Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

REMEDIAL EFFECT OF CORIANDRUM SATIVUM (CORIANDER) EXTRACTS ON LEAD INDUCED OXIDATIVE DAMAGE IN SOFT TISSUES OF SWISS ALBINO MICE

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Received: 26 Mar 2012, Revised and Accepted: 2 May 2012

ABSTRACT

The present communication attempts to evaluate the antioxidant potential on the seeds of *Coriandrum sativum*, Umbelliferae family. Administration of coriander extracts to lead induced mice countered oxidative stress as evidenced by significantly decreased lipid peroxidation and increased activity of SOD, CAT, and GSH and total protein content in the liver, kidney and testis of lead induced mice treated with coriander. Beside this, Treatment with coriander decreased the activity of AST, ALT enzyme and cholesterol level in the soft tissues of lead induced mice. Efficacy of coriander to reduce tissue lead concentration was also evaluated. Histopathological studies of kidney revealed that supplementation of coriander extract showed the tubules appear more or less normal. In conclusion, the treatment with coriander extracts ameliorated oxidative stress in lead induced mice due to the synergistic action of antioxidant phytochemicals, carotenoids, flavonoids etc. present in the extracts. From the findings of the study, the coriander is identified to possess antioxidant potential and hence it is worth to be considered as a natural chelating agent for lead intoxication.

Keywords: Lead, *Coriandrum sativum,* Biochemical changes, Liver, Kidney, Testis.

INTRODUCTION

Coriandrum sativum Linn Umbeliferae is an annual herb originating from the Mediterranean countries[1]. The seed of coriander are one of the most important spices in the world and are regularly used by Indian kitchen. In addition to its culinary value, coriander is known for its wide range of healing properties. It is generally used in gastrointestial complaints such as anorexia, dyspepsia, flatulence, diarrhea, gripping pain and vomiting[2] and as antiedemic, Antiseptic, and emmenagogue[3]. The traditional claim for its anti diabetic has been validated in streptozotacin (STZ) diabetic mice[4] and in high fat diet rats[5]. Coriander is also used in detox diet. It helps to remove toxic mineral residue such as mercury and lead, and excrete them in the urine or faeces.

Lead a soft, grey-blue heavy metal found ubiquitously is a common cause of poisoning of domestic animals throughout the world[6]. Lead poisoning is one of the foremost environmental health threats[7]. Pathogenesis of lead poisoning is mainly attributed to lead- induced oxidative stress. Chronic lead exposure is known to disrupt the pro oxidant/antioxidant balance existing within the mammalian cells[8, 9].

Liver, responsible for maintaining the body's metabolic homeostasis has been considered as the target organ for the toxic effects of Pb[10]. It is the largest repository of softy tissue Pb followed by kidney[11, 12].

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[13]. GPx, CAT, and SOD are potential targets for lead toxicity because these antioxidant enzymes depend on various essential trace elements for proper molecular structure and activity[14].

The present study reports the effect of Pb on soft tissues of male mice and the antioxidant activity and chelating property of coriander against lead induced toxicity.

MATERIALS AND METHODS

Experimental Plant Material

The plant *Coriandrum sativum* (seeds) was collected from Krishi Vigyan Kendra, Banasthali University, Rajasthan, India and was identified as a RCR 435 variety.

Preparation of aqueous extract of *Coriandrum 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 ^{0}C), the mixture was then heated for 30 min in the water bath at 65 ^{0}C. The extract was filtered, concentrated by heating over the water bath (65 ^{0}C) and dried under vacuum [4] with the yield of 5.9 % (w/ w). The extract was stored at 4 ^{0}C and used to treat animals as needed.

Preparation of alcoholic (ethanolic) extraction of *Coriandrum sativum*

The dried and powered seeds (200 g) were extracted successively with ethanol (800 ml) in a soxhlet extractor for 48 hours at 60 ^{0}C. After extraction, the solvent was evaporated to dryness at 50-55 ^{0}C by using a rotary evaporator and the extract left behind (yield was 9.8 %) was stored at 4 ^{0}C. It was dissolved in distilled water whenever needed for experiments.

Animals

Male Swiss albino mice weighing approximately 15-30 g (2 to 2.5 months) were obtained from Haryana Agricultural University, Hissar, India for experimental purpose. The Animal Ethics Committee of Banasthali University, Banasthali, India has approved the experimental protocol. All experiments were conducted on adult male albino mice (*Mus musculus* L.) weighing 25-35 g (3-4 month old). They were housed in polypropylene cages in an air conditioned room with temperature maintained at 25 °C ± 3 °C, relative humidity of 50 % ± 5 % and 12 h alternating light and dark cycles. The mice were provided with a nutritionally adequate chow diet (Hindustan lever Limited, India) and drinking water *ad libitum* throughout the study.

