İstanbul Üniversitesi
Veteriner Fakültesi Dergisi

Journal of the Faculty of
Veterinary Medicine Istanbul University

*İstanbul Üniv. Vet. Fak. Derg. / J. Fac. Vet. Med. Istanbul Univ., 41 (1), 84-91, 2015*
*doi: 10.16988/iuvfd.2015.90715*

***Araştırma Makalesi*** ***Research Article***

# Protective Effects of Vitamin C (Ascorbic Acid) in Lead Acetate Exposed Diabetic Male Rats: Evaluation of Blood Biochemical Parameters and Testicular Histopathology

*Alireza AYOUBI[1], Reza VAIZADEH[1], Arash OMIDI[2]*, Mohsen ABOLFAZLI[1]*

*[1]Department of Animal Sciences, Agriculture Faculty, Ferdowsi University of Mashhad, Mashhad, Iran*
*[2]Department of Animal Health Management, School of Veterinary Medicine, Shiraz University, Shiraz, Iran*

***Sorumlu Yazar / Corresponding Author:***
*Arash OMIDI*
*e-mail: aomidi@shirazu.ac.ir*

***Geliş Tarihi / Received:***
11 June, 2014

***Kabul Tarihi / Accepted:***
17 September, 2014

***Anahtar Kelimeler:***
Antioksidan, diyabet, kurşun, toksisite, vitamin C, Wistar sıçanlar

***Key Words:***
Antioxidant, diabetes, lead, toxicity, vitamin c, Wistar rats

**Abstract**

The aim of this study was to investigate the protective effects of vitamin C against lead toxicity by measuring the blood parameters and studying histopathology of testis in diabetic male rats. Wister rats (42) were randomly assigned into7 groups: I) healthy; II) fed lead acetate only; III) vitamin C administered only; IV) diabetic; V) diabetic rats administered by vitamin C; VI) diabetic rats given lead acetate and VII) diabetic rats received lead acetate and vitamin C. The diabetic and lead groups had higher glucose, cholesterol, LDL, triglycerides and lower insulin and HDL concentration than the control group. It was found that vitamin C administration led to a lower level of blood glucose, cholesterol, LDL and triglycerides and higher HDL concentration in diabetic rats significantly. It was concluded that the antioxidant property of vitamin C resulted in reducing the oxidative stress complications of toxic levels of lead acetate in diabetic rats.

**Özet**

**Kurşun Asetata Maruz Kalan Diyabetik Erkek Sıçanlarda Vitamin C (Askorbik Asit)'nin Koruyucu Etkisi: Kan Biyokimyasal Parametreleri ve Testis Histopatolojisinin Değerlendirilmesi**

Bu çalışmanın amacı diyabetik erkek sıçanlarda kan parametrelerinin ölçülmesi ve testisin histopatolojisinin çalışılması ile vitamin C'nin kurşun toksisitesine karşı koruyucu etkisini incelemektir. Wistar sıçanlar (42) rastgele 7 gruba ayrılmıştır I) sağlıklı; II) sadece kurşun asetat ile beslenenler; III) sadece vitamin C uygulananlar; IV) diyabetik; V) vitamin C uygulanan diyabetik sıçanlar; VI) kurşun asetat verilen diyabetik sıçanlar ve VII) kurşun asetat ve vitamin C uygulanan diyabetik sıçanlar. Diyabetik ve kurşun verilen grupların kontrol gruplara kıyasla daha yüksek glukoz, kolesterol, LDL, trigliseritler ve daha düşük insülin ve HDL konsantrasyonuna sahip oldukları tespit edilmiştir. Vitamin C uygulaması kanda glukoz, kolesterol, LDL ve trigliserit düzeylerini düşürdüğü HDL konsantrasyonunu ise arttırdığı saptanmıştır. Sonuç olarak diyabetik farelerde vitamin C'nin antioksidan özelliği, kurşun asetatın toksik seviyelerinde oksidatif stres komplikasyonlarını azaltmasına neden olmuştur.

## Introduction

Diabetes mellitus as a metabolic disorder is classified into two major subtypes; type I or insulin dependent diabetes (IDDM), type II or non-insulin dependent diabetes (NIDDM) and malnutrition-related form. IDDM results from a cellular mediated autoimmune destruction of β-cells of pancreas (Aikinson and Maclaren, 1994; Takeshi et al., 2002). NIDDM or adult onset diabetes results from development of insulin resistance and deficiency (De Fronzo et al., 1997). Hyperglycemia increase oxidative stress which contributes to development and progression of diabetic complications (Ahmed, 2005). Oxidative stress refers to an imbalance between the free radicals production and antioxidant defenses that led to tissue damage. Evidences indicate that glycemic

