: flexible resin system; hard heat-cured acrylic resin; cold acrylic; elasticity

1. Introduction:

Although clinician's skills and experience play a major role in designing and fabrication of the optimum prosthodontic restorations, the selection of denture resins is equally important, especially when the patient has been using the prostheses since long. Eighteen cases who were not satisfied with their conventional acrylic dentures were selected. They were provided flexible dentures along with a questionnaire to precisely evaluate the advantages of new material. Prosthodontic planning & observations regarding this material are discussed on various parameters .[1] Loss of teeth, which may be due to trauma, dental diseases, pathology, or otherwise not only alters the psychological thought of the patients but also disturbs the esthetics, phonetics, and functional occlusion. Replacement of missing teeth is highly essential in order to restore the defect and regain function as best as possible.

Since ages, polymethyl methacrylate (PMMA) has been used to fabricate the dentures and when facilities are available, metal cast / metal frame / metal base dentures are also fabricated to restore the defects. The acrylic denture base prostheses have their own advantages and disadvantages. Some problems with these prostheses are difficult to address, such as insertion in undercut areas, brittleness of methyl methacrylate which leads to fracture, and allergy to methyl methacrylate monomer.[2,3]

A flexite denture is a partial denture made of elastic nylon resin, more flexible than plastic of which a regular denture is made. Because the flexite denture base is fixed to the gum and can be thin, no clasp is needed and more comfortable to wear by the patient used in compelet and partial denture constracted by flexite. . Polymethyl methacrylate , the material has been used in dentistry for many years in the form of tem softporary crowns and thermal polymerized as baseplate material for partial and complete dentures. [4,5]

Innovation of the nylon-derived denture base material in the 1950s paved the way for a new type of dentures. Flexible dentures are an excellent alternative to conventionally used methyl methacrylate dentures, which not only provide excellent aesthetics and comfort but also adapt to the constant movement and flexibility in partially edentulous patients.[6,7] Dental 'D' reintroduced tooth-colored clasps using Acetal resin. The clasps were flexible, did not need periodic adjustment to keep them tight, and the tooth colored esthetics were appreciated by the patients. Pressing Dental followed in the early 1990s with an Acetal resin, (marketed in the U.S. by DENTSPLY Austenal), which in addition to tooth colored clasps, has been used for an entire partial denture framework as well as other appliances. Thermoplastic materials for dental prostheses, Valplast (Valplast Int. Corp. - USA) and Flexiplast (Bredent - Germay), were first introduced to dentistry in the 1950s. Both materials were similar grades of Polyamides (nylon plastics). Since their introduction, there has been a continued interest in thermoplastic dental materials.[8,9] Flexite M.P.-a thermoplastic acrylic, is a special blend of polymers and has the highest impact rating of any acrylic. You can bounce a Flexite M.P. denture off the floor without cracking the base. Flexite M.P. has a surface hardness of 55-65, making it popular for bruxism appliances as well as dentures. The flexible framework RPD can replace any number of teeth in a dental arch, similar to the flipper and cast metal RPD. There is, however, one type of removable tooth replacement device that can (legally) be built only out of the flexible framework variety of material.[3,4,10]

You are missing only a few teeth scattered over either arch (upper or lower teeth), or even if you have a minimum of two teeth on both sides of the arch, then you can most inexpensively replace the missing teeth with a removable partial denture (RPD). There are several types of RPD's. All of them use standard denture teeth as replacements for the missing natural teeth. The differences between them are the materials used to support the denture teeth and retain the RPD in the mouth. The application of nylon-like materials to the fabrication of dental appliances. has been seen as an advance in dental materials. This material generally replaces the metal, and the pink acrylic denture material used to build the framework for standard removable partial dentures.[5,10] The Flexite Company has six different monomer free plastics for fabricating RPD's, dentures, TMJ's, bruxism, anti-snoring devices, sports mouth guards, tooth colored clasps, Flexite-metal combination cases etc. Flexite plastics are safe, non-toxic, comfortable, biologically inert, and meets the leaching requirements for colorants. Flexite plastics are esthetically superior to other plastics. Flexite plastics are CE certified. The Flexite Quality Assurance Program is designed to meet the ISO 9000 rules. Flexite Plus "flexible" partial dentures eliminate the use of metal, providing the patients with a partial denture alternative that delivers a precise fit, tissue-colored esthetics and maximum patient comfort. Flexible dentures will not cause sore spots due to negative reaction to acrylic resins and will absorb small amounts of water to make the denture more soft tissue compatible. Flexible dentures may be used as analternate treatment plan in rehabilitating the anomalies such as ectodermal dysplasia. [3,6,7,11,12]

2. Materials and methods:

1). Tensile strength test:

In this study the Specimens were preparedfrom the three types material flexible, cold –cured and hard heat-cured acrylic resin.

Specimens were prepared according to the American Association (ADA) specification No. 12 for denture acrylic resin.[12] The tensile test specimens were dumbbell-shaped and consisted of a central bar of 18mm length and 3mm diameter which blends gradually into two larger ends with 6mm diameter for the machine grip. Specimens were divided into3 groups (100 specimens each) as follows:

Group 1: flexible acrylic resin

Group 2: hard acrylic resin.

Group3: cold acrylic resion.

2). Flexible acrylic resin:

The waxed up dumbbell shaped pattern was invested in the dental flask and wax was eliminated by boiling while the processing was carried out by using the thermoplastic injection system. The thermo-injection was performed by inserting the cartilage that contains the selected shade into the NEW PRESS that was pre-heated to 220^0c. After 20 minutes, when the flexible acrylic resin had cooled, deflasking was completed and specimens were finished and polished.

Hard acylic resin:

The waxed up dumbbell shaped pattern was fabricated. The wax elimination by boiling followed by backing the acrylic resin, curing, deflasking was completed and specimens were finished and polished.

3-Cold acrylic resin: mixed the powder and liquid according manufacture flasking was completed and specimens were finished and polished.

Dimensions examination:

All finished specimens were examined by the digital caliper.

Tension test and measuring procedures:

Tensile strength of the test specimens was determined by using the computerized material testing system model LXL plus. *Yield tensile* **strength:**

Yield tensile strength was performed according to American National Standered Institute (ANSI E8) using an offset value of 1%. The loading rate was at a cross head speed of 0.5mm/min and the head was travelled until the yield strength had been exceeded.

The transverse strength test

According to the ANSI/ADA specification NO.12 for denture base resin material, specimens with modification of their thickness. Specimens consisted of bars of 62mm length, 10mm width and 1mm thickness.

Specimens were divided into 3groups, 100 samples each.

Group 1 was made of flexible acrylic resin.

Group 2 was made of hard acrylic resin.

Group3: cold acrylic resion.

Vicker's hardness test.

For determination of the micro-hardness,100 specimens of the tested flexible acrylic resin and hard acrylic resin were prepared and subjected to transverse strength testing. To minimize the risk of misreading, due to the work hardness readings were taken away from the fracture region. Vicker's micro-hardness test was accomplished by means of a 50mg load and 30 second loading time. Readings were spaced at 50um from 40 to600um depth.

3. Results:

Mean values of ultimate strength of the hard acrylic, cold acrylic and flexible acrylic resin samples, statistically significant difference was recorded. The mean value of the ultimate tensile strength of hard acrylic resin and cold acrylic was inferior to that of flexible acrylic resin.

Mean values of the percentage elongation between the hard acrylic, cold acrylic and flexible acrylic resin samples, there was statistically significant difference. The mean value of the percentage

elongation of flexible acrylic resin was higher than that of hard acrylic resin and cold acrylic resin.

Mean values of yeild strength of the hard acrylic, cold acrylic resin and flexible acrylic resin samples, statistically significant difference was recorded. The mean value of the yeild strength of flexible acrylic resin was higher than that of hard acrylic resin and cold acrylic resin**.**

Mean values of the modulus of elasticity of the hard acrylic cold acrylic resin , and flexible acrylic resin samples, there was statistically significant difference . The mean value of the modulus of elasticity of flexible acrylic resin was higher than that of hard acrylic resin and cold acrylic resin**.** Upon comparing the mean value of the Vicker's hardness of the hard type and the flexible type, statistically significant difference was reported. The mean value of the Vicker's hardness of the flexible type was inferior to that of the two acrylic type.

Regarding the stiffness a statistically significant difference was reported whan the hard acrylic resin and cold acrylic resin were compared with the flexible acrylic resin. The mean value of the stiffness of the hard acrylic resin was higher than the flexible acrylic resin and cold acrylic resin.

Tab. (1): Means, Standered deviation and Duncan's multiple range tests of flexible and hard acrylic resin.

Material	Flexible acrylic resin			Hard acrylic resin			Cold cured acrylic resin		
test	Mean	S.D	dt	Mean	S.D	dt	mean	SD	dt
Tensile strength(MPA)	1300	9.750	A	950	7.300	B	80.0	8.0	C
Yield strength(MPA)	550	5.002	A	400	4.500	C	30.0	4.0	B
Percentage elongation	16	1.500	A	10.5	0.8	B	9.2	0.7	C
Modulus of elasticity	80.40	2.000	A	65.20	1.400	B	55.0	1.10	B
Stiffness(Kg/mm)	22	5.400	A	30.00	8.400	C	28.0	8.02	C
Vicker's hardness(Kg/mm^2)	190	4.008	A	240	7.05	C	200	6.0	B

S.D= Standered diviation. d.t= Duncan's Multiple Range Test.
Means with the same latter are not significantly different at $p<0.05$

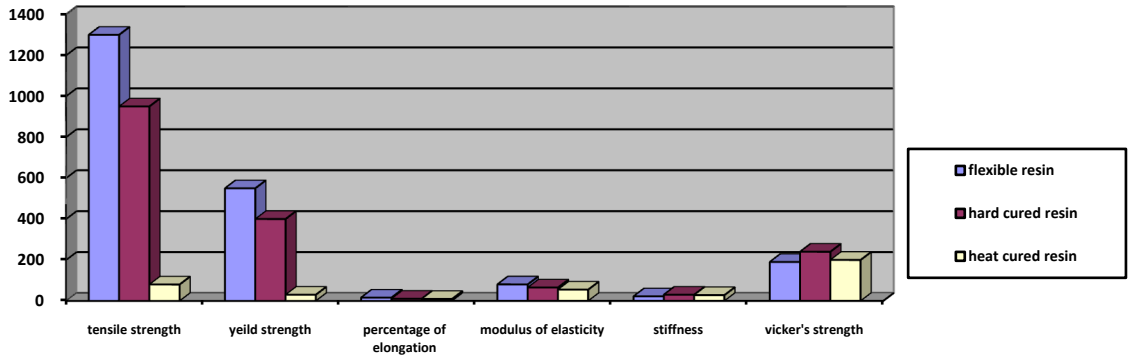

Fig (1): mean ± SD of different tests for the tested materials.

4. Discussion:

The most commonly used material for the fabrication of complete / partial dentures so far has been PMMA. This material is not ideal in every respect and it is the combination of virtues rather than one single desirable property that accounts for its popularity and usage. In spite of various advancements and research in dental materials, training, and techniques across the world, the fracture, foul smell, and allergy to PMMA could not be avoided.(2) Patients, who start wearing dentures at an early age due to various reasons, often get frustrated and start searching something better available for them. Although, cast partial denture has been a viable substitute, the requirement of high skill in preparation, technique-sensitive casting procedure, heavy weight, and visibility of metal clasp made it more difficult and cumbersome alternative and net results have not been encouraging. (1,3) Thermoplastic materials for dental prostheses, Valplast (Valplast Int. Corp. - USA) and Flexiplast (Bredent - Germay), were first introduced to dentistry in the 1950s. Both materials were similar grades of Polyamides (nylon plastics). Since their introduction, there has been a continued interest in thermoplastic dental materials.(4) Rapid Injection Systems (currently known as The Flexite Company - USA), originated in 1962, introduced the first Flexite thermoplastic which was a flouropolymer (a Teflon-type of plastic).(5,6) Valplast introduced a

flexible semi-translucent thermoplastic resin to create flexible tissue-born partial dentures. While the material was not strong enough to allow for conventional tooth born rest seat, the flexibility added to patient comfort in wearing the appliances. Flexite also was early into the dental market with flexible thermoplastic acrylic hybrid resin for removable appliances. Acetal was first proposed as an unbreakable thermoplastic resin removable partial denture material . It was during this period that Rapid Injection Systems developed the first tooth-colored clasps with a thermoplastic fluoropolymer.(2,4,7) Thermal polymerized PMMA demonstrates high porosity, high water absorption, volumetric changes and residual monomer. These properties lead to many of the problems associated with thermally polymerized acrylic versus the thermoplastic version. Thermoplastic acrylic has poor impact resistance, but has adequate tensile and flexural strength for a variety of applications. The material is easy to adjust, handle and polish. It is relineable and repairable at the chair-side. Thermoplastic acrylic is available in both tooth and gingival colors, and has both translucency and vitality, providing excellent esthetics. Like most thermoplastic resins, acrylic resin is also strong, resists fracturing, and is flexible. However, acrylic does not wear as well as acetal during occlusal forces and consequently will not maintain vertical dimension over long periods of time(3,6,8), Valplast introduced a flexible semi-translucent thermoplastic resin to create flexible tissue-born partial dentures. While the material was not strong enough to allow for conventional tooth born rest seat, the flexibility added to patient comfort in wearing the appliances. Flexite also was early into the dental market with flexible thermoplastic acrylic hybrid resin for removable appliances. Acetal was first proposed as an unbreakable thermoplastic resin removable partial denture material in 1971. It was during this period that Rapid Injection Systems developed the first tooth-colored clasps with a thermoplastic fluoropolymer.(9.10) The Flexite Company, developed and patented the first pre-formed tooth-color clasps known as Clasp-Eze. This product, made of a nylon material, is available in pink and clear color shades and currently sold worldwide. new line of thermoplastic Acetal, Acrylic, and Polycarbonate materials that can be used in most thermoplastic presses. These materials offer excellent esthetics combined with favorable physical properties and easy processing characteristics. The use of this material takes longer than the other ones. The material is monomer-free, virtually unbreakable, lightweight and impervious to oral fluids. Flexite Plus may also be combined with a metal framework to eliminate the display of metal labial clasps. (4,10,11)

Conclusion:

1- The modulus of elasticity of flexible acrylic resin was higher than that of hard acrylic resin and cold acrylic resin.

2- Flexible acrylic resin is highly material that can be used to replace the conventional hard acrylic resin because of its good aesthetic, physic-mechanical, and bio-compatibility properties.

3-flexible resin system" for their Success denture press. a flexible tissue colored thermoplastic resin for flexible partial dentures. Currently Cosmetic Dental Materials, has introduced Aesthetic.

4- The clasps were flexible, did not need periodic adjustment to keep them tight, and the tooth colored esthetics were appreciated by the patients.

5- Flexible acrylic resin is highly material reduce damage of tooth structure andsoft tissue, reduce in thickness.

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References:

1. Zarb GA, Bolender CL, Carlsson GE. 11th ed. St Louis: Mosby; 1997. Boucher's Prosthodontic Treatment for Edentulous Patients; pp. 337–42.
2. American Dental Association Specification No.12 dental base resin material 1999.
3. Jagger DC, Harrison A, Jandt KD. The Reinforcement of Dentures. J Oral Rehabil. 1999;26:185–94.
4. Donovan TE, Cho GC. Esthetic considerations with removable partial dentures. J Calif Dent Assoc 2003;31(7):551-7.
5. Negruţiu M, Sinescu C, Sandu Liliana, et al.(2oo4) Guidelines of removable partial dentures, Ed. Marineasa, Timişoara.
6. Nishigawa G, Matsunaga T, Maruo Y, Okamoto M, Natsuaki N, Minagi S.(2003) Finite Element Analysis of the Effect of the Bucco-Lingual Position of Artificial Posterior Teeth under Occlusal Force on the Denture Supporting Bone of the Edentulous Patients. J Oral Rehab;30:646–52.
7. Yunus N, Rashid AA, Azmi LL, Abu Hassan MI.(2005) Some Flexural Properties of a Nylon Denture Base Polymer. J Oral Rehabil.;32:65–71.
8. Lowe LG.(2004) Flexible denture flanges for patients exhibiting undercut tuberosities and reduced width of the buccal vestibule: a clinical report. J Prosthet Dent 2004; 92(2):128-31.
9. Phoenix RD, Mansueto MA, Ackerman NA, et al.(2004) Evaluation of mechanical and thermal properties of commonly used denture base resins. J Prosthodont 2004;13(1):17-27.
10. Beaumont AJ Jr.(2002) An overview of esthetics with removable partial dentures. Quintessence Int 2002;33(10):747-55.
11. Negruţiu M, Bratu D, Romînu M, et al.(2001) Polimeri utilizaţi în tehnologia protezelor mobile şi mobilizabile. Revista Natională de Stomatologie 2001; IV(1):30-41.
12. Dhiman RK Col, Roy Chowdhury SK.(2009) Midline Fracture in Single Complete Acrylic vs Flexible Dentures. MJAFI. 2009;65:141–5.

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Role of Heme Oxygenase -1 Induction and Type 5 Phosphodiesterase Inhibition in Hepatic Ischemia Reperfusion Injury in Male Albino Rats

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Abstract: ***Objective****:* Ischemia and reperfusion (I/R) injury is a pathophysiologic process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the compromised tissue. Both heme oxygenase producing carbon monoxide and nitric oxide synthase producing nitric oxide are involved in cytoprotection against ischemia and reperfusion. The aim of the present study was to investigate the possible hepatic cytoprotective effects of pretreatment with cobalt (III) protoporphyrin IX chloride (Copp) and sildenafil citrate during ischemia, separately and in combination on hepatic I/R injury assessed by serum alanine transaminase (ALT), a marker of hepatic IR injury, and necrotic index. ***Materials and methods:*** the study was carried out using fifty male albino rats belonging to the local strain aged eight weeks with body weight 165 to 200 g*m.* Rat were divided randomly into five groups, each included 10 rats: group I(control sham-operated), group II(hepatic I/R, ischemia for 45 minutes followed by reperfusion for 2 hours), group III(Copp pretreatment and I/R), group IV (I/R with sildenafil injection during ischemia), and group V(Copp pretreatment and sildenafil injection during ischemia). After two hours of reperfusion following ischemia, animals were killed and blood is collected for serum ALT determination and hepatic tissues were used for determining histological evidence of hepatocellular injury assessed by necrotic index. Liver samples are also used for determining HO-1 gene expression and total hepatic nitrite content. ***Results:*** Hepatic ischemia and reperfusion (group II) resulted in hepatocellular injury as revealed by significant increases ($p< 0.05$) in mean value of serum levels of ALT and necrotic index. This was accompanied by significant ($p < 0.05$) increases in the mean values of hepatic HO-1 gene expression and total hepatic nitrite content compared to the control group. Induction of HO-1, by pretreatment of rats with Copp (group III) resulted in hepatocellular protection as evident by significant decreases ($p < 0.05$) in mean values of serum level of ALT and necrotic index. This was accompanied by significant increases in the mean values of hepatic HO-1 gene expression and insignificant change ($p >0.05$) in total hepatic nitrite content compared to group II. Sildenafil citrate injection during ischemia (group IV) also resulted in hepatocellular protection as evident by significant decreases ($p < 0.05$) in mean values of serum levels of ALT and necrotic index accompanied by significant increases ($p < 0.05$) in the mean values of hepatic HO-1 gene expression and total hepatic nitrite content compared to group II. Compared to group III, sildenafil injection during ischemia produced insignificant changes ($p > 0.05$) in the mean value of serum level of ALT and necrotic index. However, HO-1 gene expressions was significantly ($p < 0.05$) decreased while total nitrite content was significantly ($p < 0.05$) increased. Compared to group II pretreatment of rats with Copp and Sildenafil injection during ischemia(group V) produced significant decreases ($p < 0.05$) in the mean value of serum levels of ALT, necrotic index while hepatic HO-1 gene expression and total nitrite content were significantly($p < 0.05$) increasd. Compared to group III and IV by pretreatment of rats with Copp and Sildenafil injection during ischemia produced significant decreases ($p < 0.05$) in the mean value of serum levels of ALT and necrotic index while hepatic HO-1 gene expression and total nitrite content were significantly($p < 0.05$) increased. *Conclusion*: Induction of HO-1 gene expression and inhibition of phosphodiesterase type 5 could have synergistic hepatoprotective effects against I/R injury. Further investigations are recommended for using agents that are not hepatotoxic and can protect the liver and other organs from I/R injury.

[Hassan M. Eissa; Mohammad E. Saleh; Laila A. Elsayed; and Hend A. Hassan. **Role of Heme Oxygenase -1 Induction and Type 5 Phosphodiesterase Inhibition in Hepatic Ischemia Reperfusion Injury in Male Albino Rats.** *Life Sci J* 2012;9(3):1711-1724] (ISSN:1097-8135). http://www.lifesciencesite.com. 250

Key words: Hepatic ischemic- reperfusion injury- Heme Oxygenase-1 – Nitric oxide – Phosphodiesterase Type 5 Inhibition.

1. Introduction

Ischemia and reperfusion (I/R) injury is a pathophysiologic process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the compromised tissue. Transient episodes of hepatic ischemia occur during solid organ transplantation, trauma, hypovolemic shock, and elective liver resection, when inflow occlusion or total vascular exclusion is used to minimize blood loss. The pathophysiology of liver I/R injury includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage resulting from activation of inflammatory pathways. Histopathologic changes include cellular swelling, vacuolization, endothelial cell disruption, neutrophil infiltration, and hepatocellular necrosis [(1, 2)].

The heme oxygenase (HO) system is the rate-limiting step in oxidative degradation of heme into bilivirdin, carbon monoxide (CO) and free iron [3]. Upregulation of Inducible HO-1 is known to be a protective response from cellular stress following I/R injury, radiation, and inflammation. Overexpression of HO-1 exerts a cytoprotective function in a number of I/R injury and liver transplants models [4]. HO-1 is an attractive target for anti-inflammatory therapies and potential candidate responsible for cell injury [5].

Nitric oxide (NO) has been shown to be released by the conversion of L-arginine to L-citrulline, a reaction catalyzed by one of three NO syntheses (NOS): neuronal NOS, endothelial NOS, and inducible NOS [6]. NO has been shown to be a mediator/protector of ischemia and reperfusion (I/R) tissue-mediated injury [7]. NO has been shown to be tissue-protective through its physiologic regulation of vascular tone, inhibition of platelet aggregation, attenuation of leukocyte adherence to the endothelium, scavenging of oxygen-derived free radicals, maintenance of normal vascular permeability, inhibition of smooth muscle proliferation, immune defenses, and stimulation of endothelial cell regeneration [8]. Delivery of NO during an ischemic insult has been shown to limit the extent of reperfusion damage to the heart [9], liver [10], lungs [11], and kidneys [12].

CO and NO share apparent similarities in structure, molecular weight and solubility [13]. Both NO and CO interact with iron (Fe) to form 5 or 6 coordinated haem complexes, which result in conformational changes and activation of the cyclic guanosine monophosphate (cGMP) / soluble guanylyl cyclase (sGC) [sGC/cGMP] pathway. Thus, many of the biological effects of CO are similar to NO, including its anti-apoptotic, anti-proliferative and anti-inflammatory mechanisms [14]. CO influences cell survival by blocking cytokine-mediated mitochondrial release of cytochrome *C* [15] and has been shown to influence hepatoprotection through the transcriptional upregulation of iNOS in the liver. Both exogenously administered and endogenously released NO stimulates HO-1 gene expression and CO production [15, 16]. Furthermore, CO and NO have been shown to participate in vasoactive cross talk, influencing growth factors, anti-inflammatory mediators, angiogenesis, and vascular remodeling [17].

Sildenafil citrate (Viagra), the first orally active and highly selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE-5), was approved for treating erectile dysfunction. It exhibited cardioprotective action against ischemia-reperfusion injury in both in situ and isolated hearts ([18, 19, and 20]). Sildenafil induced acute and delayed cardioprotection against ischemia-reperfusion injury through enhancement of nitric oxide (NO) generation by increased expression of endothelial NO synthase (eNOS)/inducible NO synthase (iNOS), activation of PKC, and opening of mitochondrial ATP-sensitive K^+ channels [21].

The aim of the present work is to study the effects of induction of HO-1, inhibiting phosphodiesterase type 5, and combined induction of HO-1 and inhibiting phosphodiesterase type 5 on ischemic reperfusion injury of the liver assessed by changes in serum alanine transaminase (ALT) and necrotic index and their effects on hepatic nitrite production and HO-1 gene expression. Also study the effect of ischemic reperfusion on hepatic NO production, indicated by hepatic nitrite production and on HO-1 gene expression.

2. Material and Methods

This study was carried out in Physiology, Histology, and Biochemistry departments, Faculty of Medicine, Cairo University. Fifty male albino rats aged eight weeks with body weight 165 to 200 gm were used in the study. Rats were placed in animal house under ordinary living conditions and were housed in wire mesh cages, 5 rats per cage, at room temperature. Rats were fed rat chow with free access to water. Rats were fasted for 12 hours before experiments and were randomly classified into the following five groups each contained 10 rats.

Group I: control group (sham operated group):

Rats were injected with 1 ml/kg isotonic saline intraperitoneally (I.P) 24 hours before sham operation. At time of sham operation, they were anesthetized then laparotomy and liver exposure were performed. 22.5 minutes later, isotonic saline (1ml/kg) was injected intravenously in rat tail.

Group II: ischemia reperfusion (I/R) group:

Animals of this group are subjected to hepatic ischemia reperfusion injury as 45 minutes of ischemia followed by 2 hours of reperfusion.

Group III: ischemia reperfusion group (I/R) with pre-operative injection of Cobalt (III) protoporphyrin IX chloride (CoPP):

Rats of this group underwent the same procedure as group II, but they were injected I.P with Cobalt (III) protoporphyrin IX chloride (CoPP) at a dose of 5mg/kg 24 hours before operation [5]. CoPP, an activator of HO-1, was purchased from (Sigma –Aldrich Egypt No. 1900) in the form of vials, each contains 500 mg. CoPP is dissolved in 0.2 mmol/L NaOH, adjusting its pH to 7.4 with 1mmol/L HCl and diluting it with isotonic saline.

Group IV: ischemia reperfusion (I/R) group with sildenafil citrate injection:

Rats of this group underwent the same procedure as group II, but they were injected intravenously with Sildenafil citrate. Sildenafil citrate (supplied from Pfizer, Egypt in the tablet form, 50 mg) was dissolved in saline to obtain a concentration of 2 µg/ml and injected I.V at a dose of 2 µg/kg 22.5 minutes after the onset of ischemia [22].

Group V: ischemia reperfusion (I/R) group with Copp and sildenafil injection:

Rats of this group underwent the same procedure as group II and were injected I.P with CoPP at a dose of 5mg/kg 24 hours before operation [5] and intravenously injected with Sildenafil citrate at a dose of 2 µg/kg, 22.5 minutes after the onset of ischemia [22].

Induction of hepatic ischemia reperfusion injury

Anesthesia was induced by intraperitoneal injection of thiopental sodium at a dose of 50 mg/kg (supplied from Eipico Egypt in the form of vials, each contains 500 mg) and maintained by repeat doses (IV) of 25 mg/ kg if necessary, based on animal movement. All the surgical procedures were performed under sterile conditions. Body temperature was maintained using a heating pad placed under the animal. I/R was produced by temporarily occluding the blood supply to the left lateral and median lobes of the liver, as described by **Zhang *et al.*** [23]. Laparotomy was carried out through a midline incision, and the ligamentous attachments from the liver to the diaphragm were severed and the liver exposed. Ischemia of the median and left lateral lobes of the liver was produced by clamping the corresponding vascular pedicle containing the portal vein and branches of the hepatic artery using an atraumatic microvascular clamp for 45 min. Other hepatic lobes were not handled during the procedure. This method produces ischemia to the left and median lobes of the liver, and leaves the blood supply to the right and caudate lobes uninterrupted. The liver was then placed back in its original position for 45 min and kept moist with sterile gauze dampened with 0.9% saline. Core body temperature was monitored by recording rectal temperature using rectal thermometer, and a heat lamp was utilized to maintain body temperature at 37 ± 0.4°C At the end of the ischemia period, the vascular clamp was removed, and the liver was reperfused for 2 hrs. After reperfusion, 3–5 ml of blood was collected from the vena cava in sterile syringes without anticoagulant and centrifuged to separate the serum. The serum samples were stored at −20°C for later analysis of ALT. Ischemic hepatic tissue samples were collected and part of the ischemic lobes used for histologic examination was fixed in alcohol – formalin – acetic acid and embedded in paraffin blocks. While samples used for HO-1 gene expression and nitrite were weighted and immediately frozen in isopentane and liquid nitrogen, then stored at −80°C for later analysis. The animals were killed by exsanguination. Sham-operated animals were treated in an identical fashion except for the omission of vascular occlusion

Measurement of serum ALT

Serum alanine transaminase (ALT), an established surrogate marker of hepatic IR injury [24] was measured using kits supplied by Lab Biotechnology (USA) catalog (Sup) according to the manufacturer's instructions.

Morphometric assessment of reperfusion injury.

Histopathology scoring was performed on randomly selected high-power fields by investigators blinded to sample identity Excised Liver samples embedded in paraffin blocks were cut in 6-µm sections and stained with hematoxylin and eosin. Stained sections were evaluated at 1200 magnification by a point-counting method for severity of hepatic injury using an ordinal scale as follows; **grade 0:** minimal or no evidence of injury, **grade 1:** mild injury consisting in cytoplasmic vacuolation and focal nuclear pyknosis, **grade 2**: moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders, and **grade 3:** severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration[25]**.** To assess necrotic index, the mean score between lobular, periportal, and perivenous necrosis was calculated for each specimen [26].

Measurement of total hepatic nitrite

The concentration of nitrites (NO_2^-) and nitrates (NO_3^-) in the liver was determined by the Griess reaction. After deproteination of liver homogenate by a solution of zinc sulfate, samples were incubated with cadmium granules to reduce nitrate to nitrite; the total nitrite was measured at 540 nm absorbance by diazotization with Griess reagent[27]**.**The results are expressed as the sum of the *N*-oxides of NO (NOx).

Measurement of HO-1 gene expression.

According to the method described by **Hoshida *et al.*** [28] Total RNA was extracted from liver tissue sing SV total RNA extraction kit supplied by (Promega Madisson USA) according to manufacturer instruction. Total RNA (5µg) was subjected to reverse transcription for cDNA synthesis at 42°C for 50min, using 1.6mM dNTPs, 10mM DTT, 176nM random hexamers (Invitrogen, Carlsbad, California, USA), 125U reverse transcriptase SuperScript II (Promega, Madisson, USA), and first strand buffer in a final volume of 30µl. The reaction was terminated by heating the samples at 75°C for 10min. For PCR, 4µl cDNA was incubated with 30.5µl water, 4µl 25mMMgCl2, 1µl dNTPs (10mM), 5µl 10×PCR buffer, 0.5µl (2.5 U) Taq polymerase and 2.5µl of each primer containing 10pmol. The oligonucleotide sequences for HO-1 were 5'- GAGCGCCCACAGCTCGACAG -3' (sense) and 5'-GTGGGCCACC AGCAGCTCAG -3' (antisense). The reaction mixture was subjected to 40 cycles of PCR amplification as follows: denaturation at 95°C for 1 min, annealing at67 °C for 1 min and extension at 72°C for 2min. PCR products were electrophoresed on 2% agarose stained with ethidium bromide and visualized by ultraviolet transilluminator. Semiquantitation was performed using gel documentation system (BioDO, Analyser, Biometra,

Gottingen, Germany). According to the amplification procedure, relative expression of each studied gene (R) was calculated according to the following the formula: densitometrical units of each studied gene/densitometrical units of b-actin.

PCR detection of b-actin

Presence of RNA in all samples was assessed by analysis of the 'house-keeping' gene b-actin. Complementary DNA was generated from 1 mg total RNA extracted with avian myeloblastosis virus reverse transcriptase for 60min at 371C. For PCR, 4ul complementary DNA was incubated with 30.5ul water, u ml 25mM MgCl2, 1ml deoxyribonucleotide triphosphates (10mM), 5ul 10x PCR buffer, 0.5ul (2.5U) Taq polymerase and 2.5ul of each primer containing 10pM. b-actin primers (forward 5-TGTTGTCCCTGTATGCCTCT-3; reverse 5-TAATGTCACGCACGATTTCC-3) The reaction mixture was subjected to 40 cycles of PCR amplification, denaturation at 95 ^{0}C for 1min, annealing at 57^{o}C for 1min and extension at 72 ^{0}C for 2mimutes

Statistical analysis

Data were expressed as mean + standard deviation (S.D.). The difference between two groups was assessed by using student t- test for unpaired data. $p < 0.05$ values are considered statistically significant. Correlation was done to show the association between two quantitative variables [(29)].

3. Results

Table 1 demonstrated that, in sham operated control group (group I), the mean value of serum level of ALT was 47.4± 5.45 U/l while the mean value of necrotic index was 0.000 (Fig.1). The mean value of hepatic HO-I gene expression was 0.001 ± 0.0003 arbitrary unit while the mean value total hepatic nitrite was 19.67 ± 2.78 pico (p) mol/mg protein.

In group II, in which rats were subjected to ischemia for 45 minutes followed by reperfusion for 2 hours, the mean value of serum level of ALT was 74.97± 5.18 U/l while the mean value of necrotic index was 2.79 ± 0.35 (Fig.2).The mean value of hepatic HO-I gene expression was 0.06 ± 0.01 arbitrary unit while the mean value total hepatic nitrite was 31.36 ± 4. 9 p mol/mg protein. These results demonstrated that ischemia and reperfusion resulted in significant increases ($p < 0.05$) in the mean values of serum level of ALT, necrotic index, hepatic HO-1 gene expression and total hepatic nitrite content compared to control group.

In group III, in which rats were pretreated with Copp and subjected to ischemia for 45 minutes followed by reperfusion for 2 hours, the mean value of serum level of ALT was 56.42± 6.8 U/l while the mean value of necrotic index was 1.9 + 0.43(Fig.3).The mean value of hepatic HO-I gene expression was 0.18± 0.04 arbitrary unit while the mean value total hepatic nitrite was 33.56 ± 5.9 p mol/ mg protein. These results demonstrated that, compared to group II, induction of HO-1, by pretreatment of rats subjected to I/R with Copp resulted in significant decrease ($p < 0.05$) in the mean value of serum level of ALT and necrotic index while the mean values of hepatic HO-1 gene expression was significantly increased. However, the mean value of total hepatic nitrite content was insignificantly changed ($p>0.05$). These results demonstrated the cytoprotective effect of HO-1 induction against I/R injury as evident from the significant decreases ($p < 0.05$) in mean values of serum levels of ALT and necrotic index compared to group II.

In group IV, in which rats were subjected to ischemia for 45 minutes followed by reperfusion for 2 hours and intravenously injected with sildenafil citrate during ischemia, the mean value of serum level of ALT was 56.86. + 6.3 U/l while the mean value of necrotic index was 2.06 + 0.33 (Fig.4).The mean value of hepatic HO-I gene expression was 0.09 ± 0.02 arbitrary unit while the mean value total hepatic nitrite was 40.3 ± 4.6 pmol/ mg protein. These results demonstrated that, compared to group II**,** Intravenous injection of sildenafil citrate during ischemia produced significant decreases in the mean values of serum level of ALT and necrotic index while the mean values HO-1 gene expression and total hepatic nitrite were significantly increased ($p < 0.05$). These results demonstrated the cytoprotective effect of sildenafil against I/R injury as evident from the significant decreases($p < 0.05$) in mean values of serum level of ALT and necrotic index. Compared to group III, the mean value of serum level of ALT and necrotic index demonstrated insignificant change ($p >0.05$), HO-1 gene expression was significantly ($p < 0.05$) decreased while total nitrite content was significantly increased ($p < 0.05$).

In group V, in which rats were pretreated with Copp and injected with sildenafil citrate during ischemia, table 1 demonstrated that the mean value of serum level of ALT was 48 84. + 4.9 U/l while the mean value of necrotic index was 0.49 + 0.11(Fig.5).The mean value of hepatic HO-I gene expression was 0.2 ± 0.03 arbitrary unit while the mean value total hepatic nitrite was 46.99 ± 5.4 p mol/ mg protein. These results demonstrated that pretreatment of rats, subjected to I/R with Copp and Intravenous injection of sildenafil citrate during ischemia produced significant decreases in the mean values of serum levels of ALT and necrotic index while the mean values of hepatic HO-1 gene expression and total nitrite content were significantly increased compared to group II. Compared to group III and group IV, data obtained from group V demonstrated significant decreases ($p < 0.05$) in the mean values of serum level of ALT, necrotic index while the mean values HO-1 gene expression and total hepatic nitrite were

significantly increased ($p < 0.05$). These results demonstrated that pretreatment with Copp and intravenous injection of sildenafil citrate during ischemia produced significant cytoprotective effect compared to pretreatment with Copp or sildenafil injection individually as evident from the significant ($p < 0.05$) decreases in mean values of serum levels of ALT and necrotic index compared to group III and group IV.

The present work demonstrated significant ($p < 0.05$) positive correlation between the mean values of serum levels of ALT and necrotic index (Fig. 6). Significant positive correlation is also demonstrated between the mean values of hepatic HO-1 gene expression and the mean values of total hepatic nitrite content (Figs. 6, 7).

Table (1): Effects of HO-1 induction and inhibition of phosphodiestarse type5 on serum alanine transaminase (ALT), necrotic index, hepatic heme oxygenase-1 (HO-1) gene expression and total hepatic nitrite contents in male albino rats subjected to hepatic ischemia and reperfusion injury**.**

		Ischemia for 45 minutes followed by reperfusion for 2 hours			
	Group I **Control group**	**Group II** **Ischemia reperfusion**	**Group III** **Pretreated with Copp**	**Group IV** **Sildenafil injection during ischemia**	**Group V** **pretreated with Copp and sildenafil injection during ischemia**
Serum ALT (U/l) Mean + SD	47.4+ 5.45	74.97±5.18 $^{@\,A}$	56.4±6.81 $^{*\,A}$	56.86+ 6.3 $^{*\,A\,\Theta\,B}$	48.84 + 4.9 $^{*\,A}$ $^{\#\,A}$
Necrotic index Mean + SD	0.000	2.799 ± 0.35 $^{@\,A}$	1.932± 0.43 $^{*\,A}$	2.06+ 0.3 $^{*\,A\,\Theta\,B}$	0.49+ 0.11 $^{*\,A}$ $^{\#\,A}$
HO-1 (arbitrary unit) Mean + S.D.	0.001±0.0003	0.063± 0.01 $^{@\,A}$	0.18±0.04 $^{*\,A}$	0.09+0.02 $^{*\,A\,\Theta\,A}$	0.2+0.03 $^{*\,A}$ $^{\#\,A}$
Total nitrite (pmol/ mg protein) Mean + S.D.	19.67±2.78	31.36±4.97 $^{@\,A}$	33.65 ± 5.9 $^{*\,B}$	40.3+4.61 $^{*\,A\,\Theta\,A}$	46.99+ 5.4 $^{*\,A}$ $^{\#\,A}$

A Significant changes ($p< 0.05$). B Insignificant changes ($p>0.05$). @ Compared to control group. * Compared to group II. ΘCompared to group III. # Compared to group III and IV

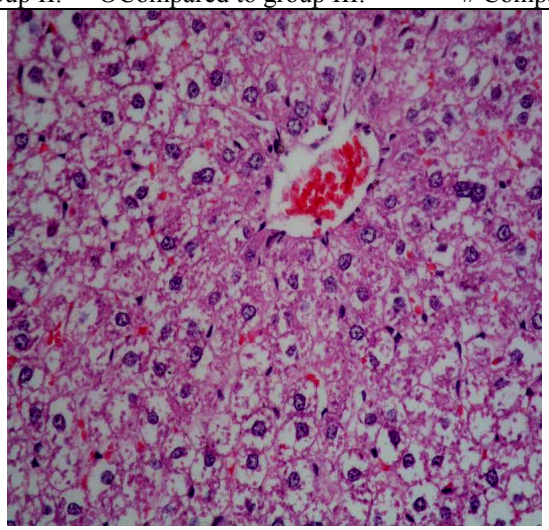


Figure 1:(Group I) This slide shows normal arrangement of cells normal nuclei and cytoplasm (necrotic index = o.oo ± , magnification power = 400).

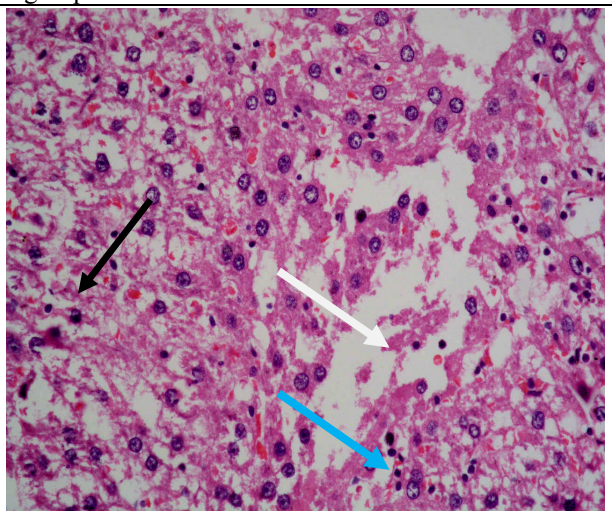


Fig. 2 (Group II): The white arrow represents disintegration of hepatic cords, the black one neutrophil infiltration and the blue arrow shows nuclear pyknosis (necrotic index 2.7±, magnification power = 400).

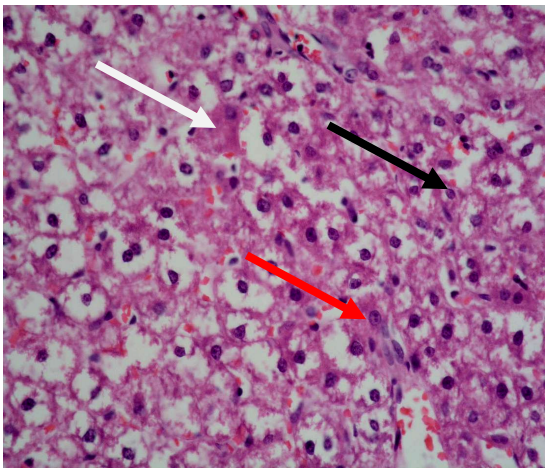


Fig. 3(Group III): The white arrow represents cytoplasmic hypereosinophilia, the blackone shows nuclear pyknosis and the red arrow represents loss of intercellular borders(necrotic index 1.9±, magnification power =400).

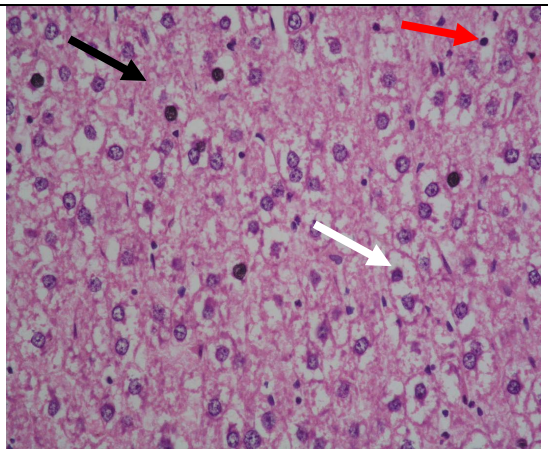


Fig. 4 (Group IV)The white arrow represents cytoplasmic vaculation ,the the red one shows nuclear pyknosis and the black arrow represents loss of intercellular border (necrotic index 2,06±, magnification power = 400)

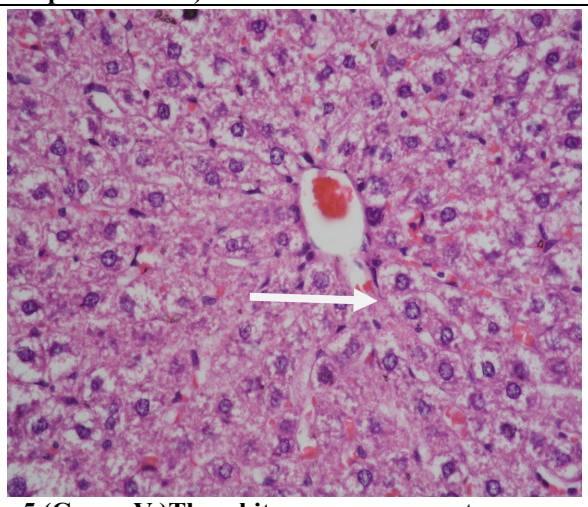
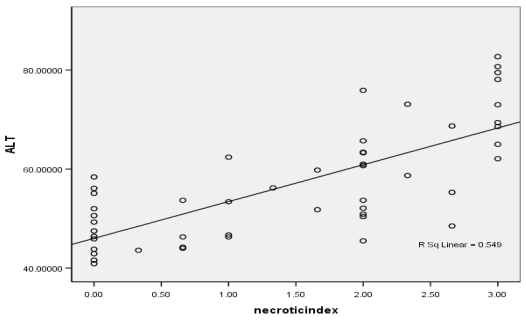
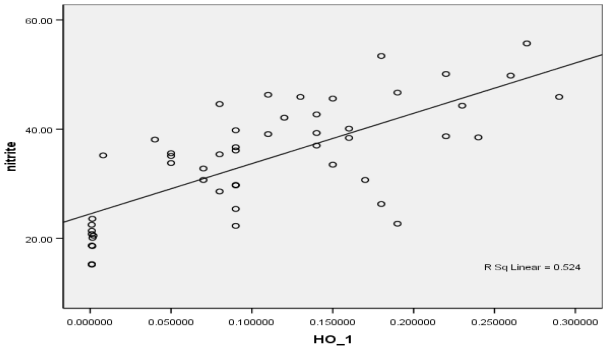


Fig. 5 (Group V)The white arrow represents cytoplasmic vaculation (necrotic index 0.49±, magnification power = 400)



Fig(6): Correlation between the mean level of serum ALT and the mean level of necrotic index.



Fig(7): Correlation between the mean level of HO-1gene expression and the mean level of nitrite in liver tissue

4. Discussion

Ischemia and reperfusion (I/R) injury is a pathophysiologic process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery to the compromised tissue. Transient episodes of hepatic ischemia occur during solid organ transplantation, trauma, hypovolemic shock, and elective liver resection, when inflow occlusion or total vascular exclusion is used to minimize blood loss. The pathophysiology of liver I/R injury includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunction and damage resulting from activation of inflammatory pathways. Histopathologic changes include cellular swelling, vacuolization, endothelial cell disruption, neutrophil infiltration, and hepatocellular necrosis **([1, 2]).**

In the present study, I/R injury of the liver was induced by exposure of the liver to 45 minutes of ischemia followed by 2 hours of reperfusion. Liver injury was assessed by estimation of serum alanine aminotransferase (ALT) and histological examination. Hepatic HO-1 gene expression and total nitrite content in the liver were also estimated after 2 hours of reperfusion.

The present work demonstrated significant increase ($p< 0.05$) in the mean values of serum level of ALT and necrotic index in group II subjected to I/R injury, compared to sham operated control group (group I). Significant positive correlation ($p<0.05$) was reported between mean values of serum ALT and mean values of necrotic index in all studied groups subjected to ischemia and reperfusion .These findings are consistent with the results of **Sepodes *et al.*** [30] who reported that liver ischemia for 30 minutes followed by reperfusion for 2 hours resulted in significant rises in serum levels of ALT. **Wang *et al.*** [31] found increased levels of ALT in mice after 45 minutes of partial hepatic ischemia. **Kim *et al.*** [32] reported that hepatic I/R caused significant hepatocellular damage as demonstrated by elevated plasma ALT level.

Blood perfusion to previously ischemic tissue induces severe tissue injury, which is called ischemia-reperfusion (I/R) injury [33]. Deprivation of oxygen to the liver during ischemia induces severe lesions but much more important ones are originated during reperfusion when oxygen entry to the organ is restored. On this case, an additional liver aggression occurs exacerbating greatly the previous injury induced by ischemia. Both facts lead to the induction of multiple and close related inflammatory processes in liver and extrahepatic organs which define the complex pathophysiology of I/R injury [34]. **Linfert *et al.*** [35] reported that interruption of blood flow to any tissue leads to inadequate tissue oxygenation and an increased cellular anaerobic pathways, and if adequate oxygenation is not restored then disruption of cellular functions and cell death results. On reperfusion, despite restoration of adequate cellular oxygenation, there is further damage caused by direct cytotoxicity from oxygen free radicals and by a secondary immunological assault upon the injured organ involving components of both the innate and adaptive immune system.

I/R injury of the liver is characterized by sinusoidal vasoconstriction, neutrophil accumulation, platelet aggregation and alterations on the capillary permeability leading to a progressive inflammatory reaction with important microcirculatory alterations, which can trigger diffuse cell death and consequent acute organ failure[33]. The progression of I/R injury depends primarily on the presence of pre-existing parenchymal alterations, such as hepatic steatosis and fibrosis, as well as the duration of ischemia period [36]**.** When oxygen supply to hepatocytes becomes insufficient as result of reduced or absent blood flow, there is inhibition of the mitochondrial oxidative phosphorylation with the subsequent reduction in adenosine triphosphate (ATP) synthesis. Depletion of cellular ATP store induces alterations in transmembrane ion transport by inhibition of the ATP-dependent Na+/K+ ATPase, leading to intracellular sodium accumulation, secondary alterations in cellular calcium homeostasis and, particularly, cell swelling and death [37].

Post-ischemic liver injury is biphasic in nature consisting of an acute or early phase and a subacute or late phase [38]**.** The early phase of injury occurs in the absence of leukocyte infiltration and is thought to be initiated by a rapid alteration in the redox state of the tissue in favor of a more oxidative environment. The late phase of injury is dependent upon the production of several different cytokines and chemokines that promote the infiltration of large numbers of polymorph nuclear neutrophils (PMNs) and lymphocytes into the liver interstitium via the up-regulation of endothelial cell adhesion molecules and formation of chemotactic gradients [39]**.** Interstitial PMNs become fully activated and release copius amounts of reactive oxygen species together with extracellular matrix degrading enzymes. The net result of this inflammatory infiltrate is an amplification of the acute injurious response resulting in extensive inflammatory tissue injury [40]**.**

The results of the present work demonstrated that hepatic heme oxygenase (HO-1) gene expression was significantly increased in group II (I/R group) compared to the control group. Induction of HO-1 by Copp (group III) resulted in significant increase in HO-1 gene expression and significant decreases in the mean values of ALT and necrotic index compared to group II. These results demonstrated the cytoprotective effect of HO-1 against I/R injury of the liver.

The heme oxygenase (HO) system is the rate-limiting step in the oxidative degradation of heme into biliverdin, carbon monoxide (CO) and free iron [3].

Three HO isoforms have been identified: inducible HO-1, also known as heat shock protein 32; constitutively expressed HO-2; and a related but less well-characterized HO-3. HO-1 has been shown to be expressed principally in Kupffer cells [4, 41]. HO-1 is induced in a variety of organs during diverse stress-related conditions and is thought to provide cytoprotection [42, 43]. HO-1 is readily induced by heme, oxidants, lipopolysaccharide, cytokines, irradiation, heavy metals, and other stressors [44]. HO-1 presents at low to undetectable levels in Kupffer cells under basal conditions, but it undergoes a rapid transcriptional activation and expresses both in Kupffer cells and hepatocytes as a response to noxious stimuli. Ho-1 induction is considered to be adaptive cellular response to survive on exposure to environmental stress [45].

HO-1 provides an important protective response from cellular stress following ischemia, preventing the deleterious effects of heme as well as mediating anti-inflammatory and antiapoptotic functions via its products [46]. It is unclear whether baseline HO-1 levels before the injury or the degree of HO-1 up-regulation following the injury is important to confer cytoprotection [47]. HO-1 overexpression by pharmacological means or via genetic engineering has been shown to exert potent cytoprotective effects in hepatic I/R injury transplant models, where both proinflammatory and apoptotic responses remain profoundly diminished in HO-1-overexpressing liver transplants [48].

Induction of HO-1 and its metabolites is protective in a large number of seemingly unrelated pathologies, including sepsis, malaria, endotoxic shock, I/R injury, organ transplant, and myocardial infarction,. This spectrum of protection is attributed to multi-level mechanisms of cytoprotection and inflammatory modulation [49]. The cobalt protoporphyrin induction of HO-1 has been shown to improve liver function and histologic characteristics [46]. Using cobalt protoporphyrin and the HO-1 antagonist zinc protoporphyrin demonstrated the protective effects of HO-1 induction during the prolonged storage of liver transplants [48]. Hypertonic saline prevented ischemia-reperfusion injury by promoting the expression of heme oxygenase-1 [50]. Induction with simvastatin preconditioning also had a protective result [51].

The beneficial effects of HO-1 are presumably mediated by the degradation of pro-oxidative heme and production of biologically active HO reaction products [52].Biliverdin and bilirubin are powerful antioxidants [53]. CO mediates the antiapoptotic, anti-inflammatory, antiproliferative and vasodilatory properties of HO-1 [54] and iron induces the synthesis of ferritin, which is also a cytoprotective molecule and sequesters free iron [55].

Overexpression of HO-1 exerts a cytoprotective function in a number of I/R injury and liver transplant models [56]. Thus, HO-1 is an attractive target for anti-inflammatory therapies and potential candidate responsible for cell injury [5].There is evidence that treatment with the products or related molecules of the HO-1 reaction is protective, including biliverdin [57], bilirubin, and carbon monoxide [58]. Specifically, exogenous carbon monoxide was protective in liver transplants [59, 60]. There is also evidence that heme oxygenase-1 mediated cytoprotection depends on and can be substituted by carbon monoxide generation [61].

The results of the present work demonstrated significant increases ($p< 0.05$) in the mean values of total hepatic nitrite in group II and group III compared to the control group. Integrated Nitric oxide production can be estimated from determining the concentrations of nitrite and nitrate end products. The measurement of total nitrate and nitrite concentration (NOx) is used as an index of NO production [62].

Nitric oxide is produced from L-arginine by nitric oxide synthase enzymes (NOS) [63] .Three NOS have been identified: two constitutive (cNOS: type 1 or neuronal and type 3 or endothelial) and one inducible (iNOS: type 2). In the liver, cNOS activity is normally detectable in Kupffer cells, whereas no cNOS is ever encoded in hepatocytes. However, hepatocytes, Kupffer and stellate cells (the three main types of liver cells) are prompted to express an intense iNOS activity once exposed to effective stimuli such as bacterial lipopolysaccharide and cytokines [64].

The process of ischemia and reperfusion is known to cause inducible nitric oxide synthase induction and activation, and there is evidence that interleukin (IL)-1β [65], IL-1 receptor [66], and IL-1 receptor along with nuclear factor-kappa beta (NF-kappa β) [67] may have an important role in that induction. During ischemia and reperfusion, both helpful and harmful effects of nitric oxide have been reported, and the nitric oxide molecule has been described as having a “janus face” [68]These conflicting results about the role of nitric oxide during ischemia reperfusion, with some studies showing beneficial results and others harmful results, have been attributed to the use of nonspecific inhibitors of nitric oxide synthase [69]. Whether nitric oxide has a helpful or harmful effect depends on several factors in the liver [70].

Lhuillier *et al.* [71] measured nitric oxide generated in the liver parenchyma during ischemia and reported that NO concentrations increased after the onset of ischemia to reach a plateau by 10 minutes. The short delay existing between the onset of ischemia and the increase in NO signal is consistent with the involvement of endothelial nitric oxide synthase (eNOS). However, nonselective NOS inhibitors administered before ischemia failed to inhibit the increase in NO, whereas NO production remained

inhibited in the control group treated with nonselective NOS inhibitors, thereby suggesting the presence of enzyme-independent sources of NO. NO stores are available in tissues and could be mobilized during ischemia**.** Another enzyme-independent source for NO might be the endogenous reduction of nitrite and nitrate resulting in the appearance of NO. This mechanism has been demonstrated in ischemic heart and could be the source of NO in the ischemic liver [(72)]. However, this NO production was dependent on the amount of nitrite–nitrate available in the tissue before ischemia. [(73)].

De Caterina *et al.* [(74)] provide evidence that NO enhances the *de novo* synthesis and/or stabilization of the natural inhibitor Ikβ-α.Furthermore, NO is known to interact with and decompose O_2^- or other reactive radicals or oxidants thereby limiting the formation of O_2^- derived H_2O_2 and preventing the downstream oxidant-induced pathways for NFkB activation [(75)]. Another possible mechanism may be that NO-dependent activation of soluble guanylyl cyclase (sGC) with the subsequent production of the vasorelaxant cyclic guanosine 5'-monophosphate (cGMP) may protect against reperfusion injury by enhancing blood flow, thereby limiting the degree of ischemia to the liver. It has been proposed that NO-mediated activation of protein kinase G via the sGC/ cGMP pathway opens mitochondrial K_{ATP} channels which reduces calcium accumulation within the mitochondria and prevents the loss of cytochrome c from the mitochondrial intermembranal space [(76)]. Alternatively or, in addition to, NO may reversibly inhibit mitochondrial respiration via interaction with complex I and/or cytochrome c oxidase. This would inhibit apoptosis, maintain small but significant amounts of O_2 during ischemia and allow for a more controlled resumption of respiration following reperfusion. Similar observations have been made with NO-dependent S-nitrosation of caspase-3 resulting in inactivation of this enzyme and inhibition of apoptosis. This would minimize free radical-mediated damage to the mitochondrial membrane and preserve cellular function [(77)]. NO may attenuate the later stages of post-ischemic tissue damage by inhibiting platelet/leukocyte-endothelial cell interactions [(78)]**.**

The results of the present work demonstrated cytoprotective effect of sildenafil citrate on liver subjected to I/R injury as evident from the significant decrease in mean value of serum level of ALT and necrotic index compared to I/R group .

Sildenafil is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5A (PDE-5). PDE-5 is responsible for the degradation of cGMP by hydrolysis to guanosine 5'-monophosphate (5'-GMP). Thereby, inhibition of PDE-5 by sildenafil preserves the cGMP pool and potentiates downstream signaling [(79)].

The basic mechanisms of NO-dependent cytoprotection are diverse and include direct neutralization of the superoxide radical [(80)] or inhibition of proapoptotic enzymes, such as caspase-3–like proteins, through *S*-nitrosylation [(81)]**.** These actions are cGMP independent and do not require increased activity of soluble guanylyl cyclase, the target enzyme of NO in many biological systems [(82)]. By contrast, endothelial protection afforded by NO against the deleterious effects of proinflammatory cytokines has clearly been shown to be cGMP dependent [(83)]**.**

NO modulates hepatocellular/tissue injury through its participation in neutrophil adhesion, platelet aggregation, and maintenance of normal vascular permeability [(84)]. Nitric oxide binds to the haem moiety of guanylate cyclase and increases its activity by 400-fold, catalyzing the conversion of guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Elevation of cGMP relaxes the smooth muscles in blood vessels, inhibits platelet aggregation and adhesion, and blocks the adhesion of white cells to the blood vessel wall, cellular necrosis and apoptosis [(85,86)]**.**

The cytoprotective effect of sildenafil against hepatic I/R injury, observed in the present work, is supported by the work of **Duranski *et al.*** [(22)] who reported that the soluble isoform of guanylyl cyclase is an important cellular target of NO. Genetic overexpression of eNOS protects against hepatic I-R injury. Direct sGC inhibition increased serum ALT levels in mice after hepatic I-R, suggesting that sGC function plays pivotal role in attenuating I-R injury. Control mice treated with sildenafil were significantly protected against hepatic I-R injury. Taken together, these data suggest that the NO-sGC-cGMP axis plays a critical role in limiting the extent of hepatic I-R injury [(22)]**.**Contrarily to the hepatic protective effect of sildenafil against I/R injury observed in the present work, ***Leão et al.*** [(87)] reported that pre-treatment with sildenafil in rats resulted in increased damage to hepatocytes in a model of hepatic ischemia/reperfusion. These controversy can be explained on the basis of different protocols used as method, dose, and timing of sildenafil administration.

The present work demonstrated a significant increase in the mean value of hepatic nitrite concentration in group IV compared to group II .**Salloum *et al.*** [(19)] reported that sildenafil up regulated both eNOS and iNOS and that inhibition of iNOS completely abolished the protective effect of sildenafil.

The present work demonstrated significant increase in the mean value of hepatic HO-1 expression in sildenafil group compared to group II (I/R group).

In addition to its action as a vasodilator, NO can regulate the expression of a variety of genes. In particular, there is solid evidence that NO regulates the expression of HO-1 [(88)]. Treating aortic smooth muscle

cells with the NO donor increases HO-1 gene transcription, resulting in increased mRNA and protein expression. This induction of HO-1 by NO occurs in a cGMP-independent manner. The pathways by which NO regulates the expression of HO-1 and other genes are complex and but appear to involve mitogen-activated protein kinase (MAPK) members such as extracellular signal-regulated kinase (ERK) and p38 [(88)]. On the other hand, the pathway, through which cGMP induces HO-1 could be a direct one, i.e., via cGMP-sensitive transcription factors, such as activator protein-1[(89)]. Alternatively, cGMP may act through secondary increases in cAMP, which were reported to occur in endothelial cells in response to NO donors and which were possibly due to cGMP-elicited inhibition of cAMP breakdown [(90)]. Because activator protein-1 and cAMP-responsive elements have been identified in the promoter region of HO-1, [(91)]. HO-1 induction by NO/cGMP may be regulated through different mechanisms, depending on species and tissue [(92)].

The results of the present work demonstrated that pretreatment of rats with both Copp and sildenafil during ischemia resulted in significant decreases in the mean values of ALT and necrotic index compared to group III and IV, and resulted in significant increases in the mean values of hepatic HO-1 and nitrites compared to group II, III, and IV. These results demonstrated that combined effects of both Copp and sildenafil have more cytoprotective effect than the effect of either copp or sildenafil alone.

CO, like NO, is a second messenger gas involved in a number of physiological processes [(93)]. Both CO and NO activate soluble guanylate cyclase to increase cyclic GMP (cGMP) levels. It is becoming increasingly clear that iNOS/NO and HO-1/CO can modulate each other's activity. These two system are linked in that NO can up-regulate HO-1 expression leading to the formation of endogenous CO [(94)], and CO can bind to the heme group in the iNOS protein and influence the production of NO [(95)]. This interaction between HO-1/CO and NO can explain the positive correlation between hepatic HO-1 gene expression and nitrite content, an index of NO production [(62)] observed in the present work

CO and NO share apparent similarities in structure, molecular weight and solubility [(96)]. Both NO and CO interact with iron (Fe) to form 5 or 6 coordinated haem complexes, which result in conformational changes and activation of the sGC/cGMP pathway. Thus, many of the biological effects of CO are similar to NO, including its anti-apoptotic, anti-proliferative and anti-inflammatory mechanisms [(14)]. Other studies have confirmed the participation of both NO and CO-mediated signaling cascades in suppression of platelet aggregation. In addition to regulating vascular cell growth, CO influences cell survival by blocking cytokine-mediated mitochondrial release of cytochrome C [(15)] and has been shown to influence hepatoprotection through the transcriptional upregulation of iNOS in the liver. Both exogenously administered and endogenously released NO stimulates HO-1 gene expression and CO production [(95)]. Furthermore, CO and NO have been shown to participate in vasoactive cross talk, influencing: growth factors, anti-inflammatory mediators, angiogenesis and vascular remodeling [(17)].

Conclusion and Recommendations

The results of the present work demonstrated that induction of HO-1 gene expression and inhibition of cGMP-specific phosphodiesterase type 5 (PDE-5) could have synergistic hepatoprotective effect against I/R injury observed in many clinical situations. Further investigations are recommended for using agents that are not hepatotoxic and can protect the liver and other organs from I/R injury.

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Reference

1. **Fondevila, C. Busuttil, R.W., and Kupiec-Weglinski, J.W.:** Hepatic ischemic /reperfusion injury: a fresh look. Exp. Mol. Pathol., 2003; **74:** 86-93.
2. **Selzner, N., Rudiger, H., Graf, R., and Clavien. P. A. :** Protective strategies against ischemic injury of the liver. Gastroenterology, 2003; **125**: 917-936.
3. **Choi, A.M., and Alam, J. :** Heme oxygenase-1: function, regulation, and implication of a novel stress-inducible protein in oxidant-induced lung injury. Am. J. Respir. Cell. Mol. Biol., 1996; **15**: 9-19.
4. **Kobayashi, T., Hirano, K., Yamamoto, T., Hasegawa, G., Hatakeyama, K., Suematsu, M., *et al.* :** The protective role of Kupffer cells in the ischemia-reperfused rat liver. Arch. Histol. Cytol. 2002; **65**: 251-261.
5. **Zeng, Z., Huang, H.F., Chen, M.Q., Song, F., and Zhang, Y.J**.: Heme oxygenase-1 protects donor livers from ischemia/reperfusion injury: The role of Kupffer cells. World J. Gastroenterol, 2010; **16(10)**: 1285-1292.
6. **Schulz, R., Kelm, M., and Heusch G**. **:** Nitric oxide in myocardial ischemia/ reperfusion injury. Cardiovasc. Res., 2004; **61**:402–413.
7. **Anaya-Prado, R., Toledo-Pereyra, L.H., Lentsch, A.B., and Ward, P.:** Ischemia/reperfusion injury. J. Surg. Res., 2002; **105**:248-258.

8. **Ohmori, H., Dhar, D.K., Nakashima, Y., Hashimoto, M., Masumura, S., and Nagasue, N. :** Beneficial effects of FK409, a novel nitric oxide donor, on reperfusion injury of rat liver. Transplantation., 1998; **66(5)**:579-585.
9. **Bolli, R. :** Cardioprotective function of inducible nitric oxide synthase and role of nitric oxide in myocardial ischemia and preconditioning: an overview of a decade of research. J. Mol. Cell. Cardiol. , 2001; **33**:1897–1918.
10. **Aiba, M., Takeyoshi, I., Ohwada, S., Kawashima, Y., Iwanami, K., Sunose, Y., *et al.* :** Novel nitric oxide donor (FK409) ameliorates liver damage during extended liver resection with warm ischemia in dogs. J. Am. Coll. Surg., 2001; **193**:264–271.
11. **Murakami, S., Bacha, E.A., Mazmanian, G.M., Détruit, H., Chapelier, A., Dartevelle, P., *et al.*: Effects** of various timings and concentrations of inhaled nitric oxide in lung ischemia-reperfusion. Am. J. Respir. Crit. Care Med., 1997; **156**:454–458.
12. **Lopez-Neblina, F., Toledo- Pereyra, L.H., Mirmiran, B., and Paez-Rollys, A.J.:** Time dependence of Na-nitroprusside administration in the prevention of neutrophil infiltration in the rat ischemic kidney. Transplantation. 1996; **61**:179-183.
13. **Ryter, S.W., Morse, D., and Choi, A.M.K.:** Carbon monoxide: to boldly go where NO has gone before. Science STKE., 2004; **Vol. 2004 (230)**: RE6.
14. **Boehning, D., and Snyder, S.**: Novel neural modulators**.** Ann. Rev. Neurosci., 2003; **26:**105-131.
15. **Durante, W:** Targeting heme oxygenase-1 in vascular disease**.** Curr. Drug Targets. , 2010; **11:**1504-1516.
16. **Liu, X., Chapman, G., Peyton, K., Schafer, A., and Durante, W.:** Carbon monoxide inhibits apoptosis in vascular smooth muscle cells**.** Cardiovasc. Res., 2002; **55:**396-405.
17. **Wanstall, J., Jeffery, T., Gambino, A., Lovren, F., and Triggle, C.:** Vascular smooth muscle relaxation mediated by nitric oxide donors: a comparison with acetylcholine, nitric oxide and nitroxyl ion. Br. J. Pharmaco., 2001; **134:** 463-472.
18. **Ockaili, R., Salloum, F., Hawkins, J., and Kukreja, R.C.:** Sildenafil (Viagra) induces powerful cardioprotective effect via opening of mitochondrial K_{ATP} channels in rabbits. Am. J. Physiol. Heart Circ. Physiol., 2002; **283**: H1263–H1269.
19. **Salloum, F., Yin, C., Xi, L., and Kukreja, R.C.:** Sildenafil induces delayed preconditioning through inducible nitric oxide synthase-dependent pathway in mouse heart. Circ. Res., 2003; **92**: 595–597.
20. **Salloum, F., Abbate, A., Das, A., Houser, J.E., Mudrick, C.A., Qureshi I.Z., *et al.*:** Sildenafil (Viagra) attenuates ischemic cardiomyopathy and improves left ventricular function in mice. Am. J. Physiol. Heart Circ. Physiol., 2008; **294**: H1398–H1406.
21. **Das, A., Ockaili, R., Salloum, F., and Kukreja, R.C.:** Protein kinase C plays an essential role in sildenafil-induced cardioprotection in rabbits. Am. J. Physiol. Heart Circ. Physiol., 2004; **286**: H1455–H1460.
22. **Duranski, M.R., Elrod, J.W., Calvert, J.W., Bryan, N.S., Feelisch, M., and Lefer, D.J.:** Genetic overexpression of eNOS attenuates hepatic ischemia-reperfusion injury. Am. J. Physiol. Heart Circ. Physiol., 2006; **291:H2980**–H2986.
23. **Zhang, W., Wang, M., Xie, H.Y., Zhou, L., Meng, X.Q., Shi, J., *et al.*:** Role of reactive oxygen species in mediating hepatic ischemia-reperfusion injury and its therapeutic applications in liver transplantation. Transplant. Proc., 2007; **39**:1332–1337.
24. **Iu, S., Harvey, P.R., Makowka, L., Petrunka, C.N., Ilson, R.G., and Strasberg, S.M.:** Markers of allograft viability in the rat. Relationship between transplantation viability and liver function in the isolated perfused liver. Transplantation, 1987; **44**:562-569.
25. **Camargo, C.A, Jr., Madden, J.F., Gao, W., Selvan, R.S., and Clavien, P.A.:** Interleukin-6 protects liver against warm ischemia/reperfusion injury and promotes hepatocyte proliferation in the rodent. Hepatology, 1997; **26***:* 1513–1520.
26. **Degli Esposti, D., Sebagh, M., Pham, P., Reffas, M., Pou, C., Brenner, C., *et al.*:** Ischemic preconditioning induces autophagy and limits necrosis in human recipients of fatty liver grafts, decreasing the incidence of rejection episodes. Cell Death and Disease, 2011; **2**, e111.
27. **Vodovotz, Y.*:*** Modified microassay for serum nitrite and nitrate. Biotechniques, 1996; **20*:*** 390-394.
28. **Hoshida, S., Nishida, M., Yamashita, N., Igarashi, J., Aoki, K., Hori, M., *et al.*:** Heme oxygenase-1 expression and its relation to oxidative stress during primary culture of cardiomyocytes. J. Mol. Cell. Cardiol., 1996; **28**:1845–55.
29. **Knapp, R.G., and Miller, M.C.:** III, "Clinical epidemiology and biostatistics", 1992 Williams & Wilkins, Baltimore, Maryland, USA.
30. **Sepodes, B., Maio, R., Pinto, R., Sharples, E., Oliveira, P., McDonald, M., *et al.*:** Recombinant human erythropoietin protects the liver from

hepatic ischemia-reperfusion injury in the rat. Transpl. Int., 2006; **19**: 919-926.

31. **Wang, H., Li, Z.Y., Wu, H.S., Wang, Y., Jiang, C.F., Zheng, Q.C., *et al.*:** Endogenous danger signals trigger hepatic ischemia/reperfusion injury through toll-like receptor 4/nuclear factor-kappa B pathway. Chin. Med. J. (Engl), 2007; **120**: 509–514.
32. **Kim, M. S., Lee, K, H., Lee, W.M., Jun, J.H., and Kim, D.H.:** CD44 Disruption Attenuates Murine Hepatic Ischemia/Reperfusion Injury**.** Korean Med. Sci., 2011; **26(7)**: 919–926.
33. **Serracino-Inglott, F., Habib, N.A., and Mathie, R.T**.: Hepatic ischemia-reperfusion injury. Am. J. Surg., 2001; **181**:160- 166.
34. **Glantzounis, G.K., Salacinski, H.J., Yang, W., Davidson, B.R., and Seifalian, A.M.** The contemporary role of antioxidant therapy in attenuating liver ischemia-reperfusion injury: a review. Liver Transpl., 2005; **11**:1031-47.
35. **Linfert, D.**, **Chowdhry, T., and Rabb, H.:** Lymphocytes and ischemia- reperfusion injury. Transplant Rev., (Orlando) 2009; **23**:1-10.
36. **Isozaki, H., Okajima, K., Kobayashi, M., Hara, H., and Akimoto H.:** Experimental study of liver injury after partial hepatectomy with intermittent or continuous hepatic vascular occlusion. Differences in tolerance to ischemia between normal and cirrhotic livers. Eur. Surg. Res., 1995; **27**: 313-322.
37. **Belzer, F.O., and Southard, J.H.:** Principles of solid-organ preservation by cold storage. Transplantation, 1988; **45**: 673-676.
38. **Fan, C., Zwacka, R.M., and Engelhardt, J.F.:** Therapeutic approaches for ischemia/reperfusion injury in the liver. J. Mol. Med., 1999; **77**:577–592.
39. **Caldwell, C.C., Okaya, T., Martignoni, A., Husted. T., Schuster, R., and Lentsch, A.B.:** Divergent functions of CD4+ T lymphocytes in acute liver inflammation and injury after ischemia-reperfusion. Am. J. Physiol. Gastrointest. Liver. Physiol., 2005; **289**: G969–G976.
40. **Jaeschke, H.:** Mechanisms of Liver Injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. Am. J. Physiol. Gastrointest. Liver Physiol., 2006; **290**:G1083–G1088.
41. **Kiemer, A.K., Gerwig, T., Gerbes, A.L., Meissner, H., Bilzer, M., and Vollmar, A.:** Kupffer-cell specific induction of heme oxygenase 1 (hsp32) by the atrial natriuretic peptide--role of cGMP. J. Hepatol., 2003; **38**: 490-498
42. **Fujita, T., Toda, K., Karimova, A., Yan, S.F., Naka, Y., Yet, S.F., *et al.*:** Paradoxical rescue from ischemic lung injury by inhaled carbon monoxide driven by derepression of fibrinolysis. Nat. Med., 2001; **7**: 598-604.
43. **Coito, A.J., Buelow, R., Shen, X.D., Amersi, F., Moore, C., Volk, H.D., *et al.*.**: Heme oxygenase-1 gene transfer inhibits inducible nitric oxide synthase expression and protects genetically fat Zucker rat livers from ischemia reperfusion injury. Transplantation, 2002; **74**: 96-102
44. **Platt, J.L., and Nath, K.A.:** Heme oxygenase: protective gene or Trojan horse. Nat. Med., 1998; **4**:1364–1365**.**
45. **Farombi E. O. and Surh, Y.J.:** Heme oxygenase-1 as a Potential Therapeutic Target for Hepatoprotection. Journal of Biochemistry and Molecular Biology (Seoul)**,** 2006; **39 (5):**479-491.
46. **Tsuchihashi, S., Zhai, Y., Fondevila, C., Busuttil, R.W., and Kupiec-Weglinski, J.W.:** HO-1 upregulation suppresses type 1 IFN pathway in hepatic ischemia/reperfusion injury. Transplant. Proc., 2005; **37(4)**:1677-1678.
47. **Geuken, E., Buis, C.I., Visser, D.S., Blokzijl, H., Moshage, H., Nemes, B., *et al.*:** Expression of heme oxygenase-1 in human livers before transplantation correlates with graft injury and function after transplantation. Am. J. Transplant., 2005; **5**: 1875-1885.
48. **Kato, H.**, **Amersi, F., Buelow, R., Melinek, J., Coito, A.J., Ke, B. *et al.*:** Heme oxygenase-1 overexpression protects rat livers from ischemia/reperfusion injury with extended cold preservation. Am. J. Transplant., 2001; **1**: 121-128.
49. **Soares, M.P., and Bach, F.H.:** Heme oxygenase-1: from biology to therapeutic potential. Trends Mol. Med., 2009; **15**:50–58.
50. **Ke, Q.H., Zheng, S.S., Liang, T.B., Xie, H.Y., and Xia, W.L.:** Effects of hypertonic saline on expression of heme oxygenase enzyme-1 in hepatic ischemia/reperfusion injury rats. Zhongguo Wei Zhong Bing Ji Jiu Yi Xue., 2006; **18(1)**:5-8. Chinese.
51. **Lai, I.R., Chang, K.J., Tsai, H.W., and Chen, C.F.:** Pharmacological preconditioning with simvastatin protects liver from ischemia-reperfusion injury by heme oxygenase-1 induction. Transplantation, 2008; **85(5)**:732-738.
52. **Peterson, S.J., Frishman, W.H., and Abraham, N.G.:** Targeting heme oxygenase: therapeutic implications for diseases of the cardiovascular system. Cardiol. Rev., 2009; **17**: 99-111.
53. **Stocker, R., Yamamoto, Y., Mc Donagh, A.F., Glazer, A.N., and Ames, B.N.:** Bilirubin is an antioxidant of possible physiological importance. Science, 1987; **235**: 1043-1046.

