

Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits

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Abstract: The objective of this study was to characterize the Lead toxicity syndrome, to assess biomarkers that may be most useful for detecting toxicant-induced reproductive dysfunction, and to determine whether supplemental vitamin C would tend to alleviate the lead toxicity in rabbits. To test the hypothesis that the level of lead exposure is associated with an adverse effect on semen quality, in terms of sperm concentration, morphology, motility to assess antioxidant as important markers of disease using total antioxidant status. Adverse effects of lead on the testes may be mediated by oxidative damage and subsequent lipid peroxidation. The effect of lead acetate administration on testicular, hepatic and renal functions and the biomarker of effect for them were investigated in the present study with a trial of treatment by vitamin C. 35 male rabbits were divided into five groups. One control group and four groups received orally low and high doses of lead acetate (10.8 and 15 mg/kg b.wt., respectively). One low and one high received, in addition, 1 g vitamin C / L in drinking water. SOD, γ -GT, AST, ALT, cholinesterase, acid phosphatase, and LDH activities were measured in both serum and semen. Also semen characteristics were measured. Results concerning all the enzymes were promising. SOD, LDH, ALT and acid phosphatase activities in serum and semen were obviously affected by lead. Vitamin C was a good antioxidant that recuperates from the normal enzymatic status in both serum and semen. In conclusion, lead levels led to testicular hypo function, which is supported by the results of semen picture. The hazardous effect of lead led to disturbance in the activities of enzymes under investigation such as SOD, γ -GT, LDH, AST, ALT, Cholinesterase, Acid phosphatase. Vitamin C proved its antioxidant effect on recuperating from the normal status of enzymes in semen and serum. LDH and prostatic acid phosphatase are shown to be biomarkers of testicular dysfunction, while LDH, ALT may be used as biomarkers for hepatic and renal dysfunction. This study established the principle that lead toxicity can be prevented and makes it worthwhile to establish an acceptable treatment or preventive regimen in the light of the present results. [El-Tohamy, M.M., and El-Nattat, W.S. Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits. Journal of American Science 2010;6(11):613-622]. (ISSN: 1545-1003).

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1. Introduction:

Lead is a male reproductive toxicant (Winder, 1989), the primary mechanism of the toxic action of lead appears to be a disruption of the hypothalamic control of pituitary hormone secretion and in turn, spermatogenesis (Sokol, 1987). Since male do not possess accessory reproductive organs, reproductive potential relates to three factors; sperm availability, quality and quantity (Tsuji and Karagatzides, 2001).

Reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of a reproductive system and in the pathogenesis of infertility. ROS may also play a role in other reproductive organ diseases. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Because assisted reproductive techniques are used extensively in the treatment of infertility, it is critical to understand the in-vitro conditions that affect fertilization and embryo

development. Treatments that reduce oxidative stress may help infertility that is caused by this imbalance. Such strategies include identifying the source of excessive generation of ROS, treating the primary cause, and in-vitro and in-vivo supplementation of antioxidants. Research is in progress to identify the mechanisms that are involved in the etiology of reproductive diseases caused by ROS, and to create effective strategies that can counteract oxidative stress (Agarwal and Allamaneni, 2004).

This effect is associated with indicators of oxidative stress. The present study showed that rabbits subjected to lead and cadmium had increased serum levels of LPO. This may be a result of either overproduction of ROS or accumulation of ROS resulting from dysfunction of antioxidants/antioxidants during lead exposures.

Exposures of experimental rabbits to lead induce oxidative stress, but to date, no examination of this phenomenon has been reported. Exposure to lead and cadmium results in decreased nitric oxide production in rabbits. And inhibition of NO synthesis

leads to a marked decrease in GSH synthesis through down regulation of the rate-limiting enzyme. Elevated serum (LPO) some possible mechanisms for the lead induced –oxidative stress is discussed. Pb exerts at a dose encountered exerts adverse effects on the male reproductive system, and this effect is associated with indicators of oxidative stress.

