Prophylactic Efficacy of *Coriandrum sativum* (Coriander) on Testis of Lead-Exposed Mice

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Abstract Lead poisoning is a worldwide health problem, and its treatment is under investigation. The aim of this study was to access the efficacy of *Coriandrum sativum* (coriander) in reducing lead-induced changes in mice testis. Animal exposed to lead nitrate showed significant decrease in testicular SOD, CAT, GSH, total protein, and tissue lead level. This was accompanied by simultaneous increase in the activities of LPO, AST, ALT, ACP, ALP, and cholesterol level. Serum testosterone level and sperm density were suppressed in lead-treated group compared with the control. These influences of lead were prevented by concurrent daily administration of *C. sativum* extracts to some extent. Treating albino mice with lead-induced various histological changes in the testis and treatment with coriander led to an improvement in the histological testis picture. The results thus led us to conclude that administration of *C. sativum* significantly protects against lead-induced oxidative stress. Further work need to be done to isolate and purify the active principle involved in the antioxidant activity of this plant.

Keywords *Coriandrum sativum* · Testis · Lead nitrate · Histology · Mice

Introduction

Coriandrum sativum (common name: coriander), belonging to family Umbelliferae, is an herb that is widely cultivated in India and is recognized for its carminative and cooling properties [1]. Both the leaves and seeds of the plant are used for medicinal purpose. Coriander contains many active principles, primarily isocoumarines [2], and the most important one is coriandrin. In the field of alternative medicine, coriander has immense value for the treatment of abdominal problems, especially stomach ulcers. The hypotensive [3] and hypoglycemic [4] properties of coriander have already been recognized. The ability of coriander to increase levels of antioxidant enzymes and to

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manipulate lipid metabolism have also been reported [5, 6]. In Ayurvedic literature, the regular use of the decotation of the seeds of coriander is considered to be effective in lowering blood lipid levels [1]. It has also been speculated that Chinese parsley may enhance the excretion of heavy metals in the urine of patients with various infections and augments the efficacy of antibiotics [7, 8].

Of all the heavy metals that contaminate the environment and pose hazards to public health, lead has been of major concern. Lead is an environmental pollutant and metabolic poison with a variety of toxic effects, among which is its adverse influence on reproduction [9]. Numerous studies [9–13] over the year have investigated the detrimental effects of lead on reproductive function. Many studies have shown that reproductive toxicity is an important feature of lead toxicity [14–16]. During lead exposure, it accumulates in testis in a dose-dependent manner [14, 15]. Lead toxicity induces a significant increase in apoptotic cell death in seminiferous tubules of young growing rats [14]. It is also associated with disruption of spermatogenesis and histoarchitecture and lowered enzyme activities in testis [15]. A direct testicular toxicity may occur via the hypothalamic–pituitary–testicular axis [17, 18].

Recent studies have proposed that one possible mechanism of lead toxicity is the disturbance of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS) [19, 20]. This can evoke the oxidative damage of critical biomolecules such as lipids, proteins, and DNA. It has been also reported that lead exposure has a dose-response relationship with changes in antioxidant enzyme levels and their activities [21]. These enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), expressions of which were found to be changed in animals and in workers exposed to lead [22–24].

Present investigation attempts to reveal the efficacy of the extracts of *C. sativum* if any, in the regulation of lead toxicity.

Material and Methods

Chemicals

Lead nitrate was purchased from Central Drug House (India). All other chemicals used in the study were of analytical reagent and obtained from Sisco Research Laboratories (India), Qualigens (India/ Germany), SD fine chemicals (India), HIMEDIA (India), and Central Drug House (India).

Experimental Plant

The plant *C. sativum* (seeds) was collected from Krishi Vigyan Kendra, Banasthali University, Rajasthan, India, and was identified as an RCR 435 variety. Coriander seeds contain moisture (6.3%), protein (11–17%), volatile oil (0.3%), nonvolatile ether extract (22%), ether extract (19.6%), crude fiber (31.5%), carbohydrates (24%), ash (5.3%), calcium (0.08%), phosphorus (0.44%), sodium (0.02%), potassium (1.2%), vitamin B_1 (0.26 mg/100 g), vitamin B_2 (0.23 mg/100 mg), niacin (3.2 mg/100 g), vitamin C (ascorbic acid; 12 mg/ 100 g), vitamin A (175 IU/100 g), flavonoid glycosides (26%) [25], and essential oil linalool (60–80%), alpha-pinene (0.2–8.5%), gamma-terpinene (1–8%), geranylacetate (0.1–4.7%), camphor (1.4%), and geraniol (1.2–4.6%) [3]. Aqueous and alcoholic coriander extracts were prepared for the experimental purpose.