54. **Peyton, K.J., Reyna, S.V., Chapman, G.B., Ensenat, D., Liu, X.M., Wang, H., *et al.*:** Hemeoxygenase-1-derived carbon monoxide is an autocrine inhibitor of vascular smooth muscle cell growth. Blood, 2002; **99**: 4443-4448.
55. **Vile, G.F., and Tyrrell, R.M.:** Oxidative stress resulting from ultraviolet A irradiation of human skin fibroblasts leads to a heme oxygenase-dependent increase in ferritin. J. Biol. Chem., 1993; **268**: (20):14678-14681.
56. **Kobayashi, T., Sato, Y., Yamamoto, S., Takeishi, T., Hirano, K., Watanabe, T., *et al.*:** Augmentation of heme oxygenase-1 expression in the graft immediately after implantation in adult living-donor liver transplantation. Transplantation, 2005; **79**: 977-980.
57. **Fondevila, C., Shen, X.D., Tsuchiyashi, S., Yamashita, K., Csizmadia, E., Lassman, C., *et al.*:** Biliverdin therapy protects rat livers from ischemia and reperfusion injury. Hepatology, 2004; **40(6)**:1333-1341.
58. **Kato, Y., Shimazu, M., Kondo, M., Uchida, K., Kumamoto, Y., Wakabayashi, G., *et al.*:** Bilirubin rinse: A simple protectant against the rat liver graft injury mimicking heme oxygenase-1 preconditioning. Hepatology, 2003; **38(2)**:364-373.
59. **Kaizu, T., Nakao, A., Tsung, A., Toyokawa, H., Sahai, R., Geller, D.A., *et al.*:** Carbon monoxide inhalation ameliorates cold ischemia/reperfusion injury after rat liver transplantation. Surgery, 2005; 138(2):229-235.
60. **Kaizu, T., Ikeda, A., Nakao, A., Tsung, A., Toyokawa, H., Ueki, S., *et al.* :** Protection of transplant-induced hepatic ischemia/reperfusion injury with carbon monoxide via MEK/ERK1/2 pathway downregulation. Am. J. Physiol. Gastrointest. Liver Physiol., 2008; **294(1)**:G236-G244.
61. **Amersi, F., Shen, X.D., Anselmo, D., Melinek, J., Iyer, S., Southard, D.J. *et al.*:** Ex vivo exposure to carbon monoxide prevents hepatic ischemia/reperfusion injury through p38 MAP kinase pathway. Hepatology, 2002; **35(4)**:815-823.
62. **Moshage, H., Kok, B., Huizenga, J. R., and Jansen, P. L.:** Nitrite and nitrate determination in plasma: a critical evaluation. Clin. Chem., 1995; **41**: 892-896.
63. **Stuehr, D.J.:** Enzymes of the L-arginine to nitric oxide pathway. J. Nutr., 2004; **134(10 suppl)**:2748S-2751S.
64. **Salkowski, C. A., Detore, G., McNally, R., Van Rooijen, N., and Vogel, S. N. :** Regulation of inducible nitric oxide synthase messenger RNA expression and nitric oxide production by lipopolysaccharide in vivo. J. Immunol., 1997; **158** :905–912.
65. **Yanagida, H., Kaibori, M., Yamada, M., Habara, K., Yokoigawa, N., Kwon, A.H, *et al.*:** Induction of inducible nitric oxide synthase in hepatocytes isolated from rats with ischemia-reperfusion injury. Transplant. Proc., 2004; **36(7)**:1962-1964.
66. **Yanagida, H., Kaibori, M., Yoshida, H., Habara, K., Yamada, M., Kamiyama, Y., *et al.*:** Hepatic ischemia/reperfusion upregulates the susceptibility of hepatocytes to confer the induction of inducible nitric oxide synthase gene expression. Shock, 2006;26(2):162-168.
67. **Teshima, S., Nakanishi, H., Nishizawa, M., Kitagawa, K., Kaibori, M., Yamada, M., *et al.*:** Up-regulation of IL-1 receptor through PI3K/Akt is essential for the induction of iNOS gene expression in hepatocytes. J. Hepatol., 2004; **40(4)**:616-623.
68. **Wink, D.A., Miranda, K.M., Espey, M.G., Pluta, R.M., Hewett, S.J., Colton, C., *et al.*:** Mechanisms of the antioxidant effects of nitric oxide. Antioxid. Redox. Signal., 2001; **3(2)**:203-213.
69. **Hines, I.N., Harada, H., Flores, S., Gao, B., McCord, J.M., and Grisham, M.B.:** Endothelial nitric oxide synthase protects the post-ischemic liver: potential interactions with superoxide. Biomed. Pharmacother., 2005; **59(4)**:183-189.
70. **Chen, T., Zamora, R., Zuckerbraun, B., and Billiar, T.R.:** Role of nitric oxide in liver injury. Curr. Mol. Med., 2003; **3(6)**:519-526.
71. **Lhuillier, F., Parmantier, P., Goudable, J., Crova, P., Delafosse, B. Annat, G., *et al.*:** Hepatic ischemia is associated with an increase in liver parenchyma nitric oxide that is in part enzyme-independent. Anesthesiology, 2003; **98**: 373–378.
72. **Zweier, J.L., Samouilov, A., and Kuppusamy, P.**: Non-enzymatic nitric oxide synthesis in biological systems. Biochim. Biophys. Acta, 1999; **1411**: 250–262.
73. **Chan, K.L., Zhang, X.H., Fung, P.C., Guo, W.H., and Tam, P.K.:** Role of nitric oxide in intestinal ischaemia-reperfusion injury studied using electron paramagnetic resonance. Br. J. Surg., 1999; **86:** 1427–1432.
74. **De Caterina, R., Libby, P., Peng, H.B., Thannickal, V.J., Rajavashisth, T.B., Gimbrone, M.A., *et al.*:** Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecules and proinflammatory cytokines. J. Clin. Invest., 1995; **96**:60–68.
75. **Grisham, M.B., Jourd'heuil, D., and Wink, D.A.:** Nitric oxide. I. Physiological chemistry of

nitric oxide and its metabolites: implications in inflammation. Am. J. Physiol., 1999; **276:** G315–G321.

76. **Dezfulian, C., Raat, N., Shiva, S., and Gladwin, M.T.:** Role of the anion nitrite in ischemia-reperfusion cytoprotection and therapeutics. Cardiovasc. Res., 2007; **75**:327–338.
77. **Lefer, D.J., Jones, S.P., Girod, W.G., Baines, A., Grisham, M.B., and Cockrell, A.S., et al.:** Leukocyte-endothelial cell interactions in nitric oxide synthase- deficient mice. Am. J. Physiol., 1999; **276:**H1943–H1950.
78. **Fisher, P.W., Salloum, F., Das, A., Hyder, H., and Kukreja, R.C.:** Phosphodiesterase-5 inhibition with sildenafil attenuates cardiomyocytes apoptosis and left ventricular dysfunction in a chronic model of doxorubicin cardio toxicity. Circulation, 2005; **111:** 1601–1610.
79. **Hassan, M.A, and Ketat, A.F.** : Sildenafil citrate increases myocardial cGMP content in rat heart, decreases its hypertrophic response to isoproterenol and decreases myocardial leak of creatine kinase and troponin T. BMC Pharmacol., 2005; 5: 10.
80. **Kukreja, R.C., Salloum, F., Das, A., Ockaili, R., Yin, C., Bremer, Y.A., *et al.*:** Pharmacological preconditioning with sildenafil: basic mechanisms and clinical implications. Vascul. Pharmacol., 2005; 42: 219–232.
81. **Beckman, J.S., and Koppenol, W.H.:** Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. Am. J. Physiol., 1996; **271:**C1424–C1437.
82. **Dimmeler, S., Haendeler, J., Nehls, M., and Zeiher, A.M**.: Suppression of apoptosis by nitric oxide via inhibition of interleukin-1beta-converting enzyme (ICE)-like and cysteine protease protein (CPP)-32-like proteases. J. Exp. Med., 1997; **185:** 601–607.
83. **Polte, T., Oberle, S., and Schröder, H**.: Nitric oxide protects endothelial cells from tumor necrosis factor-α-mediated cytotoxicity: possible involvement of cyclic GMP. FEBS Lett., 1997; **409:** 46–48.
84. **Phillips, L., Lopez-Nebllna, F., Toledo, A., Anaya-Prado, R., and Toledo-Pereyra, P.:** Nitric Oxide Mechanism of protection in ischemia reperfusion injury. Invs. Surgery, 2009; **22:**46-55.
85. **Russwurm, M., and Koesling, D.:** Isoforms of NO-sensitive guanylyl cyclase. Mol. Cell. Biochem., 2002; **230:**159-164.
86. **Das, A., Xi, L., and Kukreja, R.C.:** Phosphodiesterase-5 inhibitor sildenafil preconditions adult cardiac myocytes against necrosis and apoptosis. Essential role of nitric oxide signaling. J. Biol. Chem., 2005; **280:** 12944–12955.
87. **Leão, L.R.S., Lima, H. C.S., do Rêgo, A. C.M., Azevedo, I.M., and Filho, A.M.D.:** Sildenafil in the prevention of hepatic ischemia/reperfusion injury in rats. J. Surg. Cl. Res., 2010; **Vol.1 (1)**: 66-74.
88. **Chen, K., and Maines, M.D.:** Nitric oxide induces heme oxygenase-1 via mitogen activated protein kinases, ERK and p38. Cell Mol Biol (Noisy-le-grand), 2000; **46 (3)**:609–617.
89. **Alcaraz, M.J., Habib, A., Creminon, C., Vicente, A.M., Lebret, M., Levy-Toledano, S., *et al.*:** Heme oxygenase-1 induction by nitric oxide in RAW 264.7 macrophages is upregulated by a cyclo-oxygenase-2 inhibitor. Biochim. Biophys. Acta, 2001; **1526(1)**: 13–16.
90. **Polte, T., and Schröder, H.:** Cyclic AMP mediates endothelial protection by nitric oxide. Biochem. Biophys. Res. Commun., 1998; **251**:460–465.
91. **Immenschuh, S., Hinke, V., Ohlmann, A., Gifhorn-Katz, S., Katz, N., Jungermann, K., *et al.*:** Transcriptional activation of the heme oxygenase-1 gene by cGMP via a cAMP response element/activator protein-1 element in primary rat hepatocytes. Biochem. J., 1998; **334:**141–146.
92. **Polte, T., Abate, A., Dennery; P.A and Schröder, H.:** Heme Oxygenase-1 Is a cGMP-Inducible Endothelial Protein and Mediates the Cytoprotective Action of Nitric Oxide. *Arteriosclerosis, Thrombosis, and Vascular Biolog.,* 2000; **20:** 1209-1215.
93. **Hartsfield, C.L.:** Cross talk between carbon monoxide and nitric oxide. Antioxid. Redox Signal, 2002; **4**:301–307.
94. **Motterlini, R., Green, C.J., and Foresti, R.**: Regulation of heme oxygenase-1 by redox signals involving nitric oxide. Antioxid. Redox Signal, 2002; **4**:615–624.
95. **Lin, H.Y., Juan, S.H., Shen, S.C., Hsu, F.L., and Chen, Y.C**.: Inhibition of lipopolysaccharide-induced nitric oxide production by flavonoids in RAW264.7 macrophages involves heme oxygenase-1. Biochem. Pharmacol., 2003; **66**:1821–1832.
96. **Nathan, C.:** Nitric oxide as a secretory product of mammalian cells**.** FASEB J., 1992; **6**: 3051-3064.

Toxicological Studies of Malathion on Japanese Quail (*Coturnix Japonica*)

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Abstract: Over the last few decades, ecotoxicological impacts of organophosphorus insecticides have been accentuated by sharp increase of their use in agriculture. In this study the effect of orally administrated Malathion on male Japanese quail was investigated. The acute oral 72-hrs LD_{50} of Malathion was found to be 146.06 mg/kg B.wt. Malathion was supplied at a dose of 1/20 of LD_{50} for eight weeks. The evaluation strategy of the current investigation used observation of clinical signs and stress related alterations which were assessed by evaluating relative organ weights; hematological; biochemical and histopathological investigations. The tendency of Malathion to accumulate in selected tissues and organs of male treated quails was evaluated by detecting its level in liver, kidney and muscle. Significant decrease in red blood cell counts RBCs, hemoglobin (Hb) and packed cell volume (PCV) of treated quails were observed in comparison to their controls. Significant alterations in total and differential leucocytes counts were also observed. Treated quails showed a significant increase in liver enzyme activities (AST, ALT and ALP) as well as in total bilirubin and glucose levels. Meanwhile, they showed significant decrease in total protein, albumin, and globulin. Regarding kidney function; serum creatinine, urea and uric acid of treated quails were significantly increased in comparison to their control. Cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol levels of treated quails showed significant increase in comparison to their controls, while LDL-cholesterol levels showed a significant decrease. Malathion residue concentration in liver, kidney and muscle showed higher concentration in liver and kidney followed by muscles. Histopathological alterations were observed in treated quails. Safe use and all precautions should be followed during application of Malathion to minimize such undesirable effects.
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Key words: Quail, Malathion, Toxicology, Treatment.

1. Introduction

Pesticides have contributed from one side to dramatic increase in crop yields, and from the other side they may induce adverse ecotoxicological and hazardous health effects on a variety of living organisms, including birds. Organophosphorus pesticides are widely used in agriculture and veterinary practice to control various pests. A number of long persistent organophosphates, which have been banned or severely restricted, are still used in many developing countries (De Silva *et al*., 2006). As birds have a high trophic level, they are vulnerable of accumulating large dosage of certain chemicals (Deka and Borah, 2008). Some sub-lethal effects of pesticides were studied in birds with a view to identify characteristic biochemical response that may be useful for the monitoring of exposure to sub-lethal levels in the field (Dahamna *et al*., 2004).

Organophosphates have a remarkable acute toxicity due to inhibition of the cholinesterase enzyme and inducing acute neurological effects. Malathion is one of the most commonly used organophosphorus insecticides and is the main cause of the most acute pesticide poisoning. Its contamination may occur in poultry following its application to fruit, vegetables, grain, fiber and other crops. Contamination of poultry birds may also result from ingestion of treated cereals (Moghadamina and Abdollahi, 2002) or from the use of Malathion in the control of external parasites (Rao and Yadgirkar, 2000). Malathion degrades into more toxic metabolites in the tissues like liver, kidney and brain and consequently poses a potential threat to public health due to the presence of pesticide residues in poultry meat (Garg *et al*., 2004b).

In the last few decades, the World wide attention is focusing on the environment and how to protect it. The fragmented and incomplete studies on the toxicological and histopathological action of Malathion necessitated this study to investigate the toxicological effects of subchronic exposure of male Japanese quail (*Coutrnix coutrnix japonica*) to Malathion after estimating its LD_{50}. These effects include the clinical signs, alterations in the relative weight of some organs (liver, kidney and spleen), hematological alterations, biochemical changes, and the tendency of Malathion to accumulate in liver, kidney and muscle during the treatment period. Histopathological alterations of liver, kidney and spleen were also investigated.

2. Materials and Methods

Experimental animals:

A total of 120 apparently healthy male Japanese quail (*Coturnix coturnix japonica*) weighing from 100 to 150 g, purchased from Faculty of Agriculture at Cairo University, applied for both LD_{50} and a treatment

study. Studied animals were kept under observation for two weeks before the onset of the experiment. Quails were divided into two groups; control group and malathion-treated group. 10 quails/cage was housed in wooden cages (100 x 80 x 60cm) under suitable hygienic free pathogens for 8 weeks.

Determination of the acute oral 72-hrs LD_{50} and dose determination:

The 72-hrs LD_{50} of Malathion was determined according to Finney (1964). The treated dose was calculated to be equivalent to 1/20 of the determined LD_{50}. Daily oral intubation of Malathion was extended up to 8 weeks, while control group was orally intubated by distilled water and was kept under the same laboratory conditions.

Sampling:

At the end of the 1st, 2nd, 4th and 8th week, 5 quails from each group were kept for about 12 hrs without water and feed. Quails **were slaughtered and blood was collected**. Blood was collected **sterile containers to avoid contamination**. Collected blood was divided into two portions. One was used for hematological examination after adding EDTA as an anticoagulant and the other portion was centrifuged to obtain serum that was stored at -20°C for further biochemical analysis. Post-mortem examination was carried out and relative weights of liver, kidney and spleen were recorded. Liver, kidney and muscle were stored at -20°C to detect concentration of Malathion residues. Parts of liver, kidney and spleen were fixed in 10% formalin buffer for histopathological examination.

Hematological parameters:

Total red blood cell (RBC) and white blood cell (WBC) counts (/µl) were determined by the Natt & Herrick (1952). Hemoglobin level and values of erythrocyte indices were evaluated as described by Wintrobe (1965), while haematocrite percentage (PCV%) and differential leukocytic percentage were determined according to Dacie and Lewis (1991).

Biochemical parameters:

Hepatic aspartate aminotransferase activity (AST) and alanine aminotransaminase activity were determined kinetically according to Schumann and Klauke (2003). Alkaline phosphatase activity (ALP) was determined according to Rec (1972), while total bilirubin was determined according to Jendrassik and Grof (1938). Glucose concentration was evaluated according to Young (2001). The serum albumin, and total protein values were estimated by Biuret and Dumas method as suggested by Dumas *et al. (1971)*. Serum globulin concentration and albumin/globulin ratio were calculated according to Rojkin *et al.* (1974). Serum concentration of creatinine, urea, and uric acid were determined according to Henry (1974), Patton and Crouch (1977) and Young (2001) respectively. Finally levels of serum cholesterol, triglyceride and HDL-cholesterol were determined according to Young (2001), while VLDL-cholesterol and LDL-cholesterol concentrations were evaluated according to Norbert (1995) and Friedwald (1972) formulas respectively.

Determination of insecticide residues:

Ten grams of tissue sample was mixed with Na_2SO_4 and packed in thimble of Soxhelt extraction and then the mixture was extracted and concentrated. The extract was cleaned up through alumina chromatography columns. Elution was done according to Erney (1983), and standard curve was constructed for Malathion by known concentration in known volume followed by injection of known volume into GLC-ECD.

Histopathological investigation:

Histopathological examination of liver, kidney and spleen was carried out according to Lillie (1969).

Statistical Analysis:

Data analysis was performed by using student ANOVA test and comparing between means using LSD as outlined by PC-STAT (1995).

3. Results and Discussion

Determination of the acute oral 72-hrs LD_{50}:

LD_{50} is commonly used to express the relative hazards associated with the acute toxicity of Malathion. The principle of safety evaluation studies is to define the potential of Malathion that cause damage. Gruzdyev *et al*. (1980) reported that the lower the absolute value of LD_{50} the higher is the toxicity characterizing the formulation. In the present study, the acute oral 72-hrs LD_{50} of Malathion was found to be 164.06 mg/kg. The mentioned results confirmed the moderate toxicity of Malathion to a variety of bird species that was reported by other authors.

Clinical signs:

After two weeks of treatment with Malathion treated quails have experienced some clinical signs including; roughened feathers, weakness, dropped wings, loss of balance, hyperexcitability incoordination, convulsions, thick mucoid discharge from the mouth, wheezing and dysnea. After four weeks, neurotoxic symptoms increased gradually till the end of the treatment period. Additionally, the study bird showed abnormalities in gait and behavior. Before death ataxia, muscular convulsion and comma have occurred in some cases. The treated bird showed signs of depression, and preferred to stand still followed by zigzag movements. Observed ataxia and incoordination were followed by hypersalivation, open mouth breathing and paralysis of legs. The observed clinical signs could be explained by the inhibitory effect of Malathion on acetylecholinesterase (Gultekin *et al*., 2006 and De Silva *et al*., 2006) that lead to abnormal acetylcholine build up. Similar signs were observed by other authors, including Varsik *et al*., 2005).

Organs relative weights:

Liver, kidney and spleen relative weight of treated quails showed a significant increase when

compared to their controls. Such increase was recorded at P<0.05 from the 1st week of treatment for liver, meanwhile it was recorded at *P*<0.01 from the 4th week of treatment for kidney and from the 1st week of treatment for spleen . In all cases such significant increase showed a time dependent trend (Table 1). Such observed enlargement in the studied organs may indicate an initial effect of systemic toxicity that probably facilitates erythrocyte removal by the reticuloendothelial system (Mahmoud, 2000).

Hematological parameters:

From the 2nd week of treatment RBCs and Hb of treated quails showed a highly significant decrease (*P*<0.01), meanwhile MCV showed significant increase (*P*<0.05) when compared to their controls (Table 1). Such significant alterations of haematological parameters showed a time dependent trend, the most potent reduction or increase was recorded at the end of the experiment. The observed decrease in RBCs and Hb concentration could be due to the destruction of erythrocytes as a direct effect of Malathion treatment, or due to the indirect adverse effect of Malathion on the bone marrow (Nemi, 1993). Such decrease was concurred with splenomegally recorded in the current study. Destruction of red cells could be also due to mutagenic and hematotoxic effect of Malathion (El-Shater, 2003). After 8 weeks of treatment, a significant increase (*P*<0.01) of WBCs and neutrophils and a significant decrease (*P*<0.01) of eosinphils, monocytes and lymphocytes were observed when compared to their controls and all previously tested periods, in a time dependent manner (Table 1). The reported leukocytosis that began after the first week of treatment associated with lymphopenia and neutrophilia which began after the second week of treatment could be due to adverse effect of Malathion on the normal function of the bone marrow and/or lymphoid tissue (Rajini *et al*., 1987). In addition, the observed lymphopenia may reflect stress imposed on the immune system in response to Malathion intoxication (Garg *et al*., 2004a). It was interesting to correlate the recorded hematological changes with histopathological findings noticed in spleen of treated quails, such as hyperplastic proliferation in lymphoid tissue, angiopathy of follicular artery and depletion of lymphoid follicles.

Biochemical Changes:

Liver Function:

After 8 weeks of treatment AST, ALT and ALP activities of treated individuals showed a significant increase when compared to their activities of control groups and earlier tested periods. Such significant increase, observed after 8 weeks, was recorded at *P*<0.01 for AST and ALT and was recorded at *P*<0.05 for ALP. Significant differences were observed for the 3 enzymes in a time dependent trend as seen in table 1. Such noticed increase in AST and ALT activities could be used as an indicator of altered permeability of plasma membrane and/or cell damage (Hasheesh *et al*., 2002a). Meanwhile, the recorded increase in ALP activity may be due to the osteoblastic activity and general toxic damage to liver as reported by Garg *et al*. (2004b). Total bilirubin and glucose concentration of treated quails showed a significant increase (*P*<0.01) in comparison with their controls for all studied periods except bilirubin level of individuals treated for 1 week that sowed its significant increase at *P*<0.05. Time dependent significant increase of total bilirubin and glucose levels was also noticed at *P* <0.01 for total bilirubin of the 2nd, 4th and 8th week when compared to its value of the 1st week and at *P* <0.05 for glucose level of the 8th week when compared to its level of the 4th week (Table 2). The noticed increase in the bilirubin level may be due to haemolysis that could be caused by excessive rapid destruction of erythrocytes (Hasheesh *et al*., 2002a) and this was supported by the recorded low RBCs count observed in the present study. In general, the induced alteration of liver function could be explained by the formation of lipid peroxidation which is considered as one of the molecular mechanisms for Malathion-induced hepatic damage (Gokalp *et al*., 2003). Moreover, it was meaningful to correlate between the observed alterations of liver functions and the direct effect of Malathion on the histological features of liver of treated quails that showed focal lymphocytic infiltration, hyperplasia of the bile ducts, and desquamation of lining epithelial cells, proliferation of the fibrous tissue of the portal area, fatty changes and necrosis. The recorded increase in blood glucose concentration may be due to the accelerated glycogenolysis and increased level of lipid peroxidation (Abdollahi *et al*., 2004).

Protein profile:

From the 2nd week to the end of treatment period, total protein and albumin of treated quails showed a significant decrease (*P* <0.01) when compared to their controls (Table 2). Such significant decrease of total protein and albumin showed time dependent tendency. On the other hand significant decrease in globulin concentration of quails treated for 4 weeks was recorded at p<0.01 in comparison to their controls. Globulin concentration showed also a time dependent significant decrease (Table 2). Regarding A/G ratio, a significant decrease (*P* <0.01) was recorded for treated quails on the 2nd and 8th week of treatment in comparison to their controls. Such decrease of individuals treated for eight weeks showed a significant decrease at P<0.01 when compared to individuals treated for one week (Table 2). The noticed reduction in total protein could be related to the action of Malathion on nucleic acids (Devi, 1981) and it may indicate a physiological adaptability of quails to compensate pesticide stress. As mentioned by Garg *et al*. (2004b), it may be concluded that the observed

decrease in serum globulin could be due to reduction in synthesis by the plasma cells.

Kidney functions:

Serum creatinine and uric acid concentration of treated quails showed a significant increase (P <0.0) compared to their controls for all tested periods, with the exception of uric acid concentration of individuals treated for one week that showed non-significant increase (P >0.05) when compared to their controls. A time dependent significant increase was also recorded for both creatinine and uric acid when compared to their relevant concentrations of individuals treated for shorter time periods. Urea concentration of quails treated for two weeks showed a significant increase (P <0.05) in comparison to their controls. Such significant increase was recorded at P <0.01 on the 4th and 8th week of treatment (Table 2). The observed increase of serum creatinine may be attributed to renal insufficiency, urinary tract obstruction and impairment of renal function induced by Malathion (Hasheesh *et al*., 2002a). The same authors explained the increased level of urea by the increased nitrogen retention and/or due to corrupted renal function. The increase of uric acid usually occurs due to renal failure or toxemia induced by Malathion resulting in damage to the epithelium of the kidney tubules (Malik *et al*., 2004). In concurrent with the mentioned biochemical alterations, treatment with Malathion induced renal degenerative changes in renal tubules, progressive infiltration, degenerative changes in renal epithelial cells, in addition to atrophied renal corpuscles and focal interstitial nephritis.

Lipid profile:

Cholesterol, triglycerides, VLDL and LDL-cholesterol of treated quails showed a significant increase when compared to their controls during different treatment periods (Table 2) Such increase was recorded at P <0.01 with the exception of cholesterol that showed its increase at P <0.01. A time dependent significant increase (P <0.01) was also noted when cholesterol, triglycerides, VLDL and LDL-cholesterol levels of quails treated for 8 weeks compared to individuals exposed to Malathion for shorter periods of time. HDL-cholesterol concentration of the treated group showed a significant decrease (P <0.01) in comparison with their controls. Such decrease showed a time dependent trend. The recorded high level of cholesterol in blood of treated quails is a major risk factor as mentioned by AHA Science Advisory (2001). Such observed increase could be attributed to the blockage of liver bile duct causing reduction of its secretion (Kalender *et al*., 2005), and it could be also attributed the hyperadrenal activity that was induced because of Malathion treatment (Malik *et al*., 2004). The possible Malathion induced activation of serum enzyme activity could be considered for the observed HDL-cholesterol decrease (Ibrahim and El-Gamal, 2003). In accordance with Ibrahim and El-Gamal (2003), results of the current study suggested a change from HDL-cholesterol into LDL-cholesterol. Regarding the triglyceride level, the recorded increase in serum triglycerides of treated quails may be due to increased lipid mobilization from liver and its decreased removal from plasma (Slotkin *et al*., 2005).

Malathion residues in body tissues:

Concentration of Malathion in liver, kidney and muscle of quails treated for two and eight weeks showed a significant increase (P <0.01) when compared to their controls. Such significant increase showed a time dependent trend, as residue concentration detected in the mentioned organs showed a higher rate of increase as time of exposure extended (Fig. 9). The observed persistence of Malathion in studied tissues of treated quails could be attributed to its limited elimination and its biotransformation, despite the rapid hydrolysis of organophosphorus *in vitro* (Hasheesh *et al*., 2002b). The distribution of Malathion residues is likely to be correlated with the lipid content of the organ, being high in the organs with high lipid contents.

Histopathological Investigations:

Figure 2 (1-12) showed histopathological alterations of liver (1-5), kidney (6-8) and spleen (9-12) of quails treated with Malathion in comparison to their controls. From the first week of treatment, liver of treated quails showed focal lymphocytic aggregation and hyperplasia of bile ductules. As period of exposure extended, angiopathy and cholingitis associated with desquamation of epithelial cells and proliferation of the fibroblasts were noticed. In addition to that swollen endothelia of the branches of hepatic artery and degenerated tunica media were observed in addition to oedema. Moreover, numerous numbers of hepatic cells undergo fatty degenerative changes. Moreover, necrotic changes were observed in the hepatocytes. Kidney of quails treated with Malathion showed severe degenerative changes of the renal corpuscle and renal tubules with noticeable hydrpnephrosis and progressive infiltration of the inflammatory cells were observed after four weeks of treatment. As the period of exposure extended, focal interstitial nephritis was observed. Regarding spleen, the white pulp showed hypersensitivity of the reticular cells and the lymphoid elements showed depletion during the first four weeks of treatment. After the 8th week of treatment, the reticular cells were laden with haemosidrin. The subcapsular and medullary sinuses showed oedema and disorganization of lymphoid follicles appeared.

The observed histopathological alterations could be attributed to the direct effect of Malathion on the studied organs (Rodrigo *et al*., 2001).

Table (1): Body weight and hematological values of quails orally treated with Malathion (8.2 mg Kg b. wt $^{-1}$)

Parameter (unit)	1st week		2nd week		4th week		8th week		LSD at 5%	LSD at 1%
	C	MT	C	MT	C	MT	C	MT		
Liver (wt x 10^{-4})	156.00±6.00^{c}	178.00±3.00^{bcd}	158.00±6.00^{c}	182.00±7.00^{bc}	164.00±4.00^{cdc}	190.00±9.00^{ab}	160.00±3.00^{dc}	206.00±9.00^{a}	19.6	26.4
Kidney (wt x 10^{-4})	53.40±0.50^{c}	53.50±0.44^{c}	54.00±0.42^{c}	54.20±0.64^{c}	53.70±0.21^{c}	57.90±0.32^{b}	53.50±0.27^{c}	61.90±0.39^{a}	1.2	1.6
Spleen (wt x 10^{-4})	4.70±0.06^{d}	6.42±0.03^{c}	4.46±0.11^{d}	6.49±0.12^{c}	4.44±0.11^{d}	7.18±0.23^{d}	4.49±0.04^{d}	7.72±0.19^{a}	0.38	0.51
RBCs cells/mm^{3}	3.72±0.18^{a}	3.71±0.03^{a}	3.89±0.12^{a}	3.02±0.05^{b}	3.84±0.01^{a}	2.66±0.04^{c}	3.88±0.02^{a}	2.62±0.07^{c}	0.25	0.33
HB (g/dl)	11.80±0.38^{a}	11.46±0.14^{a}	11.46±0.27^{a}	9.54±0.12^{b}	11.74±0.25^{a}	8.72±0.07^{c}	11.60±0.15^{a}	8.60±0.11^{c}	3.05	4.11
PCV (%)	38.86±1.86^{ab}	35.94±1.10^{bc}	40.14±0.95^{a}	35.26±0.18^{c}	38.56±1.14^{ab}	30.84±0.44^{d}	40.24±1.23^{a}	30.44±0.60^{d}	3.05	4.11
MCV (Fl)	104.40±8.17^{bc}	96.85±3.53^{c}	103.14±1.39^{c}	116.72±3.39^{a}	100.34±2.81^{c}	115.96±3.19^{ab}	103.68±3.72^{c}	116.45±3.60^{a}	11.72	15.79
MCH (pg)	31.72±1.69	30.88±0.68	29.46±1.36	31.58±0.81	30.56±0.66	32.72±0.34	29.88±0.54	32.84±0.67	-----	-----
MCHC (%)	30.34±1.52^{ab}	31.90±1.06^{a}	28.58±1.04^{bc}	27.05±0.18^{c}	30.46±1.14^{ab}	28.61±0.57^{bc}	28.80±0.87^{bc}	28.26±0.30^{c}	2.67	3.6
WBCs (x10^{3})	3.42±0.06^{d}	4.54±0.19^{c}	3.39±0.13^{d}	5.84±0.21^{b}	3.52±0.06^{d}	6.30±0.26^{b}	3.43±0.04^{d}	7.72±0.10^{a}	0.47	0.63
Neutrophils (%)	2.60±0.24^{d}	2.40±0.24^{d}	3.60±0.24^{d}	7.60±0.24^{c}	2.40±0.24^{d}	15.00±0.45^{b}	2.60±0.24^{d}	20.40±1.18^{a}	1.41	1.9
Eosinophils (%)	2.60±0.24^{ab}	3.20±0.20^{a}	3.20±0.20^{a}	2.40±0.24^{b}	3.20±0.12^{c}	1.20±0.12^{c}	2.60±0.24^{ab}	00.00±0.00^{d}	0.75	1.01
Lymphocytes (%)	91.40±0.60^{a}	89.00±1.10^{ab}	90.00±2.94^{a}	85.60±0.75^{b}	91.20±1.17^{a}	81.60±0.75^{c}	92.00±1.43^{a}	78.60±0.60^{c}	0.67	0.91
Monocytes (%)	3.40±0.24^{c}	5.40±0.40^{a}	2.20±0.20^{c}	4.40±0.24^{b}	3.20±0.20^{c}	2.20±0.20^{d}	2.80±0.24^{cd}	1.00±0.00^{c}	0.67	0.91

Number of birds in each experiment was five. Data are expressed as mean ± SE.
LSD is the least significant difference.
Values which share the same superscript letters are not significantly different (P< 0.05).

Table (2): Serum Biochemical studies of quails orally treated with Malathion (8.2 mg Kg b.wt $^{-1}$)

Blood parameter (unit)	1st week		2nd week		4th week		8th week		LSD at 5 %	LSD at 1 %
	C	MT	C	MT	C	MT	C	MT		
Glucose (mg/dl)	101.80±2.06c	132.20±8030ab	103.40±4.87c	134.20±4.60ab	93.60±4.20c	122.00±1.01b	97.60±2.30c	142.00±12.50a	17.38	23.41
Protein (g%)	3.44±0.13ab	3.28±0.08b	3.54±0.06a	2.98±0.06c	3.54±0.06a	2.61±0.06d	3.50±0.05a	2.52±0.05d	0.2	0.28
Albumin (g%)	1.22±0.05ab	1.08±0.05b	1.34±0.02a	0.82±0.03c	1.20±0.02ab	0.78±0.05c	1.40±0.02a	0.68±0.03c	0.21	0.28
Globulin (g%)	2.22±0.18ab	2.20±0.03ab	2.20±0.07ab	2.10±0.03ab	2.34±0.07d	1.83±317.30ab	2.08±0.08bc	1.84±0.05cd	0.25	0.33
A/G (%)	0.57±0.03abc	0.48±0.02cde	0.60±0.03ab	0.41±0.01ef	0.51±0.03bcd	0.42±0.03def	0.66±0.04a	0.36±0.03f	0.01	0.13
Cholesterol mg%	139.80±1.03d	150.20±1.96c	135.00±1.15d	157.00±1.65c	137.60±3.10d	172.40±6.79b	141.00±2.52d	212.00±3.53a	9.19	12.37
Triglycerides mg%	99.20±3.29d	120.00±1.76c	105.40±0.75d	128.00±0.71bc	100.80±2.71d	135.60±1.04b	109.00±2.92d	153.00±8.17a	9.98	13.45
HDL- cholesterol mg%	101.3±1.29 a	65.80±0.52b	111.80±1.52a	60.50±0.22c	112.90±0.79a	59.50±1.25c	111.8±1.74a	55.50±1.52d	3.46	4.66
LDL-cholesterol mg%	19.84±0.65d	24.00±0.34c	21.08±0.15d	25.60±0.014bc	20.16±0.54d	27.12±0.20b	21.80±0.58d	30.60±01.63a	1.99	2.69
vLDL-cholesterol mg%	9.64±0.91d	60.66±5.50c	2.10±0.07d	70.8±5.15c	4.60±0.44d	85.58±6.94b	7.38±0.63d	125.90±3.45a	10.91	14.69
Creatinine mg%	0.27 ± 0.01c	0.37 ± 0.01ab	0.25 ± 0.02c	0.40 ± 0.03ab	0.22 ± 0.01c	0.36 ± 0.005b	0.26 ± 0.02c	0.43 ± 0.01a	0.06	0.08
Urea mg%	9.80 ± 0.25c	10.20 ± 0.52c	9.56 ± 0.32c	12.36 ± 0.35b	9.86 ± 0.34c	14.86 ± 1.33a	8.67 ± 0.45c	15.06± 1.37a	2.15	2.9
Uric acid mg%	2.28 ± 0.03cd	2.60 ± 0.12c	2.02 ± 0.17d	4.20 ± 0.33b	2.20 ± 0.05cd	4.66 ± 0.20ab	2.40±0.08cd	5.12±0.24a	0.52	0.7
AST (U/L)	204.0±4.10e	247.0± 9.10cd	209.0± 3.50e	255.2±21.20be	225.0±2.20de	278.6±3.10b	217.0±4.30e	325.0±20.50a	26.8	36.2
ALT (U/L)	40.0±2.80c	53.06 ± 2.41b	40.08± 3.90c	67.06 ± 6.30a	40.06± 2.90c	74.08 ± 4.70a	39.04±2.07c	73.06± 2.47a	10.57	14.24
Alkaline Phosphatase (U/L)	5667.4±201.9c	7845.60±250.50b	5637.2±324.10c	8480.4±17.50ab	5279.6±474.90c	8075.6±317.30ab	5705.8±207.60c	8838.6±157.50a	780.2	1050.7
T. bilirubin mg%	0.30±0.003b	0.33 ± 0.01b	0.33 ± 0.01b	0.44 ± 0.01a	0.30 ± 0.02b	0.43 ± 0.02a	0.31 ± 0.02b	0.48 ± 0.03a	0.06	0.08

Number of birds in each experiment was five. Data are expressed as means ± SE. LSD is the least significant difference.
Values which share the same superscript letters are not significantly different (P< 0.05).

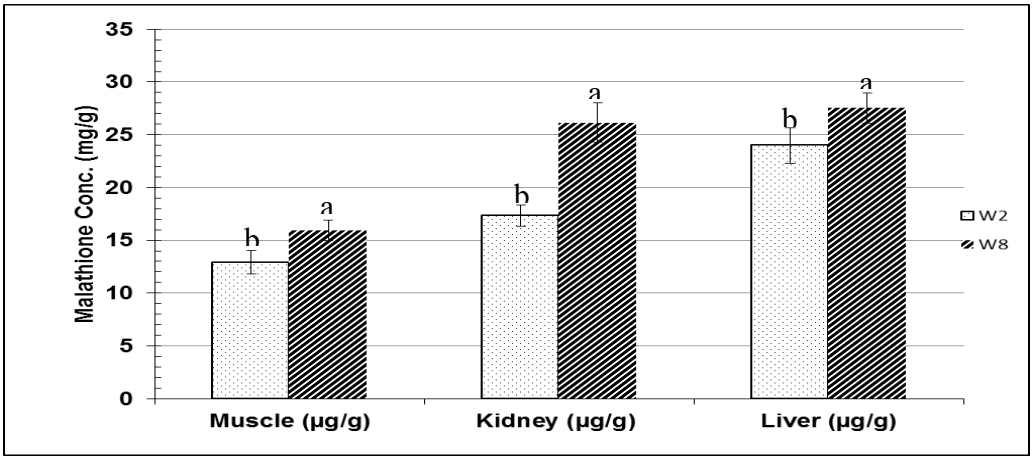

Fig. (1): Changes in residue of the Malathione concentration in toxicated tissues (g/g).

As mentioned by Gawish *et al*. (2006), the observed histopathological alterations may be due to the fact that organophosphorus insecticides generate free radicals in the biological system. Liver was reported as a target organ for Malathion toxicity (Yang *et al*., 2000 and Abdollahi *et al*., 2004). The necrotic condition observed in the liver of treated quails was in corroboration with the observed increase in AST, ALT and ALP that support the damage of liver cells observed with Malathion toxicity. Histopathological alterations of the kidney of treated quails could be caused during the course of excretion of these residues or it could be induced because of the cytotoxic effect of Malathion (Piramanayagam *et al.,* 1996). Alterations found in spleen could be based on the destructions of Vitamin A which is essential for normal growth and immunological functions (Adams *et al*., 1966).

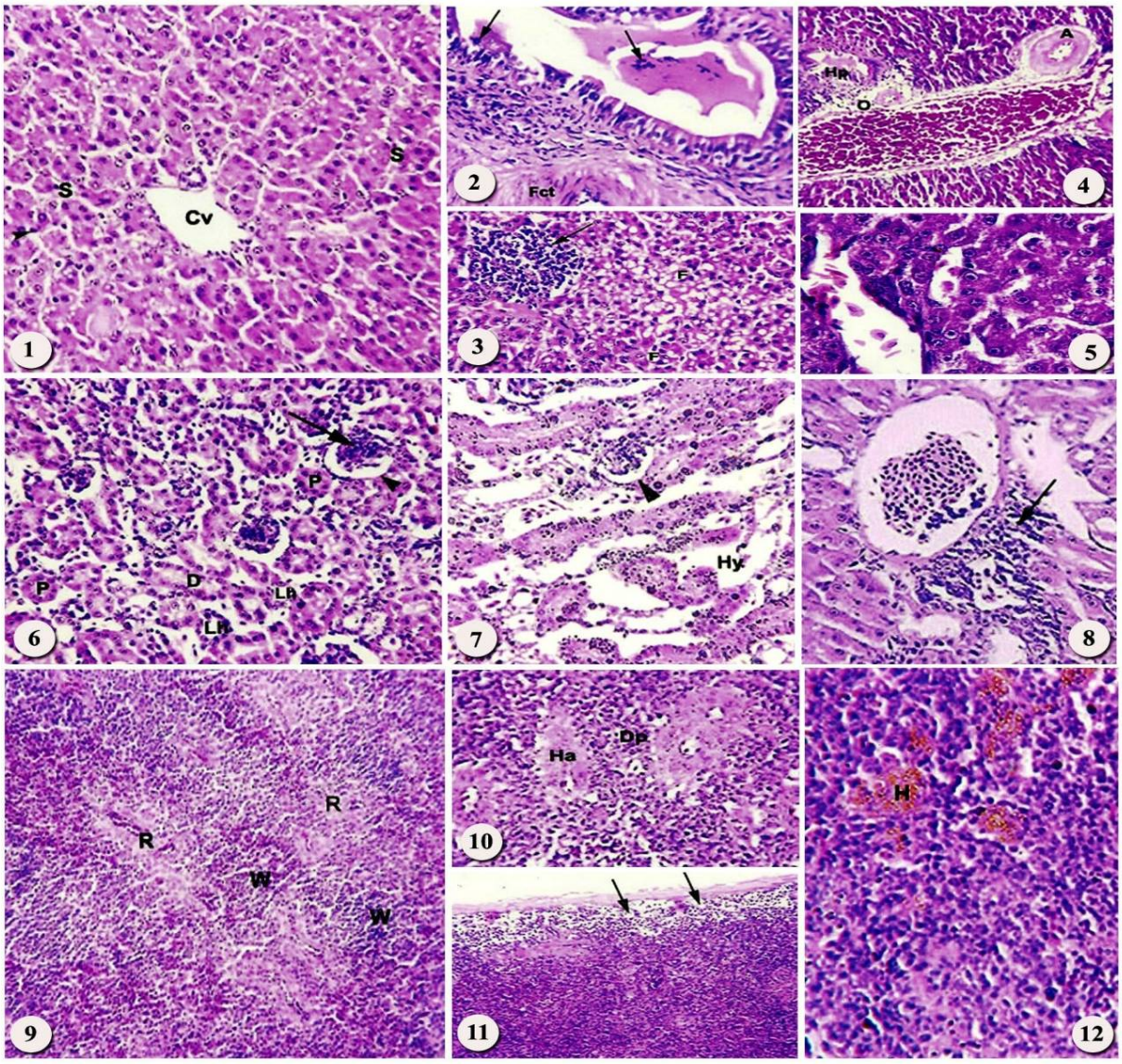

Fig. (2): Histopathological alteration of liver (1-5), kidney (6-8) and spleen (9-12) of control and treated quails.

1. Liver section of control quail showing a central vein (CV) surrounded by hepatic cords of normal hepatocytes. Notice the hepatic sinusoid (S) lined by endothelium (arrow head) and kupffer cells (H & E x400).
2. Two weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing desqumation of the epithelial cells (arrows) of the bile ductless and fibrous connective tissues (Fct) formation around the branches of the hepatic artery (H & E x400).
3. Four weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing angiopathy (A), hyperplasia (Hp) of the bile duct and edema (O) (H & E x200).
4. Four weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) showing a number of hepatocytes undergo fatty changes (F) and focal aggregation of the lymphocytes (arrow) (H & E x200).
5. Eight weeks-post treatment with malathion liver tissue section (8.2 mg Kg Bwt^{-1}) multiple necrotic changes (H & E x1000).
6. Control kidney section showing normal renal corpuscle formed of Bowman's capsule (arrow head) and renal glomerulus (arrow). Note the proximal tubules (P), distal tubule (D) and loop of Henel's (Lh) (H & E x400).
7. Four weeks-post treatment with malathion kidney tissue section (8.2 mg Kg Bwt^{-1}) showing a degenerative renal capsule (arrow head) with noticeable hydronepherosis (Hy) with a progressive infiltration with inflammatory cells (H & E x200).
8. Eight weeks-post treatment with malathion kidney tissue section (8.2 mg Kg Bwt^{-1}) showing focal interstitial nephritis (arrow) (H & E x200).
9. Control spleen section showing normal aggregation of the white pulp (W) separated by red pulp (H & E x200).
10. One week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing hyperactivity (Ha) of reticular cells and depletion of lymphoid elements (Dp) (H & E x400).
11. Eight week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing edema (arrow) of the subcapsular sinuses (H & E x200).
12. Eight week-post treatment with malathion spleen tissue section (8.2 mg Kg Bwt^{-1}) showing edema mosidrosis (H) (H & E x400).

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References

Abdollahi, M.; Donyavi, M.; Pournourmohammadi, Sh. And Saddat, M. (2004): Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following subchronic exposure to malathion. Comp. Bioch. Physiol. Part C: Toxicol. & Pharmacol., 137(4):343.

Adams, A.W.; Emerick, F.J. and Carlson, C.W. (1996): Effect of nitrate and nitrite in the drinking water of chicks, poults and laying hens. Poult. Sci., 45: 1215-1222.

AHA Science Advisory (2001): Stanol/Sterol Ester containing foods and blood cholesterol levels, #71-0201 Circulation, 103-1177.

Dacie, S.J. and Lewis, S.M. (1991): Practical hematology, 7th ed., Churchill, Livingstone.

Dahamna, S. Sekfali, N. and Walker, C.H. (2004): Biochemical indicators of hepatotoxic effects of pesticides. Commun. Agric. Appl. Biol. Sci., 69(4): 821-828.

Deka, K. and Borah, J. (2008): Haematological and Biochemical Changes in Japanese Quails

Coturnix coturnix Japonica and Chickens Due to *Ascaridia galli* Infection. International Journal of Poultry Science, 7 (7): 704-710.

De Silva, H.J.; Samarawickrema, N.A. and Wickremasingle, A.R. (2006): Toxicity due to organophosphorus compound: what about chronic exposure? Trans. R. Soc. Trop. Med. Hyg., 100(9): 803-806.

De Silva, H.J.; Samarawiskrema, N.A. and Wickremasingle, A.R. (2006): Toxicity due to organophosphorus compound: What about chronic exposure? Trans. R.soc. Trop. Med. Hyg., 100(9): 803-806.

Devi, A.P. (1981): Studied on the toxicity of endosulfan to some fresh water fish water fish with special reference to certain physiological changes induced in *Channa punctatus* (Bloch). Ph.D. Thesis, Nagarjuna University, Nagarjuna, Nagar, South India.

Dumas, B.T.; W.A. Watson and H.G. Biggs. (1971): Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chim. Acta, 31: 87-96.

El-Shater, A.A. (2003): Effects of organophosphorus insecticide parathion on the secretory activity of the thyroid gland and on some biochemical and hematological parameters of adult male rats. J. Egypt. Ger. Soc. Zool. (40A): Comp. Physiol., 447-456.

Erney, R.D. (1983): Rapid screening procedure for pesticides and polychlorinated biphenyl's in tissue: Collaborative study. J. Assoc. Anal. Chem., 66:969-974.

Finney, D.J. (1964): An International drug safe guard plan. J. Chronic Dis., 17:563-581.

Friedwald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem., 18:499-502.

Garg, U.K.; Pal, A.K.; Jha, G.J. and Jadhao, S.B. (2004b): Hemato-biochemical and immune-pathological effects of chronic toxicity with synthetic pyrethroid, organophosphate and chlorinated pesticides in broiler chicks. International Immunopharmacol., 4:1709-1710.

Gawish, A.M.; Ahmed, S.K. and Abdel Mageed, F.A. (2006): Studies on the effect of pesticide diazinon and the metrondiazole drug (flagyl) on the histology of some body organs of the rat *(Rattus norvigicus)*. J. Egypt. Ger. Soc. Zool., (50C): Histol. Histochem., 183-193.

Gokalp, O.; Gulle, K.; Sulak, O.; Cicek, E. and Altuntas, I. (2003): The effect of methidathion on liver: role of vitamin E and C. Toxicol. Indust. Health, 19:63-67.

Gruzdyev, G.S.; Zinchenko, V.A.; Kalinin, V.A. and Slovotsov, R.J. (1980): Fundamental of Agriculture Toxicology. In. Gruzdyev, G.S. (ed). The chemical protection of plants, 21-25.

Gultekin, F.; Ozturk, M. and Akdogan, M. (2006): The effect of organophosphorus insecticide chloropyriphos-ethyl on lipid peroxidation and antioxidant enzymes *(in vitro)*. Arch. Toxicol., 74:533-538.

Hasheesh, W.S.; Marie, M.A.S; Fakhary, F.M. and Mohamed, E.A.A. (2002a): Influence of organophosphorus pesticide triazophos on some biochemical aspects in male albino rats. J. Egypt. Ger. Soc. Zool., (37A): Comp. Physiol., 165-183.

Henry, R.J. (1974): Clinical chemistry, principles and technics, 2nd Edition, Harper and row, 525.

Ibrahim, N.A.; Mohamed, F.Z.; Al Zahaby, A.S. and El Kady, I.M. (1993): Acute and chronic effects of dimethoate and dursban on serum transaminase, alkaline phosphatase, cholinesterase activities and creatinine level in white rats. J. Egypt. Ger. Soc. Zool., (10A): 147-167.

Jendrassik, L. and Grof, P. (1938): Simplified photometric methods for the determination of the blood bilirubin. Biochem. Z., 297:81-89.

Kalender, S.; Oguten, A.; Uzunhisarcikli, M.; Acikogoz, F.; Durak, D.; Uusoy, Y. and Kalender,

Y. (2005): Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicol., 211(3): 197-206.

Lillie, R.D. (1969): Biological stains. 8th ED. The Williams and Wilkins Co., Baltimore. 172-175.

Mahmoud, M.M. (2000): Toxicological study of short and long-term administration of fenitrothion on male albino rats. Ph.D. Thesis, Dept, of Zoologym Fac. Sci., Cairo Univ.

Malik, G.; Dahiya, J.P. and Gera, S. (2004): Biochemical studies on chlorpyrifos toxicity in broiler chickens. Ind. J. Anim. Sci., 74(5): 4732-476.

Moghadamina A.A. and Abdollahi, M. (2002): An epidemiological study of poisoning in Northern Ialamic Republic of Iran. East Meditter. Health, J., 8:1-6.

Natt, M.P. and Herrick, C.A. (1952): A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Science, 31: 735-738.

Nemi, C.J. (1993): Essential of Veterinary Haematology. 1st Ed., P. 159, Lea and Febiger, Philadelphia.

Norbert, W.T. (1995): Clinical guide to laboratory tests, 3rd Ed. Philadilphia, W.B. Saunders Co.

Patton, C. and Crouch, S.R. (1977): Enzymatic determination of urea. Anal. Chem., 94:464-496.

PC-STAT, (1995): One way analysis of variance procedure. Georgia University.

Piramanayagam, S.; Murali Manohar, B. and Sundararaj, A. (1996): Pathology of malathion toxicity in rats. Ind. Vet. J., 73:734-737.

Rajini, P.S. and Krishnakumari, M.K. (1988): Toxicity of pirimiphos-methyl: Effect of dietary feeding on blood and urine constituents in albino rats. J. Environ. Sci. Health, Part B1B, 23:145-158.

Rao, D.D. and Yadgirkar, G. (2000): Pathology of subacute malathion toxicity in Japanese quail. Ind. J. Vet. Pathol., 24: 39-40.

Rec. GSCC (DGKC). (1972): Deutche Gesellschaft fur klinische Chemie J. Clin. Biochem., 10:182.

Rodrigo, A.F.; Hernandez, J.J.; Lopez-Caballero, F. and Gill, A.P. (2001): Immunohistochemical evidence for the expression and induction of paraxonase in rat liver, kidney, lung and brain tissue, implications for its physiological role, Chem. Biol. Interact., 137:123-137.

Rojkin, M.L.; Olguin del Mariani, M.C.; Drappo, G.A. and Ysosa, C.F. (1974): Fraccionamiento Proteico Por determination directa albumina. Bioq. Clin., VII/4:241.

Schumann, G. and Klauke, R. (2003): Clin. Chim. Acta., 327:69-79.

Slotkin, T.A.; Brown, K.K. and Seidler, F.J. (2005): Developmental exposure of rats to chloropyrifos elicits sex-selective hyperlipidemia and hyperinsulinemia in adulthood. Environ. Health Persepect., 113(10): 1291-1204.

Varsik, P.; Buranova, D.; Kondas, M; Kucera, P. Goldenberg, Z. and Pokorona, V. (2005): Chronic Poisoning neuropathy after organophosphorus poisoning in quails (*Coturnix coturnix japonica*). Bartisl Lek Listy, 106 (10): 293-296.

Wintrobe, M.M. (1965): Clinical hematology, 4th Ed. Lea and Febiger, Philadelphia.

Young, D.S. (2001): Effects of disease on Clinical Lab. Tests, 4th Ed AACC.

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Influence of Surface Temperature on Surface Fouling–Theoretical Approach

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Abstract: A theoretical approach for investigation the effect of surface temperature on surface particulate fouling has been developed. This approach is based on the basic fouling processes. As a result of this study, for each working condition, there was a specific surface temperature, defined as **critical surface temperature**, Below this temperature, i.e. the working temperature is less than the critical one, the fouling rate will increase by increasing surface temperature and it has the maximum value nearest the critical temperature. Above the critical temperature, i.e. the working temperature is greater than critical one, the fouling rate has a negative sign, and it means that some erosion of the heat transfer surface will be occurred. This erosion has maximum value nearest the critical temperature and decreases with temperature. At the critical temperature no fouling will be built up. On the light of these results, all contrary conclusions presented in literatures which concluded that; "the **increase in surface temperature may lead to increase, decrease or have no effect on the amount of material depositing at a surface** " are right conclusions. This depends on the working temperature is below, equal or above the critical surface temperature. A new formula describing the critical surface temperature with the affecting parameters has been deduced.
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Keywords: Surface fouling, surface temperature, particle sticking, mass transfer, heat transfer surface erosion.

1. Introduction

Fouling of heat transfer surface is defined as the accumulation of unwanted material on the heat transfer surface. This accumulation deteriorates the ability of the surface to transfer heat besides to the increase of the pressure drop through the heat transfer apparatus. Many investigators have studied the fouling phenomenon theoretically and experimentally. **Kern and Seaton** [1, 2] and **Ken** [3], made the groundwork of the fouling studies. Recently, **Nesta and Bennett** [4] introduced a new design of heat exchangers. They concluded that minimize wall temperature and maximize the flow velocity tends to minimize fouling; they also found that the heat exchanger material has a pronounced effect on fouling particularly when biological fouling is a concern. **Yang *et al.,*** [5] constructed a model for the fouling induction period. They found that the shorter induction periods are dealing with higher surface temperature. **Subbarao *et al.,*** [6] studied particulate fouling of glass particles under high temperatures. They concluded that the fouling layer formation is strongly dependent on the gas phase temperature and gas phase velocity and once the deposit has formed, increasing gas velocity hasn't any effect on removal of particles from the deposited layer. Mostafa ***et al.,*** [7], studied experimentally the effect of surface temperature on both the precipitation fouling and particulate fouling. They found that the fouling resistance increases with temperature in the case of precipitation fouling, where it decreases with temperature in the case of particulate fouling.

Fouling is influenced by a great number of affecting parameters, on the one hand by physical ones such as; flow velocity, temperature and the chemical nature and surface finish of the wall. On the other hand, the chemical concentration of the different compounds (solute, solvent, impurities) has to be taken into account. The great number of these parameters and their interdependence explain the difficulties encountered when predicting fouling by a theoretical approach. That is the reason why this phenomenon is essentially represented by empirical laws for each particular case. This allows optimal operating conditions and technical solutions to be obtained which avoid, or at least reduce, fouling, for a specific process. According to many previous researchers; the most important ones are the flow velocity and the surface temperature.

The effect of flow velocity is well known in which the most studies such as **Mostafa *et al.,*** [8], Dunqi ***et al.,*** [26], and Knudsen ***et al.,*** [27], indicated that the fouling rate decreases with increasing flow velocity.

Through the years many efforts have also been made on probing the phenomenon of the particulate fouling on heating surfaces. Some examples of using Fluent code for predicting fouling phenomena occurring at heating surfaces can be found in publications [9-10]. Particulate fouling of convective heat-transfer surfaces is usually assessed by empirical correlations. Nevertheless, constant progress in numerical calculation methods allows for predicting fouling phenomena occurring at heating surfaces, **Wacławiak and Kalisz** [11].

Also, many achievements have been obtained on deposition and removal models to predict particulate

fouling on heating surfaces under inertial impaction. **Thornton and Ning** [12], and **Konstandopoulos** [13] studied deposition criteria for particle inertial collision with the tube wall. **Feng *et al.,*** [14] researched the effect of influence parameters on particle-wall inertial collision deposition. **Abd-Elhady *et al.,*** [15] proposed that inertial impact speed is the main parameter of collision deposition for particles with a powdery layer. **Van *et al.,*** [16] developed a two-body collision deposition mechanism for particle impaction with a powdery layer. **Huang *et al.,*** [17] developed a numerical model for the deposition rate using macro probability statistics.

Fouling removal is another important process of the fouling growth. **Rodriguez *et al.,*** [18] reported that fouling removal is mostly depended upon gas flow velocity. **Abd-Elhady *et al.,*** [19] found that fouling removal is related to the impact speed or the contact time of the incident particles. **Polley *et al.,*** [20] concluded that fouling removal rate is proportional to the 0.8 power of the Reynolds number. Previous research in fouling mechanism has respectively focused on the process of particulate deposition or fouling removal.

Fouling growth on heating surfaces is determined by the difference between the deposition and removal of particles on and from the fouling layer. Particulate fouling is mainly influenced by physicochemical properties and transport mechanisms of suspended particles, such as particulate size, transport forces arising from the gradients of density, temperature and velocity in the flow field. An integrated fouling model was developed by **Pan *et al.,*** [21] by considering the combined suspended particles deposition and the fouling removal processes.

From the above literature review it was shown that, the effect of the surface temperature on the fouling rate has been mentioned in several studies however, these studies indicated that the role of surface temperature is not well understood and much more research needs to be carried out on the effect of surface temperature. Up to now, the effect of surface temperature on the surface fouling is not well known, where some literatures show that; the increase in surface temperature may lead to increase [6, 22], decrease [4, 23], or have no effect on the fouling rate [24]. The motivation of the present work is to improve our understanding of this problem and to provide solutions to reduce and control fouling.

2. Theoretical Approach

From the previous theoretical studies [13-38], the particulate fouling process is consisting of two sub-processes which are deposition process and removal process. Therefore the fouling rate is given by

Accumulation rate = deposition rate – removal rate, or

$$\frac{dm_f}{d\theta} = \phi_d - \phi_r \qquad (1)$$

2.1. Deposition Rate (φ_d)

In the present model, only the colloidal particles i.e. $d_p < 50$ μm, where the gravity has no effect on deposition or removal processes, will be considered. For large particles, $d_p \geq 50$ μm there is another type of fouling mechanism what is known as sedimentation fouling (i.e. deposition occurs under the effect of gravity) which can be often prevented in heat transfer equipment by pre-filtration.

The increasing rate of the fouling layer thickness (x_f) is given by

$$\left(\frac{dx_f}{d\theta}\right)_d = \frac{S \cdot N}{\rho_f A_s} \qquad (2)$$

Where the stick ability (S) is given by the Arrhenius equation as

$$S = k_s \exp\left(-E/R_g T_s\right) \leq 1 \qquad (3)$$

Where k_s is constant, known as sticking coefficient

As shown in Fig. (1), the particle flow rate toward the surface (N) is represented as

$$\begin{aligned} N &\propto \Delta C \cdot \dot{M} \\ &= k_1 (C_s - C_b) \cdot \dot{M} \end{aligned} \qquad (4)$$

Where k_1 is constant, for steady flow conditions and constant fluid properties, the constant $|k_1| = |k_D|$ where k_D is the mass transfer coefficient.

The particles concentration at the surface (C_s) is given by

$$\begin{aligned} C_s &= \frac{non-stick\ particles}{flow\ rate,\ \dot{M}} \\ &= \frac{N(1-S)}{\dot{M}} \end{aligned} \qquad (5)$$

From Eqns. (4) & (5), the particles mass flux toward the surface is given as

$$\begin{aligned} N &= k_1 \cdot \dot{M}\left[C_b - \frac{N(1-S)}{\dot{M}}\right] \\ &= \frac{k_1 C_b \dot{M}}{1 + k_1 (1-S)} \end{aligned} \qquad (6)$$

From Eqns. (2) & (6);

$$\left(\frac{d(x_f)}{d\theta}\right)_d = \frac{S \cdot N}{\rho_f A_s}$$

$$= \frac{k_1 C_b S \cdot \dot{M}}{\rho_f A_s \left[1 + k_1 (1 - S)\right]}$$

but

$$\dot{M} = \rho \cdot u \cdot F$$

Therefore

$$\left(\frac{dx_f}{d\theta}\right)_d = \frac{k_1 C_b S \rho u F}{\rho_f A_s \left[1 + k_1 (1 - S)\right]}$$

And the deposition rate is given as

$$\phi_d = \rho_f \left(\frac{dx_f}{d\theta}\right)_d$$

$$= \frac{k_1 C_b S \rho u F}{A_s \left[1 + k_1 (1 - S)\right]} \qquad (7)$$

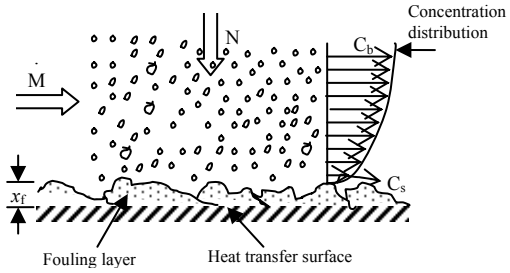

Fig. (1) The deposition process

2.2. Removal Rate (φ_r)

As shown in Figs. (2) & (3) the decreasing rate in the fouling layer thickness due to removal process $\left(dx_f / d\theta\right)_r$ is proportional to the shear stress (τ), the fouling layer thickness (x_f), and to the inverse of deposit strength (ψ), therefore

$$\left(\frac{dx_f}{d\theta}\right)_r \propto x_f \tau \frac{1}{\psi}$$

$$\left(\frac{dx_f}{d\theta}\right)_r = k_2 x_f \tau \frac{1}{\psi}$$

Where k_2 is constant and the deposit strength (ψ) is represented by the weaker force of the adhesion or cohesion forces.

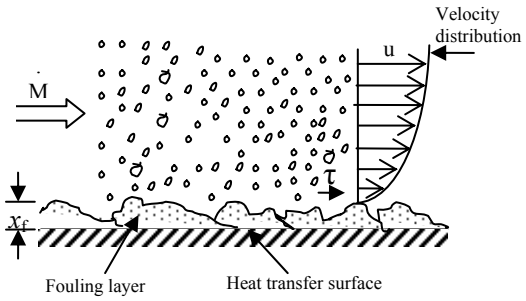

Fig. (2) The removal process

$$\tau \propto \rho u^2$$

$$= k_3 \rho u^2$$

Where $k_3 = ½ f$, and f is the friction factor, therefore

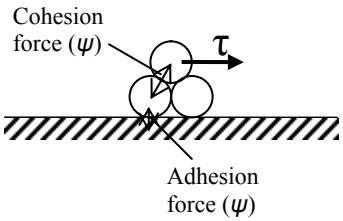

Fig. (3) The removal mechanism

$$\left(\frac{dx_f}{d\theta}\right)_r = k_2 x_f \frac{k_3 \rho u^2}{\psi}$$

$$= k_4 x_f \frac{\rho u^2}{\psi}$$

Where $k_4 = k_2 k_3$

Therefore the removal rate is given as

$$\phi_r = \rho_f \left(\frac{dx_f}{d\theta}\right)_r$$

$$= k_4 \rho \rho_f x_f \frac{u^2}{\psi} \quad (8)$$

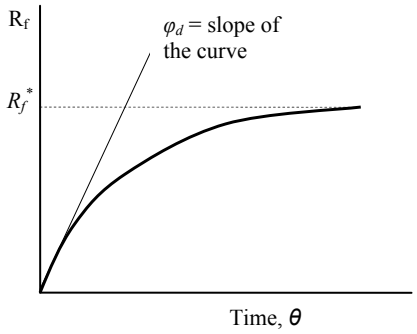

Fig. (4) The fouling curve

2.3. Fouling Factor (R_f)

From Eqn. (1), the fouling rate is given by

$$\frac{dm_f}{d\theta} = \phi_d - \phi_r$$

$$= \frac{k_1 C_b S \rho u F}{A_s\left[1 + k_1(1-S)\right]} - k_4 \rho \rho_f x_f \frac{u^2}{\psi}$$

But

$$m_f = \rho_f x_f$$

$$= \rho_f \left(\lambda_f R_f\right)$$

Where λ_f is the thermal conductivity of the fouling layer, and R_f is the fouling factor, therefore

$$\frac{dR_f}{d\theta} = \frac{1}{\rho_f \lambda_f}\left(\frac{dm_f}{d\theta}\right)$$

$$= \frac{1}{\rho_f \lambda_f}\left[\frac{k_1 C_b S \rho u F}{A_s\left[1 + k_1(1-S)\right]} - k_4 \rho \rho_f x_f \frac{u^2}{\psi}\right]$$

$$= \frac{k_1 C_b S \rho u F}{\rho_f \lambda_f A_s\left[1 + k_1(1-S)\right]} - \frac{k_4 \rho u^2}{\psi} R_f \quad (9)$$

Integrating this equation with a boundary condition; ($R_f = 0$ at $\theta = 0$), gives that

$$R_f = \frac{k_1 C_b S F \psi}{k_4 u \rho_f \lambda_f A_s\left[1 + k_1(1-S)\right]}\left[1 - \exp\left(-\frac{k_4 \rho u^2}{\psi}\theta\right)\right] \quad (10)$$

And

$$\left.\frac{dR_f}{d\theta}\right|_{\theta=0} = \frac{k_1 C_b S \rho u F}{\rho_f \lambda_f A_s\left[1 + k_1(1-S)\right]} = \phi_d \quad (11)$$

It means that the slope of the fouling curve at time zero represents the deposition rate (Φ_d), as shown in figure (4)

And at $\theta = \infty$, the asymptotic fouling factor (R^*_f) is given by

$$R_f^* = R_f\big|_{\theta=\infty} = \frac{k_1 C_b S F \psi}{k_4 u \rho_f \lambda_f A_s\left[1 + k_1(1-S)\right]} \quad (12)$$

From Eqns. (10) and (12), the fouling factor can be written as

$$R_f = R_f^*\left[1 - \exp\left(-\frac{k_4 \rho u^2}{\psi}\theta\right)\right] \quad (13)$$

Substituting by S from Eqn. (3) into Eqn. (10), the fouling factor (R_f) can be written as

$$R_f = \frac{e^{-E/R_g T_s}}{u\left[\frac{k_4\rho_f\lambda_f A_s(1+k_1)}{k_1 K_s C_b F\psi} - \frac{k_4\rho_f\lambda_f A_s}{C_b F\psi} e^{-E/R_g T_s}\right]}\left(1 - e^{\frac{-k_4\rho u^2}{\psi}\theta}\right) \quad (14)$$

This equation can be rewritten in the following form

$$R_f = \frac{e^{-E/R_g T_s}}{u\left[A' - B' e^{-E/R_g T_s}\right]}\left(1 - e^{-D' u^2 \theta}\right) \quad (15)$$

Where A', B' and D' are lumped parameters which are given as

$$A' = \frac{(1+k_1)k_4\rho_f\lambda_f A_s}{k_1 K_s C_b F\psi}$$

$$B' = \frac{k_4\rho_f\lambda_f A_s}{C_b F\psi} \quad (16)$$

$$D' = \frac{k_4\rho}{\psi}$$

These parameters can be drawn from the experimental data.

From Eqns. (12) and (16);

$$R_f^* = \frac{e^{-E/R_g T_s}}{u\left[A' - B' e^{-E/R_g T_s}\right]} \quad (17)$$

And

$$\frac{R_f}{R_f^*} = 1 - e^{-D' u^2 \theta} \quad (18)$$

2.4. Critical Surface Temperature (T_{sc})

Differentiating Eqn. (15) and equating to zero, the critical surface temperature, T_{sc}, can given as

$$T_{sc} = \frac{-E/R_g}{\ln(A'/B')} \quad (19)$$

Substituting from Eqn. (16) by values of A' and B', the critical surface temperature can be expressed as

$$T_{sc} = \frac{-E/R_g}{\ln\left(\frac{1+k_1}{k_1 \cdot k_s}\right)} \quad (20)$$

From Eqn. (20), it can be seen that the critical surface temperature, T_{sc}, depends mainly on the activation energy, E, the constant k_1 which can be represented by mass transfer coefficient, k_D, and the sticking coefficient, k_s.

3. Results and Discussion

To show the effect of surface temperature, T_s, on both the fouling factor, R_f, and the asymptotic factor, R_f^*, the values of the lumped parameters, A', B', D' and E/R_g have been drawn from the available experimental and computational data [7, 27, 28, 32, 33 and 35], and used in Eqns. (15) and (17).

3.1. Effect of Surface Temperature on Fouling Factor

From the drawn values, three cases have been selected and listed in Table (1). Using these selected values of the lumped parameters and by the aid of Eqn. (15), the R_f - θ curves have been drawn and illustrated for each case in Figs. (5 - 7) for different values of surface temperature.

Table (1) selected values of the lumped parameters

Case	A'	B'	D'	u, m/s	R_g/T_s
1	$1.864*10^{-13}$	12575.94	0.007	1.67	12657
2	$3.096*10^{-13}$	559.64	0.017	1.64	12657
3	$3.888*10^{-13}$	1363.64	0.032	1.62	12657

From figures (5 - 7), it can be seen that the fouling factor, R_f, is increased by increasing the surface temperature, T_s, until a specific value of T_s, above this specific temperature R_f has a negative values i. e., some erosion of the heat transfer surface will be occurred. This specific value of T_s is called the *critical surface temperature*, T_{sc}, which depends on the activation energy, E, the mass transfer coefficient, k_D, and the sticking coefficient, k_s, as discussed above and it is given by Eqn. (20). As shown in all figures and at $T_s = T_{sc}$, the fouling factor, $R_f = 0$ i.e., there is no fouling. For all the illustrated cases, R_f increases with time, θ, for constant T_s and constant flow velocity, u

3.2. Effect of Surface Temperature on Asymptotic Fouling Factor

By using the listed values in Table (1) and by the aid of Eqn. (17), the relation between R_f^* and T_s is illustrated in Figs. (8 - 10). As shown in these figures, it is clear that the asymptotic fouling factor, R_f^* is increased by increasing the surface temperature until the critical surface temperature, T_{sc}, in which at this T_{sc} the asymptotic factor is zero and above T_{sc} the asymptotic factor has negative values. This phenomenon satisfies the mentioned conclusion in literature, Gudmundsson [28] which dealing with the effect of surface temperature on fouling rate and stated that "increase in surface temperature may increase,

decrease or have no effect on the amount of material depositing at a surface". According to Eqn. (19) and from the above figures, the critical surface temperatures for the three cases listed in Table (1) are 53.63, 80.61 and 87.29 ^{o}C respectively. The temperatures are plotted in Celsius scale instead of the Kelvin scale to be more readable.

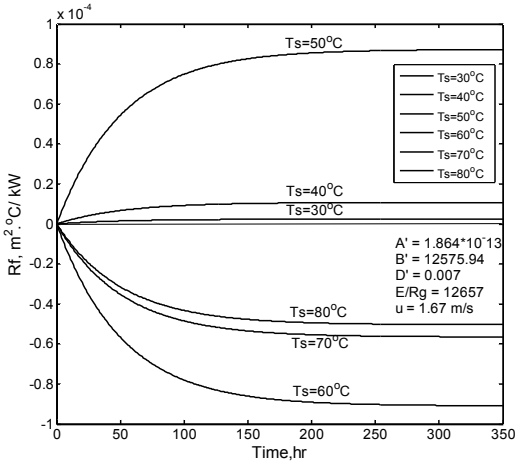

Fig. (5) R_f-θ curves for case 1

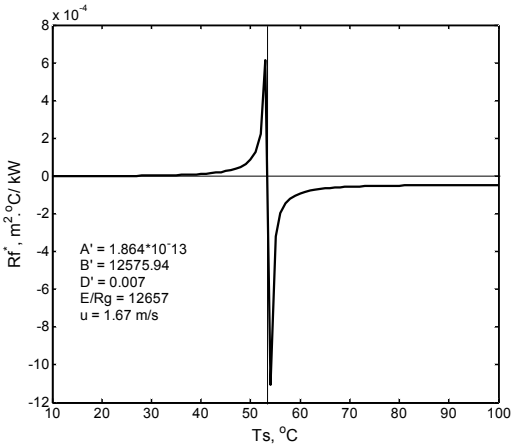

Fig. (8) R_f^*-T_s curves for case 1

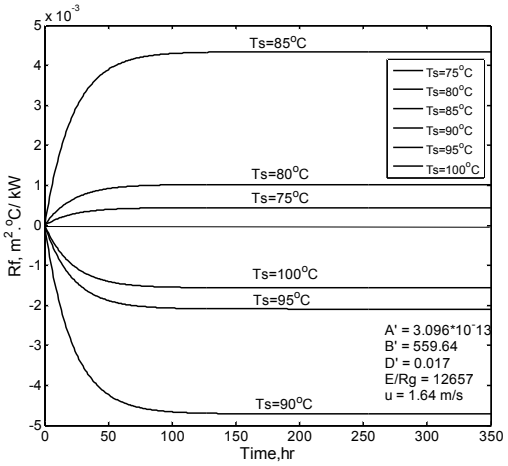

Fig. (6) R_f-θ curves for case 2

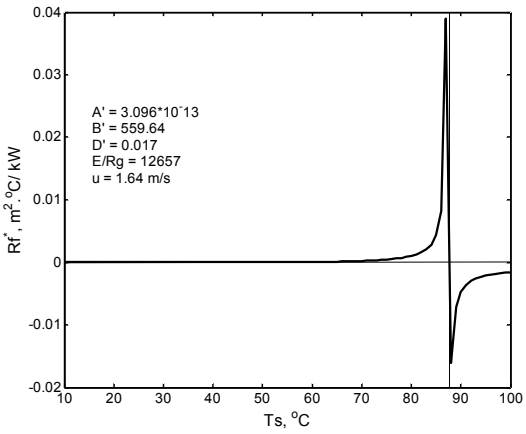

Fig. (9) R_f^*-T_s curves for case 2

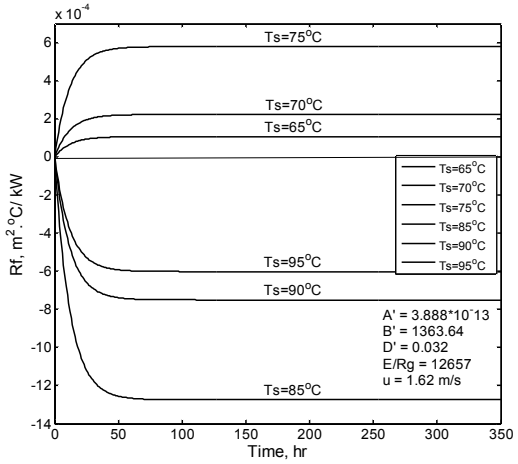

Fig. (7) R_f-θ curves for case 3

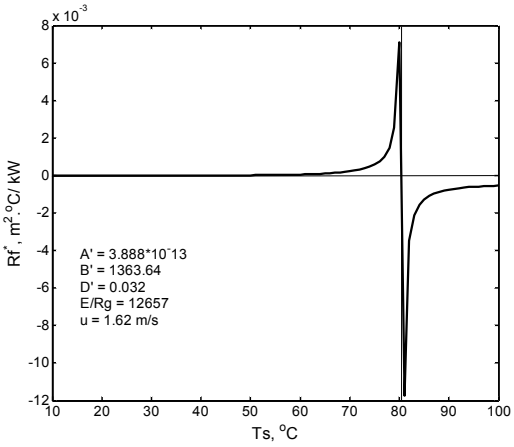

Fig. (10) R_f^*-T_s curves for case 3

The above results could interpret the phenomena of existing a very thick fouling layer at some sites of the surface of an electrical heating element which used to heat water and there is no fouling at other sites of the same surface or may be there is some erosion, this may be due to the variation of temperature over the surface. For example, the case 2 in Table (1), t_{sc} = 80.61 ^{o}C, it means that the sites of the heat transfer surface which have a temperature of 80 ^{o}C or less will face a high rate of fouling while the sites which have a temperature of 81 ^{o}C or higher will face some erosion.

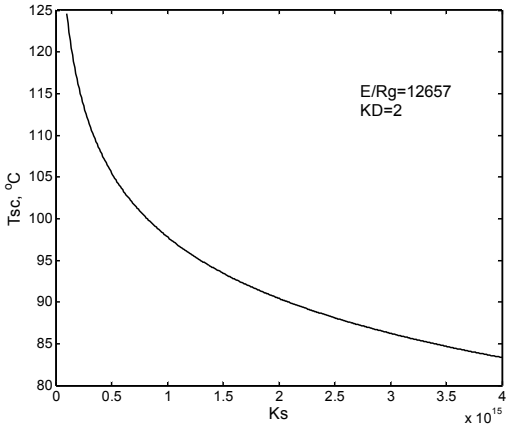

Fig. (11) Effect of sticking coefficient, k_s on the critical surface temperature, T_{sc}

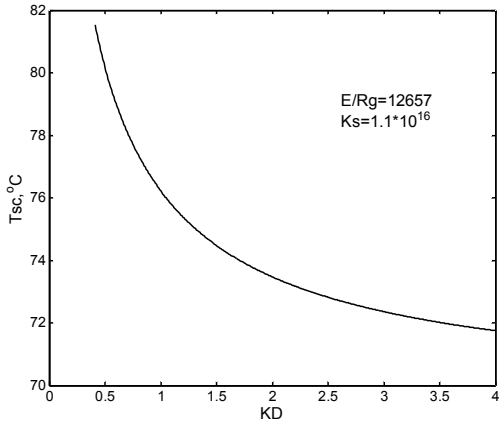

Fig. (12) Effect of mass transfer coefficient, k_D on temperature, T_{sc}

3.3. The Critical Surface Temperature

To show the relation between the critical surface temperature, T_{sc}, and the working parameters, the selected values listed in Table (1) have been exploited and used in Eqn. (20) and drown in Figs. (11, 12). From these figures, it can be seen that the critical surface temperature, T_{sc}, exponentially decreases with both of k_s and k_D coefficients. This means that by determining the sticking coefficient, k_s and the mass transfer coefficient, k_D, and controlling them, the critical surface temperature, T_{sc}, can be controlled for a specific value of the activation energy, E.

