ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

ISSN (print):2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

Effect of Vitamin C Against Lead Acetate Toxicity on Sperm Count, Sperm Morphology and Testis Tissue in the Rat Before and in Recovery Period

Gulzar Star Hama amin [1], Karim Rahman Hamad[2]

[1&2] Department of Biology, College of Education, Salahaddin University-Erbil, Kurdistan Region, Iraq.

A B S T R A C T

The present study was conducted to investigate the effect of lead acetate (LA) (30 mg/Kg B.wt/day), and vitamin C (Vit.C) (100 mg/Kg B.wt/day) against LA toxicity in adult male rats. The design of study included two experiments (exp.). In exp-I rats were divided into 3 groups. Group I: control, group II: received LA, and group III: received LA coadministrated with Vit.C, for 6 weeks. In exp-II rats were divided into 4 groups. Group I: control, group II: received LA, group III: received LA coadministrated with Vit.C, and group IV: received LA. The groups were treated for 6 weeks, then groups II, &III in order to be recover were left without treatment (as control) for additional 6 weeks. While group IV after cessation of LA received Vit.C within recovery period (for 6 weeks). At the end of each experiment rats were sacrificed. Blood samples were collected and used for determination of serum MDA. Histological sections were made from testis. Sperm characteristics included sperm count was determined from caudal epididymis and sperm abnormalities from left vas deferens. In exp-I, LA group showed significantly decreased sperm count, significant increase in sperm abnormalities and MDA, and testicular tissue damage. While in group III Vit.C against LA significantly improved sperm characteristics and testicular tissue as well. In exp-II, Group II showed almost no improvement in sperm characteristics and testicular tissue, whereas MDA was increased non-significantly from control. In group III the coadministrated Vit.C with LA, markedly improved sperm characteristics, and testicular tissue similar to Vit.C against LA in exp-I. Meanwhile, the improvement by Vit.C in group IV was occurred in lesser extent. In conclusions, Vit.C had a protective effect against LA toxicity, and it was markedly more effective when coadministrated along with LA rather than its administration after cessation of LA.

KEY WORDS: Lead acetate, Vitamin C, Testis, Sperm count, Sperm abnormality.
DOI: http://dx.doi.org/10.21271/ZJPAS.32.3.14
ZJPAS (2020) , 32(3);127-138. .

1. INTRODUCTION

Lead is a heavy metal of wide occupational and environmental contamination. Lead toxicity is associated with an increased risk of adverse effect on a variety of target organs (Abd-El-Reheem and Zaahkcuk, 2007).

It causes serious health effects which might be permanent and lead to fatality (Assi *et al.*, 2016). The reproductive system of both males and females is affected by lead (Wani *et al.*, 2015). Several surveys have linked exposure to lead with decreased sperm count and other signs of male reproductive toxicity (Bonde *et al.*, 2002). The study of (Liu *et al.*, 2008) showed toxic effect of LA on male offspring rats which exhibited disordered arrangement of germ cells and Leydig cells, a decreased spermatogenic cell layer in the seminiferous tubules, and giant cells in the

* **Corresponding Author:**
Gulzar Star Hama amin
E-mail: Gulzarstar91@gmail.com
Article History:
Received: 03/12/2019
Accepted: 15/01/2020
Published: 15 /06 /2020

lumen, however the diameter of seminiferous tubules significantly decreased. Other study demonstrated that the histopathological examination of testes obtained from rats treated with lead, showed mild degenerative changes (Hari Priya and Reddy, 2012). In another study, rats exposed to LA, revealed that lead can induce pronounced alterations on germ cells in the testis (Haouas *et al*., 2015). Previously it has been shown that lead caused significant reduction in epididymal sperm count in mice (Wadi and Ahmad, 1999). In addition, it was reported that LA significantly decreased total testicular sperm and total cauda epididymal sperm in rats (Ait Hamadouche, 2009). Furthermore, the reduction in epididymal sperm count in rats was confirmed by (Hari Priya and Reddy, 2012; Anjum and Reddy, 2014). Clinical and animal studies also indicate that abnormalities of spermatogenesis result from toxic lead exposure (Sokol *et al*., 1985), and higher percentages of immature and abnormal sperm in lead exposed workers have been reported (Telišman *et al.*, 2007). Besides that, researchers revealed the significant increase in sperm abnormality in lead acetate intoxicated rats (Allouche *et al*., 2009; Elgawish and Abdelrazek, 2014; Ramah *et al.*, 2015).

Vitamin C is a water-soluble substance (Bendich *et al.*, 1986), It has a low-molecular weight that protects the cell from oxygen-nitrogen radicals (Ogutcu *et al*., 2008). Vitamin C (ascorbic acid), has been used in the treatment of lead toxicity. It has importance in maintaining the testes physiological integrity (El-Tohamy and El-Nattat, 2010). Previous study indicated that vitamin C at a concentration (10 mg/kg body weight) which is equivalent to the human therapeutic dose significantly minimize the testicular malondialdehyde content, and a companied by increase in sperm count and significant decrease in the percentage of abnormal sperm morphology in the mice exposed to lead acetate for 5-8 weeks (Mishra and Acharya, 2004). (Ayinde *et al.*, 2012) reported that vitamin C coadministrated with lead acetate significantly increased sperm count in lead treated rats, and decreased the percentage of abnormal sperm morphology. In view of our reviewing and observations, there is little information deals with the study of protective effect of vitamin C against lead acetate toxicity on male reproductive system, therefore the present research plan aimed to study the effects of lead acetate and vitamin C against lead acetate toxicity on sperm count, sperm abnormalities and testis section before and in recovery period in the rat.

2. MATERIALS AND METHODS

2.1. Animals and housing

The male rats (*Rattus norvegicus)* of this study were obtained from inbreeding in animal house of Department of biology, College of Education, Salahaddin university-Erbil. During the entire period of experiments the rats were kept in special cages with a steel stainless wire mesh top to hold standard rodent diet (Pico Lab. Rodent Diet 20) and tap water *ad libitum*. The room temperature was kept at about 22±4 ^{o}C and the light dark cycle was 12/12 hours.

