ORIGINAL ARTICLE

Protective effect of ellagic acid against lead-induced reproductive toxicity in male rats

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Abstract

Introduction: Infertility affects many couples especially in developing countries and is a reproductive health problem worldwide. The widespread use of heavy metals like lead has led to manifold rise in the occurrence of free lead in biological systems and the inert environment. The detrimental effect on male reproductive system is one of the major evidence of occupational and environmental lead exposure. It has been documented that the presence of higher amounts of lead in the blood of exposed workers compared to control workers is associated with reduced volume of ejaculation, decreased total sperm number and motility, and reduction in semen density, as well as increased percentage of pathological spermatozoa. **Materials and Methods:** The lead-induced reproductive toxicity study was carried out in healthy adult male Wister albino rats. Rat doses of ELLAGIC acid (EA) were selected as 50 mg/kg and 25 mg/kg through oral route. After acclimatization, animals were divided into four random groups of eight animals in each; received normal saline, lead acetate, and EA high and low doses along with lead acetate, respectively, for 70 days. Serum was separated by centrifugation for the estimation of testosterone level by ELISA. Thereafter, the animals were sacrificed; testis was weighed and used for the epididymal sperm count, motility, and viability and then for preparation of homogenate to estimate antioxidants such as super oxide dismutase, catalase, and thiobarbituric acid reactive substances. Remaining testis was embedded in formaline in saline solution (10%) for histological examination. **Results, Discussion, and Conclusion:** Observed results suggested dose dependent beneficial effects for EA against lead acetate induced male reproductive toxicity and it was concluded that EA exhibited dose-dependent protection against lead-induced male reproductive toxicity.

Key words: Ellagic acid, heavy metal toxicity, lead acetate, male reproductive toxicity

INTRODUCTION

Male and female reproductive systems are one of the major target sites of lead-induced toxicities.[1] It is already proved that acute or chronic lead exposure can alter testicular functions in humans as well as wild life.[2,3] It was found that that the presence of higher amounts of lead in the blood of exposed workers was associated with reduced volume of ejaculation, semen density, total sperm number and motility, and increased percentage of pathological spermatozoa.[4] Some of the other effects of the high levels of blood lead include reduced sex drive, spermatogenesis, damage to the chromosome, infertility, and alteration in serum testosterone level. In one study, it was observed that the high levels of lead in semen reduce the sperm count, contributing to its infertility.[5]

Several reports suggest that workers exposed to lead suffered with oligospermia and asthenozoospermia, with altered sperm morphology.[6,7] It is also seen that lead toxicity can even extend to epididymis and results in altered sperm maturity.[8] Lead is also considered as endocrine disruptor modifying hormonal metabolism by altering synthesis and breakdown of testosterone, follicle stimulating hormone, and luteinizing hormone.[9] Hence, it appears that lead burden disturbs hormone-mediated spermatogenesis and steroidogenesis of male reproduction.[10]

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Received: 09-04-2022
Revised: 24-05-2022
Accepted: 06-06-2022

Polyphenolic compounds contain many phenolic groups and they are extensively available in different plants, fruits, and vegetables. They exhibit well established protection against many diseased condition affecting different major organs.[11,12] Polyphone has been already reported to be beneficial in heavy metal toxicity. Polyphenols also cause detoxification and help to remove of heavy metals.[13] They show the protection probably by scavenging reactive oxygen species, produced by lead and other heavy metals. It is also reported that they are responsible for detoxification by accumulated heavy metal removal from major organs.[11,14]

Ellagic acid (EA) is an important component of different fruits, nuts, and vegetables and is abundantly found in pomegranates, raspberries, black raspberries, blackberries, strawberries, red and white guava, beefsteak fungus, cranberries, walnuts, and almonds.[15,16] It is a polyphenolic phytochemical well known for several medicinal properties.[17] The present study has been undertaken to explore the protective effect of EA against lead-induced male reproductive toxicity.

MATERIALS AND METHODS

Chemicals

All analytical grade chemicals were purchased from standard companies. Lead acetate was purchased from Loba Chemicals, Mumbai and the biochemical kits were procured from Crest Biosystems, Goa, India.

Phyto-Chemicals

From Yucca Enterprises, Mumbai, India, EA sample was procured.

Experimental Animals

Healthy adult male Wistar albino rats approximately in the range of 170–200 g were housed in polypropylene cages, under standardized condition (12 h L: D cycles, 25 ± 5°C) with paddy husk bedding at the Institutional Central Animal House. They received standard pellet food and free access to purified drinking water. Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines from Ministry of Social Justice and Empowerment, Government of India were followed with prior permission from the Institutional Animal Ethics Committee for conducting the study.