status is associated with oxidative stress even in subjects with well-controlled type 2 diabetes (Rytter et al., 2009). Lead is known to induce a broad range of physiological, biochemical, and behavioral dysfunctions on nervous systems, haemopoietic system, cardiovascular system, kidneys, liver and reproductive systems in animals as well as humans (Goyer, 1996; Ruff et al., 1996; Ping-Chi and Yueliang, 2002). Lead is a ubiquitous environmental and industrial pollutant. Lead absorbed through the digestive and respiratory system, and skin. Some of the important sources of environmental lead exposure are petroleum products, leaded paints and drinking water. High lead levels in the environment and drinking water results to chronic lead toxicity. Antioxidants protect the cells from oxidative stress by using both enzymatic and non-enzymatic strategies. Carotenoids, vitamin E and vitamin C may protect against free radicals and lipid peroxidation thereby reducing macro-vascular complications of diabetes (Maha et al., 2012). Vitamin C is considered as the most potent anti-oxidants present in the body. Studies have shown that vitamin C apply its protective effects against stroke, hypertension, coronary heart disease and peripheral vascular diseases (Yokoyama et al., 2000). Studies have shown the inverse association between vitamin C and diabetes. It has been demonstrated that Vitamin C decreases the oxidative stress mainly at least 2 types of diabetes (Chan et al., 1994; Urakawa et al., 2003). In addition, the uricosuric effect of Vitamin C likely plays a role in its disease-preventative properties (Choi et al., 2005). The aim of this study was to investigate the protective effect of vitamin C against lead toxicity by evaluating the blood parameters and testicular histopathology characteristics in diabetic male Wistar rat.

## Materials and Methods

### Animals

Forty-two male Wistar rats with mean body weight of 182.6±2 g were individually housed in polypropylene cages in the standard rat house (22-25°C on a 12 hrs light-dark cycle) and fed a pellet diet (Javaneh, Khorasan Co, Mashhad, Iran) with free access to tap water.

### Induction of Experimental Diabetes

Streptozotocin (STZ) (Sigma, U.S.A; 55 mg/kg body weight) was dissolved in 0.1 M sodium citrate buffer at pH 4.5 just before use (Srinivasan, 1972), and injected intra peritoneally (IP). Three days after STZ administration, the diabetic rats with blood glucose concentration more than 300 mg/dl were selected and divided into 4 groups. Protocols were approved by the local experimental ethics committee.

### Treatment Schedule

Two weeks after STZ injection, healthy and diabetic rats were randomly selected and divided into seven experimental groups of 6 rats each. Experimental groups include:

1- Group I: Negative control (received distilled water).
2- Group II: Lead acetate (100 ppm lead dissolved indistilled water).
3- Group III: Vitamin C (100 mg/kg body weight Vitamin C with IP injection).
4- Group IV: Diabetic or positive control (55ppm streptozotocin injection).
5- Group V: Diabetic + vitamin C (Dia. + vit. C) (55 ppm streptozotocin, 100 mg/kg vitamin C IP injection).
6- Group VI: Diabetic + lead acetate (Dia. + L) (55 ppm streptozotocin injection, 100 ppm lead dissolved in distilled water).
7- Group VII: Diabetic + lead acetate + vitamin C (Dia. + L + vit. C) (55 ppm of streptozotocin, 100 ppmlead acetate dissolved in distilled water, 100 mg/kg Vitamin C IP injection).

The experiment period was 4 weeks and the control, diabetic control, lead acetate and diabetic+ lead acetate treatments received an equivalent volume (0.5 ml) of citrate buffer with IP injection per day.

### Biochemical and Histopathological Analysis

At the end of the experiment (day 29) the rats were anesthetized with Ketamine (50 mg/kg) after withholding food for 12 h. The blood samples were taken from the heart apex to assess lipids, enzymes and hormone concentrations. The right testis was removed after collecting the blood, washed in saline, and fixed in 10% formalin at room temperature for 72 h. After fixing the tissue, it was thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared, and embedded in soft paraffin. Tissue sections of about 5µm were obtained, stained with hematoxylin and eosin, and examined under light microscope. Twenty seminiferous tubular sections were selected from stained testes of rats and then some parameters such as seminiferous tubule diameter, lumen, cell thickness and number of Leydig cells and Sertoli cells were measured and recorded.

**Insulin and Testosterone Assay**

The insulin and testosterone level of each blood sample was measured by an enzyme-linked immune sorbent assay using a commercial kit (rat insulin ELISA and rat testosterone ELISA; DRG Instruments GmbH, Germany), according to manufacturer's instructions.

**Statistical analysis**

The significance of differences between the treatments was established by the GLM procedure of SAS statistical software. Significance set at P<0.05.

**Results**

The effect of lead and vitamin C on body weight, blood parameters, spermatogenesis and testosterone levels in the experimental rats are reported in Tables 1 to 3. The results showed that feed intake and body weight of diabetic rats decreased significantly (P<0.05) compared to the control group.