2.2. Chemicals

lead acetate trihydrate Pb $(CH_3COO)_2$ .3H2O (LA) and ascorbic acid ($C_6H_8O_6$) (Vit.C) were manufactured by (Scharlab S.L. SPAIN).

2.3. Design of the experiments

2.3.1. Experiment I

Twenty-one adult male rats were divided randomly into three equal groups, each group contains seven rats. Treatments were given for six weeks as the following:

Group I (control): Rats received 0.6 ml distilled water (D.W)/ day orally by gavage.

Group II {lead acetate (LA) group}: Rats received LA 30 mg/kg B.wt in 0.6 ml D.W/ day orally by gavage.

Group III {lead acetate + Vitamin C (LAV) group}: Rats received LA 30 mg/kg B.wt in 0.3 ml D.W/ day and Vit.C 100 mg/kg B.wt in 0.3 ml D.W/ day orally by gavage.

2.3.2. Experiment II

Twenty-eight adult male rats were divided randomly into four equal groups as the following:

Group I (control): Rats received 0.6 ml D.W/ day orally by gavage.
Group II {lead acetate- Recovery (LAR) group}: Rats received LA 30 mg/kg B.wt/ day in 0.6 ml D.W orally by gavage.
Group III {lead acetate + Vit.C- Recovery (LAVR) group}: Rats received LA 30 mg/kg B.wt in 0.3 ml D.W/ day and Vit.C 100 mg/kg B.wt in 0.3 ml D.W/ day orally by gavage.
Group IV {lead acetate- Recovery+ Vit.C (LARV) group}: Rats received LA 30 mg/kg B.wt/ day in 0.6 ml D.W orally by gavage. At the end of six weeks of treatment, in groups (II, III & IV) of the experiment the treatments were stopped. The groups II & III were remained (as control) without treatment, while group IV received Vit.C (100 mg/kg B.wt in 0.3 ml D.W/ day orally by gavage), in order to be recover for additional six weeks.

2.4. Collection of blood samples

At the end of both experiments (I&II), after fasting for 24 hours, rats were anaesthetized by ether (Kempinas *et al*., 1994). Blood samples were collected by a syringe 5 ml through cardiac puncture, and immediately placed into gel tube. The samples were centrifuged, (Sorvall RC-5B Refrigerated Super speed Centrifuge), then the sera in Eppendorf tube were stored in deep freeze.

2.5. Dissection and removal organs

After withdrawal of blood samples, animals were dissected. The left testis and left caudal epididymis (epid.) were removed, and testis was preserved in 10% formal saline for fixation. The left caudal epididymis was used in sperm counting.

2.6. Sperm count

Left caudal epididymis of each rat was cut and homogenized in 5 ml of normal saline (0.9% NaCl) by manual homogenizer. Homogenates were kept in refrigerator at $4^{o}C$ for 24 hours to allow sperm to be released from the walls. Then 1 ml of the refrigerated homogenate was added to 7 ml of Eosin (0.2 %) and the samples were placed in a Neubauer hemocytometer, using light microscope. Head of the sperms were counted in 25 squares (Yucra *et al*., 2008).

2.7. Sperm morphology

Sperms were prepared from left vas deferens according to *(*Wyrobek *et al.,* 1983), the suspensions were smeared and dried. Then stained with 1% Eosin for 5 minutes. The slides were washed by distilled water and left to dry. Then sperm morphology (normal sperms, head defect sperms and tail defect sperms) were identified under microscope (1000X).

2.8. Histological sectioning

Preserved testes samples in 10% formal saline exposed to serial processes. Then embedded in paraffin wax and cooled (Drury and Wallington, 1980). Paraffin sections were cut by rotary microtome, then stained with hematoxylin (H) and eosin (E) (Bancroft and Gamble, 2008).

2.9. Malondialdehyde

Serum malondialdehyde (MDA) level was measured spectrophotometrically, by (APEL PD-303 SPECTROPHOTOMETER, 100~240V AC 50/60Hz 15W. APEL CO., LTD. JAPAN) at 532 nm. Thiobarbituric acid reaction (TBAR) method was used, and lipid peroxidation was expressed as MDA in μmol/L.

2.10. Statistical analysis

All data were expressed as mean ± S.E and statistical analysis carried out by GraphPad Prism Eight, version 6. Data analysis was made using one-way ANOVA. Results compared by ANOVA and Tukey's multiple comparisons test to determine significance among groups. Values were considered to be significantly different when $P<0.05$.

3. RESULTS

3.1. Experiment I

3.1.1. Effect of vitamin C against lead acetate toxicity on sperm count

The sperm count in groups of control (127.1± 11.61 x10^6 sperm/epid.), LA (28.94± 7.628 x10^6 sperm /epid.) and LAV (115.5± 9.538 x10^6 sperm /epid.) are shown in table 1 and figure 1-A. The sperm count was decreased significantly (P<0.01) in LA group and non-significantly in LAV group as compared to control, while in LAV group it was increased significantly (P<0.01) as compared to LA group.

3.1.2. Effect of vitamin C against lead acetate toxicity on sperm morphology

The value of sperm morphology (including normal sperm, sperm with head defect, and sperm with tail defect) in groups of control (84.22± 3.080 %; 3.088± 0.650 %; 12.69± 2.618 %), LA (34.34± 1.678 %; 11.24± 1.896%; 54.42± 1.232%), and LAV (72.72± 2.994%; 4.577± 0.178%; 22.70± 2.851%) are shown in table 1 and figure 1-B, C&D.

The percentage of normal sperms was decreased significantly (P<0.01) in LA group as compared to control. Also, in LAV group the percentage of normal sperms significantly (P<0.05) decreased from control, while it was increased significantly (P<0.01) as compared to LA group. Sperm with head defect and tail defect sperm in LA group significantly (P<0.01) increased as compared to control, while the comparison of head defect sperm of LAV group with that of control was non-significant. Also, in LAV group the percentage of head defect sperms significantly (P<0.01) decreased as compared to LA group. The percentage of tail defect sperm in LAV group was significantly (P<0.05) increased as compared to control, while it was decreased significantly (P<0.01) as compared to LA group.

