

Hydroalcoholic Seed Extract of *Coriandrum sativum* (Coriander) Alleviates Lead-Induced Oxidative Stress in Different Regions of Rat Brain

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Abstract Lead exposure is known to cause apoptotic neurodegeneration and neurobehavioral abnormalities in developing and adult brain by impairing cognition and memory. *Coriandrum sativum* is an herb belonging to *Umbelliferae* and is reported to have a protective effect against lead toxicity. In the present investigation, an attempt has been made to evaluate the protective activity of the hydroalcoholic extract of *C. sativum* seed against lead-induced oxidative stress. Male Wistar strain rats (100–120 g) were divided into four groups: control group: 1,000 mg/L of sodium acetate; exposed group: 1,000 mg/L lead acetate for 4 weeks; *C. sativum* treated 1 (CST1) group: 250 mg/kg body weight/day for seven consecutive days after 4 weeks of lead exposure; *C. sativum* treated 2 (CST2) group: 500 mg/kg body weight/day for seven consecutive days after 4 weeks of lead exposure. After the exposure and treatment periods, rats were sacrificed by cervical dislocation, and the whole brain was immediately isolated and separated into four regions: cerebellum, hippocampus, frontal cortex, and brain stem along with the control group. After sacrifice, blood was immediately collected into heparinized vials and stored at 4 °C. In all the tissues, reactive oxygen species (ROS), lipid peroxidation products (LPP), and total protein carbonyl content (TPCC) were estimated following standard protocols. An indicator enzyme for lead toxicity namely delta-amino levulinic acid dehydratase (δ -ALAD) activity was determined in the blood. A significant ($p < 0.05$) increase in ROS, LPP, and TPCC levels was observed in exposed rat brain regions, while δ -

ALAD showed a decrease indicating lead-induced oxidative stress. Treatment with the hydroalcoholic seed extract of *C. sativum* resulted in a tissue-specific amelioration of oxidative stress produced by lead.

Keywords Lead · Brain · *Coriandrum sativum* · Reactive oxygen species · Lipid peroxidation products · Trace metals

Introduction

Lead is a soft, bluish-gray heavy metal and it is used in industries for its unusual physicochemical properties [1]. Lead is a persistent toxic metal for human beings and domestic animals and remains for long periods in the environment as well as in foods [2]. The most common symptoms of lead exposure are loss of appetite, weight loss, constipation, irritability, fatigue, and occasional vomiting. If lead concentration exceeds 10 $\mu\text{g/dL}$ in the blood, it is considered harmful and can lead to neurological disorders, cognitive impairments, and hypertension [3]. It was reported that low-level lead exposure causes a significant increase in expression of multidrug resistance protein 1 (Mrp1) and nuclear factor E2-related factor-2 (Nrf2) in rats [4]. One of the most acceptable and possible mechanisms of lead toxicity is the generation of reactive oxygen species (ROS) [5] and depletion of antioxidant enzymes, followed by lipid peroxidation, protein oxidation, and DNA damage [6], resulting in apoptosis which has been also demonstrated earlier in our laboratory [7–9].

Recent studies have revealed the role of herbal or plant-based products or active principals from plants to show protection against lead-induced oxidative stress in rats and mice. These include coriander seeds [10], green tea [11], flaxseed oil [12], naringenin (a flavonoid) [13],

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puerarin (a flavonoid) [14], bulk, and nanocurcumin [15]. Administration of antioxidant and micronutrient-rich milk reduced lead-induced oxidative damage in mice [16]. Similarly, in the present study, the protective role of *Coriandrum sativum* seed extract has been tested against lead toxicity in rats.

C. sativum (common name: Chinese parsley and coriander) belongs to *Apiaceae* (*Umbelliferae*) family, is an herb, and is cultivated throughout India. *C. sativum* leaf extract exhibited protection against gastric mucosal damage [17], removal of memory deficits [18], and sleep-prolonging effect in mice [19]. The extract was also found to show in vitro antioxidant and antibacterial properties [20]. Coriander seeds are also used for stimulation of bile secretion in rats [21], antioxidant activity [22], and antibacterial activity [23]. The hydroalcoholic extract of seeds was known for its anti-anxiety effects [24]. The seed powder showed hypoglycemic action [25], and the hydromethanolic extract exhibited cardioprotective action [26].

The seeds of *C. sativum* contain calcium, phosphorus, sodium, potassium, vitamin B₁, vitamin B₂, niacin, vitamin C, vitamin A, flavonoid glycosides [27], linalool, alpha-pinene, gammaterpenine, geranyl acetate, camphor, and geraniol [28]. Photoactive furoisocoumarins, namely coriandrin and dihydrocoriandrin, were also isolated from *C. sativum* seeds [29]. The essential oils of *C. sativum* seed belong to oxygenated monoterpenes, whereas the main compounds are linalool (74.6 %), camphor (5.9 %), geranyl acetate (4.6 %), and *p*-cymene (4 %) [30].

Chinese parsley (*C. sativum*) protected rainbow trout *Oncorhynchus mykiss* from cadmium toxicity [31]. The effect of Chinese parsley on cadmium binding to proteins from the liver and kidney of rainbow trout *O. mykiss* was also studied [32]. A sorbent made of *C. sativum* removed inorganic and methyl mercury from aqueous media [33]. The protective role of aqueous and alcoholic seed extract of *C. sativum* against lead toxicity on hematological and immunological variables was reported [34]. The prophylactic role of coriander (*C. sativum*) on testis of lead-exposed mice was studied [10]. The leaf extract of *C. sativum* protected rats from carbon tetrachloride-induced hepatotoxicity [35]. *C. sativum* leaves showed therapeutic efficacy against metronidazole-induced genotoxicity in the peripheral erythrocytes of *Channa punctatus* [36]. Recently, the protective role of the hydroalcoholic seed extract of *C. sativum* against lead toxicity in the liver, kidney, and blood of rats has been reported from our laboratory [37]. A thorough search of literature revealed that there were no studies on the ameliorative effects of hydroalcoholic extract of *C. sativum* seeds on lead-induced oxidative stress in rat brain. To fill this gap, the present investigation was undertaken.