Conclusions And Recommendations

A new theoretical model for investigation the effect of surface temperature on both the fouling rate and asymptotic fouling factor for particulate fouling was developed. The present results show that, each operating condition has its own critical surface temperature which can be predicted by the aid of the present model. Working below this temperature leads to increase the fouling rate with increasing surface temperature where working above this temperature leads to exist some erosion of the heat transfer surface which is decreased by increasing surface temperature. To avoid the high rate of fouling or high rate of surface erosion, it must to work with a temperature as far as possible from the critical surface temperature. And, to work without fouling or surface erosion, it must work exactly at the critical surface temperature, and as it is known that doing this is very difficult or say it is impossible therefore, the working far from the critical temperature is recommended. In the design of heat transfer equipments and knowing the sticking coefficient and mass transfer coefficient, the critical surface temperature can be determined and controlled.

Nomenclature

A_s	heat transfer surface area, m^2	R_g	universal gas constant, $J/mol\ K$
A'	lumped parameter, *defined by Eqn. (16)*	R_f	fouling factor (fouling resistance), m^2K/W
B'	lumped parameter, *defined by Eqn. (16)*	R_f^*	asymptotic fouling factor, m^2K/W
C	concentration of fouling material, kg_p/kg_{fl}	S	stickability, -
C_b	concentration of fouling material at fluid bulk, kg_p/kg_{fl}	T_s	heat transfer surface temperature, K
C_s	concentration of fouling material at surface, kg_p/kg_{fl}	T_{sc}	heat transfer critical surface temperature, K
D'	lumped parameter, *defined by Eqn. (16)*	u	fluid flow velocity, m/s
E	activation energy, J/mol	x_f	thickness of fouling layer, m
f	friction factor, -	**Greek Letters**	
F	fluid flow cross-sectional area, m^2	φ_d	deposition rate, kg/m^2s
k_D	mass transfer coefficient, m/s	φ_r	removal rate, kg/m^2s
		λ_f	thermal conductivity of the fouling layer, W/mK
		θ	time, s
		ρ	density of working fluid, kg_p/m^3
		ρ_f	density of fouling

k_s	sticking coefficient, -	layer, kg_{fl}/m^3	
k_1	proportional constant, -, *defined by Eqn. (4)*	τ	fluid shear stress, N/m^2
k_4	proportional constant, s^{-1}, *defined by Eqn. (7)*	ψ	strength of fouling layer, N/m^2
m_f	mass of deposited material, kg_p/m^2	**Subscripts**	
M'	fluid flow rate, kg_{fl}/s	d	deposition
N	particles mass flux toward the surface, kg_p/s	f	fouling
		fl	fluid
		p	particle
		r	removal

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References

1. Kern D.Q. and Seaton R.E., "A Theoretical Analysis of Thermal Surface Fouling", Br. Chem. Eng., 4 (5): 258-262, 1959.
2. Kern D.Q. and Seaton R.E., "Surface Fouling: How to Calculate Limits", Chem. Eng. Prog. 55 (6), 71-73:1959.
3. Kern D.Q., "Heat Exchanger Design for Fouling Surfaces", Chem. Eng. Prog., 62 (7): 51-56, 1966.
4. Nesta J. and Bennett C.A., "Fouling Mitigation by Design", 6th International Conference on Heat Exchanger Fouling and Cleaning, Germany, June 5 - 10, 2005.
5. Yang M., Young A., Niyetkaliyev A. and Crittenden B., "Modelling Fouling Induction Periods", International Journal of Thermal Sciences (51): 175-183, Elsevier Publisher, December 2011.
6. Subbarao K., Rindt C. and Steenhoven A., "Preliminary Study of Particulate Fouling in a High Temperature Controlled Experimental Facility", 8th International Conference on Heat Exchanger Fouling and Cleaning, Schladming, Austria, June 14-19, 2009.
7. Mostafa M.A., Ali Y. and Helmi G., "Effect of Surface Temperature on Particulate and Crystallization Fouling", 13th Int. Water Technology Conference, Sharm El-Sheikh, Egypt, 2009.
8. Mostafa M.A., Salem M., Helmi, G. and Asfour F., "Effect of Flow Velocity on the Surface Fouling", Mansoura Engineering Journal (MEJ), Vol.32, No(1):, pp M27-M37, March 2007.
9. Yilmaz S. and Cliffe K.R., "Particle Deposition Simulation Using the CFD Code FLUENT", J. Inst. Energy 8, pp: 65-73, 2000.
10. Kaer S.K., Rosendhal L.A. and Baxter L.L., "Towards a CFD Based Mechanistic Deposit Formation Model for Straw-Fired Boilers", Fuel, 85, pp: 833-848, 2005.
11. Wacławiak K. and Kalisz S., "A Practical Numerical Approach For Prediction of Particulate Fouling in PC Boilers", Fuel 97, : 38-48, 2012.
12. Thornton C. and Ning Z., "A Theoretical Model for the Stick/Bounce Behavior of Adhesive Elastic-Plastic Spheres", Powder Tech. 99, pp: 154-162, 1998.
13. Konstandopoulos A.G., "Particle Sticking/Rebound Criteria at Oblique Impact", J. Aerosol Sci. 37 pp: 292-305, 2006.
14. Feng X.Q., Li H., Zhao H.P., *et al.,* "Numerical Simulations of the Normal Impact of Adhesive Micro Particles with a Rigid Substrate", Powder Tech., 89, pp: 34-41, 2009.
15. Abd-Elhady M.S., Rindt C.C., Wijers J.G. *et al.,* "Modeling the Impaction of a Micron Particle With a Powdery Layer", Powder Tech. 168, pp: 111-124, 2006.
16. Van B.M., Rindt C.C., Wijers J.G. *et al.,* "Rebound Characteristics for 50-μm Particles Impacting a Powdery Deposit", Powder Tech. 165, pp: 53-64, 2006.
17. Huang L.Y., Norman J.S., Pourkashanian M. and Williams A., "Prediction of Ash Deposition on Superheater Tubes From Pulverized Coal Combustion", Fuel, 75 (3), pp: 271–279, 1996.
18. Rodriguez C. and Smith R., "Optimization of Operating Conditions for Mitigating Fouling in Heat Exchanger Networks", Chem. Eng. Res. Des. 85, pp: 839-851, 2007.
19. Abd-Elhady M.S., Rindt C.C. and Van S.A., "Contact Time of an Incident Particle Hitting a 2D Bed of Particles", Powder Tech. 191, pp: 315-326, 2009.
20. Polley G.T., Wilson D.I., Yeap B.L., *et al.,* "Evaluation of Laboratory Crude Oil Threshold Fouling Data for Application to Refinery Pre-heat Trains", Appl. Therm. Eng. 22, pp: 777–788, 2002.
21. Pan Y., Fengqi S., Zhigao Xu and Carlos E.R., "An Integrated Theoretical Fouling Model for Convective Heating Surfaces in Coal-fired Boilers", Power Technology 210, pp: 150-156, 2011.
22. Burrill K.A., "The Deposition of Magnetite Particles from High Velocity Water onto Isothermal Tubes", AECL-5308, Feb. 1977.
23. Hopkins R.M. and Epstein, N., "Fouling of Heated Stainless Steel Tubes by a Flowing Water Suspensions of Ferric Oxide in Water", 5th Int. Heat Transfer Conf., 2, pp: 180-184, Tokyo, 1974.
24. Watkinson A.P. and Epstein, N., "Particulate Fouling of Sensible Heat Exchangers", 4th Int. Heat Transfer Conf., Versailles, Sept. 1970.
25. Taborek J., Aoki T., Ritter R.B., Palen, J.W. and Knudsen, J.G., "Predictive Methods for Fouling

Behavior", Chem. Eng. Prog., Vol. 68, No.(7): , pp. 69 -78, July 1972.
26. Dunqi X.U. and Knudsen, J.G., "Functional correlation of Surface Temperature and Flow Velocity on Fouling of Cooling-Tower Water", Heat Transfer Eng., vol.7, pp:.63-71, Nov. 1986.
27. Knudsen J.G. "Apparatus and Techniques for Measurement of Fouling of Heat Transfer Surfaces", Fouling of Heat Transfer Equipment, Somerscales, E.F., and Knudsen, J.G., (eds.), 57-82, Hemisphere, N.Y., 1981.
28. Gudmundsson J.S., "Particulate Fouling", Fouling of Heat Transfer Equipment, Somerscales, E.F., and Knudsen, J.G., (eds.), 357- 387, Hemisphere, N.Y., 1981.
29. Epstein N., "General Thermal Fouling Models", Fouling Science and Technology, Melo, L.F., *et al.,* (eds.), 15-30, Kluwer Academic Publishers, Netherlands, 1988.
30. Visser J., "Adhesion and Removal of Particles part1", Fouling Science and Technology, Melo, L.F. *et al.,* (eds.), 87-104, Kluwer Academic Publishers, Netherlands, 1988.
31. Visser J., "Adhesion and Removal of Particles part2", Fouling Science and Technology, Melo, L.F. *et al.,* (eds.), 105-123, Kluwer Academic Publishers, Netherlands, 1988.
32. Kallio G.A. and Reeks M.W., "A Numerical Simulation of Particle Deposition Turbulent Boundary Layers", Int. J. Multiphase Flow, 15 (3): 433-436, 1989.
33. Beal S.K., "Correlation for the Sticking Probability and Erosion of Particles" J. Aerosol Sci., 9:455-561, 1978.
34. Abd-Elhady M.S., Rindt1 C.M., Wijers J.G. and van Steenhoven A.A., "Particulate Fouling Growth Rate as Influenced by the Change in the Fouling Layer Structure", 6th International Conference on Heat Exchanger Fouling and Cleaning, Germany, June 5 - 10, 2005.
35. Kazi S.N., Duffy G.G. and Chen X.D., "Fouling and Fouling Mitigation on Different Heat Exchanging Surfaces", 8th International Conference on Heat Exchanger Fouling and Cleaning, Schladming, Austria, June 14-19, 2009.
36. Basim O. H., Graham J. N., Peter A., Richard A. C. and Richard K., "The Effects of Temperature and Hydrodynamics on the Crystallization Fouling Under Cross Flow Conditions", Int. J. of Applied Thermal Engineering, Elsevier Publisher, December 2011.
37. Polley G.T. and Gonzales-Garcia G., "Procedure for Applying Fouling Models to Predict Overall Fouling Rates in Industrial Heat Exchangers", 8th International Conference on Heat Exchanger Fouling and Cleaning, Schladming, Austria, June 14-19, 2009.
38. Zhenhua Q., Yongchang C. and Chongfang M.A., "Experimental Study of Fouling on Heat Transfer Surface During Forced Convective Heat Transfer", Chinese Journal of Chemical Engineering, 16(4) 535-540, 2008

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PARAOXONASE 1 Gene Polymorphism Relationship with Type 2 Diabetes Mellitus

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Abstract: **Background:** Human serum paraoxonase1 (PON1) an antioxidant enzyme closely associated with high density lipoprotein (HDL) has been implicated in the prevention of low density lipoprotein (LDL) oxidation and these may provide HDL-associated protection against atherosclerosis. The aim of this study is to investigate PON1 activity and its gene polymorphism in type 2 diabetes mellitus and its potential significance in the occurrence of diabetic complications. **Patients and Methods:** This study includes 60 subjects divided into: Twenty healthy subjects as a control (group I), Twenty diabetic patients without vascular complications (group II), and Twenty diabetic patients with vascular complications (group III). Laboratory investigations included: estimation of PON1 enzymatic activity by hydrolysis of paraoxon and PON1gene polymorphism by polymerase chain reaction (PCR) followed by polymorphism specific restriction enzyme digestion , other investigations included fasting and post prandial plasma glucose (FPG and PPPG), glycosylated hemoglobin (HbA1c) and lipid profile. **Results** :It revealed that PON1 activity was significantly lower in diabetics than control ($p< 0.001$) and is lower in group III (183.6 ± 52.01) than group II (230.05 ± 59.75) . PON1 was negatively correlated to HbA1c(r = -0.540 ,$P<0.001$) . Gene and allele frequencies was significantly different in diabetics than control at 192 polymorphism ($X^2= 7.645$, $P <0.05$) but not at 55 polymorphism with QQ higher in diabetics (77.5%) and RR higher in controls (25%). In both control and diabetics QQ and MM genotypes have the lowest activity of PON1 and RR and LL genotypes have the highest activity and QR and LM genotypes have intermediate activity ,HbA1c was highest in QQ and MM genotypes, intermediate in QR and LM genotypes and lowest in RR and LL genotypes. The allelic frequency of Q and M genotypes were higher in group III than in group II and R and L genotypes were lower in group III than in group II. **Conclusion:** Paraoxonase activity is affected by PON1 genetic variability in type2 diabetic patients. The PON1 QQ and MM genotypes are associated with lower PON1 activity than RR and LL genotypes. In type 2 diabetic patients the QQ and MM genotypes are more common and associated with poor glucose control mainly in diabetic patients with vascular complication which suggest their essential roles in occurrence of diabetic vascular complications.
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Key words: PON1 gene polymorphism, diabetic complication, PON1 activity, HbA1c.

1. Introduction

The oxidative modification of low-density lipoprotein (LDL) in the artery wall is believed to be central to the pathogenesis of atherosclerosis. One such mechanism is the prevention of LDL oxidation by high-density lipoprotein (HDL). HDL appears to decrease the accumulation of lipid peroxides on LDL by a mechanism that is, at least in part, enzymatic[1].

The prevention of oxidation of LDL is largely attributable to - HDL associated enzyme – paraoxonase-1 (PON1). Besides lipid peroxides, PON has been found to hydrolyzes hydrogen peroxides, which are a major reactive species produced by the arterial wall during atherogenesis[2].

Paraoxonase-1(PON1) is part of a multi-gene family also comprising the PON2 and PON3 genes, which are also located on chromosome7[3].

The PON1 gene product is serum paraoxonase, which is expressed mainly in the liver. Serum paraoxonase circulates as sub fraction of HDL and appears to use phospholipids on both high and low density lipoprotein particles as physiological substrate [4].

Paraoxonase-1 has two genetic polymorphisms, both due to amino acid substitutions: one involving glutamine (Q genotype) and arginine (R genotype) at position 192, and the other involving leucine (L genotype) and methionine (M genotype) at position 55. These polymorphisms affect the hydrolytic activity of the PON1 isoenzymes with respect to certain substrates, such as paraoxon and lipid peroxides[1].

There is evidence that the genetic polymorphisms of PON1 least able to protect LDL against lipid peroxidation are overrepresented in coronary heart disease, particularly in association with diabetes [5].

The chronic vascular complications of diabetes are a major cause of morbidity and premature mortality. In spite of more wide-spread availability of intensive diabetes management, approximately one in three people with diabetes develop aggressive

complications and over 70% die of atherosclerosis-related diseases. Potential mediators of vascular damage may include the interrelated processes of lipoproteins abnormalities, glycation, oxidation and endothelial dysfunction. Therefore, recognition and treatment of lipoprotein related risk factors may facilitate early recognition and treatment of high risk diabetic patients [6].

The aim of the present study is to investigate paraoxonase-1 activity and its gene polymorphisms (PON1 55 & PON1 192) in patients with type 2 diabetes mellitus as well as the potential significance of these polymorphisms in the occurrence of diabetic complications.

2. Subjects and Methods:

This study was carried out at Clinical Pathology and Internal Medicine Departments, Faculty of Medicine, Zagazig University Hospitals.

Subjects: The study included 60 subjects, they give consent about the sampling they were classified into the following: Twenty apparently healthy subjects**,** their ages ranged from 38 to 68 years with a mean ± SD of 52.95±9.24 years (Group I) .Twenty patients with type 2 diabetes mellitus without vascular complications, their ages ranged from 40 to 67 years with a mean ± SD of 54.85±8.11 years(Group II) . Twenty patients with type 2 diabetes mellitus with vascular complications, Their ages ranged from 45 to 71 years with a mean ± SD of 57.55±7.10 years (Group III). They were sub-classified into Two sub- groups according to the type of vascular complications: Ten patients with micro - vascular complications Group IIIa (5 retinopathy, 3 nephropathy , 2 neuropathy) . Their ages ranged from 49 to 68 years with a mean ± SD of 56.30±6.90years, and ten patients with macro-vascular complication Group IIIb (6 coronary heart disease (CHD), 4cerebral vascular disease (CVD). Their ages ranged from 45 to 71 years with a mean ± SD of 58.80±7.44 years. These subjects had no ketoacidosis, renal failure, liver disorder, or thyroid disease, acute or chronic inflammation, or infection. Subjects taking drugs known to affect lipoprotein oxidation or lipid-lowering drug were excluded from the study.

Methods:

After overnight fasting .EDTA blood samples were taken for HbA1c and PON1 genotyping. Sera were separated for lipid profile, liver function, kidney function tests, and PON1 activity, and blood samples were collected on Na fluoride /K oxalate tubes for FPG, PPPG.

All members of this study were subjected to full history taking, thorough clinical examination, routine laboratory investigations :Fasting and post prandial plasma glucose (FPG, PPPG), Liver & kidney function tests, and Lipid profile .All previous tests (except HDL-C and LDL-C) were determined on ADVIA 1650 auto-analyzer (Siemens Medical Solutions Diagnostic, USA). HDL-C was determined using Eli-tech kit (ELI-TECH -Diagnostics, France)[7]**.** LDL-cholesterol (LDL-C) was calculated using Friedewald's equation [8]. Glycosylated haemoglobin (HbAlc) by ion exchange resin chromatography using Stanbio Glycohaemoglobin[9]. Analysis of PON1 activity using paraoxon (*O*,*O*-diethyl-*O*-*p*-nitrophenyl phosphate); Sigma-Aldrich ,Germany) as a substrate according to the method described by Mackness *et al.* [10]. Determination of PON1 genotype was conducted by PCR amplification, followed by polymorphism-specific restriction enzyme digestion and gel electrophoresis [11]. Total genomic DNA was purified from buffy coat samples using the E.Z.N.A. ™ Blood DNA Kit (Omega – biotek. Inc). Genomic DNA samples were stored at -20°C until genotyping analysis. For the polymorphism at position 192 ,sense primer 5′ TATTGTTGCTGTGGGACCTGAG 3′, Antisense primer 5′ CACGCTAAACCCAAATACATCTC 3′, For the polymorphism at position 55 ,Sense primer 5′ GAAGAGTGATGTATAGCCCCAG 3′,and Antisense primer 5′ TTTAATCCAGAGCTAATGAAAGCC 3′ were used. Amplification steps of DNA was performed by pure Taq ready –To-Go PCR Beads (Amersham Biosciences).The PCR reaction mixture contained 5 µl of template DNA ,3 µl of each primer (100 uM/L) [Opern] in a tube containing one PCR bead [200 uM of each dNTP in 10 mM Tris-HCL(pH9.0),50 mM HCL and 1.5 mM $MgCl_2$and 2.5 U Taq DNA polymerase].After denaturating the DNA for 5 min at 95 °c for 1 cycle, the reaction mixture was subjected to 46 cycles of 1 min of denaturation at 94°C, 30 sec of annealing at 61 °C, and1 min of extension at 72 °C for the polymorphism at position 192. For the polymorphism at position 55 the PCR reaction and cycling were the same as descibed above, except that 30 cycles were carried out. PCR product was digested with 8 units of restriction endonucleases Alw I and Nla III (New England Biolabs, Cambridge, MA, U.S.A.) overnight at 37°C. The digested product was separated by agarose gel electrophoresis using DNA ladder 50bp. After finishing the procedure, the gel was viewed and photographed using UV illumination for sample visualization.

At position 192, allele Q (glutamine) corresponds to the presence of non-digested fragment of 99 bp product, while allele R (arginine) corresponds to 2 digested fragments of 66 bp and 33 bp. Characteristic bands were obtained at the following molecular weights: 99 for the PON1 192QQ polymorphism; 66 and 33 for the RR

polymorphism, and 99, 66, and 33 for the QR polymorphism .

At position 55, allele L (leucine) corresponds to the presence of non-digested fragment of 170 bp, while allele M (methionine) corresponds to 2 digested fragments of 126 bp and 44 bp. Characteristic bands were obtained at the following molecular weights: 170 for the PON1 55LL polymorphism; 126 and 44 for the MM polymorphism, and 170, 126, and 44 for the LM polymorphism.

Statistical Analysis

Statistical analysis was performed with the Statistical package for social scientists "SPSS" program for windows 10 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean± standard deviation (SD), Chi-square and student-t test was used for statistical comparisons between two groups of patients' parametric data. Analysis of variance (ANOVA) and least significant difference (LSD) were done to test the difference between the different studied groups. Correlation analysis was performed with Pearson correlation test. P-values below 0.05 were considered significant.

3. Results:

As regard FPG, PPPG, HbA1c ,triglycerides, total cholesterol, HDL-C, LDL-C and PON-1activity, there were high significant difference between all studied groups ($P < 0.001$),and between each two studied groups (Table1).

As regard PON1 activity there was no significant difference in PON1 activity between groups IIIa and IIIb (t=1.612, $p > 0.05$) (Table 2).

There was no significant correlation between PON1 activity and other studied parameters in controls, diabetic patients except for HbA1c in diabetic patients. where there was a significant negative correlation between PON1 activity and Hb A1C (r = -0.540, $P < 0.001$) (Table 3).

There were no significant differences in serum level of, triglycerides, total cholesterol, HDL-C, LDL-C between PON1 192,PON1 55 genotypes among both control and diabetic patients group ($P>0.05$) (not shown in tables).

As regard PON1 genotypes distributions and allele frequencies in the studied groups, the QQ genotype (Gln/Gln) was the most common in diabetic patients (77.5%) as well as in controls(45%), while the RR genotype (Arg/Arg) was the lowest one in both (5%,25%) respectively. The genotypes distributions of the PON1 192 polymorphisms was significantly different in diabetic patients compared to controls ($X^2=7.645$, $P<0.05$).The allelic frequency of Glycine192 (Q) was higher in the diabetic patients than controls (86% vs. 60%). Significant difference between the allele frequencies for the PON1 192 polymorphism was detected in diabetic patients as compared to controls ($X^2=5.175$, $P <0.05$). Also, the LL genotype (Leu/Leu) was the most common in controls (60%) , whereas the LM(47.5%) was more common than the LL genotype in diabetic patients. The genotype distribution of PON1 55 polymorphisms in diabetic patients was not statistically significant different compared to controls ($X^2=4.901$, $P >0.05$). The allelic frequency of Methionine 55 (M) was higher in the diabetic group than controls (44% vs. 22.5%) but no statistically significant difference between controls and diabetic patients was detected ($X^2=2.353$, $P>0.05$). (Table 4).

In control group, as regard PON1 activity, there was a significant difference among different PON1 192 genotypes (F= 12.166, $P <0.01$). Also, there was a significant difference in PON1 activity among different PON1 55 genotypes (F= 7.155, $P <0.01$). PON1 activity was lower in QQ,MM, intermediate in QR,LM and higher in RR,LL genotypes.(Table 5).In diabetic patients, as regard PON1 activity, there was a significant difference among different PON1 192 genotypes (F= 4.276, $P<0.05$). Also, there was a significant difference in PON1 activity among different PON1 55 genotypes (F= 3.557, $P<0.05$). PON1 activity was lower in QQ, MM, intermediate in QR, LM and higher in RR ,LL genotypes.(Table 6).

In diabetic patients, as regard HbA1cvalues, there was a significant difference among different PON1 192 genotypes (F= 3.986, $P <0.05$). Also, there was a significant difference in Hb A1c values among different PON1 55 genotypes (F= 4.346, $P <0.05$). HbA1cvalues was higher in patient with QQ,MM , intermediate in QR, LM and lower in RR,LL genotypes .(Table 7).The genotypes distributions of the PON1 192 polymorphisms was significantly different in group II compared to group III ($X^2=7.152$, $P <0.05$). The allelic frequency of Glycine 192 (Q) was higher in the diabetic group with vascular complication than diabetic group without vascular complication ($X^2=5.175$, $P <0.05$). The genotype distribution of PON1 55 polymorphisms was significantly different in group II compared to group III ($X^2=8.322$, $P <0.05$). The allelic frequency of Methionine 55 (M) was higher in the diabetic group with vascular complication than diabetic group without vascular complication ($X^2=5.125$, $P <0.05$).(Table8).

Table (1): Biochemical findings of the three studied groups

Group / Parameter	Group 1 (n = 20) Mean± SD (range)	Group II (n = 20) Mean ±SD (range)	Group III (n = 20) Mean± SD(range)	F	*P*
FPG (mg/ dl)	91.4 ± 10.57 (73 -107)	157.55±33.25* (97- 243)	168.75 ± 30.74** (117 - 213)	48.478	< 0.001
PPPG (mg/dl)	114.9 ± 11.69 (95 – 130)	242.4 ± 52.92* (163 – 365)	265.45 ± 48.74** (172 – 367)	74.257	< 0.001
Hb A1c %	4.38 ± 0.608 (3 – 5.5)	9.11 ± 0.954* (7.4 – 10.9)	10.427± 0.764**# (8.9 – 11.5)	325.58	< 0.001
Triglycerides (mg/dl)	122.2± 24.9 (81-160)	213.45 ± 56.65* (105-305)c	267.6 ± 59.12**# (152-345)	44.241	< 0.001
Total cholesterol (mg/dl)	172.3 ± 20.89 (142-214)	223.5 ± 25.98* (163-257)	250.7± 38.66**# (173-310)	36.489	< 0.001
HDL –C (mg/dl)	52.85 ± 10.43 (40-70a,b	41.85 ± 7.84* (34-60)	39.45 ± 7.07** (30-52)	13.904	< 0.001
LDL -C (mg/dl)	95.01± 23.55 (55-135.8)	138.96 ± 25.2* (82.4-173)	157.73±43.76** (79-224.8)	20.031	< 0.001
PON1Activity (U/L)	301.7± 98.74 (150-466)	230.05 ± 59.75* (121-368)	183.6 ± 52.01**# (112-320)	13.254	< 0.001

*statistical significant with group I ** statistical significant with group I # statistical significant with group II

Table (2): Comparison of mean ± SD and range between groups (IIIa) and (IIIb) as regard PON1activity (U/L).

Group / Parameter	Group IIIa (n = 10) Mean± SD (range)	Group IIIb (n=10) Mean± SD (range)	T test	
			t	*P*
PON1 Activity (U/L)	201.6± 32.22 (153-252)	165.65 ± 62.87 (112-320)	1.612	> 0.05 NS

Table (3):Correlation between PON1 activity(U/L)and other studied parameters in the control group and diabetic subjects .

Parameters / Group	Control group (n = 20)		Diabetic patients (n = 40)	
	r	*P*	r	*P*
HbA1c%	- 0.136	> 0.05	-0.540	< 0.001.H.S
FPG(mg/dl)	0.14	> 0.05	0.242	> 0.05
PPPG(mg/dl)	0.386	> 0.05	-0.089	> 0.05
Triglycerides(mg/dl)	0.245	> 0.05	-0.275	> 0.05
Cholesterol(mg/dl)	-0.087	> 0.05	0.083	> 0.05
HDL-C(mg/dl)	0.217	> 0.05	0.259	> 0.05
LDL-C(mg/dl)	-0.225	> 0.05	0.123	> 0.05

P>0.05 non-significant

Table (4): PON1 genotypes distributions and allele frequencies in the studied groups.

Groups / Parameters	Control group (n = 20)		Diabetic patients (n= 40)		X^2	*P*
	N0	%	No.	%		
PON192genotypes						
QQ	9	45	31	77.5		
QR	6	30	7	17.5	7.645	<0.05 S
RR	5	25	2	5		
Allele frequencies						
Q		60		86	5.175	<0.05 S
R		40		14		
PON55genotypes						
LL	12	60	13	32.5		
LM	7	35	19	47.5	4.901	>0.05 N.S
MM	1	5	8	20		
Allele frequencies						
L		77.5		56	2.353	>0.05 N.S
M		22.5		44		

Table (5): PON1 activity(u/l) in control group according to their PON1 192, PON1 55 genotypes.

	Control group (n=20)		
	PON1 activity(u/l) Mean± SD (range)	F	*P*
PON192genotypes QQ(n=9) QR(n=6) RR(n=5)	 242.78±66.73 (150-363) 287±86.97 (152-381) 425.4±26.23(400-466)	12.166	<0.01 S
PON55genotypes LL(n=12) LM(n=7) MM(n=1)	 348±86.18(167-466) 266.67±47.85(152-283) 150	7.155	<0.01 S

Table (6): PON1 activity(u/l) in diabetic patients according to their PON1 192, PON1 55 genotypes.

Genotypes	Diabetic patients (n=40)		
	PON1 activity(u/l) Mean± SD (range)	F	*P*
PON192genotypes QQ(n=31) QR(n=7) RR(n=2)	 193.29±54.4(112-311) 247.29±59.94 (186-368) 275±63.64(230-320)	4.276	<0.05 S
PON55genotypes LL(n=13) LM(n=19) MM(n=8)	 231.64±64.4(131-368) 206.17±53.4(126-311) 164.88±47.37(112-260)	3.557	<0.05 S

Table (7): HbA1c % in diabetic patients according to their PON1 192, PON1 55 genotypes.

Genotypes	Diabetic patients (n=40)		
	HbA1c% Mean± SD (range)	F	*P*
PON192genotypes QQ(n=31) QR(n=7) RR(n=2)	 10.011±0.955(7.9-11.5) 9.035±0.757(8.5-10.7) 8.906±1.269 (7.4-9.6)	3.986	<0.05 S
PON55genotypes LL(n=13) LM(n=19) MM(n=8)	 9.432±1.062(7.9-11.4)a 9.612±1.060(7.4-11.1)b 10.688±0.683(9.5-11.5)	4.346	<0.05 S

Table (8): PON1 genotypes distributions in group II and III.

Groups / Parameters	Group II (n = 20)		Group III (n= 20)		X^2	*P*
	N0	%	No.	%		
PON192genotypes QQ QR RR	 12 6 2	 60 30 10	 19 1	 95 5 0	7.152	<0.05 S
Allele frequencies Q R		 75 25		 97.5 2.5	5.125	<0.05 S
PON55genotypes LL LM MM	 10 9 1	 50 45 5	 3 10 7	 15 50 35	8.322	<0.05 S
Allele frequencies L M		 72.5 27.5		 40 60	5.175	<0.05 S

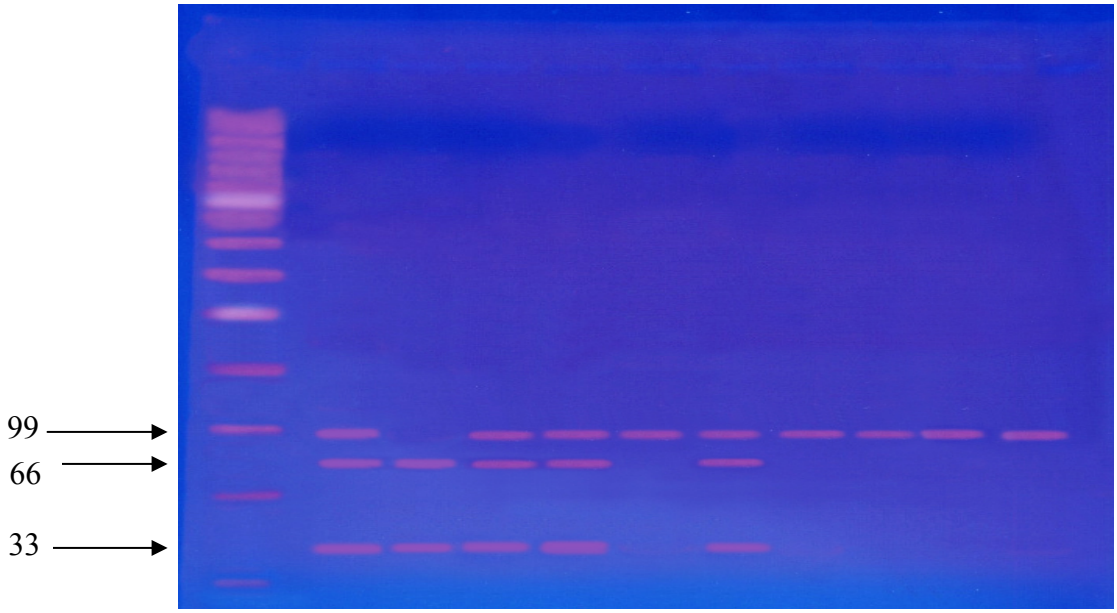

Figure (1): Agarose gel electrophoresis of the PCR products of the PON1 192 genotype in group II. Lane 1 represents 50 bp DNA marker, lanes 6,8,9,10,11 represent QQ genotype, lanes2,4,5,7 represent QR genotype, lane 3 represent RR genotype.

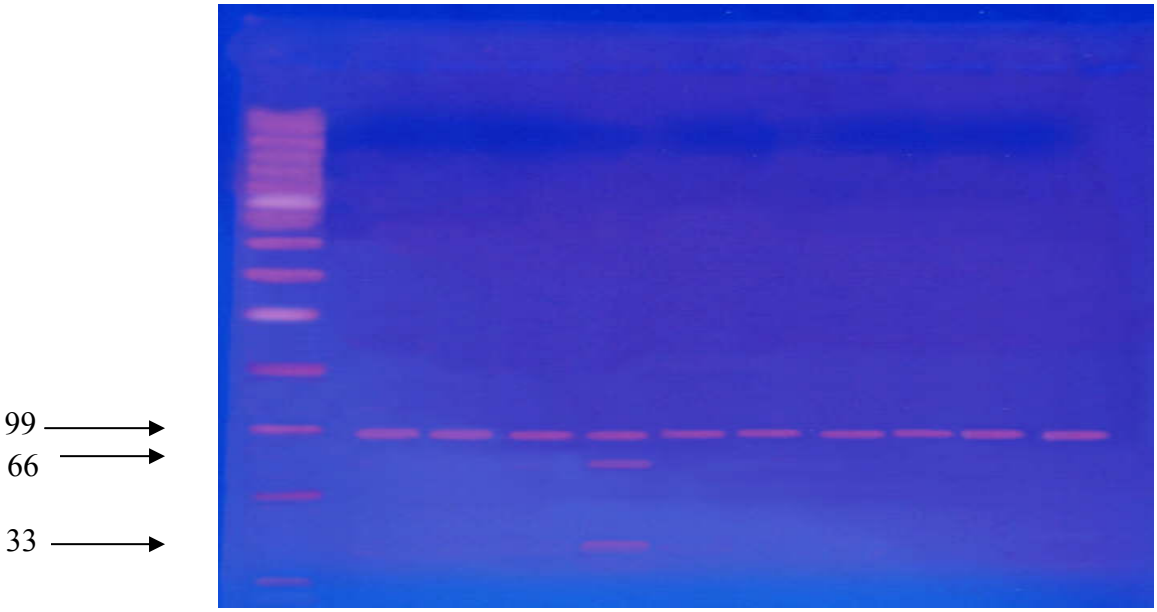

Figure (2): Agarose gel electrophoresis of the PCR products of the PON1 192 genotype in group III. Lane 1 represents 50 bp DNA marker, lanes 2,3,4,6,7,8,9,10,11 represent QQ genotype, lane 5 represent RR genotype.

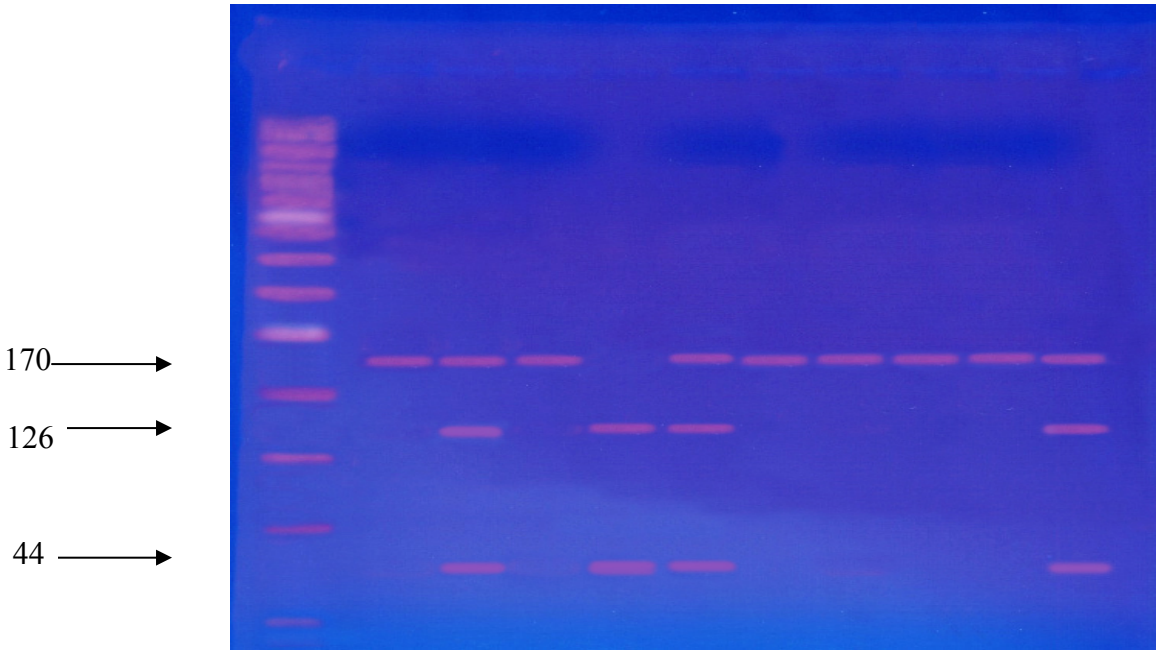

Figure (3): Agarose gel electrophoresis of the PCR products of the PON1 55 genotype in group II. Lane 1 represents 50 bp DNA marker, lanes 2,4,7,8,9,10 represent LL genotype, lanes 3,6,11 represent LM genotype, lane 5 represent MM genotype.

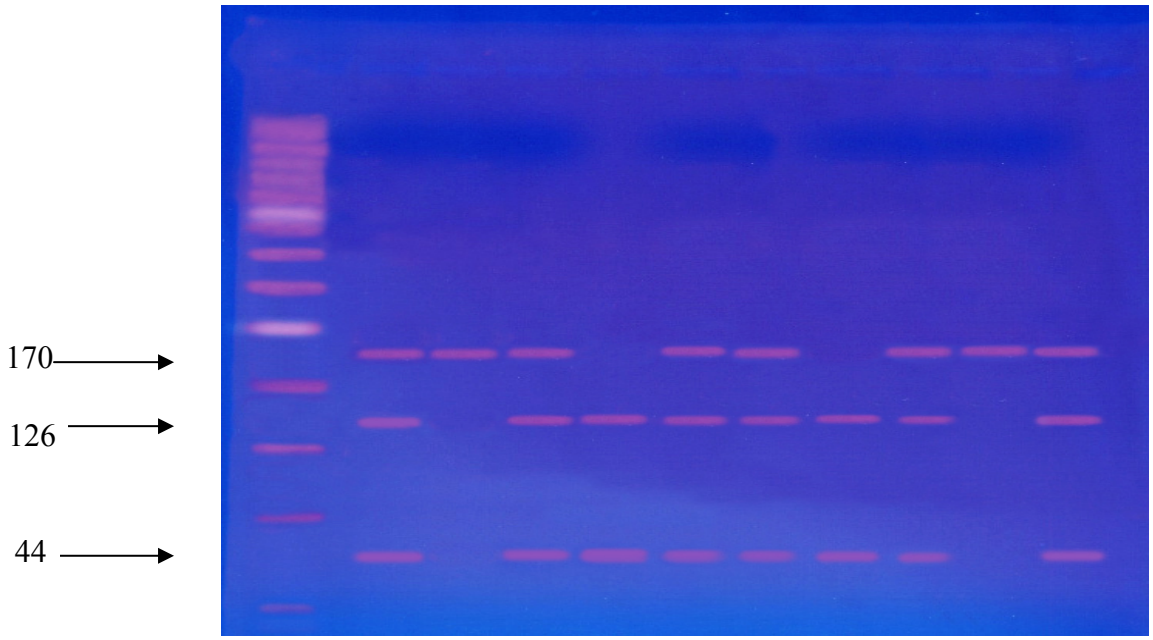

Figure (4): Agarose gel electrophoresis of the PCR products of the PON1 55 genotype in group III. Lane 1 represents 50 bp DNA marker, lanes 3,10 represent LL genotype, lanes 2,4,6,7,9,11 represent LM genotype, lanes 5,8 represent MM genotype.

4.Discussion:

It is a well established fact that diabetes is a risk factor for cardiovascular disease [12]. While microvascular complications of diabetes include nephropathy and retinopathy, macrovascualr complications resulting in atherosclerotic cardiovascular disease such as coronary artery disease, cerebrovascular disease and peripheral vascular disease which are the leading cause of death in the diabetic population [13].

Paraoxonase 1 is synthesized and secreted by the liver, it is a HDL associated enzyme capable of inhibition of atherosclerosis initiated by oxidatively modified LDL[2].

In this study PONI activity was significantly lower in diabetic patients compared to the control group ,and the diabetic group with vascular complications had a significantly lower level than those without vascular complications and no significant difference between subgroups with micro and macro- vascular complications. Also PONI activity was negatively correlated with HbA1cbut not correlated with serum lipids. These results are in agreement with **Sakai *et al.*** [14]**; Pfolil *et al.*** [15]; **Inoue *et al.*** [16] and **Mackness *et al.*** [1].

The reduced PONI activity could be explained by that PONI is bound by HDL in lesser extent in diabetic patients as compared to healthy persons and its activity is then poorly stabilized [17].

The reduced activity of PONI may predict its antioxidant properties which may take part in the development of vascular complications in diabetes mellitus[18]. PONI activity may be partially inactivated in the presence of oxidative stress as probably occurs in type 2 diabetes mellitus[19]. Low activity of PONI could explain the increased lipid peroxidation in this disease[20]. The negative correlation between PONI activity and HbA1c in diabetic patients coincide with **Flekac *et al.*** [21].The low enzyme activity is caused by glycation of the PONI protein than by reduced synthesis [22]. Glycation of HDL or direct glycation of PONI in HDL as occurs in diabetes may result in detatchment of PONI itself from HDL and PONI inactivation [23]. The non significant correlation between PONI activity and serum lipids is consistent with earlier findings of **Abbott *et al.*** [24]. The nonsignfiicant difference in serum lipids among PONI 192 and 55 genotypes was reported by **Odawara *et al.*** [25] and **Mackness *et al.*** [1]

Paraoxonase-1 has two genetic polymorphisms, both due to amino acid substitutions: one involving glutamine (Q genotype) and arginine (R genotype 192, and the other involving leucine (L genotype) and methionine (M) genotype at position 55. The R genotype 192 is more active than Q 192 and L55 has higher activity than M55 [26,27]**.**

In this study in 192 QQ polymorphism was the most common in both diabetics and control (77.5%,45%) respectively and 192 RR was the lowest in both diabetics and control group (5%,25%) respectively. Gene frequency at 192 polymorphism and allele frequency(Q) were significantly different in diabetics (86%)from control (60%). At 55 polymorphism gene frequency and allel frequency are not significantly different in diabetics from control. This results go hand in hand with **Mackness *et al.*** [28] and **Flekac *et al.*** [21]**, Agachan *et al.*** [29].

PONI gene polymorphism may influence variability of the enzyme activity and an association between cardiovascular disease and PONI gene polymorphism has been reported in diabetes mellitus [30]. Low PONI activity decreases ability to prevent lipid peroxide formation with consequent acceleration of the oxidant stress .Overproduction of the reactive oxygen species in diabetic patients may be due to chronic hyperglycemia, hyperinsulinemia, elevated fatty acids and dyslipidemia [31].

In this work the significant difference in PONI activity at 192 genotypes, PONI activity was lower in QQ, intermediate in QR and higher in RR genotypes in diabetic patients and in controls. These results are in accordance with **Agachan *et al.*** [32] and **Ikeda *et al.*** [33]. **Gaidukov *et al.*** [34] reported that Q192 alloenzyme exhibited lowerstability, lipolactonase and modulatory effect on macrophage cholesterol efflux. **Bhattacharyya *et al.*** [35] found that R 192 polymorphism individuals have higher serum PONI activity and reduction in prevalent coronary artery disease.

The significant difference in PONI 55 genotypes in this study in PONI activity was lower in MM, intermediate in LM and higher in LL genotypes in diabetic patients and controls. These results are in agreement with those of **Garin *et al.*** [36]

The HbA1c results was higher in QQ, MM genotypes than in RR, LL genotypes respectively and patients with QR, LM genotypes had intermediate diabetic control. These results are in agreement with **Mackness *et al.*** [26] and **Flekac *et al.*** [21] who concluded that QQ and MM genotypes in diabetes is associated with poorer glucose control.

This study found that the genotype distribution of 192 polymorphism and 55 genotype are significantly different in diabetic group with vascular complications than those without vascular complications in agreement with **Altuner *et al.***.[37] .Also the allelic frequencies of both 192(Q) and 55 (M) were higher in the diabetic group with vascular complications compared to diabetic group without vascular complications. Q (97.5 VS.75%), M(60%VS.27.5%) .Also there were a lower frequency of R allele(2.5% VS.25%) and L allele(40%VS.72.5) in those with vascular complications than in group without vascular complication this was hand in hand with **Agachan *et al.*** [29]**.**

These results were in agree with **Flekac *et al.*** [21]. who support that the association of MM and QQ genotypes with poorer diabetic control and more decreased enzyme activity in angiopathy relates to the

assumption that L and R carriers might be better protected against atherosclerosis.

It is probable that Pon1 activity may be partially inactivated in the presence of oxidative stress as occurred with type 2 diabetes mellitus. In both MM and QQ genotypes had higher thiobarbituric acid reactive substances(TBARS) and lower glutathione (GSH) level, whereas genotypes of PONI RR and PONI 55 LL alleles have lower TBARS and higher GSH levels and have protective effects against oxidative stress. [38]

Conclusion

Paraoxonase activity is affected by PON1 genetic variability in type2 diabetic patients and controls. The PON1 192 RR and 55 LL genotypes are associated with higher PON1 activity than QQ and MM genotypes. Higher prevalence of QQ and MM genotypes in diabetes is associated with poorer glucose control and more common in diabetic group with vascular complications. This may suggest their essential role in occurrence of diabetic vascular complications.

Conflict of interests: none.

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References

1-Mackness B., Durrington PN., Abuashia B., Boulton AJ. and Mackness MI. (2000): Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. *Clin. Sci. 98: 355-363.*

2-Sentí M., Tomás M., Fitó M., Weinbrenner T., Covas MI. and Sala J.(2003): Antioxidant paraoxonase 1 activity in the metabolic syndrome. *J Clin Endocrinol Metab., 88:5422-5426.*

3-Primo-Parmo SL., Sorenson RC., Teiber J. and La Du B. N. (1996): The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family.*Genomics 33:498–507*

4-Hegele RA., (1999):Paraoxonase genes and disease. *Ann Med. 31(3): 217-224.*

5-Durrington PN., Mackness B. and Mackness MI.(2001): Paraoxonase and atherosclerosis. *Arterioscler Thromb Vasc Biol., 21:473*

6-Jenkins AJ., Best JD., Klien RL. and Lyonss TJ.(2004):Lipoproteins, glycoxidation and diabetic angiopathy. *Diabetes Metab Res Rev 20(5):349-368.*

7-Burstein M., Scholnick HR., and Morfin R.,(1970):Rapid method of isolation of lipoproteins from human serum by precipitation with polyanion. J Lipid Res., 11 (6): 583-595.

8-Friedewald WT., Levy RI. and Fredrickson DS.(1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem., 18: 499-502.*

9-Svendsen P.(1980):Methods used for measuring FHb concentration. *Diabetologia 19:130-136.*

10-Mackness MI., Harty D., Bhatnagar D., Winocour PH., Arrol S. and Ishola M.(1991): Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis 86:193-199.*

11-Humbert R., Adler DA., Disteche CM., Hassett C., Omiecinski CJ. and Furlong CE. (1993): The molecular basis of the human serum paraoxonase activity polymorphism .*Nat Genet 3: 73-76.*

*12-L*aakso M.(1999): Hyperglycemia and cardiovascular disease in type 2 diabetes. *Diabetes 48: 937-942.*

13-American diabetes association. (2002): Summary of revisions for the 2002 clinical practice recommendations additions to the clinical practice recommendations. *Diabetes Care 25:S3.*

14-Sakai T., Matsuura B. and Onji M.(1998): Serum paraoxonase activity and genotype distribution in Japanese patients with diabetes mellitus. *Intern Med., 37:581-584.*

15-Pfohl M., Koch M., Enderle MD., Kühn R., Füllhase J. and Karsch KR.(1999): Paraoxonase 192 Gln/Arg gene polymorphism, coronary artery disease, and myocardial infarction in type 2 diabetes. *Diabetes 48:623-627.*

16-Inoue M., Suehiro T., Nakamura T., Ikeda Y., Kumon Y. and Hashimoto K.(2000):Serum arylesterase/ diazoxonase activity and genetic polymorphisms in patients with type 2 diabetes. *Metabolism, 49: 1400-1405.*

17-Baum L., Ng HK., Woo KS., Tomlinson B., Rainer TH., Chen X., Cheung WS., Chan DK., Thomas GN., Tong CS. and Wong KS.(2006): Paraoxonase 1 gene Q192R polymorphism affects stroke and myocardial infarction risk. *Clin Biochem, 39: 191-195.*

18-Dantoine T., Debord J., Merle L. and Charmes JP.(2003): From organophosphate compound toxicity to atherosclerosis: role of paraoxonase 1. *Rev Med Interne, 24: 436-442*

19-Aviram M., Rosenblat M. and Billecke S. (1999):Human serum paraoxonase (PON1) is inactivated by oxidised low density lipoprotein

and preserved by antioxidants. *Free Radicals Biol. Med. 26: 89*

20-Letellier C., Durou MR., Jouanolle AM., Le Gall JY., Poirier JY. and Ruelland A. (2002): Serum paraoxonase activity and paraoxonase gene polymorphism in type 2 diabetic patients with or without vascular complications. *Diabetes Metab. 28 297-304.*

21-Flekac M., Škrha J., Zidkova K., Lacinova Z. and Hilgertova J.(2008): Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. *Physiol. Res. 57: 717-726.*

22-Hedrick CC., Thorpe SR., Fu MX., Harper CM., Yoo J. and Kim SM.(2000): Glycation impairs high-density lipoprotein function. *Diabetologia 43:312-320.*

23-Karabina SA., Lehner AN., Frank E., Parthasarathy S. and Santanam N. (2005): Oxidative inactivation of paraoxonase - implications in diabetes mellitus and atherosclerosi s. *Biochim Biophys Acta, 1725:213-221.*

*24-A*bbott CA., Mackness MI., Kumar S. , Boulton AJ. and Durrington PN. (1995): Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins Arterioscler Thromb Vas Biol 15:1812-1818

25-Odawara M., Tachi Y. and Yamashita K.(1997): Paraoxonase polymorphism (Gln192-Arg) is associated with coronary heart disease in Japanese non-insulin-dependent diabetic patients. *J Clin Endocrinol Metab., 82: 2257-2260.*

26-Billecke S., Draganov D. and Counsell R. (2000): Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metab. Dispos. 28, 1335–1342.*

27-Blatter-Garin MC., James RW. and Dussoix P. (1997):Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J. Clin. Invest. 99: 62–66***.**

28-Mackness B., Durrington PN., Boulton AJM., Hine D. and Mackness MI.(2002): Serum paraoxonase activity in patients with type I diabetes comparedto healthy controls. *Eur J Clin Invest., 32:259-64.*

29-Agachan B., Yilmaz H., Ergen HA., Karaali ZE. and Isbir T. (2005): Paraoxonase (PON1) 55 and 192 Polymorphism and Its Effects to Oxidant-Antioxidant System in TurkishPatients with Type 2 Diabetes Mellitus . *Physiol. Res. 54: 287-293.*

30-Fortunato G., Rubba P., Panico S., Trono D., Tinto N., Mazzaccara C., Michelem D., Iannuzzi A., Vitale DF., Salvatore F. and Sacchetti L.(2003): A paraoxonase gene polymorphism, PON 1 (55), as an independent risk factor for increased carotid intima-media thickness in middle-aged women. *Atherosclerosis 167 141-148.*

31-Maritim AC., Sanders RA. and Watkins JB.(2003): Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol.,17: 24-38, 2003.*

32-Agachan B., Yilmaz H., Karaali Z. and Isbir T. (2004): Paraoxonase 55 and 192 polymorphism and its relationship to serum paraoxonase activity and serum lipids in Turkish patients with non-insulin dependent diabetes mellitus.*Cell. Biochem. Funct. 22: 163-168.*

33-Ikeda Y., Suehiro T., Inoue M., Nakauchi Y., Morita T. and Arii K.(1998):Serum paraoxonase activity and its relationship to diabetic complications in patients with non-insulin-dependent diabetes mellitus. *Metabolism 47:598-602.*

34-Gaidukov L., Rosenblat M. and Aviram M.(2006): The 192R/Q polymorphs of serum paraoxonase PON1 differ in HDL binding, lipolactonase stimulation, and cholesterol efflux.*J Lipid Res 47(11): 2492-2502.*

35-Bhattacharyya T., Nicholls SJ. and Topol EJ. (2008): Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA. 299(11): 1265-1276*

36-Garin MC., James RW., Dussoix P., Blanche H., Passa P. and Froguel P. (1997): Paraoxonase polymorphism Met-Leu54 is associated with modified serum concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. *J Clin Invest., 99:62-66.*

37- Altuner D., Suzen S.H., Ates I., Koc G.V., Aral Y. *,and Karakaya A.(2011).*: Are PON1 Q/R 192 and M/L 55 polymorphisms risk factors for diabetes complications in Turkish population? Clin. Biochem., 44 ,372-6

38- ErgunM.A., Yurtcu E., Demirci H., IlhanM.N., BarkarV. *,Yelkin I.,Menevse A* .(2001). PON1 55 and 192 gene polymorphisms in type 2 diabetes mellitus patients in a Turkish population Biochem. Genet., 49 , 1–8.

Cloning, Characterization and Expression of Human Dentin Matrix Protein1 (DMP-1)

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Abstract: This study aimed to produce dentin matrix protein 1 (DMP-1) by cloning of DMP-1 cDNA and transfecting FS 293 cells as wellas isolation and purification of the expressed protein which would be used *in vitro* and *in vivo* dental and bone experiments. DH5α competent cells were transformed with plasmid carrying human DMP-1 gene (Trueclone, catalog No TC303479, Origene) and distributed on plates containing ampicillin then incubated at 37 °C for 24 hrs. Ampicillin resistant colony was grown in sterile glass tubes containing 5 ml LB medium and incubated at 37 °C for 16-17 hrs with vigorous shaking. DNA was then isolated using an Invitrogen miniprep kit. Recovered miniprep DNA samples were digested with NOT I enzyme (Invitrogen) to release the DMP-1 or control insert and run on 1% agarose gel. One DNA vector with the correct sequence was retransformed into DH5α *E.coli* and then grown up in large quantities for DNA maxipreps. DNA samples were digested with NOT I enzyme to release the DMP-1 or control insert and run on 1% agarose gel. FS293 cells (Freestyle ™ 293 Expression System, Invitrogen) were transfected with DMP-1 cDNA for protein expression. Partial Purification of DMP 1 was done. Conditioned medium (600 ml) was applied to a 20-ml DEAE-Sephacel (Sigma) column. Absorbance of eluted proteins was monitored at 230 nm with 2.4 ml fractions collected. Those containing DMP-1 were identified by Dot Blotting. BCA protein Assay Kit (Pierce) was used to quantify the amount of protein present in the pooled fractions.Then, protein was visualized using SDS PAGE and Western Blotting. Sodium Dodecyl Sulphate-polyacrylamide gel electrophoresis was performed using NuPAGE Novex 4-12% Bis-Tris gels (Invitrogen). Two staining techniques were used, Coomassie blue to visualize the protein bands and estimate their molecular weight. The second technique is Stains All (SIGMA) was used to differentiate between acidic glycoproteins and phosphoproteins which stain blue and other classes of proteins which stain red. Samples containing DMP1 were further purified using the Hydroxyapatite column. Dot blotting the fractions using DMP-1 antibody (LF 148 Takara inc.) revealed the presence of DMP-1 in fractions which could be used in dental and bone injuries and experiments.
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Key words: DMP1, Dentine, Bone, cDNA, cloning, transfection, DH5α competent cells.

1. Introduction:

Dentin matrix protein 1 (DMP1) is a non-collagenous protein expressed in bone and dentin (George *et al.,* 1993). It is an acidic protein rich in aspartic acid, glutamic acid, and serine residues. Most of the serine residues are phosphorylated by casein kinase II (George *et al.,* 1994; . D'Souza *et al.,* 1997; MacDougall *et al.,* 1998).

DMP1 was originally thought to be found only in dentin, but later it was also detected in bone, cartilage, and non-mineralized tissues such as the brain, pancreas, kidney, and salivary glands (George *et al.,* 1995; Begue-Kirn *et al.,* 1998; Feng *et al.,* 2003; Ogbureke and Fisher 2004, 2005, 2007).

DMP1 is a multifunctional protein that has been found to regulate cell attachment to the extracellular matrix (Kulkarni *et al.,* 2000)) and cell differentiation (Narayanan *et al.,* 2001; Kalajzic *et al.,* 2004) to activate matrix metalloproteinase-9,(Fedarko *et al.,* 2004) and has been postulated to play a significant role in biomineralization.(Ye *et al.,* 2004) as well as having a role in expressing osteocalcin, alkaline phosphatase and DSPP determined by over expression studies in mesenchymal stem cells (Narayanan *et al.,* 2001). DMP1 is also involved in calcium and phosphate metabolism through the kidney.(Terasawa *et al.,* 2004).

DMP1 is highly acidic, a property that is necessary for calcium binding because it provides a microenvironment for mineral precipitation (Qin *et al.,* 2003 and Gericke et al., 2010).

In vitro, DMP1 acts as a hydroxyapatite (HA) crystal nucleator with very high calcium ion binding capability (He *et al.,* 2003). It plays an active role in nucleating the initial calcium phosphate crystals during the initial stages of dentine and bone formation. (Hao *et al.,* 2004).

DMP-1 is a low abundance protein which is difficult to characterize and it plays a role in the formation of all mineralized tissues during their development (Feng *et al.,* 2003).

It may also be involved in the regulation of phosphate homeostasis through fibroblast growth factor 23 (FGF23), a newly identified hormone that is released from bone and targeted in the kidneys; deletion of the *Dmp1* gene leads to a dramatic increase of FGF23 mRNA in osteocytes (Feng *et al.,* 2006).

The importance of DMP1 in biomineralization has been shown through mice and human genetic studies. In mice, a lack of DMP1 results in poor mineralization of bone and dentin, whereas mutations in the DMP1 gene in humans result in osteomalacia (Ye *et al.,* 2004, 2005; Feng *et al.,* 2006). Interestingly, DMP1 expression was also observed in malignant tumor cells (Fedarko *et al.,* 2001; Fisher *et al.,* 2004; Ogbureke *et al.,* 2007).

It has also been implicated in the transcription activity of other dentine specific matrix genes like DMP-2 (Narayanan *et al.,* 2003) and DSPP (Narayanan *et al.,* 2006) the candidate gene for dentinogenesis imperfecta TYPE II and III genetic disorders.

The extracellular matrix (ECM) of bone and dentin contains fragments originating from intact DMP1, namely, a 37-kDa fragment from the NH2-terminal region and a 57-kDa fragment from the COOH-terminal region of the DMP1 amino acid sequence (Qin *et al.,* 2003). NH2- terminal fragment of DMP1 in the ECM of bone and dentin also occurs as a proteoglycan (Qin *et al.,* 2006). The proteoglycan variant, referred to as DMP1-PG, possesses a single glycosaminoglycan side chain linked to the core protein via Ser74 in the rat DMP1 amino acid sequence.

In vitro mineralization studies have demonstrated that the COOH-terminal fragment promotes mineralization by acting as a nucleator for hydroxyapatite formation (Tartaix *et al.,* 2004; He *et al.,* 2005; Gajjeraman *et al.,* 2007; Gericke et al., 2010). Information regarding the biological functions of the NH_2-terminal fragment and DMP1-PG is lacking.

When DMP1 applied in situ, it induces differentiation of dental pulp stem cells into odontoblasts (Almushayt, 2006).

The aim of our study was to produce DMP-1 protein by transfecting FS 293 cells with DMP-1 cDNA which would then be used *in vitro* and *in vivo* dental and bone injuries and experiments.

2. Materials and Methods:

Materials:

1. Competent DH5α *E.coli* (Invitrogen)
2. Human cDNA clone (Trueclone, catalog No TC303479, Origene)

Accession No: NM_004407.1. Homo sapiens dentin matrix acidic phosphoprotein (DMP1). Vector: pCMV6-Neo Vector size ~ 5.8 kb Insert size (Not1 digest): 1.5 kb.

3. All chemicals for which source is not noted were of analytical grade from Sigma, Fluka, and BDH.

Methods:

1. Reparation of the plates

1%Bacto-Tryptone 10 gm
0.5%Yeast Extract 5 gm
1%Sod.Chloride 10 gm
1.5% Bacto Agar 15 gm

Dissolve in 900 ml dd water and adjust pH to 7.5 with NaOH. Complete the volume to 1000 ml. Autoclave for 25 min. Cool to about 50 ^{o} C before adding antibiotic (1000 µl of 1000x stock /litre).

Note: make sure that the temperature of the solution is about room temp. before adding antibiotic to prevent its destruction.

2.TAE Buffer

Trizma base 48.4 gm
G. Acetic Acid 11.42 ml
Di Na EDTA 7.44 gm

To be dissolved in 1000 ml d. water.

3. LB Media

Trypticase Peptone 10 gm
Yeast Extract 5 gm
NaCl 10 gm

Dissolve in 900 ml dd water and adjust pH to 7.5 with NaOH. Then complete the volume to 1000 ml and autoclave for 35 min.

4.2X YT Media

Tryptone 16 gm
Yeast Extract 10 gm
NaCl 5 gm

Dissolve in 900 ml d. water and adjust pH to 7.4 using NaOH. Then complete the volume to 1000 ml autoclave for 35 min.

5. Antibiotic – Final concentration

Ampicillin 100 µg/ml.

Make 100x stock in water and sterilize through 0.22 µ filter into microfuge tubes.

Store aliquots at -20 ^{o} C

Transformation of DH5α competent cells

Plasmid carrying human DMP-1 gene (Trueclone, catalog no TC303479, Origene) was resuspended in 10 µl deionised water and incubated for10 min at room temperature.

1. Thaw DH5α (commercially competent *E. coli* cells) on ice.
2. Thaw DNA on ice
3. Warm selected antibiotic containing plates (face down) at room temperature for 45 min.
4. To one tube (50 µl) of copetent cells add 0.5 µl plasmid DNA. Incubate on ice for 30 min.
5. Shock at 37^{o} C for 90 sec.

6. Put back into ice for 2 min. and add 100 µl of YT to give 150 µl.
7. To one plate add 10 µl of the above mixture and 40 ul of YT.
8. Add 6 sterile glass beads and rotate for 1 minute until the liquid is gone.
9. Repeat this step in another plate using 25 µl of DNA and 25 µl of YT.
10. Incubate at 37 ^{o} C overnight.

Plasmid DNA Purification (Miniprep).

1. Set up 4 sterile glass tubes, two for each plate.
2. Put 5 ml of LB into each sterile glass tube with lid.
3. Add appropriate antibiotic, 50 µl (100x ampicillin).
4. Using sterile yellow tip pick a single colony from plate and put in the tube.
5. Put the tubes in shaking water bath with vigorous shaking at 37^{o}C for 16-17 hours. .

DNA was then isolated using **Purelink Quick Plasmid Miniprep Kit (Invitrogen Inc.)** according to the manufacturer instructions. This method is comprised of three steps, DNA isolation, DNA binding and DNA elution.

N.B. We used 50 µl sterile water instead of TE buffer for elution of DNA from the column because TE buffer interferes with DNA sequencing and restriction digestion.

Quality assessment of the cDNA: Recovered miniprep DNA samples along with the sample supplied by Origene (as control) were digested with NOT I enzyme (Invitrogen) to release the DMP-1 or control insert.

Restriction digestion mixture: 5.5 ul water , 3 µl DNA, 1 µl 10x buffer (reaction buffer #3) and 0.5 µl enzyme Not 1. Incubate the mixture at 37 ^{o}C for 2 hours in a water bath. Add 1 ul dye to the mixture after digestion.

DNA Electrophoresis: 1% agarose gel electrophoresis was run to the intact DNAs (control) and NOT I digests to confirm the expected insert size. The DNA ladder (Qiagen) was used as a standard. The gel was stained with Ethidium Bromide and visualized by UV light. A picture was taken for records.

DNA Sequencing: One sample of the DMP-1 DNA was sent the sequencing Lab (Department of Biochemistry, University of Alberta) using forward and reverse primers supplied by Origene.

Plasmid DNA purification (Maxiprep)

Transformation of DH5α competent cells: One DNA vector with the correct sequence was retransformed into DH5 α E.coli. and then grown up in large quantities for DNA maxipreps.

I. First day

1. Set up 4 sterile glass tubes, two for each plate. 2. Put 5 ml of LB into sterile glass tubes with lid.
3. Add appropriate antibiotic (50 µl amp.)
4. Using sterile yellow tip pick a single colony from plate and put in the tube.
5. Put the tubes in shaking water bath for 7 – 8 hours at 37 ^{o}C with vigorous shaking.
6. At 4 pm add 100 ml sterile LB to sterile 500 ml flask, add 100 µl 1000x antibiotic stock. Mix, then add 100 µl of starter culture.
7. Grow over night at 37^{o}c in water bath with vigorous shaking.

II. Second day

1.Turn on Sovall centrifuge and cool down GSA rotor.
2. Transfer bacterial culture to sterile GSA rotor bottle and centrifuge for 30 minutes at 3500 rpm. 3. Pour off supernatant gently and discard.

N.B. Make sure that all supernatant has been drained.

DNA was then isolated using **Purelink Quick Plasmid Maxiprep Kit (Invitrogen Inc.)** according to the manufacturer instructions. This method is comprised of three steps, DNA isolation, DNA binding and DNA elution.

N.B. Air dry DNA pellet for 5-10 min. Redissolve DNA in 400 ul sterile water .

Determine the recovered DNA concentration by adding 5 µl in 495 µl water and read absorbance at 260 nm.

The recovered maxiprep DNA samples along with a sample of miniprep (as control) were digested with NOT I enzyme (Invitrogen) to release the DMP-1 or control insert (as done in miniprep), quantified and stored at -22 ^{o}C.

DNA yield from maxiprep

Sample # 1	1.55 ug/ul DNA
Sample # 2	1.12 ug/ul DNA
Sample # 3	0.88 ug/ul DNA
Sample # 4	0.98 ug/ul DNA

DNA Electrophoresis

1% agarose gel electrophoresis was run to the intact DNAs (control) and NOT I digests to confirm the expected insert size. The DNA ladder (Qiagen) was used as a standard. The gel was stained with Ethidium Bromide and visualized by UV light. A picture was taken for records.

3. FS293 cells (Freestyle ^{TM} 293 Expression System, Invitrogen) transient transfection for protein expression:

FS293 cells were grown in suspension culture according to the manufacturer's protocol (Invitrogen). The viable cell number was determined using Trypan Blue. The required number of cells were transferred from the shaker flask into 50ml sterile tube then centrifuged at 1000rpm for 5 minutes. The medium was discarded and the cells resuspended at a known concentration in FS Expression medium. The cells are vortexed vigorously for 10-30 seconds to break up clumps. Because optimal transfection requires single

cell suspension. The volume of the cell suspension needed is transferred to a sterile shaker flask. FS expression medium was added so that the cell density was approximately 1.1 x 10^6 viable cells / ml. The flask was then placed in the incubator on the shaker.

Initially, a small scale (30 ml) transfection is done to check if the protein is expressed.

A. 30µg plasmid DNA in Opti-MEM I was diluted to a total volume of 1ml and mixed gently.
B. 40µl of 293fectin in Opti-MEM I was diluted to a total volume of 1ml (added to 960µl in a sterile 15ml tube) and mixed gently then incubated for 5 minutes at RT.
C. The diluted DNA was added to the diluted 293fectin to obtain a total volume of 2ml, mixed gently, and then incubated for 30 minutes at RT to allow the DNA-293fectin complexes to form.
D. While the DNA-293fectin complexes were incubating, the cell suspension was removed from the incubator and 28 ml of suspension was aliquoted into each sterile 125ml flask. After the complex formation was complete, the 2ml were added to the shaker flask where each flask will have a 30ml volume with a final density of approximately1x10^6 viable cells/ml.
E. The complex was then incubated at 37^{o}C at 8% CO_2 on the shaker and the cells/medium was harvested 96 hours later. SDS-PAGE blot was performed on the supernatant to confirm the expression of DMP-1 and consequently a large scale (1000 ml) transfection was performed.

4. Partial Purification of DMP 1:

Conditioned medium (600 ml) was applied to a 20-ml DEAE-Sephacel (Sigma) column equilibrated in 0.05 M Tris-HCl, 0.01M NaCl, pH 7.4 (Tris buffer) at 10^{o}C at a flow rate of 53 ml/hr. The column was washed with buffer and then eluted with a linear gradient of 0.01-0.8 M NaCl in Tris buffer. It was washed with 2x30 ml 0.8M Nacl/Tris to ensure all bound proteins were removed. Absorbance of eluted proteins was monitored at 230 nm with 2.4 ml fractions collected. Those containing DMP-1 were identified by Dot Blotting.

5. Dot blotting the fractions:

10 µl of the DEAE fractions were applied to a Biorad Dot Blot apparatus on to a Nitrocellulose membrane. The dry membrane was soaked in PBS until wet and blocked with 4 % non fat milk dissolved in PBS/Tween 20 for 30-45 minutes at RT with shaking. The primary antibody (LF 148 of Takara Inc.) was then diluted to the appropriate concentration in PBS/Tw, added to the paper and was left 1-2 hours at room temperature on the shaker. After washing 4x4 with PBS/Tw. The secondary anti-rabbit IgG alkaline phosphatase conjugated antibody (Sigma) was diluted 1:2500 in PBS/Tw, and added to the paper. It was left for 30-90 minutes on shaker, washed 3 x 4 minutes with PBS/Tw. Proteins were visualized with BCIP/NBT (Roche). It was washed several times with water and then air dried. Based on the results of the dot blotting, the Antibody positive fractions containing DMP 1 were pooled and concentrated using Amicon Ultra-15 centrifugal Filter Devices (Millipore 10,000 NMWL) by centrifugation at 3750 rpm until the volume came down to 500-1000µl. The retentate was transferred to a pre-weighed microfuge tube and weighed.

6. *BCA* (bicinchoninic acid) *protein Assay Kit* (Pierce, Rockford, IL, USA) was used to quantify the amount of protein present in the pooled fractions.

7. Protein visualization using SDS PAGE and Western Blotting:

Sodium Dodecyl Sulphate-polyacrylamide gel electrophoresis was performed using NuPAGE Novex gradient 4-12% Bis-Tris gels (Invitrogen). Two staining techniques were used, Coomassie blue to visualize the protein bands and estimate their molecular weight. The gels were washed using 10% methanol for 5 minutes twice, then the stain was added and it was placed on a shaker at room temperature for one hour. Then destaining procedure was done using 10% acetic acid and 5% methanol. The gels were washed with distilled water and scanned.

The second technique is Stains All (SIGMA) was used to differentiate between acidic glycoproteins and phosphoproteins which stain blue and other classes of proteins which stain red. The staining solution is composed of 0.005% Stains All, 10% formamide, 25% isopropanol, 15 mM Trizma, 65% H_2O, HCL is added -pH 8.8. This mixture was kept in the dark for several hours. The gels were then washed with distilled water and scanned.

Following SDS-PAGE, the gel is prepared for electroblotting using a standard tank transfer. The proteins were transferred to PVDF membrane (MilliPore) using 32 volts for 1-1.5 hrs. Transferred proteins were visualized as for dot blots.

7. Final purification using HA column:

Equilibrate a 2ml Hydroxyapatite column (CHT Ceramic Hydroxyapatite, Bio-Rad) with approximately 10 column volumes of buffer A (10mM Na phosphate pH 6.8). Prepare the sample, adjusting the pH and conductivity to those of buffer A. The sample was diluted with buffer A containing 0.3 mM $CaCl_2$, then applied to the column. Wash the column with approximately 5 column volumes of buffer A, unbound material was allowed to pass

through the column. The bound proteins were eluted with approximately 20 column volumes of buffer B (10mM Na phosphate pH 6.8) linear gradient of increasing concentration of phosphate buffer (10-700 mM). After elution clean the column with approximately 5 column volumes of buffer B followed by a sanitation step using approximately 5 column volumes of 1 M NaOH. The same procedure for evaluation of fractions was performed.

3. Results:

Quality assessment of the cDNA product:

Purified full length miniprep DNA and the Not I restriction enzyme digestion were separated by agarose gel electrophoresis where their sizes compared to the DNA ladder (Quiagen).

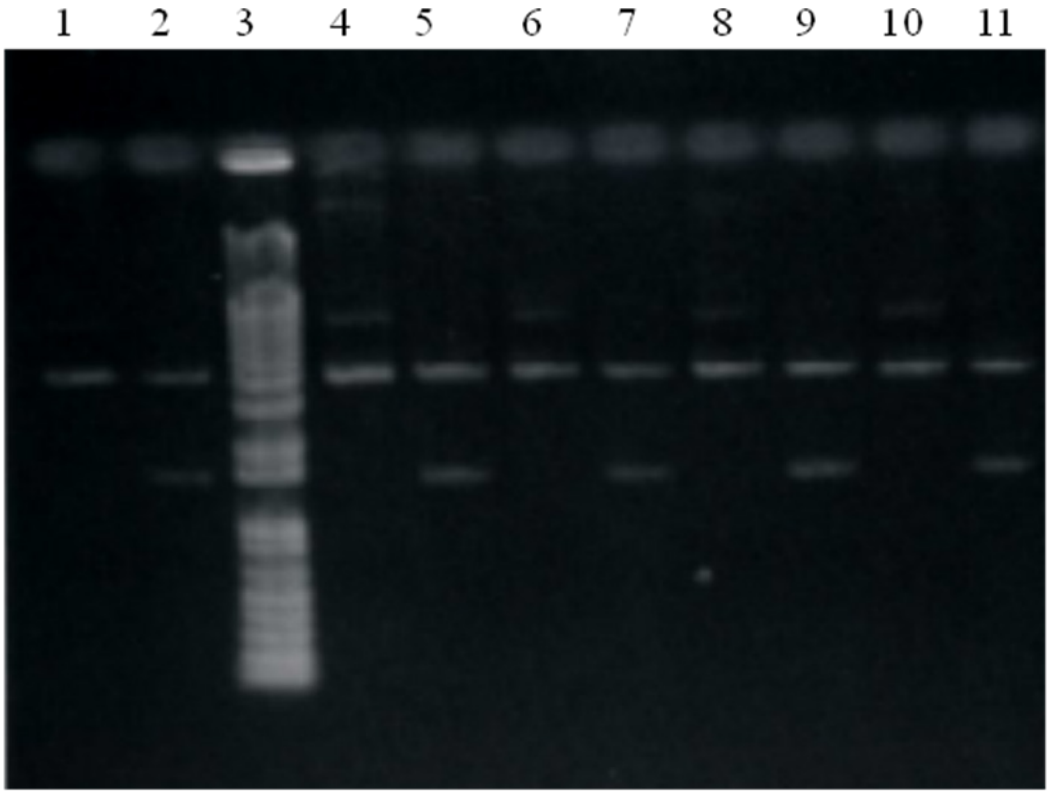

Fig (1): Miniprep products on 1% agarose gel when photographed on ultraviolet lamp using Kodak (camera) and scanned (Epson 1.3) showing lane 1 and 2 represent the intact control sample and its digest , lane 3 represent DNA ladder, lanes 4,6,8 and 10 represent the intact cDNA of DMP-1 while lanes 5, 7,9 and 11 represent the digest of the same samples. Each lane and its digest represent one different single colony. It is clearly obvious the positions of the bands are at the same level mentioned by the manufacture (5.8 and 1.5 kb) which indicates the success of the cloning procedure.

DNA minipreps sequencing:

DNA minipreps of the recombinant plasmid containing DMP1 insert were characterized by DNA sequencing in the sequencing Lab (Department of Biochemistry, University of Alberta, Canada) using forward and reverse primers supplied by Origene. The obtained DNA sequence was aligned with Human cDNA clone. Accession No: NM_004407.1. Homo sapiens dentin matrix acidic phosphoprotein. As seen in (Fig.3a,5'→3' seq. &3b, 3'→5'seq.)