The imbalance between (ROS) production and (TAC) in seminal fluid indicates oxidative stress and is correlated with male infertility. A composite ROS–TAC score may be more strongly correlated with infertility than ROS or TAC alone recent scientific evidence revealed that a condition known as "oxidative stress" may, in fact, is a common factor in some of the causes of male infertility. Oxidative stress in the semen occurs when the level of ROS (reactive Oxygen Species) is greater than the TAC (Total Antioxidant Capacity). Although low levels of ROS are needed to normal sperm function, high levels of ROS clearly impair fertility. Antioxidants are substances that protect cellular components from damaging oxidative reactions by reaction with the free radicals and other reactive oxygen species.

The specific aims of the present study are to determine the efficacy of Vitamin C to improve reproductive performance of rabbits. There is growing evidence that oxidative stress significantly impairs sperm function, and plays a major role in the etiology of defective sperm function.

This may lead to the onset of male infertility via mechanisms involving the induction of per oxidative damage to the plasma membrane; both spermatozoa and seminal plasma possess antioxidant systems capable of counteracting the harmful effects of ROS. Studies have demonstrated that infertile male is more likely than fertile ones to have depressed (TAC) and lower levels of individual antioxidants (Smith *et al.*, 1996 Lewis *et al.*, 1995). ROS is thought to be involved in many aspects of male infertility, where spermatozoa are rendered dysfunctional by lipid peroxidation and altered membrane function, together with impaired metabolism, morphology, motility, and fertility.

On the other hand, there is another category of enzymes, which act on superoxide radicals to eliminate its hazardous effects. The inhibition of these enzymes by lead causes the increase of superoxide radicals O_2^- and its accumulation inside the cell leading to its death (Mylroie *et al.*, 1986). Vitamins A, E, D and C were recorded to have antioxidant activities. Their antagonistic effects to superoxide accumulation varied from one vitamin to the other.

In this study, Vitamin C is used to investigate its antioxidant activity for decreasing the inhibitory effect of lead acetate on serum and semen

enzymes in New Zealand rabbits and monitoring the biomarker of effect on the vitality of the male.

2. Materials and methods

Experimental design:

35 mature male rabbits (2.5 kg average) were raised in the animal house at National Research Centre (NRC), they were divided into 5 equal numbered groups (n=7). The first group was adopted as a control group (distilled water treated). The second and third were dosed 10.8 mg lead acetate/kg b.wt. Orally and considered as the low dose group. The fourth and fifth groups were dosed 15 mg lead acetate/kg b.wt. and considered as the high dose group. All the groups received their dosage orally dissolved in distilled water using animal gavages. The four latter groups were treated 5 consequent days/week, and the treatment expended for 8 weeks. The third and fifth groups obtained in addition to the lead acetate doses, vitamin C in concentration of 1 g / litre drinking water every day without disturbance. All lived in animals were sacrificed on the day after the last dose.

Semen collection:

Semen was collected from bucks using a rabbit AV and a teaser female (Hafez, 1970). The collection was achieved from the end of the fourth week until the end of the experiment for the routine evaluation of both live sperm and sperm abnormalities percentage using eosin aniline stain and before the slaughter immediately for enzyme determination.

Blood sampling:

Blood was collected while sacrificing animals in sterilized capped tubes and sterilized heparinized tubes (for SOD determination). The tubes were incubated at 37°C for 10 minutes in a slope position, and then centrifuged at 3500 rpm for 10 minutes. Serum was collected and immediately tested for the enzymes.

The whole heparinized blood was treated according to the following method: 0.5 ml of whole blood was centrifuged for 10 minutes at 3000 rpm and then the plasma was aspirate off. Then erythrocytes were washed four times with 3 ml of 0.9% NaCl solution and centrifuged for 10 minutes at 3000 rpm after each wash. The washed and centrifuged erythrocytes were made up to 2.0 ml with cold redistilled water, mixed and left to stand at +4°C for 15 minutes. The lysate was diluted with 0.01 mmol/l phosphate buffer pH 7.0 (Randox, Cat. No. SD 124). so that the % inhibition falls between 10% and 60%. A 25 folds dilution of lysate is recommended (final dilution factor = 100).