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 design

Adult Swiss albino male mice were divided into 6 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(NO3)2 in 1 ml distilled water at a dose of 20 mg/ kg body weight/ day;

Group III and Group IV were administered with aqueous coriander extract at a dose of 300 mg/ kg body weight and 600 mg/ kg body weight, respectively, by oral gavage once daily for 33 days from 8 day after beginning of lead nitrate exposure to the end of the experiment.

Group V and Group VI were administered with ethanolic coriander extract at a dose of 250 mg/ kg body weight and 500 mg/ kg body weight, respectively, by oral gavage once daily for 33 days from 8 day after beginning of lead nitrate exposure to the end of the experiment.

The dose for lead nitrate was decided on the basis of experiments conducted in the laboratory. The plant doses were selected on the basis of experiments conducted in our own laboratory and on the basis of earlier published reports[15]. After the administration of last dose, the animals were given a one-day rest and were killed under light chloroform anesthesia. The hepatic, renal and testis tissues were excised and divided into two parts one part was homogenized in ice-cold buffer and utilized for various oxidative stresses and biochemical analysis and the second part stored at–20 °C before wet acid digestion with HNO3 for lead estimation. Kidney divided in an additional part which was fixed in 10% formalin for histological studies

Oxidative stress and biochemical analysis

Liver, kidney and testis were minced and homogenized (10 % w/ v) in ice-cold 0.1 M sodium phosphate buffer (pH-7.4). The homogenate was centrifuged at 10,000 rpm for 15-20 min at 4 °C twice to get enzyme fraction. The resultant supernatant was used for various biochemical assays.

LPO was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of membrane lipids[16]. SOD activity was assessed according to the method of Marklund and Marklund [17]. CAT activity was assayed following the method of Aebi[18]. GSH content was determined according to the method of Ellman[19]. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel[20].

Total protein content was estimated by the method of Lowry et al.[21] using bovine serum albumin as standard. The cholesterol level was determined by using cholesterol as a standard by the method of Zak's[22].

Metal Estimation

Lead concentration in live, kidney and testis were 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).

Histopathological examination

Kidneys were removed, washed in saline were fixed in buffered 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 Haematoxylin and Eosin and examined under light microscope.

Statistical analysis

Data are expressed as the Mean ± SEM. The data was analyzed using the Statistical Package for Social Science program (S.P.S.S. 11). Statistical analysis was done using analysis of variance (ANOVA) followed by Tukey test and the level of significance was set at $p < 0.05$.

RESULTS

Hepatic Oxidative stress and biochemical changes

Lead induced changes in hepatic oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice are shown in Table 1.

Table 1: Lead induced changes in hepatic oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice.

	LPO (n mole of MDA formed/ g of tissue)	SOD (unit/ ml)	CAT (µM of H2O2 degraded/ min/mg protein)	GSH (mg/ g of tissue)	AST (IU/ L)	ALT (IU/ L)	Total Protein (g/ dl)	Total cholesterol (mg/ g of tissue)
Group I	111.58 ± 3.23	1.13 ± .04	32.22 ± 0.47	7.62 ± 0.14	32.08 ± 0.24	48.46 ± 0.34	8.25 ± 0.26	24.48 ± 0.39
Group II	146.56 ± 0.69*	0.90 ± 0.007*	26.14 ± 0.41*	3.80 ± 0.12*	54.72 ± 1.0*	63.62 ± 0.95*	6.75 ± 0.03*	38.70 ± 0.30*
Group III	122.40 ± 0.71a	0.97 ± 0.005a	27.12 ± 0.40	4.40 ± 0.14a	43.21 ± 0.99a	55.00 ± 0.79a	6.87 ± 0.05	32.49 ± 0.52a
Group IV	120.85 ± 0.46a	1.01 ± 0.007a	28.50 ± 0.31a	4.57 ± 0.13a	41.57 ± 0.52a	53.42 ± 0.44a	7.24 ± 0.07	31.27 ± 0.45a
Group V	119.16 ± 0.64a	1.03 ± 0.006a	28.75 ± 0.43a	5.33 ± 0.09a	40.55 ± 0.66a	52.61 ± 0.41a	7.45 ± 0.10b	31.55 ± 0.35a
Group VI	117.01 ± 0.47a	1.05 ± 0.02a	29.27 ± 0.41a	5.94 ± 0.06a	37.84 ± 0.85a	51.27 ± 0.46a	7.80 ± 0.06a	29.74 ± 0.23a

Values are Mean ± S.E.M.; n= 12.

*P< 0.001 compared to normal animals.

aP< 0.001 and bP< 0.01 compared to lead nitrate exposed animals. Lead nitrate at a dose of 20 mg/kg body weight caused significant ($p < 0.001$) increase in the level of TBA-reactive product in liver and significant decrease in SOD and CAT activity, and GSH content, in comparison to control group (group I).