Table 1: Effect of vitamin C against lead acetate toxicity on sperm count and sperm morphology in the rats.

In each group n=7

Parameters / Groups	Sperm Count x10^6 / epid.	Normal sperm (%)	Head defect sperm (%)	Tail defect sperm (%)
Control	127.1± 11.61[a]	84.22± 3.080[a]	3.088± 0.650[a]	12.69± 2.618[a]
LA	28.94± 7.628[b]	34.34± 1.678[b]	11.24± 1.896[b]	54.42± 1.232[b]
LAV	115.5± 9.538[a]	72.72± 2.994[c]	4.577± 0.178[a]	22.70± 2.851[c]

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences.

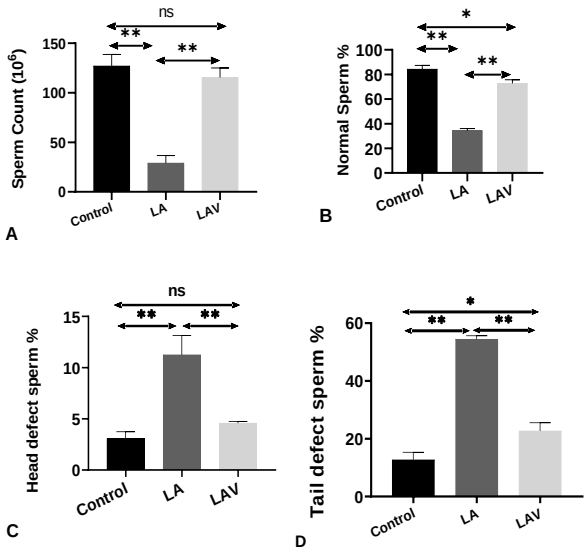

Figure 1: Effect of vitamin C against lead acetate toxicity on: A- Sperm count, B- Percentage of normal sperms, C- Percentage of head defect sperm and D- Percentage of tail defect sperm in the rats *=P<0.05 **=P<0.01.

3.1.3. Effect of vitamin C against lead acetate toxicity on serum MDA level

The serum MDA level in groups of control (4.344± 0.364 µmol/L), LA (6.706± 0.757 µmol/L), and LAV (4.865± 0.718 µmol/L) are shown in table 2 and figure 2. In LA group it was increased significantly (P<0.05) as compared to control. While non-significant change was observed in LAV group as compared to control and LA group.

Table 2: Effect of vitamin C against lead acetate toxicity on serum MDA level in the rats.

In each group n=7

Groups / Parameter	control	LA	LAV
MDA (µmol/L)	4.344± 0.364[a]	6.706± 0.757[b]	4.865± 0.718[ab]

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences.

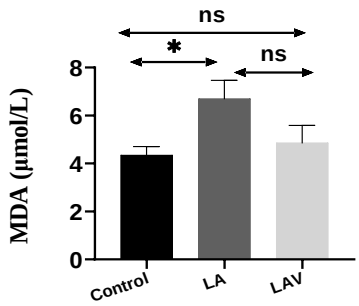

Figure 2: Effect of vitamin C against lead acetate toxicity on serum MDA level in the rats *=P<0.05.

3.1.4. Effect of vitamin C against lead acetate toxicity on histological sections of testis

The histological section of testis of control rat (figure 3) shows normal architecture of seminiferous tubules, organization of germ cells, active spermatogenesis, large number of spermatozoa in the lumen of seminiferous tubules, and large number of Leydig cells in the interstitial tissue.

The histological section of testis of rat treated with LA (figure 4) shows deformities in testicular architecture, atrophied seminiferous tubules and decline in their diameters. Degeneration in Sertoli cells and germ cell layers including all types of germ cells, indicating sever disruption in spermatogenesis. The lumen of seminiferous tubule free of spermatozoa and contains cell debris. Few spermatogonia appear shrunk with pyknotic nuclei. Depletion of Leydig cells, congested blood vessel in edematous interstium.

The histological section in testis of rat of LAV group (figure 5) shows improvement in tissue architecture of seminiferous tubules, with active spermatogenesis and organization in germ cell layers. Improvement in degenerated interstitium and Leydig cell, but still the section obviously shows reduced diameter in seminiferous tubule, and degeneration as in Leydig cells, and edema in interstitum.

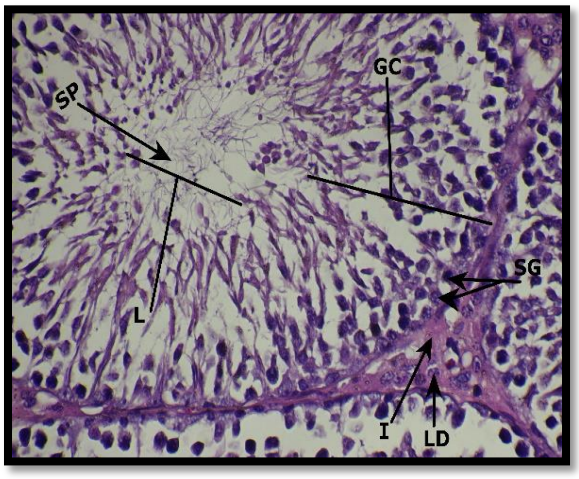

Figure 3: Section from testis of control rat, showing normal testicular architecture, seminiferous tubules, normal germ cell layers (GC) with active spermatogenesis and large amount of spermatozoa (SP) in lumen (L), and normal Leydig cells in interstitial tissue (I). (Stain: H&E. 400x).