Preparation of Aqueous Extract of *C. sativum*

Dried coriander seeds were ground to a fine powder, of which 100 g were added to 500 ml distilled water. After 24 h, maceration was done at room temperature (37°C); the mixture was then heated for 30 min in the water bath at 65°C. The extract was filtered, concentrated by heating over the water bath (65°C) and dried under vacuum [26] with the yield of 5.9% (*w/w*). The extract was stored at 4°C and used to treat animals as needed.

Preparation of Alcoholic Extraction of *C. sativum*

The dried and powered seeds (200 g) were extracted successively with ethanol (800 ml) in a soxhlet extractor for 48 h at 60°C. After extraction, the solvent was evaporated to dryness at 50–55°C by using a rotary evaporator, and the extract left behind (yield was 9.8%) was stored at 4°C. It was dissolved in distilled water whenever needed for experiments.

Animals and Treatment

Male Swiss albino mice (*Mus musculus*) weighing 15–30 g (2–2.5 months old) were obtained from Haryana Agricultural University, Hisar (India), for experimental purpose. The animals were acclimatized for 15–25 days prior to experiment. The Institutional Animal Ethical Committee has approved the animal studies. Colony-bred adult male albino mice were maintained under standard laboratory conditions at a temperature of 25°C±3°C, relative humidity of 50%±15%, and photoperiod of 12 h (12 h-light and 12 h-dark cycle). The mice were housed in polypropylene cages. Animals had free access to standard food pellet diet (Hindustan Lever Limited; metal contents in parts per million dry weight: Cu 10.0, Zn 45.0, Mn 55.0, Co 5.0, Fe 75.0) and drinking water ad libitum throughout the study. Essential cleanliness and, to the best extent, sterile conditions were also adopted according to SPF facilities.

Experimental Design

Adult Swiss albino male mice were divided into six groups of 12 mice each and treated by oral gavage as follows:

Group I-   Control (normal, untreated), received distilled water;
Group II-   Lead nitrate treated group, received freshly dissolved $Pb(NO_3)_2$ in 1 ml distilled water at a dose of 40 mg/kg body weight per day for 40 days;
Group III-   After exposure to lead nitrate (40 mg/kg body weight per day for 7 days), the animals received 1 ml of aqueous coriander extract at a dose of 300 mg/kg body weight per day and lead nitrate simultaneously for 33 days;
Group IV-   After exposure to lead nitrate (40 mg/kg body weight per day for 7 days), the animals received 1 ml of aqueous coriander extract at a dose of 600 mg/kg body weight per day and lead nitrate simultaneously for 33 days;
Group V-   After exposure to lead nitrate (40 mg/kg body weight per day for 7 days), the animals received 1 ml of ethanolic coriander extract at a dose of 250 mg/kg body weight per day and lead nitrate simultaneously for 33 days;

Group VI- After exposure to lead nitrate (40 mg/kg body weight per day for 7 days), the animals received 1 ml of ethanolic coriander extract at a dose of 500 mg/kg body weight per day and lead nitrate simultaneously for 33 days;

The concentration of lead nitrate used in the experiment was 1/56 of LD_{50} [27]. The dose for lead nitrate was decided on the basis of previously performed experiments in our own laboratory. The plant doses were selected on the basis of experiments conducted in our own laboratory and on the basis of earlier published reports [28]. After the administration of the last dose, the animals were given a 1-day rest and killed under light chloroform anesthesia. Blood was collected for serum. The testis tissue was excised and divided into two parts; half a portion of the testis from each animal was processed for biochemical variables and histological analysis and the other half portion of testis was stored at −20°C before wet acid digestion with HNO_3 for lead estimation.

Preparation of Testis Homogenate

Testes were sliced into pieces and homogenized with a blender in ice-cold 0.1 M sodium phosphate buffer (pH 7.4) at 1–4°C to give 10% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 15–20 min at 4°C twice to get enzyme fraction. The resulting supernatants were separated and used for various biochemical estimations as depicted below:

Biochemical Assays

Lipid peroxidation (LPO) was estimated by thiobarbituric acid reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids as described earlier by Nwanjo and Ojiako [29]. The testicular SOD activity was assayed according to the method of Marklund and Marklund [30]. CAT activity was assayed following the procedure of Aebi [31]. Glutathione (GSH) content was determined by the method of Ellman [32]. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel [33]. Activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined according to the protocol described in the laboratory practical manual by Sadashivam [34]. The protein content was determined by using bovine serum albumin as a standard by the method of Lowry et al. [35]. The cholesterol level was determined by using cholesterol as a standard by the method of Zak's [36].

Determination of Testosterone Level

Serum level of testosterone was assayed following the standard protocol as supplied through the respective ELISA kit.