Material and Method

Chemicals

Lead acetate (99.8 %), thiobarbituric acid (TBA), 2,7-dichlorofluorescein diacetate (DCF-DA), *meso*-2,3-dimercaptosuccinic acid (DMSA), guanidine hydrochloride, and 5-aminolevulinic acid hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The rest of the chemicals were obtained from SISCO Research Laboratories Private Limited (Mumbai, India).

Coriandrum sativum Seed Extract

The hydroalcoholic extract of *C. sativum* seed was obtained as a gift sample (Batch No. L11060631) in the form of dried powder from M/S. Laila Impex Private Limited (Vijayawada, Andhra Pradesh, India).

Animals and Treatments

The male rats of Wistar strain (100–120 g) were procured from Mahaveer Enterprises (Hyderabad, India), and they were kept in the animal facility for 4 days before they were used for experimentation. They were allowed free access to feed (Pranav Agro Industries, India) and water ad libitum. The present study was approved by the Institutional Animal Ethical Committee (IAEC/135/2011). Overall, 24 rats were treated with lead acetate (1,000 mg/L) [37] through drinking water for a period of 4 weeks, parallel controls (eight rats) were maintained on sodium acetate (1,000 mg/L) [37] and the solutions were prepared with distilled water. Group I (eight rats): the control received sodium acetate (1,000 mg/L) in water; group II (eight rats) was exposed to lead acetate (1,000 mg/L); group III (eight rats): *C. sativum*-treated 1 (CST1) received the hydroalcoholic seed extract of *C. sativum* in distilled water through oral intubation at a dose of 250 mg/kg body weight/day for seven consecutive days [37] after 4 weeks of lead exposure. Group IV (eight rats): *C. sativum*-treated 2 (CST2) received the hydroalcoholic seed extract of *C. sativum* in distilled water through oral intubation at a dose of 500 mg/kg body weight/day for seven consecutive days [37] after 4 weeks of lead exposure. The treatments and supply of food to rats were stopped 6 h before sacrifice. Sacrifice was done by cervical dislocation and the whole brain was removed initially. Different regions of the brain namely the cerebellum, hippocampus, frontal cortex, and brain stem were isolated immediately on ice. The brain regions were washed in cold normal saline (0.85 % NaCl) solution. Rat blood was drawn by cardiac puncture and preserved in heparinized vials at 4 °C for estimation of enzyme activity.

Biochemical Assays

ROS

ROS levels in the tissues were estimated using a standard method [38]; 10 % homogenate of each brain region was made in 0.32 M sucrose solution. The homogenates were centrifuged at $1,800\times g$ for 10 min. Pellet was discarded and the supernatant was centrifuged at $31,500\times g$ for 10 min to obtain the crude synaptosomal pellet (P_2). The P_2 pellet was diluted in 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) buffer (120 mM NaCl, 2.5 mM KCl, 0.1 mM $MgCl_2$, 6.0 mM glucose, 1.0 mM $CaCl_2$, 5.0 mM $NaHCO_3$, 10 mM HEPES, pH 7.4) to a concentration of 0.1 g equivalent/mL. The diluted fractions were incubated with $5\ \mu M$ DCF-DA (added from the stock solution of 0.5 mM in 10 % ethanol) at $37\ ^\circ C$ for 15 min. The fluorescence was read at an excitation wavelength of 488 nm and emission wavelength of 525 nm in a spectrofluorometer (Systronics, 152). ROS levels were given as nanomoles of DCF-DA oxidized per 15 min per milligram of protein.

Lipid Peroxidation Products (LPP)

LPP levels were determined following the spectrophotometric method [39]; 10 % homogenate of each brain region was made in 1.5 % KCl. To 1 mL of the homogenate, 2.5 mL of trichloroacetic acid (TCA) was added to precipitate the proteins. The contents were centrifuged at 3,500 rpm for 10 min at $4\ ^\circ C$. The supernatant was discarded and the obtained pellet was dissolved in 2.5 mL of 0.05 M H_2SO_4 , and to this, 3 mL of 2 M thiobarbituric acid (TBA) was added. All the contents were incubated in boiling water bath at $100\ ^\circ C$ for 30 min. The contents were cooled down to room temperature and the color was extracted into 4 mL of *n*-butanol. The pink color obtained was read at 530 nm using a spectrophotometer (Rayleigh UV-9200) against the blank. The results were shown as micro-moles of malondialdehyde (MDA) formed per gram weight of tissue.

Total Protein Carbonyl Content (TPCC)

TPCC levels were quantified using a marginally modified method [40]; 10 % brain tissue homogenate was prepared in cold buffer (50 mM phosphate buffer containing 1 mM EDTA, pH 6.7). The homogenate was centrifuged at $10,000\times g$ for 15 min at $4\ ^\circ C$, and the supernatant was precipitated with equal amounts of 20 % TCA and incubated on ice for 5 min. The tubes were centrifuged at $10,000\times g$ for 10 min at $4\ ^\circ C$ and the supernatant was discarded. The pellet was resuspended in 500 μL of 10 mM 2,4-dinitrophenyl hydrazine (DNPH) in 2 M HCl and incubated at room temperature for 1 h, with vortexing every 10 min at $4\ ^\circ C$, and the pellet was

washed three times with ethanol/ethyl acetate mixture. After the final wash, the pellets were resuspended in 500 μL of 6 M guanidine hydrochloride (pH 2.3). The contents were incubated at $37\ ^\circ C$ for 15 min and were centrifuged at $10,000\times g$ for 10 min at $4\ ^\circ C$. Carbonyl content was measured using a spectrophotometer (Rayleigh UV-9200) at 360 nm against a blank reagent. The results were expressed as nanomoles of carbonyl per milligram of protein.