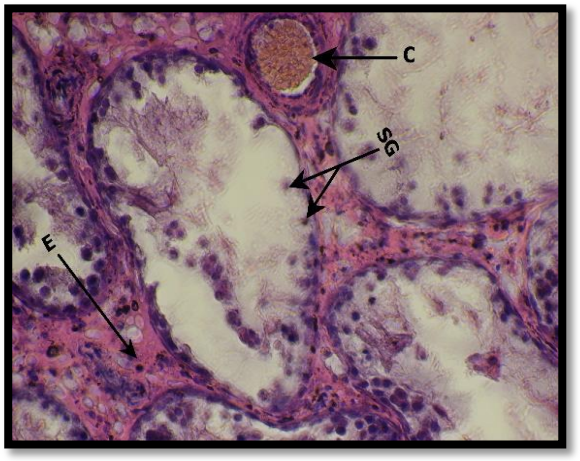

Figure 4: Section from testis of rat treated with LA, showing deformities in testicular architecture. Atrophied shrunk seminiferous tubules. With exception of irregular layer of spermatogonia (SG), almost seminiferous tubule contains no spermatogenic cells and spermatozoa. Sever edema (E) and congested blood vessel (C) in interstitial space. (Stain: H&E. 400x).

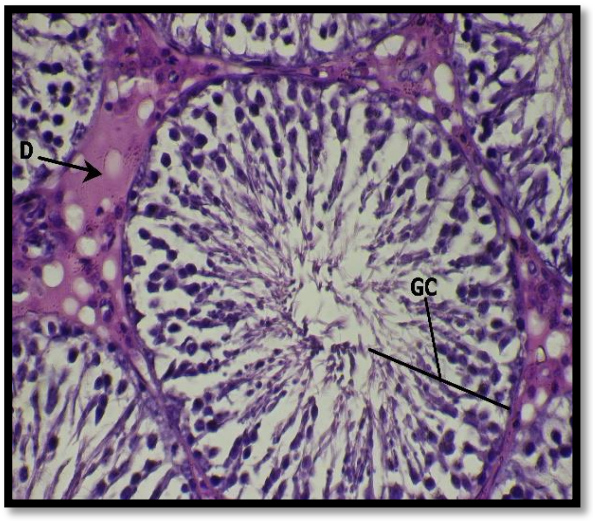

Figure 5: Section from testis of rat treated with Vit.C in coadministration with LA, showing improved seminiferous tubule with normal germ cell layers (GC) and active spermatogenesis. But still degeneration in Leydig cells (D) and mild edema are seen between seminiferous tubules (Stain: H&E. 400x).

3.2. Experiment II

3.2.1. Effect of vitamin C against lead acetate toxicity on sperm count in recovery period

Sperm counts in groups of control (112.700± 15.850 $x10^6$ sperm /epid.), LAR (29.070± 7.460 $x10^6$ sperm /epid.), LAVR (87.570± 11.520 $x10^6$ sperm /epid.), and LARV (67.760± 14.77 $x10^6$ sperm /epid.) are shown in table 3 and figure 6-A. Sperm count in LAR group was significantly ($P<0.01$) decreased as compared to control group. Both Vit.C supplemented groups (LAVR & LARV) showed non-significant reduction as compared to control. While sperm count in LAVR group was increased significantly ($P<0.05$) as compared to LAR group, and non-significantly increased as compared to LARV group. In spite of the observation of higher sperm count in LARV group compared to LAR group the difference was non-significant between them.

3.2.2. Effect of vitamin C against lead acetate toxicity on sperm morphology in recovery period

Sperm morphology (including normal sperm, sperm with head defect and sperm with tail defect) in groups of control (88.110± 3.019 %; 3.135± 0.625 %; 8.752± 2.552 %), LAR (41.070± 1.487%; 10.010± 1.133 %; 48.920± 1.837 %), LAVR (76.100± 2.375 %; 4.517± 0.288 %; 19.380± 2.241%) and LARV (57.730± 5.141 %; 7.807± 1.027 %; 34.460± 4.444 %) are shown in table 3 and figure 6- B, C & D.

The percentage of normal sperm in LAR and LARV groups were decreased significantly ($P<0.01$) as compared to control. While the percentage of normal sperm in LAVR group showed non-significant reduction as compared to control, and significantly ($P<0.01$) increased as compared to LAR and LARV. Also, in LARV group the percentage of normal sperms was significantly ($P<0.01$) increased as compared to LAR group. The percentage of head defect sperm significantly ($P<0.01$) increased in groups LAR and LARV as compared to control. While in LAVR the percentage of head defect sperm was increased non-significantly as compared to control, and significantly decreased as compared to LAR ($P<0.01$), and LARV ($P<0.05$). In addition, the percentage of head defect sperm in LARV group slightly decreased from LAR group. The percentage of tail defect sperms in LAR and LARV groups were increased significantly ($P<0.01$) as compared to control group, while LAVR group showed non-significant increase as compared to control,

and significantly (P<0.01) decreased as compared to LAR and LARV groups. Also, in LARV group the percentage of tail defect sperm was significantly (P<0.01) decreased as compared to LAR group.

Table 3: Effect of vitamin C against lead acetate toxicity on sperm count and sperm morphology in the rat in recovery period.

In each group n=7

Parameters / Groups	Sperm Count x 10^6/ epid.	Normal sperm (%)	Head defect sperm (%)	Tail defect sperm (%)
Control	112.700± 15.850[a]	88.110± 3.019[a]	3.135± 0.625[a]	8.752± 2.552[a]
LAR	29.070± 7.460[b]	41.070± 1.487[b]	10.010 ± 1.133[b]	48.920± 1.837[b]
LAVR	87.570± 11.520[a]	76.100± 2.375[a]	4.517± 0.288[a]	19.380± 2.241[a]
LARV	67.760± 14.77[ab]	57.730± 5.141[c]	7.807± 1.027[b]	34.460± 4.444[c]

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences.