Sperm Density

Sperm density was determined in testis according to the method given in modern experimental Zoology book of Gupta and Chaturvedi [37]. To 100 mg of the tissue (testis), 2 ml of normal saline was added. Then it was teased with a needle. In brief, 0.05 ml of this tissue was taken and diluted with $NaHCO_3$ up to 11 marks on WBC pipette. Then, one drop was put on the already focused slide, and sperms were counted in 16 squares.

Metal Estimation

Lead concentration in testis was measured after wet acid digestion. Lead was estimated using a hydride vapor generation system (model MHS-10, Perkin Elmer) fitted with an atomic absorption spectrophotometer (model A Analyst 100, Perkin Elmer).

Histological Analysis

Testes were removed, washed in saline, and fixed in 10% formalin at room temperature for 72 h. After fixing the tissue, it was thoroughly washed under running water and dehydrated in ascending grades of ethyl alcohol, cleared, and then embedded in soft paraffin. Tissue sections of about 6 μm were obtained, stained by hematoxylin and eosin, and examined under light microscope.

Statistical Analysis

Data are expressed as the mean±SEM. The data were analyzed using the Statistical Package for Social Science program (SPSS 11). Statistical analysis was done using analysis of variance followed by Tukey's test, and the level of significance was set at $p<0.05$.

Results

Lipid Peroxidation, Antioxidant Enzymes and GSH Level

Table 1 illustrates the effect of lead nitrate alone, and effect of treatment with *C. sativum* extracts individually during lead nitrate exposure on lipid peroxidation, activity of antioxidant enzymes (SOD and CAT), and level of nonantioxidant enzyme (GSH) in control and experimental groups of animals.

The level of lipid peroxidation was significantly higher ($p<0.001$) in lead-treated animals (group II) than that of normal untreated mice. Whereas, significant decrease ($p<0.001$) in testis SOD, CAT activity, and GSH content of mice were observed in lead nitrate-treated animals as compared with control group. After the treatment with aqueous coriander extract, a significant decrease ($p<0.001$ for both low- and high-dose groups) in the level of LPO was observed in comparison to lead nitrate-treated group. Administration of ethanolic extract of plant to animals also improved LPO level as compared with lead group ($p<0.001$ for both low- and high-dose groups).

In comparison to lead-exposed group, administration of aqueous and ethanolic coriander extract at low and high doses improved SOD and GSH content insignificantly ($p>0.05$). However, at a high dose of aqueous coriander extract, CAT activity increased significantly ($p<0.05$), but, in low-dose treated group, it increased insignificantly ($p>0.05$) in comparison to the lead-treated group. With ethanolic extract of coriander, CAT activity was also recovered in animal groups V and VI when compared with group II ($p<0.001$ for both low and high doses).

Biochemical Parameters

Table 2 represents the effect of lead nitrate alone and effect of treatment with *C. sativum* extracts individually during lead nitrate exposure on some biochemical parameters such as

Table 1 Prophylactic Efficacy of C. sativum Extracts on Lipid Peroxidation and Antioxidant-Related Variables in Testis Tissue of Lead Nitrate-Exposed Mice

Parameters	Control Mice Group (Normal, Untreated)	Treated Mice Groups				
	Group I Distilled Water	Group II Lead Nitrate	Group III Lead Nitrate+aqueous Coriander Extract, Low Dose	Group IV Lead Nitrate+aqueous Coriander Extract, High Dose	Group V Lead Nitrate+ethanolic Coriander Extract, Low Dose	Group VI Lead Nitrate+ethanolic Coriander Extract, High Dose
LPO (MDA formed nmole/g	110.39±4.44	153.86±0.84* (+39.37%)	140.77±0.63** (−8.50%)	135.04±0.38** (−12.23%)	125.16±0.62** (−18.65%)	121.35±0.67** (−21.12%)
SOD, unit/ml of tissue extract	1.12±0.03	0.89±0.02 (−20.53%)	0.91±0.005 (+2.24%)	0.93±0.007 (+4.49%)	0.96±0.006 (+7.86%)	1.06±0.15 (+19.10%)
CAT, μmoles of H_2O_2 degraded/min/ mg protein	37.21±0.81	24.94±0.36* (−32.97%)	25.06±0.47 (+0.48%)	26.51±0.41*** (+6.29%)	30.34±0.36** (+21.65%)	31.43±0.32** (+26.02%)
GSH, mg/g tissue	2.55±0.15	1.76±0.15* (−30.98%)	1.57±0.11 (+10.79%)	1.85±0.05 (+5.11%)	2.04±0.05 (+15.90%)	2.10±0.04 (+19.31%)

Values are Mean±SEM; $n=12$

* $p<0.001$ compared with normal animals

** $p<0.001$ compared with lead-exposed animals

*** $p<0.05$ compared with lead-exposed animals

Table 2 Prophylactic Efficacy of *C. sativum* Extracts on Some Biochemical Parameters in Testes Tissue of Lead Nitrate-Exposed Mice