Delta-Amino Levulinic Acid Dehydratase (δ -ALAD) Activity

Delta-ALAD activity was estimated following a spectroscopic method [41]. The blood samples from rats were collected in heparinized vials using cardiac puncture. The samples were stored at $4\ ^\circ C$ until analysis. Blood was initially hemolyzed with water for 10 min at $37\ ^\circ C$. To the blood sample, potassium phosphate buffer and amino levulinic acid (ALA) (12 mM) were added and incubated for 90 min at $37\ ^\circ C$. After incubation, the enzyme activity was stopped with the addition of 10 % TCA (10 mM $HgCl_2$). Later, the samples were centrifuged at 6,000 rpm for 10 min, and to the supernatant, 1 mL of Erlich reagent was added and incubated for 20 min. To the incubated sample, 0.5 mL of distilled water was added and read at 555 nm in a spectrophotometer (Rayleigh, UV-9200). The results were expressed as nanomoles of porphobilinogen (PPB) formed per hour per milliliter.

Metal Estimation

Metal concentration was measured following an analytical method [42]. The analysis of metal content was carried out with the wet tissue. A known quantity of the tissue was kept in a muffle furnace at a temperature of $600\ ^\circ C$ for about 4–5 h to make into ash. The ash obtained was digested with nitric acid (HNO_3) and dissolved in a known amount of 0.01 N HNO_3 . The final clear and colorless solution was used for metal estimation with an inductively coupled plasma-mass spectrometer (ICP-MS) (Agilent-7700S). Metal concentration was given as microgram of metal per gram wet weight of tissue.

Statistical Analysis

The mean values ($n=8$) with standard deviations were calculated. The significant differences between control, exposed, and treated groups were determined using one-way ANOVA at $p<0.05$ followed by Bonferroni's multiple comparison test. One-way ANOVA was performed using GraphPad Prism version 5.0 for Windows, GraphPad Software, San Diego, CA, USA (www.graphpad.com).

Results

Effect of *C. sativum* on ROS Levels in Different Brain Regions

Figure 1 represents the data on ROS in different brain regions of the control, exposed, CST1, and CST2 groups. There was a significant ($p < 0.05$) increase in the ROS in all the brain regions of the exposed group compared with their respective controls. A maximum increase in ROS was seen in exposed brain stem (39.87 %) with respect to their controls (Table 1). The cerebellum and hippocampus showed maximum recovery in ROS levels in CST1 and CST2 groups (Fig. 1). However, the CST2 group showed the highest recovery than the CST1 group (Table 1). The order of decrease for ROS when compared to exposed for CST1 was cerebellum > brain stem > hippocampus > frontal cortex, whereas for CST2, brain stem > cerebellum > hippocampus > frontal cortex (Table 2). In CST1 and CST2 groups, all the brain regions showed a significant ($p < 0.05$) decrease in ROS levels when compared to their exposed groups (Table 2).

Effect of *C. sativum* on LPP Levels in Different Brain Regions

The results on LPP are presented in Fig. 2 for different brain regions of the control, exposed, CST1, and CST2 groups. A significant ($p < 0.05$) increase was observed in LPP levels of different brain regions of the exposed group when compared to their respective controls (Fig. 2). Brain stem (37.33 %) and frontal cortex (34.9 %) of the exposed group showed a maximum increase in LPP among the brain regions (Table 1). The hippocampus of CST2 showed a significant ($p < 0.05$) recovery in LPP levels (Table 1). The order of decrease for LPP in CST1 and CST2 groups when compared to the control was hippocampus > frontal cortex > cerebellum > brain stem

(Table 1). A significant ($p < 0.05$) decrease in LPP levels was seen in all the brain regions of CST1 and CST2 groups when compared to their respective exposed groups (Table 2) and the hippocampus showed maximum recovery.

Effect of *C. sativum* on TPCC Levels in Different Brain Regions

Figure 3 depicts data on TPCC in the different brain regions of the control, exposed, CST1, and CST2 groups. The TPCC levels increased significantly ($p < 0.05$) in all the brain regions of the exposed group. However, a maximum increase in TPCC levels was observed in the hippocampus of the exposed group when compared to their respective control (Fig. 3; Table 1). The brain stem and frontal cortex showed maximum and significant ($p < 0.05$) recovery in TPCC levels followed by the hippocampus and cerebellum in the CST2 group when compared to control (Table 1). When compared to the exposed groups, CST1 and CST2 groups of all the brain regions showed a significant ($p < 0.05$) decrease in TPCC levels (Table 2). The order of decrease for TPCC in the CST2 group in comparison with the exposed was frontal cortex > brain stem > hippocampus > cerebellum (Table 2).

Effect of *C. sativum* on δ -ALAD Activity in the Blood

δ -ALAD activity (Fig. 4) of blood showed a significant ($p < 0.05$) decrease (30.47 %) in the exposed group when compared to control. However, CST1 and CST2 groups exhibited a significant ($p < 0.05$) increase in δ -ALAD activity and a maximum increase in δ -ALAD enzyme activity was seen in the CST2 group. There was also a significant ($p < 0.05$) increase in CST2 compared to the CST1 group showing dose dependency.