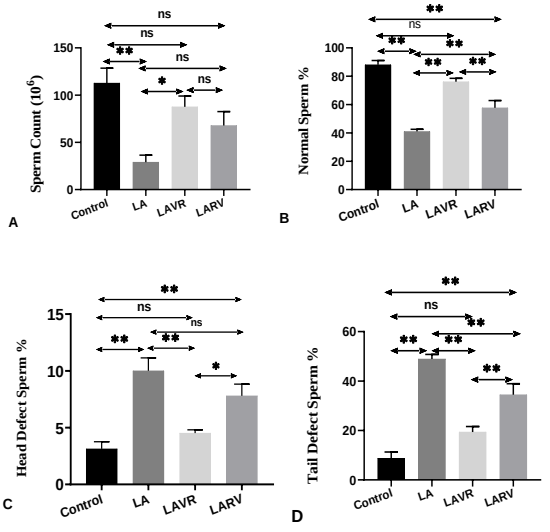

Figure 6: Effect of vitamin C against lead acetate toxicity on: A- sperm count, B- percentage of normal sperms, C- percentage of head defect sperm and D- percentage of tail defect sperm in the rat in recovery period *=P<0.05 **=P<0.01.

3.2.3. Effect of vitamin C against lead acetate toxicity on serum MDA level in recovery period

The serum MDA level in groups of control (4.350± 0.222 µmol/L), LAR (5.025± 0.438 µmol/L), LAVR (4.368± 0.367 µmol/L), and LARV (4.994± 0.485 µmol/L) are shown in table 4. Non-significant differences were observed in serum MDA level among all groups.

Table 4: Effect of vitamin C against lead acetate toxicity on serum MDA level in the rat in recovery period.

In each group n=7

Parameter / Groups	control	LAR	LAVR	LARV
MDA(µmol/L)	4.350± 0.222[a]	5.025± 0.438[a]	4.368± 0.367[a]	4.994± 0.485[a]

Data presented as mean ± S.E. The same letters mean non-significant differences while the different letters mean significant differences.

3.2.4. Effect of vitamin C against lead acetate toxicity on histological sections of testis in recovery period

The histological section of testis of control rat (figure 7) shows normal architecture of seminiferous tubules, organization of germ cells, active spermatogenesis, large number of spermatozoa in the lumen of seminiferous tubules, and large number of Leydig cells in the interstitial tissue.

Most of the testis section's area of LAR group (figure 8) shows severe degeneration and deformities in atrophied seminiferous tubule. Also, un improvement seen in degenerated germ cell layers, and interstitial tissue as well.

The histological section of testis of LAVR group (figure 9) shows that Vit.C prevented lead degenerative effect on tissue architecture, prevented damage in seminiferous tubules to a remarkable extent, decreased degeneration in germ cell layers, and increased activity in spermatogenesis, hence increased the spermatozoa in the lumen. Also improved Leydig cells, and interstium. While the histological section of

testis of LARV group (figure 10). Vit.C administration throughout the period of withdrawal treatment of LA slightly reduced testicular tissue degeneration. The section shows shrunken seminiferous tubule, lost testicular architecture, degeneration in germ cell layer, irregular layer of spermatogonia, absence of spermatocytes and spermatids in germinal epithelium, indicating loss of spermatogenesis, and the lumen contains cell debris. Also showed edematous interstitial tissue, with degeneration in Leydig cells.

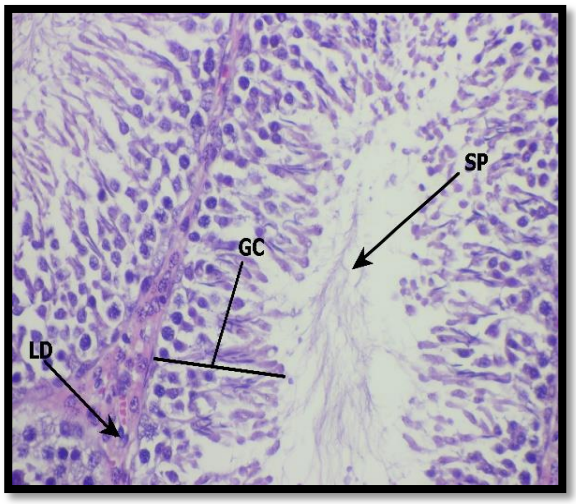

Figure 7: Section from testis of control rat, showing normal testicular architecture, seminiferous tubule, with normal germ cell layers (GC) with active spermatogenesis and large amount of spermatozoa (SP) in lumen (L). And normal Leydig cells in interstitial tissue (I). (Stain: H&E. 400x).

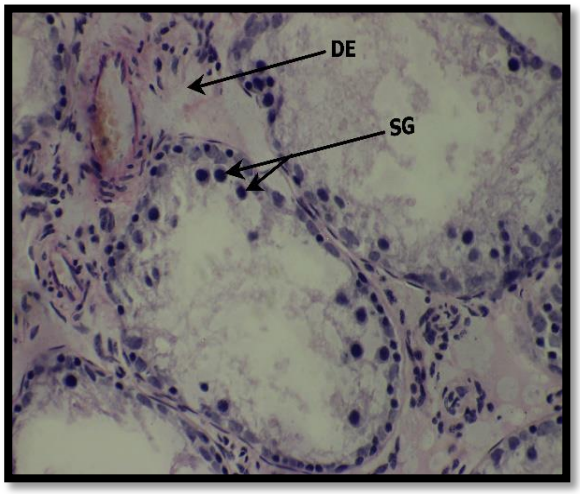

Figure 8: Section from testis of rat treated with LA left to be recovery, still showing deformities in testicular architecture. Atrophied shrunken seminiferous tubules with exception of irregular layer of spermatogonia (SG), almost seminiferous tubule contains no spermatogenic cells and spermatozoa. Sever edema (E) in interstitial space. (Stain: H&E. 400x).

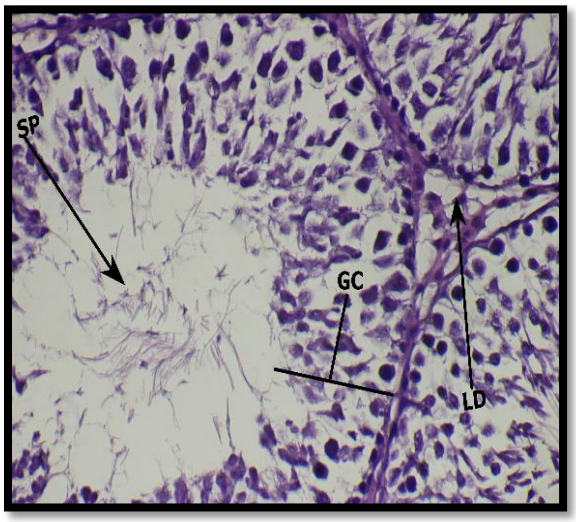

Figure 9: Section from testis of rat treated with Vit.C in coadministration with LA left to be recovery, showing improvement in testicular architecture, improved seminiferous tubule with germ cell layers (GC). The lumen contains spermatozoa. Leydig cells (D) are seen between seminiferous tubules (Stain: H&E. 400x).