However, treatment with aqueous coriander extract along with lead nitrate caused a significant reduction ($p<0.001$for both low and high dose) in LPO level when compared with lead induced group. A significant increase ($p<0.001$ for both low and high dose) in the activity of SOD, CAT and GSH were observed after the treatment with aqueous coriander extract, in comparison to lead nitrate exposed group II.

Supplementation of ethanolic extract of coriander offered significant reduction in lipid peroxidation level in both groups, compared to group II ($p<0.001$for both low and high dose) while administration of the same dose significantly elevated the SOD, CAT and GSH activity in group V and VI, compared to lead group ($p<0.001$ for both low and high dose).

It is clear from the results that treatment with lead nitrate showed a significant elevation in some biochemical parameters which include AST, ALT and total cholesterol as compared to control group animals ($p<0.001$). The mean value of total protein was significantly decreased by lead nitrate intake when compared with control ($p<0.001$).

The AST, ALT and total cholesterol levels in liver homogenate were significantly reduced by administration of aqueous coriander extract at a dose of 300 and 600 mg/kg body weight ($p < 0.001$ vs. lead nitrate intoxicated mice). In comparison to lead nitrate exposed animals (group II), total protein increased insignificantly in groups III and IV.

Compared with the lead nitrate control (group II), administration of ethanolic *Coriandrum sativum* extract at a dose of 250 and 500 mg/kg body weight resulted in significant decrease ($p < 0.001$) of hepatic AST, ALT and total cholesterol levels. The total protein content in groups V and VI significantly ($p<0.01$ and $p < 0.001$ respectively) increased in hepatic tissues, when compared with lead control values (group II).

Renal Oxidative stress and biochemical changes

Lead induced changes in renal oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice are shown in Table 2.

Table 2: Lead induced changes in renal oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice.

	LPO (nmole of MDA formed/g of tissue)	SOD (unit/ ml)	CAT (µM of H_2O_2 degraded/ min/mg protein)	GSH (mg/ g of tissue)	AST (IU/ L)	ALT (IU/ L)	Total Protein (g/ dl)	Total cholesterol (mg/ g of tissue)
Group I	99.80 ± 2.66	1.08 ± 0.08	30.43 ± 0.97	6.86 ± 0.15	22.01 ± 0.30	17.72 ± 0.30	5.75 ± 0.26	18.38 ± 0.21
Group II	128.60 ± 0.93*	0.95 ± 0.01*	21.18 ± 0.31*	4.18 ± 0.04*	37.67 ± 0.93*	30.43 ± 1.16*	3.13 ± 0.12*	30.29 ± 0.33*
Group III	112.26 ± 0.65a	0.97 ± 0.008	23.48 ± 0.28d	4.69 ± 0.08b	31.34 ± 0.96a	25.20 ± 0.67a	3.97 ± 0.04	24.45 ± 0.49a
Group IV	111.57 ± 0.70a	1.01 ± 0.007a	26.05 ± 0.31a	4.95 ± 0.04a	27.74 ± 0.71a	24.18 ± 0.51a	4.01 ± 0.03	22.62 ± 0.44a
Group V	109.42 ± 0.61a	1.00 ± 0.01a	26.55 ± 0.36a	5.09 ± 0.05a	27.00 ± 0.60a	22.06 ± 0.54a	4.57 ± 0.05a	22.21 ± 0.43a
Group VI	102.53 ± 0.66a	1.02 ± 0.01a	27.30 ± 0.32a	5.94 ± 0.07a	23.20 ± 0.66a	20.95 ± 0.28a	4.99 ± 0.02a	20.36 ± 0.44a

Values are Mean ± S.E.M.; n= 12.
*P< 0.001 compared to normal animals.
aP< 0.001, bP< 0.01 and dp<0.05 compared to lead nitrate exposed 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 renal 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 at a dose of 300mg/kg body weight and 600mg/ kg body weight, showed significant decrease ($p<0.001$) in the level of LPO was observed in comparison to lead nitrate-treated group. While the administration of same dose significantly elevated GSH content ($p<0.01$ and $p<0.001$ respectively), compared to lead nitrate treated animal (group II). In comparison to lead nitrate exposed animals (group II), SOD and CAT activity increased significantly in group IV but insignificantly in group III treated animals.

Supplementation of ethanolic coriander extract in animals registered a significant decrease ($p<0.001$for both low and high dose) in LPO, in both plant treated group, compared with lead treated group. Moreover, low and high dose of Ethanolic *Coriandrum sativum* extract treatment led to significant ($p < 0.001$) elevation in the SOD, CAT and GSH content, when compared with group II animals.