Enzymatic analysis in blood and semen:

- 1- In erythrocytes:
 - i- Superoxide dismutase (SOD) in whole blood according to Wooliams et al. (1983) using kits purchased from Randox, UK.
- 2- In serum and whole semen
 - i- Aspartate amino transferase (AST), alanine amino transferase (ALT) according to Bergmeyer (1978) and lactate dehydrogenase (LDH) according to Buhl and Jackson (1978) using kits purchased from Stanbio, Texas, USA.
 - ii- Cholinesterase according to Den Blawen et al. (1983), Acid phosphatase according to Moss (1984) and \square GT according to Szasz (1969) using kits purchased from Quimica Clinica Aplicada S.A., Spain.
 - iii- The MDH 586 method was used to determine MDA activity as described by Gerard et al., (1998) and measured at 586.
 - iv- All enzymes were measured using Shimadzu Spectrophotometer with different wavelengths specific for each enzyme.

Statistical analysis:

Data were analyzed using one-way ANOVA to determine whether the effect of lead (Pb) and vit.

C gave a significant difference than control group or not (H_0). The results were accepted at a level of 95% confidence. Statistical analysis using SPSS (Statistical package for social science) version 12, software package for data analysis (Saeys et al., 2007) was done.

3. Results and Discussion:

We previously reported that dietary exposure to lead resulted in suppressed spermatogenesis and testosterone levels without significant changes in luteinizing hormone (El-tohamy 2003). The result showed that exposure to concentrations of inorganic lead (micrograms/dl in blood impaired male reproductive function by reducing the sperm count, volume, density or changing sperm motility and morphology. Semen analysis (Table 1) revealed that the lead treated groups were significantly ($P < 0.01$) lower in mass motility, individual motility, sperm concentration / ml semen, live sperm %, while, they were significantly ($P < 0.01$) higher in the total primary sperm abnormalities %, then the control group. Vitamin C has ameliorated the hazardous effect of lead in both groups (III and V).

Table 1- The effect of lead acetate alone or in the addition of vitamin C on semen characteristics in the male rabbits.

Treatment Biomarkers	Group I (Control) (n=12)	Group II Low Lead (n=36)	Group III (lowLead+C) (n=12)	Group IV (high Lead) (n=7)	Group V (high Lead+C) (n=28)
Mass motility score (1-5)	4.5 ^b ± 0.12	3.44 ^a ± 0.13	4.00 ^{ab} ± 0.25	3.57 ^a ± 0.12	3.86 ^a ± 0.22
Individual motility (%)	91.25 ^b ± 0.90	76.25 ^a ± 1.33	81.25 ^a ± 3.09	79.64 ^a ± 1.31	80.71 ^a ± 2.32
Volume (ml)	0.48 ^a ± 0.056	0.69 ^b ± 0.055	0.47 ^a ± 0.014	0.39 ^a ± 0.021	0.52 ^{ab} ± 0.063
Concentration (x10 ⁶ /ml)	120.00 ^b ± 6.89	87.50 ^a ± 5.59	77.00 ^a ± 2.71	70.40 ^a ± 3.99	106.86 ^b ± 6.71
Total concentration (x10 ⁶)	53.80 ^{bc} ± 3.62	58.23 ^c ± 5.24	36.30 ^{ab} ± 2.22	25.83 ^a ± 1.27	62.53 ^c ± 9.33
Live %	94.33 ^c ± 0.25	82.53 ^b ± 0.32	90.26 ^d ± 0.50	74.21 ^a ± 0.51	88.83 ^c ± 0.46
Primary spr abnormalities %	15.33 ^a ± 0.55	25.16 ^c ± 0.25	21.27 ^b ± 0.41	34.31 ^d ± 1.42	19.35 ^b ± 0.78

Same superscript are non significantly different within row ($P < 0.05$) Duncan test

The results of present study showed that lead acetate significantly affected the semen characteristic in both group II and IV, while; vitamin C corrected these adverse effects (table 1). This agrees with the finding of El-Nattat et al. (2000). Low lead exposure was more consistently associated with indicators of sperm production than was semen lead. Measurement of semen leads may not be a valuable

adjunct to conventional blood lead monitoring for investigations of male reproductive system toxicity.