Parameters	Control Mice Group (Normal, Untreated)	Treated Mice Groups				
	Group I Distilled Water	Group II Lead Nitrate	Group III Lead Nitrate+aqueous Coriander Extract, Low Dose	Group IV Lead Nitrate+aqueous Coriander Extract, High Dose	Group V Lead Nitrate+ethanolic Coriander Extract, Low Dose	Group VI Lead Nitrate+ethanolic Coriander Extract, High Dose
AST (IU/L)	15.39±0.50	52.22±0.92* (+239.31%)	35.14±1.06** (−32.70%)	32.19±1.03** (−38.35%)	28.78±0.48** (−45.51%)	26.86±0.55** (−48.56%)
ALT (IU/L)	12.11±0.43	34.11±0.79* (+181.66%)	25.35±0.45** (−25.68%)	22.50±0.47** (−34.03%)	21.04±0.40** (−38.31%)	18.72±0.61** (−45.11%)
ACP, μmoles of PNP formed/min/g tissue	11.01±0.02	31.02±0.03* (+181.74%)	26.30±0.16** (−15.21%)	19.97±0.06** (−35.62%)	28.09±0.18** (−9.44%)	18.94±0.04** (−38.94%)
ALP, μmoles of PNP formed/min/g tissue	11.08±0.04	26.50±0.15* (+139.16%)	22.29±0.09** (−15.88%)	19.46±0.13** (−26.56%)	21.28±0.23** (−19.69%)	17.75±0.13** (−33.01%)
Protein, g/100 ml	4.83±0.27	1.05±0.05 (−78.26%)	1.64±0.05 (+56.19%)	1.77±0.05 (+68.57%)	2.88±0.04*** (+174.28%)	3.19±0.08*** (+203.80%)
Cholesterol, mg/g	31.37±1.95	36.29±1.78 (+15.68%)	35.97±0.29 (−0.88%)	35.81±0.36 (−1.32%)	32.87±0.51 (−9.42%)	31.09±0.56**** (−14.32%)

Values are mean±SEM; $n=12$

*$p<0.001$ compared with normal animals

**$p<0.001$ compared with lead-exposed animals

***$p<0.01$ compared with lead-exposed animals

****$p<0.05$ compared with lead-exposed animals

AST, ALT, ACP, and ALP and total protein and total cholesterol levels in testicular tissue of control and experimental groups of animals.

It is also clear from the results that treatment with lead nitrate showed a significant increase in parameters which include AST, ALT, ACP, ALP, and total cholesterol level as compared with the control group ($p<0.001$). Total protein concentration was significantly lower in lead group than in control.

Administration of aqueous extract of coriander showed significant decrease ($p<0.001$ for both low and high doses) in AST, ALT, ACP, and ALP when compared with lead nitrate-treated group. On the other hand, treatment with ethanolic coriander extract also increased these values significantly as compared with group II ($p<0.001$).

Whereas, cholesterol level diminished insignificantly in testis tissue ($p>0.05$), after the administration of aqueous plant extract in both low- and high-dose groups. Supplementation of ethanolic coriander extract decreased cholesterol level significantly in the high-dose group ($p<0.05$) but insignificantly in low-dose group ($p>0.05$), when compared with lead-induced group (II).

On administration of aqueous coriander extract along with lead nitrate, total protein increased insignificantly ($p>0.05$ for both low- and high-dose groups) as compared with the lead-treated group. Supplementation with ethanolic coriander extract significantly increased ($p<0.01$ for both low and high doses) total protein content as compared with group II.

Table 3 represents the effect of lead nitrate alone and effect of treatment with *C. sativum* extracts individually during lead nitrate exposure on serum testosterone level, sperm density, and lead nitrate concentration in testes of control and experimental group of animals.

Serum Testosterone

Testosterone level was significantly ($p<0.001$) lower in lead group than in control group. Whereas, administration of plant extracts individually in combination with lead nitrate to groups III and IV increased testosterone level, but results were insignificant ($p>0.05$). However, upon treatment with ethanolic coriander extract at low and high doses, insignificant increase ($p>0.05$) in testosterone level was observed, as compared with group II.

Sperm Density

The sperm density of mice treated with lead nitrate was significantly lower than that of control animals ($p<0.001$). Supplementation of aqueous coriander extract provoked increase in sperm density, in both aqueous plant extract-treated groups, compared with lead group. Elevation in sperm density persisted on treatment with ethanolic coriander extract, compared with lead nitrate-treated group.

