



Antioxidant Role of Vitamin C in Alleviating the Reproductive Toxicity of Lead Acetate in Male Rats

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ABSTRACT

Environmental pollution with heavy metals represents global problem. One of these heavy metals is the lead acetate that emits from many industries such as paint, ceramics, lead containing pipes and plastics led to a manifold rise in the occurrence of free lead in biological systems and the environment. Exposure to lead acetate affects most of the body's organs especially testes since it has a unique vascular system. Therefore, the present study aimed to evaluate the protective effect of vitamin C against lead acetate induced testicular toxicity in rats. Thirty male adult albino rats were used in this study. They were equally divided into three groups; group I "control group", group II "lead acetate treated group" and group III "lead acetate and vitamin C treated group". Administration of lead acetate (20 mg /kg body weight for 8 successive weeks) resulted in a significant decrease in serum level of testosterone. It also led to a significant increase in the testicular tissue homogenate concentration of MDA and a significant decrease in GSH concentration and catalase activity. Administration of vitamin C (20 mg/kg body weight) with lead acetate for 8 successive weeks succeeded in improving semen quality and antioxidant enzyme concentrations of testes. It can be concluded that lead acetate testicular toxicity in rats led to disturbance in serum level of the main male reproductive hormone and increased testicular contents of oxidative markers and decreased the antioxidant markers. The use of vitamin C improved these changes.

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Introduction

Lead is one of the earliest metals discovered by the human race. It is considered as a potent occupational toxin. Its non-biodegradable nature is the prime reason for its prolonged persistence in the environment. It is considered as the major pollutant of the environment due to its popular use in product manufacture such as paints, batteries, cosmetic products, water pipes, pottery glazing and toys (Dapul and Laraque, 2014). Lead is a multi-organ toxicant involved in various cancers, neuronal, renal damages and reproductive impairment in both human and animals (Flora et al., 2012; Shaffer and Gilbert, 2017). Lead causes a number of adverse effects on the reproductive system including reproduction of libido, abnormal spermatogenesis, chromosomal damage, infertility, abnormal prostatic function and changes in serum testosterone level.

Oxidative stress has been reported as a major mechanism of lead induced toxicity (Flora et al., 2011). Increased levels of

reactive oxygen species (ROS) are the major toxic effects of lead. Reactive oxygen species are by-products of biochemical processes in aerobic organisms and ROS concentration is regulated by antioxidants such as glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) under normal conditions. The imbalance between the production and scavenging of ROS results in the formation of oxidative stress, which could lead to ROS detoxification system impairment and consequently, increase in the production of ROS (Hanas et al., 1999; Flora et al., 2012; Szymanski, 2014). Accordingly, interest has recently grown in the role and usage of natural antioxidants like vitamin C as a strategy to prevent oxidative damage, so present study aimed to examine the meliorating effects of vitamin C on lead induced testicular toxicity in rat.

Materials and methods

Chemicals

Both lead acetate and vitamin C were purchased from sigma company, Cairo, Egypt. Malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase commercial kits were purchased from Bio-diagnostic

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Company for research kits, Egypt.

Experimental animals and design

Thirty male adult albino rats weighing (150.0±180.0 g) were obtained from the animal house, Helwan, Egypt. They were kept under suitable conditions for one week for adaptation. They were maintained in stainless steel cages in a well-ventilated animal house at normal temperature (22.0±5.0 °C) under 12:12 h light dark cycle. They were fed with standard laboratory diet and given water ad libitum. The rats were maintained under standard conditions in an animal house as per the guidelines of Beni-Suef University Committee for the Purpose of Control and Supervision on Experiments on Animals. The rats were randomly divided into three equal groups (10 rats each). Group I (control group) was administered distilled water orally by stomach tube daily for 8 successive weeks. Group II (lead acetate treated group) was administered lead acetate dissolved in distilled water at a dose of 20 mg/kg body weight (Shaban and Said, 2011) orally by stomach tube daily for 8 successive weeks. Group III (lead acetate and vitamin C treated group) was dosed orally with the same previous dose of lead acetate in addition to vitamin C at a dose of 20 mg/kg body weight (Shaban and Said, 2011) by stomach tube daily for 8 successive weeks.