The results also showed that vitamin C supplementation reduced ROS generation and improved semen quality. The beneficial effect of vitamin C in improving fertilization rate was possibly due to a reduction in lipid per oxidation potential. Seminal plasma confers some protection against ROS

damage because it contains enzymes that scavenge ROS.

High lead exposure induces oxidative stress in animal. Oxidative stress is an imbalance between the free radical production and antioxidant defense systems of the body (Yao et al., 2006 and Urso et al, 2003).

Antioxidants present in the seminal plasma are the most important form of protection available to spermatozoa against (ROS) (Aitken, 1999). They provide a defense mechanism through 3 levels of protection, prevention, interception and repair. A growing body of evidence suggests that low seminal (TAC) is related to male infertility (Sikka, 2001). Thus, it is important to ensure that any measurement of seminal TAC is accurate and reliable and easy as a diagnostic tool in the evaluation and follow up of male infertility. In a normal situation, the cellular antioxidant mechanisms present in almost all tissues and their secretions are likely to quench those ROS and protect against oxidative damage (Jones, et al., 1979). Antioxidant supplementation can theoretically protect and prevent such per oxidative damage. To evaluate this oxidative stress and determine the role of antioxidants have a great potential in therapeutic practice. Studies on how these cellular changes caused by LPO effect seminal parameters and sperm function and whether they could be reversed by antioxidants are open to further investigations.

Antioxidants, in general, are free radical scavengers that suppress the formation of ROS and/or

oppose their actions. SOD is well known biological antioxidants that convert superoxide (O) and peroxide (HO) radicals to form O₂ and H₂O. SOD protects against spontaneous O₂ toxicity and LPO (Fridovich, 1985). SOD and catalase also remove O₂ and play an important role in protecting spermatozoa. In spermatozoa, production of (MDA) an end-product of LPO induced by ferrous ion promoters, has been reported (Bell et al., 1993). Seminal plasma possesses major antioxidant defenses, including enzymatic and no enzymatic antioxidants. Chain-breaking antioxidants trap ROS directly to prevent amplification of radical formation and subsequent damage to sperm.

The results (Table 2). showed that ROS combined with total antioxidants capacity could predict fertility in male. Oxidative stress in the semen occurs when the level of ROS is greater than the TAC. Although low levels of ROS are needed to normal sperm function, high levels of ROS clearly impair fertility. Supplementation of vitamin C improved sperm motility and reproductive efficiency, and reduced the production of free radicals which can improve rabbit semen quality. The conclusion is that the antioxidants used are effective in combating cell damaging free radicals, which are known to contribute towards testicular function. Lipid peroxidation considered to be the key mechanism of ROS-induced sperm damage, which leads to loss of sperm motility.

Table 2. The effect of lead acetate alone or in the addition of vitamin C on MDA and TAC in the semen of male rabbits.

parameters Groups	Control	Low dose lead acetate(10.8mg/Kg b.wt)	High dose lead acetate(15.mg/Kg b.wt)	Low dose lead acetate + vit. C (1g/L drinking water)	High dose lead acetate + vit. C (1g/L D water)
MDA serum Nmol/ml	10.98 ±0.37 ^b	14.79±0.21 ^{ac}	18.28±0.25 ^{ac}	11.62±0.14 ^b	10.36±0.15 ^b
MDA semen Nmol/ml	11.34±0.35 ^b	16.06±0.36 ^{ac}	18.30±0.37 ^{ac}	12.36±0.39 ^b	11.83±0.37 ^b
TAC serum mmol/L	0.61±0.24 ^{bc}	0.47±0.01 ^{ac}	0.28±0.02 ^{ac}	0.70±0.02 ^{ab}	0.93±0.02 ^{ab}
TAC semen mmol/L	1.25±0.10 ^{bc}	0.83±0.02 ^{ac}	0.60±0.02 ^{ac}	1.92±0.09 ^{ab}	2.74±0.17 ^{ab}

Data were recorded as Mean ± S.E.

