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# Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats

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ABSTRACT

The aim of this study was to investigate the protective effects of cinnamon on lead acetate induced reproductive toxicities in rats. Thirty-two male rats were randomly divided into 4 groups, 8 rats in each. Control rats received distilled water, while treated rats received lead acetate (30 mg/kg), cinnamon (250 mg/kg) and lead acetate and cinnamon (30 mg/kg and 250 mg/kg) for 60 days by gavage tube. In cinnamon treated rats, the relative weights of testes, epididymis, seminal and prostate glands were significantly ($P < 0.05$) increased compared with that in lead acetate treated rats. Sperm cell concentration and viability were significantly ($P < 0.05$) reduced, while sperm abnormalities were significantly ($P < 0.05$) increased in lead treated rats. The superoxide dismutase (SOD) and catalase activities were significantly reduced ($P < 0.001$) in lead acetate treated rats compared to the other groups, while the addition of cinnamon to lead acetate improved the level of SOD compared to the lead treated group. There was a marked reduction ($P < 0.001$) in the expression of androgen receptor and significant ($P < 0.001$) increase in the level of caspase-3 protein expression in the testis of lead treated rats. In conclusion, cinnamon exhibited protective effect on reproductive system by inhibiting lead acetate induced oxidative stress and excessive cell apoptosis.



## 1. Introduction

The diverse deleterious health effects upon exposure to heavy metals in the environment are a matter of serious concern and a global issue. Lead is the most abundant toxic metal in the environment [1]. Lead occurs naturally in the environment. However, most of the high levels found throughout the environment come from human activities. Environmental levels of lead have increased more than 1000-fold over the past three centuries as a result of human activity. The greatest increase occurred between the years 1950 and 2000, and reflected increasing worldwide use of leaded gasoline [2].

Lead does not have any detectable beneficial biological role, however on the contrary its detrimental effect on physiological, biochemical and behavioral dysfunctions have been documented in animals and humans by several investigators [3,4]. Lead is a male reproductive toxicant [5]. Toxicity is manifested in male reproductive function by deposition of lead in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate. Lead has an adverse effect on sperm count, sperm motility and retarded the activity of spermatozoa [6]. The effect of lead on testis is still a matter of controversy where exposure to low dose of lead was

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found to arrest spermatogenesis [7] or to have no effect [8].

The mechanism of lead-induced oxidative stress involves an imbalance between generation and removal of ROS (reactive oxygen species) in tissues and cellular components causing damage to membranes, DNA and proteins [1]. Lead is reported to cause oxidative stress by generating the release of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides [9]. Lead acetate enhances lipid peroxidation and nitric oxide production in both serum and testes with concomitant reduction in antioxidant enzymes as catalase and superoxide dismutase [10].

The androgen receptor (AR) plays a key role in androgen action. In the male reproductive system, the testis and epididymis are major targets of androgen action, and androgen is critical for maintenance of spermatogenesis and secretory function in epididymal epithelial cells [11]. Caspases are a family of genes important for maintaining homeostasis through regulating cell death and inflammation [12].

There has been increased interest among phytotherapy researchers to use medicinal plants with antioxidant activity for protection against heavy metal toxicity [9]. Cinnamon (*Cinnamomum zeylanicum*), a medicinal plant belongs to *Luaraceae* family. This plant has many therapeutic effects. One of its most important effects is its impact on the increase of sexual ability [26]. Limited data are available on the protective effect of this substance against the toxicity of heavy metals on male reproduction. Administration of cinnamon extract before exposure to lead could reduce many of its side effects. Therefore, the present study was carried out to investigate the protective role of cinnamon extract against the effect of lead acetate on testicular functions, superoxide dismutase, expression of androgen receptor and casapase-3 in adult male albino rats.

## 2. Materials and methods

### 2.1. Preparation of materials

Lead acetate trihydrate was obtained from Oxford Lab. Co., India (CAS: 6080-56-4). Lead acetate was dissolved in distilled water at concentration of 30 mg/kg body weight of 1% solution and administrated to rats by gavage tube. For preparation of cinnamon extract, values of 10 g cinnamon was weighed and added to 100 ml of boiling distilled water. Then the solution was cleared with filter paper and was ready for administration by gavage tube. The dose of cinnamon was 250 mg/kg body weight.

### 2.2. Animals and housing

A total number of 32 adult male albino rats were used in the present study and their weight ranged between 130 and 150 g. Animals were raised at Faculty of Veterinary Medicine, Suez Canal University, Egypt. They were maintained in stainless steel cages with wood shavings. Food and water were supplied *ad libitum*. Rats were housed at a controlled temperature of $26 \pm 1\,^{\circ}C$, 60% humidity and under a 12 h light: 12 h dark schedule. The animals were divided into 4 groups. The first one ($n = 8$) were used as control and received only distilled water. The second one ($n = 8$) were administrated lead acetate at concentration of 30 mg/kg body weight of 1% solution by gavage tube. The third one ($n = 8$) were administrated cinnamon extract (250 mg/kg body weight) by gavage tube. The fourth one ($n = 8$) were administrated lead acetate at concentration of 30 mg/kg body weight of 1% solution and cinnamon extract (250 mg/kg body weight) by gavage tube for 60 days.

### 2.3. Organ relative weights

At the end of the study period, rats were euthanized and organs were dissected. Testes, tail of the epididymis, seminal and prostate glands are removed and weighed. The organ relative weights (organ weight/body weight $\times$ 100) were measured for each rat in treated and control groups.

### 2.4. Sperm concentration and morphology assay

The content of epididymis was obtained by cutting of the cuda epididymis using surgical blades then squeezed in a sterile clean watch glass. This content was diluted 5 times with 2.9% sodium citrate dihydrate solution and thoroughly mixed to estimate the sperm concentration [13]. One drop of the suspension was smeared on a glass slide and stained by Eosin Nigrosin stain to determine the viability and sperm abnormalities using the criteria of Okamura et al. [14].

### 2.5. Testicular superoxide dismutase (SOD) and catalase assay

Specimens from testis were collected from all experimental and control groups. The tissues were homogenized in 50 mM potassium phosphate (pH 7.4). The samples were centrifuged at 4000 rpm for 15 min, at $4\,^{\circ}C$ and the supernatants were stored at $-80\,^{\circ}C$ until analysis. SOD (Biodiagnostic, Egypt) was done according to Nishikimi et al. [15] at absorbance 560 nm over 5 min. The method based on the ability of the enzyme to inhibit the phenazine methosulphate–mediated reduction of nitro blue tetrazolium dye. Catalase (Biodiagnostic, Egypt) was carried out according to Aebi [16] at absorbance of 510. The method based on the reaction of catalase with a known quantity of $H_2O_2$. The reaction was stopped after one min., with catalase inhibitor.

### 2.6. Histopathology

Specimens from testis were collected from all experimental and control groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70–100%) and then prepared using standard procedures for Hematoxylin and Eosin stain as described by Bancroft et al. [17].

### 2.7. Immunohistochemistry of androgen receptor and caspase-3

The paraffin embedded testis were cut into 5 μm sections and mounted on positively charged slides for both androgen receptors and caspase-3 immunohistochemistry.