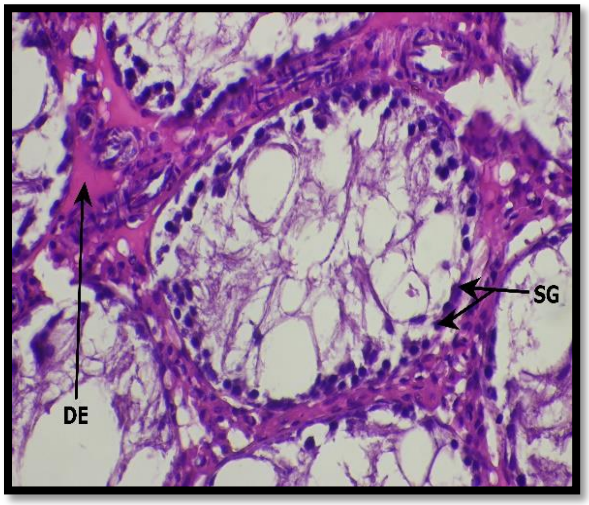

Figure 10: Section from testis of rat of LARV group, showing slightly improvement in atrophied seminiferous tubule. Seminiferous tubule contains irregular layer of spermatogonia (SG), and cell debris in lumen. Also, edema and degeneration in interstitial tissue (DE) are seen in the section (Stain: H&E. 400x).

4. DISCUSSION

In experiment I, the significant decrease of sperm count and significant increase in percentage of sperm abnormality in LA group is supported by (Pasha *et al.*, 2016) who reported significantly decreased epididymal sperm count and significantly increased sperm abnormality in rats exposed to LA. Which is confirmed by (Nasr *et al*., 2017). (Anjum *et al.*, 2016) reported that the increased oxidative stress induced by lead could damage the sperm membrane, DNA, and protein. This may explain the reduced sperm reserves and sperm membrane integrity in rats.

The damaged testis tissue in LA group is supported by (Al-Omair *et al*., 2017) who showed degenerations, and deformities in the testis architecture. Also, testis damage by LA was confirmed in the rat by (Mabrouk, 2018; Ali and Al-Derawi, 2018).

The protection of sperm count, sperm morphology and improvement of testicular architecture by Vit.C against lead acetate toxicity in LAV group is supported by (Sharma, 2013) who showed higher sperm count, lower percentage of abnormal sperm and improvement of testis in mice treated with Vit.C against lead acetate toxicity. The testis improvement might be due to inhibition of lead absorption in intestine (Dawson *et al*., 1999), early chelation of lead upon the first stages poisoning (Raafat *et al.*, 2009), of course such mechanism minimize lead storage to low extent. Also, might be due to scavenging the reactive oxygen and nitrogen species before they induce damage in the organ (Ambali *et al.*, 2011). In addition, Vit.C increase antioxidant content, and reduce lipid peroxidation product in the lead treated rats (Ayinde *et al*., 2012)**.**

In the present work the significantly increased serum MDA level in LA group in agreement with the study of (El-Nekeety *et al*., 2009) who showed that rats treated with LA, revealed significant increase in MDA level. Also, the present study is supported by (Ahmad Nisar *et al.*, 2013; Ali and Al-Derawi, 2018) who confirmed that LA caused a significant increase in lipid peroxidation level in rat. The reduction in MDA of LAV group, indicating improvement towards the normal, and supported by the study of (Ahmad Nisar *et al.*, 2013) who demonstrated the protective effect of Vit.C against oxidative stress induced by lead in rat. Also accordance with the present work (El-Tantawy, 2016) showed that treatment with Vit.C along with LA resulted a significant decrease in MDA in rats.

In experiment II, the significant decrease of sperm count and significant increase in percentage of sperm abnormality in LAR group most probably is attributed to limited ability of rats in repairing of damaged testis tissue (figure 8) which is indicating the impairment of testis in supplying the epididymis by spermatozoa. In addition, the ability of lead to accumulate in the testis and epididymis (Fahim *et al*., 2013), which is slowly released from body compartment (Flora and Agrawal, 2017), make the organ in which lead is accumulated even in recovery period continuously affected by LA toxicity.

The significant increase of sperm counts and significant decrease in percentage of sperm abnormality in LAVR group from LAR group indicating improvement by Vit.C most probably occur through elimination of lead by chelation of lead ions as reported by (Raafat *et al*., 2009), and scavenging the reactive oxygen and nitrogen species before they could cause damage to the organs as reported by (Ambali *et al*., 2011). It was reported that, the increased oxidative stress induced by lead could damage the sperm membrane, DNA, and protein. This may explain the reduced sperm reserves and sperm membrane integrity in rats (Anjum *et al*., 2016). So, removing of lead by Vit.C decreasing oxidative stress, hence increasing sperm reserves and sperm membrane integrity.

Since the recovery in sperm count and sperm abnormalities by Vit.C in LAVR group markedly is more than in LARV group. So, the present study displays the limited ability of Vit.C in LARV group to remove the degenerative effect of lead toxicity which left behind before administration of Vit.C.

The MDA level was improved toward normal in recovery period in LAR group. While (Omobowale *et al*., 2014) reported that given 0.5 and 1.0 mg/ml of LA for 6 weeks resulted in significantly increased MDA level in the liver of rats, and after withdrawn of LA for another 6 weeks rats exposed to 1.0 mg/ml LA did not recover.