**Table 1.** Effects of lead and vitamin C on average daily feed intake and body weight of Wistar male rats in a 4 weeks feeding trial.

**Tablo 1.** Wistar ırkı erkek sıçanlarda 4 haftalık besleme denemesinde kurşun ve vitamin C'nin ortalama günlük yem tüketimi ve canlı ağırlıkları üzerine etkileri.

| Treatment[1] | Feed Intake (g) | Body Weight (g) |
|---|---|---|
| **Healthy control** | 92.32 ± 3.2$^{a}$ | 219.3 ± 5.6$^{a}$ |
| **Lead acetate** | 84.47 ± 5.1$^{ab}$ | 195.6 ±4.9$^{b}$ |
| **Vitamin C** | 88.01 ±2.8$^{a}$ | 213.4 ± 5.7$^{a}$ |
| **Diabetic control** | 68.18 ± 3.7$^{b}$ | 143.4 ± 3.8$^{c}$ |
| **Diabetic + Vit. C** | 74.47 ± 4.2$^{b}$ | 158.3 ± 5.4$^{c}$ |
| **Diabetic + Lead** | 58.01 ± 3.6$^{c}$ | 148.6 ±4.9$^{c}$ |
| **Dia.+ L + Vit. C** | 62.13 ± 5.2$^{c}$ | 156.4 ± 3.6$^{c}$ |

[1]Values are given as mean ± S.D for 6 rats in each group.

$^{a, b, c:}$ Values not sharing a common superscript letter differ significantly at P<0.05

Dia.+L+Vit. C: diabetic rats treated with lead and supplemented with vitamin C.

**Table 2.** Effects of vitamin C and lead acetate on glucose, insulin, ALT and AST concentrations in serum of diabetic male rats.

**Tablo 2.** Diyabetik erkek sıçanların serum glikoz, insülin, ALT ve AST konsantrasyonlarına vitamin C ve kurşun asetatın etkileri.

| Treatment[1] | Glucose (mg/dl) | Insulin (ng/ml) | ALT (U/ml) | AST (U/ml) |
|---|---|---|---|---|
| **Healthy control** | 98.6 ± 25.6$^{c}$ | 0.63 ± 0.16$^{a}$ | 74.3 ± 11.4$^{b}$ | 243.1 ± 24.3$^{c}$ |
| **Lead acetate** | 142.8 ± 27.1$^{c}$ | 0.30 ± 0.07$^{bc}$ | 86.0 ± 10.5$^{b}$ | 284.2 ± 23.6$^{b}$ |
| **Vitamin C** | 106.5 ± 30.5$^{c}$ | 0.58 ± 0.17$^{a}$ | 63.6 ± 12.5$^{c}$ | 235.5 ± 32.9$^{c}$ |
| **Diabetic control** | 427.8 ± 26.6$^{a}$ | 0.18 ± 0.08$^{c}$ | 135.3 ± 12.3$^{a}$ | 272.4 ± 41.1$^{b}$ |
| **Diabetic + Vit.C** | 315.4 ± 32.7$^{b}$ | 0.21 ± 0.16$^{c}$ | 88.6 ± 10.4$^{b}$ | 253.6 ± 33.7$^{c}$ |
| **Diabetic + Lead** | 484.6 ± 27.5$^{a}$ | 0.17 ± 0.17$^{c}$ | 142.8 ± 11.6$^{a}$ | 323.5 ± 25.4$^{a}$ |
| **Dia.+ L + Vit.C** | 433.6 ± 27.4$^{a}$ | 0.27 ± 0.07$^{bc}$ | 136.8 ± 10.4$^{a}$ | 281.7 ± 23.9$^{b}$ |

[1]Values are given as mean ± S.D for 6 rats in each group.

$^{a, b, c:}$ Values not sharing a common superscript letter differ significantly at P<0.05

Dia. + L + Vit.C: diabetic rats treated with lead and supplemented with vitamin C.AST, Aspartate Amino Transferase; ALT, Alanine Amino Transferase.

**Table 3.** Effects of vitamin C and lead acetate on the lipid profile of the experimental Wistar rats (mg/dl).
**Tablo 3.** Vitamin C ve kurşun asetatın Wistar ırkı sıçanlardaki lipid durumuna etkileri (mg/dl).