Sampling and biochemical analyses

Rats were sacrificed after 8 weeks and blood samples were collected in clean dry centrifuge tubes. They were left 20 minutes at room temperature to clot, and then centrifuged at 1000 Xg for separation of blood serum. The serum samples were separated in eppendorf tubes and stored at -20°C until used for the biochemical assays. Serum level of testosterone was measured by using enzyme-linked immunosorbent assay (ELISA) kits from Kamiya Biomedical Company (Washington, USA) following the instructions of the manufacturer.

The testes were removed and dissected free from the surrounding fat and connective tissue. 0.5 g of testes was homogenized in 5 ml of phosphate buffered saline (pH 7.4), centrifuged at 5000 rpm for 10 min. at 4 °C and the supernatant was used for quantitative determination of reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase according to Beutler et al. (1963); Satoh (1978); Marklund and Marklund (1974) and Aeb (1984) respectively.

Sperm functions analysis

Sperm count

The Neubauer counting chamber was used in counting the total number of spermatozoa. About 10 ml of the diluted sperm suspension with phosphate buffer saline (pH 7.2) was transferred to each counting chamber of the hemocytometer and was allowed to stand for 5 min then counted under a

binocular light microscope (Raji et al., 2005).

Sperm viability

Sperm viability was investigated using the eosin stain. The staining was conducted with one drop of freshly collected semen and two drops of eosin solution. Quantitative viability expressed as a percentage was determined by counting viable and nonviable spermatozoa per chamber. Viable spermatozoa cannot absorb eosin stain while nonviable spermatozoa can absorb the stain. Sperm viability was defined as the percentage of dead sperm cells (Raji et al., 2005).

Statistical analysis

The values are expressed as Mean ±SEM. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test using Graph Pad Instate software (version 3). Differences were considered significant at P<0.05.

Results

Effect of administration of vitamin C with lead acetate on sperm count, sperm viability percentage and serum testosterone level

The results in Table 1, reported that administration of lead acetate at a dose of 20 mg/kg b.w for 8 weeks led to a significant decrease in sperm count, sperm viability% and serum testosterone concentration in rat group (group II) in comparison to control group (group I). Administration of vitamin C with lead acetate significantly increased the sperm count and testosterone serum concentration in rat group (group III) in comparison to lead acetate group (group II).

Antioxidant effect of vitamin C administration with lead acetate on testicular tissue homogenate concentrations of MDA, GSH, SOD and catalase in different rat groups

Results in Table 2, showed the oxidative effect of lead acetate administration for 8 weeks as there was a significant increase in MDA concentration and a significant decrease in GSH and catalase concentration in testicular homogenate in group II of rats in comparison to control group (group I), while the SOD activity was not changed. Vitamin C administration with lead acetate significantly increased the level of GSH and catalase in testicular homogenate in group III of rats and significantly decreased the MDA level in the same group.

Discussion

Lead toxicity is a common public health threat in developing countries due to human activities such as mining and farming. Exposure to lead have numerous effects on the reproductive system both in human and animals, which includes reduced libido, abnormal spermatogenesis, infertility, changes

Table 1. Sperm count, viability percentage and serum testosterone concentration in different rat groups.

	Group I	Group II	Group III
Sperm count (x10 ⁶ /ml)	18.60±1.80 ^a	10.70±1.36 ^b	17.40±1.24 ^c
Sperm viability (%)	85.00±2.24 ^a	46.00±5.09 ^b	57.00±7.00 ^a
Testosterone (pg/ml)	5.07±0.46 ^a	0.51±0.13 ^b	3.44±0.34 ^c

The data are presented as Mean±S.E., in each row, different superscript means significantly different at p < 0.05.

Group I: Control; Group II: Lead acetate group; Group III: Lead acetate and Vit. C group

Table 2. Testicular homogenate concentrations of MDA, GSH, SOD and catalase in different rat groups.