The alteration in serum MDA level in all experimental groups (LAR, LAVR, & LARV) was non-significant as compared to control. However, in LAVR group its value reduced more than in other two groups and reached to control. This may be due to early chelation of lead ions (Raafat *et al*., 2009). And also, may be partly due to the antioxidant role of the vitamin resulted in scavenging the reactive oxygen and nitrogen species before they could induce damage in the organs (Ambali *et al*., 2011).

The damaged testis tissue in LAR group proportionally similar to that of LA treated group in exp-I. Unchange in testis tissue damage after stoppage of lead administration in recovery period most probably is attributed to limited ability of rat in repairing of damaged testis tissue. In addition, the tissue degeneration may be in part is attributed to stored lead in the testis as reported by (Mudipalli, 2007) that absorbed lead stored in soft tissue. Also (Ali and Al-Derawi, 2018) reported that lead accumulation in testicular tissue leads to oxidation and damage.

In LAVR group, Vit.C protected testicular tissue against LA most probably due to inhibition of lead absorption in intestine (Dawson *et al*., 1999), early chelation of lead (Raafat *et al*., 2009), and scavenging the reactive oxygen and nitrogen species before they could induce damage in the organs (Ambali *et al*., 2011). It seems that, the interstitial tissue involving Leydig cells was improved in LAVR group better than in LAV group-exp.I, may be due to self-recovery in interstitial tissue of testis after Vit.C treatment in recovery period. In contrast to LAVR group, the damaged tissue in LARV group was slightly improved, and indicating that Vit.C protect testis tissue through chelation of lead and scavenging radicals before lead inducing damage, and limitedly it repairs the damaged testis tissue induced by lead toxicity.

CONCLUSIONS

In the present study**,** the coadministration of Vit.C with LA left to recovery (exp.II) showed improvement in sperm counting, sperm abnormality, and in testicular tissue, similar to that of coadministration Vit.C with LA of (exp.I). Whereas, the improvement by Vit.C provided after cessation of LA treatment (exp.II), markedly was less than that of coadministration of Vit.C with LA left to be recover. On the other hand, almost there is no improvement in all measurements in LA group of recovery (exp.II) other-than MDA which is proportionally related to oxidative stress and indicates to lowering of LA in these animals.

References

ABD-EL-REHEEM, A. & ZAAHKCUK, S. 2007. Protective effect of Vitamin C and selenium against the toxicity induced by lead acetate on some physiological parameters in blood of male albino rats. *Bulletin of the Physiological Society of Egypt,* 27**,** 59-76.

AHMAD NISAR, N., SULTANA, M., ASHRAF WAIZ, H., AHMAD PARA, P., AHMAD BABA, N., AHMAD ZARGAR, F. & HUSSAIN RAJA, W. 2013. Experimental study on the effect of vitamin C administration on lipid peroxidation and antioxidant enzyme activity in rats exposed to chlorpyriphos and lead acetate. *Veterinary World,* 6.

AIT HAMADOUCHE, N. 2009. Reproductive toxicity of lead acetate in adult male rats. *Am. J. Sci. Res.,* 3**,** 38-50.

AL-OMAIR, M. A., SEDKY, A., ALI, A. & ELSAWY, H. 2017. Ameliorative Potentials of Quercetin against Lead-Induced Hematological and Testicular Alterations in Albino Rats. *Chin J Physiol,* 60**,** 54-61.

ALI, S. & AL-DERAWI, K. 2018. Al monsour NAA "Testicular toxic effect of lead acetate on adult male rats and the potential protective role of alcoholic extract of ginseng (histological, histomorphometrical and physiological)". *Sci. J. Med. Res,* 2**,** 87-92.

ALLOUCHE, L., HAMADOUCHE, M. & TOUABTI, A. 2009. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone

in albino rats. *Experimental and Toxicologic Pathology,* 61**,** 503-510.

AMBALI, S., ANGANI, M., ADOLE, A., KAWU, M., SHITTU, M., AKANDE, M. & OLADIPO, O. 2011. Protective effect of vitamin C on biochemical alterations induced by subchronic co-administration of chlorpyrifos and lead in Wistar rats. *Journal Environment Analytic Toxicol,* 1**,** 108.

ANJUM, M. R. & REDDY, P. S. 2014. Recovery of lead- induced suppressed reproduction in male rats by testosterone. *Andrologia,* 47**,** 560-567.

ANJUM, M. R., MADHU, P., REDDY, K. P. & REDDY, P. S. 2016. The protective effects of zinc in lead-induced testicular and epididymal toxicity in Wistar rats. *Toxicology and industrial health,* 33**,** 265-276.

ASSI, M. A., HEZMEE, M. N. M., HARON, A. W., SABRI, M. Y. M. & RAJION, M. A. 2016. The detrimental effects of lead on human and animal health. *Veterinary world,* 9**,** 660.

AYINDE, O. C., OGUNNOWO, S. & OGEDEGBE, R. A. 2012. Influence of Vitamin C and Vitamin E on testicular zinc content and testicular toxicity in lead exposed albino rats. *BMC Pharmacology and Toxicology,* 13**,** 17.

BANCROFT, J. D. & GAMBLE, M. 2008. *Theory and practice of histological techniques,* Elsevier Health Sciences.

BENDICH, A., MACHLIN, L., SCANDURRA, O., BURTON, G. & WAYNER, D. 1986. The antioxidant role of vitamin C. *Advances in Free Radical Biology & Medicine,* 2**,** 419-444.

BONDE, J. P., JOFFE, M., APOSTOLI, P., DALE, A., KISS, P., SPANO, M., CARUSO, F., GIWERCMAN, A., BISANTI, L. & PORRU, S. 2002. Sperm count and chromatin structure in men exposed to inorganic lead: lowest adverse effect levels. *Occupational and environmental medicine,* 59**,** 234-242.

DAWSON, E. B., EVANS, D. R., HARRIS, W. A., TETER, M. C. & MCGANITY, W. J. 1999. The effect of ascorbic acid supplementation on the blood lead levels of smokers. *Journal of the American College of Nutrition,* 18**,** 166-170.

DRURY, R. & WALLINGTON, E. 1980. Preparation and fixation of tissues. *Carleton's histological technique,* 5**,** 41-54.