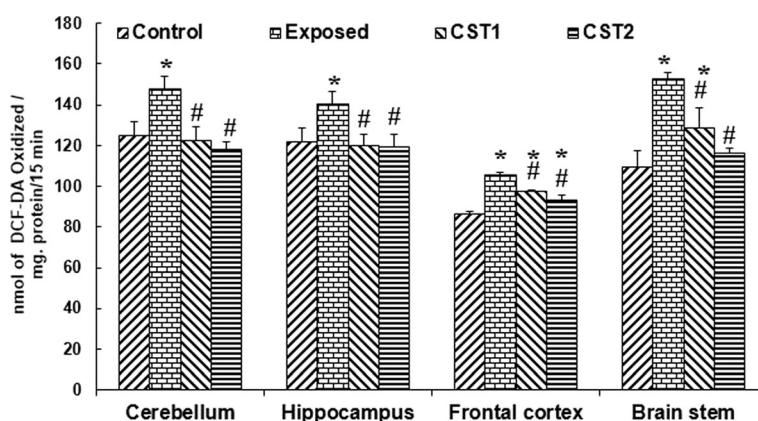


Fig. 1 Reactive oxygen species (ROS) in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units and abbreviation used: nanomoles of DCF-DA (dichlorofluorescein diacetate)

oxidized per milligram of protein per 15 min. * $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

Table 1 Percent change over their respective controls and levels of significance in different brain regions of the exposed, CST1, and CST2 groups

	Exposed	CST1	CST2
ROS			
Cerebellum	+17.92***	-2.19	-5.56
Hippocampus	+14.97***	-1.5	-1.97
Frontal cortex	+22.25***	+12.93***	+2.96***
Brain stem	+39.87***	+17.75**	+6.18
LPP			
Cerebellum	+30.68***	+12.12***	+7.18***
Hippocampus	+30.13***	-4.7	-9.44**
Frontal cortex	+34.90***	+2.71	-0.7
Brain stem	+37.33***	+16.04**	+14.86**
TPCC			
Cerebellum	+108.82***	+25.73***	+24.26***
Hippocampus	+141.97***	+27.76***	-1.5
Frontal cortex	+106.00***	+8.5	-35.0*
Brain stem	+71.64***	-24.62***	-40.29***
Lead			
Cerebellum	+1123.81***	+564.28***	+351.58***
Hippocampus	+515.77***	+326.17***	+322.81***
Frontal cortex	+144.44***	+94.44***	+15.55
Brain stem	+402.27***	+105.45	+42.72
Copper			
Cerebellum	-26.58*	+40.76***	+84.66***
Hippocampus	-30.35***	+13.74*	+50.96***
Frontal cortex	-32.43**	-11.46	+2.13
Brain stem	-36.21**	+5.40	+54.56***
Zinc			
Cerebellum	-47.42***	-46.04***	-32.95***
Hippocampus	-25.39***	+0.24	+10.73***
Frontal cortex	-39.76***	-12.25	+39.04***
Brain stem	-47.82***	-28.23***	+54.77***
Iron			
Cerebellum	-25.70***	+5.58	+9.61
Hippocampus	-34.96***	+20.24**	+22.69**
Frontal cortex	-38.55***	-12.37	+3.95
Brain stem	-21.35*	+19.68*	+22.79***
Chromium			
Cerebellum	-54.68***	-36.71***	-17.96***
Hippocampus	-58.54***	-32.72***	-17.45***
Frontal cortex	-36.36***	-32.72***	-1.8
Brain stem	-30.53***	+6.59	+45.91***
Delta-ALAD activity	-30.47***	+34.71***	+58.71***

+ indicates increase and - indicates decrease

* $p < 0.05$, significantly different from the control; ** $p < 0.01$, significantly different from the control; *** $p < 0.001$, significantly different from the control

Table 2 Percent change over exposed and levels of significance in different brain regions of the CST1 and CST2 groups

	CST1	CST2
ROS		
Cerebellum	-17.06***	-19.92***
Hippocampus	-14.41***	-14.73***
Frontal cortex	-07.61***	-11.68***
Brain stem	-15.81***	-24.09
LPP		
Cerebellum	-14.20***	-17.98***
Hippocampus	-26.84***	-30.41***
Frontal cortex	-23.86***	-26.40***
Brain stem	-15.49***	-16.35***
TPCC		
Cerebellum	-39.78***	-40.49***
Hippocampus	-47.19***	-59.29***
Frontal cortex	-47.52***	-68.59***
Brain stem	-56.08***	-65.21***
Lead		
Cerebellum	-45.71***	-63.09***
Hippocampus	-30.79***	-31.33***
Frontal cortex	-20.45***	-52.72***
Brain stem	-59.09***	-71.58***
Copper		
Cerebellum	+91.72***	+151.52***
Hippocampus	+63.31***	+116.76***
Frontal cortex	+31.04***	+51.17***
Brain stem	+65.24***	+142.30***
Zinc		
Cerebellum	+2.62	+27.52
Hippocampus	+34.37***	+48.43***
Frontal cortex	+45.68***	+130.79
Brain stem	+37.54***	+196.64
Iron		
Cerebellum	+42.11***	+47.59***
Hippocampus	+84.90***	+88.67***
Frontal cortex	+42.61***	+69.19***
Brain stem	+52.24***	+56.18***
Chromium		
Cerebellum	+39.65***	+81.03***
Hippocampus	+99.12***	+62.28***
Frontal cortex	+5.71	+54.28***
Brain stem	+53.45***	+110.06***
Delta-ALAD activity	+95.45***	+131.18***

+ indicates increase and - indicates decrease

* $p < 0.05$, significantly different from the exposed; ** $p < 0.01$, significantly different from the exposed; *** $p < 0.001$, significantly different from the exposed