5′→3′ sequence Frame 2

```
>ref|NM_004407.1|  Homo sapiens dentin matrix acidic phosphoprotein (DMP1), mRNA
Length=2682

 Score = 1229 bits (665),  Expect = 0.0
 Identities = 717/740 (96%), Gaps = 12/740 (1%)
 Strand=Plus/Plus

Query  125  ATGAAGATCAGCATCCTGCTCATGTTCCTTTGGGGATTATCCTGTGCTCTCCCAGTAACC  184
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Query  185  AGGTATCAAAATAATGAATCTGAGGATTCTGAAGAATGGAAGGGTCATTTGGCTCAGGCA  244
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Sbjct  160  AGGTATCAAAATAATGAATCTGAGGATTCTGAAGAATGGAAGGGTCATTTGGCTCAGGCA  219

Query  245  CCAACACCACCCTTGGAGAGCAGTGAGTCATCAGAAGGCAGTAAAGTTAGCTCAGAGGAA  304
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Sbjct  220  CCAACACCACCCTTGGAGAGCAGTGAGTCATCAGAAGGCAGTAAAGTTAGCTCAGAGGAA  279

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Sbjct  280  CAGGCAAATGAAGACCCCAGTGACAGCACTCAGTCAGAGGAGGGCCTGGGCTCTGATGAT  339

Query  365  CATCAATACATTTATAGGCTAGCTGGTGGCTTCTCCAGGAGCACAGGAAAAGGAGGAGAT  424
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Query  425  GATAAAGATGACGATGAAGATGACAGTGGAGATGACACCTTTGGTGACGATGACAGTGGC  484
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Query  485  CCAGGGCCCCAAAGACAGACAAGAAGGAGGAAACTCCAGACTGGGAAGTGATGAGGACTCT  544
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Query  545  GATGACACCATACAAGCCAGTGAAGAGAGTGCCCCACAAGGG-CAAGACAGTGCCCAAGA  603
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Query  604  TACCACCAGTGAGAGCAGGGAACT-GACAATGAGGACCGGGTGGACAGCAAGCCTGAGGG  662
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Query  839  AG-GAATC-GG-GAAAACAG  855
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Sbjct  817  AAAGAATCTGGAGAAAACAG  836
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3′→5′ sequence Frame 3

```
>ref|NM_004407.1|  Homo sapiens dentin matrix acidic phosphoprotein (DMP1), mRNA
Length=2682

 Score = 1219 bits (660),  Expect = 0.0
 Identities = 730/761 (95%), Gaps = 20/761 (2%)
 Strand=Plus/Minus

Query  154   GACAGCTGATGCTAATAGCCGTCTTGGCAGTCATTGTCATCTTGGTCCCCAATGGGTTTG  213
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Sbjct  1652  GACAGCTGATGCTAATAGCCGTCTTGGCAGTCATTGTCATCTTGGTCCCCAATGGGTTTG  1593

Query  214   TTGTGATAGGCATCAACTGTTAATTTCCGGCTCTCTATCTCAATGTTTTTCAACTGGCCA  273
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Sbjct  1592  TTGTGATAGGCATCAACTGTTAATTTCCGGCTCTCTATCTCAATGTTTTTCAACTGGCCA  1533

Query  274   TCTTCCTCACTGCTTGATTTGCTCTCCGTGGAGTTGCTATCTTCTTTGGATCTGCTGCTG  333
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Query  334   TCTTGAGAGTCACTGTCGTCTTCCTCAGAATGGCTTTCCTCGCTCTGACTCTCTGCTGAG  393
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Query  394   CTGCTGTGAGACTGGAGGCCCTCCTGGCTGGAGCTGTTCTCATCCTCAGGGGACTCCGGG  453
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Query  454   CTTTCCTCTGAGAAGTTGAGGCTCTCACTGGATTCGCTGTCTGCTTGCTCCTCTCTGGAT  513
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Query  514   TCACTTTTTGAGTGGGAGAGTGTGTGCGAGCTGTCCTCCTCGCTGGAGTCACTGTCTTCC  573
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Sbjct  1292  TCACTTTTTGAGTGGGAGAGTGTGTGCGAGCTGTCCTCCTCGCTGGAGTCACTGTCTTCC  1233

Query  574   TGGTCTTCTACATAACTAGTTGTGGGGTCGGGGTTATCTCCCCTGGACTCACTCACCACC  633
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Query  805   TTTTCTGTA-AGCTC-C-CTTGACCTCT-CCATTGTGGTGTTGNCATCACGCTC-CTTCT  859
             ||||||||| || || | |||||| ||| |||||||| ||||| ||||| |||||||||||
Sbjct  992   TTTTCTGTAGAG-TCACTCTTGACTTCTTCCATTGTGTTGTTGTCATCAAGCTCGCTTCT  934

Query  860   GTCATCTTCC-CTGAAAT-C-AGAACTTCCTAAAA-TTTTC  896
             |||||||||| |||| || | ||| |||||||||| |||||
Sbjct  933   GTCATCTTCCTCTGAGATGCGAGA-CTTCCTAAAAATTTTC  894
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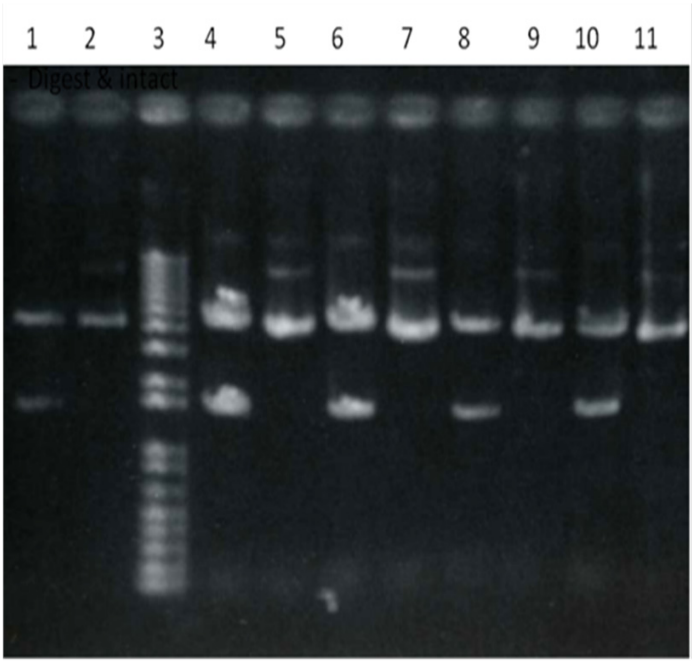

Fig (2): Maxiprep products on 1% agarose gel when photographed on ultraviolet lamp using Kodak (..) and scanned (Epson 1.3) showing lane 2 and 1 represent the intact control sample and its digest , lane 3 represent DNA ladder, lanes 5,7,9 and 11 represent the intact cDNA of DMP-1 while lanes 4, 6,8 and 10 represent the digest of the same samples. Each lane and its digest represent one different single colony. It is clearly obvious the positions of the bands are at the same level mentioned by the manufacture (5.8 kb and 1.5 kb) which indicates the success of the cloning procedure.

Evaluation of the transfection procedure

Western blotting was used to evaluate the conditioned medium collected 96 hours post transfection 20µL (DMP-1) medium and the control (E.V.) medium were blotted into PVDF membrane. The takara® antibody (Fig. 4) was used for immunostaining the conditioned medium from the DMP-1 transfection and showed bands in consistent with the position of the DMP-1 (~57 kda).Medium of the control group didn't show any bands indicating successful transfection and secretion of the DMP-1 protein.

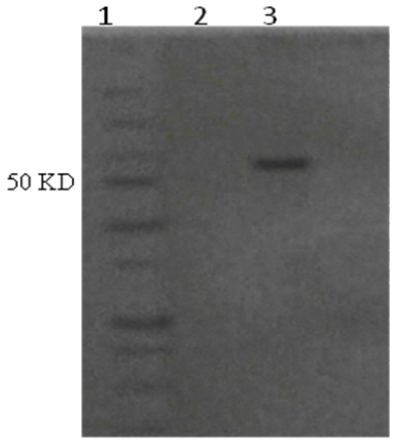

Fig (4): The scanned blotted PVDF membrane using the DMP-1 antibody, showing the presence of DMP-1 (lane 3) in the conditioned medium post transfection while the absence of this protein in the control E.V. group (lane 2). Lane 1 contains protein standard (Bio Rad).

Purification using DEAE column:

The DEAE column was connected to the holochrome monitor 230 nm and 0-2 A. 85 fractions were produced from 600 ml of the conditioned medium. Each fraction contains ~2.4ml of protein in 0.05 Tris. The chromatography showed two peaks: the first from fractions (#1) 10-30 while the second (#2) was from fractions 33-65. Fig (5).

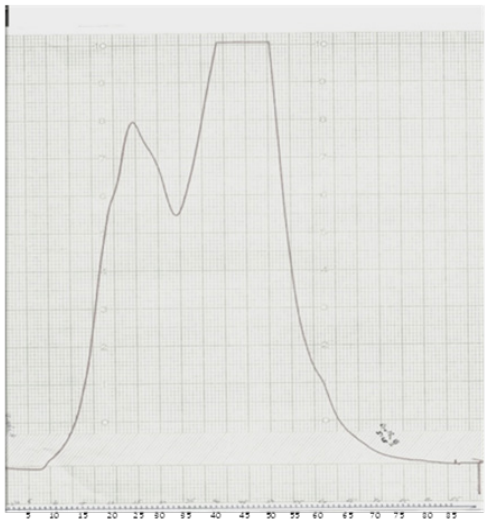

Fig (5): The DEAE elution profile of proteins.

Dot Blotting the fractions using *Takara* Antibody was performed in order to exclude all non DMP-1 containing fractions. Fig (6).

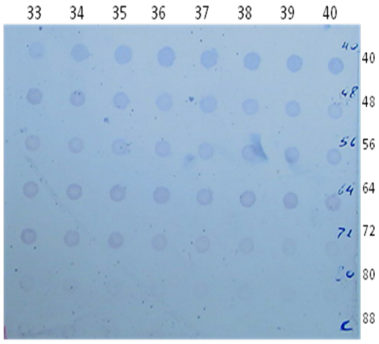

Fig (6): Positive fractions were from number 33 to 68 as seen on the nitrocellulose membrane after blotting the fractions using DMP-1 antibody (LF 148 – Takara inc.)

BCA protein assay was performed to quantify the concentration of protein available in these samples. Table (1) showing the alignment of the samples in response to the standard (D1-E3) with a gradual increase of 1 µl each three wells and the samples (A4-F5) of 2 µl each in three wells each sample. These data gave the concentration µg/2µl. The concentration (µg/µl) was calculated and tabulated in table (2).

Table 1: Calculated concentrations sheet (Raw data of BCA protein assay)

	1	2	3	4	5
A	EMPTY	5.061	9.694	8.109	4.360
B	EMPTY	6.067	9.084	7.316	4.329
C	EMPTY	5.945	11.370	9.115	4.055
D	2.653	5.091	11.827	13.656	1.038
E	2.562	8.353	11.492	12.772	1.312
F	1.891	7.987		14.448	1.495
G	6.250	9.724		EMPTY	EMPTY
H	4.878	9.785		EMPTY	EMPTY

Table 2: Concentration of proteins in samples 1- 4

Sample number	Concentration (µg/µl)
1	4.0895
2	6.607
3	2.135
4	0.587

Two SDS pages were run and stained with Commassie blue (Fig 7) to visualize acidic proteins which stain blue and Stains All (Fig 8) to differentiate between glycoprotein and phosphoprotein which stain blue and other proteins which stain red. Our targeted protein is an acidic phosphoprotein, that's why these two types of stains were chosen, where the results indicated according to the estimated molecular weight, its presence on both types of stains but with a higher concentration in sample 2.

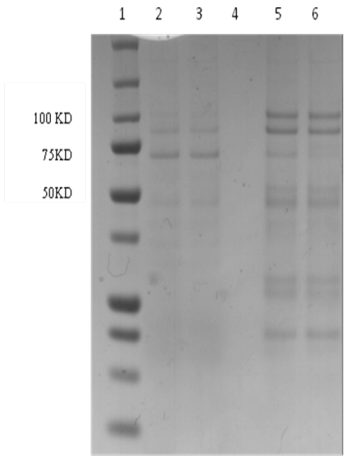

Fig (7): A photograph showing SDS page stained using Commassie blue. Lane 1 standard (Bio-Rad) lane 2 sample 1, lane 3 sample 2, lane 4 was left empty, lane 5 sample 3 and lane 6 sample 4. Note that the presence of a stain at the level from 50-75 KD in samples 1,2 only.

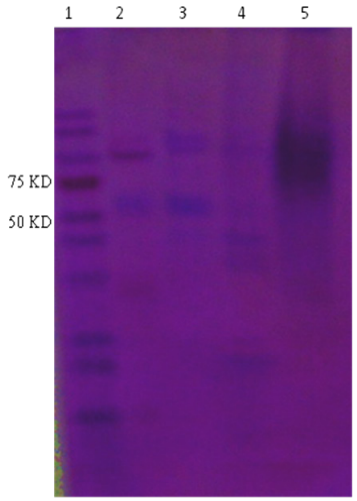

Fig (8): A photograph showing SDS page stained using Stains All. Lane 1 standard (Bio-Rad) lane 2 sample 1, lane 3 sample 2, lane 4 sample 3 and lane 5 sample 4. Note that the presence of a blue stain at the level from 50-75 KD in samples 1,2.

Purification using Hydroxyapatite column: Samples 1 and 2 containing DMP1 were further purified using the Hydroxyapatite column .

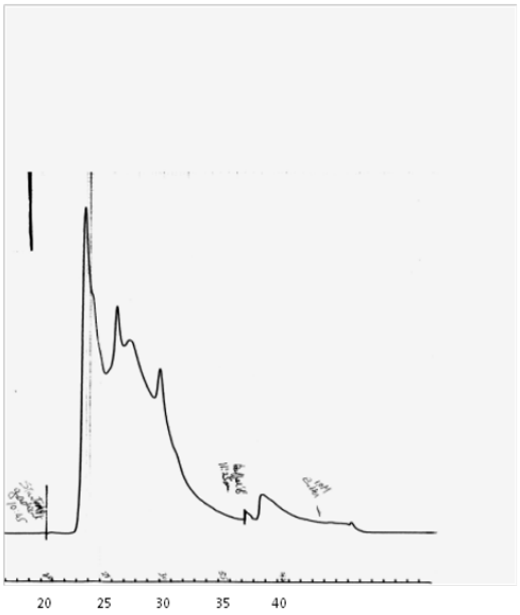

Fig (9): represents the protein in question relative to the 25-34 fractions.

Dot blotting the fractions (Fig 10) using the *Takarra* Antibody revealed the presence of DMP-1 in fractions (26-34).

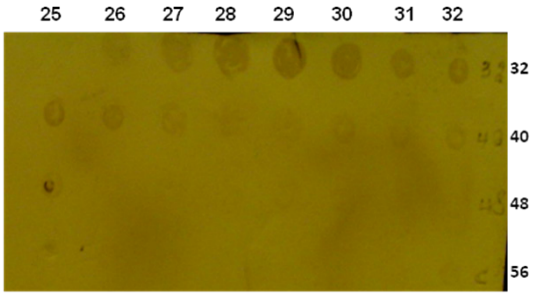

Fig (10): Nitrocellulose membrane of the eluted fractions from the HA column, notice the dots in response to the reaction to the DMP-1 antibody (LF 148 Takara inc.) in 26th to 34th fraction.

4. Discussion:

Dentin matrix protein-1 (DMP1), a member of the Small Integrin-Binding Ligand, N-linked Glycoprotein (SIBLING) family of extracellular matrix proteins (Qin *et al.*, 2003), occurs predominantly as (1) a 37K N-terminal fragment, (2) a 57K C-terminal fragment, and (3) glycosaminoglycans (mainly chondroitin 4sulfate) are linked to the NH_2-terminal 37-kDa fragment of DMP1 via Ser^{74}, located in the Ser^{74}-Gly^{75} dipeptide and referred to as the dentin matrix protein-1 proteoglycan fragment (Qin *et al.,* 2006).

DMP1 was originally thought to be found only in dentin, but later it was detected in bone, cartilage, and non-mineralized tissues such as the brain, pancreas, kidney, and salivary glands (George *et al.,* 1995; Begue-Kirn *et al.,* 1998; Feng *et al.,* 2003; Ogbureke and Fisher 2004,2005,2007).Non-human DMP-1 has been cloned and sequenced from a number of animals (George *et al.,* 1993, Hirst *et al.,* 1997a, Macdougall *et al.,* 1998 Toyosawa *et al.,* 2000) as well as human DMP-1 (Hirst *et al.,* 1997b) .

DMP1 is a multifunctional protein that has been found to regulate cell attachment (Kulkarni *et al.,* 2000), cell differentiation (Narayanan *et al.,* 2001 and Kalajzic *et al.,* 2004) to activate matrix metalloproteinase-9,(Fedarko *et al.,* 2004) and has been postulated to play a significant role in biomineralization (Ye *et al.,* 2004). *In vitro*, DMP1 acts as a hydroxyapatite (HA) crystal nucleator with very high calcium ion binding capability (He *et al.,* 2003). DMP1 also is involved in calcium and phosphate metabolism through the kidney (Terasawa *et al.,* 2004 and Gericke et al., 2010).

Recombinant bacterial DMP1 has been used in several studies (Kulkarni *et al.,* 2000, Narayannan *et al.,* 2003, Fedarko *et al.,* 2004), while to our knowledge no one produced it from transfection of a eukaryotic cell line. The FS 293 cells are human embryonic kidney cells that have been adapted

(Graham *et al.,* 1977) to serum free suspension culture producing high levels of protein. (*Invitrogen* Catalogue, 2002). Western blotting for the conditioned medium of this transfected cell line revealed the presence of DMP-1 which indicated the success of the transfection procedure while DMP-1 was not produced by cells transfected with the empty vector). Two types of transfection are commonly known, the permanent one (stable transfection) and the transient type. The stable transfection produce a consistent amount of protein while in the transient one, the level of protein is unknown. The consistent protein release from the stably transfected cell line (HT1080 unpublished data) can be used as co-culture in cell culture experiments. Our results indicated the success of both types of transfection and their consistency.

Column chromatography is one of the most common methods of protein purification. The acidic nature of the DMP-1 making it easily isolated using the DEAE column. Further purification of DMP-1 containing fractions detected by antibody staining was performed using a hydroxyapatite column and the results were in accordance to another study which used the same type of column in purifying the DSP. (Yamakoshi *et al.,* 2005)

Two types of stains were used to stain the SDS gels, the first was the Comassie Blue which detects proteins by their visualization as blue bands. The second stain was Stains All which differentiates between blue bands representing acidic glycoprotein and phosphoproteins (DMP-1), and red bands representing other proteins. (Kim *et al.,* 2006)

DMP1 is present in bone and dentin as proteolytically processed fragments; those are the 37-kDa fragment from the NH2-terminal portion and a 57-kDa fragment from the COOH-terminal region (Qin *et al.,* 2003). Cleavage of rat DMP-1 at the NH2 terminal leaves four aspartic acid residues. This cleavage pattern is similar to that of Dentin Sialo-Phosphoprotein (DSPP) where the fragment having two aspartic acid residues at the NH2 terminal is dentin sialoprotein DSP, and that from the COOH terminal is dentin phosphoprotein (Qin *et al.,* 2001). These cleavage explain the different forms observed for the DMP-1 using two types of antibody (Dr Larry Fisher's and Takara's inc.). The (57-KDa) fragment was seen in all our fractions using both types of Antibody. According to studies of in vitro mineralization, the COOH-terminal 57-kDa fragment has been shown to promote mineralization by acting as a nucleator for the formation of hydroxyapatite (Tartaix *et al.,* 2004; Lu et al., 2009). The existence of different forms is also supported by its presence at (95-KDa) as reported by Yu *et al.* (2006).

Further results obtained from the DEAE column seemed to contain a GAG chain which is in consistent with Yamakoshi *et al.,* (2005) who found this type of chain in porcine DSP, a similar acidic glycoprotein extracted from dentin. The availability of these chains may promote the hydration of the tooth as well as facilitate the molecular interaction between the different growth factors during dentin mineralization (Yamakoshi *et al.,* 2005).

It has been reported that in addition to its direct role in the formation and/or growth of hydroxyapatite crystals, DMP1, acting as a transcription factor, may be involved in regulating other genes associated with dentinogenesis and osteogenesis (Narayanan *et al.,* 2003 and Narayanan *et al.,* 2006).

DMP1 is highly expressed in the osteocytes embedded in bone matrix, and is associated with maintenance of the lacunarcanalicular system of these cells (Toyosawa *et al.,* 2001; Rios *et al.,* 2005; Feng *et al.,* 2006). DMP1 was increased in osteocytes in loaded bone, perhaps functioning in the mechanical response (Gluhak-Heinrich *et al.,* 2003; Yang *et al.,* 2005; Foster *et al.,* 2012). DMP1-null background resulted in osteomalacia, abnormal osteocyte maturation. The biological activity of DMP-1 in osteocyte maturation is mediated by its 57-kDa COOH-terminal fragment (Lu *et al.,* 2011).

DMP1 possesses endogenous biological activity that can be released by proteolytic cleavage. Matrix metalloproteinases (MMPs), which are able to cleave DMP1 into peptides of various molecular sizes, represent good candidates for regulating the function of extra cellular matrix (ECM) molecules during dentin development or during pulp repair after an injury such as carious decay. In addition, *in vivo* confirmation of the capacity of the C-ter peptide to promote cell differentiation opens a therapeutic interest of using this peptide to regenerate dentin after an injury. (Chaussain *et al.,2009).*

It was reported that exogenous DMP1 added to exposed dental pulp could act as a morphogen trigger and/or promoter of the differentiation of undifferentiated ectomesenchymal cells in the pulp toward the odontoblast lineage (Narayanan *et al.*, 2006).

Furthermore, it was reported that DMP1 is primarily localized in the nuclear compartment of undifferentiated osteoblasts, implying that DMP1 could act as a

transcriptional component for the activation of osteoblast/odontoblast-specific genes, like osteocalcin (Narayanan *et al.*, 2003). Qin *et al.* (2006) studies has revealed the role of DMP-1 as a key player in the control of mineralization and Pi homeostasis, hence, its importance in osteogenesis and dentinogenesis.

The produced DMP1 protein will be used in dental and bone injuries. DMP1 protein osteogenic and dentinogenic effects will be the main goal in our future *in vivo* and *in vitro* studies which will shed new light on the manner in which DMP1 controls osteogenesis and dentinogenesis in both healthy individuals and those with disease.

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References

1. Almushayt A, Narayanan K, Zaki AE, George A (2006): Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts. Gene Ther., **13**: 611-620.
2. Begue-Kirn C, Krebsbach PH, Bartlett JD, Butler WT (1998): Dentin sialoprotein, dentin phosphoprotein, enamelysin and ameloblastin: tooth-specific molecules that are distinctively expressed during murine dental differentiation. Eur J Oral Sci., 106: 963–970.
3. Chaussain, C.; Eapen,A.S., Huet, E., Floris, C., Ravindran,S., Hao, J., Suzanne Menashi,S., and George, A. (2009): MMP2- cleavage of DMP1 generates a bioactive petide promoting differentiation of dental pulp stem progenitor cells European Cells and Materials.18: 84 - 95.
4. D'Souza RN, Cavender A, Sunavala G, Alvarez J, Ohshima T, Kulkarni AB, and MacDougall, M. (1997): Gene expression patterns of murine dentin matrix protein 1 (Dmp1) and dentin sialophosphoprotein (DSPP) suggest distinct developmental functions in vivo. *J Bone Miner Res*. 12:2040–2049.
5. Fedarko NS, Jain A, Karadag A, Fisher LW (2004): Three small integrin binding ligand N-linked glycoproteins (SIBLINGs) bind and activate specific matrix metalloproteinases. FASEB J., **18:**734–736.
6. Fedarko NS, Jain A, Karadag A, Van E, Man MR, Fisher LW (2001): Elevated serum bone sialoprotein and osteopontin in colon, breast, prostate, and lung cancer. Clin Cancer Res., 7:4060–4066.
7. Feng JQ, Huang H, Lu Y, Ye L, Xie Y, Tsutsui TW, Kunieda T, *et al.* (2003) The dentin matrix protein 1 (Dmp1) is specifically expressed in mineralized, but not soft, tissues during development. J Dent Res., 82:776–780.
8. Feng JQ, Ward LM, Liu S, Lu Y, Xie Y, Yuan B, Yu X, Rauch F, Davis SI, Zhang S, Rios H, Drezner MK, Quarles LD, Bonewald LF, White KE. (2006): Loss of DMP1 causes rickets and osteomalacia and identifies a role for osteocytes in mineral metabolism. Nat Genet, 38:1230–1235.
9. Fisher LW, Jain A, Tayback M, Fedarko NS (2004): Small integrin binding ligand N-linked glycoprotein gene family expression in different cancers. Clin Cancer Res 10:8501–8511
10. Foster BL, Nagatomo KJ, Nociti FH Jr, Fong H, Dunn D, *et al.* (2012): Central Role of Pyrophosphate in Acellular Cementum Formation. PLoS ONE 7(6): e38393.
11. Gajjeraman S, Narayanan K, Hao J, Qin C, George A. (2007): Matrix macromolecules in hard tissues control the nucleation and hierarchical assembly of hydroxyapatite. J Biol Chem; 282:1193–1204.
12. Gericke, A., Qin, C., Sun,Y., Redfern, R., Redfern, D., Fujimoto, Y., Taleb, H., Butler, W.T., and Boskey, A.L. (2010): Different Forms of DMP1 Play Distinct Roles in Mineralization. *J Dent Res* 89(4):355-359.
13. George A, Gui J, Jenkins NA, Gilbert DJ, Copeland NG, Veis A(1994): In situ localization and chromosomal mapping of the AG1 (Dmp1) gene. *J Histochem Cytochem*. 42:1527–1531
14. George A, Sabsay B, Simonian PA, Veis A (1993): Characterization of a novel dentin matrix acidic phosphoprotein. Implications for induction of biomineralization. J Biol Chem., 268:12624–12630
15. George A, Silberstein R, Veis A (1995): In situ hybridization shows Dmp1 (AG1) to be a developmentally regulated dentin-specific protein produced by mature odontoblasts. Connect Tissue Res., 33:67–72
16. Gluhak-Heinrich J, Ye L, Bonewald L, Feng J, MacDougall M, *et al.* (2003): Mechanical loading stimulates dentin matrix protein 1 (DMP1) expression in osteocytes *in vivo*. J Bone Miner Res., 18: 807–817.
17. Graham, F. L., Smiley, J., Russell, W. C. & Nairn, R. (1977): Characteristics of a human cell line transformed by DNA from human adenovirus 5. Journal of General Virology 36, 59-72.
18. Hao J, Zou B, Narayanan K, George A (2004): Differential expression patterns of the dentin matrix proteins during mineralized tissue formation. Bone 34:921–932
19. He G, Dahl T, Veis A, George A (2003): Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein 1. Nat Mater., **2:**552–558.
20. He G, Gajjeraman S, Schultz D, Cookson D, Qin C, Butler WT, Hao J, George A (2005): Spatially and temporally controlled biomineralization is facilitated by interaction between self-assembled dentin matrix protein 1 and calcium phosphate nuclei in solution. Biochemistry 44:16140–16148.
21. .Hirst KL, Ibaraki-O'Connor K, Young MF, Dixon MJ. (1997a): Cloning and expression analysis of the

bovine dentin matrix acidic phosphoprotein gene. J Dent Res., 76:754–760.
22. Hirst KL, Simmons D, Feng J, Aplin H, Dixon MJ, MacDougall M. (1997b):Elucidation of the sequence and the genomic organization of the human dentin matrix acidic phosphoprotein 1 (DMP1) gene: exclusion of the locus from a causative role in the pathogenesis of dentinogenesis imperfecta type II. Genomics. 15: 42(1):38-45.
23. Kalajzic I, Braut A, Guo D, Jiang X, Kronenberg MS, Mina M, Harris MA, Harris SE, Rowe DW (2004): Dentin matrix protein 1 expression during osteoblastic differentiation, generation of an osteocyte GFP-transgene. Bone, **35:**74–82.
24. Kim J-W, Yamakoshi Y, Iwata T, Hu YY, Zhang H, Hu JC-C, Simmer JP.(2006): Porcine dentin matrix protein 1: gene structure, cDNA sequence, and expression in teeth. Eur J Oral Sci., 114: 33–41.
25. Kulkarni GV, Chen B, Malone JP, Narayanan AS, George A (2000): Promotion of selective cell attachment by the RGD sequence in dentine matrix protein 1. Arch Oral Biol., **45:**475–484.
26. Lu Y, Qin C, Xie Y, Bonewald LF, Feng JQ (2009). Studies of the DMP1 57-kDa functional domain both *in vivo* and *in vitro*. *Cells TissuesOrgans* 189:175-185.
27. Lu Y, Yuan B, Qin C, Cao Z, Xie Y, Dallas SL, *et al.* (2011): The biological function of DMP-1 in osteocyte maturation is mediated by its 57-kDa COOH-terminal fragment. *J Bone Miner Res*. 26:331–340.
28. MacDougall M, Gu T, Luan X, Simmons D, Chen J. (1998):Identification of a novel isoform of mouse dentin matrix protein 1: spatial expression in mineralized tissues. J Bone Miner Res., 13:422–431.
29. Narayanan K, Gajjeraman S, Ramachandran A, Hao J, George A (2006): Dentin matrix protein 1 regulates sialophosphoprotein gene transcription during early odontoblasts differentiation. J Biol Chem., 281:19064–19071.
30. Narayanan K, Ramachandran A, Hao J, He G, Park KW, Cho M, George A.(2003): Dual functional roles of dentin matrix protein 1. Implications in biomineralization and gene transcription by activation of intracellular Ca2+ store. J Biol Chem., 278: 17500–17508.
31. Narayanan K, Srinivas R, Ramachandran A, Hao J, Quinn B, George A (2001): Differentiation of embryonic mesenchymal cells to odontoblast-like cells by overexpression of dentin matrix protein 1 Proc Natl Acad Sci USA **98:**4516–4521.
32. Ogbureke KU, Fisher LW (2004): Expression of SIBLINGs and their partner MMPs in salivary glands. J Dent Res 83:664–670
33. Ogbureke KU, Fisher LW (2005): Renal expression of SIBLING proteins and their partner matrix metalloproteinases (MMPs). Kidney Int 68:155–166
34. Ogbureke KU, Fisher LW (2007): Sibling expression patterns in duct epithelia reflect the degree of metabolic activity. J Histochem Cytochem 55:403–409
35. Ogbureke KU, Nikitakis NG, Warburton G, Ord RA, Sauk JJ, Waller JL, Fisher LW (2007): Up-regulation of SIBLING proteins and correlation with cognate MMP expression in oral cancer. Oral Oncol 43:920–932.
36. Qin C, Brunn JC, Cook RG, Orkiszewski RS, Malone JP, Veis A, Butler WT.(2003): Evidence for the proteolytic processing of dentin matrix protein 1. Identification and characterization of processed fragments and cleavage sites. J Biol Chem;278:34700–34708.
37. Qin C, Brunn JC, Jones J, George A, Ramachandran A, Gorski JP, Butler WT.(2001): A comparative study of sialic acid-rich proteins in rat bone and dentin. Eur J Oral Sci 109:133–141.
38. Qin C, Huang B, Wygant JN, McIntyre BW, McDonald CH, Cook RG, Butler WT. A (2006): chondroitin sulfate chain attached to the bone dentin matrix protein 1 NH2-terminal fragment. J Biol Chem;281:8034–8040.
39. Rios H, Ye L, Dusevich V, Eick D, Bonewald L, et al. (2005) DMP1 is essential for osteocyte formation and function. J Musculoskelet Neuronal Interact 5: 325–327.
40. Tartaix PH, Doulaverakis M, George A, Fisher LW, Butler WT, Qin C, Salih E, Tan M, Fujimoto Y, Spevak L, Boskey AL. (2004): *In vitro* effects of dentin matrix protein-1 on hydroxyapatite formation provide insights into *in vivo* functions. J Biol Chem, 279:18115–18120.
41. Terasawa M, Shimokawa R, Terashima T, Ohya K, Takagi Y, Shimokawa H (2004): Expression of dentin matrix protein 1 (DMP1) in nonmineralized tissues. J Bone Miner Metab 22**:** 430–438.
42. Toyosawa S, Sato A, O'hUigin C, Tichy H, Klein J.(2000): Expression of the dentin matrix protein 1 gene in birds. J Mol Evol. 50: 31–38.
43. Toyosawa S, Shintani S, Fujiwara T, Ooshima T, Sato A, *et al.* (2001): Dentin matrix protein 1 is predominantly expressed in chicken and rat osteocytes but not in osteoblasts. J Bone Miner Res 16: 2017–2026.
44. Yamakoshi Y, Hu JC, Fukae M, Iwata T, Kim JW, Zhang H, Simmer JP (2005): Porcine dentin sialoprotein is a proteoglycan with glycosaminoglycan chains containing chondroitin 6-sulfate. J Biol Chem, 280(2):1552-1560.
45. Yang W, Lu Y, Kalajzic I, Guo D, Harris M, et al. (2005): Dentin matrix protein 1 gene cis-regulation: use in osteocytes to characterize local responses to mechanical loading in vitro and in vivo. J Biol Chem 280: 20680–20690.
46. Ye L, MacDougall M, Zhang S, Xie Y, Zhang J, Li Z, Lu Y, Mishina Y, Feng JQ (2004): Deletion of dentin matrix protein-1 leads to a partial failure of maturation of predentin into dentin, hypomineralization, and expanded cavities of pulp and root canal during postnatal tooth development. J Biol Chem, 279:19141–19148.
47. Ye L, Mishina Y, Chen D, Huang H, Dallas SL, Dallas MR, Sivakumar P, Kunieda T, Tsutsui TW, Boskey A, Bonewald LF, Feng JQ. (2005): Dmp1-

deficient mice display severe defects in cartilage formation responsible for a chondrodysplasia-like phenotype. J Biol Chem, 280:6197–6203.

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Trust Region Algorithm for Multi-objective Transportation, Assignment, and Transshipment Problems

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Abstract: In this paper, we present a trust-region globalization strategy to solve a multi-objective transportation (MOT) problem, which is one of great interest to many researchers and several local methods have been proposed to solve it. A weighting approach is used together with an active set strategy and a multiplier method to transform (MOT) problem to unconstrained optimization problem and we used a trust-region algorithm to solve it. In this work, the effect of changing weights on (MOT) problem is studied to show the degree of satisfaction of each objective. We also make a comparative study between our proposed approach and different approaches treated the multi-objective transportation problem before. The proposed approach is carried out on two multi-objective transportation test problems.

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Key Words: Multi-objective Optimization, Trust Region, Transportation and Assignment Problems

1. Introduction:

In real-world cases transportation problems can be formulated as multi-objective transportation problems because the complexity of a social and economic environment requires explicit considerations of criteria other than cost. Examples of additional concerns include: average delivery time of commodities, reliability of transportation, accessibility to the user, product deterioration, and many others. Thus, multiple penalty criteria may exist concurrently, which leads to the research work on multi-objective transportation (MOT) problems.

The multi-objective transportation problem is of great interest to many researchers and several local methods have been proposed to solve it [1, 2, 11, 12, 14, 15, 18, 19].

Most of the methods which are used to solve (MOT) problem are local methods. By local method we mean that the method is designed to converge to optimal solution from closest starting point whether it is local or global one. For a local method, there is no guarantee that it converge if it starts from remote.

In this paper we will use a trust-region globalization strategy to solve (MOT) problem. Globalizing strategy means modifying the local method in such a way that it is guaranteed to converge at all even if the starting point is far away from the solution.

In this work, we convert the multi-objective transportation problem to a single-objective constrained optimization problem with equality and inequality constraints (SCOEI) problem, by using a weighting approach. The weighting approach is considered as one of the most useful algorithms in treating multi-objective optimization problems to generate a wide set of optimal solutions (pareto set), for more detail see [17]. Here, an active set strategy is used to convert (SCOEI) problem to a single-objective equality constrained optimization problem (SECO) problem and a multiplier method is used to convert (SECO) problem to unconstrained optimization problem.

The trust-region strategy for solving (SCOEI) problem, (SECO) problem, and unconstrained optimization problem has proved to be very successful, both theoretically and practically [6-10]

In this current study, the effect of changing weights on (MOT) problem was studied to show the degree of satisfaction of each objective. We also make a comparative study between our proposed algorithm and different approaches treated the multi-objective transportation problem before.

Subscripted functions denote function values at particular points; for example,

$$f_k = f(x_k),\ \nabla f_k = \nabla f(x_k),\ l_{k+1} = l(x_{k+1}, \mu_{k+1}, \upsilon_{k+1}),\ \nabla_x l_k = \nabla_x l(x_k, \mu_k, \upsilon_k)$$

and so on. However, the arguments of the functions are not abbreviated when emphasizing the dependence of the functions on their arguments. The matrix H_k denotes the Hessian of the Lagrangian function $l(x_k, \mu_k, \upsilon_k)$ or an approximation to it. Finally, all norms are l_2 -norms.

This paper is organized as follows: In section two we introduce the mathematical form of multi-objective transportation problem and how (MOT) problem transform to unconstrained optimization problem. In section three we give a detailed discussion of the trust region algorithm for solving (MOT) problem. Furthermore, we then discuss in detail the two test problems with all their possible solutions in section four. Finally, the conclusion is discussed in section five, and we come to acknowledgments in section six.

2 Mathematical Formulation of (MOT) Problem.

The mathematical model of (MOT) problem can be stated as follows:

$$\text{minimize } f^{\hat{k}}=\sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij}, \quad \hat{k}=1,2,...,p$$

$$\text{subject to} \quad \sum_{j=1}^{n} x_{ij}=b_i, \qquad (2.1)$$

$$\sum_{i=1}^{m} x_{ij}=c_j,$$

$$x_{ij}\geq 0,$$

where m and n stands for the number of sources and the number of destinations, respectively, and $\hat{k}=1,2,...,p$. Positive constants b_i are the amount of homogeneous product for i^{th} origin which are transported to n destinations. Positive constants c_j represent the demand of homogeneous product for the j^{th} destination. Positive constants $a_{ij}^{\hat{k}}$ represent the coefficients of the $\hat{k}^{th}$ objective functions which are associated with transportation of unit of the product from source i to destination j . Variables x_{ij} are the unknown quantity to be transported from origin i to destination j .

The first set of constraints $\sum_{j=1}^{n} x_{ij}=b_i$ stipulates that the sum of shipments from the source must equal its supply and the second set of constraints $\sum_{i=1}^{m} x_{ij}=c_i$ requires that the sum of the shipments to the destination must satisfy its demand. Since the total supply is equal to total demand, this formulation is called a balanced transportation problem. In this paper, we study the case of balanced transportation problem because the unbalanced transportation problem can be converted to balanced transportation problem after including a dummy origin or a dummy destination.

Definition 2.1 :(Nondominated solution). A feasible vector x^0 in a feasible region S , yields a nondominated solution of (MOT) problem, iff there is no other vector such that $x\in S$

$$\sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij} \leq \sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij}^{0} \qquad \forall \hat{k}=1,2,...,p,$$

and

$$\sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij} < \sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij}^{0} \qquad \text{for some } \hat{k}.$$

For more detail see [16].

Definition 2.2:(Efficient Solution). A point $x^0\in S$ is efficient, iff there does not exist another $x\in S$ such that

$$\sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij} \leq \sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij}^{0} \qquad \forall \hat{k}=1,2,...,p,$$

and

$$\sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij} \neq \sum_{i=1}^{m}\sum_{j=1}^{n} a_{ij}^{\hat{k}} x_{ij}^{0} \qquad \text{for some } \hat{k}.$$

Otherwise x^0 is an inefficient solution. For example, the point $x^0\in S$ is efficient if its criterion vector is not dominated by the criterion vector of another point in the feasible region S . In this paper we will use efficient solution. [17].

Definition 2.3:(Compromise Solution). A feasible vector $x^*\in S$ is called a compromise solution of (MOT) problem iff $x^*\in E$ and $f(x^*)\leq \min\{f(x)\forall x\in S\}$ where E is the set of efficient solutions. [13].

From the above definition a compromise solution must meet two conditions

The solution should be efficient. 1-

2- The feasible solution vector x^* should have the minimum deviation from the ideal point than any other point in S .

The compromise solution which maximizes the underlying utility function is the closest one to the ideal solution. While knowledge of the set of efficient solutions E is not always necessary in real world cases, the decision maker's preferences should be considered in the determination of the final compromise solution.

Definition 2.4:(Preferred Compromise Solution).

The solution is called the preferred compromise solution if the compromise solution satisfies the decision maker's preferences.

By using the weighting approach, the multi-objective optimization problem (2.1) is converted to the following single-objective constrained optimization problem with equality and inequality constraints (SCOEI) problem.

$$\begin{aligned} &\text{minimize } f(x)=\sum_{\hat{k}=1}^{p}\sum_{i=1}^{m}\sum_{j=1}^{n} w^{\hat{k}} a_{ij}^{k} x_{ij}, \\ &\text{subject to } \sum_{j=1}^{n} x_{ij}=b_i, \qquad (2.2) \\ &\qquad \sum_{i=1}^{m} x_{ij}=c_j, \\ &\qquad x_{ij}\geq 0, \end{aligned}$$

where $\sum_{\hat{k}=1}^{p} w^{\hat{k}}=1$ and $w^{\hat{k}}\geq 0$ *for all* $\hat{k}$.

The above problem can be written as follows:

$$\begin{aligned} &\text{minimize } f(x) \\ &\text{subject to } y(x)=0, \qquad (2.3) \\ &\qquad z(x)\leq 0, \end{aligned}$$

$j=1,2,...,n.$, $z(x)=[x_{ij}]^T$, $i=1,2,...,m$,

and where $y(x)=[\sum_{j=1}^{n} x_{ij}-b_i, \sum_{j=1}^{n} x_{ij}-c_j]^T$

The functions $f(x):\Box^{n\times m}\to\Box$, $y(x):\Box^{n\times m}\to\Box^{n+m}$, *and* $z(x):\Box^{n\times m}\to\Box^{n\times m}$ are twice continuously differentiable.

The Lagrangian function associated with problem (2.3) is the function

$$l(x,\mu,\upsilon)=f(x)+\mu^T y(x)+\upsilon^T z(x), \qquad (2.4)$$

where $\mu\in\Box^{n+m}$ and $\upsilon\in\Box^{n\times m}$ are the Lagrange multiplier vectors associated with equality and inequality constraints, respectively.

Following [6], we define a 0-1 diagonal indicator matrix $U(x)\in\Box^{(n\times m)\times(n\times m)}$,

whose diagonal entries are

$$u_e(x)=\begin{cases}1 & \text{if } z_e(x)\geq 0,\\ 0 & \text{if } z_e(x)<0,\end{cases} \qquad (2.5)$$

where $e=1,2,...,m\times n.$

Using the above matrix, we transform problem (2.3) to the following equality constrained optimization problem

$$\begin{aligned} &\text{minimize } f(x) \\ &\text{subject to } y(x)=0, \qquad (2.6) \\ &\qquad \frac{1}{2}z(x)^T U(x) z(x)=0. \end{aligned}$$ U

sing a multiplier method, we transform the equality constrained optimization problem (2.6) to the following unconstrained optimization problem

$$\begin{aligned} &\text{minimize } \Phi(x,\mu,\upsilon;s;r)=l(x,\mu,\upsilon)+\frac{s}{2}\|U(x)z(x)\|^2+\frac{r}{2}\|y(x)\|^2 \\ &\text{subject to } x\in\Box^{n\times m}, \qquad (2.7) \end{aligned}$$

where s is the positive parameter and $r>0$ is a parameter usually called the penalty parameter.

A detailed description of the main steps of the trust-region algorithm for solving the above problem and it's an algorithmic framework is presented in the following section.

3. Trust Region Algorithm Outline

This section is devoted to presenting the detailed description of the trust-region algorithm for solving problem (2.7).

3.1. Computing a Trial Step

We compute the trial step d_k by solving the following trust-region sub problem

$$\begin{aligned} &\text{minimize } l_k+\nabla_x l_k^T d+\frac{s_k}{2}\|U_k(z_k+\nabla z_k^T d)\|^2+\frac{r_k}{2}\|y_k+\nabla y_k^T d\|^2, \\ &\text{subject to } \|d\|\leq\delta_k, \qquad (3.1) \end{aligned}$$

where H_k is the Hessian matrix of the Lagrangian function $l(x_k,\mu_k,\upsilon_k)$ or an approximation to it. Since our convergence theory is based on the fraction of Cauchy decrease condition, therefore a dogleg method can be used to compute the trial step. [5].

3.2. Testing the Step and Updating δ_k

To test the step, estimates for the two Lagrange multipliers μ_{k+1} and υ_{k+1} are needed. Our way of evaluating the two Lagrange multipliers μ_{k+1} and υ_{k+1} is presented in Step 5 of Algorithm (3.1) below.

To test whether the point $(x_k+d_k,\mu_{k+1},\upsilon_{k+1})$ will be taken as a next iterate, an actual reduction and a predicted reduction are needed and defined as follows:

The actual reduction in the merit function is defined as

$$Ared_k = l(x_k,\mu_k,\upsilon_k) - l(x_{k+1},\mu_k,\upsilon_k) - \Delta\mu_k^T y_{k+1} - \Delta\upsilon_k^T z_{k+1} + \frac{s_k}{2}[z_k^T U_k z_k - z_{k+1}^T U_{k+1} z_{k+1}] + \frac{r_k}{2}[\|y_k\|^2 - \|y_{k+1}\|^2], \quad (3.2)$$

where $\Delta\mu_k = (\mu_{k+1} - \mu_k)$ and $\Delta\upsilon_k = (\upsilon_{k+1} - \upsilon_k)$.

The predicted reduction in the merit function is defined to be

$$Pred_k = q_k(0) - q_k(d_k) - \Delta\mu_k^T(y_k + \nabla y_k^T d_k) - \Delta\upsilon_k^T U_k z_k + \frac{r_k}{2}[\|y_k\|^2 - \|y_k + \nabla y_k^T d_k\|^2], \quad (3.3)$$

where

$$q_k(d) = l_k + \nabla_x l_k^T d + \frac{1}{2} d^T H_k d + \frac{s_k}{2}\|U_k(z_k + \nabla z_k^T d)\|^2. \quad (3.4)$$

After computing a trial step and updating the Lagrange multipliers, the penalty parameter is updated to ensure that $Pred_k \geq 0$. To update r_k, we use a scheme that has the flavor of the scheme Proposed by El-Alem [7]. This scheme is described in Step 6 of Algorithm (3.1) below. After that, the step is tested to know whether it is accepted. This is done by comparing $Pred_k$ against $Ared_k$.

If $\frac{Ared_k}{Pred_k} < \eta_1$ where $\eta_1 \in (0,1)$ is a small fixed constant, then the step is rejected. In this case, the radius of the trust region δ_k is decreased by setting $\delta_k = \alpha_1\|d_k\|$, where $\alpha_1 \in (0,1)$ and another trial step is computed using the new trust-region radius. If $\frac{Ared_k}{Pred_k} \geq \eta_1$ then the step is accepted.

Our way of evaluating the trial steps and updating the trust-region radius is presented in Step 7 of Algorithm (3.1) below. After accepting the step, we update the parameter s_k and the Hessian matrix H_k. To update s_k, we use a scheme suggested by Yuan [20]. This scheme is described in Step 8 of Algorithm (3.1) below.

Finally, the algorithm is terminated when either $\|d_k\| \leq \varepsilon_1$ or $\|\nabla_x l_k\| + \|\nabla z_k U_k z_k\| + \|y_k\| \leq \varepsilon_2$, *for some* $\varepsilon_1, \varepsilon_2 > 0$.

3.3. Main Algorithm

A formal description of the trust-region algorithm for solving problem (2.7) is presented in the following algorithm.

Algorithm 3.1. (The Main Algorithm)

Step 0. (Initialization)

Given $x_1 \in \mathbb{R}^{n\times m}$. Compute U_1. Evaluate μ_1 and υ_1 (see Step 5 with $k = 0$ and $\mu_0 = (0,0,...,0)^T$). Set $s_1 = 1$, $r_0 = 1$, $\sigma_1 = 1$, and $\beta = 0.1$. Choose $\varepsilon_1 = \varepsilon_2 = 10^{-8}$, $\alpha_1 = 0.05$, $\alpha_2 = 2$, $\eta_1 = 10^{-4}$, and $\eta_2 = 0.5$ such that $\varepsilon_1 > 0$, $\varepsilon_2 > 0$, $0 < \alpha_1 < 1 < \alpha_2$, and $0 < \eta_1 < \eta_2 < 1$. Set $\delta_{\min} = 10^{-3}$ and $\delta_{\max} = 10^5\delta_1$ such that $\delta_{\min} \leq \delta_1 \leq \delta_{\max}$.

Set $k = 1$.

Step 1. (Test for convergence) If $\|\nabla_x l_k\| + \|\nabla z_k U_k z_k\| + \|y_k\| \leq \varepsilon_2$, then terminate the algorithm.

Step 2. (Compute a trial step)

a) Compute the step d_k by solving (3.1)(

b) Set $x_{k+1} = x_k + d_k$.(

Step 3. (Test for termination)

If $\|d_k\| \leq \varepsilon_1$, then terminate the algorithm.

Step 4. (Update the active set)

Compute U_{k+1}.

Step 5. (Compute the Lagrange multipliers μ_{k+1} and υ_{k+1})

a) Compute μ_{k+1} by solving(

$$\begin{aligned} &\min imize && \|\nabla f_{k+1} + \nabla y_{k+1}\mu_k + \nabla z_{k+1}U_{k+1}\upsilon\|^2 \\ &subject\ to && U_{k+1}\upsilon \geq 0, \end{aligned}$$

and set the rest of the components of υ_{k+1} to zero.

$\mu_{k+1} = \mu_k$. then set $\|\nabla f_{k+1} + \nabla y_{k+1}\mu_k + \nabla z_{k+1}U_{k+1}\upsilon_{k+1}\| \leq \varepsilon_1$,

(b) If

Else, compute μ_{k+1} by solving

$$\min imize \quad \|\nabla f_{k+1} + \nabla y_{k+1}\mu + \nabla z_{k+1}U_{k+1}\upsilon_{k+1}\|^2.$$

End if.

Step 6. (Update the penalty parameter r_k)

If $Pred_k \leq \frac{r_k}{4}[\|y_k\|^2 - \|y_k + \nabla y_k^T d_k\|^2]$,

then set

$$r_k = \frac{4[q_k(d_k) - q_k(0) + \Delta\mu_k^T (y_k + \nabla y_k^T d_k) + \Delta\upsilon_k^T U_k z_k]}{\|y_k\|^2 - \|y_k + \nabla y_k^T d_k\|^2} + \beta.$$

End if.

Step 7. (Test the step and update the trust-region radius)

If $\frac{Ared_k}{\Pr ed_k} < \eta_1$.

Reduce the trust-region radius by setting $\delta_k = \alpha_1 \|d_k\|$ and go to step 2

accept the step $x_{k+1} = x_k + d_k$. Else if $\eta_1 \le \frac{Ared_k}{\Pr ed_k} < \eta_2$, then

Set the trust-region radius: $\delta_{k+1} = \max(\delta_k, \delta_{\min})$.

Else, accept the step: $x_{k+1} = x_k + d_k$.

Set the trust-region radius: $\delta_{k+1} = \min\{\delta_{\max}, \max\{\delta_{\min}, \alpha_2 \delta_k\}\}$.

End if

Step 8. (To update the parameters s_k and σ_k)

(a) Set $s_{k+1} = s_k$ and $\sigma_{k+1} = \sigma_k$.

(b) Compute $Tpred_k = q_k(0) - q_k(d_k) - \Delta\mu_k^T (y_k + \nabla y_k^T d_k) - \Delta\upsilon_k^T U_k z_k$.

(c) If $Tpred_k < \sigma_k \|\nabla z_k U_k z_k\| \min\{\|\nabla z_k U_k z_k\|, \delta_k\}$,

then set $s_{k+1} = 2s_k$ and $\sigma_{k+1} = \frac{1}{2}\sigma_k$.

End if.

Step 9. Set $k = k + 1$ and go to Step 1

In the following section, we introduce two multi-objective transportation test problems to obvious the goal of our paper.

4. Multi-objective Transportation Test Problems

In this section, we introduce two test problems for the multi-objective transportation optimization problem. The proposed algorithm was implemented on 2.7 MHZ PC using MATLAB 7 to confirm the effectiveness of the algorithm. The two multi-objective transportation optimization test problems are presented in the following subsections.

4.1. Multi-objective Transportation Test Problem 1

Let us consider the following numerical example presented by many [1-4, 16, 21]

to illustrate the application of the proposed algorithm. The problem has the following characteristics:

supplies: $b_1 = 8$, $b_2 = 19$, and $b_3 = 17$.

demands: $c_1 = 11$, $c_2 = 3$, $c_3 = 14$, and $c_4 = 16$.

penalties: $a^1 = \begin{pmatrix} 1 & 2 & 7 & 7 \\ 1 & 9 & 3 & 4 \\ 8 & 9 & 4 & 6 \end{pmatrix}$ and

$a^2 = \begin{pmatrix} 4 & 4 & 3 & 4 \\ 5 & 8 & 9 & 10 \\ 6 & 2 & 5 & 1 \end{pmatrix}$.

This problem could be written as follows:

$$\begin{aligned}
\min imize\ f_1 &= x_{11} + 2x_{12} + 7x_{13} + 7x_{14} \\
&\quad + x_{21} + 9x_{22} + 3x_{23} + 4x_{24} \\
&\quad + 8x_{31} + 9x_{32} + 4x_{33} + 6x_{34}, \\
\min imize\ f_2 &= 4x_{11} + 4x_{12} + 3x_{13} + 4x_{14} \\
&\quad + 5x_{21} + 8x_{22} + 9x_{23} + 10x_{24} \\
&\quad + 6x_{31} + 2x_{32} + 5x_{33} + x_{34}, \\
subject\ to\ & x_{11} + x_{12} + x_{13} + x_{14} = 8, \\
& x_{21} + x_{22} + x_{23} + x_{24} = 19, \\
& x_{31} + x_{32} + x_{33} + x_{34} = 17, \\
& x_{11} + x_{21} + x_{31} = 11, \\
& x_{12} + x_{22} + x_{32} = 3, \\
& x_{13} + x_{23} + x_{33} = 14, \\
& x_{14} + x_{24} + x_{34} = 16, \\
& x_{ij} \ge 0, \quad \forall i = 1,2,3, \quad j = 1,2,3,4.
\end{aligned}$$

4.2.1Results and Discussions of (MOT) Test Problem

A weighting approach is used together with the trust-region algorithm (3.1) to solve the above problem at several values of weighting values based on $w^1 = \{0, 0.1, ..., 1\}$ and $w^2 = \{1, 0.9, ..., 0\}$. By discussing the effect of changing weights on the two objective functions f_1 and f_2, we note from Figure (1) that the best value of w^1 is 0.4 and w^2 is 0.6, which give $f_1 = 173$ and $f_2 = 173$ as the best compromise solution.

To evaluate the performance of the suggested approach we show a schematic comparison in table (1) between our results and the results of researchers who have used other approaches (Interactive approach [16], Fuzzy approach [1], Fuzzy approach [4] and IFGP approach [2]). It becomes evident from the table that the value of $f_2 = 173$ is the best result,

whereas the value of $f_1 = 173$, while still acceptable, is not the best

Table 1. Comparison between different approaches.

The name of approach	f_1	f_2
Interactive approach [16]	186	174
Fuzzy approach [1]	170	190
Fuzzy approach [4]	160	195
IFGP approach [2]	168	185
Proposed approach	173	173

4.3. Multi-objective Transportation Test Problem 2

Let us consider the following numerical example presented by Aneja and Nair[3] ; Ringuest and Rinks [16] to illustrate the goal of our paper. The problem has the following characteristics

supplies: $b_1 = 5$, $b_2 = 4$, $b_3 = 2$, and $b_4 = 9.$

demands: $c_1 = 4$, $c_2 = 4$, $c_3 = 6$, $c_4 = 2$, and $c_5 = 4.$

penalties: $$a^1 = \begin{pmatrix} 9 & 12 & 9 & 6 & 9 \\ 7 & 3 & 7 & 7 & 5 \\ 6 & 5 & 9 & 11 & 3 \\ 6 & 8 & 11 & 2 & 2 \end{pmatrix},$$

$$a^2 = \begin{pmatrix} 2 & 9 & 8 & 1 & 4 \\ 1 & 9 & 9 & 5 & 2 \\ 8 & 1 & 8 & 4 & 5 \\ 2 & 8 & 6 & 9 & 8 \end{pmatrix}$$

and

$$a^3 = \begin{pmatrix} 2 & 4 & 6 & 3 & 6 \\ 4 & 8 & 4 & 9 & 2 \\ 5 & 3 & 5 & 3 & 6 \\ 6 & 9 & 6 & 3 & 1 \end{pmatrix}.$$

This problem could be written as follows:

$$\begin{aligned}
\min imize\ f_1 &= 9x_{11} + 12x_{12} + 9x_{13} + 6x_{14} + 9x_{15} \\
&+ 7x_{21} + 3x_{22} + 7x_{23} + 7x_{24} + 5x_{25} \\
&+ 6x_{31} + 5x_{32} + 9x_{33} + 11x_{34} + 3x_{35} \\
&+ 6x_{41} + 8x_{42} + 11x_{43} + 2x_{44} + 2x_{45,} \\
\min imize\ f_2 &= 2x_{11} + 9x_{12} + 8x_{13} + x_{14} + 4x_{15} \\
&+ x_{21} + 9x_{22} + 9x_{23} + 5x_{24} + 2x_{25} \\
&+ 8x_{31} + x_{32} + 8x_{33} + 4x_{34} + 5x_{35} \\
&+ 2x_{41} + 8x_{42} + 6x_{43} + 9x_{44} + 8x_{45,} \\
\min imize\ f_3 &= 2x_{11} + 4x_{12} + 6x_{13} + 3x_{14} + 6x_{15} \\
&+ 4x_{21} + 8x_{22} + 4x_{23} + 9x_{24} + 2x_{25} \\
&+ 5x_{31} + 3x_{32} + 5x_{33} + 3x_{34} + 6x_{35} \\
&+ 6x_{41} + 9x_{42} + 6x_{43} + 3x_{44} + x_{45,} \\
subject\ to\quad & x_{11} + x_{12} + x_{13} + x_{14} + x_{15} = 5, \\
& x_{21} + x_{22} + x_{23} + x_{24} + x_{25} = 4, \\
& x_{31} + x_{32} + x_{33} + x_{34} + x_{35} = 2, \\
& x_{41} + x_{42} + x_{43} + x_{44} + x_{45} = 9, \\
& x_{11} + x_{21} + x_{31} + x_{41} = 4, \\
& x_{12} + x_{22} + x_{32} + x_{42} = 4, \\
& x_{13} + x_{23} + x_{33} + x_{43} = 6, \\
& x_{14} + x_{24} + x_{34} + x_{44} = 2, \\
& x_{15} + x_{25} + x_{35} + x_{45} = 4, \\
& x_{ij} \geq 0, \quad \forall i = 1,2,3,4, \quad j = 1,2,3,4,5.
\end{aligned}$$

4.4.2 Results and Discussions of (MOT) Test Problem

Similar to the (MOT) test problem 1, the weighting approach is used together with the trust-region algorithm (3.1) to solve the above problem and discuss the effects of changing weights on it. As one weight is changed linearly in each case, the other two weights are generated randomly, such that $\sum_{\hat{k}=1}^{3} w^{\hat{k}} = 1$ and $w^{\hat{k}} \geq 0$ for all $\hat{k} = 1,2,3.$ The values of the weights which are used for three cases are illustrated in three tables (2-4).

Figures (2-4), show the objective functions obtained from six solutions corresponding to the six weights compared to the weights for three cases. We observe that the best compromise solutions are $f_1 = 144$, $f_2 = 104$, and $f_3 = 73$ which are occur at $w^1 = w^2 = w^3 = 0.6.$

When we compare the results of our suggested approach and the results of researchers (Interactive approach [2] and Fuzzy approach [1]) who have used other approaches it becomes evident from table (5) that the value of $f_3 = 73$ is the best result, the value of $f_2 = 104$ is in agreement with the value obtained by using the Interactive approach [2] and better than the result of Fuzzy approach [1], whereas the value of $f_1 = 144$, is comparatively higher than the results obtained by other approaches.

Table 2. Different weights (w^1 is changed linearly)

Run	w^1	w^2	w^3
1	0.0000	0.5721	0.4279
2	0.2000	0.6205	0.1795
3	0.4000	0.2118	0.3882
4	0.6000	0.1636	0.2364
5	0.8000	0.1759	0.0241
6	1.0000	0.0000	0.0000

Table 3. Different weights (w^2 is changed linearly)

Run	w^1	w^2	w^3
1	0.6028	0.0000	0.3972
2	0.5676	0.2000	0.2324
3	0.4573	0.4000	0.1427
4	0.2718	0.6000	0.1282
5	0.1468	0.8000	0.0532
6	0.0000	1.0000	0.0000

Table 4. Different weights (w^3 is changed linearly)

Run	w^1	w^2	w^3
1	0.7477	0.2523	0.0000
2	0.5994	0.2006	0.2000
3	0.4576	0.1424	0.4000
4	0.1354	0.2646	0.6000
5	0.1076	0.0924	0.8000
6	0.0000	0.0000	1.0000

Table 5.Comparison between different approaches.

The name of approach	f_1	f_2	f_3
Fuzzy approach [1]	112	106	80
Interactive approach [2]	127	104	76
Proposed approach	144	104	73

5. Conclusions

In the present work we propose a new approach by using the trust-region globalization strategy to solve a multi-objective transportation (MOT) problem, which is interested to many researchers and several local methods have been proposed to solve it. Globalizing strategy means modifying the local method in such a way that it is guaranteed to converge at all even if the starting point is far away from the solution. The trust-region strategy for solving (SECOP) and unconstrained optimization problem has proved to be very successful, both theoretically and practically.

A weighting approach is used together with an active set strategy and a multiplier method to transform (MOT) problem to unconstrained optimization problem and we used a trust-region algorithm to solve it.

We have arrived at the conclusion that this new numerical technique has shown itself to be suitable for the numerical and parametric study of (MOT) problem after having been tested in the work with two test problems. Also, this approach consider as interactive approach, because it allows the decision maker to specify the weights of the criterion importance which show the degree to which the objectives have been satisfied. Finally, the success of our approach on most of the test problems not only provides confidence, but also stresses the importance of numerical parametric studies to investigate the best weighting values of each objective function which leads directly to the best compromise solution in solving multi-objective transportation problems.

6. Acknowledgements

First and foremost, we give thanks to God for the grace and mercy he has shown us. We depend on Him and we could not have accomplished this work without Him.

Words cannot express our deep gratitude to The Deanship of Scientific Research, Taibah University, KSA for supporting this project by grant No. 1432/1415

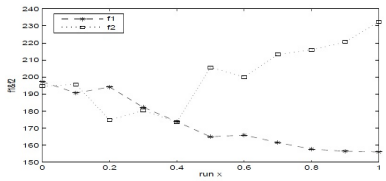

Figure 6.1: Plot showing the values of f_1 and f_2 as w_1 or w_2 changes linearly.

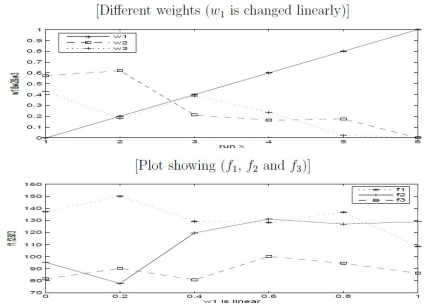

Figure 6.2: Plot showing the values of f_1, f_2 and f_3 solution for different weights in 6 runs of table 2.

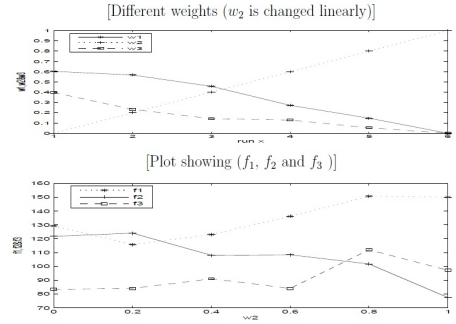

Figure 6.3: Plot showing the values of f_1, f_2 and f_3 solution for different weights in 6 runs of table 3.

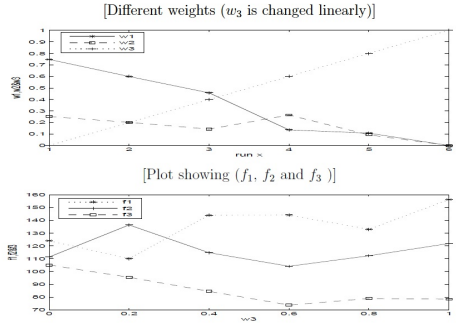

Figure 6.4: Plot showing the values of f_1, f_2 and f_3 solution for different weights in 6 runs of table 4.

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References

[1] Abd El-Wahed W. F., A Multiobjective Transportation Problem under Fuzziness, Fuzzy Sets and Systems, Vol. 117, No. 1, 2001, pp.: 27-33.

[2] Abd El-Wahed W. F. and S. M. Lee, Interactive Fuzzy Goal Programming for Multiobjective Transportation Problems, Omega, Vol. 34, No. 2, 2006, pp:. 158-166.

[3] Aneja Y. P. and K. P. K. Nair, Bicriteria Transportation Problems, Management Science, Vol.25, No. 1, 1979, pp.: 73-80.

[4] Bit A. K., M. P. Biswal, S. S. Alam, Fuzzy Programming Approach to Multicriteria Decision Making Transportation Problem, Fuzzy Sets and Systems, Vol. 50, No. 2, 1992, pp.: 35-41.

[5] Dennis J. and R. Schnabel, Numerical Methods for Unconstrained Optimization and Nonlinear Equations, Prentice-Hall, Englewood cliffs, New Jersey, 1983.

[6] Dennis J., M. El-Alem, and K. Williamson, A Trust-Region Approach to Nonlinear Systems of Equalities and Inequalities, SIAM J Optimization, Vol. 9, 1999, pp.: 291-315.

[7] El-Alem M., A Global Convergence Theory for A Class of Trust-Region Algorithms for Constrained Optimization, PhD Thesis, Department of Mathematical Sciences, Rice University, Houston, Texas, 1988.

[8] El-Sobky B., A Global Convergence Theory for An Active Trust-Region Algorithm for Solving the General Nonlinear Programming Problem, Applied Mathematics and Computation Archive, Vol. 144 No.1, 2003, pp.: 127-157.

[9] El-Sobky B., A Global Convergence Theory for Trust-Region Algorithm for General Nonlinear Programming Problem, International Journal of Mathematical Modeling, Simulation and Applications, Vol. 5, Issue 1, 2012.

[10] El-Sobky B., Y. Abo-Elnaga and H. Zahed, Utilization of Trust Region Algorithm in Solving Reactive Power Compensation Problem, Applied Mathematical Sciences, Vol. 6, No. 54, 2012, pp.: 2649-2667.

[11] Gen M., K. Ida Kono and Y. Z. Li, Solving Bi-Criteria Solid Transportation Problem by Genetic Algorithm, Proceeding of the 16th International Conference on Computers and Industrial Engineering, San Antonio, 2-5 October 1994, pp. 572-575.

[12] Gen M., Y. Z. Li, and Kenichi Ida, Solving Multiobjective Transportation Problem by Spanning Tree-Based Genetic Algorithm, IEICE Transactions on Fundamentals, Vol. E82-A, No. 2, 1999, pp.: 2802-2810.

[13] Leberling H., On finding compromise solutions in multicriteria problems using
the fuzzy min-operator, Fuzzy Sets and Systems, Vol.6, 1981, pp.: 105-118.

[14] Michalewicz Z., G. A. Vignaux and M. Hobbs, a Non-standard Genetic Algorithm for the Nonlinear Transportation Problem, INFORSA Journal on Computing, Vol. 3, No. 4, 1991, pp.: 307-316.

[15] Mousa A. A., Using Genetic Algorithm and TOPSIS Technique for Multiobjective Transportation Problem: A Hybrid Approach International Journal of Computer Mathematics, Vol. 87, No. 13, 2010, pp.:3017-3029.

[16] Ringuest J. L. and D. B. Rinks, Interactive Solutions for the Linear Multiobjective Transportation Problems, European Journal Operational Research, Vol. 32, No. 1, 1987, pp.: 96-106.

[17] Steuer R., Multiple Criteria Optimization Theory, Computation and Application, New York, Wiley, 1986.

[18] Vignaux G. A. and Z. Michalewicz, A Genetic Algorithm for the Linear Transportation Problem, **IE**EE Transactions on Systems, Man and Cybernetics, Vol. 21, No. 3, 1991, pp.: 445-452.

[19] Yang L. and Y. Feng, A Bicriteria Solid Transportation Problem with Fixed Charge under Stochastic Environment, newblock Applied Mathematical Modelling, Vol. 31, No. 12, 2007, pp.: 2668-2683.

[20] Yuan Y., On the Convergence of a New Trust Region Algorithm, Numer. Math. Vol. 70, 1995, pp.: 515-539.

[21] Zaki S. A., A. A. Mousa, H. M. Geneedi and A. Y. Elmekawy, Efficient Multiobjective Genetic Algorithm for Solving Transportation, Assignment and Transshipment Problems, Applied Mathematics, Vol. 3, 2012, pp.: 92-99.

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Determine the Proper Level of Yeast with Different Levels of Roughages to Improve the Nutritive Value of Lamb's Ration

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Abstract: The aim of this study was to evaluate the effects of supplemented two levels of yeast culture (YC) to two rations of different roughage levels on animal performance, nutrient digestibility, nitrogen balance, nutritive value and rumen fermentation of growing lambs. Sixty Ossimi male lambs of an average being 37.5Kg body weight and 9 months age were randomly assigned to six nutritional groups. Animals were fed two basal rations differ in roughage ratios (Control 1 or 2) without supplementation or supplemented with 0.1 or 0.2% dry yeast containing 10^8 cells of *Saccharomyces cerevisiae* per gram (Yea- Sacc^{1026}). The growth experiment lasted 120 days. A digestion trail was carried on and samples of rumen liquor were collected at the end of the growth experiment. The results showed that addition of YC to the basal ration increased DMI, TDNI, DCPI, feed conversion ratio and did significantly ($P<0.05$) improve lambs average daily gain (ADG). On the other hand, YC supplementation improved digestion coefficients of DM, OM, CP and CF. Nitrogen balance, TDN and DCP also significantly ($P<0.05$) increased by the addition of YC. Ruminal pH value increased ($P<0.05$) and ammonia concentration decreased ($P<0.05$) for in animals fed YC supplemented rations compared to the control rations. However, total VFA's concentration not significantly affected by YC supplementation. Supplementation of YC to control 2 of higher roughage content significantly ($P<0.05$) increased acetate concentration and decreased of propionate concentration in rations 5 and 6 compared with other rations. While, the butyrate concentration were significantly ($P<0.05$) decreased with supplement control ration 1 and 2 by both two levels of YC (rations 3, 4, 5, and 6). It could be concluded that addition of YC to sheep rations containing different levels of roughages improved growth performance, crude protein and crude fiber digestibility, nitrogen balance and some rumen parameters.

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Keywords: Yeast, Roughages, Nutritive value, Growing lamb, Rumen fermentation

1. Introduction:

The goal of any feeding program is to provide the correct amount and balance of nutrients to animals at the proper time to achieve optimum productive and reproductive efficiency and profitability (Gaafar, 2010).

The use of probiotics for farm animals has increased considerably over the last 15 years. Probiotics are defined as live microorganisms which can confer a health benefit for the host animals. It also used in a wide range of nutritional techniques in order to support the host organism to reduce stress (Chaucheyars-Durand and Durand, 2010).

Yeast culture, mainly *Saccharomyces cerevisiae* may also improve ruminal fermentation (Newbold *et al.*, 1990) and therefore provide another enhancer for microbial growth. It can help in the stability of rumen fermentation by consuming oxygen in the rumen. Also, live yeast supplement can release essential enzymes, vitamins and amino acids during digestion which are thought to have a positive influence on the rumen media.

Rationary factors such as forage- to- concentrate ratio and forage type are important in determining the response to yeast culture supplementation (Piva *et al.*,1993).This may explain the contradiction found in the results of the previous studies.

Several researches (Mathieu *et al.*, 1996; Jouany *et al.*, 1998, Robinson and Grrett, 1999; Arcos Gascia *et al.*, 2000; Abd El- Ghani, 2004; Kamel *et al.*, 2004 and Mohrrery and Asad, 2009) reported that supplementation of YC to ruminant rations may improve feed intake, milk production, weight gain, digestion, numbers of anaerobic and celluletic bacteria, ruminal pH value and alter the patterns of volatile fatty acids.

Also, Ahlam (2011) concluded that supplementation of dried yeast to rations of growing goat kid's improved daily gain, feed efficiency, digestibility coefficients, rumen fermentation and utilization and absorption of minerals consequently improving animal performance under desert condition of southern Sinai.

The present study aimed to investigate the effect of yeast culture supplementation level in fattening

lambs rations contained two levels of roughage on growth performance, nutrient digestibility, nitrogen balance, nutritive value and rumen parameters.

2. Material and Methods

This study was carried out at Animal Production Farm, Faculty of Agriculture, Al-Azhar University. Chemical analyses were carried out at Laboratories of Animals Production Department, National Research center, Egypt.

Sixty Ossimi male lambs aged about nine months with an average live body weight being 37.5 Kg were randomly divided into six experimental groups (10 lambs each) in a feeding trial lasted for 120 days. Formulation and chemical composition of mixed experimental rations are presented in Tables 1 and 2, in which the basal rations (control 1 or 2) were differ in roughage ratio. The two basal rations were supplemented with either 0.1 or 0.2 % dry yeast contains 10^8 cells of *Saccharomyces cerevisiae* per gram (Yea-Sacc^{1026}).

Ingredients were mechanically mixed in 10 mm pelleted form and offered to lambs for *ad libitum* consumption with free access to water. At the end of the experiment a conventional digestion trial was conducted using four animals per each group in order to justify rations digestibility, nutritive value and nitrogen balance. Digestion trial consisted of 14 days primarily period for adaptation followed by 5 days as collection period using digestion cages.

During the collection period, feces and urine were quantitatively collected from each animal once a day at 8.0 am before feeding. Actual quantity of feed intake was recorded and water consumption was also recorded. Representative samples of the experimental feed and feces were analyzed for dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE), ash and urinary nitrogen were determined according to A.O.A.C (1995). Nitrogen free extract (NFE) was obtained by difference.

At the end of the digestion trials, rumen samples were collected from animals using stomach tube 4 hrs post feeding and filtered through four layers of cheese cloth for determining different rumen parameters. The pH value was immediately recorded using digital pH Metter, while samples were stored at -20 ^{o}C until chemical analysis. Ruminal ammonia nitrogen (NH_3-N) concentrations were determined applying NH3 diffusion technique using kjeldahle distillation method as described by A.O.A.C. (1995). Ruminal total volatile fatty acids (TVFA's) concentrations were determined by steam distillation procedure according to Warner (1964).

Volatile fatty acid fractions were determined according to Erwin *et al.* (1961) using gas liquid chromatography.

Data were statistically analyzed for analysis of variance (One-way) using the General Liner Model (SAS, 1990)**.** Differences among means were compared ($P< 0.05$) using Duncan's new multiple rang test (Duncan, 1955).

3. Results and Discussion

The basal rations (Rations 1 and 2) differed only in percentage of roughages (30 or 45% bean straw, respectively). These basal rations were supplemented with either 0.1 or 0.2% dried yeast (YC). Formulation of the experimental rations and its chemical composition are shown in Tables 1 and 2. Crude fiber content of rations 2, 5 and 6 were higher than that in rations 1, 3 and 4. This was mainly due to increasing level of bean straw on these rations (45%) compared to rations 1, 3 and 4 which contained 30% bean straw. The values of other nutrients were nearly similar in all the experimental rations.

Lambs Performance:-

Data of average daily gain (ADG), dry matter intake (DMI), TDNI, DCPI and feed conversion are presented in Table 3. Lambs fed YC supplemented rations (3, 4 and 6) showed higher ADG ($P<0.05$) compared with those fed on control rations 1 or 2 and 5. The ADG showed the least value for R2 contained higher CF content. Addition of YC improved ($P<0.05$) ADG. The pronounced effect of YC supplementation was observed for ration low CF content supplemented with 0.1% YC (R3) followed by R4 and R6 which supplemented with 0.2% YC. The highest values of ADG was recorded for ration 3 (192g/day) and the lowest values was recorded for ration 2 (150g/day). Addition of YC showed slight ($P>0.05$) improvement in daily DMI. However, animals fed ration of high CF content without YC supplementation (R2) recorded the lowest daily DMI among the other groups. The same trend was obtained for TDN and DCP intake. Also minor enhancement ($P>0.05$) was obtained in values of feed conversion ratio determined as Kg DMI or TDNI/ Kg gain.

The obtained results on performance agreed with those of Abou Ward (2001) who found that animals fed YC supplemented rations had higher DMI, TDNI and DCPI and lower feed conversion ratio compared with those fed the un-supplemented rations. Similar results were reviewed by many authors (Williamas *et al.*, 1991; Philips and Von Tungeln, 1985 and Cole *et al.*, 1992) who noticed an improvement in performance of heat stressed lambs, when YC was added to their rations. Also, Abou ward (2001) reported that lambs fed rations supplemented with 0.1% YC recorded significant increase in ADG than lambs fed un-supplemented ration. The resulted increase in the dry matter intake with added YC to the

ration may be due providing stimulating factors to rumen celluloytic bacteria (Williams, 1989; Wholt *et al.*, 1988; Williams *et al.*, 1991; Erasmus *et al.*, 1992; Piva *et al.*, 1993; Putnam *et al.*, 1997 and Wholt *et al.*, 1998).

Fallon and Hart (1987) attributed the improvement occurred in animal performance of lambs raised on rations supplemented with YC to the increased palatability of supplemented feeds which lead to an increase in animals feed intake. Chademana and Offer (1990) explained the variable DM intake responses of YC to differences in the nature of rations used, particularly their different contents of readily fermentable carbohydrates. Results of Haddad and Goussous (2005) demonstrated that 3g/d of YC supplementation to finishing Awassi lambs fed high energy rations improves weight gain, ADG and feed: gain ratio.

Digestibility and Nutritive Values:

Results concerning nutrients digestibility, nutritive values and nitrogen utilization are presented in Table 4. Digestion coefficient values for DM, OM, EE, NFE were nearly similar and were not influenced by supplementing the control 1 or 2 with YC at both the two tested levels (rations 3, 4, 5 and 6).

On the other hand, CP and CF digestibility values improved ($P<0.05$) when YC was supplemented to both control1 and 2. Rations 3, 4, 5 and 6 showed higher CP and CF digestibility values compared to the un-supplemented rations. Theses results are in agreement with the previous findings of Gado *et al.* (1998), Harris *et al.* (1992), Abou Ward (2001), Allam *et al.* (2001) and El-Ashry *et al.* (2001). Improving crude fiber digestibility may be attributed to increasing the number of rumen cellulolytic bacteria as a result of yeast supplementation (Williams, 1989 and Gomez-Alarcon *et al.*, 1990). Wiedneier *et al.* (1987) and Newbold *et al.* (1990) who reported that addition of *Saccharomyces cerevisiae* culture to sheep rations did improve the digestibility of dry matter, crude protein and hemicellulose which in turn lead to increase degradability of protein and flow of microbial nitrogen to post ruminal.

On the other hand, Chademana and Offer (1990) found that supplementation of yea- sacc^{1026} did not affect the apparent digestibility of DM, OM, NDE, and CP of hay plus concentrate of different rations. Previous studies (Dowson *et al.*, 1990 and Williams *et al.*, 1991) have reported that the stimulation of cellulose degradation by yeast culture is associated with a decreased log time which results in increased initial rates of digestion, but not in increased extent of digestion by ruminal microorganisms. Williams *et al.* (1991) reported that yeast culture stimulated DM digestion in the rumen of hay fed steers when barley was absent. They attributed this difference to stabilization of ruminal pH by yeast culture in animals receiving barley.