In comparison to normal control mice, a significant ($p < 0.001$) increase in the total cholesterol level and activities of marker enzymes such as AST, ALT were recorded in lead nitrate exposed mice. A significant ($p < 0.001$) decrease in total protein level followed by lead nitrate exposure was also noticed in group II, as compared to control animals (group I).

Treatment with low and high dose of aqueous coriander extract significantly ($p < 0.001$) reduced lead nitrate induced increase in the levels of total cholesterol, AST and ALT as compared to lead nitrate treated animals (group II). On the other hand, lead nitrate-induced depletion in protein content was insignificantly prevented by treatment with aqueous coriander extract at a dose of 300 mg/kg body weight and 600 mg/kg body weight, when compared with lead treated group II animals.

Administration of ethanolic coriander extract at 250 and 500 mg/kg body weight significantly ($p < 0.001$) suppressed the increased levels of total cholesterol, AST and ALT, when compared with lead nitrate treated animals (group II). Total protein level recovered significantly ($p < 0.001$) in response to 250 and 500 mg/kg body weight of ethanolic coriander extract, in comparison to lead intoxicated mice (group II).

Testis Oxidative stress and biochemical changes

Lead induced changes in testis oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice are shown in Table 3.

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).

It is also clear from the results that treatment with lead nitrate showed a significant increase in parameters which include AST, ALT 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 and ALT 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: Lead induced changes in testis oxidative stress parameters and marker enzymes and their response to administration of coriander extracts in mice.

	LPO (nmole of MDA formed/ g of tissue)	SOD (unit/ ml)	CAT (µM of H2O2 degraded/ min/mg protein)	GSH (mg/ g of tissue)	AST (IU/ L)	ALT (IU/ L)	Total Protein (g/ dl)	Total cholesterol (mg/ g of tissue)
Group I	110.39 ± 4.44	1.12 ± 0.03	37.21 ± 0.81	2.55 ± 0.15	15.39 ± 0.50	12.11 ± 0.43	4.83 ± 0.27	31.37 ± 1.95
Group II	133.86 ± 0.84*	0.97 ± .02	28.64 ± 0.36*	1.96 ± 0.15*	38.22 ± 0.92*	25.11 ± 0.79*	2.05 ± 0.05	34.29 ± 1.78
Group III	127.77 ± 0.63a	0.99 ± 0.005	29.06 ± 0.47	1.97 ± 0.11	25.14 ± 1.06a	22.35 ± 0.45a	2.64 ± 0.05	34.97 ± 0.09
Group IV	125.04 ± 0.38a	1.0 ± 0.007	29.51 ± 0.41d	1.98 ± 0.05	24.19 ± 1.03a	20.50 ± 0.47a	2.77 ± 0.05	33.81 ± 0.36
Group V	123.16 ± 0.62a	1.02 ± 0.006	30.34 ± 0.36a	2.03 ± 0.05	24.78 ± 0.48a	20.04 ± 0.40a	2.98 ± 0.04b	33.37 ± 0.51
Group VI	120.35 ± 0.67a	1.04 ± 0.15	31.43 ± 0.32a	2.09 ± 0.04	22.86 ± 0.55a	18.72 ± 0.61a	3.29 ± 0.08b	32.09 ± 0.56d

Values are Mean ± S.E.M.; n= 12.

*P< 0.001 compared to normal animals.

aP< 0.001, bP< 0.01 and dp<0.05 compared to lead nitrate exposed animals

Concentrations of Lead in Tissues

The lead concentration in liver, kidney and testis of mice are listed in Table 4. The hepatic and renal lead level in lead nitrate exposed group were significantly (p < 0.001) higher than in the control group. In contrast lead levels in the liver and kidney were significantly reduced in group III, IV, V, and VI supplemented with the coriander extract as compared to lead exposed group (p < 0.001).

The lead concentration in testis of lead exposed group was shown significant (p < 0.001) increased compared to the control. Testis lead level in groups supplemented with coriander extracts were not significantly decreased compared with lead exposed group.

Table 4: Lead concentrations in liver, kidney and testis tissues of mice

	Liver (µg/ g of wet tissue)	Kidney (µg/ g of wet tissue)	Testis (µg/ g of wet tissue)
Group I	0.052±0.006	0.92±0.004	0.69± 0.019
Group II	3.41±0.029*	8.71±0.03*	2.22±0.10*
Group III	2.79±0.005a	7.38±0.037a	2.08±0.08
Group IV	2.04±0.028a	6.17±0.05a	1.96±0.10
Group V	2.51±0.008a	5.25±0.04a	2.00±0.10
Group VI	1.99±0.016a	4.18±0.041a	1.90±0.1

Values are Mean ± S.E.M.; n= 12.

*P< 0.001 compared to normal animals.

aP< 0.001 compared to lead nitrate exposed animals.

Histopathological Studies

Lead induced changes on renal histological images and remedial effect of coriander extracts on that changes were examined (Fig. 1-6).