Dose Selection

EA

Based on the earlier literature, rat doses of EA were selected as 50 mg/kg and 25 mg/kg through oral route and termed as high and low dose, respectively.[18]

Experimental Protocol

After 1 week of acclimatization, the animals were randomly divided into six groups of eight animals in each. Group I served as normal control and received normal saline 2 ml/kg, *p.o.* through oral route. Group II served as toxic control and animals were treated by 0.1% lead acetate in drinking water for 70 days.[19] Group III and Group IV animals received EA 50 mg/kg and 25 mg/kg *p.o.,* respectively, for 70 days along with that lead acetate was administered same as in Group II. Food was withdrawn 12 h before lead acetate administration.

After 24 h of the last treatments, the animals were subjected for aesthesia with ketamine (75 mg/kg, i.p.) and xylazine (8 mg/kg, i.p.) and blood sample was withdrawn by retro-orbital puncture. Serum was separated by centrifugation to estimate testosterone level by ELISA. Soon after that, the animals were sacrificed; testis was weighed and subjected for the epididymal sperm count, motility, and viability and then for homogenate preparation for the estimation of antioxidants such as superoxide dismutase (SOD), catalase, and thiobarbituric acid reactive substances (TBARS).[20,21]

Evaluation of Sperm Parameters of Testicular Functions

The epididymis was minced with anatomical scissors to obtain epididymal spermatozoa in Ham's F12 medium, 5 ml and subjected for incubation for 2 min at 32°C. A little quantity was removed and taken in Neubauer hemocytometer and using light microscope at ×400 magnification motile sperms were determined. Before counting total sperm, non-motile sperm numbers were first counted. The sperm motility was expressed from the total number of sperm as a percent of motile sperm.[20]

Preparation of Testicular Tissue Homogenate (TTH)

The testes were removed gently, rinsed with physiological saline solution (0.9% NaCl) for removal of other debris adhering them. Then, weight of each testis was taken and quickly subjected for homogenization in 50 mM potassium phosphate (pH 7.4) to obtain 10% homogenate. Then, the homogenate was subjected for centrifugation at 4000 rpm for 15 min at 4°C. The supernatants were used for the estimation of SOD, catalase, and TBARS.[21]

Statistical Analysis

Results were expressed as mean ± SEM. The assessment of statistical significance was done using one-way analysis of variance followed by Tukey-Kramer multiple comparison tests. $P < 0.05$ was considered significant.

RESULTS

Effect on Serum Testosterone [Table 1]

Marked reduction in plasma testosterone level was observed in lead treatment group compared to control group after 70 days exposure to 0.1% lead acetate. The treatment groups significantly ($P < 0.01$) restored the plasma testosterone level compared to toxic control group.

Effect on Antioxidants in TTH

Effect on SOD and Catalase [Table 2]

Toxic control group reported extremely significant ($P < 0.001$) decrease in SOD and catalase activity compared to normal control.

Experimental groups EA 50 and EA 25 demonstrated extremely significant increase ($P < 0.001$) in SOD and catalase values compared to toxic control group.

Effect on TBARS [Table 2]

Toxic control group demonstrated extremely significant ($P < 0.001$) increase in TBARS activity compared to normal control. EA 50 treatment group showed extremely significant ($P < 0.001$) where as for EA 25 treated group, it was found to be moderately significant ($P < 0.01$) decrease in TBARS activity compared to toxic control group.

Effect on Sperm Parameters of Testicular Functions [Table 3]

Oral administration of lead to male rats caused a significant ($P < 0.05$) decrease in sperm count, sperm motility, and viability in toxic control group compared with the control group. On the other hand, pre-treatment of male rats with EA 50 and EA 25 caused a statistically extremely significant ($P < 0.001$) increase in sperm count, sperm motility, and viability in a dose-dependent relation when compared with the toxic control group.

Effect on Testis Weight [Table 4] in TTH Against Lead Acetate-induced Male Reproductive Toxicity

Toxic control (only lead acetate treated) group demonstrated extremely significant ($P < 0.001$) decrease in testis weight compared to normal control. Treatment groups such as EA 50, EA 25 showed extremely significant ($P < 0.001$) increase in testis weight compared to toxic control.