Different superscript in rows are significantly different at P<0.05.

a: Significant change when comparing all groups with the control group

b: Significant change when comparing all groups with lead acetate

c: Significant change when comparing all groups with lead acetate + vit.C

The results showed that supplementation of vitamin C had beneficial effects on semen characteristics. The beneficial effects of vitamin C can be attributed to the fact that vitamin C is a very

efficient antioxidant and a scavenger of oxygen free radicals who are toxic byproducts of many metabolic processes. Vitamin C is important in maintains the physiological integrity of the testes. Supplementation

of vitamin C improved sperm motility and reproductive efficiency and reduced the production of free radicals which can improve rabbit semen quality. The study required to determine whether vitamin C and other naturally occurring compounds can function as effective free radical trapping antioxidants, thereby preventing testicular peroxidative injury.

Vitamin C is essential to the body as both an antioxidant and as a nutritional supplement. Numerous antioxidants have proven beneficial in treating male infertility, such as Vitamin C and E, glutathione, Zinc and Selenium (Sinclair, 2000). Antioxidants can protect against the damaging effect of leukocyte-derived reactive oxygen species on sperm movement and may be of clinical value (Baker et al., 1996). The production of ROS is a normal physiological event in various organs, including the testes. Overproduction of ROS, can be detrimental to sperm, which associated with male infertility (Akiyama, 1999).

The results of the present study showed that lead acetate significantly affected the semen characteristic in both group and IV, while vitamin C corrected these adverse effects (table 1). The role of some enzymes markers are greatly influencing by heavy metals. Most of them contain a sulfhydryl group. Such group is a target for metals. Others like some metal-enzymes have a prosthetic group that may be replaced by these metals leading to an inhibition of enzyme activity (WHO, 1993). These suppression masks their physiological actions specially those enzymes related to superoxide radicals O_2^- that accumulates inside the cells leading to the deterioration and death of the cells. SOD is one of these enzymes (Patra et al., 1999). The results indicated that lead acetate induced an exaggerated inhibitory effect for the SOD. As lead mimics Cd in replacing Zn in its sites, this suggested the interaction of lead with the Cu, Zn and Mn, moieties such as interaction has been demonstrated where lead replaces Zn to form Cu-Pb-SOD (Bauer et al., 1980) Vitamin C ameliorate the inhibitory action of lead by removing ROS once formed, thus preventing radical chain Reactions. This observation supports the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and glutathione peroxides (GSH-Px) Allen and Balin, (1989) generated by the inhibitory action of lead; on the other hand, the antioxidants refresh the enzyme's activity and antagonize the inhibitory effect of lead.

Lactate dehydrogenase plays an important role in the intermediary metabolism as a link between

amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate (Table 3). The serum lactate dehydrogenase is cytosolic, and in cellular damage the liver, lung, muscle, kidney, testicles or heart releases it into systemic circulation (Bhargava et al., 1978). In the present study, there was a decrease in the activity of the LDH in serum due to the lead intoxication. This agrees with the findings of Yagminas et al. (1990), while, it recuperates its activity values around the control LDH activity due to the treatment with vitamin C in both semen and serum. This decrease in LDH activity may be due to an inhibitory effect induced by the dose levels of lead, although an increase in the enzyme leakage may be present. On the other hand, in the semen the leakage from testicular and glandular tissues was significant, approximately twice to four times than the treated groups with vitamin C and triple the control group. Gulvik (1989) recorded a reduced activity of LDH in the testicular tissue. This result agrees with our findings that LDH is a sensitive and convenient biosensor for detection of heavy metal salts (Fennouh et al., 1998).