| Treatment[1] | Cholesterol | LDL | HDL | Triglycerides |
|---|---|---|---|---|
| **Healthy control** | 68.8 ± 4.31$^{b}$ | 41.2 ± 2.2$^{b}$ | 33.4 ± 2.5$^{a}$ | 47.5 ± 17.4$^{c}$ |
| **Lead acetate** | 58.1 ± 5.33$^{c}$ | 60.5 ± 3.4$^{a}$ | 29.3 ± 2.7$^{ab}$ | 53.8 ± 23.1$^{b}$ |
| **Vitamin C** | 51.0 ± 5.24$^{c}$ | 36.3 ± 3.0$^{c}$ | 34.7 ± 4.0$^{a}$ | 45.3 ± 16.8$^{c}$ |
| **Diabetic control** | 75.8 ± 3.71$^{a}$ | 44.6 ± 2.4$^{b}$ | 28.4 ± 2.4$^{b}$ | 63.6 ± 27.2$^{b}$ |
| **Diabetic + Vit. C** | 46.2 ± 5.45$^{c}$ | 39.7 ± 2.0$^{c}$ | 33.6± 3.1$^{a}$ | 45.0 ± 18.4$^{c}$ |
| **Diabetic + Lead** | 76.8 ± 4.32$^{a}$ | 66.7± 4.2$^{a}$ | 22.3± 2.6$^{c}$ | 72.8 ± 23.6$^{a}$ |
| **Dia.+ L+ Vit.C** | 74.2 ± 4.61$^{a}$ | 62.3 ± 3.6$^{a}$ | 27.4 ± 3.4$^{b}$ | 66.2 ± 17.5$^{a}$ |

[1]Values are given as mean ± S.D for 6 rats in each group.
[a, b, c:] Values not sharing a common superscript letter differ significantly at P<0.05.
Dia.+ L + Vit.C: diabetic rats treated with lead and supplemented with vitamin C.

**Table 4.** Effects of vitamin C and lead acetate on spermatogenesis and testosterone level in male diabetic rats.
**Tablo 4.** Vitamin C ve kurşun asetatın diyabetik erkek sıçanlarda spermatogenezis ve testosteron seviyesine etkileri.

| Treatment[1] | Cell wall thickness (μ) | Sertoli cells (number) | Spermatocyte cells (number) | Testosterone (ng/l) |
|---|---|---|---|---|
| **Healthy control** | 269.8 ± 6.4$^{a}$ | 65 ± 0. 8$^{a}$ | 75.3 ± 2.3$^{a}$ | 7.3 ± 0.5$^{a}$ |
| **Lead acetate** | 243.7 ± 6.5$^{ab}$ | 47 ± 0.7$^{b}$ | 63.4 ± 1.4$^{ab}$ | 4.9± 0.6$^{b}$ |
| **Vitamin C** | 265.4 ± 4.8$^{a}$ | 67± 1. 3$^{a}$ | 73.4 ± 1.7$^{a}$ | 6.9± 0.7$^{ab}$ |
| **Diabetic control** | 211.8 ± 5.7$^{b}$ | 48 ± 0.5$^{b}$ | 58.6 ± 2.3$^{b}$ | 3.8 ± 0.5$^{c}$ |
| **Diabetic + Vit. C** | 248.3 ± 4.5$^{ab}$ | 38 ± 0.7$^{c}$ | 62.4 ± 3.6$^{ab}$ | 4.6 ± 0.6$^{b}$ |
| **Diabetic + Lead** | 196.9 ± 3.8$^{c}$ | 27 ± 1.2$^{c}$ | 43.8 ± 1.8$^{c}$ | 3.2 ± 0.5$^{c}$ |
| **Dia.+ L+ Vit.C** | 223.4 ± 6.3$^{b}$ | 33 ± 0.6$^{c}$ | 47.5 ± 2.5$^{c}$ | 3.6 ± 0.7$^{c}$ |

[1]Values are given as mean ± S.D for 6 rats in each group.
[a, b, c:] Values not sharing a common superscript letter differ significantly at P<0.05.

**Blood parameters**

Effects of lead acetate exposure and vitamin C on some blood parameters are presented in Tables 2 and 3. Lead acetate injection to the diabetic rats increased the blood glucose concentration sharply (P<0.05). Insulin level in these groups of rats reduced significantly (P<0.05) (Table 2). A significant differences were seen in aspartate amino transferase (AST) and alanine amino transferase (ALT) levels (P<0.05) in the diabetic and normal rats (Table 2).Administration of Vitamin C also decreased AST and ALT activities significantly (P<0.05) in the diabetic rats. Cholesterol, LDL and triglyceride concentrations increased in the diabetic rats in comparison with the healthy control rats. However, the cholesterol, LDL and triglyceride levels in the treated rats with vitamin C decreased compared to diabetes + lead group. The same trend was observed in blood glucose levels.

**Testicular indices**

The effects of vitamin C and lead acetate on spermatic cells and testosterone levels in diabetic male rats are given in Table 4. The results showed that the cell wall thickness in diabetic rats decreased significantly (P<0.05) compared to healthy control rats. This reduction was exacerbated in diabetic rats exposed to lead acetate (P<0.05). The number of sertoli cells and spermatocytes in diabetic + lead acetate rats showed a reduction trend. The production of testosterone was decreased in diabetes +lead acetate rats compared with the control group (P<0.05).