	Group I	Group II	Group III
MDA (nmol/g tissue)	30.98±3.19 ^a	57.24±2.82 ^b	27.18±3.38 ^c
GSH (mg/g tissue)	21.3±0.52 ^a	6.92±1.13 ^b	19.54±1.84 ^c
SOD (U/g tissue)	254.48±15.43 ^a	230.28±1.92 ^a	243.6±4.32 ^a
Catalase (U/g tissue)	2.52±0.15 ^a	0.33±0.06 ^b	2.56±0.26 ^c

The data are presented as means±S.E., in each row, different superscript means significantly different at $p < 0.05$.

Group I: Control; Group II: Lead acetate group; Group III: Lead acetate and Vit. C group

MDA: Malondialdehyde; GSH: Glutathione; SOD: Superoxide dismutase.

in serum testosterone and abnormal prostatic function (Flora et al., 2012). Lead directly targets testicular spermatogenesis and also the sperms in the epididymis inducing reproductive toxicity (Wadi and Ahmad, 1999). In lead treated groups of rats; suppression of serum testosterone, intratesticular sperm counts, and sperm production were reported (Thoreux-Manlay et al., 1995; Apostoli et al., 1998). That was achieved in the obtained results as recorded in Table 1, which revealed that sperm count, viability percentage and serum testosterone level were significantly reduced in lead administrated group (group II) for 8 weeks. Dapul and Laraque (2014) explained that lead interfere with the cytochrome P450 enzymes in the liver, which affects hormone synthesis, and cholesterol synthesis, the latter acts as a precursor of steroid hormones biosynthesis. Results from this study were supported by Wang et al. (2007), who reported that exposure to lead exhibited a significant decrease in sperm count and motility. Administration of vitamin C significantly improve sperm count and viability as supplemented ascorbic protects the testes (Azari et al., 2014), and maintains the genetic architecture of the sperm cells (Fraga et al., 1991). Thus, decrease in the concentration of testicular ascorbic acid content may predispose the testes to toxic injury.

In the present study, there was a significant increase in MDA concentration and a significant decrease in reduced glutathione concentration in testicular homogenate (Table 2) in lead acetate rat group (group II), which indicated a state of lipid peroxidation and increased oxidative stress (Brochin et al., 2008; Flora et al., 2012; Ebuehi et al., 2012). Lead acetate toxicity inactivate antioxidant enzymes like catalase and SOD enzymes as lead can replace the zinc ions that serve as important co-factor for these enzymes (Flora et al., 2007). Reduction in catalase activity impairs scavenging of superoxide radicals, which enhances the oxidative stress of lead toxicity. Results from this study revealed that catalase activity was significantly decreased in the testicular homogenate in group (II) in comparison to control group (group I), while SOD activity was not changed between different rat groups. Studies have shown that uptake of certain nutrients like mineral elements, flavonoids and vitamins can provide protection from the environmental lead toxicity. These nutrients play a pivotal role in restoring the imbalanced pro-oxidant/oxidant ratio that arises due to oxidative stress (Hsu and Guo, 2002). Vitamin C has the ability to normalize alteration of oxidative stress biomarkers initiated by lead (Chang et al., 2011; Jewo et al., 2012; Ghanwat et al., 2016), so supplementation of ascorbic acid could be the best chelation therapy for lead intoxication (Flora et al., 2003; Wang et al., 2007; Seven et al., 2010). The obtained results (Table 2) confirmed that administration of vitamin C (20 mg/kg b.w.) for 8 weeks for rats resulted in significant increase in testicular homogenate concentration of GSH, catalase and a significant decrease in MDA concentration. The antioxidant effect of vitamin C is due to hydrogen atoms which pairs up with un-

paired electron of the free radicals, converting them to non-free radicals. Vitamin C indirectly quenches free radicals by virtue of regenerating some important antioxidants such as GSH, and vitamin E (Jagetia et al., 2003). The latter is capable of terminating lipid peroxidation chain reactions (Nimse and Pal, 2015).

Conclusion

Administration of lead acetate to rats for 2 months has a bad effect on testes by causing decline in sperm count and viability. It also decreases the antioxidant levels in testicular tissue as glutathione, superoxide dismutase and catalase, and results in increased lipid peroxidation. Administration of vitamin C as a natural antioxidant in combination with lead acetate for 2 months improve semen quality, testosterone level and antioxidant status.

Conflict of interest

Authors declared no conflict of interests exists.

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