In a subsequent study, Newbold *et al.* (1995) reported that some yeast culture increased the number of total and cellulolytic bacteria in the rumen and in some cases increased cellulose degradation. They also suggested that *Saccharomyces cerevisiae* culture stimulated the rate rather than the extent of fiber digestion by ruminal microorganisms.

El-Waziry *et al.* (2002) observed that N degradability was slightly increased by the addition of yeast. The improvement of digestion coefficients may be attributed to increase in the number of rumen cellulolytic bacteria (Williams, 1989, Gomez-Alarcon *et al.*, 1990).

The results of the present study was also in agreement with those of Erasmus *et al.* (1992) who reported that CP digestibility was significantly increased with yeast culture supplementation. Wholt *et al.* (1998) found that CP digestibility improved by cows fed a ration supplemented with YC. They suggested that such improvement may be due to the greater DM intake by the experimental cows. Wiedmeier *et al.* (1987) reported significant higher CP digestibility values in dairy cattle fed YC supplemented rations. This supplementation could probably provide stimulatory factors toward proteolytic bacteria that significantly higher CP digestibility.

However, Aramble and kent (1990), Williams *et al.* (1991), Mir and Mir (1994) found little or no effect on ration digestibility. Williams and Newbold (1990) suggested that YC may alter the site of digestion and total tract digestibility studies do not give an accurate representation of effects of YC in the rumen.

The nutritive value of the tested rations expressed as TDN and DCP showed insignificant improvement due to supplementing the control rations 1 or 2 with YC at the two tested levels. Theses results may reflected the improvement occurred in nutrients digestibility and the slightly higher feed intake of lambs fed rations supplemented with YC.

Addition of 0.1 or 0.2 % YC significantly ($P<0.05$) increased the resulted values of N-balance for rations contained the low CF content (rations 3 and 4). Addition of 0.1 % YC to the high dietary CF level did not enhance N-balance value (ration 5). However, when such high CF content ration was supplemented with 0.2 % YC, N-balance significantly ($P<0.05$) increased. This means that lambs fed rations 3 and 6 supplemented with 0.1 and 0.2% YC, respectively, retained significant ($P<0.05$) more N than lambs received the control 1 and 2. This might

be attributed to the improvement of crude protein digestibility. This finding agreed with that of Cole *et al.* (1992) who reported that lambs raised on rations supplemented with YC had greater N-balance than the control. Similar trend was reported by Abou Ward (2001), Allam *et al.* (2001) and El-Ashry *et al.* (2001).

The increase in N-balance may be due to the possible increased production of microbial protein synthesis or increased presence of fermentable energy (Tagari *et al.*, 1976), the variability in nitrogen that might escape ruminal fermentation or an increased utilization of ammonia in the rumen (Holzer *et al.*, 1986).

Rumen liquor Parameters:-

Table 5 summarized values of ruminal pH, TVFA's concentration, the molar proportion of individual VFA's and also NH_3-N concentration 4 hrs post feeding. Data showed that pH values significantly increased ($P<0.05$) when the ration was supplemented with YC at 0.1 or 0.2%. This elevation was probably related to the reduction occurred in ruminal lactic acid concentration. These result agreed with those obtained by Newbold *et al.* (1990) and Williams *et al.* (1991) who pointed out a significant reduction in ruminal lactate concentrations accompanied with small elevation in ruminal pH value when YC was added to sheep ration.

Abou Ward (2001) showed that inclusion of YC with the basal ration resulted in a non-significant increase in either pH or TVFA's values. The results of this study agreed with those of Abd El-Ghani (2004) who found that bucks fed YC had higher pH values ($P< 0.05$) at 3 h post feeding compared to the control group. Also, Kamra *et al.* (2002) showed that pH value increased ($P< 0.05$) in rumen liquor of the YC supplemented group.

In contrary, Harrison *et al.* (1988) showed that pH value decreased in the rumen of animals received yeast supplements, while others reported little or no effect of yeast on ruminal pH (Adams *et al.*, 1981; Wiedmeier *et al.*, 1987, Gado *et al.*, 1998 and Zelenak *et al.*, 1994).

Ammonia N concentration significantly ($P<0.05$) decreased due to YC supplementation. These results are in agreement with those of Dawson (1993), Harris *et al.* (1992), El-Waziry *et al.* (2000), Abou Ward (2001) and Kamra *et al.* (2002) who found that addition of YC increased number of anaerobic and cellulitic bacteria.

Yeast culture supplementation tended to reduce ruminal NH_3-N (Harrison *et al.*, 1988 and Newbold, 1990). Lower ammonia concentrations in the rumen of animal fed yeast may increase transformation of ammonia into microbial protein (Harrison *et al.* 1988). According to Wiedmeier *et al.* (1987) the greater concentrations of total anaerobic bacteria in the rumen, might explain why ruminal ammonia concentration are lowered since ammonia is the preferred source of N for large proportion of the ruminal microbial population (Bryant and Robinson 1963). As a conclusion, the lower ammonia concentration in rumen liquor of lamb fed rations supplemented with YC may reflect an accelerate synthesis of microbial protein from ammonia, which in turn would be reflected on fiber digestibility and lamb performance.

Data of rumen TVFA's concentration showed no significant decrease due to supplementing the control rations with YC at both the yeasted levels at 4 hrs post feeding.

Similar results were obtained by Wiedmeier *et al.* (1987) who stated that although YC had to change patterns of VFA's produced by ruminal bacteria, no difference in ruminal TVFA's concentration was detected.

The results of the present study showed significant differences ($P<0.05$) on TVFA's concentrations among treatments.

The reported effect of yeast supplementation on TVFA's concentrations in rumen was inconsistent. In this study, yeast stimulated the production of acetate at the expense of propionate. Similar results were reported by Chademana and Offer (1990), Mutsvangwa *et al.*, (1992), Kumar *et al.* (1994), Zelenok *et al.*, (1994), Newbold *et al.* (1995) and El-Waziry *et al.* (2000). Results of molar proportion of acetate significantly increased ($P<0.05$) while propionate and butyrate molar decreased ($P<0.05$) when the control rations (1 and 2) were supplemented with both levels of YC.

Similar results were obtained by Mir and Mir (1994) when the VFA data were analyzed, yeast supplementation resulted a decreased production of propionic acid when the steers were fed either the corn silage ration or the high grain ration.

However, other authors had found either an increase of propionate of expense of acetate (Adams *et al.*, 1981; Harrison *et al.*, 1988; Newbold *et al.*, 1990; Eramuset *et al.*, 1992; Plata *et al.*, 1994; El-Hassan *et al.*, 1996, El-Badway *et al.*, 1998 and Abou Ward, 2001), or no effect of yeast on VFA concentration (Dawson *et al.*, 1990; Callaway and Martin, 1996 and Kung *et al.*, 1997).

Table 1: Formulation of the experimental rations.

Ingredients	**Control$_1$ (1)**	**Control$_2$ (2)**	**Control$_1$ +%0.1yeast (3)**	**Control$_1$ +%0.2yeast (4)**	**Control$_2$ +%0.1yeast (5)**	**Control$_2$ +%0.2yeast (6)**
Yellow corn	30	30	30	30	30	30
Un-decorticated cottonseed meal	10	10	10	10	10	10
Soya bean meal	15	6.0	14.9	14.8	5.9	5.8
Wheat bran	7.5	-	7.5	7.5	-	-
Urea	-	1.5	-	-	1.5	1.5
Molasses	5.0	5.0	5.0	5.0	5.0	5.0
Bean Straw	30	45.0	30	30	45.0	45.0
Lime stone	1	1	1	1	1	1
Salt	1	1	1	1	1	1
Vit & Min Mix	0.5	0.5	0.5	0.5	0.5	0.5
Yeast culture (yea-sacc) (YC)	-	-	0.1	0.2	0.1	0.2

Table 2: Chemical composition of the experimental rations

Rations Item	**Control$_1$ (1)**	**Control$_2$ (2)**	**Control$_1$ +%0.1yeast (3)**	**Control$_1$ +%0.2yeast (4)**	**Control$_2$ +%0.1yeast (5)**	**Control$_2$ +%0.2yeast (6)**
Dry matter	89.79	90.10	89.81	89.85	90.26	89.88
Ash	10.10	11.42	10.51	10.60	11.35	11.17
Organic matter	89.90	88.58	89.49	89.40	88.65	88.83
Crude protein	13.95	14.00	14.10	14.22	13.99	14.19
Ether extract	3.52	3.00	3.38	3.53	3.11	2.97
Crude fiber	17.21	20.79	17.66	17.74	20.91	20.65
N- Free extract	55.22	50.79	54.35	53.91	50.64	51.02

Table 3: Performance of male lambs fed finishing rations with different levels of roughage supplemented by different levels of YC.

Items	**Control$_1$ (1)**	**Control$_2$ (2)**	**Control$_1$ +%0.1yeast (3)**	**Control$_1$ +%0.2yeast (4)**	**Control$_2$ +%0.1yeast (5)**	**Control$_2$ +%0.2yeast (6)**
Body weight (Kg)						
Initial	37.8±2.9	37.5±2.5	37.7±3.0	38.0±3.2	37.3±3.1	38.0±2.6
Final	57.8±2.1	55.5±2.5	60.7±1.8	60.0±2.2	57.1±1.9	60.0±1.7
Total gain	20	18	23	22	20	22
ADG (gm./h/day)	167^{b}±4.9	150^{c}±4.2	192^{a}±6.0	183^{a}±5.1	167^{b}±5.3	183^{a}±4.8
DM Intake:						
gm/animal/day	1800	1730	1860	1890	1810	1856
TDN Intake:						
gm/animal/day	1118	1017	1190	1204	1104	1165
DCP Intake:						
gm/animal/day	148	131	173	178	156	176
Feed conversion:						
Kg.DMI/Kg. gain	10.8	11.5	9.7	10.3	10.8	10.1
Kg.TDN/Kg. gain	6.7	6.8	6.2	6.7	6.6	6.4

a, b and c Means in the same raw with different superscripts differ (P<0.05)

Table 4: Effect of YC level on nutrients digestibility, nutritive values and rationary nitrogen utilization for rations contained different levels of roughage

Digestibility%	**Control$_1$ (1)**	**Control$_2$ (2)**	**Control$_1$ +%0.1yeast (3)**	**Control$_1$ +%0.2yeast (4)**	**Control$_2$ +%0.1yeast (5)**	**Control$_2$ +%0.2yeast (6)**
Dry matter	66.7±3.1	64.5±3.3	67.3±3.2	66.8±2.8	65.6±3.2	67.9±3.1
Organic matter	68.8±2.8	66.2±2.1	69.4±3.6	68.9±3.9	68.9±3.3	69.9±3.4
Crude protein	58.9^b±2.1	54.4^c±2.9	66.1^a±2.2	66.2^a±2.6	61.3^a±2.4	67.1^a±2.1
Ether extract	83.8±3.4	80.4±3.6	82.4±3.6	82.2±4.0	80.0±4.1	82.3±4.1
Crude fiber	48.3^c±2.4	47.6^c±2.5	57.8^a±2.3	58.1^a±2.7	53.8^b±2.8	58.4^a±2.8
N-free extract	72.1±4.0	70.7±4.6	71.2±4.4	70.5±4.4	70.7±4.5	69.9±5.1
Nutritive values						
TDN	62.1	58.8	64.0	63.7	61.0	62.8
DCP	8.2	7.6	9.3	9.4	8.6	9.5
N-Utilization (gm/h/day)						
Intake	40	39	42	43	41	44
Faecal	16	18	14	15	16	14
Digestable	24	21	28	28	25	30
Urinary - N	21.4	18.6	24.6	24.8	22.3	26.7
Nitrogen balance (gm/h/day)	2.6^b	2.4^b	3.4^a	3.2^a	2.7^b	3.3^a

a, b and c means in the same raw with different superscripts differ (P<0.05)

Table 5: Effect of YC level on rumen liquor parameters of lambs fed different levels of roughage (4 hrs post feeding).

Items	**Control (1)**	**Control (2)**	**Control$_1$ +0.1% yeast (3)**	**Control$_1$ +0.2% yeast (4)**	**Control$_2$ +0.1% yeast (5)**	**Control$_2$ +0.2% yeast (6)**
pH Value	6.1^b ±0.1	6.2^b ±0.2	6.7^a±0.1	6.7±0.1	7.0^a±0.2	7.1^a±0.2
NH3- N mg/dL	35.9^a ±2.0	40.1^a±3.1	25.4^b±2.8	26.6^b±2.3	25.6^b±3.7	25.2^b±2.3
Total VFA's mcq/dL	12.4^a±0.7	10.1^b±0.8	11.9^a±0.5	12.1^a±0.4	9.4^b±0.3	9.1^b±0.9
Acetate, molar%	53.1^b±1.3	55.6^b±2.5	56.3^b±1.4	55.4^b±2.3	60.9^a±1.2	62.4^b±2.5
Propionate, molar%	25.9^a±0.8	22.3^a±1.0	23.0^a±0.7	23.8^a±0.8	18.4^b±0.6	17.1^b±0.7
Butyrate, molar%	17.5^a±0.6	16.4^a±0.4	14.9^b±0.2	15.1^b±0.4	15.2^b±0.3	15.0^b±0.3

a,b Means in the same raw with different superscripts differ (p<0.05)

Conclusion

From the obtained results and the forgoing discussion, it could be concluded that addition of YC to sheep rations containing different levels of roughages improved growth performance determined as daily gain, feed intake and feed conversion ratio. Such supplementation increased crude protein, crude fiber digestibility, nitrogen balance and some rumen parameters. The higher level of inclusion (0.2%) was recommended for higher dietary roughage content.

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References

1. Abd El-Gani, A. A. (2004). Influence of diet supplementation with yeast culture (*Saccharomyces cerevisiae*) on performance of Zaraibi goats. Small Ruminant Research, 52: 223-229.
2. Abou ward, G.A. (2001). Supplementing finishing rations with yeast culture (Yea-Sacc^{1026}) and its influence on lamb's performance. J. Agric. Sci. Mansoura Univ., 26 (5): 2677-2686.
3. Adams, D.C.; M. L. Galyean; H. E. Kiesling; J. D. Wallace and M. D. Finkner (1981). Influence of yeast culture, sodium bicarbonate and monensin on liquid dilution rate, rumen fermentation and feedlot performance of growing steers and digestibility in lambs. J. Anim. Sci., 53:780.
4. Ahlam R. Abdou (2011). Utilization of *Saccharomyces cerevisiae* supplementation for feeding goats in South Sinai. Egyptian J. Nutrition and Feeds, 14 (2):169-181.
5. Allam, M.; K. El- Shazly; B. E. A. Borhami; M. A. Mohamed (2001). Effect of Baker's yeast (*Saccharomyces cerevisiae*) supplementation on digestion in sheep and milk response in dairy cows. Egypt. J. Nutr. & Feed: 4 (special Issue), 315-323.

A. O. A. C. (1995). Official Methods of Analysis. 16th edition. Association of Official Analytical Chemists. Arlington, VA, USA.
6. Arambel, M. J. and A. Kent (1990). Effects of yeast culture on nutrient digestibility and milk yield response in early to mid-lactation dairy cows. J. Dairy. Sci., 73: 1560-1563.
7. Arcos-Garcıa, J. L.; F.A. Castrejon,, G.D. Mendoza, E.P. Perez-Gavilan (2000).Effect of two commercial yeast cultures with *Saccharomyces cerevisiae* on ruminal fermentation and digestion in sheep fed sugar cane tops. Livestock Production Science 63 (2000) 153–157
8. Bryant, M. P. and L. M. Robinson (1963). Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria J. Dairy Sci., 46: 150.
9. Callaway, T. R. and S. A. Martin (1996). Effects of *Saccharomyces cerevisiae* culture on ruminal bacteria that utilize lactate and digest cellulose. J. Dairy Sci., 80: 2035-2044.
10. Chademana, I. and N. W. Offer (1990). The effect of dietary inclusion of yeast culture on digestion in the sheep. Anim. Prod., 50: 483.
11. Chaucheyars-Durand, F and H. Durand (2010). Probiotics in animal nutrition and health beneficial microbes, 1(1):3-9.
12. Cole, N. A.; C. W. Purdy and D. P. Hutcheson (1992). Influence of yeast culture on feeder calves and lambs. J. Anim. Sci., 70: 1682.
13. Dawson, K.A. (1993). Current and future role of yeast culture in animal production. a review of research over the last seven years. page 269 in: "Biotechnology in the feed industry". T.P. Lyons, ed. Altech Tech. Pub., Nicholasville, Ky.
14. Dawson, K. A.; K. E. Newman and J. A. Boling (1990). Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. J. Anim. Sci., 68: 3392.
15. Duncan, D. B. (1955). Multiple Range and Multiple F Tests. Biometric, 11: 1- 42.
16. El-Ashry, M. A. , Zeba A. Motagally and Y. A. Maareck (2001). Effect of live dried eakers yeast and yeast culture on performance of growing buffalo calves. Egypt. J. Nutr. and Feed, 4: 607-617.
17. El- Badawy, A. Y.; H. M., Gado and M. A. Tawila (1998). Influence of rationary yeast culture on the lactation performance of goats. Arab. Univ. J. Agric. Sci., 6: 111-121.
18. El-Hassan, S. M., C. J. Newbold, L. E. Edwards, J. H. Topps and R. J. Wallace (1996). Effect of yeast culture on rumen fermentation microbial protein flow from the rumen and live –weight gain in hulls given high cereal diets. Anim. Sci., 62: 43.
19. El-Waziry, A. M., Kamel, H. E. M. and M. H. M. Yacout (2000). Effect of baker's yeast (*Saccharomyces cerevisiae*) supplementation to berseem (*Trifolium alexandrium*) hay ration on protein digestion and rumen fermentation of sheep. Egypt. J. Nutr. and Feed, 3: 71-82.
20. Erasmus, L. J.; P. M. Botha and A. Kistner (1992). Effect of yeast culture supplement on production, rumen fermentation duodenal nitrogen flow in dairy cows. J. Dairy Sci., 75: 3056.
21. Erwin. E. S.; G. J. Marrco and E. M. Emery (1961). Volatile fatty acids analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci., 44:1768.
22. Duncan, D. B. (1955). Multiple Range and Multiple F Tests. Biometric, 11: 1- 42.
23. Fallon, R. J. and F. J. Hart. (1987). The effect of Yea Sacc inclusion in calf concentrate diets on calf performance. Ir. Grassl. Animal Prod. Assoc. J. 21: 156.
24. Gaafar, H. M. A.; E. M. Abdel-Raouf and K. F. A. EL-Reidy (2010).Effect of fibrolytic enzyme supplementation and fiber content of total mixed ration on productive performance of lactating buffaloes. Slovak J. Anim. Sci., 43:147-153.
25. Gado, H. M.; A.Y. Badawi.; F.L.H. Helal and Sohair A. Nasr (1998). Effect of yeast culture supplementation level on the growth performance of growing goats. Arab Uni. J. of Agric Sci., 1: 123.
26. Gomez- Alarcon, R. A.; C. Dudas and J. T. Huber (1990). Influence of culture of Aspergillus oryzae on rumen and total tract digestibility of rationary components. J. Dairy Sci., 73: 703-710.
27. Haddad , S. G. and Goussous , S. N. (2005) Effect of yeast culture supplementation on nutrient intake, digestibility and growth performance of Awassi lambs. Animal Feed Science and Technology. 118: 343–348.
28. Harris, B. Jr.; D. E. Dominey; W.A. Smith; H. H. Van Hom and C.J. Wilcox (1992). Effects of feather meal at two protein concentrations and yeast culture on production parameters in lactating dairy cows. J. Dairy Sci., 75: 3525.
29. Harrison, G.A.; R.W. Hemken; K.A. Dawson; R. J. Hamon and K.B. Baker (1988). Influence of addition of yeast culture supplement to rations of lactating dairy cows on ruminal function and microbial populations. J. Dairy Sci., 71:2967-2975.
30. Holzer, Z; D. levy and V. Samule (1986). Interactions between supplementary nitrogen source and ration performance and nitrogen utilization in growing and fattening male cattle. J. Anim. Prod., 42: 19.
31. Jouany, J. P.; F. Mathieu; J. Senaud; J. Bohatier; G. Bertin and M. Mercier (1998). The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on the digestion of the cell wall fraction of a mixed diet defaunated and refaunated sheep rumen. Reprod. Nutr. Dev., 38: 401-416.
32. Kamel, H.E. M; Sekine, J.; El-Waziry, A. M. and Yacout, M. H. M (2004). Effect of *Saccharomyces cerevisiae* on the synchronization of organic matter and nitrogen degradation kinetics and microbial nitrogen synthesis in sheep fed Berseem hay (*Trifolium alexandrinum*). Small Ruminant Research, 52:211-216.
33. Kamra, D. N.; L. C., Chaudhury; N., Agarwal; R., Singh and N. N., Pathak (2002). Growth performance, nutrient utilization, rumen fermentation and enzyme activities in calves fed on *Saccharomyces cerevisiae* supplemented ration. Indian J. Anim. Sci., 72: 472-475.

34. Kumar, U.; V. K. Sareen and S. Singh (1994). Effect of *Saccharomyces cerevisiae* yeast culture supplement on ruminal metabolism in buffalo calves given a high concentrate ration. J. Anim. Prod., 59: 209-215.
35. Kung, L. Jr.; E. M. Kreck and R. S. Tung (1997). Effects of a live yeast culture and enzymes on *in vitro* ruminal fermentation and milk production of dairy cows. J. Dairy Sci., 80: 2045-2051.
36. Mathieu, F., Jouany, J. P., Senaud, J., J. Bohatier; G. Bertin and M. Mercier (1996). The effect of *Saccharomyces cerevisiae* and *Aspergillus oryzae* on fermentations in the rumen of faunated and defaunated sheep, protozoal and probiotic interactions. Reprod. Nutr. Dev., 36: 271-287.
37. Mir, Z. and P. S. Mir (1994). Effect of addition of live yeast (*Saccharomyces cerevisiae*) on growth and carcass quality of steers fed high- forage or high-grain and on feed digestibility and *in situ* degradability. J. Anim. Sci., 72: 537.
38. Moharrery, A. and E. Asad (2009). Effect of supplementing malate and yeast culture (*Saccharomyces cerevisiae*) on the rumen enzyme profile and growth performance of lambs. J. Anim. and feed Sci., 18:283-295.
39. Mutsvangwa, T.; I.E. Edwards; J.H., Topps and G.F.M. Paterson (1992). The effect of rationary inclusion of yeast culture (Yea- Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. Anim. Prod., 55: 35-40.
40. Newbold, C. J.; P. E.V. Williams; N. Mc kain; A. Walker and R. J. Wallace (1990). The effects of yeast culture on yeast numbers and fermentation in the rumen of sheep. Proc. Nutr. Soc., 49: 47A.
41. Newbold, C.J.; R. J. Wallace; X.B. Chen and F.M.Mc. Intosh (1995). Different strains *Saccharomyces cerevisiae* differ in their effects on ruminal bacterial numbers *in vitro* and in sheep. J. Anim. Sci., 73:1811.
42. Phillips, W. A. and D. L. Von Tungelin (1985). The effect yeast culture on the post- stress performance of feeder calves. Nutr. Rep. Int., 32:287.
43. Piva G.; S. Belladonna; G. Fusconi and F. Sicbaldi (1993). Effect of yeast on dairy cow performance, ruminal fermentation, blood component and milk manufacturing properties. J. Dairy Sci., 76: 2717-2722.
44. Plata, F. P.; G. D. Mendoza; M. J. R. Barcena-Gama and M. S. Gouzalez (1994). Effect of yeast culture (*Saccharomyces cerevisiae*) on neutral detergent fiber digestion in steers fed oat straw based ration. Animal-Feed Sci. Technol., 49: 203-210.
45. Putnam, D. E.; C. C. Schwab; M. T Socha; N. L. Whitehouse; N. A. Kierstead and B. D. Garthwaite (1997). Effect of yeast culture in diet of early lactating dairy cows on ruminal fermentation and passage of nitrogen fraction and amino acid to the small intestine. J. Dairy Sci., 80, 374-384.
46. Robinson, P. H. and J. E. Garrett (1990). Effect of Yeast Culture (*Saccharomyces cerevisiae*) on Adaptation of Cows to Postpartum Diets and on Lactational Performance. J. Anim.Sci., 77: 988-999.
47. SAS Institute (1990). SAS® / STAT User's Guide: Statistics. Version 6, 4th Edition. SAS Institute Inc, Cary, NC.
48. Tagari, H., D. Levy; Z. Holzer and D. Jlan (1976). Poultry litter for intensive beef production. Anim. Prod., 23: 317.
49. Warner, A. C. I. (1964). Production of volatile fatty acids in the rumen: methods of measurements. Nutr. Abst. Rev., 34: 339.
50. Wholt, J. E.; T.T. Corcione and P. K. Zajac (1998). Effect of yeast on feed intake and performance of cows fed rations based on silage during early lactation. J. Dairy Sci., 81: 1345.
51. Wiedmeier, R. D.; M. J. Arambel and J. L. Walters (1987). Effects of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestion. J. Dairy Sci., 70: 2063.
52. Williams, P. E. and G.J. Newbold (1990). Rumen probiosis: The effects of novel microorganisms on rumen fermentation and ruminant productivity. In: S. W. Haresign and D. J. A. Cele (ed). Recent Advances in Animal Nutrition, P 211. Butterworth, London.
53. Williams, P. E.; G. A. G. Tait; G. M. Innes and G. J. Newbold (1991). Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* Plus growth medium) in the ration of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci., 63:3016.
54. Williams, P. E. (1989). The mode of action of yeast culture in ruminal rations. A review of the effect on rumen fermentation patterns. Biotechnology in the feed Industry, Altech-Tech. Publishers. Nicholasville, Kentucky, USA, pp.65.
55. Wohlt J. E., Corcione, T. Tand Zajac P. K. (1988).Effect of yeast on feed intake and performance of cows fed diets based on corn silage during early lactation. J Dairy Sci., 81(5):1345-52.
56. Zelenak, I.; D. Jalc; V. Kmet and P. Siroka (1994). Influence of ration and yeast supplement on *in vitro* ruminal characteristics. Anim. Feed Sci. and Tech., 49:211.

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Intelligent Fault Detection of Ball bearing Using FFT, STFT Energy Entropy and RMS

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Abstract: According to the non-stationary characteristics of ball bearing fault vibration signals, a ball bearing fault detection method using FFT, STFT energy entropy and root mean square is put forward. In this paper, first, original rushing vibration signals are transformed into a frequency domain, then, the STFT transformation is calculated in the way that first the frequency resolution and then the time resolution has been assumed to be high. Then the theory of energy entropy mean and root mean square is proposed. The analysis results from energy entropy and root mean square of different vibration signals show that the energy and root mean square of vibration signal will change in different frequency bands when bearing fault occurs. Therefore, to diagnose ball bearing faults, we run the test rig with faulty ball bearing in various speeds and loads, and collect vibration signals in each run; then, we calculate the energy entropy mean and root mean square which are indicators of the type of faults. The analysis results from ball bearing signals with three different faults in various working conditions show that the diagnosis approach based on the utilization of, STFT and FFT for extracting the energy and root mean square of different frequency bands can identify ball bearing faults accurately and effectively. We have optimized signal decomposition levels with the use of analysis, and then, interestingly enough, we have introduced a new method to effectively diagnose different faults of rolling bearings.
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Keywords: Ball bearing fault diagnosis, components, root mean square, Energy entropy mean.

1. Introduction

The vibration signals of a ball bearing operating with faults will present non-stationary characteristics, and how to extract the fault characteristic information from the non-stationary vibration signals is the crux of the ball bearing fault diagnosis [1-3]. This is performed in traditional diagnosis techniques with the waveforms of the fault vibration signals in the time domain, and thus, construct the criterion functions to identify the working condition of roller bearings. However, because the nonlinear factors such as loads, clearance, friction, stiffness and soon have distinct influence on the vibration signals due to the complexity of the construction and working condition of ball bearings, it is very difficult to make an accurate evaluation of the working condition of roller bearings through the analysis in time domain only [4,5]. FFT analysis have had extensive use in diagnosis of faults of roller bearings, as they are both capable of extracting time and frequency local features of the signal [6-8]. Due to the limitation of the length of the FFT bases, energy leakage will occur in FFT transformations. Moreover, Fourier analysis has been the most widely used analysis method of signals for the detection of bearing faults. However, there are some crucial restrictions of the Fourier transform [6]: the signal to be analyzed must be strictly periodic or stationary; otherwise, the resulting Fourier spectrum will make little physical sense. Unfortunately, the vibration signals of rolling bearings have often non-stationary nature, and indicate non-linear processes; moreover, their frequency components can vary over time. Therefore, the Fourier transform often fails to pretty successfully diagnose the type of faults occurred in rolling bearings. On the other hand, since in time–frequency analysis methods the one-dimensional signal is mapped to a two-dimensional time–frequency plane, the information of both of time and frequency domains of a signal can be simultaneously produced. Therefore, in the later studies, the time–frequency analysis methods are widely used to detect the faults in bearings since they can determine not only the time of the impact occurring but also the frequency ranges of the impact location, and hence can determine not only the existence of faults but also the causes of faults [9].

In this paper, FFT, STFT is applied to the ball bearing fault diagnosis. First, the original acceleration vibration signals is transformed into a frequency, STFT domain, then the concept of energy entropy mean and root mean square is proposed, which defined by calculating the mean value of the vibration signal entropies and root mean square of a bearing with a fault in different various speeds a loads. By studying the energy entropy means and root mean square of different working condition signals we illustrate that it will change when different bearing fault occurs. Similarly, the original signal is decomposed by the wavelet packet, and then the energy entropy mean are extracted accordingly from the time series that are obtained after the wavelet coefficients are reconstructed. To diagnose ball bearing faults, we run the test rig with faulty ball bearing in various speeds and loads and collect vibration signals in each run, and then calculate the energy entropy mean in frequency domain and root mean square in frequency domain which indicate the fault types.

1. Experimental Procedure

Three data sets each containing twenty data files were collected from three bearings which are the same but with different faults. The first data file was collected from each test bearing when the loading was zero and the bearing was running at the highest speed (2000 rpm).The load was then increased step by step, the speed was kept at 2000rpm, and four other data files were collected. The load was then brought back to zero and speed was decreased by 1000 rpm and the next five data files were collected under five different loads similar to the first five data files. This procedure was continued until all twenty five sets of data were collected. The sampling frequency was chosen as 41.67 kHz, this sampling frequency along with the data record size of 4098 guarantees that the sampling procedure covers at least 1.6 revolutions of shaft at the lowest speed.

2.1- Test Bearings

An impact impulse is generated every time a ball hits a defect in the raceway or every time a defect in a ball hits the raceway. Each such impulse excites a short transient vibration in the bearings at its natural frequencies. Each time this defect is rolled over an impact is produced the energy of this impact depends on the severity of the defect Many failure modes of a rolling element bearing produce such a discontinuity in the path of the rolling elements, Moreover the majority of rolling element bearing failure cases begin with a defect on one of the raceways. In this research defects on inner raceway (IRD), outer raceway (ORD), balls (BLD) and abrasive in cage (ABR), poor lubrication (PRL) defect were introduced in the form of scratches. These scratches provide the aforementioned discontinuity in the path of rolling elements. Therefore a rolling element bearing with a nick or a fatigue spall or even a brindled bearing affects the time domain signal very similar to a bearing which has a scratch on one of its components.

2.2- Fast Fourier transform (FFT)

A Fast Fourier transform (FFT) is an efficient algorithm to compute the discrete Fourier transform (DFT) and its inverse. There are many distinct FFT algorithms involving a wide range of mathematics, from simple complex-number arithmetic to group theory and number theory. An overview of the available techniques and some of their general features has been presented in this article.

A sequence of values is decomposed into different frequency components through using a DFT. Though this operation is effective in many fields (see discrete Fourier transform for properties and applications of the transform), it is often too time-consuming to be practically computable from the definition. On the other hand, an FFT is able of quickly computing the same result; that is, the computation of a DFT of N points with the use of the definition takes O (N^2) arithmetical operations, while an FFT computes the same result with only O (N log N) operations. The computation speed in these two methods is substantially different, particularly for long data sets with N of the order of thousands or millions; thus, the computation time in such cases can be practically reduced by several orders of magnitude, and also, the improvement is approximately proportional to N/log (N). This huge improvement has made many DFT-based algorithms practical; FFTs are of great importance to a wide variety of applications, from digital signal processing and solving partial differential equations to algorithms for quick multiplication of large integers [10,11].

3. Short-Time Fourier Transform

The Short-Time Fourier Transform (STFT) (or short-term Fourier transform) is a powerful tool for the purpose of signal processing which characterize a specifically useful class of time-frequency distributions which can indicate complex amplitudes versus time and frequency for any signal. The following formula gives the definition of the STFT transformation:

$$sf(b.w) = \int_{-\infty}^{+\infty} f(t)g(t-b)e^{-iwt}dt \qquad (1)$$

As it can be seen in E. (1), STFT is a time-frequency transformation; that is, it represents all the information of time, frequency and domain of the signal simultaneously. STFT is an indication of energy conservation law which states that:

$$\int_{-\infty}^{+\infty} \left|g(t)\right|^2 dt = 1 \qquad (2)$$

With the use of Parsoval equation 2, the following relation can be obtained:

$$\int_{-\infty}^{+\infty} \left|f(t)\right|^2 dt = \frac{1}{2p}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} \left|sf(b.w)\right|^2 dbdw \qquad (3)$$

Using the short-time Fourier transformation, the signal can be revised as the following equation:

$$f(t) = \frac{1}{2p}\int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} sf(b.w)g(t-b)e^{iwt}dbdw \qquad (4)$$

The weakness of STFT it is impossible to have high resolution in both time and frequency domains.

The Discrete Fourier Transform (DFT) is an invertible transform and an important tool widely used in signal processing and analysis. DFT can be computed with the use of stable efficient algorithms known as Fast Fourier Transform (FFT) algorithms. Its applicability is for cases with discrete time and frequency variables. Let x_n and X_k respectively represent the discrete time signal and the discrete frequency transform function. The DFT is given by

$$X_k = \sum_{n=0}^{N-1} x_n e^{-j2pkn/N},\ k = 1,\ 2,\ldots,N \qquad (5)$$

Where

$$x_n = \frac{1}{N}\sum_{k=0}^{N-1} X_k e^{j2pkn/N} \quad (6)$$

Root Mean Squared (RMS) AND Energy entropy

The RMS value of a signal is directly related to the energy or destructive ability of the signal. Energy and root mean square of a signal are obtained in equation (7) and (8)

$$E = \sqrt{\sum_{i=1}^{n} |x(i)|^2} \quad (7)$$

$$X_{rms} = \sqrt{\frac{1}{N}\sum \left[x(n)\right]^2} \quad (8)$$

Where x (i) the amount of vibration signals have sampling point i and n total number of samples used to are. If the vibrations signal to the original components of the analysis, we formed m components separately and we will calculate the energy of each component to the set the energy distribution reached. Because each component Posts Contents are different frequency, energy distribution consisting of a space frequency can be

$$E = \{E_1, E_2, ...E_m\} \quad (9)$$

Energy and entropy as defined in [20]:

$$H_{EN} = -\sum_{i=1}^{m} P_i \log pi \quad (10)$$

Where $P_i = \frac{E_i}{E}$ is the percent of the energy of ith in the whole signal energy $E = \sum_{i=1}^{m} E_i$.

4. Results and discussion

4.1- FFT Energy Root Square and Energy Entropy

In this stage, the original signal was transformed into the frequency domain; then, three dominant frequency band widths were observed. These three ranges are shown in Tables (1). Thus the values of energy root mean squares (RMS) in each of these ranges were calculated according to Eq. (8); moreover, the sum of RMS in all three ranges was obtained. Thereafter, the ratios of the energies of each of these ranges to the total energy were calculated; furthermore, with the use of Eq. (10), the entropy energies for each of three types of faults were obtained. Here, from the values shown in the table, it can be simply inferred that both of these criteria; i.e., entropy energy and RMS, can be utilized for the purpose of fault diagnosis.

Also in this section, the original acceleration vibration (Fig.1), frequency spectrum (Fig.2-4) of signal for three type faults at 2000 rpm speed and 1000N load are shown. The FFT energy Root mean squares and energy entropy for three different faults at 2000 rpm speed and 1000N load are shown in table 1 and 2.

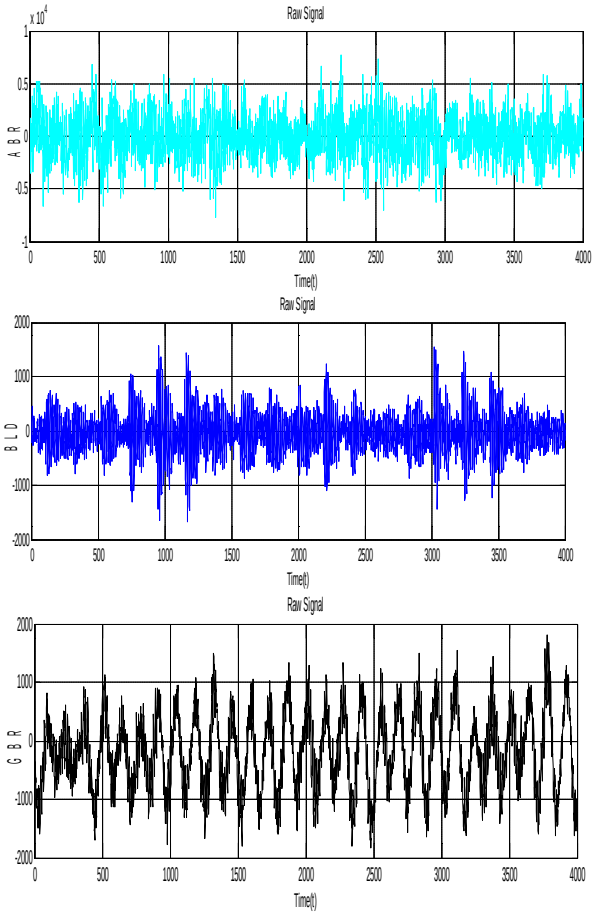


Fig.1. Original acceleration vibration of the signal for three different faults

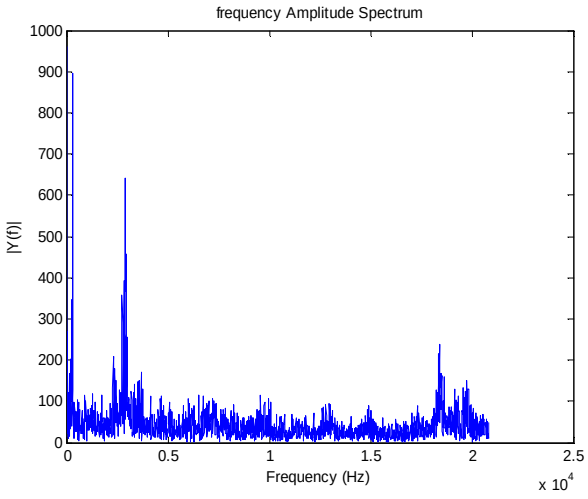


Fig. 2. Amplitude spectrum of the signal for ABR faults at 2000 rpm speed and 1000N load.

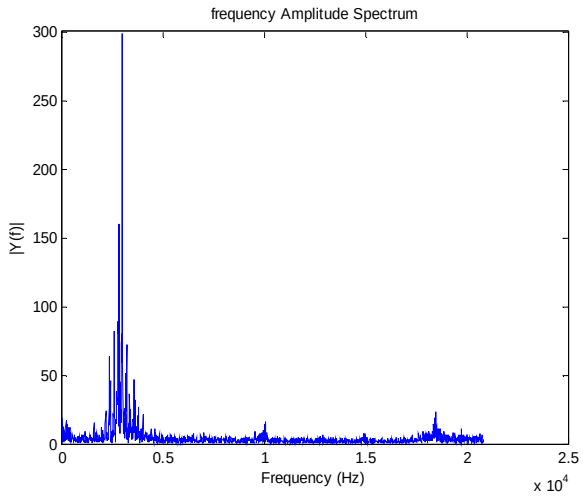


Fig. 3. Amplitude spectrum of the signal for BLD faults at 2000 rpm speed and 1000N load.

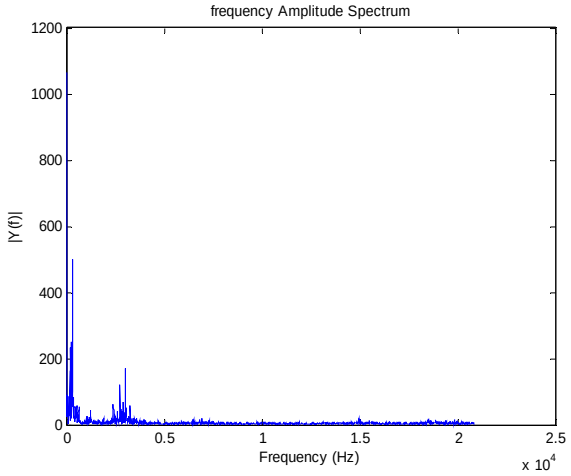


Fig. 4. Amplitude spectrum of the signal for GBR faults at 2000 rpm speed and 1000N load.

Table 1. The FFT energy root mean square for three different faults at 2000 rpm speed and 1000N load.

frequency-band (HZ)	ABR	BLD	GBR
10_325	26.1151	1.0483	17.3036
2760_3035	28.7072	9.9035	6.4114
18330_18560	12.9435	1.1062	0.7805

Table 2. The FFT energy entropy for three different faults at 2000 rpm speed and 1000N load

ABR	BLD	GBR
0.9761	0.9998	0.5149

.

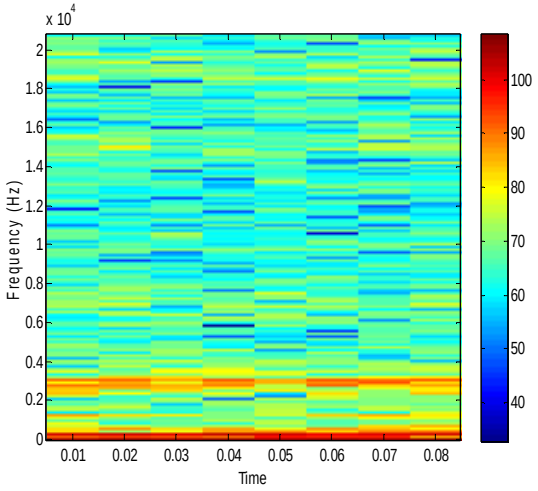
4.2- STFT Energy Root Mean Square and Energy Entropy

In this stage, we have used two approaches to choose the window. In the first approach, time resolution has been increased; thus, frequency resolution would have been decreased. Furthermore, the second approach has been considered to be the opposite of the first approach.

In the first approach, a matrix with the dimensions 417*8 has been obtained whose frequency and time axes have been divided into 417 and 8 parts, respectively. The column windows can be clearly seen form Fig. (5) Which are, indeed, the time windows. Moreover, as it has been illustrated in Fig.(6), in the second approach, a matrix with the dimensions 417*8 has been obtained whose frequency and time axes have been divided into 10 and 398 parts, respectively.

Thereafter, at the next stage, the corresponding values of RMS for one of the column windows of Fig. (5), including the frequency range of [2000-3000], and also for one of the row windows of Fig. (6), containing the range of frequencies of [100-1200], have been calculated with the use of Eq.(8). Then, these calculated values have been divided by the value of RMS of the total signal. Moreover, the entropy energy has been calculated for the row window of Fig. (6), and the obtained results have be tabulated in Tables (3, 4, and 5), respectively. It should be pointed out that we have chosen these frequency ranges in view of their higher values of amplitudes.

(a)



(b)

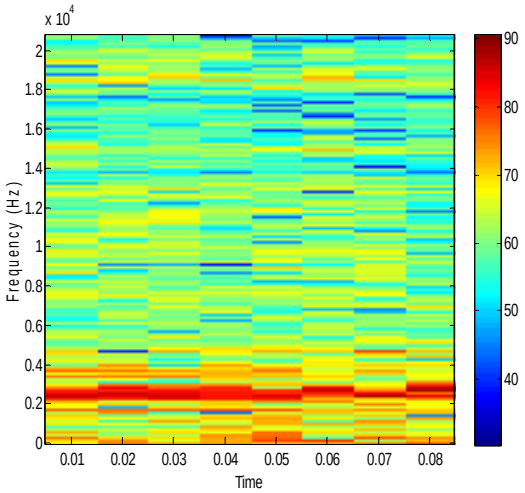
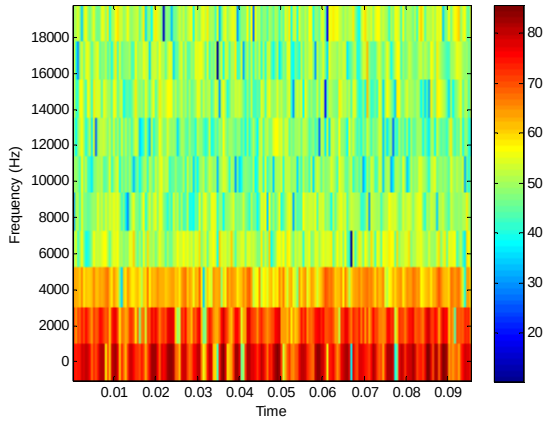


Fig. 5. STFT of the signal for two different faults at 2000 rpm speed and 1000N load (frequency resolutions are increased): (a) GBR, (b) BLD.

(c)



(d)

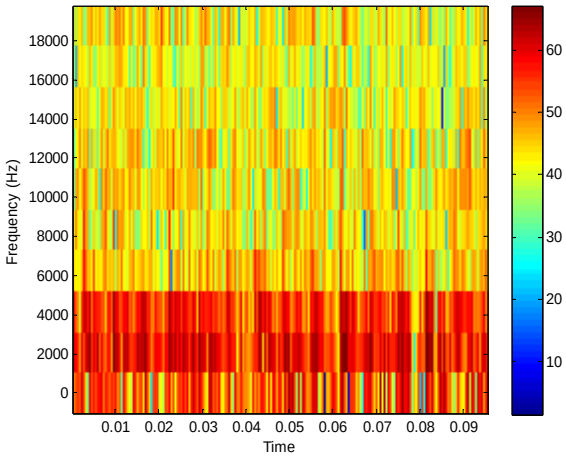


Fig. 6. STFT of the signal for two different faults at 2000 rpm speed and 1000N load (time resolutions are increased): (a) GBR, (b) BLD.

Table 3. The STFT energy root mean square for row window for three different faults at 2000 rpm speed and 1000N load

ABR	BLD	GBR
0.0139	0.0552	0.0023

Table 4. The STFT energy root mean square for column window for three different faults at 2000 rpm speed and 1000N load.

ABR	BLD	GBR
0.015	0.0022	0.0001

Table 5. The STFT energy entropy for three different faults and 1000N load.at 2000 rpm speed

ABR	BLD	GBR
0.3569	0.3390	0.1420

References:

[1] N. Tandon and A. Choudhury, "A review of vibration and acoustic measurement methods for the detection of defects in rolling element bearings," *Tribology Int.*, 32 pp.469–480,1999

[2] D. Ho, R.B. Rand, "Optimization of bearing diagnostic techniques using simulated and actual bearing fault signal," *Mechanical Systems and Signal Processing* 14 (5) (2000) 763–788.

[3] Z. Peng, F. Chu, Y. He, "Vibration signal analysis and feature extraction based on re-assigned wavelet scalogram," *Journal of Sound and Vibration* 253 (5) (2002) 1087–1100.

[4] C.J. Li, S.M. Wu, "On-line detection of localized defects in bearings by pattern recognition analysis,"

ASME Journal of Engineering for Industries 111 (1989) 331–336.

[5] P.W. Tse, Y.H. Peng, R. Yam, "Wavelet analysis and envelope detectionfor rolling element bearing for rolling element bearing fault diagnosistheir affectivities and flexibilities," *Journal of Vibration and Acoustic* 123 (2001) 303–310.

[6] J. Ling, L. Qu, "Feature extraction based on Morlet wavelet and its application for mechanical fault diagnosis," *Journal of Sound and Vibration* 234 (1) (2000) 135–148.

[7] F. Tavakkoli, M. Teshnehlab, "A ball bearing fault diagnosis method based on wavelet and EMD energy entropy mean," *International Conference on Intelligent and Advanced Systems* 2007.

[8] D.E. Newland, "Wavelet analysis of vibration," part I: theory. *J Vib Acoustics* 1994;116:409–16.

[9] DE Newland, " Wavelet analysis of vibration, part II: wavelet maps", *J Vib Acoustics* 1994;116:417–25.

[10] N. Brenner and C. Rader, 1976, A New Principle for Fast Fourier Transformation, IEEE Acoustics, Speech & Signal Processing 24: 264-266.

[11] Brigham, E.O. (2002), The Fast Fourier Transform, New York: Prentice-Hall

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Study on the biology and epidemiology of Uncinula necator the causal agent of grape powdery mildew disease

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Abstract: This study was carried out during 2007-2009 in the vineyards of Ardabil province of Iran to study the biology and the epidemiology of Uncinula necator the causal agent of grape powdery mildew disease. The study concentrated on the survival and the initiation of primary inoculum of the fungal causal agent. Results of histopathological experiments indicated that U. necator survived as mycelium in the dormant buds of the grapes during winter season. Results of study on the effect of environmental factors on fungus biology showed that the pathogenic activity of the fungus began when the temperature was between 16-19°C with a relative humidity more than 50%. It was also found that optimum temperature and relative humidity for the sporulation of U. necator was 20-25°C and 50-100% respectively. According to the results, fungal conidia were trapped during formation of 5-6 true leaves and first disease symptoms were observed on the clusters on late June after fruit formation. Fungal cleistothecia were observed abundantly at the end of season on the leaves, petioles and twigs but they were not able to survive during winter. Formation of ascospores on young leaves was proved but their role as the primary inoculum was not supported by the results of this study. Results of this study and the new findings on the biology and epidemiology of U. necator may be of national and international interests for the management of powdery mildew disease which is one of the most destructive diseases around the world including Iran.

[H. Karbalaei Khiavi, H. Shikhlinskiy, A. Babaei Ahari & Mohammad Akrami. **Study on biology and epidemiology of Uncinula necator the causal agent of grape powdery mildew disease.** *Life Sci J* 2012;9(3):1787-1792] (ISSN:1097-8135). http://www.lifesciencesite.com. 258

Key Words: Grape, Powdery mildew, Uncinula necator, Biology, Epidemiolog

1. Introduction:

Grape is one of the most important fruits around the world and it is believed that has been cultivated in many regions of the world since thousands years ago. Over the time different pests and diseases have been spread to grape vineyards and have become one of the most important yield reducing factors in the world vineyards **[3]**.

Powdery mildew disease caused by plant pathogenic fungus, Uncinula necator is one of the most important and destructive diseases of grape in many countries of the world including Iran **[1, 3, 5, 9, 15, 20]**. It can cause serious damages and loses to grape production in conducible environmental conditions, affect the grape production and the yield quantitatively and qualitatively and increase the production cost significantly **[3, 4, 7, 13]**. The disease was reported from Iran grape vineyards in 1946 for the first time and since then it has been observed in many grape growing provinces of the country **[3]**. It is believed that U. necator the causal agent of the disease has first been identified in North America and then it has been spread to Europe before 1840,s and has officially been reported from Europe in 1945. In general almost all Vitis vinifera cultivars and its hybrids are susceptible to powdery mildew disease **[13]**. Studies on the life cycle of the fungal agent of the disease have shown that it usually survives in the dormant buds of the plant as mycelium **[12, 14, 16]**. Most researchers believe that the sexual stage of the fungus does not play an important role in disease cycle **[12, 14, 16]**. Sexual form of the fungus as cleistothecium on older leaves and twigs has been reported from Iranian vineyards **[1]**. However, their roles and importance in disease biology and epidemiology have not studied yet. Some studies have investigated the biology and epidemiology of U. necator previously. For example **Brunelli [2]** reported that the fungus has two biotypes and is capable of overwintering as cleistothecium and mycelium. Formation of cleistothecium at the end of season on grape branches and leaves have been reported by several studies conducted in USA **[2]**, France **[18]**, Australia **[17]** and Iran **[1]**. In Italy and USA, researchers have reported cleistothecia as the primary inoculum of the disease **[2, 14]**. Germination of ascospores of U. necator was reported for the first time in 1895 **[9]**. **Galloway [10]** reported that fungal ascospores did not play important roles in powdery mildew disease incidence. They also believed that

production of ascs in disease cycle was not pathologically important.**Cortesi et al. [5**] reported that fungus survival in the winter (overwintering) was not well known and it may survive as ascocarp or mycelium.

In Aedebil province of Iran where this study was carried out, primary inoculum of U. necator for disease occurrence in vineyards is not well known. This study was therefore conducted and executed during 2007–2009 to study and investigate the biological and epidemiological aspects of the disease including survival, overwintering and primary inoculums formation in relation with environmental conditions and plant phenology.

2.Materials and Methods:

2.1.Visiting provincial vineyards for disease monitoring

During March-November 2007-2009, different vineyards in the province were visited routinely and fungus survival (overwintering), disease incidence and symptoms development in relation with plant phenology were investigated. Major grape varieties grown in these regions included Keshmeshi, Rasmi and Shahani belonging to Vitis vinefera species.

2.2.Investigation of fungal conidia and ascosporic release

In this experiment, for determination of primary and secondary infection sources, in each vineyard, 20 wooden stands with the height of 25, 50, 75 and 100 cm respectively were placed between the rows in five selected locations and microscopic slides covered with Vaseline were carefully placed on the upper and side surface of each stand for spore trapping. Slides were carried to the laboratory every 3 days, were stained with cotton blue-lacto-phenol solution and the number of trapped spores were determined and recorded using a light microscope.

2.3.Examination of dormant twigs for survival of fungal mycelium

During March 2007-2009, and before budding stage, grape dormant buds were collected in experimental sites and were carried to the laboratory for further processing. Collected samples were then examined for the presence of U. necator mycelium by fixation, staining, molding in paraffin and microtome profiling using procedure described by **Gee et al. [11]**. Permanent profiles were then prepared from leaf fragments using glycerol gel and were examined under a light microscope for the detection of fungal mycelium.

2.4. Investigation of fungal ascospore release

During the month of October 2007-2009, infected grape leaves containing fungal cleistothecia were collected from experimental vineyards, were cut to 5-cm pieces, were placed in cloth bags and were hanged on woody stand for ascospore release. During November through May 2007-2009 the samples were examined every two weeks for the release of ascospores according to the procedures described by Pearson and Gaudry **[13]**. For the collection of cleistothecia, 10 peaces of the leaves were placed in a flask containing 100 ml of distilled water and were shaken for release of the cleistothecia. Distilled water in the flask containing cleistothecia was then screened twice using micro screens and fungal ascocarps were collected and were placed on paper disks. Paper disks were then placed in a moist petri dish containing a microscope slide. Release of fungal ascospores was determined after 24 hours by examining the slides under a light microscope.

2.5.Investigation of cleistothecia survival during the season

During October to May 2007-2009 infected twigs, leaves and surface soil were collected every months using the procedure described by **Cortesi et al. [5]**. Fungal ascocarps were examined under a light microscope and viability of ascospores was determined in fluorecin diacetate solution after 5 minutes according to the method described by **Gee et al. [11]**. Viability determination of ascocarps was based on the production of green fluorescent pigment by at least 50% of the ascospores **[11]**.

2.6.Evaluation of pathogenicity of fungal ascospores

To investigate the pathogenicity of U. necator, infected leaves revealing disease symptoms were collected from experimental vineyards and were taken to the laboratory for further processing. Infected leaves were placed in 9-cm diameter Petri dishes, were surface sterilized and were then transferred into other Petri dishes containing 25 ml water agar (WA) medium and 100 ppm benzimidazole fungicide. Four moist paper disks each containing 20 fungal cleistothecia were placed inside the lid of each Petri dish. Leaves were then examined for the infection caused by fungal ascospores **[8]**.

2.7.Investigation of the effect of environmental factors on U.necator sporulation

The impact of environmental factors including temperature and moisture on the germination of fungal conidia in invitro condition was evaluated according to the procedure described by Spencer **[19]**. The impact of weather conditions including rain and

air temperature on the release of fungal spores and disease incidence were also investigated using data obtained from provincial metrological service.

Results

Powdery mildew disease symptoms on the grape plants did not appear in the experimental vineyards until mid June. However, various disease symptoms including fungal conidia, tissue discoloration and fruit deformation appeared on the upper surface of the leaves, on the underside of the leaves, on the fruit and on the shoot. Fungal asexual and sexual structures are are on the leaves, fruits and twigs. According to the results of cytological tests in our study, it was shown that the survival of the causal agent of the disease (U. necator) during winter (overwintering) took place as mycelium in the dormant buds of the grape plants (Fig. 1). Results of the study also indicated that fungal conidia were released from late May to early September. These conidia were shown to be the primary inoculum of the disease. First conidia were trapped around late May in our experiments.

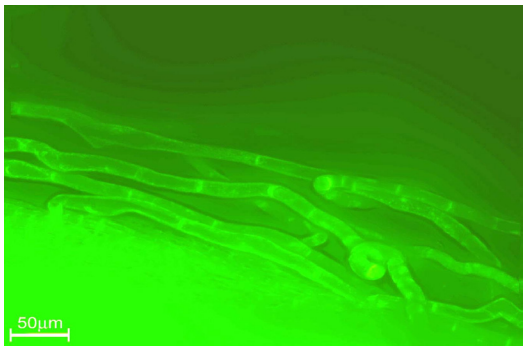

Figure 1. Hyphae of powdery mildew in h of a grape dormant bud

According to the results, first disease symptoms were observed on leaf and fruit in mid June and sexual forms of the fungus (ascocarps) were detected on the infected plants in early October (Fig. 2).

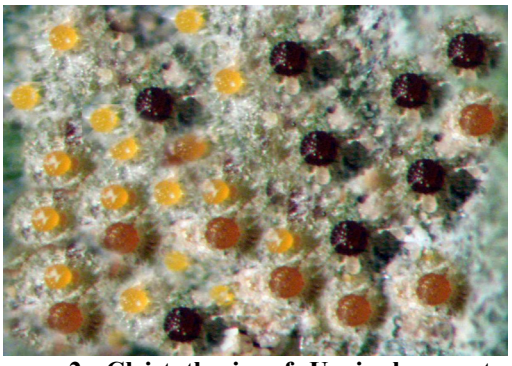

Figure 2. Cleistothecia of Uncinula necator in various stages of maturity on grape leaf

Results of the study on the effects of environmental factors on sporulation of U. necator indicated that in experimental vineyards conidia release began in late May when temperature was about 16-19°C and the maximum conidia release was observed when air temperature reached 20-25°C (Table 1). The optimum temperature and relative humidity for fungal conidia germination were 25°C and 40-100% respectively (Table 1 and 2). In temperature below 20°C conidia release was gradually reduced. The maximum temperature for conidia germination was found to be 34°C and it was stopped when temperature exceeded 34°C (Table 2).

Table 1. Effects of temperature in the germination of Uncinula necator conidia at different time intervals

No.	Temperature (°C)	Time before germination begins (hr)	Germination (%)	Maximum germination (%)	Time before maximum germination occurs (hr)
1	7	19	0.24	5	29-34
2	10	18	1.23	7	29-34
3	13	11	1.60	11	27-29
4	16	11	2.95	13	22-25
5	19	2	5.30	28	21-25
6	22	2	15.00	62	18-22
7	25	2	18.50	78	14-18
8	28	2	15.75	63	11-15
9	31	2	6.10	27	6-10
10	33	2	0.65	-	0-2
11	34	-	-	-	-

Table 2. Effects of relative humidity on the germination of Uncinula necator the causal agent of powdery mildew disease

No.	Relative humidity (%)	Conidia germination (%)
1	10	5
2	20	13
3	40	68
4	60	75
5	80	76
6	100	79

4. Discussion:

Results of our study in the role of cleisthothecia in the survival and overwintering of U. necator showed that cleistothecia were formed abundantly on the leaves and branches at the end of season, but these cleistothecia could not resist and survive during the winter. These findings did not support the roles of cleistothecia in fungal survival and pathogenesis. Our results agree with those of previous studies which have indicated that cleistothecia do not play important roles in the occurrence of powdery mildew disease **[9, 11, 18]**. In these studies winter surviving cleistothecia on the leaves were tested for the pathogenicity, but they failed to cause disease by lack of symptoms induction **[8, 9, 11,18]**. We obtained the similar results in this study and our test cleistothecia did not induce any disease symptoms on the young leaves when the leaves were inoculated with these cleistotehcia.

In a previous study, **Gadouri and Pearson [14]** proved that when grape leaves carrying ascocarpes were buried in the soil, all ascocarps lost their viability **[8]**. The results of our experiments in this regard agree with those of above-mentioned study. In our study it was also found that no viable ascocarp was detected in the leaves which were collected from the vineyards soil in early spring.

The results of our study on the impact of environmental factors on fungal sporulation agree with those of previous studies carried out by **Delp [6], Built and Lafon [3], Pearson and Goheen [15] and Spencer [19]**. According to our results and their findings environmental factors including temperature and relative humidity are very important and play critical roles in fungal conidia and ascospore release.

The overall results of this study on biology and epidemiology of U. necator the causal agent of grape powdery mildew disease provide some novel information for better understanding of the interactions among environmental factors, the host plant and the pathogen. To select and choose more effective control methods for a certain disease on a given plant and in a given area, study of the interactions among the above factors is very important. The outcomes and findings of this study may therefore be used effectively in formulation of management strategies to combat and overcome the powdery mildew disease which is one of the most destructive and damaging diseases of the grape around the world including Iran.

5. Conclusion:

Generally, Results of histopathological experiments indicated that U. necator survived as mycelium in the dormant buds of the grapes during winter season. Results of study on the effect of environmental factors on fungus biology showed that optimum temperature and relative humidity for the sporulation of U. necator was 20-25°C and 50-100% respectively. According to the results, fungal conidia were trapped during formation of 5-6 true leaves and first disease symptoms were observed on the clusters on late June after fruit formation. Fungal cleistothecia were observed abundantly at the end of season on the leaves, petioles and twigs but they were not able to survive during winter.

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References

1. Banihashemi Z, Parvin S, The occurrence of ascigenous stage of Uncinula nector var. necator in fars. Iran.Plant Pathol. 1995;31:1- 4.
2. Brunelli A, Cortesi P, Faretra F, Population biology of Uncinula necator. Faretra Francesco, University of Bari, Res. Prog. year. 2004;23:234-245.
3. Built J, Lafon R. Powdery mildew of the vine. In: The Powdery Mildew. 3rd ed. Spencer DM, Academic Press. New York, USA. 1978;pp.525-548.
4. Calonnec A, Cartolaro P, Poupot C, Dubourdieu D, Darriet A, Effects of Uncinula necator on the yield and quality of grapes (Vitis vinifera) and wine. Plant Pathol. 2004;53:434-445.
5. Cortesi P, Bisiach M, Ricciolini M, Gadoury DM, Cleistothecia of Uncinula necator an additional source of inoculum in Italian vineyards. Plant Dis. 1997;81:922-926.
6. Delp CL, Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathol. 1954;44:515-525.
7. Evants KJ, Whisson L, Scott ES, An experimental system for characterizing isolates of Uncinula necator. Mycol. Res. 1996;100(b):675-680.
8. Gadoury DM, Pearson RC, Initiation, development, dispersal and survival of cleistothecia of Uncinula necator in New York vineyards. Phytopathol. 1988;78:1413-1421.
9. Gadoury DM, Seem RC, Pearson RC, Wilcox WF, Effects of powdery mildew on vine growth, yield and quality of Concord grapes. Plant Dis. 2001; 85:137-140.
10. Galloway BT, Observation on the development of Uncinula spiralis. Bot. Gaz. 1895;20:488-493.

11. Gee L, Stummer BE, Gadoury D, Biggins LE, Scott ES,. Maturation of cleistothecia of Uncinula necator and release of ascospores in Australia. Austr. J. Grape Wine Res. 2000;6(1):13-20.
12. Naumova NA, Testing of seeds for fungous and bacterial infections. Method of phytopathological examination of seeds. Keter Press. Wiener Binding LtD, Yerusalam. 1972; 320pp.
13. Pearson RC, Gadoury DM, Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathol. 1987;77:1509-1514.
14. Pearson RC, Gartel W, Occurrence of hyphae of Uncinula necator in buds of grapevine. Plant Dis. 1985;69:149-151.
15. Pearson RC, Goheen AC, Compendium of Grape Diseases. Am. Phytopathol. APS Press St Paul MN. USA. 1990;273pp.
16. Pool RM, Pearson RC, Welser MJ, Jokson AN, Seem RC, Influence of powdery mildew on yield and growth of rosette grapevine. Plant Dis. 1984;68:593-595.
17. Sall MA, Wrysinski J, Presentation of powdery mildew in buds of grapevine. Plant Dis. 1982;66:678-679.
18. Schnathorst WC, Environmental relationships in the powdery mildews. Phytopathol. 1956;3:343–366.
19. Spencer DM, Powdery mildew of strawberries. In: The powdery Mildews. ed. Spencer DM, Academic press. New York, USA, 1978; pp.355 – 358.
20. Wicks TJ, Magarey P, First report of Uncinula necator cleistothecia on grape in Australia. Plant Dis. 1985;69:727.

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Allium hirtifolium Boiss: Radical scavenging property and the lowering effects on blood fibrinogen and factor VII

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Abstract: Enhancement of blood fibrinogen or factor VII increases cardiovascular diseases. *Allium hirtifolium* Boiss (Mosir) has been shown to have cardioprotective effect. This study, therefore, aimed to evaluate the effects of *Allium hirtifolium* Boiss on factor VII and fibrinogen blood levels. Its radical scavenging property was also measured. Twenty four NewZealand male rabbits were randomly designated into 3 groups of 8 and were fed for 60 days with normal diet, hypercholestrol (1%) diet or hypercholestrol (1%) diet+ Mosir. At the beginning and 60 days after the start of the study, the blood fibrinogen and factor 7 were measured and compared in different groups. The Mosir radical scavenging property was measured using the beta-carotene linoleate method. The blood fibrinogen and factor 7 were higher in hypercholesterolemic group (26.7 ± 329.22 and 17.1 ± 277.7mg/dl) compared to normal diet group (13.7 ± 287.25 and 18.2 ± 230.0 mg / dl, respectively) ($P<0.05$), at the end of the experiment. The amount of blood fibrinogen and factor 7 were decreased in hypercholesterol+Mosir group (23.9 ± 180.00 and 53.3 ± 237.0 mg / dl) compared to hypercholesterol diet group ($P<0.05$). radical scavenging activity of Mosir extract was 52.1 ± 3.3%. Mosir may have beneficial effect on heart by decreasing blood fibrinogen and factor 7 as cardiovascular risk factors. These effects of Mosir should be considered carefully in patients with hemostatic disorders.

[Asgari. S, Rafieian-kopaei. M , Pourgheysari. B, Ansari-Samani. R , Deris F , Shahinfard. N , Hojjati. MR and Salimi. M . **Allium hirtifolium Boiss: Radical scavenging property and the lowering effects on blood fibrinogen and factor VII.** *Life Sci J* 2012;9(3):1793-1798] (ISSN:1097-8135). http://www.lifesciencesite.com. 259

Keywords: Rabbit, Fibrinogen, Factor 7, Hypercholesterolemia, Allium hirtifolium Boiss

Introduction:

Cardiovascular diseases, especially atherosclerosis, are growing rapidly and are considered the first cause of death in industrialized societies (1, 2). Studies done in America and Europe have estimated occurrence range of cardiovascular diseases at 3 to 8 percent. According to surveys conducted by the Ministry of Health and Medical Education, 5.38 percent of deaths in Iran occur due to coronary artery disease (1). Studies have shown that regulation of hemostatic and Thrombogenic factors potential by simple changes in lifestyle can be considered as primary or secondary prevention (3).

Today, the factor VII and fibrinogen activity (Hemostatic factors) have been recognized as risk factors involved in cardiovascular diseases. Now, different procedures and medications including supplements of vitamins E, folic acid, B6 and B12 vitamins, and if necessary, anticoagulant drugs are used for the control of these risk factors and prevention of vascular disease (4, 5). However despite the use of these supplements, it seems that outbreak of atherosclerosis, progression of acute coronary syndromes and brain ischemia or ischemia in other tissues can be still prevented by inhibiting the coagulation and inflammation activity.

Factor VII is a vitamin K dependent factor that is produced by hepatocytes and is released in the blood stream. High plasma level of factor VII is associated with a risk increase in development of arterial thrombosis. 25 percent increase in factor VII activity increases risk of fatal coronary artery disease by 55 percent (6). It also has been identified that factor VII levels is associated with serum triglyceride levels. Nutrition with a high fat diet caused increase in the lipoproteins and triglycerides concentration and lipolysis of large lipoprotein particles causes increase in activated factor VII (7). Factor VII activity levels in people who have a high fat diet is 16 percent more than those who consume low-fat diet (8).

Fibrinogen is a protein with a high molecular weight produced by the liver and its normal average level is 250mg/dl. Plasma fibrinogen levels increase in inflammatory, malignant and liver diseases (9). Plasma

fibrinogen increase could be prelude to clot formation increase (10). Although the increase in plasma fibrinogen associated with other risk factors for coronary artery disease such as age, smoking, high blood pressure, elevated blood fats, diabetes and obesity can be seen, but fibrinogen as an independent risk factor also plays a role in the development of atherosclerosis disease (11, 12). Fibrinogen by affecting on plasma viscosity fibrinogen, platelet aggregation and the fibrin level which it forms provides the risk of developing coronary artery disease (14, 15).
Several studies have shown the relationship between plasma fibrinogen level and severity of coronary artery disease in the angiography. Most of these studies consider this matter mainly due to the obstruction of the vessel lumen which is a sign of this subject that plasma fibrinogen increase is a thrombogen factor (16). Reports show correlation between components of the clotting system (fibrinogen and factor VII) and fibrinolytic factors such as tissue plasminogen activator (TPA) and plasminogen activator inhibitor (PAI) and atherosclerosis (17).
Studies show that flavonoids and phenolic compounds in plants have many biological effects including antioxidant properties, inhibition of free radicals and anti-inflammatory effects (18). According to increasing prevalence of heart disease and reduction in age of getting this disease, investigating for new drugs and herbs for control and prevention of atherosclerosis plaque and reduction in cardiovascular disease risk factors is important. Shallot (*Allium hirtifolium* Boiss) is a species of tulips large family consisting of about 500 known different species. The family in addition to shallot includes other important species known as garlic, onions and leeks which have food and drug application throughout the world (20, 19). Allium plants use has been common as local traditional drug since ancient times and such plants have been used for centuries because of its medicinal value and properties. These plants are rich in flavonols and organosulfur compounds and have shown the anticancer properties in *in vitro* studies (21). Shallot is a traditional plant like garlic, but its chive is darker than garlic and is often used as a food flavoring in the diet. Heretofore, numerous studies have been conducted on the properties and characteristics of shallot which among them we can mention Hypocholesterolemia effects (22), having activity of inhibiting hemolysis and depletion of glutathione due to stress in human erythrocyte (23), hypoglycemia and hypercholesterolemia effects (24, 25), antibacterial effects (26), high antioxidant potential (27) and the Hematological effects (28). Moreover, some of effective compounds in this plant such as antifungal peptide askaline (29) and mannose-specific lectin (30) have been identified and isolated. The results showed that edible prescription of alcoholic extract of shallot for 20 days cause decrease in total cholesterol level and LDL and increase in HDL and prescription of this plant has no effect on serum triglycerides level (31). Also it has been proven that prescription of shallot extract in male rats could reduce lipid peroxidation due to having antioxidant (31). As for the existing compounds in shallots and its hypocholesterolemic effects and effect of blood hemostatic potential on atherosclerosis and cardiovascular diseases, if this plant causes decrease in hemostatic factors, it could be very helpful in cardiovascular diseases. Since the plant was proposed as a powerful antioxidant compound and some reports suggest its protective effect on cardiology system, therefore the aim of this study was to evaluate the shallot effect on fibrinogen and Factor VII in rabbits consuming high cholesterol. The Mosir radical scavenging property was also measured using the beta-carotene linoleate method.

Methodology

Extraction

In this experiment, the collected shallots from Koohrang area in Chaharmahal & Bakhtiari province was approved by Medicinal Plants Research Center of Shahrekord University of Medical Sciences and the extract obtained from it were used intraperitoneally in rabbits. During the period, body weight and food intake were measured.
After drying shallot in the shade and crushing, act of the extraction was performed at temperature of 20 -15 with 80 percent ethanol. For this purpose, the powder was soaked in solvent. 100 g of plant powder was poured in 500 ml of 80% ethanol. 48 hours later, after filtering the extract, extraction repeated twice and the collected plant extract was transferred to the vacuum distillation unit and concentrated. Then it was dried at a temperature of 40 ° C (32).

Grouping and rabbits treatment

In an interventional study, 24 New Zealand male rabbits weighing 9/12 ± 2010 g were purchased from Razi Institute, Karaj and were kept in animal kennel of Medicinal Plants Research Center, Shahrekord University of Medical Sciences for two weeks at an appropriate temperature and humidity, hours of natural darkness and light and standard basal diet and then were treated. Rabbit nourishment was done by standard prepared grain food purchased from Pars feed company (Tehran) which contains 15% protein, 40-50% carbohydrates, 2% vegetable fat and 15 to 25% fiber. In order to prepare food after scientific confirmation, the obtained powder after grinding cholesterol with weight ratio of 0.01 was mixed with the powdered and standard food and animal feed was produced again. During the experiment period the animals had access to

enough food and water. Rabbits were randomly divided into 3 groups of 8 as described below and were under the various regimes for 60 days as follows: Basal diet, high cholesterol diet (1%), high cholesterol diet (1%) + shallot extract (1g/kg BW) (32). Shallot extract was injected intraperitoneally once a day (about 11 am) for 60 days.

Biochemical factors measurement:

Before the beginning and end of the study, the animals were fasted for 12 hours, and blood samples of rabbits were taken from the central ear artery. Blood taken from rabbits was poured in two separate tubes to prepare serum and plasma. Tubes with specific number and date were centrifuged for 20 minutes at 3500 rpm in order to prepare serum and plasma. Fibrinogen level was measured by Mahsayaran kit and factor VII was measured by STA-Deficient VII kit (15).

Measurement of the antioxidant activity by beta-carotene-linoleate method

Beta-carotene-linoleate method was used for measuring antioxidant capacity (33). Two milligrams of beta-carotene was solved in 2/0 ml of chloroform. 20 mg linoleic acid and 200 mg Tween 40 were added to the emulsion. 40 ml of water saturated with oxygen was added to the above materials. Shallot extract was prepared at a concentration of 2/0 milligrams per liter in pure ethanol. 4 ml of the prepared solution was added to 2 ml of the prepared extract and control (ethanol). Antioxidant activity of extract based on Beta-Carotene Bleaching rate at a wavelength of 470 nm and at 180 minutes (15 minute intervals), and by using the following formula was calculated and the mean of obtained values was considered as the percent of antioxidant activity of the extract.

Formula 1:

$$AA = 100\ [1 - (A_o - A_t) / (A^o_o - A^o_t)]$$

A_o and A^o_o represent the absorbance of light at time zero, A_t A^o_t represent the absorbance of light at different times during 180 minutes for the sample and control (33).

Measurement of plasma antioxidant capacity:

Plasma antioxidant capacity was measured by using Tripyridyl Triazine (*TPTZ*) (Sigma Co, USA) and based on dr Heydarian et al method. Reduction of ferric to ferrous ions in the presence of antioxidants creates colored ferrous tri-pyridyl-triazine complex which has maximum absorbance of light at 593 nm. $FeSO_4$ in the range of 100 to 1000 mM was used in order to draw the standard curve. In this method, first a working solution was prepared with a ratio of 10:1:1 from 10 mM triazine in 40mM chloridric acid, 20 mM solution of FeCl3 (Merck, Germany) and 300 mM acetate buffer (buffer solution 10 volume, each of triazine and FeCl3 1 volume). Then 25 µl of each sample's plasma was poured in clean tubes and 1/5 ml of working solution was added to each tube (One test tube was prepared for blank that instead of sample, distilled water was added to it). The tubes were incubated for 10 minutes in water bath at 37°C and subsequently each sample's absorbance of light relative to blank at a wavelength of 593 nm was read (34).

Statistical Analysis:

Results were analyzed using Instat 3 software. To investigate the biochemical results and comparison of the experimental groups, Kruskal - Wallis and Dunn tests were used and $P<0.05$ was considered statistically significant.

Results:

The results of this study showed that at the beginning of the period, biochemical factors values among the study groups were not significantly different ($P> 0.05$). Comparison of blood's fibrinogen level in the basal diet (normal) and high-cholesterol diet (1%) groups indicated that fibrinogen level in high-cholesterol group compared to basal diet group had significant increase ($P<0.05$). Also significant reduction was observed in high-cholesterol+shallot group compared to high-cholesterol diet ($P<0.05$) (Table 1). Factor VII level in high-cholesterol group had significant increase compared to basal diet ($P<0.05$) and significant decrease in high-cholesterol+shallot group compared to high-cholesterol diet was observed ($P<0.05$) (Table 1).

Table 1: Mean (mg/dl) of fibrinogen and factor VII levels in the diet groups under study

	diet	Fibrinogen ± SD	Factor VII ± SD
1	Basal (normal)	287/25±13/7	230/0±18/2
2	High-cholesterol (1%)	329/22±26/7a	277/7±17/1a
3	High cholesterol (1%) + shallot	180/00±23/9b	237/0±53/3b

a:$P<0.05$,compared with the basal diet group

b:$P<0.05$,compared with high-cholesterol diet group

In this study, the antioxidant capacity of shallot extract with concentration of 0.2 g / L, equivalent to 52.1 ± 3.3%, was obtained. The antioxidant capacity of plasma in shallots + high-cholesterol group was obtained 943.907 ± 249.51μM that was far more than the basal (normal) group, 629.675 ± 130.73μM. The results showed that there is a significant correlation between the antioxidant capacity of shallots + high-cholesterol group (943.907 ± 249.51μM) and antioxidant capacity of samples before the intervention (440.089 ± 99.99μM) (P <0.001).