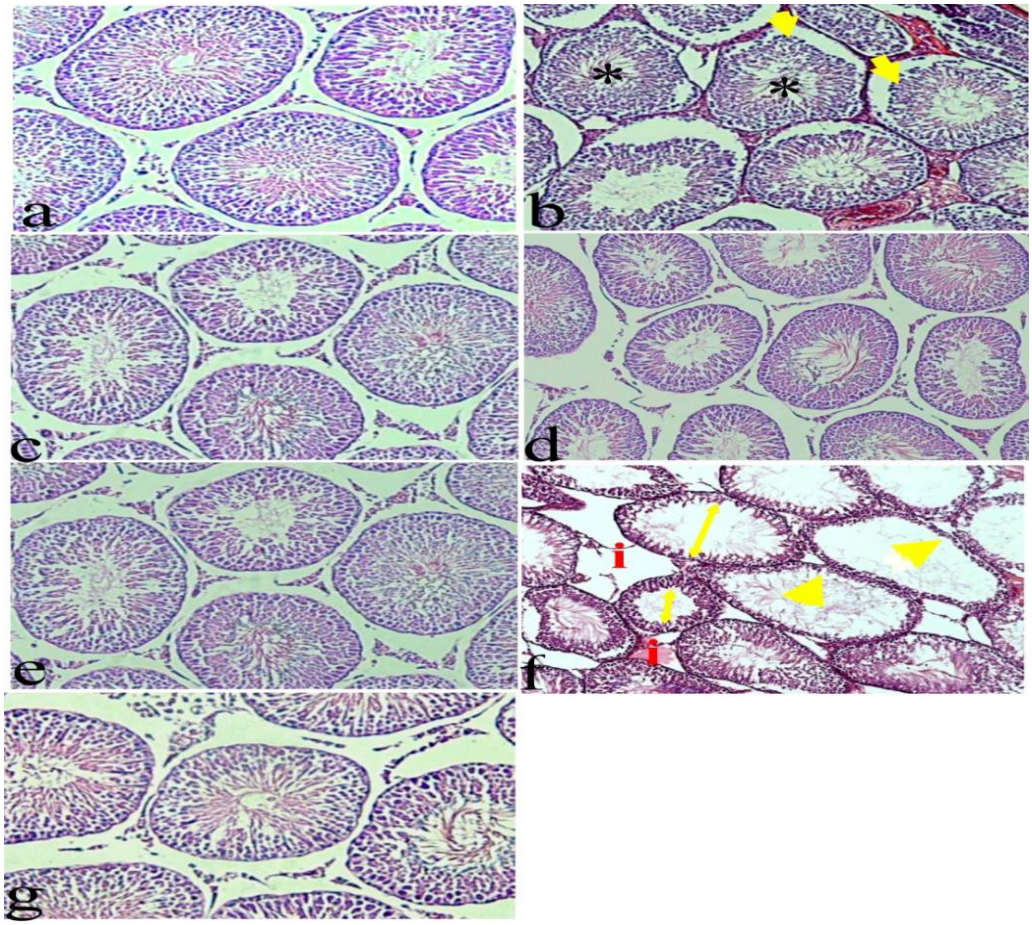


**Figure 1.** H&E staining of *transverse section* of rat *testis* of control and treatment groups; (×100); (a): Normal histological structure of the seminiferous in the control group, (Group I); (b):Presence of other spermatogenic cells in the central lumen (star), decay cell layer, disorganized basement membrane and degradation in germinal cells (arrows) in lead acetate treated rats, (Group II);(c): Regular seminiferous tubules with normal germinal epithelium and existence of sperm in lumen of vitamin C treated rats, (Group III); (d):Existence of sperm in lumen, and the intact basement membrane in diabetic rats received distilled water (Group IV); (e): Well-organized seminiferous tubules, and thickened basement membrane in diabetic + vitamin C, (Group V); (f):Shrunken and narrow thickened basement membrane, sharp decrease in seminiferous cell wall thickness (triangles), increased the diameter of the lumen (arrow), interstitial tissue destruction (i) and reducing the number of layers of spermatogenesis in diabetic + lead acetate, (Group VI); (g): Shrunken but organized seminiferous than diabetic and lead treatment in diabetic + lead acetate + vitamin C rats, (Group VII).