ELGAWISH, R. A. R. & ABDELRAZEK, H. M. 2014. Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicology Reports,* 1**,** 795-801.

EL-NEKEETY, A. A., EL-KADY, A. A., SOLIMAN, M. S., HASSAN, N. S. & ABDEL-WAHHAB, M. A. 2009. Protective effect of Aquilegia vulgaris (L.) against lead acetate-induced oxidative stress in rats. *Food and chemical toxicology,* 47**,** 2209-2215.

EL-TANTAWY, W. H. 2016. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of traditional and complementary medicine,* 6**,** 327-331.

EL-TOHAMY, M. & EL-NATTAT, W. 2010. Effect of antioxidant on lead-induced oxidative damage and reproductive dysfunction in male rabbits. *J Am Sci,* 6**,** 613-622.

FAHIM, M. A., TARIQ, S. & ADEGHATE, E. 2013. Vitamin E modifies the ultrastructure of testis and epididymis in mice exposed to lead intoxication. *Annals of Anatomy-Anatomischer Anzeiger,* 195**,** 272-277.

FLORA, S. J. & AGRAWAL, S. 2017. Arsenic, cadmium, and lead. *Reproductive and developmental toxicology.* Elsevier.

HAOUAS, Z., ZIDI, I., SALLEM, A., BHOURI, R., AJINA, T., ZAOUALI, M. & MEHDI, M. 2015. Reproductive toxicity of lead acetate in adult male rats: Histopathological and cytotoxic studies. *Journal of Cytology & Histology,* 6**,** 1.

HARI PRIYA, P. & REDDY, P. S. 2012. Effect of restraint stress on lead- induced male reproductive toxicity in rats. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology,* 317**,** 455-465.

KEMPINAS, W., FAVARETTO, A., MELO, V., CARVALHO, T. L., PETENUSCI, S. & OLIVEIRA- FILHO, R. 1994. Time-dependent effects of lead on rat reproductive functions. *Journal of Applied Toxicology,* 14**,** 427-433.

LIU, H., NIU, R., WANG, J., HE, Y. & WANG, J. 2008. Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. *Fluoride,* 41**,** 184-191.

MABROUK, A. 2018. Therapeutic effect of thymoquinone against lead- induced testicular histological damage in male Wistar rats. *Andrologia,* 50**,** e13014.

MISHRA, M. & ACHARYA, U. R. 2004. Protective action of vitamins on the spermatogenesis in lead-treated Swiss mice. *Journal of Trace Elements in Medicine and Biology,* 18**,** 173-178.

MUDIPALLI, A. 2007. Lead hepatotoxicity & potential health effects. *Indian Journal of Medical Research,* 126**,** 518-528.

NASR, N. E., ELMADAWY, M. A., ALMADALY, E. A., ABDO, W. & ZAMEL, M. M. 2017. Garlic Powder Attenuates Apoptosis Associated with Lead Acetate-Induced Testicular Damage in Adult Male Rats. *Alexandria Journal for Veterinary Sciences,* 54.

OGUTCU, A., SULUDERE, Z. & KALENDER, Y. 2008. Dichlorvos-induced hepatotoxicity in

rats and the protective effects of vitamins C and E. *Environmental Toxicology and Pharmacology,* 26**,** 355-361.

OMOBOWALE, T. O., OYAGBEMI, A. A., AKINRINDE, A. S., SABA, A. B., DARAMOLA, O. T., OGUNPOLU, B. S. & OLOPADE, J. O. 2014. Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats. *Environmental toxicology and pharmacology,* 37**,** 1202-1211.

PASHA, H. F., REZK, N. A., SELIM, S. A. & EL MOTTELEB, D. M. A. 2016. Therapeutic effect of spermatogonial stem cell on testicular damage caused by lead in rats. *Gene,* 592**,** 148-153.

RAAFAT, B. M., SHAFAA, M. W., RIZK, R. A., ELGOHARY, A. A. & SALEH, A. 2009. Ameliorating effects of vitamin C against acute lead toxicity in albino rabbits. *Australian J. Basic Appl. Sci,* 3**,** 3597-3608.

RAMAH, A., EL-SHWARBY, R. M., NABILA, M. & EL-SHEWEY, E. A. 2015. The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil. *Benha Veterinary Medical Journal,* 28**,** 43-52.

SHARMA, D. N. 2013. Ascorbic Protects Testicular Oxidative Stress and Spermatozoa Deformationsin Male Swiss Mice Exposedto Lead Acetate. *Universal Journal of Environmental Research & Technology,* 3.

SOKOL, R. Z., MADDING, C. E. & SWERDLOFF, R. S. 1985. Lead toxicity and the hypothalamic-pituitary-testicular axis. *Biology of reproduction,* 33**,** 722-728.

TELIŠMAN, S., ČOLAK, B., PIZENT, A., JURASOVIĆ, J. & CVITKOVIĆ, P. 2007. Reproductive toxicity of low-level lead exposure in men. *Environmental research,* 105**,** 256-266.

YUCRA, S., GASCO, M., RUBIO, J., NIETO, J. & GONZALES, G. F. 2008. Effect of different fractions from hydroalcoholic extract of Black Maca (Lepidium meyenii) on testicular function in adult male rats. *Fertility and sterility,* 89**,** 1461-1467.

WADI, S. A. & AHMAD, G. 1999. Effects of lead on the male reproductive system in mice. *Journal of Toxicology and Environmental Health Part A,* 56**,** 513-521.

WANI, A. L., ARA, A. & USMANI, J. A. 2015. Lead toxicity: a review. *Interdisciplinary toxicology,* 8**,** 55-64.

WYROBEK, A. J., GORDON, L. A., BURKHART, J. G., FRANCIS, M. W., KAPP, R. W., LETZ, G., MALLING, H. V., TOPHAM, J. C. & WHORTON, M. D. 1983. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals: A report of the US Environmental Protection Agency Gene-Tox Program. *Mutation Research/Reviews in Genetic Toxicology,* 115**,** 1-72.