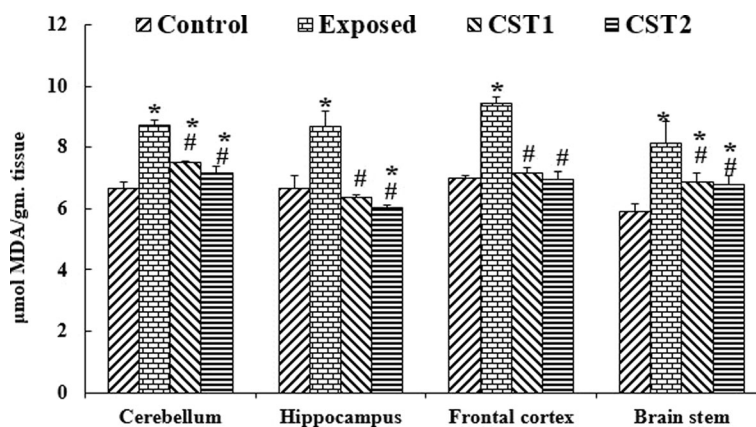


Fig. 2 Lipid peroxidation products (LPP) in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units and abbreviation used: micromoles of malondialdehyde per gram of tissue.

* $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

Effect of *C. sativum* on Lead Concentration in Different Brain Regions

The data on tissue lead concentration in different brain regions of the control, exposed, CST1, and CST2 groups are presented in Fig. 5. A significant ($p < 0.05$) increase in lead concentration was seen in all the exposed brain regions compared to their respective controls (Fig. 5). The order of increase for lead concentration in exposed brain regions was cerebellum > hippocampus > brain stem > frontal cortex (Table 1). Both CST1 and CST2 groups showed a similar order of decrease for lead concentration in brain regions after treatment, i.e., frontal cortex > brain stem > hippocampus > cerebellum with respect to their controls (Table 1). When compared to the exposed, the order of decrease for the CST2 group was brain stem > cerebellum > frontal cortex > hippocampus (Table 2). All the brain regions in CST1 and CST2 showed a significant ($p < 0.05$) decrease in lead concentration when compared to the exposed group.

Effect of *C. sativum* on Copper Concentration in Different Brain Regions

The concentration of copper in different brain regions of the control, exposed, CST1, and CST2 rats was presented in Fig. 6. There was a significant ($p < 0.05$) decrease in copper concentration in the exposed brain regions when compared to their respective controls (Fig. 6). A maximum decrease (36.21 %) in copper concentration was seen in brain stem (Fig. 6), and the order of decrease for copper concentration in other regions was frontal cortex > hippocampus and cerebellum (Table 1). However, the CST2 group restored the copper levels back to normal and the increase was significant ($p < 0.05$) in the case of the cerebellum, brain stem, and hippocampus except for the frontal cortex when compared to their respective controls (Table 1). The order of restoration for copper in comparison with the exposed was cerebellum > brain stem > hippocampus > frontal cortex (Table 2). When compared to the exposed, CST1 and CST2 groups of all the brain regions showed a significant ($p < 0.05$) increase in copper levels.

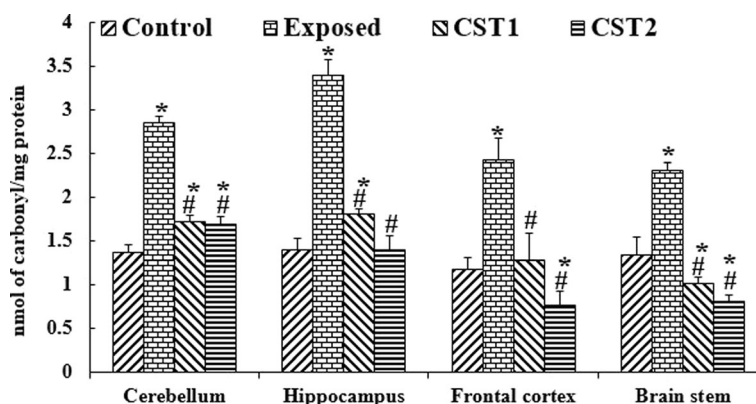


Fig. 3 Total protein carbonyl content (TPCC) in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: nanomoles of carbonyl per milligram of protein. * $p < 0.05$, significantly

different from their respective controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

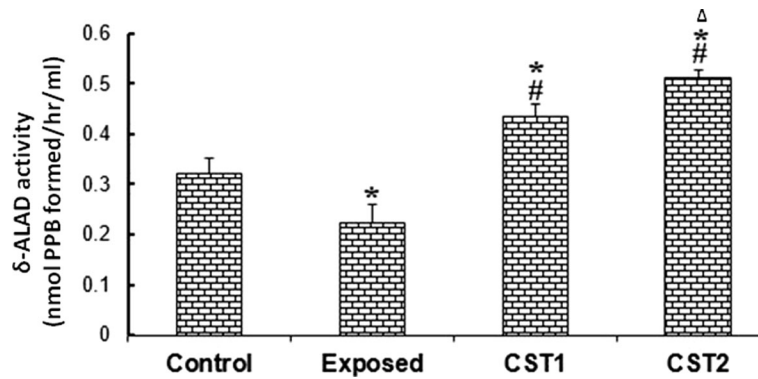


Fig. 4 Delta-amino levulinic acid dehydratase (ALAD) activity in the blood of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: nanomoles of porphobilinogen (PPB) formed per hour per

milliliter of blood. * $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from the exposed group; Δ $p < 0.05$, significantly different from CST1 as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

Effect of *C. sativum* on Zinc Concentration in Different Brain Regions

Figure 7 shows the data on zinc concentration in different brain regions of the control, exposed, CST1, and CST2 groups. Zinc concentration showed a significant ($p < 0.05$) decrease on lead exposure in different brain regions when compared to their respective controls (Fig. 7). The order of decrease for zinc in exposed brain regions was brain stem > cerebellum > frontal cortex > hippocampus (Table 1). However, the order of restoration for zinc in the CST2 group when compared to the control and exposed was similar, i.e., brain stem > frontal cortex > hippocampus > cerebellum (Tables 1 and 2). All the brain regions except the cerebellum in CST1 and CST2 showed a significant ($p < 0.05$) increase in zinc concentration when compared to the exposed group (Table 2).