**Şekil 1.** Kontrol ve deney gruplarındaki sıçanlarda testislerin enine kesitinin H & E boyaması ; (×100); (a):Kontrol grubunda seminiferusun normal histolojik yapısı, (Grup I); (b): Kurşun asetat uygulanan sıçanlarda sentral lümendeki diğer spermatogenik hücrelerin varlığı (yıldız), bozulan hücre tabakası, dağınık bazal membran ve germinal hücrelerde bozulma (oklar); (c): Vitamin C uygulanan sıçanlarda normal germinal epitelyumlu ve lümende sperma bulunan düzenli seminiferus tübülleri (Grup III); (d): Distile su uygulanan diyabetik farelerde lümende sperm varlığı ve bozulmamış bazal membran (Grup IV); (e): Vitamin C uygulanan diabetik farelerde iyi organize seminiferus tübülleri ve kalınlaşmış bazal membran (Grup V); (f): Büzüşmüş ve daralıp kalınlaşmış bazal membran, seminifer hücre duvar kalınlığında keskin azalma (üçgenler), lumenin çapında artış (ok), interstisyel doku yıkımı; (i): ve kurşun asetat uygulanan diyabetik farelerde spermatogenezisin katman sayısının azalması (Grup VI); (g): Kurşun asetat ve vitamin C uygulanan diyabetik farelerde diyabetik ve kurşun asetat uygulananlara göre büzüşmüş fakat organize seminiferus (Grup VII).

**Discussion**

Both feed intake and body weight decreased in the diabetic rats in comparison with the control group, but vitamin C supplementation towered these losses significantly probably due to basal lost of energy and protein. According to Owu et al. (2006) higher basal metabolic rate in diabetic rats led to increase in lipid and protein degradation. Generally, vitamin C administration had an ameliorating effect on harmful effects of diabetes and lead poisoning on body weight. Gamal et al. (2012) reported that vitamin C supplementation decreased the elevated levels of blood glucose, cholesterol, triglycerides, and LDL in diabetic rats significantly. A noticeable improvement in glucose and lipid metabolism and an increasing level of HDL-cholesterol have been observed in patients treated with vitamin C (Shargorodsky et al., 2010). Hyperglycemia increases oxidative stress through the overproduction of ROS, which is related to body system injuries (Upasani et al., 2001). It has been accepted that oxidative damage is generally worsen in diabetic patients (Bechara et al., 1993). The increase in oxygen free radicals in diabetes could be due to raise in blood glucose levels, which upon auto oxidation generate free radicals. It has been shown that free radical production resulted in loss of weight, and higher cases of polydipsia, polyuria, glucosuria, polyphagia, hypoinsulinemia, and hyperglycemia (Hakim et al., 1997). On the other hand, lead can apply its toxic effects on various parts of the body, including liver, kidney, brain and haemopoietic systems (Hilderbrand et al., 1973). It has been concluded that the main mechanism of lead toxicity is in the form of oxidative stress damage (Dorman et al., 2004). The generation of reactive oxygen species (ROS), causes lipid peroxidation (LPO) (Masuda et al., 2002). Various studies have established the ability of vitamin C to scavenging the hydroxyl and superoxide radicals and singlet oxygen (Adeniyi et al., 2008; Beckman et al., 2001; Ting et al., 1996). The antioxidant activity of vitamin C is considered to be the major defense mechanism, in the aqueous phase, against the harmful effects of free radicals (Gaur and Dixit, 2012). Moreover, previous studies suggest that the vitamin C levels in the plasma and tissues of diabetic patients and animals are lower than in those of normal groups (Goldenberg, 2003; Will and Byers, 1996). The liver is plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes (Subbiahet al., 2006). Furthermore, the increase in ALT activity in diabetes is usually due to hepatocellular damage and is usually accompanied by an increase in AST activity (Sekar et al., 1990). The AST and ALT activity are indicators of liver damages (Hearse, 1979). The administration of lead acetate in this study resulted in a significant ($P<0.05$) increase in plasma level for ALT and AST activities. The reduction of AST and ALT by vitamin C is in agreement with Rekka et al. (1992), who found that serum transaminases returned to normal activities with the healing of tissue parenchyma and regeneration of hepatocytes and renal tissues. Vitamin C induced suppression of increased ALT and AST activities. Thus, administration of this vitamin C revealed protective activity against the toxic metabolites of diabetes (Hussein et al., 2012). Administration of vitamin C revealed protective activity against the toxic metabolites of diabetes. Lipid peroxides are generally higher in diabetic patients compared to the healthy controls (Mahboob et al., 2005). Increased lipoprotein peroxidation in diabetes is partly linked to the low antioxidant status of blood and tissues associated with increased oxidative stress (Dierckx et al., 2003). Impaired insulin action not only stimulates lipolysis, but also increasing delivery of FFA to the liver and consequently increasing production of hepatic triglyceride (Owu et al., 2006). The preventive activity of vitamin C may relate to its antioxidant efficacy that inhibits lipid peroxidation enhanced by lead acetate (Upasani et al., 2001). Histopathologic studies revealed testicular damages that were ameliorated by administration of vitamin C. Diabetic and lead exposure rats induced significant decreasement in the cell thickness and number of spermatocytes and sertoli cells compared to control. Testosterone levels were reduced in lead acetate and diabetic rate compared to the control, but vitamin C treatment was increased concentration of testosterone. Lead poisoning induced damages to the germinal epithelium has been associated with sterility (Rytter et al., 2009). Based on histological findings, the activity of spermatogenic tissue was affected by diabetes treatment. Seminiferous cell wall thickness was reduced by diabetes; however this decrease was less in rats treated with vitamin C. Decreasing seminiferous cell wall thickness is probably due to the effects of lead oxidation through increasing free radical production in the testes. Kumar et al., showed that the number of seminiferous cell layers and spermatids significantly decreased by lead levels (Kumar et al., 2013). Testosterone levels in diabetic and diabetic + lead treatments decreased; however in vitamin C treatment the decline was lesser. Antioxidant activity of vitamin C and binding with lead acetate decrease