The role of some enzyme markers is greatly influenced by heavy metals. Most of them contain sulfhydryl group (-SH) at their site of the action (Keogh, 1992), such group is a target for metals. Others like some metal-enzymes have a prosthetic group that may be replaced by these metals leading to an inhibition of enzyme activity (WHO, 1993). These suppression masks their physiological actions, especially those enzymes related to superoxide radicals O_2^- that accumulate inside the cells leading to the deterioration and death of the cells. SOD is one of those enzymes (Patra et al., 1999).

The results indicated that lead acetate induced an exaggerated inhibitory effect for the SOD. As lead mimics Cd in replacing Zn in its sites, this suggested the interaction of lead with the Cu, Zn and Mn moieties. Such interaction has been demonstrated where lead replaced Zn to form Cu-Pb-SOD (Bauer et al., 1980). Vitamin C ameliorates the inhibitory action of lead by removing ROS once formed, thus preventing radical chain reactions. This observation supports the hypothesis that SOD activity is stimulated by an increased superoxide radical generation associated with the decline of SOD and glutathione peroxidase (GSH-Px) (Allen and Balin, 1989) generated by the inhibitory action of lead, while, on the other hand, the antioxidant refreshes the enzyme activity and antagonizes the inhibitory effect of lead.

Table 3- The effect of lead acetate alone or in the addition of vitamin C on some enzymes biomarkers in blood of male rabbits.

Treatment Biomarkers	Group I (Control) (n=5)	Group II (Low Lead) (n=7)	Group III (Low lead+C) (n=6)	Group IV (high Lead+) (n=7)	Group V (high Lead+C) (n=4)
Superoxide dismutase (units SOD/ml blood)	115.43 ^a ± 18.27	210.64 ^{bc} ± 25.89	187.58 ^b ± 7.82	262.81 ^c ± 25.89	109.78 ^a ± 10.53
□-GT (U/L)	9.65 ^{ab} ± 0.51	11.39 ^{bc} ± 1.95	7.78 ^a ± 0.80	14.09 ^c ± 0.47	10.26 ^{ab} ± 0.76
Lactate dehydrogenase (U/L)	518.66 ^b ± 22.87	349.19 ^a ± 12.30	559.71 ^b ± 21.04	355.32 ^a ± 7.68	532.61 ^b ± 20.60
AST (U/L)	81.33 ^a ± 3.55	95.37 ^b ± 2.95	80.82 ^a ± 3.07	81.86 ^a ± 4.47	72.79 ^a ± 1.44
ALT (U/L)	88.04 ^{bc} ± 2.84	100.09 ^c ± 11.50	78.30 ^{ab} ± 2.60	133.78 ^d ± 9.06	61.46 ^a ± 4.25
Choline esterase (U/L)	1049.44 ^b ± 109.82	838.04 ^{ab} ± 162.24	1051.23 ^b ± 128.86	479.25 ^a ± 67.69	1090.89 ^b ± 166.16
Total acid phosphatase (U/L)	59.51 ^a ± 2.40	66.72 ^b ± 1.97	84.91 ^d ± 2.16	66.68 ^b ± 1.26	76.84 ^c ± 2.83
Prostatic acid phosphatase(U/L)	1.82 ^a ± 0.33	3.31 ^a ± 0.43	8.00 ^b ± 0.56	3.00 ^a ± 0.43	7.45 ^b ± 1.56

Same superscript are insignificantly different within row (P<0.05) Duncan test.

Certain enzymes, proenzymes, and their substrates are present at all times in the circulation of normal individuals and perform a physiological function in blood. Pseudo cholinesterase is one of these enzymes (functional enzymes). They are synthesized in the liver but present in blood in equivalent or higher concentrations than in tissues (table 4). While, nonfunctional plasma enzymes

which have the unknown physiological function in blood, only provide valuable diagnostic and prognostic clinical evidence in the case of dysfunction and diseases. These nonfunctional plasma enzymes include those in exocrine secretions and true Intracellular enzymes. Prostatic acid phosphatase is one of those categories (Rodwell, 1991).