Group I (Control, Untreated, Normal animals)

A section of the control mice showed normal structure of both the renal corpuscles and tubules. Control mice showed normal rounded glomeruli and did not show any signs of damage. Renal tubules are lined with typical thick cubic epithelium. The tubules had a relatively regular distinct lumen. The tubules were well arranged and uniformly stained (Figure 1).

Group II (Lead nitrate exposed animals)

The pattern which emerges in the lead nitrate exposed mice is that of dilation of tubules; sloughing of epithelium indicated advanced disintegration of tubules. At places, casts (dead tubule's remains) was also seen. Glomeruli showed shrinkage, widened urinary space of the Bowman's capsule; however, at places they showed complete disintegration. A few proximal convoluted tubule cells were vacuolated and swollen. Inflammatory cells were observed in the intertubular spaces. Most of the cells of the convoluted tubules were highly swollen and their lumens were nearly obliterated. Some blood sinusoids appeared to be filled with erythrocytes (Figure 2).

Group III, IV (Lead nitrate + Aqueous extract of Coriander) and Group V and VI (Lead nitrate + Ethanolic extract of Coriander)

Glomeruli appeared normal. They do not show damage at any spot. Casts are absent. Tubules were compact, rounded and at places thin-walled but neither dilated nor damaged. No inclusion of blood cells was evident (Figure 3 and 5). However, in Group III and V slight oedema and vacuolation of the tubular cells appeared (Figure 4 and 6). These findings also suggest that the coriander extracts were helpful in bringing about functional improvement of mesangial cells. The remedial effect of coriander extracts was also confirmed by histological observations.

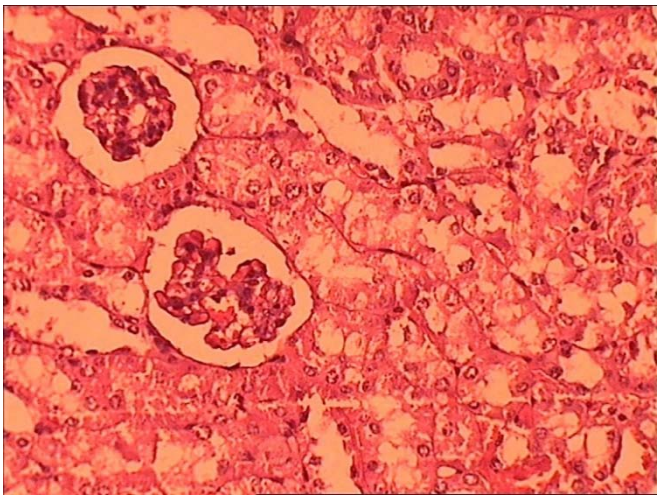

Fig. 1: T. S. of renal cortex of control (40X)

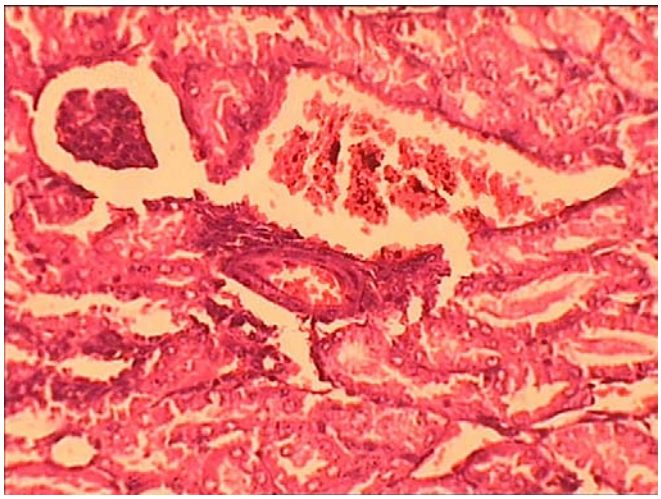

Fig. 2: T. S. of renal cortex of lead nitrate treated group (II) (40X)

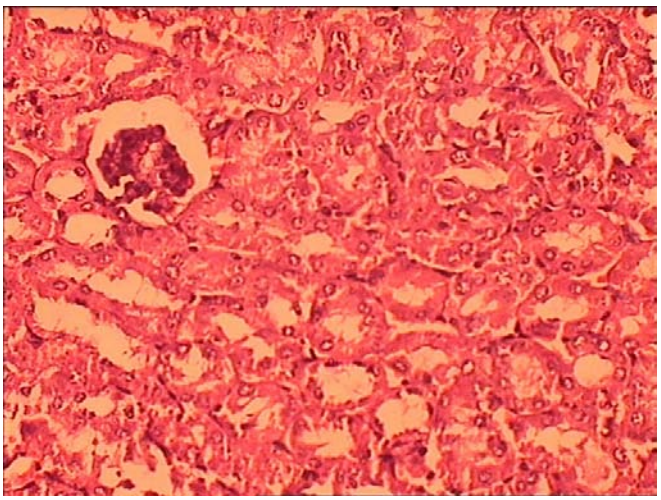

Fig. 3: T. S. of kidney of mice ingested to lead nitrate plus aqueous extract of *Coriandrum sativum* (low dose) (40 X).