Lead Concentration in Testis Tissue

A significant increase ($p<0.001$) in lead content was observed in testis tissue of lead nitrate-treated group when compared with control group. Administration of aqueous and alcoholic extracts of coriander during the experiment resulted in Pb excretion from the tissues, in comparison to lead-treated group. But decrease in lead content was found to be insignificant ($p>0.05$) in testis tissue after the treatment with both plant extracts.

Table 3 Prophylactic Efficacy of *C. sativum* Extracts on Testosterone Level, Sperm Density, and Tissue Lead Burden in Testes of Lead-Intoxicated Mice

Parameters	Control Mice Group (Normal, Untreated)	Treated Mice Groups					
	Group I Distilled Water	Group II Lead Nitrate	Group III Lead Nitrate+aqueous Coriander Extract, Low Dose	Group IV Lead Nitrate+aqueous Coriander Extract, High Dose	Group V Lead Nitrate+ethanolic Coriander Extract, Low Dose	Group VI Lead Nitrate+ethanolic Coriander Extract, High Dose	
Testosterone, ng/ml	41.19±0.26	28.18±0.13* (−31.58%)	28.86±0.14 (+2.41%)	29.29±0.11 (+3.93%)	29.24±0.10 (+3.76%)	29.78±0.11 (+5.67%)	
Sperm density, million/ml	8.32±0.19	1.55±0.13* (−81.37%)	1.95±0.22 (+25.80%)	2.58±0.14[b] (+66.45%)	2.10±0.15 (+35.48%)	2.60±0.14** (+67.74%)	
Lead level, ppm	0.71±0.014	2.25±0.01* (+216.90%)	2.10±0.08 (−6.66%)	1.96±0.10 (−12.88%)	2.00±0.10 (−11.11%)	1.90±0.14 (−15.55%)	

Values are mean±SEM; $n=12$

*$p<0.001$ compared with normal animals

**$p<0.01$ compared with lead-exposed animals

Histological Results

Histological evaluations of testis tissue in different groups were done in the sections stained with hematoxylin and eosin.

In group I, tunica albugenia and blood vessels were normal; seminiferous tubules were richly populated and gave healthy appearance (Fig. 1). There is thin basement membrane. All the cells of the spermatogenic series such as spermatogonia, spermatocyte, spermatids, and spermatozoa, even Sertoli cells, could be identified in the tubules. Lumen could easily be delineated in almost all the tubules, and majority of them were occupied by mature spermatozoa. Interstitial cells of Leydig were present in between the tubules.

In group II animals, which were poisoned with lead, tunica albugenia of their testes was thickened and blood vessels were sparse and collapsed (Fig. 2). A majority of seminiferous tubules were shrunken and had a wavy outline. The basement membrane was thickened and hyalinized. Debris of shed cells occupied most of the lumen of the seminiferous tubules. Most of the tubules contained spermatogonia and spermatocytes, which were large in size and contained darkly stained nuclei. In some cells, the nuclear membranes had been ruptured and were accompanied by fragmentation of nucleus (karyorrhexis). Some of the tubules contained only spermatogonia, which were scanty in number, bigger in size, and had eccentrically placed dark nuclei. The Sertoli had gained more prominence due to disappearance of other cells populating their neighborhood. The blood vessels in the interstitium were sparse and collapsed. The interstitial cells of Leydig were also reduced in number and their characteristic tendency of clumping together to form groups was also reduced. All these features were suggestive of atrophy of the testes.

In groups III, IV, V, VI, which were treated with lead plus *C. sativum*, the partial recovery was shown when compared with lead-treated group (Fig. 3a, b). Recovery includes an accumulation of increased spermatozoa in the luminal areas, normal seminiferous tubules, and thin basement membrane along with partial amelioration of lead-induced changes.

Discussion

We investigated the efficacy of *C. sativum*, which is considered both a traditional natural medicine and an edible vegetable, against the toxicological disorders induced by lead