Table 4- The effect of lead acetate alone or in the addition of vitamin C on some enzymes biomarkers in the semen of male rabbits.

Treatment Biomarkers	Group I (Control) (n=5)	Group II (Lead) (n=7)	Group III (Lead+VitC) (n=6)	Group IV (high Lead) (n=7)	Group V (high Lead+C) (n=4)
Lactate dehydrogenase (U/L)	954.75 ^a ± 52.98	3850.83 ^c ± 39.45	1646.90 ^b ± 30.45	4817.65 ^d ± 390.60	1788.63 ^b ± 5.07
AST (U/L)	420.79 ^a ± 62.50	1614.77 ^c ± 104.17	247.13 ^a ± 26.13	773.99 ^b ± 1.49	330.81 ^a ± 48.23
ALT (U/L)	43.32 ^b ± 2.12	44.20 ^b ± 7.56	38.60 ^b ± 3.15	23.18 ^a ± 4.87	16.50 ^a ± 0.93
Choline esterase (U/L)	195.50 ^c ± 25.60	119.91 ^a ± 4.93	156.40 ^b ± 3.41	101.66 ^a ± 3.41	106.87 ^a ± 7.88
Total acid phosphatase (U/L)	124.74 ^a ± 5.40	113.86 ^a ± 12.24	107.01 ^a ± 9.53	171.20 ^b ± 14.70	99.76 ^a ± 10.84
Prostatic acid phosphatase (U/L)	12.63 ^a ± 0.95	16.57 ^a ± 2.74	16.06 ^a ± 1.19	24.88 ^b ± 2.37	15.48 ^a ± 3.28

Same superscript are insignificantly different within row (P<0.05) – Duncan test.

The γ -glutamyl transferase, an enzyme that supports the transfer of certain amino acids into the GSH, which reduces, peroxides accumulation into the

RBCs and other cells. It is presented in abundant in the plasma membrane of renal tubular cells and in the endoplasmic reticulum of the hepatocytes (Murray,

1991). In the present study, the results showed a significant increase in the enzyme activity in case of high dose of lead acetate (14.09 U/l) than the control group. This indicates that there is a destruction of the enzyme in the store cells, and release in the blood serum (Murray, 1991). Therefore, the activity in the store cells like hepatocytes and renal tubular cells is decreased (Sivaprasad et al., 2002). Murray (1991) reported that high malonylaldehyde levels (a compound indicator on lipid peroxides) along with lowered activities of catalase, SOD, glutathione peroxidase, glutathione metabolizing enzymes (glutathione reductase, glucose-6-phosphate dehydrogenase, glutathione-S-transferase) indicate that the cells are under deteriorating stress factors including heavy metals. He recorded that a powerful antioxidant can reverse the oxidative damage by bringing about an improvement in the reductive status of the cell. The present study proved that vitamin C has stored the integrity of cells and reduced the leakage of enzymes outside the damaged cells, in addition, to its antioxidant effect via an indirect way. Moreover, vitamin C may help in decreasing the superoxide radical's accumulation inside the cells, although the SOD activity is inhibited by the hand make of lead, , it conserves the soundness of the cells in return, no leakage of enzymes and no increased enzyme activity in the serum than the normal. This is in agreement with the proposal of Sivaprasad et al. (2002). Upasani and Balaraman (2001) concluded that vitamin C had a significant antioxidant activity thereby protecting the organs from the lead-induced toxicity.