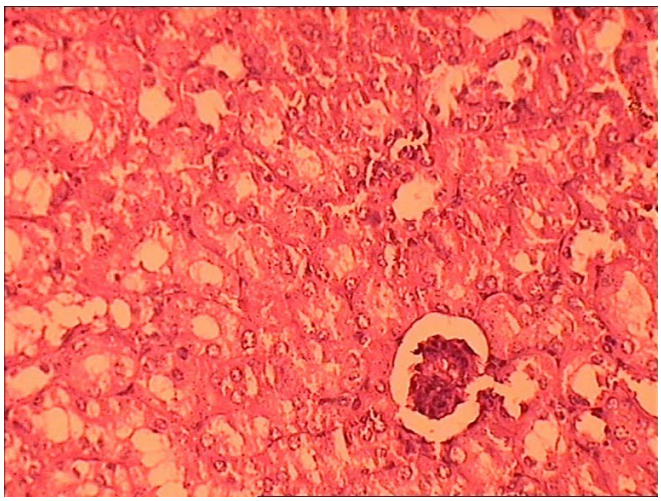

Fig. 4: T. S. of kidney of mice ingested to lead nitrate plus *Coriandrum sativum* aqueous extract (high dose) (40X).

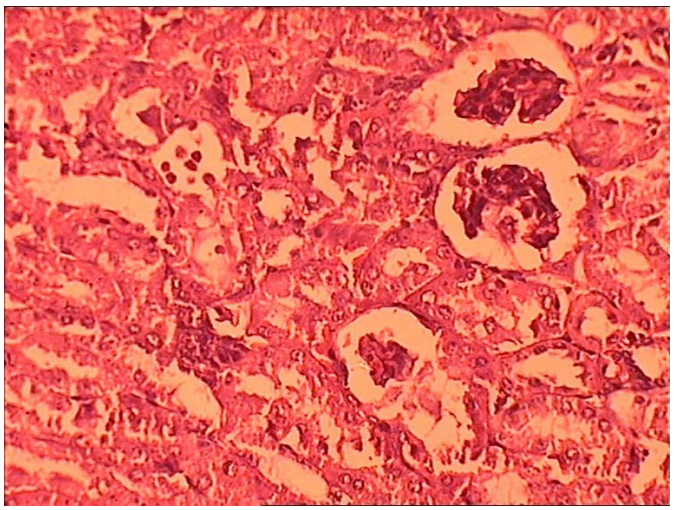

Fig. 5: T. S. of kidney of mice ingested to lead nitrate plus *Coriandrum sativum* ethanolic extract (low dose) (40 X)

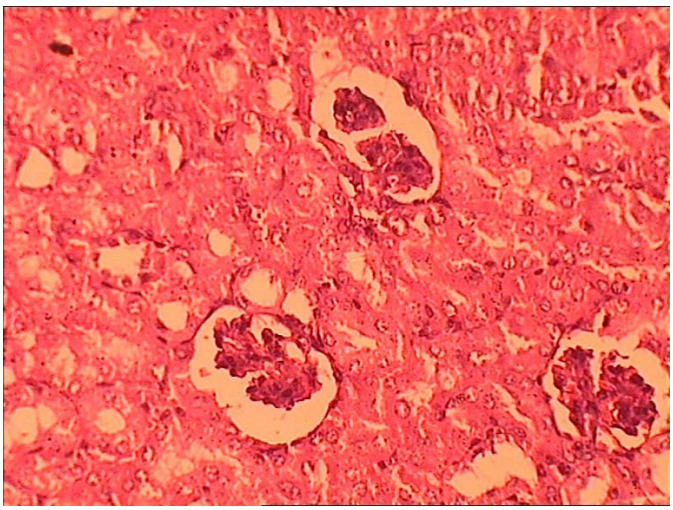

Fig. 6: T. S. of kidney of mice ingested to lead nitrate plus ethanolic extract of *Coriandrum sativum* (high dose) (40 X).

DISCUSSION

Lead is known to cause oxidative damage in various tissues by bringing about imbalance in the generation and removal of reactive oxygen species[23]. Although the exact mechanisms by which lead induces oxidative stress in various tissues are not completely understood, evidence indicates that multiple mechanisms may be involved. Numerous plant products have been shown to have high potent antioxidant activity. Recently, bioflavonoid and polyphenols of plant origin have been used extensively for free radical scavenging and to inhibit lipid peroxidation[24].