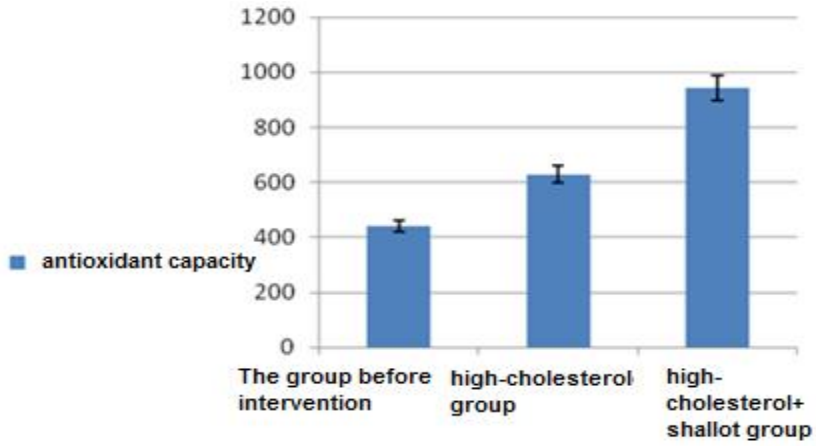

Figure 1: Comparison of the antioxidant capacity of plasma samples in tested groups (μM)

Discussion:

The results of this study showed that groups treated with shallots had significant decrease in fibrinogen and factor VII levels compared to the high-cholesterol group. Disorders of fibrinogen and coagulation are related to the development of cardiovascular diseases such as coronary artery disease, hypertension and ischemic shock (34).

Some vegetarian diets are effective in reducing concentration of clotting factors or increasing fibrinolysis and are involved in this process by reducing blood clotting with reducing fibrinogen, inhibition of platelet aggregation and increasing prothrombin time (35). The results have shown that edible prescription of alcoholic extract of shallot for 20 days cause decrease in total cholesterol level and LDL and increase in HDL (25). HALL studies in 1996 showed that there is a relationship between HDL and fibrinogen. People who have high HDL levels have low fibrinogen levels. The inhibitory effect of HDL on factor X activates proteins C and S (these proteins may inhibit coagulation factor V) that these factors reduce production of fibrinogen. HDL also inhibits platelet aggregation. Therefore, decrease in fibrinogen could also be due to an increase in HDL. Effect on reducing blood cholesterol is done by inhibiting production of HMG-CoA reductase. This enzyme causes conversion of hydroxymethyl glutaryl-CoA to mevalonic acid and its revival which is a one-way reaction and occurs in endoplasmic reticulum of cells (36). Studies have shown that garlic powder has anti-thrombotic effects due to having allicin, level of thrombin-antithrombin(III) complex have been decreased to normal level (37). Allicin is the major factor for its beneficial effects against blood lipids, blood pressure and blood clotting (36). It's recognized that long-term and edible use of shallot causes reduction of free radicals and protecting cells against chemical damage, reduction of lipid peroxidation and protecting liver against a variety of stresses that its main reason is its high level of antioxidants (38). Therefore, the use of the plant applies protective effects on body tissues and works toward reduction of oxidative stress (39). Flavonoids such as coarsetin, compferrol, myristin are inhibitors of platelet aggregation. Given that Shallot has several medicinal effects such as antioxidant property (39) make polyphenols rapidly absorbed and cause increase in the blood concentration to induce expression increase of mRNA and fibrinolytic proteins such as t-PA and setup reduction of PAI (40), which could partly explain the anti-inflammatory effects and improve blood lipid profiles and reduce lipid peroxidation in hypercholesterolemic individuals (8). Treatment with antioxidant factors such as Allium ampeloprasum with high doses of cysteine sulfoxide compounds can be effective in this regard (41). Furthermore it has been recognized that the antioxidant effect of this group of materials is applied by

increasing enzymes level related to dismutase and catalase antioxidant systems. On the other hand, this material is able to reduce the production of end products of lipid peroxidation such as malondialdehyde and hydroproxside (42). On the other hand, part of observed effects of the plant use in the present study should be attributed to the high percentage of anthocyanins with protective properties (41). Thus, by inhibition of coagulation and inflammatory activities of platelets, atherosclerosis and acute coronary syndromes and brain ischemia or ischemia in other tissues can be likely prevented.

Conclusion:

By considering the use of shallot in modern societies and according to its antioxidant effect and its certain role in the occurrence and development of various chronic diseases including cardiovascular disease, its preventive role in the incidence of these diseases is so important. In addition, according to researcher's interest for the discovery and use of drugs with natural origin because of fewer side effects to treat these diseases, shallot plant can be more considered in this field. Of course, finding probable effective compound or compounds and their affecting mechanism in cardiovascular disease is an issue that its clarification requires further and more quantitative research.

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References:

1.Braun W. Heart disease, a textbook of cardiovascular medicine. 5th ed. WB Saunders Co. 2001; 1210-15.

2.Valentin Fuster H. The Heart. 10th ed. McGraw Hill. 2001; 1065-95.

3.Ridker PM, Cushman M, Stampfer MJ. Inflammaltion, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Eng J Med. 1997; 337(5):336.

4. Perna AF, De Santo NG. Homocysteine. In: Kopple JD, Massary SG, editors. Kopple and Massary's Nutritional Management of Renal Disease. 2nd ed. Philaderphia: Lippincott Williams & Wilkins. 2004; 117-23.

5. Majerus PW, Tollefsen DM. Anticoagulant, thrombolytic and antiplatelet drugs. In: Hardman JG, Limbird LE, editors. Goodman & Gilman's the Parmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill.2001; 1519-38.

6. Bialecka M. The effect of bioflavonoids and lecithin on the course experimental atherosclerosis in rabbits. Ann Acad Med Stetin. 1997; 43: 41-56.

7. Varga Z, Czompa A, Kakuk G and Antus S. Inhibition of the superoxide anion release and hydrogenperoxid formation in PMNLs by flavonolignans. Phythoter. Res. 2001; 15 (7): 608

8.Utterman G. The mysteries of lipoprotein (a). Science 1989; 246: 904-10.

9. Podolsky DK , Isselbacher KJ. Derangements of hepatic metabolism .In: Fauci AS, Braunwald E, Lsseldacher KJ et .al(eds). Harrison,s principles of internal medicine .14th ed. New York, McGraw Hill. 1998; 1667-72.

10.Mich E, Baller D, Gleichmann U et al. Fibrinogen and leukocyte number in coronary heart disease: correlation with angiography and clinical degree. Z Kardiol .1995; 84(2):92-7.

11.Ernst E. Plasma fibrinogen: an independent cardiovascular risk factor. J Intern Med. 1990; 227(6): 365-72.

12. Ernst E and Resch KL. Fibrinogen as a cardiovascular risk factor: a meta analysis and review of the literature. Ann Intern Med. 1993; 118(12): 956-63.

13.Sumeray MS. Montgomery HE and Humphries SE. Beyond coagulation: fibrinogen as a cause of cardiovascular surgical disease. Cardiovasc Drug Ther. 1998; 12(3): 261-5.

14.Mead TW. Fibrinogen in ischaemic heart disease. Eur Heart J. 1995;16 suppl A: 31-5.

15.Smith FB, Lee AJ, Fowkes FG, Price JF, Rumley A and low GD. Hemostatic factors as predictors of ischemic heart disease and stroke in the Edinburgh artery study. Arterioscler Thromb Vasc Bio. 1997; 17(11): 3321-5.

16.Kienast J. Fibrinogen and coronary heart disease. Versicherung Smedizine 1995;47(4):122-6.

17. Gensini GF, Comeglio M, Colcila A. Hemostatic factors, atherogenesis and atherosclerosis. Biomed Pharmacother.1996; 50(8):395-405.

18. Khajehdehi P. Turmeric: Reemerging of a neglected Asian traditional remedy. J Nephropathology. 2012; 1(1):17-22.

19. Bianchini F, Vainio H. Allium vegetables and organosulfur compounds: do they help prevent cancer? J Environ Health Persp. 2001; 109(9): 893-902.

20. Fattorusso E, Iorizzi M, Lanzotti V, Taglialatela-Scafati O. Chemical composition of shallot (Allium ascalonicum Hort.). J Agr Food Chem. 2002; 50 (20): 5686 -90.

21. Mubarak AM, Kulatilleke CP. Sulfur constituents of need seed volatiles: a revision. J Phytochem. 1990; 29: 3351-2.

22. Nishimura H, Higuchi O. Antioxidative activity of sulfur- containing compounds in Allium species for human LDL oxidation in vitro. J Bio Factors. 2004; 21: 277-80.
23. Leelarungrayub N, Chanarat N, Rattanapanone V. Potential activity of Thai shallot (Allium acalonicum L.) extract on the prevention of hemolysis and glutathione depletion in human erythrocyte from oxidative stress. CMU J. 2004; 3: 225-34.
24. Jalal R, Majid Bagheri S, Moghimi A, Behnam Rasuli M. Hypoglycemic effect of aqueous shallot and garlic extracts in rats with fructose-induced insulin resistance. J Clin Biochem Nutr. 2007; 41(3): 218-23.
25.Fallahi F, Roghani M, Bagheri A. Time-dependent hypoglycemic and hypolipidemic effect of Allium ascalonicum L. feeding in diabetic rats. J Babol Univ Med Sci 2010; 12(1): 16-23.
26. Adeniyi BA, Anyiam FM. In vitro anti-Helicobacter pylori potential of methanol extract of Allium ascalonicum Linn. (Liliaceae) leaf: susceptibility and effect on urease activity. J Phytother Res. 2002; 18(5): 358-61.
27. Asgari S, Setorki M, Rafieian-kopaei M, Heidarian E, Shahinfard N, Ansari R and Forouzandeh Z. Postprandial hypolipidemic and hypoglycemic effects of *Allium hertifolium* and *Sesamum indicum* on hypercholesterolemic rabbits. Afr J Pharm Pharmacol. 2012;6(15):1131 – 5.
28. Owoyele BV, Alabi OT, Adebayo JO, Soladoyea AO, Abioyeb AIR, Jimohb SA. Haematological evaluation of ethanolic extract of Allium ascalonicum in male albino rats. J Fitoterapia. 2004; 75: 322-6.
29. Wang HX, Ng TB. Ascalin, a new anti-fungal peptide with human immunodeficiency virus type 1 reverse transcriptase-inhibiting activity from shallot bulbs. J Peptides. 2002; 23: 1025-9.
30. Mo HQ, Vandamme EJM, Peumans WJ, Goldstein IJ. Purification and characterization of a mannose-specific lectin from shallot (Allium ascalonicum) Bulbs. J Arch Biochem Biophys. 1993; 306(2): 431-8
31. Wongmekiat O, Leelarugrayub N, Thamprasert K. Beneficial effect of shallot (Allium ascalonicum L.) extract on cyclosporine nephrotoxicity in rats. Food Chem Toxicol. 2008; 46(5):1844-50.
32. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation Between Antioxidant Activity of Garlic Extracts and WEHI-164 Fibrosarcoma Tumor Growth in BALB/c Mice. J Med Food. 2011 Sep; 14(9):969-74.
33. Akhlaghi M, Shabanian G, Rafieian-Koupaei M, Parvin N, Saadat M, Akhlaghi M. Citrus aurantium Blossom and Preoperative Anxiety. Revista Brasileira de Anestesiologia 2011; 61(6):702-12.
34. Jafarian A, Ghannadi A, Elyasi A. The effects of Allium hirtifolium Boiss. On cell –mediated immune response in mice. Iran J Pharmaceutic Res. 2003;2:51-5
35- Heidarian E, Soofiniya Y, Hajihosseini R. The effect of aerial part of Cynara scolymus extract on the hyperlipidemia, plasma antioxidant capacity, and super oxide dismutase activity in diabetic rats. J Sharekord Univ Med Sci. 2011 Dec, Jan; 13(5): 1-10. Persian
36. Noto D, Barbagallo CM, Cefalu AB.FactorVII activity is an independent predictor of cardiovascular mortality in elderly woman of a Sicilian population:results of an 11-year follow –up. Thromb Haemost .87:2002;206-10.
37. Scalbert A, Manach M. Dietary polyphenols and the prevention of disease. Crit Rev foot Sci Nutr. 2005; 45;287-306.
38. Xiao D, Pinto JT, Soh JW, Deguchi A. Induction of apoptosis by the garlic- derived compound S-allylmercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH (2)-terminal kinase 1activation. Cancer Res 2003;63(20):6825-37.
39. Knowles LM, Milner JA, Dially L. disulfide inhibits P34(cdc2) Kinase activity through changes in complex formation and phosphorylation. Carcinogenesis.2000; 21(6):1129-34.
40. Grenett HE, Abou Agag LA, Parks DA, Booyse FM. Ethanol and polyphenols (cat,quer) increase expression of fibrinolytic protein mRNA in vivo in aortic endothelium. Biol Res 2004; 37(2): 342.
41. Khezri S. Sh. Encyclopedia of Medicinal Plants. Rostamkhani Publication. 2003; 568.
42. Stajner D, Canadanović-Brunet J, Pavlović A. Allium schoenoprasum L. as a natural antioxidant. Phytother Res. 2004;18(7):522-4.

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The Long Run Relation between Inflation and Economic Growth in Iran

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Abstract: Relation between inflation and economic growth in various countries has been always struggled among economists with theoretical and experimental discussions in this field. Studying most of such issues indicates that one may not achieve an absolute result for influence of inflation on economic growth and this depends on the conditions and properties of related country. This study aims to deal with effects of inflation on economic growth in Iran by asking this question: how is the relation between inflation and economic growth in Iran? As Iran economy has now encountered with problems in both inflation and development, so general analysis of inflation and economic growth is advantageous. Therefore, such long run relation between inflation and economic growth has been evaluated by ARDL method during 1974 – 2007. Results indicate that first, there is a stability and balance between inflation and economic growth; second, there is a negative relation between both variables; third, estimating the error correction model indicates adjustment speed of 40%, i.e. each year, 40% of imbalance is adjusted.
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Keywords: Inflation; economic growth; long run relationship; ARDL; error correction model

1. Introduction

Regarding to the importance of economic growth for increased social welfare, it is important to study the factors influencing economic growth. Studying the economic literature indicates that inflation is an effective factor on economic growth. Studies indicate that there are different views for such relation between both variables. Some views state that inflation can have a positive effect on economic growth; while others state its negative effect and some others prove that there is no relation between those variables. Some other views are also stating that the relation between inflation and economic growth is non-linear, i.e. inflation to a rate can have a positive or neutral effect to economic growth and then has a negative effect. But most studies resulted that high level inflation has negative and stable effects on economic growth. Thus, recent years most central banks increasingly stressed on price stability and monetary policies have been applied for low inflation with stability to not incur inflation costs, because it seems that inflation has considerable costs and burdens; some of such costs depend on mean inflation rate and some others depend on variable and unreliable inflation. Experimentally and theoretically studying the relation between inflation and economic growth, therefore, can be such important that both deal with mentioning different views for relation between both variables and experimental test of such relation can examine such views. This study intends to study the effects of inflation on economic growth in Iran and indeed answering this question that how is the relation between inflation and economic growth in Iran and as Iran economy has now encountered with problems in both inflation and development, so general analysis of inflation and economic growth is advantageous.

This study initially reviews different views and related literatures. Part two deals with methodology. Part three introduces variables and explains the model using econometrics methods and interpreting the experimental results and finally we conclude with some political recommendations.

It is admitted to start this discussion by found Philips chart because this chart significantly deals with relation between inflation and development. According to Philips chart, there is a kind of negative relation between inflation and unemployment. As due to monetary phantom it has not been anticipated the effects of price changes by workers, and consequently by increasing the costs, wages may not be increased, therefore real wages will be decreased, institutions will employ more, so production and employment may be increased. So, it can be said that (according to the content of mentioned theory) there is a negative relation between inflation and unemployment and positive relation between inflation and production.

Entering the inflation expectations in Philips chart, Freedman and Phelps indicated

that such relation exists only for short run, but in long run, adjusting the inflation expectations, Philips chart will move perpendicularly, therefore (against traditional model) its slop is not negative, so there is no reverse relation between inflation and unemployment, but inflation and unemployment can be increased (decreased) proportionally. So the relation between inflation and production will be void. Robert Lucas and other theoreticians have views beyond this. They state that if monetary policies conducted expectedly and informed, Philips chart may be perpendicular even in short run so economical policy has no effect on production and employment. Therefore there is no positive relation between inflation and production neither in short run nor in long run.

There can be seen different views about relation between inflation and economical growth in development theories. One of them is Sidrauski view (1967) in which, using improvement method for behavior of economic factors and considering the real balance of money in the desirability function, indicated that the influence of monetary inflation on development is neutral. The second view is James Tobin (1965). Assuming that money is an alternative for capital, he proved the positive effect of inflation of development. Thirs view is Stockman (1981) study which limits the influence range of Tobin. By Stockman point of view, the negative effect of inflation on development is mainly related to in-cash down payment models. He considers the money as a supplement for capital.

According to different views about relation between inflation and economic growth, experimentally studying these variables can indicate the inconsistency of consistency of each theory based on specific conditions of studied countries. Such studies can be grouped into two classes:

First class includes studies in which there are used data from several countries (on the other hand combined data). Studies by DeGregorio (1992), Barro (1995) and Alexander (1997) are of such type. Second class includes time series studies in which there is studied the influence of inflation on economic growth in a specific country. In this case, for example, one can mention Joao and Franscisco (2001) study about Brazil economy as well as other studies in Iran. The results of such studies stressed non-linear results of inflation on economic growth, i.e. inflation to a rate can have a positive or neutral effect to economic growth and then has a negative effect. Here we deal with some dimensions of such studies:

In an experimental work, DeGregorio (1992) studied the relation between inflation and economic growth in 12 countries of Latin America during 1950 – 1980 and concluded that high inflation is one of the main hindrances of economic growth during this period. Studying the influence of inflation on economic growth, Alexander (1997) used a neoclassical development equation and concluded that inflation and its changes have negative and significant effect on economic growth. In a study called "crisis of long run inflation and development", Bruno and Easterly (1998) proved that economical development will be severely reduced during high inflation periods and after lowering the inflation, it will be improved fast. This study conducted for 1994-1961 using time series study for 31 countries with high inflation. Girijansker and Anis (2001) studied inflation and economical development for four Asian countries (Bangladesh, India, Pakistan and Sri Lanka). Results indicate that in long run there is a positive relation between GDP and inflation rate for all studied countries. There is also a considerable feedback relation between inflation and economical development for those countries.

Considering the results of above mentioned studies, one can enumerate a series of considerable policy making issues. One, a mild inflation is advantageous for development. On the other side, attempting to attain a fast economical growth may actuate the economy to a place where the inflation might not be controlled and make instability in the economy. Thus such countries are on a sword edge. Joao and Francisco (2001) studied the influence of inflation on development and production during 1980- 1995 for Brazil- a country with very high inflation experienced for long run. Results indicated that short run inflation has negative effect on production, but long run inflation has no effect. Gilman et al (2002) in their paper "Inflation and Development: some theories and evidences", studied non-linear relation between inflation and economical development in OECD and APEC courtiers based on inborn development monetary model during 1961-1997. Results indicate that for OECD countries, there is a negative non-linear relation such that in low inflation level, there is a positive effect on economical development but in higher inflation level, more than 10%, this relation is increasingly negative.

2. Material and Methods

This study is of type casual-inferential discovering the relations between variables. On the other side, it is an applicable study and its results can be used to attain suitable policies. Data of this study collected based on studies from 1974 to 2007. Such data are annual-based and obtained from databank of Central Bank of Islamic Republic of Iran.

Data of variables discussed in this study are based on time series. Using these data and applying the econometrics method, the relation between economical development and factors influencing it can by analyze based on a long run framework between economical growth and inflation. Because, however time series in economy are mostly unstable, so applying common econometrics methods like Original Limit Square (OLS) method for unstable time series in most cases may result in inaccurate interpretation. Therefore, in this study there has been used new econometrics methods or evaluating and analyzing the data. Initially, based on theory and previous studies by Microfit software using ARDL method, there estimated the long run and short run relation between approximate indices and then using Error Correction Model (ECM) the adjustment rate was estimated and results analyzed by different tests.

By a classical model framework, this study dealt with studying the relation between inflation and economical development. Among different models with function forms and different variables, following model has been selected for studying the effect of inflation on economical development which is a linear model:

$$Y_t = \beta_0 + \beta_1 IN + \beta_2 K + \beta_3 G + \beta_4 X + \beta_5 P + \beta_6 D_{57} + \beta_7 D_{59}$$

Where;

Y is annual GDP growth rate, IN is inflation rate, K is private sector investment to GDP ratio, G is State Expenditures to GDP ratio, X is Exports to GDP ratio, and P is the population growth rate. D_{57} is the dummy for Revolution variable (years 1977 and 1978= 1 and other years=0) and D_{59} is dummy for War variable (for 1980 – 1988=1 and other years=0). The fundamental assumption for this model is negative effects of inflation of economical development which can be defended for Iran, because inflation rate is always more than normal state (2 – 3%).

In this model, there was used annual data of macro economy during 1974 – 2007 to determine the kind of relation between inflation and economical development. It must be mentioned that following data have been used for estimating the model.

Y (Per Capita GDP Growth Rate) is a dependent variable based on billion Rials;

IN (Inflation Rate) - it is obtained based on index of retailing cost and used services (CPI_{76}) and it is expected that has a reverse relation with economic growth.

RK (Private Sector Investment to GDP Ratio)- one of the fundamental resources of production if investment, here we used investment to domestic gross production ration for 1997.

RG (State Expenditures to GDP Ratio)- great share of Iran economy depends on government. Undoubtedly, one of the effective economical factors is state expenditures and here it has been considered the real state expenditures to GDP ratio for 1997 and it is expected that it has positive effect on economical development in long run.

POP (Population Growth Rate)- country's population as a man factor can influence on economical development, considered based on million person.

DU_{57} (A dummy for Revolution variable)- it has been considered for 1977 and 1978 and other years it is zero.

DU_{59} (A dummy for War variable)- it has been considered for 1980 – 1988 and for other years it is zero.

3. Results and discussion

Presence of long run relation is proved by different tests and here we use test provided by Pesaran et al (1996)[1]. Using calculation of statistics F, the presence of long run relation between variables is tested for significance of levels with variables in the error correction form. The important point is that mentioned F distribution is not standard. Pesaran et al calculated the proper critical value correspondent to the number of regressions and considering whether this model includes abscissa and round. They provided two groups of critical values; one is such that all variables are stable and another one is such that all are unstable (and became stable once differencing). If calculating F locates out of this boundary, it will be made an absolute decision with need to knowing that variables are I(0) or I(1). If calculating F locates beyond upper range, the H_0

[1] Tashkini (2005), PP. 48-147

as stating the lack of long run relation will be rejected and consequently this theorem will be proved and dynamic model moves towards long run equilibrium model. Related statistics after calculation is 4.74 and comparing mentioned statistics with critical quantity, we conclude that dynamic model moves towards long run equilibrium. On the other hand, there is a long run equilibrium relation between model variables.

For final determination, there is used Augmented Dickey Fuller test. To test unique root, software package determines the number of stops of dependent variable necessary to remove the self-correlation between disruption sentences in the regression, by Aquaic (AIC), Shouartz- Bizin (SBC) and Hanan- Queen (HQC) models. Maximum amount of any above models determines the number of optimal pauses. SBC usually saves number of pauses, therefore the number of pauses selected based on SBC mode. Before estimating the model, avoiding the insignificant regressions, one must assure about stability or instability of data. As indicated in table (2), inflation rate (IN) and Exports: GDP ratio (RX) and stable variables and per capita GDP growth rate (Y), population growth rate (POP), investment to GDP ratio (RK) and state expenditure to GDP ratio (RG) are unstable variables. It must be mentioned that in sample size less than 100, there is usually used Shuartz- Bizin model to avoid losing more freedom degree (Tashkini (2005), P146).

Table 1. Comparing the statistical value, F, with critical values, Pesaran et al.

Statistics F	Limits of Critical Values	Result
4.7437	(2.649) – (3.805)	Presence of long run relation (95%)

Source: study results

Table 2. Augmented Dickey Fuller and Philip – Prawn test

Values of ADF and PP statistics and critical values of each variable in 5%				
Variable name	**ADF value with abscissa**	**PP value with abscissa**	**Critical value with abscissa**	**Result**
Y	-2.82	-2.82	-2.95	I(1)
IN	-3.33	-3.21	-2.95	I(0)
RK	-0.52	-2.34	-2.95	I(1)
RX	-3.88	-3.90	-2.95	I(0)
RG	-0.31	-0.31	-2.95	I(1)
POP	-0.80	-0.80	-2.95	I(1)

Source: study results

According to the mentioned theoretical basics, proposed model is as follows:

$$Y_t = c + \sum_{i=1}^{n} \alpha_{1i} IN_{t-i} + \sum_{i=1}^{n} \alpha_{2i} RK_{t-i} + \sum_{i=1}^{n} \alpha_{3i} RX_{t-i}$$

$$\sum_{i=1}^{n} \alpha_{4i} RG_{t-i} + \sum_{i=1}^{n} \alpha_{5i} POP_{t-i} + DU_{57} + DU_{59}$$

Where;
Y: per capita GDDP growth rate
C: abscissa
IN: inflation rate
RK: Private investment to GDP ratio, on 1997
RX: real exports changes to GDP ratio, on 1997
RG: real state expenditures to GDP ratio, on 1997
POP: population growth rate
DY_{57}: Revolution virtual variable that is 1 for 1977 and 1978 and other years it is zero.
DU_{59}: War virtual variable that is 1 for 1980 – 1988 and for other years it is zero.

As indicated, estimated vectors in the model are variable indices coincidence with economic theoretical principles. There is a negative relation between inflation rate and per capita GDP growth rate. It must be mentioned that relation between POP and per capita GDP is negative as well; because population increase will positively influence the economic development when it enjoys necessary training and skill, but unfortunately working force in Iran has less skills and experience. Therefore, population growth will negatively influence on GDP. As expected, variables like investment, exports and state expenditures positively influenced on Per Capita GDP growth rate. The DY_{57} and DU_{59} also influences negatively.

$Y= -0.11 - 0.15N + 0.77RK + 0.43RG + 0.41RX – 1.12POP – 0.2D_{57} – 0.06 D_{59}$
T (-2.37) (-3.11) (3.03) (2.68) (2.66) (-2.33) (-2.98) (-2.26)

This model indicates that by changing 1% in IN, the Per Capita GDP will be increased to 15%. 1% change in RK and RG will increase Per Capita GDP about 0.77% and 0.43% respectively. 1% change in RX will increase per capita GDP to 0.41% and of course 1% change in POP will reduce per capita GDP to 1.12%.

Results in long run indicate negative relation between two main variables (economic development rate and inflation rate), such that increased inflation will reduce economic growth. It must be mentioned that such negative relation is not different from economic theoretical principles stating that inflation is a necessity for economic growth, but it has been considered mild inflation, but sever increased inflation and moving towards two figures has no positive effects on economy either, but results in forming the inflation expectations influencing negatively on economy such that Iran's economy encountered with two digit inflation during this period, for this reason, its negative effects can be indicated for economic growth.

Attaining the short run relation in the model needs summing the error sentences of co-addition regression for above mentioned relation with a pause time as an explanatory variable with first order difference of other model variables together with their pauses, then estimate the model coefficient wit ordinary limit square (OLS) method. Table 3 indicates short run relation of model. Such short run relation can be estimated by ARDL methodology as indicated in table 3.

$DY= -0.11 – 0.09DIN + 0.46DRK + 0.18DRG + 0.8DRX – 1.12DPOP – 0.29ECM (-1)$
T (-2.37) (-2.11) (1.85) (3.14) (3.69) (-2.74) (-3.99)

$R^2= 0.86$ $\bar{R}^2 = 0.78$ F= 11.2095 DW= 1.98

Table 3. long run coefficients estimated by ARDL method

Regresses Y dependent variable	coefficient	T- Statistic	probability
C	-0.11654	-2.3761	0.028
DIN	-0.09512	-2.1116	0.042
DRK	0.18434	1.8509	0.068
DRG	1.8999	3.1431	0.0005
DRX	0.80034	3.6932	0.002
DPOP	-1.1216	-2.7469	0.011
ecm (-1)	-.042153	-3.9971	0.000

Source: study results

Error correction model indicates the amount of adjustment rate as well as the percentage of remedy for imbalance in each period. We of course know that this coefficient must be negative and significant. In above mentioned relation, D indicates the first order difference of regresses. In this relation, ECM coefficient equals with -0.42; i.e. each year 40% of inequality or imbalance will be remedied.

Recognition tests are used for studying the self-correlation, side form, normality, variance conformity and structural stability in the model. According to Hashem Pesaran (2002) and Bahmani Oskouei (2001), this test can indicate whether estimated model is stable. Stability and recognition test is mostly used for time series data particularly when author is uncertain when structural failure occurs. When studying the long run and short run stability coincidently, there are used CUSUM and CUCUMQ forms. Choosing related choice, this test presents cumulative recursive residuals and square of cumulative recursive residuals between two smooth lines (95% confidence). If provided diagram is in the confidence interval, H_0, stating the lack of structural failure will be accepted and if chart is out of confidence interval (crossed the confidence interval), H_0, stating the lack of structural failure will be rejected and presence of structural failure will be accepted. Statistics CUSUM and CUSUMQ provide the possibility of structural stability test (CUSUM statistic is used for finding the systematic changes in regression coefficients and CUSUM OF SQUARE statistic used when deviation from regression coefficients is accidental). Table 4 indicates the recognition statistics.

Table 4. Recognition Tests

Test	objective	Coefficients
LM	Studying the self-correlation of error sentences	0.437
RAMESY RESET	Recognizing the model explanation problem	0.306
NORMALITY	Recognizing the normality of error sentences distribution	0.122
WHITE	Recognizing the inconsistency of error sentences variance	0.289

Source: study results

LM used for studying the presence or lack of self-correlation o error sentences, Ramsey test is used for recognizing the model explanation problem, Normality test is used for recognizing the normality of error sentences distribution, and White test is used for recognizing the inconsistency of error sentences variance. Because probability level of calculated error for all tests must be more than 5% error level, the result is that estimated model lack error sentences self-correlation problem, model explanation problem, lack of normal distribution of error sentences problem and variance inconsistency problem in error sentences and has a reliable estimation. As explained before, CUSUM and CUSUMQ tests are used for studying the presence of structural failure in the estimating model. As diagrams indicated in figure 1 are located between determined intervals (not cut the intervals), so $H_{0,}$ stating the lack of structural failure, will be accepted.

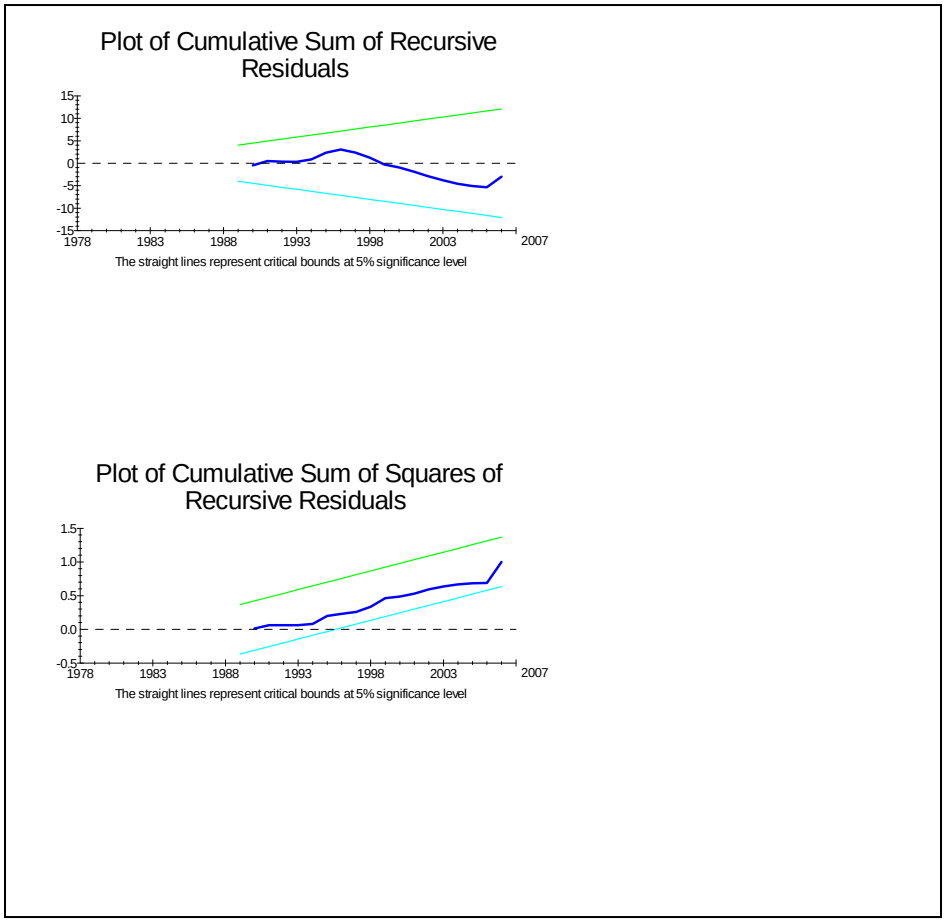

Figure 1. CUSUM and CUSUMQ tests

Results of this study could be summarized as below:

1- Short run relations indicate there is a negative relation between inflation and GDP such that if there is 1% change in the inflation rate will reduce per capita GDP to 0.09%.

2- Short run relations indicate there is no significant relation between investment and GDP, but change in export and state expenditures will increase per capita GDP to 0.8% and 1.89% respectively.

3- Estimation model for ECM indicates that the coefficient of error correction sentence is negative and significant. Significance ECM coefficient means presence of a casual long run relation from model variables to GDP. The size of such coefficient estimated to -0.42 and this indicating that if we move from period t to t+1, 40% of deviation in GDP by its long run route will be corrected by model variables.

4- Long run relations indicate negative and significant relation between inflation and economic growth such that increasing 1% in inflation will reduce GDP to 0.15%.

5- Long run relations indicate that investment variables, state expenditures and exports have positive and significant relation with GDP such that 1% increase in investment ratio and state expenditure ration will increase the GDP to 0.77% and 0.43% respectively. Increasing 1% in export changes will also increase GDP to 0.41%.

4. Conclusion

Regarding to the importance of economic development for increased social welfare, it is important to study the factors influencing economic growth. Studying the economic literature indicates that inflation is an effective factor on economic growth. Generally, results of experimental studies indicate that estimated effects of inflation on growth are negative and this can be indicated both in short run and long run. Therefore, increasing the inflation results in reduced GDP with some fluctuations. Exports and state expenditure changes will also influence positively on GDP both in long and short runs. Therefore, increasing the exports and state expenditures will increase GDP. Investment in long run can apply its positive effects on GDP, i.e. increasing the investment will increase GDP. Therefore, due to presence of negative effects of inflation on economic growth in short and long runs requires controlling the inflation as an initial objective. Thus, to make a development, the inflation needs to be reduced or at least kept in a level that can remove its damaging effects, for this reason, it is necessary to recognize the factors related to inflation and economic growth to reduce or control the inflation and increase economic growth.

This study indicated some factors that can change the relation between there variables, of which include how to supply the budget shortfall, how to allocate the budget, non performance in investment,… therefore, independency of state expenditures to oil incomes and supplying the needed financial sources will be beneficent by making the tax system efficient to attain sustainable development. Modifying the general structure of country's general budget can be a good step for increasing the economical development and controlling the inflation, for this reason, government's current credits needs to be controlled. Thus, lack of optimal systems for controlling the inflation rate and keeping it stable for at least one year can make instability in the economy and this will increase the inflation and reduced economic growth. Because during inflation, there is no production investments or investments making value added and dominated by false activities and playing exchange, and attract such investments. Economic instability will also reduce foreign investments in the country, on one side, and escape of capital from manufacturing part towards false activities, on the other side.

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References

1. Aghevali B.B and M.Khan (1978); "Government Deficits and Inflation Process in D.Cs" IMF staff papers, Vol.25.
2. Alexander, WR.J (1997); "Inflation and Economic Growth: Evidence From a Growth Equation", Applied Economic, Vol.29.
3. Bange .m & et al. (1997); "The Effect of Inflation on the Natural Rate of Output: Experimental Evidence", Applied Economics, Vol.29.
4. Barro, R, J. and Xavier Sala-Martin (1999); "Economic Growth", London, MIT Press.
5. Bruno, M and W. Easterly (1998); "Inflation Crises and Long-rum Growth", Journal of Monetary Economics, Vol.42.

6. De Gregorio, j. (1992); "The Effect of Inflation on Economic Growth", European economic review, Vol.36.
7. Erol T.and s.v Wijnbergen (1997), "Real Exchange Rate Targeting And Inflation In Turkey An Empirical Analysis With Policy Credibility" World Development, Vol 25, No10.
8. Girijanskar. M and Anis, (2001); "Inflation and Economic Growth: Evidence from Four South Asian Countries", Asia-Pacific Development Journal, Vol.8.
9.Gilman. M & et al (2002); "Inflation and Growth: Some Theory and Evidence", 10th International Conference on panel Data, Berlin, July 5-6.
10. Joao. R and Francisco. G (2001); "Does High Inflation Affect Growth in the Long and Short run?", Journal of Monetary Economic, Vol.8.
11. Morgan Theodore (1952), "Income and Employment", Englewooll cliffs,Newjersey: prentice Hall.
12. Sidrauski. M (1967); "Rational Choice and Patterns of Growth in a Monetary Economic", American Economic Review, Vol.57.
13. Slitcher (1961), "Economic Growth in the untied states", Baton Rouge; Louisiana university Press.
14. Stockman. A.C (1981); "Anticipated Inflation and the Capital Stock in a Cash in- Advance Economy", Journal of monetary Economics, Vol.8.
15. Tashkini. A (2005); "Applied Econometrics with Microfit" Vol1.
16. Tobin. J (1965); "Money and Economic Growth", Econometrica, Vol.32.

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Nature of Iranian traditional media in political communication process

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Abstract: In Iran political communication culture, due to high tension in its history affected by aliens` invasion and rulers` dictatorship , a kind of insecurity are appeared in Iran political culture that consequently is intensified by entering modernism from West into Iranian cultural sphere during the contemporary period of time which produce cultural dichotomy in political communication process. Since the communicative - verbal culture is more common in Iranian absolutist mentality due to a sense of insecurity, so the traditional- religious medias or traditional-national media have been promoted so they play a significant role in information interchanging in Islamic revolution victory, even though after its victory, the traditional- religious media are also strengthening the intermediaries between people and government in political communication process till after three decades modern media joint with IT development could influence the content of these media such as mosques, religious site (Hoseineia) by representing the traditional-religious media so that the governmental radio and television turn into a virtual mosques and religious sites ... as well as lead to weaken the traditional- religious media.
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Keywords: Species richness; beta-diversity; taxonomic diversity; forest

1. Introduction

It should be reiterated that by Iran's Islamic Revolution victory (1979 AD) the revolutionaries had lacked the modern media such as radio, television and publications, thus in that period of time traditional media communicate information through networks such as mosques, religious sites (Hossainia), Holy shrines and the public thought was mobilized against monarchy, since after the victory, clergymen could lay their secular rivals aside and surround Iran political governing by dominating the traditional media. More importantly, the political communication process about the relationship between the political government and people are linked mostly through traditional media till the modern media gradually spread into traditional media content and attached the process from traditional media to itself, therefore today the modern media have grown increasingly after three decades of Islamic revolution victory so it could digest and absorb traditional media in such a way that they cannot play role as same as before (especially in the first decade of the revolution) in the political communication process between the general public and the political - religious system(*Howard, P.* (2009) . Anderson, A. 1997 says on culture: "Culture consists of all plans have been designed for life over the history and always exist as a potential guide for people behaving obviously or obscurely, intellectually and anti-rationally and injudiciously."

Jenkins, H. 2006 also states on culture as: "A culture in a society is the way of its citizens` life [also] is a set of beliefs and habits which are learnt, shared and handed over through generation".

In Iranian culture, because of stressful and anxiety raised by alien`s invasion and political tyranny, a kind of insecurity has been formed at the heart of Iranian culture and undesirably has been continuing through the history from the past to present era and has been maturing as a defense mechanism in the form of cultural duality, so that the issue has been internalizing in their religious beliefs in various forms of "dissimulation" (religious or political self-mealy mouthed), as an example. In addition, the white lie is the other instance of self- mealy mouthed that cannot be seen in other religions and faiths formally. The other sign of high using of "lie" as a defense mechanism is swearing too much in Iranian dialogues, because the person has to invoke to "oath" to prove his legitimacy and gain other confidence. The three factors that caused duality in Iranian culture was the Arab new- Muslims invasion to Iran that made the Iranian in negative struggle and self-mealy mouthed mode in two early H.A centuries based on Arabs dominance on their lands.

Second, historically Iranian society had been attacked by foreigners, especially after Arabs invasion, Iran had been subject of great massive invasion by Seljukians, Mongols, and Afghans and..... The issue forms the great part of Iran history after Islam entrance. Third, the arrival of the Western culture from Europe to Iran in the late of 19th centuries crystallized in Constitutional Revolution. Although Iranian culture and identity, maintaining its ancient cultural heritage, could interact with western

modern culture, the interaction did not existing between Iranian religious identity and Western modern culture. In this regard, *Kelly, J. & Etling, B.* (2008) says:

"Cultural duality and communication consist of the first contacts between Iran and west …".

"Fargang Rajai" writes on Iranian`s identity problems:"Today, Iran is a nation who seeks its identity. Iran is a society fluctuating in modernity democratic ideals and suppressing aspects of tradition". Meanwhile the fourth factor can be taken into account which today is more than abovementioned factor; it is the political government tyranny over Iran history which causes mental-cultural despotized. Today, the significant part of duality in Iranian people culture is raised from worries of public people for the process of their political communication with political government in addition to historical and cultural issues which will lead to imbalances within the society and outside of it. Naturally, the problem feed the political and even cultural duality, if this imbalance continues inside and outside of people and political government relationship, undoubtedly, the way of political- social movement will be prepared. Because the system does not meet the outside and hidden expectations of the society, also it is likely against them. Especially, new Iranian generation lacks historical memory toward Islamic Revolution after three decades. Since, the generation actively enters to information-global community domain. Therefore, the political sovereignty is not so capable to impact this new arrived generation public thought. According to "Hippocrates" and Freud, "character is future destiny", overall, new generation cannot adapt itself to such a dual space like its father, altogether it is not satisfied with present status for having numerous national resources in any sense. So it tries to elevate its social base, sometime if it fails, it will attempt to emigrate from the country. For example, rural people who immigrates to cities for their needs to social mobility or citizens who know society as an obstacle for their social mobility, thus they prefer to immigrate to developed countries. Notwithstanding, the majority of the generation are not able to immigrate because of their fixation to their own society and other prohibitions, but in comparing to the first generation of revolution lack the memory of religious-revolutionary ideals because their identity formation is affected more by modern media rather than religious traditional centers. Thus, they may live natively, but they think by membership in global information community (Retamal, G. and Aedo-Richmond, R. (eds) (1998)). Nevertheless their religious beliefs foundation will be formed by modern media. Today, even Islamic fundamentalist are without identity without modern media, thereby when the Islamist prejudiced terrorists want to do a suicide attack, they produce videos and photos to be broadcasted and covered from media after operation. Apparently, this indicates the religious and faith manifestation through media; however the most religious conflicts start from the media circumstance and finally spread into real environments. Today there are several types of churches, mosques, synagogues and other religious centers which are active online on Internet and media channels. Because of that religion turns from private mode to public one. So that the boundaries between media and faith have been removing and collapsing. Hence there is many evidence that show the religion and media live together in contemporary period of time with not complete but high level of convergence, media widening was a cause the religious phenomenon will be publicized more than before, because the high impact of media and information technology on contemporary religion has led to decay "reward power" of faith, means that buying obedience and devotion to the religion is being undermined, so the submission and obedience to religion is more for "Conditional power Faith ", not because of its spiritual influence. It seems, the media weakens the public relations with organized religion and probably despite of convergence between media and religion, media get itself upper hand as expressing and representing people mentality, since today religionists are concern about the case media predominate on religion and define itself dominantly and occupy religion place as well as induce its special culture as a value system and educational factor.

According to Wedeen, Lisa. 2002, "Television and media try to act as a basis for common belief such as traditional and religious faith." Since the nature of Iran's Islamic government mass media (including radio and television and etc) is modern, regularly the media influence the religious content, i.e. the modern media impose themselves on religious concept so that there will be a conflict between the media and content, Gradually, experimentally, the modern media will desanctify religious content and will reduce ideological aspects because it is in religion place and consequently religious message will be transmitted to the audiences in a way that the modern media read and interpret it by itself (Rahimi, Babak.2003). Therefore, the Islamic government of Iran as a religious government seeks to convey Shi'ite concepts through modern media, it is not only failed but the effects were opposing. But modern media compared to traditional one are mostly influenced by society`s general beliefs and ideas(Chagay, Ram.1992). So the traditional media of any society and nation have their own particular identity and

circumstance and it distinguishes the traditional media of cultures and nations to each other. Traditional media can operate more completely in some culture than modern ones with respect to communication mechanism and are more effective than new media. Means that presenting "feedback" and "reaction" of message receiver to sender can be conducted fast and promptly perhaps and it may be due to the its bilinear nature, in the other word the sender is "the first side" of the process and the receiver is the "second side", as well as traditional media is preferred for short time and place distance and for face to face communication between sender and receiver. Inevitably, a kind of emotional and sentimental cognition will be created between sender and receiver, in addition the function of traditional media can involve social, political and economical because of their historical, social, cultural source also traditional media is trusted more than modern one due to their historical and cultural roots. For example, traditional media in Iran including mosques, religious sites, tea shops, gymnasium in public culture are considered as media bases that represent Iran identity and function, more importantly some traditional media in Iran could influence modern media for its approaching to religious government ideology after Islamic revolution. At first, the Clergymen ruling in Iran using traditional media (with religious nature) could spread Islamic Revolution in public thought and bring it to victory, secondly the social- political origin of Clergymen was in the heart of traditional-religious media, such as seminaries, mosques, etc religious sites and so on, thirdly the expansion of verbal culture due to the insecurity culture signifies the traditional media, because the traditional media mechanisms often form the communication process relying on human memory, because insecurity in the public culture does not allow communicator to present the message content in a written and recorded form. Nevertheless, verbal memorized content in the form of rhythmic poetry, stories, simple myths, symbols and so on will preserve the real message. According to scholars in this field, oral culture is rooted from closed cultures and cultures in which there is a fear and insecurity that ultimately leads to duality in social -political communication. So the governments are not as able as modern media to control and censor the traditional media why the traditional and verbal communications are considered as a best political-communicative process, because have almost formal and informal nature. The [traditional] communities more tend to attempt to retain what they are and what they have, and their transformation depends on preserving the culture and memorizing the current knowledge and pervious statements. So [The changes] would be so slowly that the innovation and creating would not be encouraged". Consequently, the traditional communication process in society like Iran will opposed to modern media if it is not surrounded by it. Therefore the Islamic government of Iran wants to backward to social and traditional-religious historic models. Because there is a past-orientation in the form of religious traditions in Iran Islamic system, so there it is trying to organize its official modern media affected by traditional media culture. Nevertheless, the modern media-affiliated to Islamic State of Iran almost try to represent the traditional-religious medias, such as mosques, religious places, and Clergymen lectures pulpits, seminaries, shrines, mourning groups and.... .

Clergymen in Islamic revolution of 1979, clearly could impoverish the king`s (Pahlavi II) modern communicative networks by support of traditional media. Even non-religious revolutionaries inevitably turned to the traditional media because of not accessing to modern media, but after the revolution victory, the traditional media could not be exploited due to lack of intellectual conformity to Shia and Sunnite religious clergymen, thus they could not have a share in political power. Today, Islamic broadcast (IRIB) and other modern media depending to Iranian Islamic regime want to reflect the content of traditional- religious media as a mirror, so these media becomes virtual mosques virtual religious places, virtual lectures pulpit, Virtual seminary schools but their modern property have less conformity with religious content, as well as weaken the traditional-religious media. Because when the person can do a rituals in front of the TV show, he/she does not tend to go to mosque and it is especially true for old people and new generation after revolution, so the modern media have weaken the social function of traditional-religious media, yet the process of political relations between sovereign and people that has been strengthened by traditional - religious media is consequently weakened. Professor Hamid Mowlana, the International communication professor in USA and Ahmadinejad`s consulate in the first period of his presidency, optimistically writes:"Iranian television is a powerful media, but not because its own technology but also due to durable cultural factors that legitimizes it ..... It is mainly said that TV has ritual function which is comparable to religion if TV in United States and Europe is religion. In Iran, it is the religion that makes provision for the TV. In Iran, it is the media's legitimacy that depends on traditional channels not vice versa ...[From] the other hand, ritual functions may cause legitimacy and why it is considered higher than communicative function of a media. Overall, traditional media in Iran

proportional to Iranian culture and history are divided to two main streams:

1 - The traditional Shi'a media, such as mosques, religious places, traditional markets, seminaries, Clergymen lecture pulpit, Imams shrines, religious cities like Qom, Mashhad and ...

2 - The traditional - national media, such as hospice, the gym, the coffee houses, especially Persian literature and poetries...

Traditional Shiite media are often supported financially by traditional markets and controlled or driven by Shia Clergymen but the features of such traditional - Shiite media is socially common among the poor and weak classes of people in combination with popular shi`I religious and its vulgar aspect will be considered as the most important index of the media.

Traditional - national media are socially belonging to the middle classes and educated people of society (especially poetry and Persian literature). In general, traditional- national media is different to traditional- Shiite media because it enjoys the civilization and nobility which transformed from the pre-Islamic era to post Islamic period flexibly and could move alternatively and interactively with traditional-shi`I media despite of differences.

Since Sufism was considered as an "attitude" of the middle classes and elicits consequently, looking at religion from Sufism perspective has spiritual nature is not so much vulgar. Naturally entering into Sufism is not relatively simple because needs to pass certain steps why the most important media is Persian poetry and literature for that social class and is full of secrets and mysteries. Poets in their own period of time were doing the same task as press today. They were people`s spokesman and interpreter of their feeling and perception of people and on the other hand they serves the powerful men.

Traditional- national media always seeks to preserve the glorified Iranian past legacy. Although there is flexibility and interaction with other culture in the nature of this media and the reason of secrets and mysteries in “Persian literature" admit the story, although Persian poetry seeks to interact and associate with shie`i and its own media. As a result, the duality element is more subtle in traditional-national media in medium class especially ″ Sofia”.

Rudaki (d. 329 AH) used less Arabic words in his poetry, and is lenient against prejudice; he exploited ancient Zoroastrian elements more than Iranian and Islamic factors and pay more attention to sensory aspects in religious issues. Hafez poetry as traditional- national media (on elicits) have a structure and style according to insecure and unstable situation of the society, and in the meaning sense has ambiguity and contrast. The secret and mystery in "Bayhaqi" word indicates the frightening and insecure circumstance which surrounded him, he praising the Ghaznavi kings, have to maintain his culture and identity by interacting with political power and preserve his own culture and legacy under its power. He says: “My goal was to write fundamental history and raise a great construction and immortalize its memory throughout the world”. So he repeated in his book continually:"Wise men know ...", "wise men admire..." or "wise men learn a lot from this." Ferdowsi also as a scholar tells about his poems:

"*I founded a high palace by poem,*
Neither wind nor storm can abolish it».

Turmoil, social tension and insecure feeling among educated people, caused they resort to poetry as a mysterious communication. Hafez and Saadi`s poetries was crystallized as a middle class media in insecure and unstable Mongol captured Iran, but it did not mature because concentration and relatively stability was rules in safavid and Ghajar era. There is no poet and poem like Hafez and Sa`adi but also the poem and literature in Safavid period was strict and artificial with great exaggeration and magnification was very common in the era. So that national poets have to leave their hometown to India and Othman or sometimes Guilan in the manner the poetry and literature had been an advertizing media for Safavid kings. The elite literature was not so much durable in Persian society and immigrates to aboard with its poets, and replaced by "epic – faith”, but “bacchanalian verse" that had indicated human suffering in sixth century was begun in the Safavid era (tenth century AH). In addition to popular religious attitude toward Persian poetry, Turkish poem was growing in Safavid kings palace and may be a foreground the media that is driven by elites and intellectuals lose its advertising use. During this period, the poetry media become two streams of national (Sufi), who were forced to migrate and religious (advertising) that is selected from Safavid superficiality. Subsequently, Safavids in response to the Ottoman and Uzbek prejudiced Sunni empire, provide a new reading of Shiism and tried to revive governmental Shia Revival and to strengthen Shia media or even import religious rituals from East of Europe, creates a ministry entitled "tragedian and commemoration” to complete the project, so that the minister traveled to Europe and add a ceremonies like Kotal Keshi, reading a commemoration fron Karbala martyrs, elegizing in group, music (cymbal) and sign bringing (as same as cross) which were adopted from Christendom in 16 and 17 centuries to Shi`ei ceremonies. Qajar kings followed the Safavids,

magnified the third Shiite Imam martyrdom as a tools for strengthening their political sovereignty to conventionalize the Karbala event and stupefy the Shiite and prevent people offend toward their government. By arriving modernity from Europe to Iran and Constitutional Revolution (1906 AD) the first modern European literature was founded in Iran.

A phenomenon could interact with the national media - particularly the traditional national poetry and affect thinking at the beginning of modernity.

The first Persian novels were generally critical, emotional and love story were written imitating from Europe literature.

Writhing the collection of "charand and Parand' by A.Dehkhoda is a sign of entering Iran literature into new area and simplification is its main characteristic. Specially, story transformation into western style was because of rationalism in contract to Romanism in literary poems.

Naturally that traditional media ″ today strongly are influenced by modern and traditional media and strongly affect on as traditional-religious media more than past and gradually lost their independent identity in political communication. So any mosque or religious place in Iran as traditional media depends on Internet and other new media.

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References

1. Anderson, A. 1997. *Media, Culture and the Environment*, London, UCL Press.
2. Chagay, Ram.1992. *Islamic symbolism: The ideology of the Islamic Revolution in Iran as reflected in Friday communal sermons,1979-1989.* New York University.
3. *Howard, P.* (, 2009). Inside the Cyberwar for Iran's Future. Miller-McCune. Available at http://www.miller-mccune.com/culture/inside-the-cyberwar-for-irans-future-6535.
4. Jenkins, H. 2006.*Convergence Culture: Where old and new media collide*, New York,New York University Press.
5. *Kelly, J. & Etling, B.* (2008). Mapping Iran's Online Public: Politics and Culture in the Persian Blogosphere. Internet & Democracy Case Study. Berkman Center Research Publication No. 2008-01.
6. Rahimi, Babak.2003. "Cyberdissent: The Internet Culture in Iran." *Middle East Review of International Affairs* 7, no. 3.
7. Retamal, G. and Aedo-Richmond, R. (eds) (1998). *Education as a Humanitarian Response,* London: Cassell/IBE.
8. Wedeen, Lisa. 2002. "Conceptualizing Culture: Possibilities for Political Science." *American Political Science Review* 96: 713-728.

Review of Ombrothermic Curve Graphs in the Interpretation of Drought (A Case study in Esfahan Province)

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Abstract: Drought is one of the most chronic natural disasters and is a gentle environmental phenomenon, so that it is more visible in the arid and semiarid regions. The intensity of dried periods in a 12-month scale was studied and analyzed and after obtaining precipitation and temperature statistics, Ombrothermic curves were drawn and studied using Excel software. Results of this study showed that in 2005, the duration of drought in majority of cities especially in Golpayegan was at least 2 months more than the long period. Therefore this article tires to study droughts in Esfahan in a 12-month scale for the statistic periods of 1992-2005 through the use of SPI "Standardize Precipitation Index".

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Keywords: Drought, Ombrothermic , SPI , Esfahan

1. Introduction

Review of the history of human life on the Earth shows that man has always been exposed to natural disasters , part of which that is related to geological and tectonic features such as earthquakes , volcanoes , and so on , is referred to as geological disasters. Another part of natural disasters caused by climate changes and fluctuations such as flood, drought, storms, lightning, is called climatic and ecological disasters (Hare, F.K., 1983). Climatic and ecological disasters are very worrying but noticeable in Iran since it is located on a global dry belt. Sometimes, drought causes great damage to ecological environments. At the same time, even a rainfall during the drought may cause irreparable damage. Today, water supply management and rational analysis of existing conditions play a vital and undeniable role in determining less harmful and more useful ways of dealing with natural disasters such as floods and droughts and mitigating their harmful irreparable damage. Also due to the increasing development of human societies and the increasing demands and utilization of natural resources the need for careful review and adoption of a rational policy towards significant natural resources has been raised. Obviously, the logical analysis of natural phenomena, requires a relatively accurate estimation and as much actual simulation as possible of existing conditions. Given the random characteristics of natural events and their absolutely random behavior, any analysis of them would be quite uncertain. Therefore, prediction of any factors will be at various levels of probability.

1.1. Drought

Drought is a creeping environmental phenomenon that gently swallows a geographical area and may last several months to several years and causes a lot of damage. Climate change is the main cause of droughts in a global scale.

All regions of the world are temporarily, but irregularly suffering from repeated drought conditions, but this situation is more visible in the regions which are erratically affected by various weather patterns. The main consequence of drought is a long period of below-normal low rainfall. Reduction of soil moisture, surface water and underground water is the next consequence of low rainfall. Drought is a disaster that results from the lack of water (rainfall) and its absence means the destruction of life. This phenomenon is one of the main hazards associated with the weather. This natural Disaster will influence all aspects of our lives. There isn't a single Definition of drought at international level which is acceptable to all sides. Generally, drought occurs when water severely depletes in a special place and time. There are many definitions of the word 'drought' which are constantly changing specially with regard to its effect on natural and social environment. Drought is caused by numerous physical and spatial factors which can mainly be studied within the framework of general circulation of atmosphere and climate changes. Different studies represent researchers efforts to know drought more as a management tool since the far past. But the influence of various climate factors on drought has prevented a clear and comprehensive definition of it. Drought, is unusual dryness and continuous lack of rainfall in comparison to its long lasting average which, based on its severity and duration, might have different effects (Wihite,D. A. and Glants, M. H. 1985). To know the quantity of droughts, the use of indices developed based on precipitation has been considered by the researchers (Palmer,N. and K. J. Holmer. 1988). Lack of rainfall

will have negative effects on underground waters, water supplies and resources, soil moisture and the flow of the rivers. Understanding this problem has led to conducting many researches and studies by different researchers (Correia, F. N, Santos, M. A, Rodrigues, R. R, 1991).

2.1. Drought characteristics

Each drought is known by three special features: intensity, duration, and the width of affected area. Quantitative measurement and expression of events are the requirements for knowing and comparing them. Therefore, to study and compare drought at different times, it must be monitored. According to Dracup , drought can be classified into 3 groups. In advanced stages of drought, water supplies and resources are facing severe shortages.
This means that groundwater reaction to droughts is too important. It should be noted that the impacts of low rainfall on soil moisture, water reservoirs, surface flow of rivers, and groundwater level will be shown at different time scales. However, for quantitative analysis of droughts, it's essential to have a definite index to determine dry and wet periods accurately (Silva. V. P. R. 2003). The review of determining indices of drought could be necessary to predict drought as the most important strategy to deal with it and to reduce its damage (Dracup J. A. Lee K. S and Paulson E. G. 1980). According to Tase Norio, years are divided to five categories of severely dry, dry, moderate, wet, and very wet. The performance of drought monitoring systems is seriously affected by the accuracy of index selection which is a description of subjective and objective conditions of drought. Each one of these indices is necessarily related to one kind of drought (agricultural, meteorological, hydrological, economic-social).

Table 1: Methods of studying drought

Methods of studying drought	Indices of studying drought
Water balance	Torrent White
Flow analysis	Palmer
Groundwater analysis	Minimum flow
Determining threshold level	Surface water supply index(SWSI)
Synoptic analysis	
Remote evaluation	
Multivariate correlation	
Using geomorphologic and historical information	

3.1. Standardized Precipitation Index (SPI)

Lack of rainfall, has different effects on groundwater, water resources, soil moisture and river flow. Understanding this issue has led to the conduction of various studies and researches by scientists and researchers. To determine the possibility of drought, the standardized precipitation index was developed. SPI has been designed to determine the lack of rainfall at different time scales. Standardized precipitation index can be calculated for any time and scale and at different time scales which depends on the rainfall probability (Mckee, T. B. ,N. J. Doesken, and J. Kleist,1993). Moreover, it can be an early warning about drought and a help to measure its severity. This method was presented by Mckee with regard to various effects of rainfall shortage on groundwater, surface water supplies and resources, soil moisture, and water flow. This index is use to quantify the lack of rainfall in a scale of several months and reflects the effects of drought on the rainfall anomalies in a relatively short time scale. However, it should be noted that the flow of groundwater and surface water reservoirs are reflecting the anomalies of long-term precipitation. That's why the standardized precipitation index (SPI) is basically calculated for 24, 12, 6, 3 and 48-month time scales. SPI is calculated by subtracting the amount of rainfall from the average in a certain time scale and then dividing it by standard deviation. SPI average in a time scale in a position equals zero and its standard deviation equals one. It's an important advantage of this index; since SPI has become normal. Therefore drier and more humid climates can be shown in the same way. In addition to drought periods, high rainfalls can also be studied by SPI. A drought happens when SPI is constantly negative and reaches to -1 or less. It ends when SPI becomes positive. Therefore each drought has a time period and will be defined by its beginning and end and its density is calculated for each month as long as it continues.

4.1. The Understudy Area:

The area which is studied in this research is Esfahan Province with an area of 106179 square kilometers. This province lies at latitude 30°42' - 34°30' north and at longitude 49°36' - 55°32' east in centaral part of Iran. This city has always been noticed by state managers due to its proper geographical position which is located in the heart of Iranian plateau. It is about 1580m above the sea level and is located in the east of Zagross Mountains. Esfahan is surrounded by dessert from north and east and its western and southern part is limited ro Zagross Mountains.

2. Material and methods

Since statistical period is needed to calculate SPI, synoptic stations which contained statistical periods were selected in the studied area. A 14-year period was chosen because of the lack of statistics. Then the statistics of rainfalls and temperature of Ardestan, Daran, East Isfahan, Golpayegan, Isfahan, KabootarAbad, Kashan, Khurbiabank, Nain, Natanz, and Shahreza stations were obtained from the meteorological office.
Then Ombrothermic curves were drawn separately for each station through Excel software and were finally used for analyzing data. After that, SPI was calculated for a12-month time scale.

Table 2: The following table briefly shows the results of Ombrothermic curves in 2005

	Ardestan	Daran	East of Isfahan	Golpayegan	Isfahan	Kabootarabad	Kashan	Khorbiyabanak	Naein	Natanz	Shahreza
Drought months	11	7	10	Station9	10	10	9	12	10	8	10
Jan								*			
Feb	*	*	*		*	*		*			*
March	*		*	*	*	*	*	*	*	*	*
April	*		*		*	*	*	*	*	*	*
May	*	*	*	*	*	*	*	*	*	*	*
June	*	*	*	*	*	*	*	*	*	*	*
July	*	*	*	*	*	*	*	*	*	*	*
August	*	*	*	*	*	*	*	*	*	*	*
September	*	*	*	*	*	*	*	*	*	*	*
October	*	*	*	*	*	*	*	*	*	*	*
November	*		*	*	*	*	*	*	*		*
December	*			*				*	*		

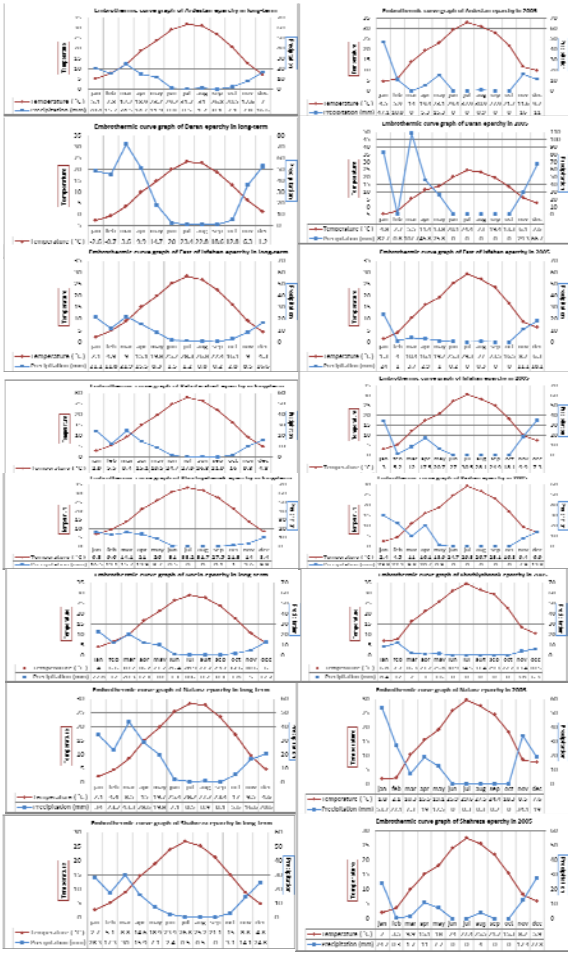

Figure 1

Figure 2.

Table 3: The results of Ombrothermic curves for a long period of time:

Station	Ardestan	Daran	East of Isfahan	Golpayegan	Isfahan	Kabootarabad	Kashan	Khorbiyabanak	Naein	Natanz	Shahreza
Drought months	9	6	8	6	8	8	8	11	9	8	8
Jan											
Feb	*							*	*		
March								*		*	
April	*		*		*	*	*	*	*	*	*
May	*	*	*		*	*	*	*	*	*	*
June	*	*	*	*	*	*	*	*	*	*	*
July	*	*	*	*	*	*	*	*	*	*	*
August	*	*	*	*	*	*	*	*	*	*	*
September	*	*	*	*	*	*	*	*	*	*	*
October	*	*	*	*	*	*	*	*	*	*	*
November	*		*	*	*	*	*	*	*		*
December								*			

Table 4: SPI tables are as following:

Eparchy / Year	Ardestan	Daran	East Of Isfahan	Golpayegan	Isfahan
1992	Moderately wet	Near normal	Near normal	Near normal	Near normal
1993	Near normal	Very wet	Extremely wet	Very wet	Very wet
1994	Severely dry	Moderately wet	Near normal	Near normal	Severely dry
1995	Near normal	Severely dry	Near normal	Moderately dry	Near normal
1996	Extremely wet	Near normal	Near normal	Near normal	Near normal
1997	Moderately dry	Near normal	Near normal	Near normal	Near normal
1998	Near normal	Near normal	Near normal	Near normal	Near normal
1999	Near normal	Moderately dry	Near normal	Near normal	Near normal

Table 5

Eparchy / Year	Kabotarabad	Kashan	Khorbiyaban	Naein	Natanz	Shahreza
1992	Near normal	Very wet	Near normal	Very wet	Moderately wet	Severely dry
1993	Very wet	Near normal	Near normal	Near normal	Near normal	Near normal
1994	Near normal	Near normal	Near normal	Near normal	Near normal	Near normal
1995	Near normal	Moderately dry	Near normal	Near normal	Near normal	Moderately wet
1996	Near normal	Very wet	Very wet	Moderately wet	Very wet	Near normal
1997	Near normal	Moderately dry	Moderately dry	Near normal	Moderately dry	Near normal
1998	Near normal	Near normal	Near normal	Near normal	Near normal	Near normal
1999	Near normal	Near normal	Moderately wet	Very wet	Near normal	Near normal
2000	Severely dry	Near normal	Near normal	Near normal	Near normal	Moderately dry
2001	Severely dry	Moderately dry	Near normal	Moderately dry	Extremely dry	Near normal
2002	Near normal	Moderately wet	Near normal	Near normal	Moderately wet	Near normal
2003	Near normal	Near normal	Near normal	Moderately dry	Near normal	Near normal
2004	Very wet	Near normal	Moderately wet	Near normal	Near normal	Extremely wet
2005	Near normal	Near normal	Severely dry	Moderately dry	Near normal	Moderately dry

Table 6

Station	Ardestan	Daran	East of Isfahan	Golpayegan	Isfahan	Kabootarabad	Kashan	Khorbiyabanak	Naein	Natanz	Shahreza
Drought months in 2005	11	7	10	9	10	10	9	12	10	8	10
SPI	Near normal	Near normal	Severly dry	Near normal	Near normal	Near normal	Near normal	Severly dry	Moderately dry	Near normal	Moderately dry

3. Discussions

The results obtained from Ombrothermic curves in 2005 indicate that the number of drought months is as the following for different cities respectively:

Khurbiabanak 12, Ardestan 11 , East of Isfahan, Isfahan, Kabootarabad, Nain and shahreza 10, Golpayegan and Kashan 9, Matanz 8, Daran 7 and for a long period of time are respectively as the following:

Khurbiabanak 11, Ardestan 9, East of Isfahan, Isfahan , Kabootarabad, Kashan, Nain, Natanz and Shahreza 8, Golpayegan and Daran 6. Therefore, the results show that drought duration in 2005 in most cities specially in Golpayegan is at least two months more than the long term duration.

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References

1. Correia,F. N,Santos,M. A,Rodrigues,R. R,1991,Reliability in Regional Drought studies,Drought Mitigation in Europe, Vogt JV,soma F (eds) Kluwer : Dordrecht : 161 – 166.
2. Dracup J. A. Lee K. S and Paulson E. G. 1980. On the definition of droughts. Water Resource Research, 16: 297 – 302.
3. Mckee,T. B. ,N. J. Doesken,and J. Kleist,1993. ; The relationship of drought frequency and duration to time scales. Preprints, 8th conference on Applied climatology ،17-22 Junuray,Anaheim,CA,PP. 179 – 184.
4. MendicinoG. ,Alfonso,S. , Pasquale monitoring and forecasting in a Mediterranean climate,Journal of Hydrology,282-302.
5. Palmer,N. and K. J. Holmer. 1988. Operational guidance during droughts: expert system approaches. J. Water Resource. plan. Mange. 114 (6) : 647 – 666.
6. Silva. V. P. R. 2003, on climate Variability in northeast Brazil,Journal of Arid Environment,54 (2),256-367.
7. Grytnes JA, Vetaas OR. Species richness and altitude: A comparison between null models and interpolated plant species richness along the Himalayan altitudinal gradient, Nepal. The Am Nat 2002;159(3):294-304.
8. Wihite,D. A. and Glants, M. H. 1985. understanding the drought phenomenon : The role of definitions –water International, 10 : 111-120.
9. Wihite,D. A. and Glants, M. H. 1985. Understanding the drought phenomenon: The role of definitions –water International, 10 : 111-120.

Discourse and Translation: A Case Study

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Abstract: The present article mainly focuses on translation analysis from the perspective of discourse analysis (DA) at micro level. In order to do that, the researcher applied the framework of Farshidvard (1984) and that of Shafaie (1984) to analyze the stylistic devices and synthetic patterns, respectively in the Persian novella "The Blind Owl" written by Sadegh Hedayat (1937) and English translations of that, done by Iraj Bashiri (1937) and D.P. Costello (1957). By carrying out this qualitative, quantitative, descriptive, corpus-based research, the researcher aimed at, first, finding the probable differences and similarities between the two English translations in terms of elements in each model, and second, finding out which translator has saved Sadegh Hedayat's style more. The results showed that the frequency of stylistic devices and synthetic patterns use have obviously influenced the translation products, in turn, making a considerable difference between the two English translations; therefore, both hypotheses were rejected at the end.
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Keywords: translation analysis, stylistic devices, synthetic patterns, discourse analysis at micro level

1. Introduction

The term translation itself has different meanings, although generally it can be defined as a process in which there are three main categories; the subject field, the product (the text that has been translated), and the process (the act of producing the translation). Moreover, the process of translation between two different languages involves the translator changing an original written text (the source text, or ST) in the original verbal language (the source language or SL) into a written text (the target text, or TT) in a different verbal language (the target language or TL) (Munday, 2010: 5). This definition connotes that translation is not a single activity. In other words, there is always a context from which the process of translation starts and another one in which the act of translation takes place. What makes the process of translation more complicated is the issue of meaning. In fact, meaning in translation can be defined as the load that is transmitted from one text to another one. Therefore, it can be concluded that similar to the process of translation, meaning is never created alone. Finding out how complex is the process of translation and transmitting the meaning through it, the need of being equipped with a useful means for analyzing the texts before and after translation is evidently felt. In other words, if a translator has the chance to own a tool, which enables him/her to analyze the texts thoroughly before and after translation, the translator might be more successful to complete the hard mission of translation and the quality of translation product might be much better. Discourse analysis (DA) is one of the tools that let the users to analyze the texts at both micro and macro level. The flexibility of discourse analysis makes this tool advantageous. The flexible nature of discourse analysis diversifies the users as well. The users or the addressees of discourse analysis are analysts, translators, and translation teachers. The analysts can take the advantage of DA by analyzing the different translated texts to find the strengths and weaknesses. Moreover, the translators take the advantage of DA, by analyzing the texts before translation, and it helps them to have a closer connection with the texts. Finally, Translation teachers might also take the advantage of DA by teaching how the Translation students can be the self-analyzers of their own works. Regarding the above-mentioned issues, the present article is aimed at applying two models of discourse analysis, which are developed by two Persian scholars, Khosro Farshidvard (1984) and Shafaie (1984), to analyze two English translations of a Persian Masterpiece "The Blind Owl" written by one of the most significant Persian authors "Sadegh Hedayat" at micro level. In addition, through investigating and comparing the two translated texts with each other and with the source text, the researcher is going to show how the models applied can be practiced and how they can help the analyzers to find the weaknesses and strengths of the translated texts. Furthermore, by analyzing the novel and the translated versions at micro level, the researcher is going to provide the readers with some suggestions for further researches in this field of study and with the same corpus. There are different scholars (Joshua 2008, as cited in Ray, 2008, and Munday, 2010) who have shown their interests to the history of translation by carrying out various researches on this subject. Munday (2010, 9-15) believes that writing on the subject of translation goes

far back in the recorded history, and as he continues the starting point of this movement begins with the discussions of Cicero and Horace (first century BCE), and St. Jerome (forth century CE), whose writings influenced the workings up to the twentieth century. Munday goes on and describes that the study of the translation field developed into an academic discipline in the second half of the twentieth century, and before that translation had normally been an element of language learning in modern language courses. In furtherance, he discusses each period and describes the characteristics of translation at that era. For example, Munday discusses that in the late eighteenth century; 1960s, translation was used to be taught at secondary schools through grammar-translation method, the aim of which was translating Holy Bible. In 1970s, the contrastive analysis has got out, and the linguistics-oriented 'science' of translation has continued strongly in Germany. The late 1970s and the 1980s, witnessed the rise of a descriptive approach that had its origins in comparative literature and Russian Formalism. Yet, the 1990s saw the incorporation of new schools and concepts, with Canadian-based translation and gender research led by Sherry Simon, the Brazilian Cannibalist School promoted by Else Vieira. This has continued space in the first decade of the new millennium, with special interest devoted to translation, globalization, and resistance. On one hand, the importance of translation issue, which has been the field of different researchers' interest (Briceno Iragorry, 1985: 145 as cited in Bastin and Bandia, 2006: 1, Lieven D'hulst, 2001: 21 as cited in Bastin and Bandia, 2006: 1-2, and Bastin and Bandia, 2006: 2-3). For example according to Lieven D'hulst (2001: 21, as cited in Bastin and Bandia, 2006: 1-2) "the history of translation has not received the attention it merits in terms of research and cannot be compared to any other type of research in translation studies." On the other hand, the issue that is significant in the field of translation and in general in the context, is the issue of discourse, the branch, which can be considered as the field of linguistics, has won a variety of researches' (Harris, 1952, Van Dijk, 1983, Brown and Yule, 1983) attentions and interests.