Fig. 1 Transverse section of testis of control group showing normal seminiferous tubules (×40)
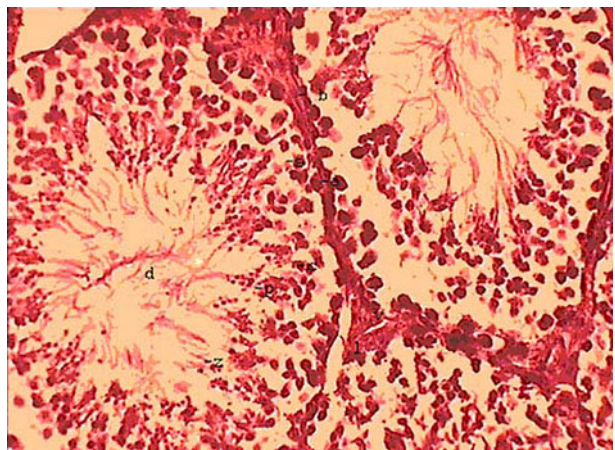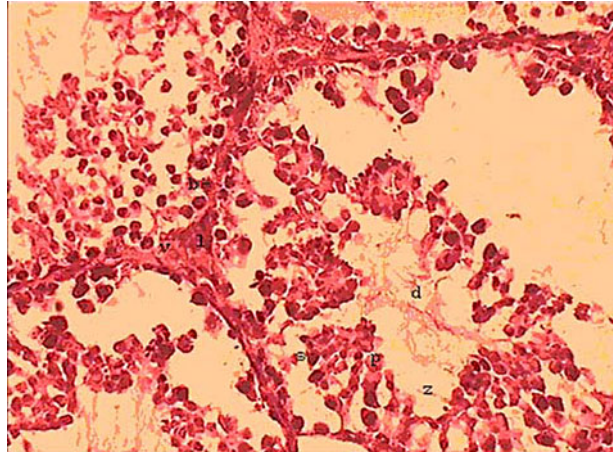
Fig. 2 Transverse section of testis of lead-treated group IIA mice, showing narrow seminiferous tubules, thickened basement membrane, degenerating spermatogonia, big spermatocyte undergoing karyolysis, relatively spared Sertoli cells, lumen filled with debris of degenerating cells, scanty Leydig cells, and collapsed blood vessels (×40)


nitrate using a mice model. It is evident from the results of the present investigation that supplementation of *C. sativum* aqueous and ethanolic extracts with lead nitrate protected animals to some extent from toxic effects of lead in general and oxidative stress in particular. This study is in confirmation with earlier reports that suggest the preventive effects of *C. sativum* (Chinese parsley) on localized lead deposition in male ICR mice [38].

Mice administered with *C. sativum* significantly restored the altered levels to some extent suggests that the active ingredients in the coriander possess antioxidant properties and protects against lead-induced oxidative stress. Typically, such an aqueous and alcoholic extract of coriander contains linalool and glucosides, such as various β-D- glucopyranosides. Long-chain (C_6-C_{10}) alcohols and aldehydes are common, and they may also contain phospholipids, phytosterols, flavonoids, and active phenols [39]. Such an extract can function as a primary or secondary chelator for mercury, as well as can be used to prepare a primary chelator blend. Positive correlations were already found between total phenolic content in the extracts and antioxidant activity [40]. Phytonutrients, flavonoids, and active phenolic acid compounds of coriander help to control blood sugar, lower cholesterol, and fight inflammation and free radicals [41].

In the present study, increase in LPO and depletion of GSH content, and SOD and CAT activities have been observed following lead exposure.

Lipid peroxidation, a basic cellular deteriorative change, is one of the primary effects induced by oxidative stress and occurs readily in the tissues due to presence of membrane rich in polyunsaturated, highly oxidizable fatty acids [42]. Although the source of prooxidant during lead-induced oxidative stress is not known, it is suggested that autooxidation of excessively accumulated aminolevulinic acid due to inhibition of amino levulinic acid dehydratase may result in formation of highly reactive cytotoxic compounds like oxidative free radicals like superoxide and hydrogen peroxide [43, 44]. The most abundant oxidative free radicals generated in living cells are superoxide anions and derivatives, particularly the highly reactive and damaging hydroxyl radical which induces peroxidation of cell membrane lipids [45]. The improper balance between reactive oxygen metabolites and antioxidant defense results in "oxidative stress" [46]. Participation of iron in Fenton reaction in vivo, leading to production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 [47] results in increased lipid peroxidation. This might be one of the reasons for significant alteration in LPO and significant changes in the activity of antioxidant enzymes observed in the present study.