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[25]. Although the source of prooxidant during lead induced oxidative stress is not known, it is suggested that autooxidation of excessively accumulated amino levulinic 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[26]. 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[27]. Gibanananda & Hussain[28] observed that the improper balance between reactive oxygen metabolites and antioxidant defense results in "oxidative stress". Participation of iron in Fenton reaction *in vivo*, leading to production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2[29] 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.

Treatment with lead nitrate significantly decreased the activities of superoxide dismutase, glutathione peroxidase, glutathione S-transferase and total antioxidants level. These results are in agreement with our previous finding[30, 31]. Lead nitrate is known to cause free radical damage in tissues by two mechanisms: Increased generation of ROS, including hydroperoxides, singlet oxygen and hydrogen peroxides, and by causing direct depletion of antioxidant reserves[32]. Superoxide dismutase, glutathione peroxidase and glutathione S-transferase enzymes take part in maintaining glutathione homeostasis in the tissues. These antioxidant enzymes are involved in the defense system against free radical mediated tissue or cellular damage after lead exposure[33]. The observed decrease in circulating antioxidants confirms the lead nitrate induced depletion of antioxidants depletion.

CAT decomposes H2O2 to H2O and O2 whereas superoxide dismutase dismutates superoxide into H2O2, and needs copper and zinc for its activity. A decrease in SOD was explained by direct blocking action of the metal on -SH group of the enzyme[34]. However, a few studies show that superoxide radicals can also inhibit the catalase (CAT) activity and the increase in H_2O_2 levels resulting from CAT inhibition could finally inhibit the SOD activity. CAT activity in tissues (liver, kidney and testis) of lead treated mice showed a dip compared to the control group. This may be due to the inhibitory action of Pb on CAT[26].

GSH is synthesized in the cytoplasm of the liver cells and then distributed through the circulatory system into different organs[35]. GSH plays a crucial role in both scavenging ROS and in detoxification of chemical compounds. Therefore, perturbation in the redox status of GSH cannot only impair cell defenses against toxic compounds, but also result in enhanced oxidative stress and tissue injury[36]. Lead has very high affinity for SH group and therefore results in lead exposed decrease in GSH level.

Liver enzymes such as ALT, AST, ACP and ALP are marker enzymes for liver function and integrity[37]. These enzymes are usually raised in acute hepatotoxicity or mild hepatic cellular injury, but tend to decrease with prolonged intoxication due to damage to the liver[38]. The present available data suggest that lead exerts possible toxic effects as the increase in ALT, AST, ACP and ALP suggest tissue damage. This study was similar to the observations of Sharma et al[39] and Ige et al[40]. Lead is known to bind to the sulfydryl groups of enzymes containing cysteine, and found to form complexes with amino acids and protein. Since ALT is liver enzyme, lead will alter the level of ALT activity in the tissues by disrupting their membrane. Consequently, there will be discharge of the cell content into the blood stream and ALT activity is known to increase only in heavy metal poisoning, toxic hepatosis, and muscular dystrophy[41]. Total protein level is a rough measure of protein status but reflects major functional changes in kidney and liver functions. One of the main targets of lead poisoning is the kidney. Chronic poisoning can lead to kidney failure, and acute poisoning to tubulopathy with Toni-Debre-Fanconi syndrome. β-2- microglobinuria and enzymuria were reported in lead toxicity in children[42].

Proteinuria due to kidney impairment in lead toxicity may be a cause of protein loss among these animals because inhibitory role of lead in protein synthesis is not yet reported. Protein loss in lead toxicity might decrease the level of specific proteins such as albumin, hormones, hormone and metal binding proteins, drug binding proteins, enzymes etc. and thereby disturb the homeostasis and rate of metabolic activities. Moreover, Pb+2 disturbs intracellular Ca^{+2} homeostasis[43] and damage the endoplasmic reticulum, which results in decrease in protein synthesis. The increase concentration of cholesterol could result in relative molecular ordering of residual phospholipids resulting in a decrease in membrane fluidity[44].

In the present study, administration of aqueous and ethanolic extract of *Coriandrum sativum* significantly increased the antioxidant enzymes in lead nitrate treated animal.