"Dictionary of language and linguistics" defines Discourse analysis as the following:

*Cover term for various analyses of **discourse**. Motivated by linguistic terminology and theory (**formal logic, structuralism, transformational grammar**) it is used synonymously with **text analysis,** with a particular interest in wellformedness (**coherence, cohesion**) and deductive rules (e.g. rules for **speech acts**). While in this strand of research, texts are mainly taken to be static products (**Discourse grammar, text linguistics**), there is another strand influenced by **functional grammar, psycholinguistics,** and approaches to cognitive science that emphasizes the dynamic character of discourse as construction and interpretation processes by the speaker/ writer and the listener/ reader (see Brown and Yule, 1983). (1998: 352)*

Although discourse analysis is not a very old field of study since it was first introduced and defined by Harris (1952), many scholars and theorists (Levinson, 2003; and Van Dijk, 1985) to name a few, have considered this field of study in a variety of social science disciplines, such as semiotics, linguistics, anthropology, cognitive psychology, and translation studies. Lemke (2004) talks about the origin of discourse analysis and believes that Aristotle, Cicero, and Longinus and followers find the root of discourse analysis in the classical Rhetorical theory. Genc et al (2006: 135) states, "critical thinking about the analysis of situations/texts is as ancient as mankind or philosophy itself." As Bressler (2007) believed discourse analysis was re-born in the sixties. In other words, the modern discourse analysis derives initially from the formalists, structuralist work of Propp on the methodology of Russian folktales, the pioneer of Genre Theory. Then it was developed by the Russian and Prague schools of functional text analysis, both for literary and non-literary genres of writing, and to a smaller extent for speech. Stubbs (1983, 10-11) explains that discourse analysis is used to refer both to the study of language above the sentence (more accurately, above the clause), and to the study of naturally occurring language. Stubbs (1983:10) believes that there are three different decisions, which have to be made in deciding how much idealization is necessary or justifiable in the study of language:

> *(a) the size of units to be studied: basically smaller or larger than sentences; (b) whether these consequences are to be contrived by the linguist or to be naturally occurring; and (c) whether non0linguistic factors of the context are to be studied or not.*

According to Van Dijk (1985), discourse analysis has become a new cross-disciplinary field of analysis since the early 1970s. It is of interests to disciplines such as anthropology, and sociolinguistics, artificial intelligence, cognitive science, philosophy of language, and text linguistics. Van Dijk (1983) believes that discourse is analyzed first at different levels, and each level has its own sub-levels. For example, as Van Dijk states, phonology is regarded as one main level for analyzing discourse, but at this level, there are different sub-levels such as intonation, the structure of sounds, and so on. Therefore, it can be concluded that discourse analysis has a comprehensive look at the details and not only studies the main levels which can be considered as the macro level of analysis, but also discourse analysis at micro level, scrutinize the sub-categories of its main category. Discourse analysts

believe that the units and structures of discourse analysis are completely different from those of languages in which "sentence" is regarded as the biggest units of the language. Now seeing discourse analysis from this point of view, it can be said that in linguistics, language is considered as a completely abstract system in which the functions of the rules will be studied, but in discourse analysis, language in its actual use has to be considered. In other words, in discourse analysis the pragmatics side of language, based on the language functions in communicating is studied. McCarthy (2005: 144-152) compares the spoken and written discourses and believes that spoken discourse types can be analyzed for their typical patterns and the linguistic realizations that accompany them, and the periodical literature of discourse analysis abounds in detailed studies of a vast range of types. However, letters are a good example of a discourse type where the receiver is usually a specified individual or group. Moreover, McCarthy believes that although, sentence was dismissed as being dubious value as a unit of discourse in speaking, it is more obvious as a grammatical unit in writing, not exactly in all kinds of writing signs and notices. In fact, McCarthy talks about the different types of discourse and divides them under the two categories of spoken and unspoken discourses. Yet, Georgakopulou et al (1977: 3-4) talk over the units of discourse analysis and in order to do that, they identifies the differences between "text" and "discourse." As they believe considering the notion of text, in the area of discourse analysis involves the material aspect of language communication. Then, they continue and state that although these two terms have been used interchangeably in different literatures, they might be completely different. According to them, text is used in the written sense of discourse, while discourse can be meant the spoken or the written form. Therefore:

> *Discourse analysis is, in some accounts, regarded as concerned with spoken texts (primarily conversation). Text linguistics, as a different discipline, has mainly been associated with written texts. In our view, the terms do not refer to different domains (speech and writing), but reflect a difference in focus. (p. 4)*

So, discourse analysis can be regarded as an umbrella term in their viewpoints for either spoken or written communication beyond the sentence. Text is the basic means of this communication. Discourse is thus a more embracing term that calls attention to the situated uses of text: It comprises both text and context. Nazari (2010: 5) explains that the scholars after Harris (1952) believed that discourse analysis includes structure analysis of the spoken language and text analysis of the written language. This current group believes that discourse analysis mostly considers the pragmatics or the structure of the sentence. Alba-Juez (2009) believes that text-internal elements constitute the text, while text-external elements constitute the context. Schiffrin (1994) as cited in Alba-Juez (2009), states that discourse analysis takes both text and context into consideration. Therefore, as stated above discourse analysis involves the study of both text and context, so one might conclude that discourse analysis is more completed than linguistic analysis why the former analyzes both texts and contexts, but the latter studies only texts. Moreover, it should be added to the above-mentioned facts that Hatim and Mason define the context in terms of text focus. This model of context includes the general categories of genre, tenor, and mode, as well. As specific discourse classification and text type (Hatim and Mason, 1990). Schiffrin et al., as cited in Alba-Juez (2009), states that all the definitions of discourse analysis fall into these three categories:

1. Anything beyond the sentence
2. Language use

A broader range of social practice that includes non-linguistic and non-specific instances of language (2001: 1). Although for Harris (1952) discourse was a higher level than sentences, he used discourse in its expanded meaning. Instead for them discourse was an umbrella term which involved not only the propositional content, but also social, cultural and contextual contexts. It is also interesting to know that "discourse analytical approaches take as their starting point the claim of structuralist and poststructuralist linguistic philosophy that our access to reality is always through language." (Jorgenson et al., 2002:4). Yet, McNabb (2008: 393) expresses that discourse analysis has a "triple concern" with the themes of *action,* and *variability* in the message. In the following, he states that discourse analysis is regarded by the rhetorical or argumentative organization of texts and talks. At the end, he mentions the objective of discourse analysis and tells that discourse analysis "is to take the focus of analysis away from question of *how* a text version relates to reality to ask instead how the version is designed to compete successfully with one or more alternative versions." (McNabb, 2008: 393). Solhjoo (1998) also believes that discourse, includes larger units than sentences. Solhjoo believes that if the rules of discourse are applied, the sentences in a paragraph can be stated more concretely, which make the reader and the hearer move from one sentence to another easier. After introducing some approaches to discourse analysis, may be this question comes to mind that why analyzing discourse is important or in other words what kinds of application it can have. In order to answer such a question some possible application of discourse analysis can be provided. As it is written in Jorgenson's et al. article: "It can be used for analyzing the national

identity: how can we understand national identities and what consequences do the division of the world into nation states have?" (2002: 2). Sanders and Maat (2006) declares that discourse shows connectedness and they state that the central objective of linguists who work on discourse analysis is characterizing this connectedness. In addition, Halliday and Hasan (1976) consider this connectedness and explain the text connectedness in terms of reference, substitution, ellipsis, conjunction, and lexical cohesion. According to Halliday and Hasan (1976: 13) as cited in Sanders and Maat (2006: 591) "these explicit clues make a text a text." Halliday and Hasan (1976: 4) as cited in Milagros Del Saz Rubio (2007: 24) state that cohesion "occurs when the interpretation of some element in the discourse is dependent on that of another." Having investigated the theoretical issues in discourse analysis, the researcher now will refer to the practical aspects of the related issue. It should be recalled that in this part, it has been tried to take a though look at some of the researches (articles, theses, dissertations, etc) which have been conducted on discourse analysis in different fields of study, literature preferably, narrative genre specifically. Rahimian *et al.* (2003) have analyzed one of the Jalal Al Ahmad's stories, "the school principal", from the discourse analysis point of view. They have applied the frameworks of Hatch (1992) to analyze the text at macro level and that of Halliday and Hasan (1976) to analyze the text at micro level. By analyzing the text at micro an macro level, they have shown how much cohesively the text is written, and if the text is a normal narration based on Hatch's model. Another research similar to the above-mentioned one has been conducted by Susan Nirmala (2009) in which she analyzed the discourse in "the man-eater of Malgudi" narrative. She applied a linguistic view on discourse, and explained the linguistic relations and its sub-categories such as anaphoric, cataphoric, cohesion, redundant, exclamations, repetitive phrases, all of which were used in the novel. After that, she investigated the dialogue discourse and finally she concluded that the language that the author has used in the novel was simple and enjoyable.

The other research, which was conducted in the field of discourse analysis is that of Labov *et al.* (1967, as cited in Schiffrin, 2001) which were done about "PEN" (Personal Experienced Narrative). In this research, they have gathered fourteen stories people narrated about their personal experiences mostly the embarrassing ones, and then they analyzed the discourse in these narratives based on a "formal" approach. Based on what they reported a clause in PEN can have two functions; referential and evaluative; referential clauses, have to do with what the story is about events, characters, and setting. Evaluative clauses, on the on the other hand, have to do with why the narrator is telling the story and why the audience should listen to it. In another research, Navas Brenes (2005) conducted a research on analyzing an oral narrative using discourse analysis tools. The researcher used a narrator, who told an anecdote about a danger-of-death experience, then he analyzed the narration from different dimensions; characteristics of spoken texts, formulaic expressions, subordination with all their sub-categories and finally discourse analysis. Then he showed that how discourse analysis could provide EFL students with key tools in order to show them how spoken language works in authentic contexts.

1.1. The significance of *"The Blind Owl"*

There were multiple reasons for choosing the novella for this study. The reasons are as following: This novella, which is authored by Sadegh Hedayat (1937), who is regarded as Iran's foremost modern writer of prose fiction and short stories (Shamissa, 2000), is the most enduring work of prose and a major literary work of 20th century Iran (Shamissa, 2000). As shamissa (2000: 18) believes various scholars have written books on the novella as well as various movies that have been produced based on it. Moreover, scholars such as Andre Breton, the surrealists' leader, have talked over the novella. Moreover, this novella is rated as the #7 most significant Persian novels by Guardian bookshop (2011). Besides, the importance of the novella, another reason for choosing this novella, was that, this book is among the few Persian novels that have been translated into English by both English and Persian translators. In other words, each of these two translators had some advantages. In fact, since the source text is in Persian, the Persian translator could take the advantage of better understanding of the source text; however, although the English translator doesn't have the equal chance for understanding the Persian language much better than the Persian translator, he could take the advantage of better understanding of the target language. Since Bashiri is fluent in English, (Wikipedia, 2012), and Costello had written different works in English including; the Oxford Russian-English Dictionary, The rag tree: A novel of Ireland (bookfinder, 2012) the researcher aimed at finding out whether there is any difference between the two translations in terms of keeping the stylistic devices and synthetic patterns used by the original writer.

2. Material and Methods

In order to carry out this research, the researcher has applied the frameworks of Shafaie (1984) and that of Farshidvard (1984), to figure out the synthetic patterns and stylistic devices in the Persian novella and the English translated ones, respectively. The synthetic patterns include the type of sentences, ranging simple, compound, and complex sentences,

form of the sentences including assertive, interrogative, imperative, exclamation, conditional, active, and passive sentences. Moreover, the nominal and verbal sentences are analyzed as well. Yet, in explaining the micro level, the stylistic devices used in the story, are very important. The most important devices include description, simile, slang, and colloquial prose. In order to make the aims of the research more clear, the following questions and hypotheses are going to be raised here, all of which are considered the major focus of this research:

1. Are there any difference between the Bashiri's (1974) and Costello's (1957) English translations of "The Blind Owl" in terms of keeping the stylistic devices of the source text from the perspective of discourse analysis at micro level put by Farshidvard (1984)?
2. Are there any difference between the Bashiri's (1974) and Costello's (1957) English translations of "The Blind Owl" in terms of keeping the synthetic patterns of the source text from the perspective of discourse analysis at micro level put by Shafaie (1984)?

$H_0$1: There is no difference between the two English translations of "The Blind Owl" in terms of keeping the stylistic devices of the source text from the perspective of discourse analysis at micro level put by Farshidvard (1984).

$H_0$2: There is no difference between the two English translations of "The Blind Owl" in terms of keeping the synthetic patterns of the source text from the perspective of discourse analysis at micro level put by Shafaie (1984).

1.2. Corpus

The corpuses under the study included: 55 pages equals to 3237 sentences selected randomly out of 98 pages of Sadegh Hedayat's novella "The Blind Owl" *[Boof-e-Koor]* written in 1937, along with two of its English Translations one done by Iraj Bashiri (1974) and the other by Costello (1957).

2.2. Data Collection

This study basically aimed at contrasting the Persian source text and the two English translated texts to look for the synthetic patterns and stylistic devices. In this regard, any manipulation in the English translations, comparing to those in the original Persian novella, were precisely scrutinized within the DA frameworks at micro level proposed by Shafaie (1984) and Farshidvard (1984). As mentioned above, in order to do that, the researcher studied 55 pages or more exactly 3273 sentences of the Persian novella to look for the synthetic and stylistic devices under the investigation and then scrutinized the same number of sentences in the two English versions, to find the same item as well. Finally, she provided some tables in which, the frequency and percentage of each item for both the original and the translated texts were inserted, based on which, the researcher made the conclusion.

3. Results

1.3. The Analysis of the Synthetic Patterns

The following table shows the most significant synthetic patterns that were found in the source and target texts. The analysis of table 1 is as the following:

Table1: synthetic patterns in the Persian version of "The Blind Owl" and the English translations done Bashiri (1974) and Costello (1957)

Hedayat's Novella (1937)	Bashiri's Translation (1974)	Costello's Translation (1957)
نمونه 1: من فقط برای سایه خودم می نویسم که جلو چراغ به دیوار افتاده است، باید خودم را بهش معرفی بکنم. (ص. 4) *[Man faghat baraye sayeye khodam minevisam ke joloye cheragh be divar oftadeh ast. Bayad khodam ra behesh moarefi bokonam.(p. 4)]*	Sample 1: I write only for my shadow, which is cast on the wall in front of the light. I must introduce myself to it. (p. 2)	Sample 1: I am writing only for my shadow, which is now stretched across the wall in the light of the lamp. I must make myself known to him. (p. 3)
نمونه 2: سه ماه-نه- دو ماه و چهار روز بود که پی او را گم کرده بودم، ولی یادگار چشمهای جادویی یا شراره کشنده چشمهایش در زندگی من همیشه ماند. چطور می توانم او را فراموش بکنم که آنقدر وابسته به زندگی من است؟ (ص. 5) *[Se maah-na-do maah va chahar rooz boob ke paye oo ra gom karde bodam, vali yadegare cheshmhaye jadoye ya sharareye koshandeye cheshmhayash dar zndegie man hamishe mand. Chetor mitavanam oo ra faramoosh bokonam ke anghadr vabasteh be zengedie man ast? (p. 5)]*	Sample 2: It was three months, no, it was two months and four days since I had lost her, but the memory of her enchanting eyes, no, the attractive malice of her eyes, remained in my life forever. How can I forget one who is so pertinent to my life? (p. 2)	Sample 2: It is three months- no, it is two months and four days-since I lost her from sight but the memory of those magic eyes, of the fatal radiance of those eyes, has remained with me all times. How can I forget her, who is so intimately bound up with my own existence? (p. 4)
نمونه 3: تمام شب را به این فکر بودم. چندین بار خواستم از روزنه دیوار نگاه بکنم ولی از صدای خنده پیرمرد میترسیدم. (ص. 10) *[tamame shab ra be in fekr bodam. Chandin bar khastam az rozaneye divar negah bokonam vali az sedaye khandeye piremard mitarsidam (p. 10)]*	Sample 3: I thought about this throughout the night. Several times I wanted to go to the hole in the wall and look, but I was afraid of the old man's laughter. (p. 6)	Sample 3: All that night I thought about these things. Again and again I was on the point of going to look through the aperture in the wall, but fear of the old man's laughter held me back. (p. 12)

In Sample one of the Persian novella, there are two sentences: the first sentence is a complex sentence, and the second one is a simple sentence. Moreover, there are two assertive sentences, both of which are active and nominal sentences. This categorization is similar to sample one of Bashiri's, and Costello's translations. Therefore, in sample one there is no difference between sentences regarding the synthetic patterns. In sample two of the Persian novella, there are three sentences, the first sentence is a compound complex; therefore, it is regarded as two assertive sentences. Moreover, the final sentence is an interrogative sentence, all the three sentences are active, and except the interrogative one, there are nominal sentences. In sample two of Bashiri's translation; however, there are four sentences. In addition, there are one simple sentence, two compound sentences, and one complex sentence. Therefore, there are five assertive sentences, and one interrogative sentence. Furthermore, the assertive sentences are

nominal, except the interrogative sentence, and active sentences. The categorization is the same in Costello's translation. Thus, in sample 2, the English translations have a bit difference with the source text according to the number of synthetic patterns. In sample three of the Persian novella, there are two sentences. The first sentence is simple, but the second sentence is compound. Therefore, there are three assertive sentences, three nominal and three active sentences. However, in Bashiri's English translation, there are three sentences, the first sentence is simple, the second one is compound, and the third one is compound as well. Therefore, there are four assertive, four active, three nominal, and one verbal sentence. On the other hand, there are three sentences in Costello's English translation. The first sentence is simple, and the second sentence is compound. Therefore, there are three assertive, three active, two nominal, and one verbal sentence. Regardless of samples one and two, it can be stated that based on sample three, Costello's translation is much closer to the source text according the use of synthetic patterns.

Table 2: The Frequency and percentage of *Synthetic Patterns* in the source and translated texts

Type of sentence		Hedayat's novella (1937)	Bashiri's translation (1974)	Costello's translation (1957)
Simple	Frequency	320	336	331
	Percentage	10%	10%	10%
Compound	Frequency	332	239	308
	Percentage	10%	7%	10%
Complex	Frequency	32	185	217
	Percentage	1%	6%	7%
Assertive	Frequency	773	644	762
	Percentage	24%	20%	24%
Interrogative	Frequency	51	44	45
	Percentage	2%	1%	1%
Imperative	Frequency	4	5	3
	Percentage	0%	0%	0%
Exclamatory	Frequency	3	3	3
	Percentage	0%	0%	0%
Conditional	Frequency	15	10	13
	Percentage	0%	0%	0%
Nominal	Frequency	773	684	787
	Percentage	24%	21%	24%
Verbal	Frequency	80	89	50
	Percentage	2%	3%	2%
Active	Frequency	826	729	676
	Percentage	26%	23%	21%
Passive	Frequency	28	43	42
	Percentage	1%	1%	1%
Total Number		3237	3237	3237

Moreover, table (2) shows the frequency and percentage of each synthetic pattern in the Hedayat's "The Blind Owl" and the two English translations. Regarding the above table, it can be stated that the percentages of simple and compound sentences were equal in the source novel, but the percentage of complex sentences was much less than the other two kinds of sentences. However, although the percentage of simple sentences were the same among both English translation, the percentages of compound and complex sentences are more in Costello's translation than that of Bashiri's. Moreover, the percentage of assertive sentences was more than interrogatives in the source text; however, although the percentage of interrogative sentences was the same in both English translation, the percentage of statements are more in Costello's translation than in Bashiri's. Furthermore, the percentages of imperative, exclamatory, and conditional sentences were the same in both source and translated texts. In addition, although the percentages of nominal sentences were more than the percentages of verbal sentences in both source and translated text, it should be stated that the percentages of nominal sentences were equal in both English translated texts, but the percentage of verbal sentences is more in Bashiri's English translation than in Costello's one.

Table 3: samples of stylistic devices used in the source and translated texts

Hedayat's Novella (1937)	Bashiri's Translation (1974)	Costello's Translation (1957)
نمونه 1: به هر حال عمویم پیرمردی بود قوز کرده که شالمه هندی دور سرش بسته بود، و سر و رویش را با شال گردن پیچیده بود،یخه اش باز بود و سینه پشم آلودش دیده میشد. پلک های ناسور سرخ و لب شکری داشت. (ص. 7) *[sample one: be har hal amoyam pirmardi bood ghooz karde ke shalmeye hendi dore saresh basteh bod, va sar o royash ra ba shal garden pichede bood, yakheash baaz bood vaa sineye pashm aloodash dide mishod. Pelkhaye nasoor sorkh va labe shekari dasht (p. 7)]*	Sample 1: In any case, my uncle was a stooped old man who wore an Indian shalma around his head and a yellow torn cloak on his shoulders. He had covered his head and face with a scarf. His collar was open and his hairy chest could be seen. One cold count the hairs of his thin bread as it protruded through his scarf. With his red, fistular eyelids and leprous lip, … (p. 4)	Sample 1: At all events my uncle was a bent old man with an Indian turban on his head and a ragged yellow cloak on his back; his face was partly concealed by a scarf wrapped around his neck; his shirt was open and revealed a hairy chest. (p. 7)
نمونه 2: لبخند مدهوشانه و بی اداره ی کنار لبش خشک شده بود، مثل اینکه به فکر شخص غایبی بوده باشد. از آنجا بود که چشمهای مهیب افسونگر، چشمهایی که مثل این بود که به انسان سرزنش تلخی میزند، چشمهای مضطرب، متعجب، تهدید کننده، و وعده دهنده او را دیدم و پرتو زندگی من روی این گودیهای براق پر معنی ممزوج و در ته آن جذب شد. (ص. 8) *[sample 2: labkhande mahdoshaneh va bi eradeye kenare labash khoshk shode bood, mesle inke be fekre shakhse ghayebi boodeh bashad. Az anja bood ke cheshmhaye mahibe afsoongar, cheshmhaye moztareb, moteajeb, tahdid konande,va vade dahandeye oo ra didam, va dar parto zendegi man roye in godihaye baragh por mani mamzooj va dar tahe aan jazb shod (p. 8)*	Sample 2: and an unconscious, involuntary smile had dried to the corner of her lips; it seemed as though she was thinking of an absent person. It was from the stool that I saw her dreadful charming eyes, eyes, which were enchanting and reproachful at the same time. It was to the shining and dreadful balls of those worried, threatening, and inviting eyes that my single beam of life was attracted, and it was to the depth of those same eyes that my life was drawn and in them annihilated. (p. 5)	Sample 2: she wore on her lips a vague, involuntary smile as though she was thinking of someone who was absent. It was then that I first behold those frightening, magic eyes, those eyes, which seemed to express a bitter reproach to mankind, with their look of anxiety and wonder, of menace and promise-and the current of my existence was drawn towards those shining eyes charged with manifold significance and sank into their depths. (p. 9)
ولی من،من که بی ذوق و بیچاره بودم، یک نقاش روی جلد قلمدان، چه میتوانستم بکنم؟ با این تصاویر خشک و براق و بی روح که همه اش به یک شکل بود چه میتوانستم بکشم که شاهکار بشود؟ (ص. 18) Vali man! Man ke bi zogh o bichareh bodam, yek naghashe roye jelde ghalamdan, che mitanavestam bokonaam? Be in tasavire khsohk o baragh o bi rooh ke hameash be yek shekl bood che mitanavestam bekesham ke shahkar beshavad? (p. 18)	Sample 3: But I, I who was devoid of talent and who was poor, a painter of pencase covers, what could I do? With these dry, glistening and lifeless pictures, all of which were the same, as models, what could I paint that would become a masterpiece? (p. 12)	Sample 3: But I, listless and helpless as I was, I, the decorator of pencase covers, what could I do? What means had I of creating a masterpiece when all that I could make were my lifeless, shiny little, each of them identical with all the rest? (p. 23)

Table 4: frequency and percentage of *stylistic devices* in the source and translated texts

The Element		Hedayat's novella (1937)	Bashiri's Translation (1974)	Costello's Translation (1957)
Description	Frequency	336	290	332
	Percentage	85%	96%	89%
Simile	Frequency	39	10	58
	Percentage	10%	3%	15%
Slang	Frequency	13	0	0
	Percentage	3%	0%	0%
Colloquial prose	Frequency	6	1	0
	Percentage	2%	0%	0%
Total Number		394	301	390

2.3. The Analysis of Stylistic Devices

In this part, the researcher examined the frequency and percentages of the stylistic devices introduced by Farshidvard (1984). However, in the beginning and, she has provided the most significant samples of these devices in the source and translated texts in table 3. The analysis of table 3 is as the following:

In sample one of the Persian novella, there are eight descriptions, all of which are the descriptions of the narrator's uncle; however, in the sample one of Bashiri's English translation there are up to ten descriptions, and in Costello's English translation there are also eight descriptions. Therefore, this number is the same as Persian novella. In sample two of the Persian novella, there are ten descriptions, and two similes. However, in the English translation of Bashiri, there are twelve descriptions, and there is only one simile. In the English translation of Costello, there are ten descriptions and one simile. In sample three of the Persian novella, there are five descriptions. However, in Bashiri's English translation, there are seven descriptions. Moreover, in Costello's English translation, there are six descriptions. According to the results achieved from the above table, it can be stated that Costello's use of stylistic devices is closer to the source text, than Bashiri's English translation. Furthermore, table 4 shows the frequency and percentage of stylistic devices applied in the Persian novella "The Blind Owl" and the two English translations. According to the above table, it can be stated that hedayat has used the most number of stylistic devices than the two translations. Moreover, as the results show, the percentages of description and simile were more in Costello's translation than in Bashiri's; In addition, the percentages of slang and colloquial prose were the same in both translations.

4. Discussions

Based on the data achieved from analyzing the synthetic patterns put by Shafaie (1984), among the 3237 items analyzed in both the source and translated texts, it should be stated that the percentages of simple, compound, assertive, nominal, and verbal sentences in Costello's translation have been much closer than the percentages of Bashiri's to the source text. On the other hand, the percentages of complex, and active sentences in Bashiri's translation were much closer to the source text than the percentages of Costello's. Furthermore, it should be stated that the percentages of interrogative sentences was the same between both translations. Finally, according to the data, it can be stated that the percentages of simple, imperative, exclamatory, conditional, and passive sentences were the same among all the texts under the study. Regarding the above-mentioned facts, since the data achieved from analyzing the Costello's translation was much closer to the source text than that of Bashiri's, it can be claimed that Costello has kept the synthetic patterns more than Bashiri in his translation. In other words, according to synthetic patterns, Costello's translation has been more successful in saving the originality of the source text. On the other hand, according the data achieved from analyzing the stylistic devices put by Farshidvard (1984) it should be stated that Hedayat's use of stylistic devices has been more than the translators. However, as the data show, Costello has used neither slang, nor colloquial prose in his translation, while Hedayat has used these two elements. Moreover, according to the data, Costello's frequency of description and simile use has been much closer to that of Hedayat; therefore, it can be stated that Costello has been more successful in saving the originality of the source text and that he has kept the similarity of the use of stylistic devices more than Bashiri, considering the source text. In addition, as the results indicate, Costello's translation has been more successful in keeping the original text stylistic devices and synthetic patterns. Of course, it should be mentioned that, this study aimed at comparing the two English translations of a Persian novel to find out which English translation has saved the synthetic patterns and stylistic devices used by the original writer more than the other. Therefore, based on the results, one cannot conclude that Costello's translation has been more successful than that of Bashiri in any other terms except the ones that were studied in this article.

5. Conclusion

This study analyzed Hedayat's "The Blind Owl" and the two English translations of that, by applying two frameworks of discourse analysis introduced by Shafaie (1984) and Farshidvard (1984), at micro level, with the aim of finding out if there is any difference between the two English translations according to each framework; therefore, two hypotheses were stated. The data achieved from the analysis of corpus indicated that regarding the Shafaie's model (1984) for analyzing synthetic patterns, Costello's translation was closer to the source text; therefore, the hypothesis one was rejected. Furthermore, the data also indicated that regarding the Farshidvard's model (1984) for analyzing stylistic devices, it turned out that Costello's translation has

been more successful than that of Bashiri's. In other word, in comparison with Bashiri, Costello's translation has kept the style of Hedayat in his translation more. Therefore, hypothesis two was rejected as well.

At the end, the researcher has provided the readers with some suggestions;

- ✓ According to Fairclough's framework (1989) what are the similarities and differences between the frequency of omissions and additions in the two English translations of "The Blind Owl?"
- ✓ According to Hatch's model (1992) what are the similarities and differences between the constitutional elements in the English translation of "The Blind Owl?"

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References

1. Alba-Juez, L. (2009). *Perspectives on Discourse Analysis: Theory and Practice.* UK: Cambridge Scholars Publishing .
2. Bashiri, I. & Hedayat, S. (1974). The Blind Owl. [Literal Translation]. In Bashiri, ed., *Hedayat's Ivory Tower,* Minneapolis: Manor House.
3. Bastin, L. G, & Bandia, F. P. (2006). *Charting the Future of Translation History*. Canada: University of Ottawa Press.
4. *Bookfinder.* (2012). Retrieved May, 25, 2012 from.
5. Bressler, Ch. E. (2007). *Literary Criticism: An Introduction to Theory and Practice. Fourth Edition.* New Jersey: Pearson.
6. Brown, G., & Yule, G. (1984). *Discourse Analysis.* Cambridge: Cambridge University Press.
7. Costello, D. P. (1957). *The Blind Owl.* USA: John Calder (Publishers) Ltd.
8. Del Saz Rubio, M. M. (2007). *English Discourse Markers of Reformulation.* Germany: Peter Long AG, International Academic Publishers.
9. Farshidvard, Kh. (1984). *Darbareye Adabiat va Naghd-e-Adabi. [About Literature and Literary Criticism].*vol, 1 & 2. Tehran: Amir Kabir.
10. Genc, B., & Boda, E. (2006). Oral Narrative Discourse of Anaphoric References of Turkish EFL Learners. *Reading Matrix, Vol. 6, No. 2.* Retrieved November 25, 2010, from. http://www.readingmatrix.com/articles/genc_bada/article2.pdf.
11. Georgakopoulou , A., & Goutsos, D. (1977). *Discourse Analysis, An Introduction*. England: Edinburg University Press.
12. *Guardian Book shop*. (2012). Retrieved May, 10, 2012 from. http://www.guardian.co.uk/books/2011/jul/20/kamin-mohammadi-top-10-iranian-books.
13. Halliday, M.A.K, & Hassan, R. (1976). *Cohesion in English.* London: Longmans.
14. Harris, Z. (1952). Discourse analysis. *Language*, Vol. 28, No. 1. USA: Linguistic society of America.
15. Hatch, Evelyn. (1992). *Discourse and Language Education*. Cambridge: Cambridge University Press.
16. Hatim, B., & Mason, I. (1990). *Discourse and the Translator*. London: Longman.
17. Hedayat, S. (1937). *Boof-e-Koor. [The Blind Owl]*. Tehran: Moassesseye Matboati-e Amir Kabir.
18. Jorgenson, M., & Philips, L. (2002). *Discourse Analysis as Theory and Method*. Sage Publication: London. Thousand Oaks. New Delhi.
19. Lemke, J. L. (2004). *Important Theories for Research Topics on the Website Discourse Analysis.* Retrieved November 25, 2010, from. http://academic.brooklyn.cuny.edu/education/jlemke/theories.htm#Discourse%20Analysis.
20. Levinson, Stephen, C. (2003). *Pragmatics*. England: Cambridge University Press.
21. McCarthy, M. (2005). *Discourse Analysis for Language Teachers*. UK: Cambridge University Press.
22. McNabb, D. (2008). *Research Methods in Public Administration and Nonprofit Management, Qualitative and Quantitative Approaches* (2nd ed.). USA: Library of Congress Cataloging-in-Publication Data.
23. Munday, J. (2010). *Introducing Translation Studies: Theories and Applications*. USA & Canada: Taylor & Francis e-library.
24. Navas Brenes, C. A., (2005). *Analyzing an Oral Narrative Using Discourse Analysis Tools: Observing How Spoken Language Works.* Retrieved November 23, 2010.
25. Nazari, F. (2010). Ashenaee Moghadamati ba Gofteman, Tahlile Gofteman va Tahlile Enteghadi Gofteman. [Basic Acquaintance with Discourse, Discourse Analysis and Critical Discourse Analysis]. Retrieved November25, 2010.
26. Nirmala, S. (2009). *A Discourse Analysis of R. K. Narayan's: The Man-Eater of Malgudi.* Vol. 9, 35-45.
27. Rahimian, j., & Momeni, A. (2003). Tajziye va Tahlile Goftemani Dastan E Modire Madrese. [A Discourse Analysis of the School Principal]. *Nashriyeye Daneshkadeye Adabiat va Oolume Ensani Daneshgahe Shahid Bahonar Kerman.[Journal of Kerman Shahid Bahonar University; Human and Art Faculty.* No. 12, 43-66.
28. Ray, M. K. (2008). *Studies in Translation; Translation: Its Brief History and Theory* (2nd rev. Enlarged ed.). India: Nice Printing Press, Delhi.
29. *Routledge Dictionary of Language and Linguistics. (1998). London and New York: Routledge.*
30. Sandres, T., & Maat, H. P. (2006). *Cohesion and Coherence: Linguistic Approaches.* Netherlands: Elsevier.
31. Schiffrin, D., Tannen, D., & Hamilton, H. E. (2001). *The Handbook of Discourse Analysis.* UK: Blackwell Publishers.
32. Shafaie, A. (1984). *Mabani-e- Elmi-e- Dastouri-e-Zaban-e-Farsi [Scientific Principles of Persian Grammar].* Tehran: Entesharat Novin.
33. Shamissa, S. (2000). *Dastan-e Yek Rooh [The Story of One Soul]. Tehran: Entesharate Ferdos.*
34. Solhjoo, A. (2009). *Goftman va Tarjomeh.[Discourse and Tranlastion].*Tehran: Nashr Markaz.
35. Stubbs, M. (1983). *Discourse Analysis: The Sociolinguistics Analysis of Natural Language.* Chicago: The University of Chicago Press.
36. Van Dijk, T. A. (1983). Discourse Analysis: Its Development and Application to the Structure of News, *Journal of Communication*. Vol. 33. No. 2. pp. 20-43.
37. Van Dijk, T. A. (1985). Dimensions of Discourse. *Handbook of Discourse Analysis, Vol. 2.* London: Academic Press.
38. *Wikipedia*. (2012). Retrieved May, 25, 2012 from http://en.wikipedia.org/wiki/Iraj_Bashiri.

8/3/2012

Reproduction biology in *Chondrostoma regium* (Heckel, 1843) in Gamasiab river in Kermanshah province, Iran*

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Abstract: In this study reproduction characteristics of *Chondrostoma regium*, which were caught from Gamasiab river, Kermanshah province,Iran were determined. Of 309 *C. regium* (146 males and 151 females and 9 unknown) were captured between August 2010 to July 2011 by gill nets of various mesh sizes. The observed sex ratio was 1.03:1 (females/males). Totally the age composition of the specimens ranged between 1-5 age groups. Total lengths and weights ranged from 117 to 261 mm and 12.2 to177.6 gr. The mean of GSI for all fishes and males and females were (5/476, 1/421, 8/68) .The max of GSI for males was in March and April and females was in April, March and February.The mean of relative fecundity (into fork length and weight) were (46/74,96/424). Mean absolut fecundity in femals was 9422/76(rang 1367-19016) and observed positively related to total length (r^2=0.86) and weight(r^2=0.88). Egg size varied monthly and egg diameters ranged from 0.5 mm to 2 mm in March. Egg size correlated negatively with the number of eggs in the ovaria.In according to GSI, absolut fecundity, diameters egg of *Chondrostoma regium* in Gamasiab river , reproduction season was reported March to May.
[Keyvan ghanbary, Mojgan khodadadi, Mehran javahri baboli, Gholamhosyn mohamadi. **Reproduction biology in *Chondrostoma regium*(Heckel,1843) in Gamasiab river in Kermanshah province, Iran**. **Taxonomic Diversity of Understorey Vegetation in Kumaun Himalayan Forests**. *Life Sci J* 2012;9(3):1825-1830] (ISSN:1097-8135). http://www.lifesciencesite.com. 264

Keywords: Chondrostoma regium,reproduction,biology,Kermanshah province,Iran

1. Introduction

There are about 140 fish species in the inland waters of Iran, which generally belong to three families: *Cyprinidae*, *Balitoridae*, and *Cobitidae* (Wossughi and Mostagir 1976). The cyprinid species exhibit a wide range of geographical distribution, life histories, and reproductive styles (Winfield and Nelson 1991). Cypriniformes or carps are a group of freshwater fishes with 6 families, 321 genera and about 3268 species found throughout the world except Australia and South America (Nelson 2006). *Chondrostoma* is one of them.There are about 26 species of *Chondrostoma* which two are known for Iran including *Chondrostoma regium* (Heckel, 1843) and *C.cyri* (Kessler, 1877) (Coad 2010). The king nase, *Chondrostoma regium.*

River in Kermanshah province 90 km long river and its catchment area is over 12,000 square kilometers (Rahimzade et al.2010). This river during the warm months of the year (late April to early November) with increasing temperature and decreasing rainfall has the lowest water volume (Biokani 2004). Understanding the reproductive biology of exploited fish stocks is important for developing stock assessment and population-viability models used for fisheries management (Kenndy et al. 2006;Nitschke et al. 2001) and knowledge of the reproductive cycle and the factors affecting it are important issues in fish and fisheries biology (Tomkiewicz et al . 2003).

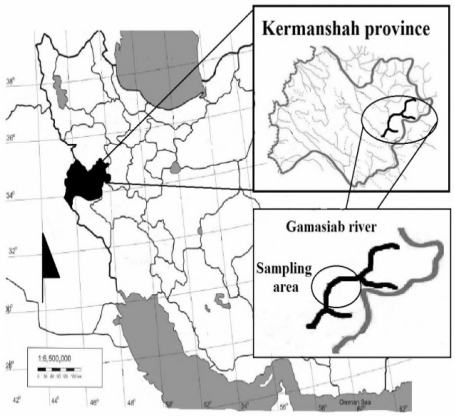

Fig 1.Situation of Gamasiab river in iran and sampling region.

Kara et al in 1998 ,Oymak in 2000 ,Zulfu in 2002 ,Ozcan in 2006 respectively in the dam lake Karasu, Ataturk, Keban and state of Hatay, Turkey, age, growth and reproduction in *C.regium* were examined. The calculation of the number of larvae from eggs and egg survival in natural environments is not possible, there by determining the fecundity rate, an estimate of future generations and the condition makes it possible (Pitcher 1996). *Chondrostoma*

regium has a wide dispersion, but there is little information on its biology in Iran. The main objective of this study is determaint of absolut fecundity, relative fecundity; gonadosomatic index, egg diameters and breeding season of *C.regium* inhabit the Gamasiab river and the fecundity relationships with other variables. We believe that information on the reproductive biology of this native fish could be important for conserving its stock.

2. Material and Methods

Fish samples were monthly collected in Gamasiab river was performed using by Cast net and Gillnet invarious mesh sizes of (1,2,3,4,5)cm and by 10,20,30,40 m lenght between August 2010 to July 2011. The water temperature ranges from 8 °C to 32 °C throughout the year. A total of 309 individuals (151 females, 146 males and 12 undetermined sexes) were sampled. Fish samples were preserved into formaldehyde 10% and taken to laboratory. The total lengths (±0.1 cm) and weight (±0.1 g) of fish were recorded (King 1995). Age determination was carried out from the scale by the method of Lagler et al(Lagler 1966). For this purpose, 15-20 scales were taken from a region under the dorsal fin (Lagler 1966). The scales were kept in 5% KOH and then in water cleaned and age was determined under a microscope. The fish were dissected laterally and sex was ascertained macroscopically and microscopally and their gonads were removed and weighed (mg). Ovaries were fixed in Gilsons fluid (Bagenal and Braun 1978). For fecundity was estimated by the gravimetric method (Zulfu and Sen 2002). For this purpose, three 0.1 gr subsamples (front, middle and caudal sections) from each ovary were taken and the number of eggs was counted in each subsample and then the total fecundity (F) was estimated using the equation:

$$F = \frac{Gw \times En(\text{in subsample})}{Sw}$$

GF=gonad weight,En=egg number in the subsample ,Sw=subsample weight (Wootton 1998). The diameters of 10 oocytes from each subsample were measured by a micrometer lamella under a microscope for determining egg size. Gonadosomatic index (GSI) was determined by the equation:

$$GSI = \frac{Gw \times 100}{Tw}$$

Gw = weight of gonad (g),Tw=total weight of fish(g) (Wossughi et.al.1978). Differences were examined by t-test and x^2 .A value of ($p < (0.05$ and $0.01)$) was considered to represent statistical significance (Kara and Solak 1998).

3. Results

During the 12 months sampling of river waters of Gamasiab river, minimum and maximum and mean range of each of physico chemical properties of water, respectively. Both figure 2 and table 1 show temperature and physico chemical properties changes in the years 2010 to 2011 in Gamasiab River. In this study, 309 fish were caught. 146 specimens were male and 151 specimens were female and 12 specimens were unlimited.

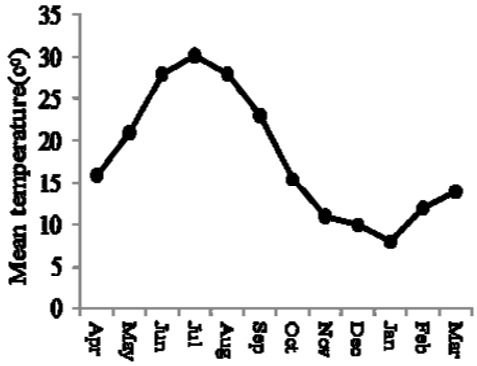

Fig2. The monthly temperature(c°) values of Gamasiab River in 2010-2011

Table 1.The mean,min,max and range for five physico chemical properties of Gamasiab river in 2010-2011

	Mean	Min	Max	Range
Tempeature(c°)	18.63	7.5	30.1	22.6
Ph	6.5	6.2	7	0.8
Hardness	183.2	153	232.2	79.2
EC	1260.06	824	1864	1040
O_2 (ppm)	9.4	9.3	10	0.8

Sex ratio of male to female fish 1: 1/03, respectively. Fish caught in five age groups (1-5) were classified. Figure 3 show numbers of specimens of *C.regium* in different age groups

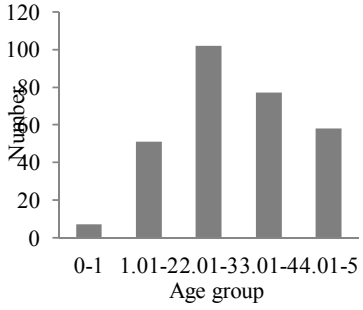

Fig3. Number of specimens of *C.regium* in different age groups in 2010-2011

Both table 2 and 3 show the profile length and weight of fish. The mean GSI for the total population was 5/476 and in male and female fishes were 1/421 and 8/68, respectively. GSI showed a significant difference between male and female fishes (p<0/01). GSI of male and female fishes separately, showed significant differences (p<0/01), (p<0/01).

Table 2.The mean,min,max and range of fork length(mm) in *C.regium* in Gamasiab river in 2010-2011

	Mean	Min	Max	Range
Male	175.9	105	226	121
Female	190.6	121	240	119
Male+Female	182.66	105	240	235

Table 3.The mean,min,max and range of total weight(gr) in *C.regium* in Gamasiab river in 2010-2011

	Mean	Min	Max	Range
Male	73.63	12.2	142.12	129.92
Female	96.6	26	177.6	151.6
Male+Female	88.32	12.2	177.6	165.4

Figure 4 show the GSI changes of the male and female fish during the months of the year 2010-2011. Comparison mean of gonad weights in males and females of different ages showed that with increasing fish ages, gonad weight Increases and this difference for females (p<0/05)and males (p<0/05) was Significant. Mean absolute fecundity in *C.regium* in Gamasiab river was 9422/76 and the minimum and maximum of this were 1367, 19016 respectively. Egg number and age showed significant differences (p<0/01).

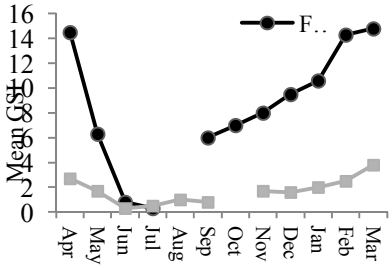

Fig 4. The monthly GSI values of females and male of *C. regium* samples from Gamasiab river in2010-2011. No data were available for female in Aug and male in Oct.

A significant correlation was observed between egg number and age (r^2=0/675),(p=0/05). Figure 5 showed a significant correlation between weight and absolute fecundity (r^2=0/842),(p=0/05). Figure 6 shows that there is significant correlation between fork length and absolute fecundity of fish(r^2=0/782) ,(p=0/05). Gonad weight and absolute fecundity also showed significant correlation (r^2=0/3922), (p=0/01). Average, minimum and maximum relative fecundity (than fork length) and relative fecundity (than weight), were obtained respectively (46/64, 7/02, 108/32) and (96/924, 10/14, 343/23).

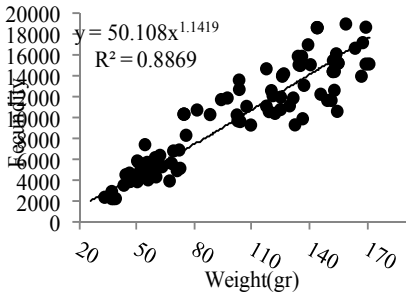

Fig 5.Relationships between fecundity and weight for 151 specimen *C. regium* from Gamasiab River in 2010-2011.

Compared mean of the relative fecundity (than fork length and total weight) of different age showed that the difference between relative fecundity (than fork length) with the age is significant (p<0/01), but the relative fecundity (than total weight) with age does not show any significant difference (p>0/05).

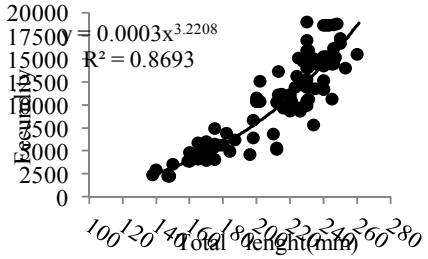

Fig 6. Relationships between fecundity and total lenght for 151 specimen *C. regium* from Gamasiab River in 2010-2011

But in general it was observed that with increasing age increases the relative fecundity. Figure 7 shows the Correlation between absolute fecundity with age

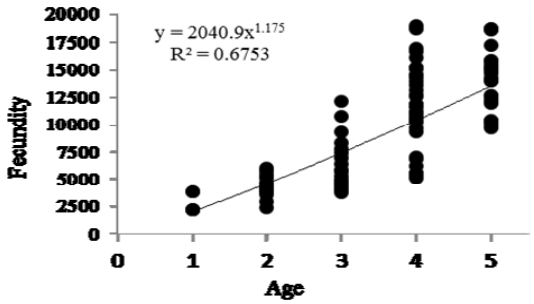

Fig 7. Relationships between fecundity and age for 151 specimen *C. regium* from Gamasiab river in 2010-2011.

Average diameter of eggs was 1/065 mm and minimum and maximum 0/54 and 2 mm were observed. Figure 8 show the changes in egg diameter during the months of the year 2010-2011.

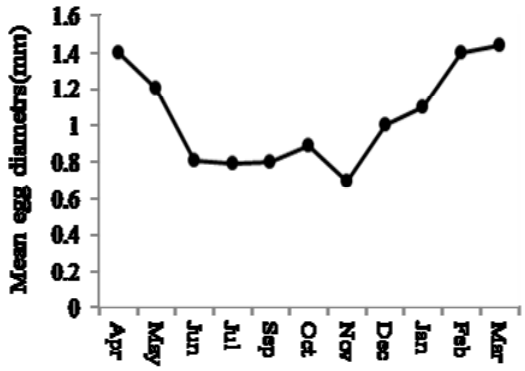

Fig8. The monthly egg size values for 151 specimen *C. regium* from Gamasiab River in 2010-2011. No data were available for Aug.

Average diameter of eggs in different monthe in 2010-2011 showed that the diameter of the egg have started to growth in December and reaches its peak in April. Egg diameter in relation to age was studied and was observed that with increases absolute fecundity and age, the egg diameter decreased. Also significant differences were observed between age and egg diameter ($p<0/01$).

4. Discussions

Gamasiab River was 5/589 and for males 1/41 and the females 8/12 .The max of GSI was observed in males in March and minimum in July and its max for female in April and in July it was at least. In Keban Dam Lake, the average GSI for *C.regium, in* male and female were 0/789, 3/47 and the max was in February and June (Zulfu and Sen 2002). In Sir Dam Lake in males and females, respectively, 1 and 4/57 was estimated and reported that the max is seen in May months (Kara and Solak 1998). Dauod in the 1999(Coad 2010), Oymak in 2000, Sevick in 1997, Balaci in 1990 , Ozcan in 2006, Oymak in 2001 reported that the max for this index of the male and female respectively in months (March - May) (April - July), (April - March), (April - March), (May- May) and (May - July). Water nitrate levels in freshwater and rainy seasons and dry years are the sequence of events that led to the creation of different effects in is reproductive in different samples (Wooton 1995). The seasonal reproductive patterns can be occur caused by weather conditions and species interactions (Gougnrad et al. 1987). Gamasiab river volume in 2008-2009 was low because rainfall was decline and a also due to pressure from the fishing industry ,Gamasiab river fishes feel threatened about their habitat and fishes increase produce sex cells and GSI. Average, minimum and maximum of absolute fecundity,in *C.regium* in Gamasiab river were respectively 9422/76, 1367 and 19 016. It was observed that the absolute fecundity increases with age but be reduced egg diameter, this could indicate that efforts to increase the quantity of fish reproduction and in contrast, it appears that reduce of quality of reproduction is of main maintain the sequence. Absolute fecundity of fish living in the Diyala River, Iraq (Coad 2010) and Tigir River (Coad 2010) were reported 6800 and 13280, the minimum and maximum absolute fecundity of fish living in Anatolia region (Ozcan 2006), Keban Dam Lake (Zulfu 2002), Saivor river (Zulfu 2002) and Ataturk dam lake (Oymak 2001) were respectively 6800,13800and 1904,16800 and1780,11340 and1074 ,15492 numbers. in comparison absolute fecundity with other regions, this is visible of fish inhabit the Gamasiab river have the more eggs with a diameter less and higher GSI, due to stress and pressure on their environment, these fish have a greater effort to reproduce and release reproductive cells, actually the sudden increase in female GSI and reach to maximum of 16/93, prove this theory. It should also be noted that the difference in fecundity in species in different regions is related to genetic profile of the different species and environmental factors such as supply and availability of food and its size, species and varieties (Zulfu 2002). The fecundity have difference for a specified size and in a population from year to other year and even different populations of a species (Nikolskii 1963). Average, minimum and maximum diameter of eggs in fish living in Gamasiab were observed 1/06, 0/54 and 2 mm. Minimum and maximum diameter of eggs in fish living Anatoly, Keban Dam Lake,Ataturk Dam Lake and Saivor river respectively, 0/75 - 1/79, 0/2 - 1/63, 0 / 66 - 1/63, 0/966- 1/939 mm have been reported. Varley in 1967 reported the egg diameter in the major freshwater fish is between 1/3-3/2 mm

(Varley 1967).The fish that have lived at 8-34 C°, are named eurytherm who often spawning at temperatures above 15 C° (Varley 1967). Temperature range in 2010-2011 in Gamasiab river was 7/5-30 C°. Temperature in this river began to rise from February and GSI in male and female fish during the months of March and April reach to peak and be low in May and will fall rapidly in June, therefore they began the breeding season for *C.regium* in Gamasiab river can be from March and max in April and May. It should be noted that the max diameter of the eggs was recorded in April and May. Temperature range was reported 15-25 C° in May and April. The factors such as temperature, ecological conditions, flooding rivers, changes in ion concentrations, changes in water quality, changes in pH and oxygen and carbon dioxide levels and environmental factors affect on the stimulation of sexual maturation (Kamali and Valinasab 2003). (Harsh dry to mesic). Moreover, basal forms of Violaceae showed affinity to mesic and cold conditions under the oak forest. Few species are able to tolerate the entire spectrum of environment and range throughout the gradient (Brown, 2001). Our study showed that perennials gained dominance over annuals in oak forest as well as pine forest (Figure 1). Perennial have ability to conserve soil and with their extensive root systems of perennial grasses they also add more organic matter to the soil than annuals which can be more favourable for plant growth. Singh and Singh (1987) observed that annuals colonize and dominate the early stages of succession. Annuals to perennials species ratio are higher at primary successional site than climax stage. Species richness generally increases during secondary succession when environmental and edaphic conditions are favourable with low fluctuations. The above results indicate that the oak forest makes climax stage for succession. The evenness and β-diversity showed similar values in sub-sites of oak as well as pine forests. The high values of beta-diversity indicate that the species composition varied from one stand to another. Equitability/evenness varied in pine forest with respect to sub-site from 27.3 (HB) to 31.4 (HT) (Table 3). This was because of the conditional presence or absence of functional relationship of species. Comparatively higher value of equitability in pine forest with respect to oak forest indicated that the individual herb species distribution is higher. This may perhaps due to intermediate level of disturbance.

The allocation of species in the Kumaun Central Himalaya is mainly governed by moisture and temperature gradients that incorporate the effect of many physical factors. Moustafa (1990) found that the association of community types is the result of the performance of the species in response to the environmental conditions that prevail in a particular forest type. Tewari (1982) assumed that the temperature gradient is the net product of elevation and aspect; while moisture gradient is a function of slope degree, soil texture and nature of soil surface.

In addition to that, hierarchical diversity concerns taxonomic differences at other than the species level. Pielou (1975) and Magurran (1998) suggested that hierarchical (taxonomic) diversity would be higher in an area in which the species are divided amongst many genera as opposed to one in which most species belong to the same genus, and still higher as these genera are divided amongst many families as opposed to few. The families, genera and species ratio was observed maximum in the pine forest as compared to the oak forest in the present study (Table 4), indicating diverse taxonomic vegetation in the pine forest.

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References

1. Biokani S (2004) Survey of fishes in Gamasiab river in iran.Gilan university:30-47.
2. Balaci K,Unlu E (1990) A study on growth characteristics some Cyprinidae living in Saivor River. National Congress of Biology Erzurum. Turke: 283-295p.
3. Bagenal TB, Braun R (1978) Eggs and early life history. In: Methods for assessment of fish and production in freshwaters. 3rd ed. T.Bagenal (Ed.). Blackwell, Oxford:165-201.
4. Coad BW (2010) Freshwater fishes of iran. Homepage. http://www.briancoad.com.
5. Gougnrad I,Poncin P, Ruwet JC, Philippart JC (1987) The spawing behavior in culture barbels influence of number of courting males .Cah . Ethol . Appl .3 :293-302.
6. Kennedy AJ, Sutton TM, Fisher BE (2006) Reproductive biology of female shovelnose sturgeon in the upper Wabash River. Indiana. J. Appl. Ichthyol. 22.177–182.
7. Kmali A, Valinasab B (2003) Reproduction in fishes. Translated in Center of research of aquatics, Iran.83-93.

8. Kara C, Solak K (1998) Some biological properties of Chondrostoma regium in habititng Sir dam lake .Ksu jurnal of sience an engineering 7(2)-2004.
9. King M (1995) Fisheries biology assessment and mangment .fishing news book.324.
10. Lagler Kf (1966) Freshwater fishery biology. W.M.C. Brown company Iowa.310.
11. Nelson JS (2006) Fishes of the world .John Wiley And Sons J, Inc, 601.
12. Nikolskii GV (1963) the ecology of fishes, Acadmic press.london.
13. Oymak A (2001) the reproduction biology of Chondrostoma regium in Ataturk dam lake, S.D.Ünv. Fen Bilimleri Enstitüsü Dergisi. 1-3.
14. Ozcan G (2006) Reproduction biology of the endemic and threatened Menderes nase Chondrostoma meandernes in Western Anatolia . Zoology In Middle East. 2009:61-67.
15. Pitcher TJ, Hart PJB (1996) Fisheres ecology .champman and hall. London.
16. Rahimzade Z, Nadrian P, Abrifam M (2010) Kermanshah geogeraphy. Edition teaching book, Iran. 13-20.
17. Sevick R (1997) A study on the growth properties of Chondrostoma regium flour in Syrian border waters between the Ataturk Dam . Mediterranean Fisheries Congress, Izmir, Turkey,. 555-561.
18. Tomkiewicz J, Tybjerg L, Jespersen A (2003) Micro andmacroscopic characteristics to stage gonadal maturation of female Baltic cod. Journal of Fish Biology 62: 253-275.
19. Varley RJ (1967) Britthish freshwater fishes .Fishing news (Books) limited . 14.
20. Wossughi GH (1987) Die Sueswasserfische des Hamun Sees. Journal of the Veterinary Faculty, University of Tehran, 41:83-97p.
21. Wossughi GH, Mostagir B (1976) Fishes in freshwater in Iran. Edition Tehran university. 200-203.
22. Wootton RJ (1998) Ecology of Teleost Fishes. Chapman and Hall. London. 404.
23. Winfield IJ, Nelson JS (1991) Cyprinid Fishes: Systematics. Biology and Exploitation. Chapman and Hall, London.
24. Zulfu M, Sen D (2002) The reproduction peculiarities of Chondrostoma regium living in Keban dam lake . Scince and eng jor firat univ 18(1).41-48.

Occurence of aflatoxin M1 in two dairy products by ELISA in central part of Iran

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Abstract: Aim: Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agent in human hepatic and extra hepatic carcinogenesis. The aim of this study was conducted to investigate the pasteurized milks and yoghurt contamination with M1 aflatoxin(AFM1) products of main factories that provide some Qom city dairy needs, in terms of contamination with this mycotoxin. **Materials and Methods:** 103 (75 pasteurized liquid milk, 28 yoghurt) sample produced by seven dairy factories were randomly selected during two cold (winter 2009) and warm (summer 2009) seasons and their AFM1 concentration was determined by a competitive Enzyme-Linked Immuno Sorbent Assay (ELISA) method. The main difference analyzed using Excel 2007 in software environment. **Results:** All of the examined samples were contaminated with AFM1 by measurable amounts. Mean of the M1 aflatoxin in whole pasteurized liquid milk samples was 22.44 ng/kg ranging from 8 to 64 ng/kg and in whole yoghurt samples mean of the M1 aflatoxin was 13.55 ng/kg ranging from 5 to 36 ng/kg . AFM1 contamination was higher than Iran National Standard (50ng/kg) only in 8.33% of the summer milk. **Conclusion: High** prevelance of AFM1 contamination in pasteurized milk and yoghurt samples is worrying and notifies the necessity of preventing measures to reduce entrance of B1 aflatoxin to dairy animal's feed and more controlling measures on milk distribution.

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Keywords: Aflatoxin M1, pasteurized milk, yoghurt, ELISA

1. Introduction

Aflatoxins are extremely toxic compounds produced by certain species of aspergillus, especially aspergillusflavus, A. parasiticus, and Anomius that contaminate plants and its products. A. flavus produces only B aflatoxins, while the others produce both B and G aflatoxins(1 Pei SC, Zhang YY, Eremin SA, Lee WJ. 2009).Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agent in human hepatic and extra hepatic carcinogenesis (Oveisi M-R.2007). Aflatoxin M1 (AFM1) is the monohydroxylated of AFB1 metabolized by cytochrome p450 enzyme system in liver and excreted into the milk of lactating livestock which consumed AFB1 contaminated diet (Murphy PA.et.al.2006). It could be appeared in milk whitin 12 h after the first ingestion of AFB1. Following the withdrawal of contaminated source, AFM1 disappeared within 72 h. there is a liner relationship between the AFM1 content in milk and the consumption of AFB1 via foodstuffs(Sassahara M.et.al.2005). It has been estimated that about 0.3-6.2% of AFB1 present in animal feed pass as AFM1 in milk(Creppy EE. 2002).Although the toxicity of AFM1 is less than AFB1, its cytotoxic, genotoxic and carcinogenic effects is well demonstrated. Hence the IARC of WHO initially categorized AFM1 as a group 2 human carcinogen(IARC.1993), but has transferred it to group 1 according to recent investigations(IARC.2002). As milk is the main nutrient for infants and children who are considered to be more susceptible to adverse effect of mycotoxins, the presence of AFM1 in milk is a concern. Infants usually use pasteurized milk more than adults (per kilogram of body weight). Infants in Iran usually consume pasteurized milk after breast weaning, up to three years of age as the main food, so the problem seems to be more important in this age group. On the other hand milk is not consumed as liquid milk, but also utilize for the preparation of infant formula, yoghurt, cheese, and milk based confectionaries including chocolate and pastry. Therefore, it is important to determine AFM1 levels in milk and dairy products in order to protect consumers in various age groups, from its potential hazard(Oveisi M-R.2007)2). The purpose of this study was to determine naturaloccurance and level of AFM1 in pasteurized liquid milk and yoghurt consumed in Qom, Iran.

2. Material and Methods

103 (75 pasteurized liquid milk, 28 yoghurt) samples produced by seven dairy factories were randomly selected during two cold (winter 2009) and warm (summer 2009) seasons from Qom, Iran.

2-1. Sample preparation:

Add to 10g yoghourt samples 100 ml of warm (20-25ºC) demonized water and shacked for 10 min with shaker in speed of 250min^{-1}. Subsequently, these samples as well as liquid milk samples were centrifuged at 3500 g for 10 min at 4ºC. Aflatoxins are water soluble (desponded 2), so the upper creamy layers were completely discarded and the lower phases were further diluted 20 times (v/v) with demonized water and then were used for the quantitiveteste.

2-2. Analysis of AFM1 in samples by competitive ELISA

The quantity of AFM1 was determined by RIDAscreenaflatoxin M1 test (R-Biopharm GmbH prepared from Rocket International Co. Ltd) which is a competitive enzyme immunoassay based on antigen-antibody reaction. The wells in the micro titer strips were coated with specific antibodies to AFM1. 100 µl of sample solution + 100 µl of standard 10ng/kg (standard addition method was used because of detection limit 5ng/kg) were added to the wells to occupy the binding sites proportionately, then mixed gently and incubated for 60 min at room temperature in the dark. Then the liquid samples were poured out of the wells and the wells were filled with 250 µl distilled water and poured out the liquid. Then other steps were done by the kit instruction and ultimately each well was washed for four times by washing buffer. At most after one hour, light absorption was read at 450 nm by ELIZA reader. The standard curve was used for determination related to the kit and the pasteurized milk and yoghourt samples by competitive ELISA. All of information after study analyzed by using Excel 2007 in software.

3. Results

The sample of pasteurized liquid milk (n= 75) and yoghourt (n=28) showed that the incidence of contamination with AFM1 is 100%, the presence of AFM1 in each group was 100%, ranging between 5-64ng/kg respectively. Iran national standard prescribe the limit of 50 ng/kg in milk and yoghourt.theoccurance and levels of AFM1 in milk and yoghourt samples are depicted in tables 1-4.

Table 1: Occurrence of AFM1 in milk and yoghourt samples in winter

Sample	n	Positive samples	AFM1 contamination	
			Range(ng/kg)	Mean±SD
Milk	39	39(100)	8-43	18/05±8/2
Yoghourt	15	15(100)	5-23	11/8±5/53

Table 2: Levels of AFM1 in winter milk and yoghourt samples.

Sampledistribution of samples n (%)					
	<5ng/kg	5-10ng/kg	10-25ng/kg	25-50ng/kg	>50ng/kg
Milk	0(0)	10(25.64)	4(26.4)	25(64.4)	0(0)
Yoghourt	0(0)	0(0)	9(60)	6(40)	0(0)

Table 3: Levels of AFM1 in summer milk and yoghourt samples

Sample	n	Positive samples	AFM1 contamination	
			Range(ng/kg)	Mean±SD
Milk	36	36(100)	9-64	26.83±14.99
Yoghourt	13	13(100)	5-36	15.3±8.38

Table 4: Occurrence of AFM1 in milk and yoghourt samples in summer

Sampledistribution of samples n (%)					
	<5ng/kg	5-10ng/kg	10-25ng/kg	25-50ng/kg	>50ng/kg
Milk	0(0)	1(2.8)	20(55.6)	12(33.3)	3(8.3)
Yoghourt	0(0)	3(23.0)	9(69.2)	1(7.7)	0(0)

4. Discussions

Iran national standard prescribe the limit of 50 ng/kg in milk and milk products(INA. 2001). There are differences in maximum permissible limit of AFM1 in various countries. For example European Communities and Codex Alimentations prescribe a limit of 50ng/kg(Commission E. 2001) and US regulation fixed the limit to a maximum of 500ng/kg for milk(Food U. Drug Administration, 1996). The occurrence and distribution of AFM1 concentration

obtained are presented in tables 1-4. Almost all of the samples were below the Iranian limit but the incidence of contamination even below standard limit is a serious problem for public health. Aflatoxicosis cause anemia, reduction of immune function, hepatotoxicosis, hemorrhage, teratogenesis, carcinogenesis and mutagenesis. The most prevalent symptoms of aflatoxicosis in animals are reduced growth rate and poor intellectual and behavioral performance. The liver is considered a target organ for the toxic and carcinogenic effects of aflatoxin(Kav K.et.al.2011). Milk and dairy products provide major nutrition's for human because many people especially children, frequently include them in their diets (Baskaya R. 2006).by consider to high toxicity and carcinogenic properties of AFM1, it's presence in milk is a concern. AFM1 is resistant to thermal inactivation, pasteurization. Autoclaving and other varieties of food processing procedures(Park DL. 2002). So to produce high quality milk, it is essential to keep feeds free from contamination by AFB1 (AFM1 mother molecol). The concentration of AFB1 in animal feed can be reduced by goob manufacturing practice and good storage practices. If preventive measure fails, however, AFB1 can be reduced in feed that has lower concentrations or by chemical, physical or biological treatment (Signorini M. 2011-Fallah AA.et.al.2009). The new approach to solve this Problem is the use of non-nutritionally inert adsorbents that can seqester the aflatoxins and reduce the absorption of these toxins from the gastro-intestinal tract(Oveisi M-R.2007). These finding show a potensial risk for consumers, especially in the absence of strict hygiene control. So finding new safe thecniqes for decontamination AFM1 from milk can be good alternatives for this problem .on the other hand, increasing the intake of antioxidants, and vitamins with the diet in order to prevent carcinogenesis should be involved in the prevention strategies.

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References

1. Baskaya R. 2006. Aydın A, Yıldız A, Bostan K. Aflatoxin M1 levels of some cheese varieties in Turkey. Medycyna Weterynaryjna. 2006;62(7):778-80
2. Commission E. 2001.Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Communities L.;77:1-13.
3. Creppy EE. 2002.Update of survey, regulation and toxic effects of mycotoxins in Europe. Toxicology letters.;127(1-3):19-28.
4. Fallah AA, Jafari T, Fallah A, Rahnama M. 2009. Determination of aflatoxin M1 levels in Iranian white and cream cheese. Food and chemical toxicology;47(8):1872-5.
5. Food U. Drug Administration, 1996, Sec. 527.400 whole milk, low fat milk, skim milk-aflatoxin M1 (CPG 7106.210). FDA Compliance Policy Guides. 1996:219.
6. INA. 2001.Maximum validity Mycotoxins in human food. Tehran Institute of Standard and Industrial Research of Iran.
7. IARC.1993. Some naturally occurring substances: Food items and constituents hetrocyclic aromatic amines and mycotoxins. IARC monographs on the evaluation of carcinogenic risk to humans International Agency for Research on Cancer. p. 451-89.
8. IARC.2002. Some mycotoxins, naphthalene and styrene. IARC monographs on the evaluation of carcinogenic risk to humans: International Agency for Research on Cancer;. p. 171-300.
9. Kav K, Col R, Kaan Tekinsen K. 2011.Detection of aflatoxin M1 levels by ELISA in white-brined Urfa cheese consumed in Turkey. Food Control.
10. Murphy PA, Hendrich S, Landgren C, Bryant CM. 2006. Food mycotoxins: an update. Journal of food science.;71(5):R51-R65.
11. Oveisi M-R, Jannat B, Sadeghi N, Hajimahmoodi M, Nikzad A.2007. Presence of aflatoxin M1 in milk and infant milk products in Tehran, Iran. Food Control. [doi: 10.1016/j.foodcont.2006.07.021].;18(10):1216-8.
12. Park DL. 2002.Effect of processing on aflatoxin. Mycotoxins and Food Safety:173-9.
13. Pei SC, Zhang YY, Eremin SA, Lee WJ. 2009.Detection of aflatoxin M1 in milk products from China by ELISA using monoclonal antibodies. Food Control.;20(12):1080-5.
14. Sassahara M, Pontes Netto D, Yanaka E.2005. Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food and chemical toxicology. 2005;43(6):981-4.
15. Signorini M. 2011.Gaggiotti M, Molineri A, Chiericatti C, Zapata de Basílico M, Basílico J, et al. Exposure Assessment of Mycotoxins in Cow´ s Milk in Argentina. Food and chemical toxicology.

Analyzing and Explaining the Process of Nostalgia in Nima Youshij's Letters

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Abstract: The present article is a research about nostalgia is the Nima Youshij's letters. Since the Nima's letters are regarded as the valid documents of his life, their analysis makes the aspects of his life and poems clear. This issue has been studied as one of the psychological and unconscious behaviors of the human in the collection of Nima's letters. In the contemporary literature, nostalgia, seems to be unavoidable, due to the social frustrations, and has much more manifestations in the works of such poets as Akhavan, Forogh, and especially Nima. In this article, after defining the nostalgia, its creating factors, are going to be explained by the psychologists including regrets over the past, sorrow caused by the loss of family members, recalling the memories of the childhood and adolescence, suffering from the pain and hardship of travel and migration, and the sorrow of getting old and recalling the death, and then the Nima's letters, which form the significant part of his written works, different evidences are going to be stated for each issue..
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Keywords: Nima Youshij, the letters, Nostalgia, homesickness

1. Introduction

Nostalgia is in fact a psychological term that has been entered the field of literature, first in the contemporary era. This issue is emerged in the existence of the poet or the writer as one of the human's psychological behaviors that exists in the unconscious, and has been as the source for creating many of the valuable literal works around the world; because nostalgia is the general, instinctive, and natural emotion of the human's existence, which been crystallized in the poet or the writer, in the expression and definition of nostalgia, it is written that it is a kind of homesickness, which is caused by being away from homeland, so as whenever the person pays attention to the past in his mind, or becomes depressed by reviewing his past, and feels somehow regretful on his past, he can be stated to be influenced by nostalgia, which is interpreted as the homesickness in Persian, today. The main factors of creating nostalgia in different people can be the following issues: regret over the past, and complain of the status quo, losing one of the family members, recalling the childhood and adolescence memories, feeling homesickness due to travelling, and its hardships and pains, the sorrow of getting old, and its hardships, wishing for the utopia, and etc. In the contemporary literature, nostalgia, is indicative in the two types of individual and social, the individual type of which can be divided into two instantaneous and continuous type, regarding time. In the individual and instantaneous nostalgia, the poet or the writer indicate a moment from the past moments in his poem or writing, but in the continuous individual nostalgia, the homesickness is clear in all his works from the beginning to the end, and since the social frustrations in the contemporary era, is one of the basic factors of creating this individual-collective emotion, therefore, nostalgia can be felt and observed in the contemporary literature. This issue, exists is the poems of such poets as Nima Youshij, Forogh Farokhzad, Akhavan Sales, and etc. for example, Nima believes that: I'm the one who is away from my homeland, like a bird from the nest/ like my age, my today has been forgotten (Karimi-Hakkak Ahmad, Kamran Talattof.2004). Or Forogh says: "those days have gone/those good days/those overfilled days/ those heavens, full of spangles (Ghanoonparvar M. R. 2009). Or Akhavan Sales who say: "it's been said that "hope, and despair!" don't know/ I'm the elegy speaker of my dead homeland. In this article, the author has tried to explain the concept of nostalgia generally, and this issue in Nima's letter specifically, so that, to analyze the creating factor of this emotion would be analyzed in Nima's existence, and present some evidences for better understanding of his poems from this regard.

1.1. The Meaning of Nostalgia

This term has been created by combining the two words with the Greek base *nostos*, which meaning coming back home, and *algia*, which means pain and suffer. In fact, this term is an expression in the psychological field, and in fact, is a mental and unconscious treatment, which takes place as the result of returning the human's mind to the past. Some believe that nostalgia is a form of homesickness, which is the product of being away from the homeland. While some other, know it as a combination of "returning and pain."In fact, nostalgia

is a natural, instinctive, and a general feeling that exists mostly among all the races and tribes, which is strengthened and indicated, when the individual separates from his past, and remembers his past time, and is regretful. When the past is full of beauty, happiness, and power for the person, this feeling even gets stronger. The past which is lost and is not returnable. During his life, when the poet or the writer faces with some problem or loses one of his dear members, or when he is affected by a disease, or when the snow of old age sits on his head, he is looking for a remedy, naturally, but when there is no postern for him, he gets a nostalgia, and he wishes the glorious time, that would never be achieved by him, and it is the exact concept we are looking for. Based on this description, nostalgia can be defined as: "the emotional tension and sadness due to being away from the homeland, and the homeland's pain, is the pain that is produced because of the desire to meet the homeland; the regrets of the past, and homesickness" (Jameson, Fredric. (1989)). "Homesickness, and the desire to return home (Pour Afkari, 1994: 1011), wishing for something, that has been recalled from the past, homesickness due to being away from the homeland, or the homesickness resulted from beautiful and sweet past times" It should be stated that, this term, has been translated as "homesickness, and the past regrets" in Persian (Jameson, Fredric. (1989). Therefore, in this type of feeling, the poet or the writer, remembers a beautiful, sweet, and shining past and depicts it painfully and regretfully with his own pen.

2.1. Nostalgia in Nima's Letters

Since, the people around the world suffer from the emotional disturbances from the environment and the time due to the machinery life, and its consequences, nowadays, nostalgia appears his face much more. Being away from purities, good ethics, and in one word, being away from human dignity in the today's world, is the factor, which wins the writer or the poet's attention to his own glorious past times to review in the world of imagination, the happy past time, and travels to the spaces or other times, and rebuilds and revives his honorable past times.

3.1. Nostalgia (Homesickness)

One of the main reasons that cause the sense of nostalgia to be created by the poet or the writer is the homesickness. Homesickness and the sorrow caused by it, has an extensive reflection on the different era's literature including mystic literature. In the classical mystic literature, poems are found abundantly, the content of which are the human's wishes in reaching the main position. In"Ney Nameh *[Song of the Reed]*," Rumi says: everyone who was away from his origin/ seeks his connection times again (Karimi-Hakkak Ahmad, Kamran Talattof. 2004). Moreover, he says in his lyrics:

I'm the bird of Heavenly garden; I'm not from the earth
I've been kept in the cage of my body, for some days
Blessed is the day, when I fly toward the friend
Fly for the love of his place (Rumi, 1990: 201).

Or Hafez, who says:

The veil on the soul's face becomes the mist of my body
Blessed is the moment, when I throw the veil from the face
Such a cage is not the punishment of such a tuneful man
I'll go to the Rizvan rose-bed, since I'm the bird of that sward

However, in the contemporary era, the nostalgia or homesickness is indicative in another way in the poets' works. In 1993, when Akhavan came from Tous to Tehran, described the hardships of travel and his homesickness and living in Tehran as the following:

Since I'm separated from my fellows and home
You're right if you say I'm in the predicament
In the Heaven, like a thirsty traveler

I've fallen on the melting origin land.

Or in the poem "Complain from Rey", in which he complains the exile, and knows himself a youngster, the nostalgia has placed him in a bad predicament:

Who am I? A strange of youth
I was born with a predicament
At an angle in Tehran
I've fallen into a predicament (ibid., 129)
Furthermore, Nima has composed in this way:
From the two township bread
Painful memory of the mountain
That unfortunately in your city
I got old, and I'm suffering
My house, my jungle, where they are, where?
They're now miles away from me
See, what the doom fate is doing with me?
It keeps me away from my homeland… (Nima Youshij, 1997).

Since Nima, the poet, was born in the green nature of the north, this feeling gets stronger in him. Nima remembers his homeland, in each and every place of his own letters. In a letter to his father, on 26th, February, 1925, Nima describes his love and interest in visiting the homeland in this way:

The wild bird, which flies are your son who knows the hunter very well. I scope, I will not take the refuge to nowhere, but the homeland.

Everything is my favorite there. When it will possible to have all your favorite things? When we will gather in one place? One tree shadow on us?. Just us, and our country and our villager friend (Youshij, 1997).

Nima cannot tolerate to be far from his homeland, and knows the city as a big prison for him, and says:

When I see the birds, jumping over the branches, when it is raining, and the Alborz peak is covered with snow and ice, I remember my own mountain." Nima wishes: "I wish I were a bird, and could move freely! I wish I were a cloud, I could travel in the infinite space! ...I'm indeed like a desert bird, who is depressed as a result of being far from my own mountain (Youshij, 1997).

Nima claims that:

My homeland was the best place where I grew up with my brother and sister! The quiet mountainous village from where I'm far, unfortunately, and I'm still alive! Therefore, how I can have fun? I sigh in the remembrance of the night of living in my homeland, continuously" (Nima Youshij, 1971: 36). In a letter to one of his friends, on January 30th, 1929, with regrets Nima advertises "his excessive wish about the beautiful sceneries of the homeland"

(Youshij, 1997)

In another letter, he writes: "No one understands my feeling, what I'm talking about, and what is affecting my thought? Overall, I have fun; however, I cannot reject that I gloom of the regretful reflection of the past, especially about my homeland" (Youshij, 1997). In a letter, to his brother, Ladbun, Nima describes the peak of his sorrow as the following: "my dear brother, I went, and maybe you don't see me again, and I farewell all my wishes and hopes. …. Be kind to my little sister, instead of me. When she grew up, tell about me to her, and tell her that I always had sorrow" (Nima Youshij, 1975: 24).

In another letter titled "my student" Nima says:

What reason should we give, not to love the place where we grew up and had fun? This place is the homeland, unless some events had made us hate this place. This patriotism feeling is very high in me. I'm habited to it. Habit is the rule of life. If it were not, living would have an absolutely bitter and frowned face (Youshij, 1997).

Nima loves his homeland so much, that he says:

Of course, you know that. I'm not against these urban guests who have come here. However, I like every little stone of my dear homeland, the value of which is still unknown to my people and father, and I don't like putting it at the service of strangers (Youshij, 1997).

Generally, Nima has the following feeling of nostalgia:

"My dear sister, I go to sleep, I burn, I wake up, I cannot stand, my homeless heart is really into flying to the homeland" (ibid. 63).

4.1. Nostalgia as a Result of Social Frustration and Failures

Nima is the poet, who pays attention to the society and the human's issues. In his idea "the human and his will is standing at the height of the history, and has made it; therefore, he is always the message of freedom" (Akbar Beyragh, 2002: 96). Moreover, it can be stated that he is a political poet, and he uses symbolism to state these issues. Anyway, Nima, "is a self-centered man who has searched in the horizon of objects, and sometimes, he shows such a strong sensitivity to the social issues, that introduces himself more sociable in comparison with the other contemporary poets, without entering any harm to the depth of his poem" (Barahani, 1995: 674). Therefore, it can be stated that Nima is a poet who knows the pain and suffering very well, and he is hurt by the society, he is homeless, and has an absolutely bitter life. He says about his burning heart and tearful eyes:

The poor condition of life hurts me as well, I wish I could see the spring like them, happily. However, my heart is like a flame, that as much as I get busier, burns me much more!. My eyes are like a piece of cloud, that have never got tired of rain… I think it's the sky, which is crying.the flower have become as red as the color of my hearts. The winds are groaning, and the violet, which has nodded her head, is as sad as me (Youshij, 1997).

In a letter to his brother Ladbun, on October (Pisces), 17th, 1924, describes his separation from him, as the darkness of disturbed dawns, and says:

Among the definitions of this riddle, one is your homelessness, and the other is my separation, and you are ragging like the storm, and I get disturbed like the darkness of the dawn …. I'll never forget you at the oment of travel. The last look of your farewell in that burning cold weather in the winter, from that black chariot, is still shaking me (ibid. 104).

Passing the time is not his favorite. He says: "I pass the time like the star under the cloud. The time is passing but not in the way we like" (ibid. 117).

Nima's life is so hard and bitter, that he rejects telling that to his sister; because, she is not able to feel it at all.

I've just woken up with the flank pain… Tonight I'll walk alone in a dark passage with a walking stick. I'll count the stars over my head. My life is very bitter. I won't explain it. You'll not able to imagine it at all (ibid. 461).

5.1. The Nostalgia of Recalling the Childhood

Recalling the childhood and adolescence is among the issues that arises the poets' emotions and feelings at an older age, continuously, and sometimes even brings tears to their eyes, because recalling those memories, creates a kind of enjoyment in the writer or the poet, and then results into homesickness, or nostalgia, and this feeling seems more resistable. In a letter on March 17th, 1930, Nima writes to one of his friends: "just recalling the past, attacks sometimes to my heart. I feel sad of passing the time and the death of creatures" (Youshij, 1997). On September (Virgo) 15th, 1923, in a letter to his sister, Nikta, Nima writes:

These days, I walked in the quiet corners of the city so much, that I'm getting crazy… what stories the owls have, that are moving and flying inside the city, and whenever I hear their voices, stop working. These little things even, have transferred my memories of the happy times of my childhood and the mountain. They arise a little buzz in my heart (Nima Youshij, 1979).