free radicals and reduce damage to the testes (Ting et al., 1996). It can be concluded that vitamin C supplementation protect rats from some harmful effects of lead toxicity in form of lead acetate or other lead containing chemicals although more study are needed.

**Acknowledgements**

The authors are grateful to the Ferdowsi University of Mashhad (Grant number: 21184) for supporting this research.

**REFERENCES**

**Adeniyi, T.T., Ajayi, G.O., Akinloye, O.A., 2008.** Effect of ascorbic acid and allium sativum on tissue lead level in female Rattus navigicus. Nigerian Journal of Health and Biomedical Sciences 7, 38-41.

**Ahmed, N., 2005.** Advanced glycation end products-role in pathology of diabetic complications. Diabetes Research and Clinical Practice 67, 3-21.

**Aikinson, M.A., Maclaren, N.K., 1994.** The pathogenesis of insulin dependent diabetes. New England Journal of Medicine 331, 1428-1436.

**Bechara, E.J.H., Medeiros, M.H.G., Monteiro, H.P., Hermes-Lima, M., Pereira, B., Demasi, M., 1993.** A free radical hypothesis of lead poisoning and inborn porphyri asassociated with 5-aminolevulinic acid overload. Quimica Nova 16, 385-392.

**Beckman, J.A., Goldfine, A.B., Gordon, M.B., Creager, M.A., 2001.** Ascorbate restores endothelium-dependent vasodilation impaired by acute hyperglycemia in humans. Circulation 103, 1618-1623.

**Chan, J.M., Rimm, E.B., Colditz, G.A., Stampfer, M.J., Willett, W.C., 1994.** Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. Diabetes Care 17, 961-969.

**Choi, S.W., Benzie, I.F., Lam, C.S., Chat, S.W., Lam, J., Yiu, C. H., Kwan, J.J., 2005.** Inter-relationships between DNA damage, ascorbic acid and glycaemic control in Type 2 diabetes mellitus. Diabetic Medicine 22, 1347-1353.

**De Fronzo, R.A., Bonadonna, R.C., Ferrannini, E., 1997.** Pathogenesis of NIDDM, International Text book of Diabetes mellitus, 2nd ed. Chichester, John Wiley, England, pp. 635-712.

**Dierckx, N., Horvath, G., van Gils, C., Vertommen, J., van de Vliet, J., De Leeuw, I., Manuel-y-Keenoy, B., 2003.** Oxidative stress status in patients with diabetes mellitus: relationship to diet. European Journal of Clinical Nutrition 57, 999-1008.

**Dorman, H.J.D., Bachmayer, O., Kosar, M., Hiltunen, R., 2004.** Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. Journal of Agricultural and Food Chemistry 52, 762-770.

**Gamal, B., Samir, B., Hossam, E., Mohamed, M., 2012.** Douaa S. Vitamin C supplementation reconstitutes polyfunctional T cells in streptozotocin-induced diabetic rats. European Journal of Nutrition 51, 623-633.

**Gaur, G.,S, Dixit, A.K., 2012.** Comparative study of vitamin C on serum lipid profile in healthy male and female human subjects. Journal of Scientific Research 4, 775-781.

**Goldenberg, H., 2003.** Vitamin C: from popular food supplement to specific drug. Forum of Nutrition 56, 42-45.

**Goyer, R.A., 1996.** Results of lead research: prenatal exposure and neurological consequences. Environmental Health Perspectives 104, 1050-1054.

**Hakim, Z.S., Patel, B.K., Goyal, R.K., 1997.** Effects of chronic ramipril treatment in streptozotocin-induced diabetic rats. Indian Journal of Physiology and Pharmacology 41, 353-360

**Hearse, D.J., 1979.** Cellular damage during myocardial ischaemia: Metabolic changes leading to enzyme leakage. In: Hearse DS, Leiris J, Loisance D (eds). Enzymes in Cardiology. John Wiley and Sons, Chichester, pp. 1-21.

**Hilderbrand, D.C., Raymond, D., Griffin, W.T., Fahim, M.S., 1973.** Effect of lead acetate on reproduction. American Journal of Obstetrics and Gynecology 115, 1058-65.