Table 1: Effect on plasma testosterone level in lead acetate induced male reproductive toxicity

Treatment	Plasma testosterone levels (nmol/l)
Normal control	05.82±2.91
Toxic control	2.84±1.35***
EA 50	4.67±1.84##
EA 25	3.83±1.42##

All values are mean±SEM, *n*=8, ***P<0.001 when compared to normal control; ##P<0.01 compared to toxic control group

Table 2: Effect on antioxidants in TTH and histological score against lead acetate-induced male reproductive toxicity

Treatment	Testicular tissue homogenate (units/mg of protein)		
	SOD	Catalase	TBARS
Normal control	40.3±1.3	16.5±1.9	2.9±0.01
Toxic control	16.8±0.7***	8.8±0.2***	6.3±0.4***
EA 50	36.5±0.4###	14.8±0.2###	3.6±0.5###
EA 25	34.3±0.6###	13.6±0.4###	4.2±0.6##

All values are mean±SEM, n=8, ***P<0.001 when compared to normal control, ###P<0.001, ##P<0.01 compared to toxic control group

Table 3: Estimation of epipidymal sperm count, motility, and viability

Treatment	Epididymal sperm count (10^6/mL)	Sperm viability (%)	Sperm motility (%)
Normal control	35.5±1.5	90.5±1.3	80.2±2.3
Toxic control	15.5±2.3***	45.3±2.8***	40.5±3.3***
EA 50	30.7±1.3###	80.8±1.6###	65.7±1.3###
EA 25	28.3±2.5###	73.5±1.5###	62.3±3.0###

All values are mean±SEM, *n*=8, ***P<0.001 when compared to normal control, ###P<0.001 compared to toxic control group

Table 4: Effect on testis weight and antioxidants in TTH against lead acetate-induced male reproductive toxicity

Group	Weight (gram) of testes
Normal control	2.6±0.4
Toxic control	2.0±0.2***
EA 50	2.4±0.1###
EA 25	2.3±0.2###

All values are mean±SEM, *n*=8, ***P<0.001 when compared to normal control, ###P<0.001, ##P<0.01 compared to toxic control group

DISCUSSION

Lead is one of environmental contaminants and ubiquitous industrial pollutant in the ecosystem, producing severe organ

damage in animals and humans. Lead is a harmful metal with no known physiological benefit. The present study was designed for the investigation of beneficial role of EA against lead acetate-induced male reproductive toxicity. Observed results suggested dose dependent beneficial effects for EA against lead acetate-induced male reproductive toxicity.

Many studies on male animal reproductive system have shown lead as a toxicant for reproductive functions. The reason can be explained as lead may have a direct negative effect on sperm and histological structure of testis. The reduction in sperm motility and quality may be due to indirect effects of lead, such as increased generation of reactive oxygen substances (ROS) in sperm cells. ROS can alter the cellular membrane integrity and the fluidity by lipid oxidation. The cell membrane is very important for motility of sperm, the structural integrity, and also for sperm viability. The results obtained here clearly gave evidence that sperm count, sperm motility, and sperm viability were significantly decreased in rats exposed to lead. In the result, the testis weight was significantly reduced in lead treated rats in comparison with the control group.[22,23]

Reduction in testosterone levels found in lead-treated rats is one indicators of the chemical toxicity on reproductive system.[24] The adequate bioavailability of testosterone plays an important role to maintain structural and functional integrity of reproductive organs.[25] Inadequate circulating male hormone may be responsible for decrease in weights of testes. Testosterone is essential to maintain the structure and function of the male accessory sex glands such as prostate and seminal vesicle. These require androgen for development, differentiation, and maintenance of epithelial cells.[26] It can be said that decrease in prostate weight in lead-treated rats may be due to reduced levels of testosterone.

From the result, it was evident that the SOD and catalase levels are markedly decreased in rats treated with lead acetate. Lead acetate may be responsible for free radical mediated tissue damage by two different ways: One is by causing direct reduction of antioxidant reserves and another is by increased generation of singlet oxygen, hydroperoxides, and hydrogen peroxides (ROS). Apart from that, decrease in serum total antioxidants and circulating antioxidants authenticate the lead acetate-induced reduction of antioxidants. There is a significant decrease in antioxidant enzymes like SOD and catalase in mitochondrial and post-mitochondrial testicular fraction of rats treated with lead. Significant decrease in antioxidant enzymes levels such as SOD and catalase had been evident in the testes of lead exposed rats. In this study, when the EA and lead acetate were administrated to rats, the level of SOD was increased compared to its level in rats treated only with lead.[27]

CONCLUSION

It can be assumed from the performed study that EA exhibited dose-dependent protection against male reproductive toxicity induced by lead through their antioxidant mechanisms from the oxygen-derived free radicals. Findings of this study may be beneficial for the people chronically exposed to the high level lead and EA in the form of formulation or through the dietary source can keep their reproductive system healthy and safe. The future studies can be designed to clinically prove the fact.

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Source of Support: Nil. **Conflicts of Interest:** None declared.