Fig. 3 a b Transverse sections of
testis of group treated with lead
plus *C. sativum*, showing normal
seminiferous tubules more or less
as control (×40). Abbreviations: *a*
spermatozoa, *b* basement mem-
brane, *c* spermatids, *d* lumen of
seminiferous tubules, *l* Leydig
cells, *p* spermatocytes, *s* Sertoli
cells, *v* blood vessels, and *z*
mature spermatozoa
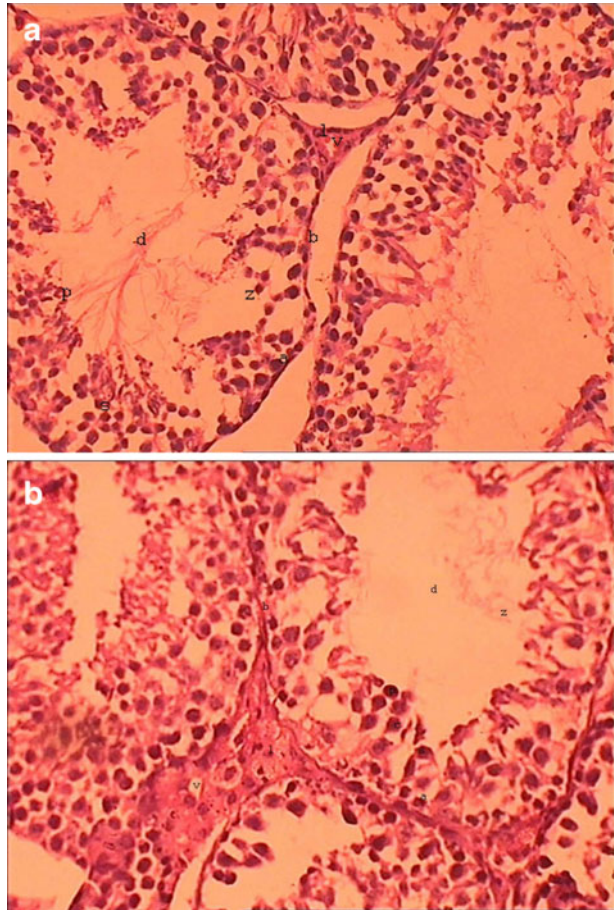
CAT and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying the peroxides (OH, H_2O_2) and superoxide anion. CAT decomposes H_2O_2 to H_2O and O_2 whereas superoxide dismutase dismutates superoxide into H_2O_2 and needs copper and zinc for its activity. In our study, we observed a decrease in CAT and SOD activities in group II compared with controls.

Superoxide anion (O^{2-}) itself directly affects the activities of catalase and peroxidase by affecting intracellular enzyme [48], creatine phosphokinase [49]. Decreased activity of Cu–Zn SOD observed may be caused by the interaction between Pb and Cu, a metal necessary for the proper functioning of the SOD cytosol enzyme [50]. A decrease in SOD was explained by direct blocking action of the metal on –SH group of the enzyme [51]. CAT activity in testis tissue of lead-treated mice showed a dip compared with the control group. This might be due to the inhibitory action of Pb on CAT [44].

GSH is one of the most important compounds, which helps in the detoxification and excretion of heavy metals. The present study showed a marked decrease in the tissue GSH level during lead toxicity. Similar observation was also noticed by earlier workers [52, 53]. Reduced GSH concentration in the present study suggests the utilization of glutathione by glutathione peroxidase. The GPx catalyzes the oxidation of GSH to GSSG [48]. This oxidation reaction occurs at the expense of (H_2O_2). Direct coupling of lead to GSH, which

results in the formation of a GSH–lead complex that is subsequently excreted in the bile, has been demonstrated in vivo [54–56]. Indirect depletion of GSH may occur when lead inhibits the enzyme and delta-aminolevulinic acid dehydratase (ALAD) before it can catalyze the condensation of two molecules of delta-aminolevulinic acid (δ-ALA) to porphobilinogen [57]. When the activity of ALAD is impeded, an effect of lead exposure that has been confirmed experimentally by several authors, the amount of δ-ALA increases [58, 59]. Since δ-ALA itself is known to be a potent inducer of LPO and ROI formation both in vivo and in vitro, its accumulation may facilitate the depletion of GSH from lead-burdened cells [60–63].

In the current study, we have seen that coriander extract treatment decreased the LPO level in testis tissue of experimental animals. Thus, it appears that the orally administered aqueous and ethanolic extract of *C. sativum* protects against lead nitrate-induced toxicity possibly through the inhibition of increased LPO level in tissue. In contrast, plant extracts elevated the SOD enzyme activity, which could explain the decrease in LPO levels. Increase in SOD activity might accelerate the removal of the ROS. It is well known that flavonoids and polyphenols are natural antioxidants [64–71]. This compound acts as promoter for SOD and catalase [68] and causes the expression of SOD and catalase [70]. The currently noted elevated levels of both SOD and CAT with *C. sativum* extracts could be due to the influence of flavonoids and polyphenols. There is another class of bioactive substances called phthalides, which possess anticarcinogenic potential. These are found in umbelliferous plants like celery, parsley, cumin, dill, fennel, and coriander. The phthalides are known to increase the glutathione-*S*-transferase level [72]. This could thus be attributed to the possibility that coriander might be providing some recovery in GSH level.

In this study, administrations of lead showed elevation in tissue AST, ALT, ACP, ALP activities, and cholesterol level and conversely decreased protein level. The present available data suggest that lead exerts possible testicular toxic effect as the increase in ALT, AST, ACP, and ALP suggests testis damage. Lead is known to bind to the sulfhydryl groups of enzymes containing cysteine and found to form complexes with amino acids and protein. Since AST and ALT is a tissue marker enzyme, lead will alter the level of AST and ALT activities in the tissues by disrupting their membrane. Consequently, there will be a discharge of the cell content into the bloodstream, and AST and ALT activities are known to increase only in heavy metal poisoning [73]. This might be the reason for increase AST and ALT activities in the tissue. Moreover, the increased activity of testicular acid phosphatase and alkaline phosphatase in lead nitrate-treated mice reflects testicular degeneration, which may likely be a consequence of suppressed testosterone and indicative of lytic activity [74].