Nima describes the happy past time when he was playing next to his mother and sister, in this way:

It's the happy sound of the past, which is not returnable. It's the sound of the memories that has poured the wishes and the regrets to the mouth of flowers and narrates them with the hearts that shake well. In the place, where you are alone, our mother fostered us. She weaved baskets with desert grass here. And made bunches of flowers, and we played together. We sit under the trees and threw our kind hearts in front of those flowers. With this broken hearts I'm very similar to the wreckage, narrating the bloody events (Nima Youshij, "A", 1971: 17-18).

Nima describes his regrets about losing the happy time of his youth as the following:

Unfortunately, I passed the first part of my youth without considering this issue. Now, I'm recalling bitterly, in the mountains and caves of the remote homeland of mine, and blame myself: what made me waste some part of my nonreturnable youth, and add to the regrets the nature has provided for me certainly.

Yet, in another place, he says:

"If I started my youth again, I would learn the order of agriculture science, or in one of the centers, I would study industry, or medication, to be a doctor" (Youshij, 1997).

6.1. The Nostalgia of losing dears

Nima is highly affected by the death of those he loves. In such a space, Nima's poem is a burning elegy, indicating the inside hot mark, which erupts like a volcano. In this mood, the sorrow and grief is clear from every word of his poems including Nima's elegy are the poems that he has composed in his father's separation. In the poem, "my father" he describes a tragic space (Nima Youshij, 2007: 348). In the father's loss, he knows himself released in grief and sorrow. This poem shows the sorrow and grief governing on Nima's heart like a mirror.

Similar to you, who went, he went soon
And left me in his sorrow
He covered himself and traveled lightly
To destroy me by his sorrow (ibid. 351).

In the sorrow of losing his father, during a letter to his brother Ladbun, on June, 16th, 1926, he writes:

Where should I start from Ladbun, how can I collect the homesicknesses? I do not know the way to do it. I woke up one night near the dawn, the window shook me heavily. I asked why you don't leave this poor poet alone. On the stairs, a familiar voice called me. I ran out of the room hastily. Alas! It was just imagination. Ladbun, where can imagination take the place of him? How the father will return? If the flame of one torch turned off, what would be the remedy? Ladbun, father has been somebody. You have to be a father some some people. Your orphan brother and friend: Nima (Youshij, 1997: 146).

In a letter, on June, 15th, 1926, Nima shows his appreciation to his father's love, under the title of "kind and honorable governor, Mr. Nezam Aldoleh,:

I've heard that the kind and honorable governor had held the obit for my father three days in Rasht. The poet does not know how, when, and in what language he should reply this sympathy and compassion. What language? Poetry, music, and each and every kind of figures and special materials are unable to reveal the latent human's conscious … here I finish the paper, with inability, and I won't talk about the uselessness of supplication against fate (ibid. 145).

Nima expresses his grief and sorrow with his friend about the loss of his father as the following:

I say hello to you, who think about a painful poet. The father's sorrow is a new hole that is

added to the wreckage of this dam. To tell you the truth, I have no fun. I'm hungry, I'm a captive, and I'm trying hard for captive and hungry people. The old who wins over hatred is not called the winner, the winner is the one, who attacks to destroy his existence. The death attacks are only irresistible (ibid., 149)

In another letter to his sister Nakta, he describes his tears in losing his father as a cloud whose job is raining.

Do you want to know what I do? The dam that was built in front of the tears, is broken again. I do not know here this flood is rolling me? I'm the cloud. Look, Nakta., you cry instead of me in the grass of "Taliv," when the sun sets, but an eternal sunset!!! (ibid: 150-151) also (Ref. ibid., 148, 160, 234, 263, 299).

7.1. Nostalgia Resulted from Weakness, and Senescence

During a letter on January 12nd, 1929, he writes to Parviz Natel Khanlari:

I'm counting the number of my past times. When I review the disappeared events one by one, my head starts shaking from the serious accidents. I think I'm getting too old, and it seems that every little insidious thing created, is going to steal something from the inside of me. The heart desires are just excuses in this regard! (ibid. 213).

In another letter to Khanlari, he knows the thoughts and the tastes as the remedies for each stage of the age, and says:

When you reach my age, and waste some part of your age like me with useless thought, you will write the same thing, and will induce that in each stage of your life, we suffer from one kind of disease. The thoughts, opinions, and tastes are the treatments that are given for that special disease (ibid. 270)

Nima believes that what is giving encouragement and power in his elderly is the nature of the mountain, which exists in him. He says: "I've lost youth, and I'm living in the old age. The only thing that induces me, I'm still young is the mountainous nature that can be interpreted as the wickedness and cordiality of the scamps of my homeland" (ibid. 279). Therefore, he believes that going to Youssh, his own village is very useful for him to rest and get ready for work. "In fact, I'm a little weak. I'd like to go to Youssh for one or two months, and live in there. The nature gives me more success, and I should get ready for work, since I've been refreshed" (ibid. 213).

Nima laments the past, he feels homesick, and believes that its compensation is nothing except action.

Whenever, I remember the past, I lament in all respects. I think, I've lost some part of my age, and I was not really useful for my people and myself. Now against the age that has been passed, I cannot give any compensation except action (ibid. 316).

Yet, in another place, he says:

But I have to be really sorry, by looking at my white hair, each of which is regarded as the death's courier, and give me the message of returning. Through counting the days lost of my age, most of which as I see has gone, and the little part has been left, and I have not done with enthusiasm and what I had to do, I feel homesick. What have I done? Nearly nothing (ibid. 344).

At this moment Nima feels defeated and dead of everything, and he has turned into a statue of sorrow and grief: "everything smells bone and shroud. Everything recalls defeat and death"(ibid., 472). " I'm really rebuffed. I was the one who was the cause of happiness in every gathering, but today, I'm the statue of sorrow and grief" (Ibid. 483).

In a letter, on June, 1953, he writes to Jalal Al Ahmad:

However, I've become really old. The situation of the stars in this month proves it. As much as I try to read all the lines of your letter, I cannot. When I wear my glasses, they fall down from my eyes to the ground similar to a false crystal bowl; it seems that they are making faces for me. They tell me now write if you can (ibid. 505).

Yet, in another letter, addressing Naghash Bashi, Nima believes that old age is the reason of absent-mindedness, and impatience, and says:

As soon as one got old, absent-mindedness and impatience come to visit him. Moreover, I've got more impatient and gloomy. I complete all my properties daily. Anyway, it is very painful, to keep waiting to listen to the bell of the caravan, and I do not like to be asked about the reason, because as much as I'm thinking about the deliverance way for myself, I suffer (ibid., 532)

8.1. The Nostalgia of Staying away from the Lover

Nima's romantic letters to his wife, in addition to stating his viewpoints about the poet, are full of expressing love, friendship, and moreover, it's full of beautiful idioms and expressions. In a letter, Nima writes to Alieh:

I've always past the flowers like disturbed breezes. I've not had any power to make them shake. I've been shining at nights like the moonlight on them. I didn't want their beauties to be hidden. Which of these flowers can shelter a weird bird on their laps? I put my nest (my

heart) on its hand! When can it split the dark clouds, remove the darknesses, and save the most inconvenient hearts? Alieh! You! You can. How much I love your self-esteem and popularity. The lovable beautiful flower of mine (Nima Youshij, "B," 1971: 17).

Yet, in another letter, to Alieh, Nima has suggested studying the history and reading the poems of great poets, so that she believes that the human's heart is the origin of everything, and only poets are able to spend sensitivity. He suggests Alieh: "shake hand with the hand, which keeps your hand. Put your foot where it won't shake under your foot." Nima believes that although the waves are very beautiful and graceful, in the moon and sunrise, nobody trusts in them. Although the strong mountain seems rough, all the flowers are placed on them. Therefore, by stating these beautiful metaphors to Alieh, who is like a flower for him, he knows the poet as a creature, whose wonders are not understood by the others, and treats the people's descriptions about the poets, as just a spiritual proximity. In this writing, he is disturbed and he is uncertain, if he is able to continue his life or not. He says that he is the patient because of his beloved, and recommends him: "Alieh, Alieh, I'm tired of everything. The only thing that can save me is your beauty. Please be kind with your patient" (ibid. 33). In another letter, to Alieh, Nima writes: "separation is sweet! It decreases enmity, and increases the friendship. Moreover, it calms the disturbed heart. So let me cry since I have nobody and my hope is interrupted, and sleep in the tears" (Youshij, 1997). According to Nima, two things are unforgettable. He says: "I do not know how to mislead, and I cannot close the wing of my imagination…. everything is forgotten here, except the attractive memories of the past and the beloved, who is far from the person" (ibid. 190). In a part of his speech in the first congress of the Iranian writers on July, 1946, Nima says: "the result of my investigation, after getting separated from the school, and passing the romantic times, ends to where it is possible to be seen in the poem of "Afsaneh, *[Legend]*" (Nima Youshij, 1978), and according to Aryan Pour's words:

The poet seeks the corner of his heart in this youth era (Afsaneh poem). And retells the narration of his love and failures, describes his life's pessimism and hardships. Moreover, he states his understanding about the instability, fleeting of life, colors, lusts, and wishes deceptions, and wherever he finds a chance, he imagines some beautiful scenes and perspectives of the past and youth times (Aryan Pour, 2003: 590)

2. Discussions

Nostalgia is in fact psychological terms that is synonymous with the regret, and indicates the person's tendency to the past times, in whose ideas, are very bright and outstanding. Nostalgia means the homesickness, and regret to the past times. In the contemporary era, this term entered the field of literature due to the frequency and severity of the social frustrations, and manifested in the poems of the contemporary poets such as Nima. Nima, who has the title of the Persian She'r-e-No' father, is in general a sad, pessimistic, and reserved, and the nostalgic concepts are clear in his poems. He says: "my main source is my suffering. I compose poems for me and the others (Nima Youshij, 1979) these writings are usually memorable, and pathetic. Therefore, "silence, darkness, boredom, and loneliness…"are felt in every part of his poems and writings. In order to relax himself, writes continuously, and recalls the bright and sweet past times such as his childhood and adolescence in his writings, and he wishes permanently to return to the pristine and beautiful nature of Youshij, where is his homeland and birthplace, on which he emphasizes.

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References

1. Ghanoonparvar M. R. 2009.The Neighbor Says: Nima Yushij and the Philosophy of Modern Persian Poetry (Ibex Studies in Persian Literature). Ibex Pub.204.
2. Jameson, Fredric. (1989)."Nostalgia for the Present." *The South Atlantic Quarterly,* 88.2: 527. 60.
3. Karimi-Hakkak Ahmad, Kamran Talattof. 2004. Essays On Nima Yushij: Animating Modernism In Persian Poetry. BRILL, - 267 pages.
4. Yushij Nima. 1979. *Harfhaye Hamsaye*. Tehran: Entesharate Donya.
5. Yushij Nima. 1997. *Namehaye Nima Yushij*. Tehran: Entesharate Negah.

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Family medicine and patients' satisfaction in Iran

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Abstract: Since the second half of 2005, a market oriented reform known as family medicine and rural health insurance scheme was introduced to primary health care network in Iran. The core objectives of this reform were to improve accessibility, quality and utilization of health care services. The assessment of patients' satisfaction, as an outcome quality indicator, was the purpose of this study. This was a cross-sectional study conducted among patients attending health centers in the district of Sari. A self-administered questionnaire, from five different parts of the city, was filled out by 400 attendees during one month of data collection in February 2010. The level of customer satisfaction was far below the level that is expected. Respondents were more satisfied with those items related to the physician than those related to the regulatory aspects of referral system or the duty of health authorities. Villagers' attendance in health centers does not reflect their satisfaction. In fact, they tend not to express their real evaluation of the quality of health centers since they know neither voice nor their choice is to be aptly taken into account.
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Key words: Family medicine, customer satisfaction, health insurance, primary health care, health sector reform

1.1. Introduction

For decades, financing and providing primary health care system have been the policy of Iran's government to address the essential health needs of its entire population. The distribution and coverage of primary health care network in Iran have been almost exhaustive in rural and remote areas and performed actively in these areas in particular (Shadpour 1994). In the region, this system has been one of the best in terms of health outcomes. As reports highlighted, vast improvements both in the health care services and health outcomes were the achievements of this system (Asadi et al. 2004; Mehryar 2004). Nonetheless, from the outset, there were increasingly more reports about primary health care network in Iran which were indicative of many problems such as inefficiency, underutilization of facilities, unavailability of health workers particularly physicians, lack of adequate resources, and staff as well as customer dissatisfaction are few of those to be mentioned (Shadpour 1994; Schieber & Klingen 1999). These problems were mostly related to curative care than preventive care. On the one hand, staff fixed salary and life-long employment in the state owned primary health care network, and the dominant of private sector outpatient curative care providers who mostly are the employees of public sector on the other side, low coverage of curative care was the main concern of policy makers and managers in the ministry of health in Iran (Shadpour 1994; Rouhani 2007). Since the second half of 2005, a market oriented reform known as family medicine and rural health insurance scheme was introduced to the primary health care network in this country. The core objectives of this reform were to improve accessibility, quality and utilization of health care services (MOH 2010).

There are few reports about the achievement of this reform, and most of which take into account the service utilization and accessibility of more staff, particularly physicians, (RHIOM 2010; Motlagh et al. 2010); however, there is no indication about the quality of this newly implemented scheme. We have used customer satisfaction as a quality outcome indicator to assess the customer point of view and provide possible information for appropriate decision or change.

Literature supports the market-based reform and variable payment mechanism under which the provider will face with a more competitive environment and will provide services to meet customer needs (Jegers et al. 2002). Competition for attracting customers is an important factor for the health care settings based on market mechanism so that non-competitive providers would most probably be driven out of the market (Kinney 2005). Experts believe that there is a direct relationship between customer value and satisfaction and organizational performance and productivity (Lothgren & Tambour 1999; Garver & Gagnon 2002; Mihelis et al. 2001). As customer satisfaction is influenced by perceived quality of services (Pascoe 1983), then it can

influence the effectiveness of care through persuading patients to comply positively with treatment regimes (Gilson et al. 1994). The arguments support this view that the impact of customer satisfaction increases demand and purchasing resulting in more profitability (Matzler et al. 2004). Particularly in an increasing market-oriented health care reform and competitive environment of health care providers, customer satisfaction is crucial to the health care providers and it can enable them to increase their share in the health care market (Etter & Perneger 1997; Kujala & Ahola 2005). While, the voice of patients has an important role in the health care delivery system, health care providers, particularly in developing countries, have often ignored patients' perceptions about health services (Andaleeb 2001). In this regard, there is a lack of evidence showing the efficient use of customer information in the decision-making process (Kujala & Ahola 2005). This means that the entire process of customer satisfaction practice, which creates valid and reliable information indicating to what extent consumers' needs are met; and use of that information in decision-making process and re-planning in accordance with organisation core objectives, has not been followed (Kujala & Ahola 2005). The underlying factors of this situation could be due to the monopolistic position or non-competitive environment of health care providers hence lack of appropriate choice available to customers, public awareness, and lack of appropriate system dealing with patient rights.

Customer satisfaction survey is a universally exploited method of getting external feedback concerning the extent to which the suppliers and providers of services have been able to meet the needs and expectations of consumers. This modern quality-based approach, as explained by authors (Mihelis et al. 2001), provides immediate, meaningful and objective feedback on customers' expectation and satisfaction. Given the reform implemented in Iran's primary health care network, which is in rural areas and small towns within which there are usually limited access to alternative health care providers, the purpose of this article is to indicate to what extent the patients are satisfied with the newly reformed primary health care facilities.

1.2. Background

Although the state owned primary health care network in Iran was successful in improving the health outcomes particularly through provision of preventive care (Shadpour 1994; Asadi et al. 2004; Schieber & Klingen 1999), the assessment of some interventions as alternative primary health care settings has shown that market efficiency could even provide better achievements both in terms of inputs and outputs at primary health care level in Iran over the past years (Rouhani 2007; Sadeghi et al. 2003).

By the approval of Iran's parliament, family medicine and rural insurance scheme got the agreement to be implemented by ministry of health and medical education, and ministry of welfare to which national health organisations are affiliated. The reform was implemented in all rural areas as well as towns with less than 20000 residents. In this reform payment mechanism as well as the method of employment in primary health sector significantly changed. In this newly created situation, the team of family medicine has the possibility to boost its income through either enrolling a bigger size of population in a designated area, or improving their performance on predetermined criteria which will be assessed and scored by the insurer. Hence, the teams of family medicine are not paid directly from the income generated, but based on the criteria mentioned. According to this scheme, all residents who are living in the areas in which the reform is implemented are insured against the curative care. A benefit package has been introduced for those who follow the terms and conditions of scheme. A general premium rates for these residents are paid per capita of enrolled population with family medicine by government to the national health insurance and are transferred to regional health authorities affiliated to ministry of health. Typically, each physician should cover a population of 4000 from the outreach area of rural health centres. The insured has to pay the cost of services partially as co-payment which varies between10% (for GP visits) to 30% (for drugs or diagnosis tests). To be entitled for the financial benefit of rural health insurance, patients require following the referral system; otherwise, the utilization of curative care is subject to full payment. Family physicians are limited to refer a maximum of 10% of their patients to secondary health care providers and specialists who are listed in advance. In such cases, patients with a signed and stamped referral letter from their family medicine will enjoy the befit of paid inpatients, outpatients curative as well as Para-clinic services from secondary health care providers just by paying the co-payment.

Ministry of health, as the only primary health care provider and even almost the only health care provider in the area of target population, has agreed to provide the service package through signing an annual contract with the teams of family medicine. Payment to these service providers is fixed per enrolled population and variable based on the level of performance. A family physician with the performance level of 90%, based on determined criteria will get 80% of full payment, and for each

percent of improved performance, will get another 2%. In the same way, they will get less per percentage of weak performance but the performance level of lower than 70% with no items less than 50% is not accepted and could lead to termination of contract, if it is not improved in subsequent two months.

2. Methods

This was a cross-sectional study conducted among the patients attending the health centres in Sari district, the capital city of Mazandaran province, in north of Iran with a typical primary health care network. Gaining from the literature, some relevant criteria were chosen for customer satisfaction survey based on which we have provided a self-administered questionnaire. Relevant to the context of study, we have included items of patient satisfaction for those attending the health centres about waiting time, physician communication, patient referral, rural insurance scheme, cost of care, and overall quality of care plus some individual characteristics of patients. To assess the level of respondents' satisfaction, a 5 scale Likert was used. Questionnaires were handed over directly to patients who were accepted the offer for participation. Assistant was given to those who were illiterate. 400 questionnaires from five different parts of the city were filed subsequently during a month of data collection period in February, 2010. After collecting the data, we analysed them using Microsoft Excel and SPSS package.

3. Results

The results of this study have shown that the majority (67%) of respondents were female. In terms of respondents' job, 60% of them were housekeepers, about 18% agricultures and labours, 8% students, and remaining 14% were from other occupations. Just 5.3% of respondents had university education, while 18% were illiterate and the rest had education between primary to high school level. Based on respondents' self-ratings in terms of their economic situation, 4% were good, 72% moderate and 23% weak. 87.5% of respondents had household number of 4 or less. The majority of respondents (63.2%) were visited by a female GP. The majority of respondents (51.7%) came to the health centres by walking and 14% via own vehicle and the remaining 34.3% by public means of transportation. About 20% of the respondents were visiting family physician for the first time, 24% for the second time , 29% for the third time, and 27% were attended the family physicians for 4 or more times in the last three months. 82.8% of respondents had rural insurance coverage and the remaining had other types of insurance. Still 38% of respondents had no medical record with family physician.

Regarding the satisfaction of respondents, Figure 1 compares the degree of patients' satisfaction against the selected items.

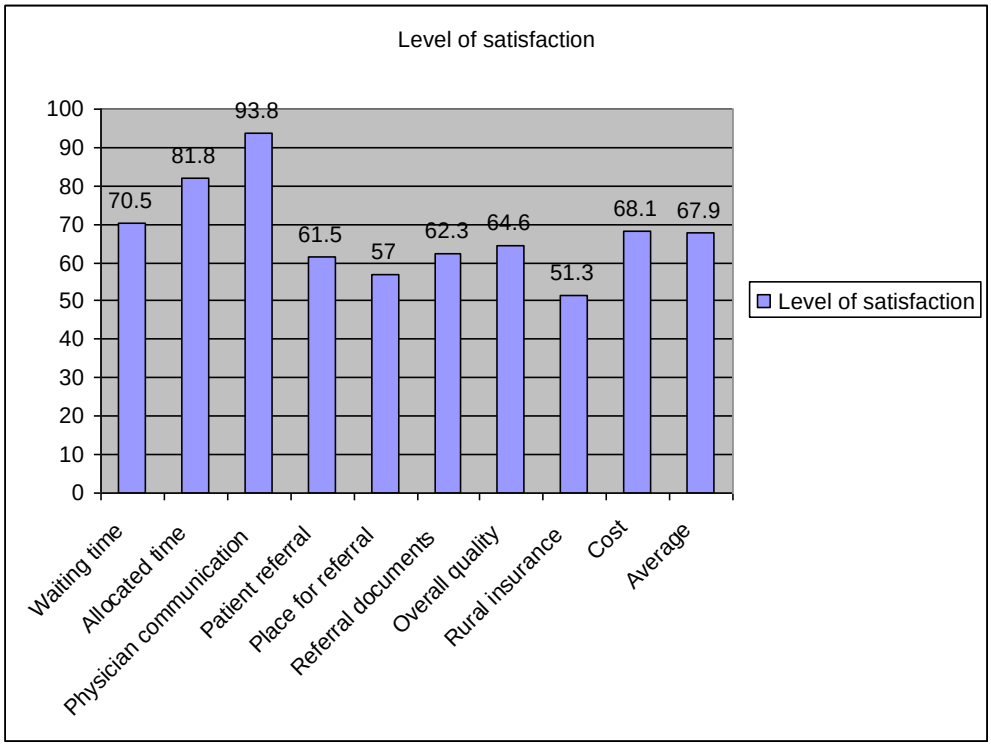

Figure 1: The level of itemised patient satisfaction about family medicine in Iran- 2010

As Figure 1 shows on average, the level of customer satisfaction was 67.9% which is below the level (90%) that is expected. Respondents were more satisfied with physician communication in comparison to other items. Satisfaction about the rural insurance in overall had the lowest rate (51.3%). As the above Figure indicates, higher levels of satisfaction were about the physician personal activities (communication, taking adequate time) than other items like the regulation of scheme (aspects of referral) or those items that are related to the health authority (cost and overall quality) as an intermediate contractor which are far below the objected score of 90%.

Other results of this study revealed that just 49% of prescribed drugs were available to the patients. Regarding to the access to physicians, only 42% of patients mentioned that they have access to them when they are seeking care at the health facility. These later findings had statistically significantly positive correlations with patients' satisfaction. Also, there was statistically significant negative correlation between the amount of payment and the level of satisfaction.

Moreover, other results of this study have indicated that in overall 61.2% of respondents mentioned that they will attend the health centre in the future if they feel sick.

We had access to research findings of customer satisfaction with the alternative primary health care settings (Rouhani 2007; Zakery 2003) conducted before in Iran. A comparison is made between the results of this study and other findings regarding some identical aspects of customer satisfaction as depicted in Figure 2.

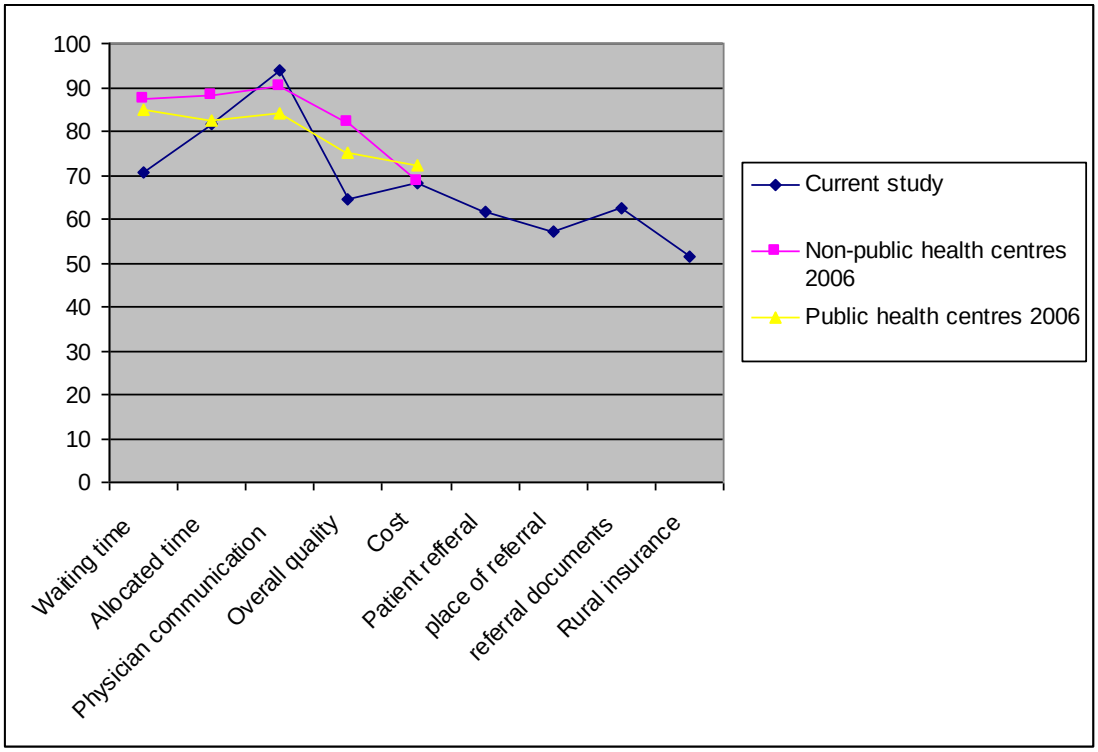

Figure 2: A comparison between the level of customer satisfaction among the patients of family medicine and the results of other research findings at primary health care level in Iran

As Figure 2 indicates, except for physician communication, for other measures of customer satisfaction used in both studies, family physicians had lower level of customer satisfaction; however, in the previous study, satisfaction with that item was relatively high. Again, concerning the other items which were particularly relevant to the family medicine, the level of customer satisfaction was below the level of other criteria.

In terms of the average level of customer satisfaction found in these research findings, a comparison is made in Figure 3.

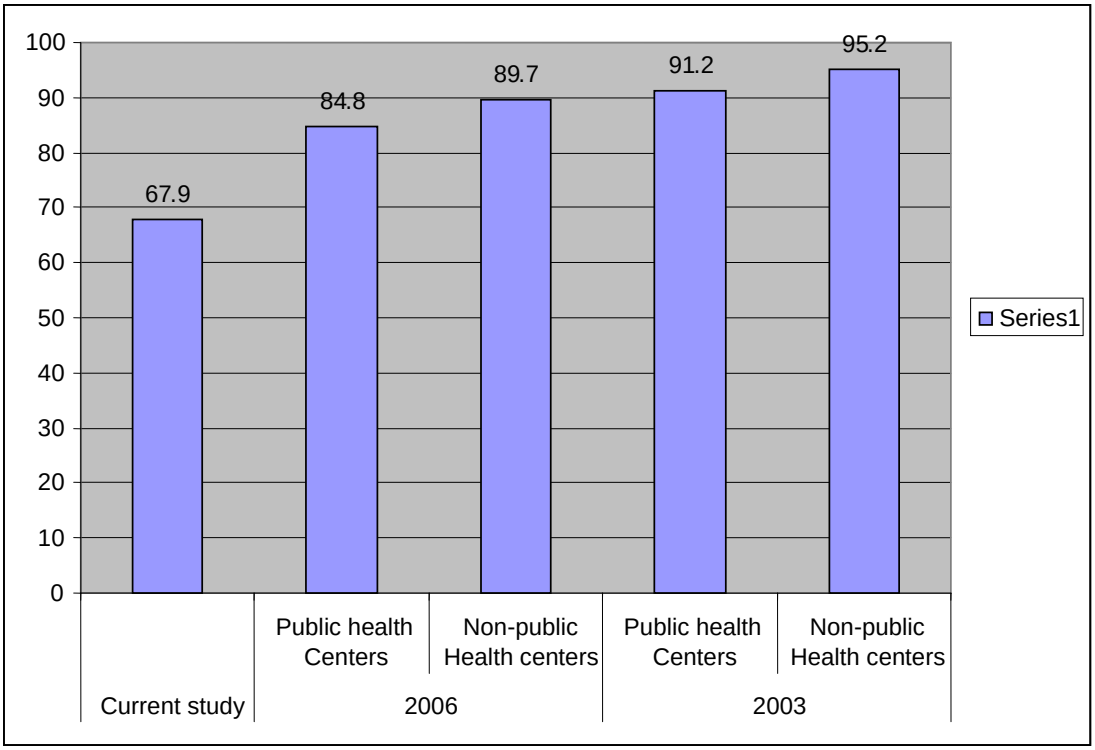

Figure 3: A comparison between the average levels of customer satisfaction on three different sets of primary health care settings in Iran

As Figure 3 reveals, the average level of customer satisfaction found in this study is the lowest one compared which attendees of alternative primary health care settings even public health centres that were publicly financed and provided. Those health centres were located in urban areas which thought they were performing passively and inefficiently as the weakest parts of primary health care system in Iran (Shadpour 1994; World Bank 2007). Also, they normally do not have monopoly position particularly for outpatient curative care in urban areas.

4. Discussion

The results of this study have shown that the level of customer satisfaction, except for the item of physician communication, is far below the level which is expected. Based on Iran's family medicine terms and conditions, the renewal of contract with the physicians in the following years is subject to achievement of an average score of 70% on different aspects of family physician performance including customer satisfaction of which none of those items is below 50% (MOH 2010). But it does not seem to be applicable, practically given the health centres dispersed across the remote areas and lack of adequate accessibility to them together with the shortage of staff in rural insurance department in Iran (RHIOM 2010). Even the level of satisfaction achieved by family medicines in overall is relatively below the level of this indicator in the health centres that were designed in a pilot study in one province that started alternative primary health care settings in Iran a few years ago, as well as public health centres in urban areas in Iran (Rouhani 2007; Zakery 2003).

Patients are more satisfied with the physician personal activities itself that might be the result of family physicians on their part to perform more friendly and responsively to achieve better score, but in those areas that are not directly related to the physicians but to the health authority or regulations set, the level of satisfaction was significantly lower.

The result of this study is in line with those of other reports (RHIOM 2010; Motlagh et al. 2010). For instance, these reports mentioned that patients are charged more than 30 percents as co-payment, three times more than the level agreed by health authorities. Also, they highlighted that the percentage of patients referred is higher than the amount permitted.

Each of those under performance, which have relation with consumer dissatisfaction, could be explained based on their influencing factors. As mentioned earlier, ministry of health has a monopolistic position as the only health care provider in the areas under reform; therefore, health

authorities, by taking advantage of such market position and as interest of profit maximization, may lower the quality or increase the cost for patients as pointed out by Adam Smith (1776), when there is no real alternative choice available to the patients or real competition among providers. As the reports indicated, those people, who are charged more than the amount allowed, do not have accessibility to the services agreed (RHIOM 2010; Motlagh et al. 2010), and in some circumstances are not allowed to be referred (MOH 2010). These could somehow explain the low level of customer satisfaction even lower than the level prior to the reform. This is happening even when a fully paid insurance premium with services planed at patients neighboring is in place with more than fifty percents of patients coming to the health centers on foot.

To explain the referral item, it is worth to mention that a cap of 10% is much far below the reality. It seems that the family physicians are attempting, just by enforcing the regulation, to prevent patients demand for referral, but they are still beyond the amount permitted as reports revealed (RHIOM 2010; Motlagh et al. 2010). In this regard, the main role is played by district health authorities in terms of providing the minimum services agreed that is not achieved so far. For instance, concerning unavailability of prescribed drugs, 70% of its cost should be paid by health authority from the resources being paid by financial package of insurance company. Again, in such circumstances where the services are not available, there is no real risk to health authorities as they still charge patients for 30 percents even to those attendees who just have got a signed and stamped referral letter for secondary care providers without any guarantee that they will be seen by those service providers at secondary level. With this explanation, the risk of patients whose needs are not provided at health centre level, is to the insurance company or patients themselves. Then, what can bring the primary health centres to provide adequate health care to patients and prevent the unnecessary referrals? In other words, if health centres even fail to address patients needs, they can still have their income for referring patients and also save the cost of drugs and other services not being given to patients and then there is no incentive to bring the amount of referral down. Also, given their monopolistic power at rural areas, there is no alternative choice available to the insurance company for having alternative competitors to win the contract. This is perhaps the only reason for not having a bidding procedure in selecting alternative health care providers. Just setting a cap for referral rate could not solve the problem as was not achieved so far; given the insurance company has not in reality the possibility of monitoring the referral rate properly and continuously (RHIOM 2010). It is quite acceptable if people are not treated adequately and not referred on demand; then, they will be dissatisfied not only in terms of the referral itself but also regarding to the rural insurance completely. This could be the best explanation about a surprising result of lowest level of customer satisfaction (51.3%) among the items about a fully paid premium rural health insurance in Iran with planned health services near living areas of rural population.

Having lower level of customer satisfaction from the level which is expected, patients still continuing attendance in the rural health centres in Iran could be interpreted as unavailability of alternative choices to the rural population either financially or geographically. Concerning this issue, the results of current study have shown that those people who were attending the health centres more frequently, had rural-insurance compared with other patients who had other types of health insurance with freedom of choice in selecting their health care provider available in the country, locally or nationally, without any requirement to follow referral system. In other words, low level of customer satisfaction with subsequent attendance in the rural health centres should be a concern of unavailability of choice to be insured under rural insurance scheme. Again, given the level of customer satisfaction together with no freedom of choice for alternative health care providers and no more freely provided curative care and financial and geographical circumstances of rural population, the issues of unmet needs and then the decision of no care as highlighted by Propper (2000) should be a concern when speaking about the performance of newly implemented reform in primary health care network in Iran.

Given the risk related to the monopolistic power of health care providers on the quality or cost of care (Smith 1776), we have found that both the quality, in terms of customer satisfaction, has decreased on the one hand and the cost to the patients in terms of direct payment as well as lack of adequate services and referring them to the other service providers has been increasing on the other hand.

5. Conclusions

By implementing market-oriented reform in primary health care system in Iran, customer satisfaction is not achieved at the level which is expected. This is a surprising result seeing people less satisfied with a completely paid insurance premium by government than in a situation where they were not insured in using the same sort of facilities. Patients are more dissatisfied with the

regulatory aspects of referral system that rule physicians to prevent patients going for secondary care if they want to use the financial benefit of rural insurance. Satisfaction is also lower for those aspects that its improvement is related to the regional health authority. Given the monopolistic position of regional health authorities at rural areas in providing health care, requirements of having referral letter for using secondary care, have left patients with no choice in real terms in using the benefit package of rural insurance in Iran. Attending the health centres but still dissatisfied, means neither voice nor choice of customers has been considered appropriately. If they cannot have access to the expected and appropriate care and cannot be referred to the alternative health care providers, what would be their decision for such scenarios? This could be a risk to the health of population in rural areas as there is the possibility that given the performance of newly reformed health centres, as have found in this study, as well as other studies, could leave people with the decision of no care regarding their felt needs. Rejecting such a hypothesis requires a full assessment of peoples' view points at the household level that may provide appropriate information about the utilization of health care services in general and in different socio-economic groups in particular.

After five years since the reform has been implemented, anticipated services are not in place, and people are charged three times higher than the amount permitted. There is not adequate choice available to them, and hence they are not satisfied. There are big concerns about perceived low-quality services. Probably such a situation has led to an increase in unmet health needs. Accordingly, it can be concluded that the reform has not fully achieved its objectives. And the quality of primary health care in rural areas in Iran, as the main health care available to these people, needs major improvement particularly on curative care.

References

1- Shadpour K. *The PHC experience in Iran*, The Council for expansion of PHC networks. MOHME, UNICEF-Tehran. 1994
2- Asadi LM, Sayyari AA, Akbaric ME, Graya D. Public health improvement in Iran—lessons from the last 20 years. *Public Health*, 2004; 118: 395–402
3- Mehryar A. Primary health care and rural poor in the Islamic Republic of Iran, scaling up poverty reduction: *A global learning process and conference Shanghai*, May 25-27, 2004
4- Schieber G, Klingen N. *Health financing reform in Iran: principles and possible next steps*. The High Council Research of Social Security Insurance. Tehran. 1999.
5- Rouhani S. *The relative efficiency of public and non-public health centres in Iran*. Ph.D Thesis. University of Keele. UK. 2007 .
6- Centre for management of primary health care network. *Handbook of family medicine and rural Insurance in Iran*. Version 11. Ministry Of Health. Tehran. Iran. 2010.
7- Regional health insurance organization of Mazandaran.. *An assessment of health care delivery at the primary health care facilities for insured rural population in Mazandaran Province*. A research based managerial report. Sari. Iran. 2010.
8- Motlagh ME, Nasrollahpour SSD, Ashrafian AH, Kabir MJ, Shabestari MA, Nahvijoy A. Satisfaction of Family Physicians (FPs) about Effective Factors on Activation of FP Program in Medical Universities. *Journal of Gilan Universities of Medical Sciences*, 2010; 76:48-55.
9- Jegers M, Kesteloot K, DeGraeve D, Gilles W. A typology for provider payment systems in health care. *Health Policy*, 2002; 60: 255-273.
10- Kinney W. A simple and valuable approach for measuring customer satisfaction. *Otolaryngology-Head and Neck Surgery*, 2005; 133: 169-172.
11- Lothgren M, Tambour M. Productivity and customer satisfaction in Swedish pharmacies: A DEA network model. *European Journal of Operational Research*, 1999; 115: 449-458.
12- Garver SM, Gagnon BG. Seven keys to improving customer satisfaction programs. *Business Horizons*, 2002;. September-October: 35-42.
13- Mihelis G, Grigoroudis E, Siskos Y, Polotis Y, Malandrakis Y. Customer satisfaction measurement in the private bank sector. *European Journal of Operational Research*, 2001; 130: 347-360.
14- Pascoe CG. Patient satisfaction in primary health care: A literature review and analysis. *Evaluation and Program Planning*, 1983; 6(3-4): 185-210.
15- Gilson L, Alilio M, Heggenhougen K. Community satisfaction with primary health care services: An evaluation undertaken in the Morogoro region of Tanzania. *Social Science & Medicine*, 1994; 39(6): 767-780.
16- Matzler K, Bailom F, Hinterhuber HH, Renzl B, Pichler J. The asymmetric relationship between attribute-level performance and overall customer satisfaction: a reconsideration of the importance-

performance analysis. *Industrial marketing management*, 2004; 33: 271-277.
17- Etter FJ, Perneger VT. Validating a satisfaction questionnaire using multiple approaches: a case study. *Social Sciences & Medicine*, 1997; 45(6): 879-885.
18- Kujala J, Ahola T. The value of customer satisfaction surveys for project-based organisations: symbolic, technical, or none. *International Journal of Project Management*, 2005; 23: 404-409.
19- Andaleeb SS. Service quality perceptions and patient satisfaction: a study of hospitals in a developing country. *Social Sciences & Medicine*, 2001; 52: 1359-1370.
20- Sadeghi H, Nikniaz A, Sehati S, Koshavar H. *The comparison between non-public and public health centres in the delivery of health care for children under one year in Tabriz.* MSc dissertation, Tabriz University of medical sciences- faculty of nursing and midwifery. Iran. 2003.
21- Zakery A. *Health institutions, a report on the cooperative health centres projects in East Azerbaijan province*. Provincial health authority East Azerbaijan Province. Tabriz-Iran. 2003.
22- The World Bank Group. Islamic Republic of Iran, *Health Sector Review*, Volume I: Main Report. 2007.
23- Smith A. *An inquiry into the nature and causes of the wealth of nations* Vol. 1, Oxford University Press. 1776
24- Propper C. The demand for private health care in the UK. *Journal of Health Economics*, 2000; 19: 855–876.

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A survey on postural deviation and flexibility of blind and sighted girls when compared with the normal situation

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Abstract: Purpose: Blindness is a factor that limits the body movements and it may cause sedentary complications in blind people. The goal of this study was to evaluate postural status and muscles flexibility in the blind and sighted girls. **Material and Method:** This cross-sectional comparative study was carried out on 40 blind and sighted girls with the range of 20 to 30 years. The group of blind was included 20 blind girls who were studying in the Khazaneh Rehabilitation Center, and they agreed to attend in the study. The other group was included 20 student sighted girls whose BMI were matched with the blind group. To evaluate postural status and muscles flexibility, a number of physical tests carried out such as: lordosis, kyphosis, scoliosis, shoulder depression, forward head posture, and passive knee extension and illiotibial band. For statistical analysis data were analyzed using Chi-square test. **Results:** The findings showed that 30% of the candidates of the two groups did not have any disorders. 20% of the blind subjects showed one or more disorders. 5% of the sighted subjects showed one or more disorders. There were significant differences in some of the tests between the blind and sighted subjects including lordosis, kyphosis, scoliosis and hamstring shortening (P>0.05) However in the other tests there were not significant differences between the two groups. **Conclusion:** Based on this study flexibility and postural status demonstrated to be affected by vision and in some of the tests such as kyphosis there is a significant differences between the two groups, in addition, the pattern of changes and disorders are different in the two groups, that is, the common disorders in the blinds was kyphosis. **Ethical approval:** This study was approved by the ethics committee of Semnan University of Medical Sciences.

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Key words: blinds, posture, flexibility, skeletal disorders, kyphosis, Lordosis, scoliosis.

Introduction

Physical condition and flexibility are the main physical fitness indices that help human health and its reduction would increase vulnerability of the motion system and causes skeletal anomalies. It was reported that changes in muscle movement and direction would increase abnormal biomechanis pressure on the joint and in such condition, blood flow is reduced and tissue restoration will be longer in damages condition (1).

Sensory systems such as vision play an important role in movement skills and overall situation and shape of body. Studies have shown that central nervous system is involved in maintaining proper body position and doing movement skills according to the information which receives from proprioception, visual, tactile and auditory senses (2, 3). Impairment in various senses such as vision causes movement and coordination performance degradation, and given the general decline in physical mobility in the blind than sighted people, the blind are more likely to have physical adverse conditions (4).

Kisner and Colby (2002) have suggested that muscles which are stretched or shortened in the body's normal condition are weakened and provide the context for postural changes in the individual (5). Studies on various individuals indicate factors related to creation and start of postural disorders.

In a study on the blind, it has been demonstrated that movement patterns in the chronic blind (those that so many years have passed form their blindness) is different from the blind in recent years (6). In 2010, Nicolas et al have reported that visual stimuli are effective on the posture (7).

It has been in the sighted people that if superficial and deep neck muscles are not activated and used equally, it will affect neck stability and cause neck posture weakness (8).

In another study which was conducted on two groups of blind and sighted people in order to compare their dynamic postural stability, postural

stability difference was reported in the two groups. This study has also shown that dynamic postural stability is greatly affected by vision (9). People who do not use visual stimuli, pressure to the soles are more and cause early fatigue in the neck muscles (10).

Although there are many studies on flexibility and normal and abnormal physical conditions in the sighted people (1, 2), there are few studies on posture and flexibility in the blind; so we sought to study physical condition on blind people. The aim of this study is to evaluate physical condition and flexibility of muscles in the blind compared with the sighted people.

Methodology

This is a descriptive-comparative study which was conducted on 40 blind and sighted girls with 20-30 age range. The girls were chosen because of their better accessibility and cooperation, while testers were female. The blind group was 20 of blind girls of Tehran department of treasury who had the inclusion criteria and was willing to participate in this study; and the sighted group was 20 female students who aligned with the blind group in terms of Body Mass Index (BMI). 20 people were chosen as the sample because of limitation in the selection of blind samples that were eligible to participate in this study and also willing; moreover, non-random simple sampling was used due to limitation in the sampling method.

All girls with visual disorder training in the treasury rehabilitation center were enrolled in the study; the sighted group was selected according to the study condition among volunteer girl students and staff of the dormitory of the Medical Sciences University of Semnan. The dormitory girls were selected through announcement in the dormitory and their referral. Also diseases and disorders that may affect normal physical condition, for example people who had a history of heart disease or suspected of cardiovascular disease or movement disorders in the two groups were excluded from the study. It is noteworthy that sighted people were selected such that can be matched with the blind in terms of Body Mass Index (BMI).

All volunteers signed consent forms; meanwhile the permit for this study was received from the organization of social welfare and Treasury Rehabilitation center.

To compare the two groups, some effective indices in mobility and flexibility skeletal structure were regulated based on a questionnaire and the following measurements were performed; these indices were:

Forward head posture (FHP)
Kyphsis
Lordosis
Scoliosis
Right & left shoulder depression
Right & left illiotibial band
Right & left hamstring

Measurements were done by two experienced physiotherapists who were not interested in this study. Valid and common methods were used to measure each of indices (11, 12, and 13). The method is described as follows.

The test results were recorded when they were confirmed by the two physiotherapists for further validity, and suspected and disputed causes were eliminated.

Testing procedures:

Forward head posture (FHP)

1. Based on observation and measurement of the neck from the wall, the person stands such that her back was in contact with the wall. And tester measured the distance from the deepest part of the neck to the wall; normally, it was 4-8 centimeters.

Kyphosis

Based on observation and using plummet line, kyphosis was determined in individuals. In these people if the main dorsal was behind the plummet line, kyphosis was recorded.

Lordosis

Based on observation and using plummet line, lordosis was determined in individuals. The person was at the side of plummet line, if major part of lumbar from the side was ahead of plummet line, it was recorded as lordosis.

Scoliosis

For scoliosis, observation, forward bending and lateral bending was used; so that when bending forward if there were non-symmetric parts including waist and shoulders folds and shoulders bulge, and if the person in standing position with straight knees bent to the sides, and also if bending movement range to the sides was not identical, it was recorded as scoliosis.

Left and right shoulder depression

Observation was used in studying shoulders depression, and non-symmetric cases were recorded; i.e. if shoulders were not on one level and a shoulder was lower, it was recorded as shoulder depression.

Illiotibial band and tensor facia lata

The flexibility of the Illiotibial band and tensor facia lata was conducted based on Ober test. Such that the person was in the side; the lower knee was slightly bent to stabilize the hip joint and pelvis was fixed by the examiner, then passively the upper leg was taken to the abduction and extension while bending or straightening the knee, leg was dropped keeping hip

extension and without any rotation on it to fall on the bed passively. If there is a short, leg remained in abduction, illiotibial band is loosed by bending the knee and flexibility of facia lata was revealed.

Right and left hamstring

Passive knee extension test: the subject in the supine position, while a small pillow is under her head lying on the bed, then femur trochanter, femur external epichondil, knee joint line and ankle external malleolus were marked by a marker. When measuring a limb, the opposite limb was taken completely flat by a different person. The tested limb was taken at 90^0 angles between tight and knee. Then leg was lifted passively until tightness is felt and was considered normal up to 20 degrees less than complete opening, and more than it was recorded as short.

Minitab statistical software was used for data analysis. To compare the indices between the two groups, chi-square test was used. Also the two groups of sighted and blind people were volunteers for conducting this study. Frequency percentage of skeletal and frequency anomalies were investigated in the two groups based on a number of determined indices; and number of people in each group having one or more anomalies mentioned in the questionnaire was recorded and anomalies in both groups were compared statistically.

Results

Number of participants in the blind group having postural and flexibility disorders based on indices of this study was compared with the sighted group. Chi-square test was used to compare indices in the two groups and the following results were obtained. The results of these tests have been listed in Tables 1, 2 and 3.

The prevalence of kyphosis, scoliosis, and short left and right hamstring in the blind group were more than the sighted group.

The two groups were compared to investigate kyphosis index and results demonstrated that number of people that have kyphosis in the blind group is significantly higher than the sighted group ($p<0.05$). kyphosis was most common among the blind.

In terms of having scoliosis, results showed that number of blind having scoliosis is significantly higher than sighted people ($p<0.05$).

To investigate lordosis index, the two groups were compared with each other and results demonstrated that the blind group has a significant difference with the sighted group in terms of having lordosis; and number of participants in sighted group having lordosis is significantly higher than the blind group ($p<0.05$). Also lordosis was most common among sighted girls.

In terms of left and right shoulder depression, number of sighted people having shoulder depression in both sides was more than the blind but the difference was not significant.

In terms of having short left and right hamstring, there was a significant difference between the two groups; and number of sighted people having short left and right hamstring was significantly higher than the sighted group ($p<0.05$).

In terms of short illiotibial band, number of the blind was more than the sighted group, but the difference between the two groups was not significant. Also none of the blind group had short illiotibial band.

Moreover, results of this study demonstrated that 30% of volunteers in each group didn't have any type of structural disorders. 20% of the blind had at least one or more structural disorders; and 5% of the sighted group had at least one or more structural disorders.

Table 1: frequency distribution of musculoskeletal dysfunction in the blind and sighted people

Type	blind		sighted	
	Frequency	Percent	Frequency	Percent
FHP	4	20%	5	25%
Kyphsis	14	70%	3	15%
Lordosis	8	40%	15	75%
Scoliosis	5	25%	1	5%
Rt shoulder depression	6	30%	7	35%
L. shoulder depression	5	25%	8	40%
Rt illiotibial band	2	10%	0	0
L. illiotibial band	2	10%	0	0
Rt. hamstring	10	50%	4	20%
- L. hamstring	12	60%	3	15%

Table 2: chi-square test results related to skeletal disorders in the two blind and sighted groups

Type	Chi-sq	*df*	P-Value
FHP	0.14	1	0.075
Kyphsis	12.38	1	0.000*
Lordosis	5.01	1	0.025*
Scoliosis	4.33	1	0.037*
Rt shoulder depression	0.11	1	0.736
L. shoulder depression	1.03	1	0.311

* P<0/05 was considered significant.

Table 3: chi-square test results related to muscular flexibility in the two blind and sighted groups

Type	Chi-sq	*df*	P-Value
Rt illiotibial band	**2.11**	1	**0.147**
L. illiotibial band	**2.11**	1	**0.147**
Rt hamstring	**3.96**	1	**0.047***
L. hamstring	**8.64**	1	**0.003***

* P<0/05 was considered significant.

Discussion

This study that was conducted to compare physical condition and muscle flexibility in the blind and sighted girls demonstrated that in both studied groups, there are some degrees of anomaly and movement limitation and totally it's higher in the blind.

The results of this study are consistent with some of previous studies conducted on healthy people: a study conducted in 2004 on healthy girl students in Semana indicated that majority of guidance girls in the statistical population had structural disorder at least in one area of the body (14). In the present study, it was also indicated that most of sighted girls had structural disorder at least in one area of the body. In this study, the most common structural disorders in the blind girls were kyphosis and in the sighted girls was lordosis. It is likely that these disorders are due to overall body condition. This study is consistent with some other studies; in the study by Gerr on the sighted people, it has been reported that fixed physical position may cause change in the posture (15). Moreover, proper physical activity will prevent from inappropriate physical condition (16).

It seems that in the blind given that their overall body condition is protective and leaning forward, the possibility of kyphosis development is higher in them; and in the sighted people, wrong habits and weight pressure may cause lordosis development in them (1).

In the present study, the least statistics of postural disorders is related to short ITB which is 10% in the blind girls and zero in the sighted girls.

Several previous studies confirm findings of the present study that blindness affects posture of individuals. In a study in Turkey conducted on two groups of blind athletes and sighted non-athletes to evaluate and compare dynamic postural stability, it was reported that dynamic postural stability is affected by vision. They also stated that there is a significant difference between the two blind and sighted groups. Also blind athlete group has higher postural stability than sighted non-athlete group (9).

Moreover, in another study on elderly sighted people, it was reported that vision is effective in creating muscular stability (17).

A study conducted in Japan on patients confirms part of the present study. In that study the severity of common lumbar lordosis and sacrum tilt has been reported (18). In the present study, the most common complication in the sighted people has also been reported to be lordosis. The reason of the difference in the methodology and features of statistical population may be relevant.

In a study by Morris et al (1992) on young patients having spinal and extremity pain, right shoulder depression was the most common. Results of this study are not consistent with any findings of the two blind and sighted groups in the present study. The reason may be related to different features of statistical population including that in the present study none of the two groups had musculoskeletal problems (19).

Pitt-Brook has described mechanisms of posture change as: skeletal disorders related to a continuous isometric contraction in a specific muscular group that causes extreme fixed posture in the person (20). Recent studies have also demonstrated that visual stimuli affect posture of healthy people and neck pain (17).

In the present study, although different postural pattern have been reported in the two blind and sighted groups, there has also been abnormal posture in both groups, which is probably due to the issue that vision has an important role on other sensory receivers in human, and has a significant impact on human's balance (21, 22). Moreover, balancing system of body can adapt itself with new positions, and movements and body position are done based on the new positions (23). In addition one acquired disorder (in age of two years) may affect completely the body structure (24).

Limitations: in doing some tests for accurate diagnosis and grading, radiology and photography were required which weren't done due to lack of volunteers' consent and other limitations; and given the existing tools and common practices only disorders were detected and their rate wasn't determined.

For selecting volunteers, given the administrative limitations, cooperation of students in Smenan was used in the framework of standards of this study. Methods like BMI were also used for more compatibility between the two groups and reducing error.

Conclusion:

According to results of this study, flexibility and posture are affected by vision; and in some tests such as kyphosis, there was a significant difference between the two groups. Moreover, the results demonstrated that changes and postural disorders pattern is different in the two blind and sighted groups; i.e. kyphosis is most common in the blind and lordosis in the sighted people.

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References:

1- Weiss RC, Personal fitness trainer: basic concepts and applications, In; Your Career in Physical Medicine, Philadelphia, W B Saunders Company, 1997, PP.327-350.
2- Kamen G, The neural control of movement, In; Kamen G., Foundation of Exercise Science, USA, Lippincott Williams & Wilkins, 2001, P.235-254.
3- Magill RA, Motor Learning and Control: Concepts and Application, 8th Edi. New York, MacGrow-Hill Companies, 2007.
4- Badke MB and Di Fabio R, Facilitatnion: new theoretical perspective and clinical approach, In; Basmajian JV and Wolf SL, Therapeutic Exercse, 5th Edi. USA, Williams & Wilkins, 1990, PP. 77-91.
5- Kisner C and Colby LA, Therapeutic Exercise Foundation and Techniques, 4th edi. F.A. Davis Company, Philadelphia, 2002.
6- Imbriba LA, Rodrigues EC, Magalhaes J and Vargas CD, Motor imagery in blind subjects: the influence of the previous visual experience, Neuroscience Letters, 2006; Vol.400, 181-185.
7- Nicolas P. Benjamin B Yannick S. Nicolas V. Effects of Vision and Tactile Stimulation of the Neck on Postural Control During Unperturbed Stance and Cervical Joint Position Sense in Young Asymptomatic Adults, Spine: 2010 : 35 (17): 1589-1594.
8- Falla D, Jull G, Russell T, Vicenzino B and Hodges P, Effect of neck exercise on sitting posture in patients with choronic neck pain, Physical Therapy, 2007; 87(4), 408-417.
9- Aydog E, Aydog ST, Cakci A and Dorsal MN, Dynamic postural stability in blind athletes using the biodex stability system, 1st World Congress of Sports injury prevention, British Journal of Sports Medicine, 2005; 39,P.373-408.
10- Nicolas V. Nicolas P and Jacques V Postural control during quiet standing following cervical muscular fatigue: effects of changes in sensory inputs, Neuroscience Letters, 2005; 378, (3): 135-139.
11- Hertling D and Kessler RM, Management of Common Musculoskeletal Disorders, 4th Ed, Lippincott Williams & Wilkins, USA, 2006.
12- Dziedzic K, Ankylosing spondylitis, in David C and Lloyd J, Rheumatological Physiotherapy,

Mosby Internaltional Limited, UK, 1999; PP. 97-114.
13- Kendall, FP, Kendall McCreary, E, Provance, PG. Muscles: Testing and function with Posture and Pain. 4th ed. Williams & Wilkins. 1993. Baltimore.
14- Kia N, A survey on skeletal structure of the secondary students girl in Semnan, Semnan, Educational and Training Organization of Semnan, 2004.
15- Gerr F, Marcus M, Ortiz D, White B, Jones W, Cohen S, et al. وComputer users' postures and associations with workstation characteristics. AIHAJ. 2000; 61(2): 223-30.
16-Lynch SS, Thigpen CA, Mihalik JP, Prentice WE, and Padua D, The effects of an exercise intervention on forward head and rounded shoulder postures in elite swimmers. Br J Sports Med 2010;44:376–81.
17- Nicolas V, Vincent N and Jean-Michel P Can vision compensate for a lower limbs muscular fatigue for controlling posture in humans? Neuroscience Letters, 2001; 308, (2): 103-106.
18- Utsumi Y. Hanaoka E. Yamagata M. Changes in lumbar lordosis in young patients with low back pain during a 10-15 years period. J Orthop. Sci. 2004, Vol.7(6), P. 618-622.
19- Griegel-Morris P, Larson K, Mueller-Klaus K, and Oatis CA, Incidence of common postural abnormalities in the cervical, shoulder, and thoracic regions and their association with pain in two age groups of healthy subjects, Physical Therapy, 1992; 72(6), PP.420-432.
20- Pitt-Brook J, Neuromusculoskeletal enviroment; Interaction concepts, In; Tidswell M, Orthopedic Physiotherapy, London, Mosby, 1998; PP.229-243.
21- Schmid M, Nardone A, Marco De Nunzio A, Schmid M and Schieppati M, Equilibrium during static and dynamic tasks in blind subjects: no evidence of cross-modal plasticity, Brain 2007 130(8):2097-2107.
22- Shumway-Cook A, Woollacott M. Attentional demands and postural control: the effect of sensory context. J Gerontol Med Sci 2000; 55A: 10-16.
23- Riley MA, Wong S, Mitra S, and Turvey MT, Common effects of touch and vision on
24- Gori M, Tinelli F, Sandini G, Cioni G and Burr D, Impaired visualsize-discriminationin children with movement disorders, Neuropsychologia, 2012, 50; 1838-1842.

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Strategic Analysis of the Presence of Corporate Venture Capital in Iranian Science and Technology Parks and incubators

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Abstract: Corporate Venture Capitals (CVCs) are important financial innovations used for financial resourcing of new high-risk and high-tech organizations. In order to reduce existing risks, CVCs have focused on developing firms, science and technology parks and incubators by their main twofold functions. The first function is providing the capital needed for the commercialization of ideas, plans and designs of entrepreneurs and the second one is preparing a good market for CVC. In present study, it is attempted to detect the internal strengths and weaknesses of science and technology parks and incubators using one of the most important strategic management instruments, i.e. SOWT model as well as to analyse environmental opportunities and threats confronting CVCs in science and technology parks and incubators and to represent the strategies in four strategic groups, such as SO, ST, WO, and WT. All strategies are prioritized using TOPSIS technique among these four groups which the most important strategies are using the experiences of pioneer countries in the field of CVCs, science and technology parks and incubators. The main purpose of this study is finding and sharing the benefits or losses of the developing companies and also developing activities associated with establishing relationship between investors and holders of new ideas such as market technology.

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1. Introduction

Nowadays, the importance of science and technology is clear more than ever. Industries have found that effective usage of science and technology is the main key to achieve higher efficiency and benefits. Governments have concluded that competition in world markets and maintaining national security is impossible without science and technology. Societies have discovered that their welfare and accomplishment is depends on how they employ science and technology appropriate in satisfying their national needs. All these issues have challenged governments in providing suitable models. Science and technology parks and incubators are considered in these models [Keshavarz et al., 2004].

An incubator or a science park provides resources like space, goals, marketing, management, structure and financing to new technology-based firms (NTBFs) [Aaboen, 2009]. In other words, one of the objectives of Science Park or incubator's establishment in most countries is to provide an infrastructure of technical, logistic and administrative support that a young firm needs in the process of struggling to achieve a profound stand in a competitive market [Chan et al., 2005]. They could also be seen as a part of resources transfer that enables the development of firms based on the innovations made at the university [Aaboen, 2009]. So, it is particularly important to those industrialized economies whereby small high tech firms are encouraged in their start up stage [Chan et al., 2005].

In addition to preparing all supports science and technology parks and incubators, it could be assuredly said that creating mechanisms for financial resourcing of innovative activities is the most important factor in fulfilling the designated technology development goals. There are different mechanisms for financial resourcing of innovative activities which are depending on the conditions and elements of the economical system of a country. But corporate venture capitals in the economical system have the most importance due to the latent riskiness of entrepreneurship. In addition, innovative activities, natural and long-term investment returns and financial-credit mechanisms are not suitable ways to finance them. In other words, traditional financing mechanisms which are based on short-term loans could not be considerable help to establish and develop the small and incipient firms. Therefore, functioning of such funds is the main prerequisite to supporting these incipient firms and improving entrepreneurship and innovation in the national innovation system [Mahbubi et al., 2003]. In the following, reserchers will present definition of CVCs and their relationship with science and technology parks and incubators, SWOT Model, explenation of TOPSIS technic and finally weaknesses, strenghths,

opportunities and threats confronting CVCs in science and technology parks and incubators and to present the strategies in four strategic groups of SO, ST, WO, and WT. All strategies are prioritized using TOPSIS technique.

2. CVCs and their relationship with science and technology parks and incubators

Since the early 1990s, US corporations have invested billions of dollars in young entrepreneurial companies (start-ups). By the late 1990s, corporate venture capital (CVC) accounted for nearly 15% of total venture investment in the US economy based on their investment activity. CVCs' parent corporations are often active players in new technologies and products markets in which start-ups are positioned; they appear to be natural candidates to engage in venture investment activity [Masulis et al., 2009].

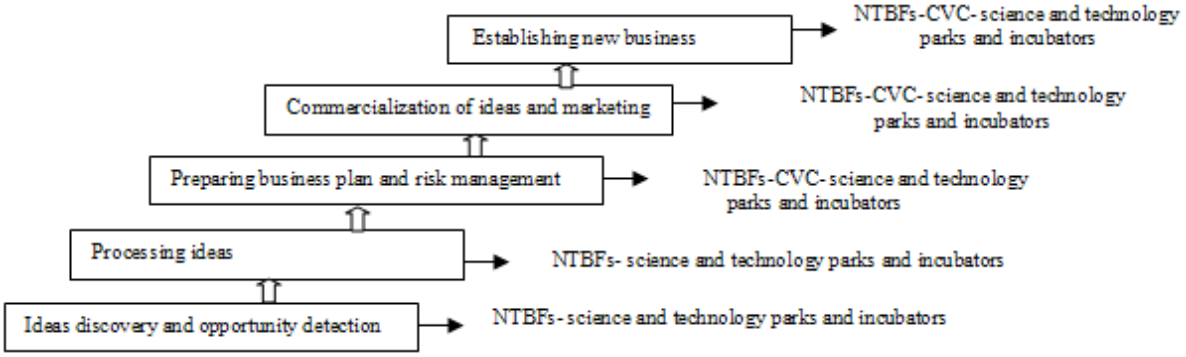
CVC is defined as any equity investment made by non-financial corporations in entrepreneurial companies for both financial and strategic objectives. That is, besides achieving financial objectives similar to independent VC investments, CV investments have a variety of strategic implications. Particularly, CVC programs have been used as effective routes to achieve these strategies [Yang et al., 2009].

CVCs invest in firms could bring high payback in a period of 5 or 7 years. Risking investors examine thousands of firms before any investment and choose a few firms which have good investment opportunities. These firms are financed by retirement funds, companies, wealthy people, and foreign investors or by themselves. A summary of the features of corporate venture capitals have been mentioned follow:

1- They finance in new, small and rapidly growing firms.
2- They share the ownership of these firms.
3- They help to develop new products and services.
4- Increase stock value with their active participation.
5- They risk higher, in order getting high profit.
6- They pursue long-term purposes [Mahbubi et al., 2004]

It might be questioned that what relation could exist between science and technology parks and incubators and CVCs? In other words, what vital role do science and technology parks and incubators play in the process of a venture capital? Chart 1 summarizes the role of incubators and science and technology parks in the process of a venture capital [Kanani et al., 2004].

Chart 1- relationship between CVC and science and technology parks and incubators



As it can be seen, in the first two stages, incubators and science and technology parks help new firms to identify and process their ideas. In the third and fourth stages, incubators and science and technology parks establish a relationship between CVCs and firms in order to design their business plan, manage threats and operationalize and market ideas. In the last stage, incubators and science and technology parks stay aside while firms establish new business with the aid of CVCs.

In fact, incubators and science and technology parks provide the necessary capital for the commercialization of ideas, plans and programs of the entrepreneurs and also provide suitable market for the owners of venture capitals in order to pick out the best investment opportunities by having a list of the best options of firms and innovative people. The above chart shows that in regard with the intricacies and ambiguities of investment in innovative activities, professional mediating institutions such as incubators and science and technology parks are a necessity in the interactive process with CVCs.

3. SWOT Model

SWOT analysis is one the most important instruments of strategic management for compromising between internal strengths and weaknesses and external opportunities and threats. SWOT analysis provides a systematic analytic

method for detecting these factors and choosing a strategy that makes the best compromise between them [Fisher, 1989]. Based on perspective of this model, an appropriate strategy should maximize strengths and opportunities and minimize weaknesses and threats. For this reason, internal strengths and weaknesses and external opportunities and threats are represented in four general states of WT, ST, WO and SO and then strategy options are created and chosen [Harrison et al., 1994]. In SO strategy, it is attempted to utilize environmental opportunities by relying on internal strengths. In WO strategy, the aim was to reduce internal weaknesses by utilizing existing opportunities. In ST strategies it is attempted reduce impacts of the external environment using the internal strengths. Finally, in WT strategy which is considered to be the worst state, the aim was reduce internal weaknesses and avoid external threats [David, 2000].

In Table 1, SWOT matrix is shown. This matrix consists of 9 cells. As it's mentioned, four cells contain the main factors; the cells represent strategies and one cell is blank or empty. In order to create a SWOT matrix the following 8 stages should be followed:

1- List the external opportunities
2- List the external treats
3- List the internal strengths
4- List the internal weaknesses
5- Match internal strengths with external opportunities and record the resultant SO Strategies
6-Match internal weaknesses with external opportunities and record the resultant WO Strategies
7- Match internal strengths with external threats and record the resultant ST Strategies
8- Match internal weaknesses with external threats and record the resultant WT Strategies [David, 2000].

Table 1 – SWOT matrix

	Strengths(S) List Strengths	Weaknesses (W) List Weaknesses
Opportunities (O) List Opportunities	SO Strategies Use strengths to take advantage of opportunities	WO Strategies Overcome weaknesses by taking advantage of opportunities
Treats (T) List Treats	ST Strategies Use strengths to avoid threats	WT Strategies Minimize weaknesses and avoid threats

4. Methodology

This is a practical study. Also, this study is empirical and descriptive regarding data collection. The methodology comprised of 3 stages. First, the researcher prepared a list of strengths, weaknesses, opportunities, and threats of Iranian science and technology parks and incubators for the presence of CVC. In the second stage, SO, WO, ST and WT strategies were proposed. These two stages were performed by a complete examination of literatures, studying books, articles, previous researches, and interviewing 30 experts. Finally in third stage, questionnaires were used to prioritize strategies using TOPSIS technique.

The questionnaire was spread among 20 experts. The idea of TOPSIS would be expressed in a series of steps.

1. Quantification and normalizing of decision matrix (N): for normalizing, norm non-scale is used.
2. Obtaining the balanced normalized matrix (V): the normalized matrix (N) is multiplied by the diagonal matrix of weights ($W_{n\times n}$), $V = N \times W_{n\times n}$
3. Determining positive and negative ideal solutions: positive and negative ideal solutions are defined, respectively:

(Matrix vector of the best values of each index V) = positive ideal solution (V_j^+)

(Matrix vector of the worst values of each index V) = negative ideal solution (V_j^-)

4. Evaluation the distance between each option and positive and negative ideals:

Euclidean distance of each option and the positive ideal (d_j^-) is calculated according to the following formulae:

$$d_i^+ = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^+)^2}, \qquad i = 1.2....., m$$

$$d_i^- = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^-)^2}, \qquad i = 1.2...., m$$

5. Determining the relative closeness (CL) of an option to the ideal solution:

$$CL_i^* = \frac{d_i^-}{d_i^- + d_i^+}$$

6. Ranking options: every option with a bigger CL is the better [Momeni, 2006].

5. Findings

5.1. Strengths, weaknesses, opportunities, and threats

The mean of strengths is merits and abilities of science and technology parks and incubators and

also the mean weaknesses is existence of restrictions and shortages in science and technology parks and incubators for the presence of CVCs. Strengths and weaknesses are true about those factors which are under the managing control of science and technology parks and incubators and they could plan an important role for their operation. Environmental opportunities and threats confronting the presence of CVC in science and technology parks and incubators are another part of SWOT model. The mean of opportunities is desirable and suitable environmental conditions for the presence of CVCs in science and technology parks and incubators. Finally, threats mean that those undesirable or unsuitable environmental conditions which affect science and technology parks and incubators adversely and impede their development. In table 5, strengths and weaknesses, and opportunities and threats from the viewpoint of commentators are presented.

Table 2. Strengths, weaknesses, opportunities and threats of science and technology parks and incubators

Threats	**Opportunities**
- Low investment security in the country and lack of tendency in CVCs to invest in Iran - Increased number of elite and student migrations and decreasing probability of the emergence of creative ideas - Non-institutionalization of entrepreneurial culture in society and universities - Shortage of financial encouragement and support from entrepreneurs and risk-taking investors - Lack of financial support of CVCs - Lack of profitable capital markets suitable for investment return and collecting the profit gained from investments - Scarcity of experienced and well-trained managers for running CVCs - Regulations controlling bank, insurance companies and retirement funds which undermine their role in capital venture - Structure of the Iranian government and a decrease of the investments made by private sector - Low activity of foreign risk-taking investors in Iran - Insufficient development of advanced technologies and industries	- Embargos and the possibility of producing the embargo products by domestic innovation - Government support of entrepreneurial activities and developing them - people's tendency to consume high-tech new goods, particularly among the youth - Possibility of higher exports in case new and internationally comparable products are produced - Government's approach to privatization and enforcement of its regulations - Higher population of the educated people and hence higher probability of proposing creative ideas - The possibility of high profitability from the existing creative ideas in science and technology parks and incubators - Government's attention to the important role of CVCs in the entrepreneurship development process.
Strengths	**Weaknesses**
- Employing a strong and knowledgeable personnel to offer legal, management, financial, marketing, sales, and technical advices - Founding most of the science and technology parks and incubators next to universities - Credit and budgets to support production of new products in science and technology parks and incubators - Presence of educated people in management and administrative section of science and technology parks and incubators - Arrangement for the accommodation of newfound firms in science and technology parks and incubators - Arranging for professional training programs for newfound firms.	- Lack of any well-designed plan for the presence of science and technology parks and incubators - The governmental structure of science and technology parks and incubators - Insufficient advertisement of the services science and technology parks and incubators provide in society - Little cooperation of science and technology parks and incubators with industry and private institutions - Lack of a good, comprehensive information system for proclamation of the latest inventions and achievements of the firms in science and technology parks and incubators - Dependence of science and technology parks and incubators income on government - Not linking science and technology parks and incubators with CVCs, industries, research resources, private institutions and other resources in a network - Science and technology parks and incubators not sharing in the benefits and losses of firms in them

5.2. Strategies

In this section, strategies and approaches for confronting threats, using opportunities, eliminating weaknesses and enhancing strengths for the presence of CVCs in science and technology parks and incubators are shown in table 3, in regard with internal weaknesses and strengths and environmental opportunities and threats. These strategies are also prioritized using TOPSIS technique. The results of TOPSIS technique and strategies are shown on table 3 In the order of their importance suggested by experts and professionals. It's noteworthy that implementing some of these strategies is possible only by assistance of administrative and governing departments.

Table 3. Presentation and prioritization of strategies using TOPSIS technique

Strategy Type	Strategy	d_i^+	d_i^-	CL_i
ST	Using the experiences of advanced countries in the field of CVCs	0.0359	0.0008	0.813
WO	Science and technology parks and incubators sharing benefits and losses of newly-developed firms	0.0340	0.010	0.776
WO	Developing activities related to establishing relationship between investors and holders of new ideas such as market technology	0.0339	0.010	0.775
ST	Establishing comprehensive copyright system in Iran	0.031	0.012	0.714
WO	Establishing common industry and university information to achieve new ideas and needs	0.029	0.013	0.691
ST	Using different advertising tools for introducing capacities and advantages of the country to foreign and domestic investors	0.031	0.016	0.656
ST	Granting financial exemption and other arrangements to CVCs	0.027	0.016	0.620
SO	Establishing strategic committee for the presence of CVCs with the participation of the directors from all science and technology parks and incubators of Iran	0.031	0.020	0.606
SO	Granting special funds to support practical researches and organizing training courses for the academics	0.025	0.017	0.596
ST	Creating free and open economic atmosphere for more extensive presence of CVCs in economy of Iran	0.025	0.018	0.0590
ST	Training entrepreneurship and CVC management in management and engineering faculties	0.026	0.019	0.578
WO	Designing strategic plans for the presence of CVCs in parks and incubators	0.028	0.022	0.513
ST	Modifying the ownership and management structure of banks and retirement funds and using modern financial instruments to provide bank services	0.025	0.021	0.543
ST	Using appropriate financial initiatives such as granting loans and assistance to improve entrepreneurship and investment	0.023	0.021	0.521
SO	Introducing investable university proposals to industry (Industries and mines chamber, chamber of commerce, etc.) and vice versa.	0.023	0.022	0.513
WT	Accelerating privatization and pruning government and state-run firms	0.023	0.024	0.498
WT	Establishing relationship with accredited entrepreneurship and business management faculties of successful countries to transfer their knowledge end experiences	0.022	0.023	0.492
SO	Studying society and industry and assessing their needs and directing individuals to produce new products with respect to the needs of the society and industry	0.022	0.025	0.468
WO	Improving and increasing advertisement about supports science and technology parks and incubators offer	0.022	0.25	0.467
SO	Studying international markets and evaluating their feasibility in order to develop exports and encouraging people to produce products which are able to compete in the international market	0.021	0.026	0.456
ST	Using various financial tools in capital market	0.020	0.024	0.453
ST	Joining international copyright conventions and treaties	0.019	0.024	0.447
WT	Improving the quality of education in universities and providing people and on-site groups in science and technology parks and incubators with welfare and laboratorial facilities	0.021	0.027	0.442
ST	Facilitating admission of newfound firms in the stock market in order to cash the investment made in newfound firms	0.019	0.029	0.401
SO	Increasing the number of entrepreneurship training programs and producing creative ideas among academics and the public	0.017	0.026	0.392
ST	Supporting the development of modern industries and technologies, in particular informational, computer and internet technologies	0.017	0.031	0.360

As it's mentioned in table 6, the strategy of using the experience of pioneer countries in the field of CVCs, science and technology parks and incubators would share benefits and disadvantages of new firms, developing activities related to establishing relationship between investors and holders of new ideas such as market technology, establishing comprehensive copyright system in Iran and creating information bank for industries and universities in order to achieve new ideas and needs which proposed by the experts as the most important strategies.

6. Conclusion

Development of entrepreneurship and emergence of entrepreneurs in a society requires certain conditions and necessities such as establishment of underlying structures and institutions. Science and Technology parks and incubators are considered as supporting and underlying institutions for the development of entrepreneurs. Dynamic and developed firms received various supports including financial support from technology and science and technology parks and incubators. Currently the most important financial support resources of parks and incubators are funded by the government. Persistence of this trend and absolute dependence on government funds in the long run is neither appropriate nor feasible.

But CVCs could gain their targeted payments to increase the value of firm. This goal is much different from other financial support tools such as loans in which only profit is received. Therefore, CVCs have a purpose similar to certain owners of a firm. Therefore, measures must be taken to have corporate venture capital in parks and incubators.

In the present study, researchers examined the internal (strengths and weakness) and environmental (opportunities and threats) conditions and proposed strategies for the presence of CVCs in science and technology parks and incubators of Iran by reviewing the theoretical basis, interviewing professional and employing one of the most powerful strategic management instruments, i.e. the SWOT model. Findings of these analyses are presented in tables 1 to 3. In sum, 6 strengths, 8 weaknesses, 11 threats, 8 opportunities, 6 SO strategies, 12 ST strategies, 6 WO strategies and 3 WT strategies were detected in this study. Finally, strategies were prioritized using TOPSIS technique and it was concluded that using the experience of pioneer countries in the field of CVCs, science and technology parks and incubators sharing in the benefits and losses of newfound firms, developing activities related to establishing relationship between investors and holders of new ideas such as market technology, establishing comprehensive copyright system in Iran and creating information bank common to industry and university in order to achieve new ideas and needs, using various advertising tools in order to introduce capacities and advantages of the country to foreign and domestic investors, granting special financial exemptions and assistance to the CVCs and establishing strategic committee for the presence of CVCs by participation of directors from all science and technology parks and incubators of Iran are considered as the most important strategies.

References

[1] Keshavarz, E., Montazeri , M ., "Venture capital and it's relation with incubators", *the frist national conference of venture capital industry* , 2004

[2] Aaboen, L., " Explaining incubators using firm analogy ", *Journal of Technovation*, Vol. 29, pp. 657-670, 2009.

[3] Chan. K.F., Lau, T., " Assessing technology incubator programs in the science park: the good, the bad and the ugly", *Journal of Technovation* , Vol. 25, pp1215-1228 , 2005.

[4] Mahbubi, J., Bagheri, K., "*Venture capital* ", tomorrow expansion entity , 2003.

[5] Masulis,R, W., Nahata, R., " Financial contracting with strategic investors: Evidence from corporate venture capital backed IPOs ", *journal of Financial Intermediation*, Vol. 18, pp. 599-631, 2009.

[6] Yang, Y., Narayanan, V.K., Zahra, S., " Developing the selection and valuation capabilities through learning: The case of corporate venture capital", *Journal of Business Venturing* , Vol 24, pp. 261-273, 2009.

[7] Mahbubi, J., Bagheri, K.," Examination of venture capital industry substructures in Iran", *the frist national conference of venture capital industry* , 2004

[8] Kanani, M., Musavi, H., Asad zamaneh, K., " Analysis role of science and technology parks and incubators for providing venture capital for SMEs ", *the frist national conference of venture capital industry* , 2004

[9] Fisher,C *., "Current and Recurrent Challenges in HRM, Journal of Management",* Vol 15. no. 2, pp 157-180, 1989.

[10] Harrison, J., Caron,K., *"Strategic planning; Industrial management; Case studies ",* West Pub. Co., 1994

[11] David, F.R., "Strategic management" prentice hall, 2000

[12] Momeni, M*., "New topics in operation research",* university of tehran., 2006

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