Effect of *C. sativum* on Iron Concentration in Different Brain Regions

The concentration of iron in different brain regions of the control, exposed, CST1, and CST2 rats was presented in Fig. 8. A significant ($p < 0.05$) decrease in iron concentration was seen in the exposed brain regions when compared to their respective controls (Fig. 8). The order of decrease for iron was frontal cortex > hippocampus > cerebellum > brain stem (Table 1). In the CST2 group, the hippocampus and brain stem showed a significant ($p < 0.05$) recovery when compared to control (Table 1). When compared to the exposed, the restoration for iron was maximum in the hippocampus followed by the frontal cortex, brain stem, and cerebellum (Table 2). In CST1 and CST2 groups, all the brain regions showed a significant ($p < 0.05$) increase in iron levels when compared to their exposed groups (Table 2).

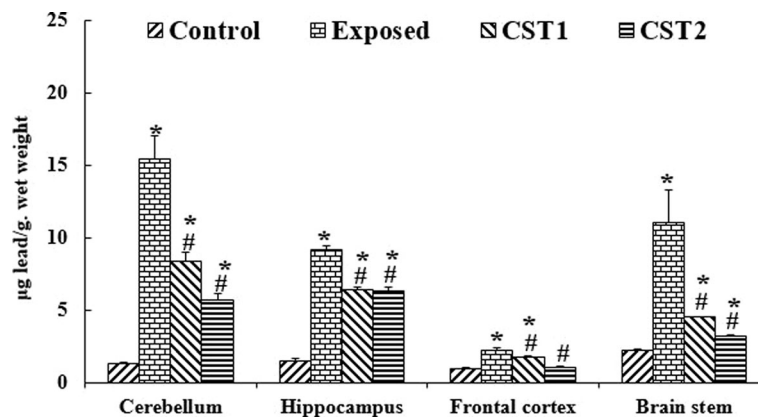


Fig. 5 Lead in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: microgram lead per gram wet weight.

* $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

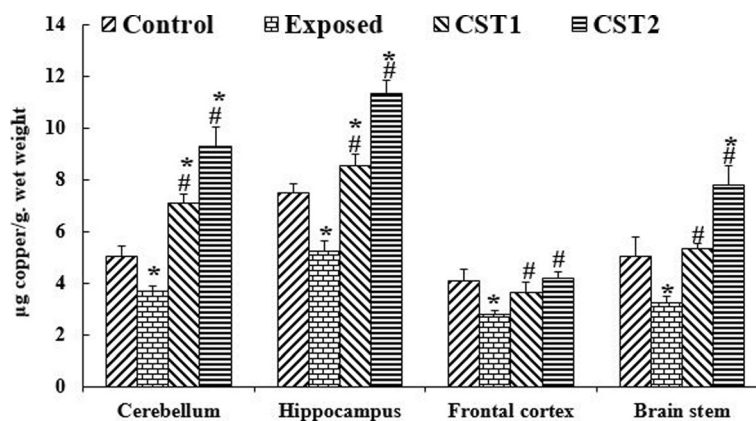


Fig. 6 Copper in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: micrograms of copper per gram wet

weight. * $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

Effect of *C. sativum* on Chromium Concentration in Different Brain Regions

Chromium concentration in different brain regions of the control, exposed, CST1, and CST2 rats was given in Fig. 9. However, a significant ($p < 0.05$) decrease in chromium concentration was observed in the exposed group when compared (Fig. 9) to their respective controls, and the order of decrease was hippocampus > cerebellum > frontal cortex > brain stem (Table 1). When compared to control, the order of restoration for chromium was brain stem > frontal cortex > hippocampus > cerebellum (Table 1). The order of recovery for chromium in comparison to the exposed was brain stem > hippocampus > cerebellum > frontal cortex (Table 2). Except for the frontal cortex of CST1, all the brain regions in CST1 and CST2 groups showed a significant ($p < 0.05$) increase in chromium concentration when compared to the exposed group (Table 2).

Discussion

The present study was aimed to investigate the ability of *C. sativum* seed extract to give protection against lead-induced oxidative stress in rat brain as *C. sativum* is well known for its prophylactic properties. The increase in lead concentration in different brain regions following lead exposure was associated with increased oxidative stress, which might be responsible for lead-induced noxious effects indicated by increase in ROS, LPP, and TPCC.

Heavy metal toxicity causes mitochondrial degeneration, resulting in the generation of excessive free radicals. Lead toxicity causes free radical damage by increasing the levels of ROS including hydroperoxide, singlet oxygen, hydrogen peroxide, and direct depletion of antioxidant reserves [43]. The primary target of oxidative damage produced by xenobiotics is on the cellular membrane [44], and the ROS attack the cellular components particularly polyunsaturated fatty acids of phospholipids which are sensitive to oxidation [45].