Lactate dehydrogenase plays an important role in the intermediary metabolism as a link between amino acid metabolism and the citric acid cycle where it converts lactate into pyruvate. The serum lactate dehydrogenase is cytosolic, and in cellular damage the liver, lung, muscle, kidney, testicles or heart releases it into systemic circulation (Bhargava et al., 1978). In the present study, there was a decrease in the activity of the LDH in serum due to the lead intoxication. This agrees with the findings of Yagminas et al. (1990), while, it recuperates its activity values around the control LDH activity due to the treatment with vitamin C in both semen and serum. This decrease in LDH activity may be due to an inhibitory effect induced by the dose levels of lead, although an increase in the enzyme leakage may be present. On the other hand, in the semen the leakage from testicular and glandular tissues was significant, approximately twice to four times than the treated groups with vitamin C and triple the control group. Gulvik (1989) recorded a reduced activity of LDH in the testicular tissue. This result agrees with our findings that LDH is a sensitive and

convenient biosensor for detection of heavy metal salts (Fennouh et al., 1998).

Many authors have discussed the effect of lead on AST and ALT in the serum (Randhawa et al., 1995; and El-Nattat, 1997). While, in liver cell culture Gutierrez et al. (1992) found that LC₅₀ of lead acetate (100 micromole) caused significant leakage of ALT and AST into the medium. This concludes that leakage cytoplasmic enzymes appear to be a sensitive indicator of cellular injury produced by heavy metals. In the current study, lead acetate executed the leaked enzymes leading to increase in activity in the serum and semen, while, on offering vitamin C the hazardous effect was removed, and the leakage was decreased. This was obvious via the decrease in ALT activity in the serum and semen.

Cholinesterases are enzymes, which hydrolyze esters of choline to give choline and the acid. Two types have been distinguished, true and pseudo. True cholinesterase is thought to be responsible for the destruction of acetylcholine.

The neuromuscular junction and is found in nerve tissue and in the red blood cells. Pseudocholinesterases are found in various tissues such as liver, heart muscle and intestine and also present in plasma or serum (Varley, 1976). The heavy metal ions (mercury, lead, cadmium, arsenic and some others) and their organic compounds belong to noncompetitive inhibitors of enzymes. They may block the -SH groups that make part of the catalytic site of the enzyme (Stroev, 1989). A significant reduction in the activity of cholinesterase was found in the serum of bucks received a dose of 15 mg/kg b.wt. This indicates that lead had reduced the cholinesterase in the serum. This may affect the role of pseudocholinesterase in the serum and liver in case of hypnotic's administration, e.g. succinylcholine (muscle relaxants). The same results were obtained in the semen, which indicates that the enzyme behaves like that in the serum and liver.

The acid phosphatase is present in high concentration in the prostate gland, erythrocytes, platelets, reticuloendothelial cells, liver, spleen and kidney. Its increase in the serum may be due to a carcinoma in the prostate, particularly if the cancer has spread beyond the capsule of the gland or has metastasized (Krupp et al., 1987). In the present study, the total and prostatic acid phosphatase activity, in the serum of lead and lead + vitamin C treated groups, were significantly increased than in the control serum. On the other hand, the lead groups treated with vitamin C showed a significantly higher level of the enzyme activity than the lead groups alone, which indicates that a tumor is present in the prostate gland or in the hemopoietic system as the vitamin could not cure it or ameliorate the case.

Moreover, the semen of those groups didn't show significant change only in the high lead dose. This agrees with the findings of Othman and El Missiry (1998).

In conclusion, lead exposure led to testicular disturbed hypo function, which is supported by the results of semen picture. The hazardous effect of lead led to disturbance in the activities of these enzymes under investigation, SOD, □□GT, LDH, AST, ALT, cholinesterase, acid phosphatase, Vitamin C proved its antioxidant effect on recuperating from the normal status of enzymes in semen and serum thus counteracts the hazardous oxidant effect of lead inside the different organs. LDH and the prostatic acid phosphatase are shown to be biomarkers for testicular dysfunction, while the ALT and LDH may be used as biomarkers for hepatic and renal dysfunction. Lead exposure may acutely induce oxidative stress and enhance antioxidant defenses and decreased lipid peroxidation in animal.

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