The pathogenesis of lead toxicity is responsible for the depletion of protein observed in the present study. Lead is multifactorial and directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body.

In the present study, lead nitrate intake increased the mean values of cholesterol significantly in testis. Lead nitrate-mediated development of hypercholesterolemia involves the activation of cholesterol biosynthetic enzymes (i.e., 3-hydroxy-3-methyglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase, CYP51) and the simultaneous suppression of cholesterol-catabolic enzymes such as 7α-hydroxylase [75]. The increase concentration of cholesterol could result in relative molecular ordering of residual phospholipids resulting in a decrease in membrane fluidity [76].

Treatment with *C. sativum* during lead nitrate exposure significantly reduced the activities of ALT, AST, ACP, and ALP when compared with the mice treated with lead. The

reduced concentrations of AST and ALT as a result of plant extract administration observed during the present study might probably be due to the presence of flavonoids in the extract. It is well documented that flavonoids and glycosides are strong antioxidants [77]. The increased activity of plasma LCAT enhanced hepatic bile acid synthesis, and the increased degradation of cholesterol to fecal bile acids and neutral sterols appeared to account for its hypocholesterolemic effect [78]. The observed decrease in these enzymes shows that aqueous and alcoholic coriander extract preserve the structural integrity of the organs from the toxic effect of lead.

The present study showed deterioration in sperm density in the lead-exposed group. This observation was in collaboration with the earlier observations of Chowdhury et al. [79]. Furthermore, most of the testicular germ cells might have been destroyed either due to membrane damage or macromolecular degradation incurred by ROS leading to a significant decline in sperm count and ultimately, testicular weight loss. There was a decline in testosterone level of the lead-treated group, which is in accordance with Sokol et al. [80].

The concentration of lead in tissues from mice exposed to lead was higher than it was in tissues from mice of control. *C. sativum* suppresses the deposition of lead by chelating the metal [38]. A sorbent prepared from coriander was found to have good efficiency in removing organic and methyl mercury from aqueous solutions [81]. Phitic acid (PA), a major phosphorus storage compound in most seeds and cereal grains, is known as a natural chelating agent. PA has strong ability to chelate multivalent metal ions. The binding of metals with PA can result in the formation of very water-insoluble salts that are poorly absorbed from gastrointestinal tract and results in poor bioavailability [82]. It is possible that coriander may contain a similar type of chelating agents.

Lead exposure produced pronounced testicular histopathology evidenced by histological alternations in testis include degeneration of seminiferous tubules, thickening of basement membrane, and condensation of the stroma. These findings correspond with the observations made by some researchers that lead acts as a spermicidal agent in case of high exposure [9, 11]. Seminiferous tubules decreased in size and had a wavy outline. The Sertoli cells were relatively spared because of their resistance to various obnoxious agents [83]. The number of blood vessels in the interstitial tissue was reduced, and most of them were collapsed as was evident by their reduced diameter. The population of Leydig cells had dispersed, and they were rarely seen in groups or clumps. The nucleoli in majority of Leydig cells, rich in rRNA, had disappeared suggesting an atrophy of these cells. These findings are agreed with that of some previous investigators [9, 84–87]. All these scientists described variety of toxic changes induced by lead in the testes depending upon dose and duration of the treatment.

Lead causes lipid peroxidation by generation of ROS. This peroxidation may cause rupture of cell as well as nuclear membrane. This might be responsible for the observed necrosis and disarray in cellular organization in histological section Fig. 2. Evidence suggests that lead induces free radical formation and thus the generated ROS react with the polyunsaturated fatty acid-rich spermatozoa, specially the mid-spermatozoa and results in peroxidation which finally leads to destruction in spermatozoa causing reduced motility and viability [88]. Supplementation of coriander extract along with lead nitrate reveals that the decrease in sperm count, motility, and viability due to toxic effects of lead is minimized to some extent. As a possible mechanism, it could be stated that coriander extract have a recovery role on lead nitrate-mediated toxicity by inducing an antioxidant effect against the oxidative stress. The antioxidant properties of coriander have been established [40].

These findings of the present investigation support coriander seed to be an effective agent in lead intoxication. But the mechanism of its effect is not clear. Further investigation

is required in order to clarify antioxidant potential of this plant. It is thus concluded that the aqueous and ethanolic extracts of *C. sativum* may show prophylactic effect against lead toxicity.

Conflict of Interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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