Supplementation of *Coriandrum sativum* caused increase in SOD, CAT activity and GSH content and decrease in LPO level in lead treated mice tissues (liver kidney and testis), supporting the antioxidant effect of both aqueous and ethanolic plant extracts. The antioxidant property of coriander extract could be directly linked to both the scavenging activity against ROS and elevation of antioxidant make up. Antioxidants generally decrease the level of oxidation by transferring the hydrogen atom to the free radical structure[45]. A previous study has shown that the formation of lipid peroxides declined whereas activities of antioxidant enzymes (catalase, glutathione peroxidase) increased in rats treated by *Coriandrum sativum*[5]. The antioxidative property of coriander seed is related to the large amounts of tocopherols, carotenoids and phospholipids[46], which act through different mechanisms. The active components of coriander could act as electron donors, which can react with free radical to form more stable products and therby terminate the radical chain reaction. Carotenoids act as primary antioxidants by trapping free radicals and as secondary antioxidants by quenching singlet oxygen[47]. Tocopherols and sterols interact with oil surfaces and release hydrogen, inhibiting the propagation step of radical reactions[47]. Synergetic effects were evidenced with combinations of carotenoids and tocopherols[47]. Although the exact mechanism of antioxidative action of phospholipids is not still fully established, these substances would synergistically act with tocopherols, would form barrier for O2 between air/oil interfaces, would favor formation of Mallard like compounds with oxidation products or would chelate pro-oxidant metals with phosphate groups[48].

There is another class of bioactive substances called phthalides, which have anticarcinogenic potential. They are found in umbelliferous plants like celery, parsley, cumin, dill, fennel, and coriander. The phthalides are known to increase the glutathione-S-transferase level[49]. This could thus be attributed to the possibility that coriander might be providing some recovery in GSH level.

The coriander mediated suppression of the increased AST and ALT activities and cholesterol level suggests the possibility of the extract to give protection against hepatic, renal and testicular injury upon lead induction. Co-administration of aqueous and alcoholic coriander extracts significantly increased total protein content. The efficiency of Coriander was due to presence of several pharmacological effects such as as antifertility[50], antihyperglycemic[51], anti-hyperlipidemic[5], antiproliferative[52], hypotensive[53] and digestive stimulant[54]. The lowering in cholesterol levels of tissues by the administration of coriander would seem to be mediated through its increased rate of degradation to bile acids and neutral sertols.

Therefore improvement of antioxidant enzymes and biochemical changes by coriander extracts could be implicated in the utility of this plant in ameliorating the pathology of lead nitrate.

The concentration of lead in tissues from mice exposed to lead was higher than it was in tissues from mice of control. *Coriandrum sativum* suppresses the deposition of lead by chelating the metal[55]. A sorbent prepared from coriander was found to have good efficiency in removing organic and methyl mercury from aqueous solutions[56]. 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[57]. It is possible that coriander may contain a similar type of chelating agents. From the results of current study, lead exposure produced marked histological alternations in kidney include dilation of tubules; sloughing of epithelium indicates advanced disintegration of tubules. The results of the some previous investigation showed that subtoxic chronic lead exposure resulted in progressive tubular, glomerular and interstitial alterations. Some of these findings are in agreement with some results of previous investigations[58]. Fowler et al[59] found that rats exposed to low doses of lead (0.005 % and 0.025 %) for nine months developed proximal tubular changes consisting of intranuclear inclusion bodies, swollen dysfunctional mitochondria, and numerous electrondense lysosomes, but no interstitial fibrosis. These results suggest that the kidney may be a major target organ of lead toxicity, and that the epithelial cells of proximal convoluted tubules and Bowman's capsule seem to be more sensitive to lead induced nephrotoxicity. Many recent studies have provided experimental evidences that lead exposure can result in the generation of ROS and cause cell damage or death through the ROS signaling pathway[60]. The results of the present work showed that the tubular damages were more prominent in the proximal convoluted tubules in comparison to that in the distal ones. This could be due to the fact that the proximal convoluted tubules are the primary sites of reabsorption and active transport leading to higher concentration of lead in the epithelial lining of these tubules. Tubular vacuolization, necrosis and dilation found in the present studies due to lead intoxication were reported by other workers[61]. These tubular alterations due to lead toxicity might be a result of a hydrolic changes in the renal tissue and suggest that lead intoxication yields to a partial failure in the ion pump transport of tubules cells which in turn produces tubular swelling and causes necrosis and vacuolization of the tubules. Also, these changes might indicate incapability of the renal cells to deal with the accumulated residues resulting from metabolic and structural disturbances caused by lead. The presence of hyaline casts in the lumen of the damaged tubules might be an indication of glomerulonephritis and or partial failure of tubular reabsorption due to lead intoxication. Kidney of mice ingested to lead plus *Coriandrum sativum* extracts shows the tubules appear more or less normal. Thus coriander extract produced protective effects in renal tissue against lead toxicity.

In conclusion the current study suggests that aqueous and ethanolic extracts of *Coriandrum sativum* can prevent or slow down the oxidative damage induced by lead in mice. The effect of lead on LPO level, GSH concentration, antioxidant enzyme activity and some biochemical variables were reversed by treatment with plant extracts. Further studies are needed to evaluate its pharmacokinetics and toxicity profile to determine its clinical dose and isolation and characterization of bioactive components.

ACKNOWLEDGMENT

The authors are thankful to the authorities of Banasthali University for providing support to the study.

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