**Hussein, H. K., Elnaggar, M. H., Al-Dailamy, J. M., 2012.** Protective role of Vitamin C against hepatorenal toxicity of fenvalerate in male rats. Global Advanced Research Journal of Environmental Science and Toxicology 1, 060-065.

**Kumar, B.A.A., Reddy, A.G.G., Kumar, P.R.R., Reddy, Y.R.R., Rao, T.M.M., Haritha, C., 2013.** Protective role of N-Acetyl L-Cysteine against reproductive toxicity due to interaction of lead and cadmium in male wistar rats. Journal of Natural Science, Biology, and Medicine 4, 414–419.

**Maha, B., Fiona, M., Nessar, A., 2012.** Effect of a high monounsaturated fatty acid diet alone or with combined vitamin E and C, or lycopene intake on oxidative stress in patients with type 2 diabetes mellitus. British Journal of Diabetes & Vascular Disease 12, 81-86.

**Mahboob, M., Rahman, M.F., Grover, P., 2005.** Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Medical Journal 46, 322-324.

**Masuda, T., Toi, Y., Bando, H., Maekawa, T., Takeda, Y., Yamaguchi, H., 2002.** Structural identification of new curcumin dimers and their contribution to the antioxidant mechanism of curcumin. Journal of Agricultural and Food Chemistry 50, 2524-25.

**Owu, D.U., Antai, A.B., Udofia, K.H., Obembe, A.O., Obasi, K.O., Eteng, M.U., 2006.** Vitamin C improves basal metabolic rate and lipid profile in alloxan-induced diabetes mellitus in rats. Journal of Biosciences 31, 575-579.

**Ping-Chi, H., Yueliang, L.G., 2002.** Antioxidant nutrients and lead toxicity. Toxicology 180, 33-44.

**Rekka, E., Kourounakas, P., Shahidi, F., Janitha, P.K., Anasundara, P.D., 1992.** Phenolic antioxidants. Critical Reviews in Food Science and Nutrition 32, 67-103.

**Ruff, H. A., Markowitz, M. E., Bijur, P. E., Rosen, J. F., 1996.** Relationships among blood lead levels, iron deficiency, and cognitive development in 2-year-old children. Environmental Health Perspectives 104, 180-185.

**Rytter, E., Vessby, B., Asgard, R., Johansson, C., Sjodin, A., Abramsson-Zetterberg, L., Moller, L., Basu, S., 2009.** Glycaemic status in relation to oxidative stress and inflammation in well-controlled type 2 diabetes subjects. British Journal of Nutrition 101, 1423-1426.

**Shargorodsky, M., Debby, O., Matas, Z., Zimlichman, R., 2010.** Effect of long-term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q10 and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risk factors. Journal of Nutrition and Metabolism 7, 55.

**Sekar, N., William, S., Balasubramaniyam, N., Kamarajan, P., Govindasamy, S., 1990.** Optimization of sodium orthovanadate to treat streptozotocin-indued diabetic rats. Journal of Biosciences 15, 67-75.

**Srinivasan, M., 1972.** Effect of curcumin on blood sugar as seen in a diabetic subject. Indian Journal of Medical Sciences 26, 269-270.

**Subbiah, R., Kasiappan, R., Karuran, S., Sorimuthu, S., 2006.** Beneficial effects of Aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. Clinical and Experimental Pharmacology and Physiology 33, 232–237.

**Takeshi, K., Shoichi, N., Yasunori, K., Yasuhiko, I., 2002.** Report of the committee on the classification and diagnostic criteria of diabetes mellitus, (The Committee of the Japan Diabetes Society on the diagnostic criteria of diabetes mellitus). Diabetes Research and Clinical Practice 55, 65-85.

**Ting, H.H., Timimi, F.K., Boles, K.S., Craeger, S.J., Ganz, P., Craeger, M.A., 1996.** Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. Journal of Clinical Investion 97, 22-28

**Upasani, C.D., Khera, A., Balaraman, R., 2001.** Effect of lead with Vitamins E, C, or Spirulina on malondialdehyde: conjugated dienes and hydroperoxides in rats. Indian Journal of Experimental Biology 39, 70-74.

**Urakawa, H., Katsuki, A., Sumida, Y., Gabazza, E.C., Murashima, S., Morioka, K., Maruyama, N., 2003.** Oxidative stress is associated with adiposity and insulin resistance in men. Journal of Clinical Endocrinologyand Metabolism 88, 4673-4676.

**Will, J.C., Byers, T., 1996.** Does diabetes mellitus increase the requirement for vitamin C? Nutrition Research Reviews 54, 193-202.

**Yokoyama, T., Date, C., Kokubo, Y., Yoshiike, N., Matsumura, Y., Tanaka, H., 2000.** Serum vitamin C concentration was inversely associated with subsequent 20-year incidence of stroke in a Japanese rural community. The Shibata study. Stroke 31, 2287-2294.