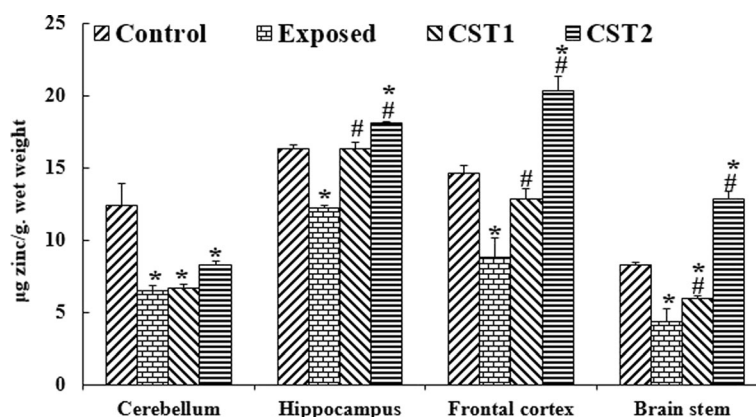


Fig. 7 Zinc in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: micrograms of zinc per gram wet

weight. * $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

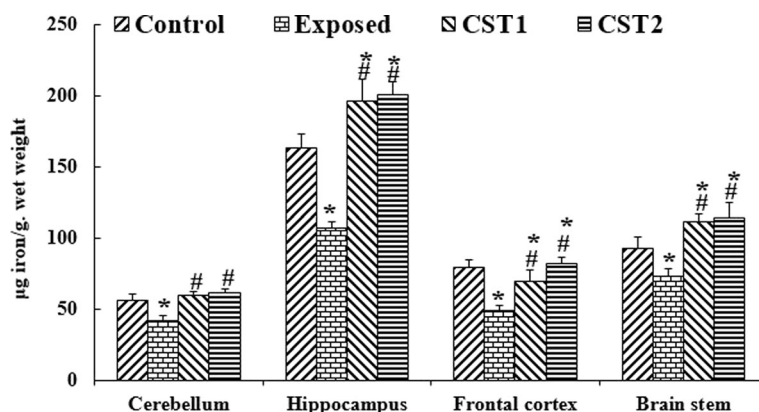


Fig. 8 Iron in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: micrograms of iron per gram wet

weight. * $p < 0.05$, significantly different from their respective controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

Flavonoids such as quercetin 3-glucuronide, isoquercetin, and rutin that were identified in coriander seeds [46] are reported to offer protection against these free radicals. Studies on the antioxidant properties of flavonoids from various plant extracts showed stimulatory action on antioxidant enzymes [47], and they also showed stimulatory action on transcription and gene expression of certain antioxidant enzymes [48]. The decrease in ROS levels in CST1 and CST2 can be attributed to the protective role of *C. sativum*. Administration of *C. sativum* seed extract restored the levels of ROS back to normal in all the brain regions tissue-specifically.

MDA is the main reactive aldehyde produced during the peroxidation of polyunsaturated fatty acids present in biological membrane. Generally, MDA levels are considered as indicators of tissue oxidative stress involving a series of chain reactions [39]. It is well known that lead binds to enzymes and reduces enzyme activities leading to disturbances even in the protein synthesis of cells [49]. Lead induces the free radical formation indirectly and influences the processes of lipid

peroxidation through damage of the protective antioxidant barrier [50]. Lead possesses a strong affinity to thiol groups of amino acids, especially cysteine. Lead may affect the antioxidant barrier via inhibiting the functional thiol groups of enzymes such as superoxide dismutase (SOD) and glutathione S-transferases (GSTs) [51, 52]. Human lymphocytes pretreated with polyphenols isolated from *C. sativum* showed protection against oxidative damage induced by hydrogen peroxide. Polyphenols extracted from coriander seeds showed a significant decrease in the concentration of thiobarbituric acid reactive species (TBARS) in human lymphocytes compared with the hydrogen peroxide (H_2O_2) control [53]. Similarly, in the present investigation, the flavonoids present in *C. sativum* might have protected the brain regions from the oxidative injury induced by lead. The volatile compounds present in coriander seed oil attenuate lipid peroxidation [54, 55]. Similar studies using coriander seed extract also showed a positive correlation between phenolic content and antioxidant activity. The seed extract was found to show concentration-

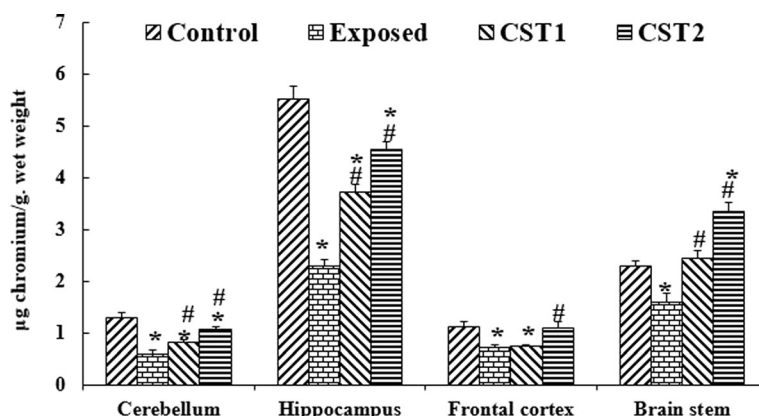


Fig. 9 Chromium in different brain regions of the control, exposed, CST1, and CST2 rats. Bars represent means of eight animals and vertical lines represent standard deviation. Units: micrograms of chromium per gram wet weight. * $p < 0.05$, significantly different from their respective

controls; # $p < 0.05$, significantly different from the exposed group as determined by one-way ANOVA followed by Bonferroni's multiple comparison test

dependent inhibitory activity towards 15-lipoxygenase and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity [22]. While pretreating the carbon tetrachloride (CCl₄)-exposed rats with *C. sativum*, normalization of TBARS and glutathione in different tissues was noticed signifying the efficacy of the plant extract to act as an antioxidant [56]. Administration of *C. sativum* significantly decreased the elevated serum enzymes (SGOT and SGPT) induced by CCl₄, indicating improvement of the functional status of the liver [35]. Quercetin, a flavonoid present in *C. sativum* seeds, could inhibit apoptotic damage by reducing lipid peroxidation and enhancing the activities of antioxidant enzymes [57]. This may be one of the possible mechanisms of protection against lead-induced lipid peroxidation in the present study.

Introduction of carbonyl groups to proteins is a hallmark of oxidative stress. Oxidative damage to proteins leads to reduction in the normal function of proteins [40]. The extent of oxidative damage to protein is generally determined by measuring the carbonyl content. These TPCC levels were found to increase in lead-exposed rat brain regions in the present investigation. Similarly, along with ROS and LPP, there was recovery in TPCC levels also after administration of *C. sativum*. This shows the protective role of *C. sativum* in reducing TPCC levels. A recent study from our laboratory on lead-induced oxidative stress in rat brain showed an increase in TPCC and treatment with aqueous extract of *Bacopa monniera* showed a decrease in TPCC [58].

Lead is well known to inhibit delta-aminolevulinic acid dehydratase activity, and this is an indicator enzyme for lead toxicity by binding to its active center [59]. This enzyme catalyzes the asymmetric condensation of two molecules of delta-aminolevulinic acid to porphobilinogen in the initial steps of heme biosynthesis. Lead toxicity increases the excretion of accumulated ALA into urine [60, 61] and decreases the production of PPB. But administration of *C. sativum* decreased the concentration of urinary ALA, suggesting that Chinese parsley has efficacy against lead poisoning [62]. Inhibition of δ -ALAD activity leads to accumulation of delta-aminolevulinic acid (δ -ALA), which undergoes auto-oxidation inducing free radicals and, in this way, induces lipid peroxidation [59]. Similarly, in the present study, there was a decrease in δ -ALAD activity and an increase in the levels of ROS and LPP due to lead toxicity. Treatment with *C. sativum* showed reduction in the levels of ROS, LPP, and TPCC. In contrast to this, there was an increase in δ -ALAD activity in CST1 and CST2 groups indicating restoration to normal levels. A similar decrease in δ -ALAD activity was noticed in rats exposed to lead [5, 63]. However, treatment with *C. sativum* restored the δ -ALAD enzyme activity in a dose-dependent manner in the present investigation.

It is clear from the results of the present investigation that administration of the hydroalcoholic extract of *C. sativum* protected brain regions against lead-induced oxidative stress.

The results of the present study corroborate well with the early reports that showed the protective effects of *C. sativum* on localized lead deposition in male imprinting control region (ICR) mice [62]. A similar prophylactic efficacy of *C. sativum* (Coriander) was reported on the testis of lead-exposed mice [10].

Lead concentration significantly increased in the exposed rat brain region specifically. However, upon treatment with *C. sativum*, there was region-specific decrease in lead levels in CST1 and CST2 rats. Micronutrients such as zinc, copper, iron, and chromium levels decreased in the lead-exposed group, but treatment with *C. sativum* showed restoration near to control in CST1 and CST2 rats. Flavonoids and phenolic compounds potentiate the removal of lead from the body by their metal-chelating ability [64]. Administration of *C. sativum* also prevented the accumulation of cadmium in the fish *O. mykiss* [65]. However, *C. sativum* is known to increase tissue glutathione levels, and there is a possibility that lead might interact with glutathione forming a complex that can be excreted [10]. This may be one of the possible mechanisms of lead chelation in different regions of rat brain in the present investigation as have been explained earlier [10]. The removal of lead in different tissues observed in the present investigation was not uniform, and this might be because of region-specific responses of the brain to amelioration therapy by *C. sativum*. Further investigations are needed to demonstrate this in detail.

Micronutrients such as copper, zinc, and iron are essential for myelin synthesis, structure, and maintenance of oligodendrocytes [66]. Trace metal homeostasis is a vital feature for proper functioning of the brain. Neurons in the central nervous system may be injured due to functional loss of metalloproteins and disruption in trace metals homeostasis by oxidative damage [67]. Toxic heavy metals are known to compete or interfere with essential elements [68], and this may result in the loss of homeostasis of micronutrients [69]. Increased or decreased levels of micronutrients in the brain may disturb the normal physiological functions and this leads to nervous system disorders [70]. In the present study, in contrast to increased lead concentration, there was a decrease in the levels of copper, zinc, chromium, and iron in the brain regions. Copper is an essential part of various enzymes including cytochrome C-oxidase, SOD, lysyl oxidase, tyrosinase, and ceruloplasmin [71], indicating its primary role in the basic biochemistry of the human nervous system. An increase in liver and kidney copper concentration on exposure to toxic metal (arsenic) was already reported [72]. However, sub-chronic arsenic toxicity resulted in the rise of copper concentration in the cerebrum and cerebellum of mice [73]. Copper homeostasis is usually maintained by biliary excretion [72], and lead exposure might have decreased biliary excretion of copper causing its accumulation. Further studies are required to support this hypothesis. However, coriander was reported to

increase bile secretion in rats [21]. This might have resulted in normalization of copper concentration in CST1 and CST2 rats.

Zinc is bound to proteins in the brain. Stable zinc levels in the brain are essential for homeostasis and this reduces neurological disorders. Oxidative stress or pathology alters the levels of copper and zinc, and this might be due to damage caused to their homeostatic mechanism that is responsible for maintenance of tissue micronutrient levels. However, administration of *C. sativum* showed restoration of copper, zinc, chromium, and iron back to physiological levels. This might be due to the protective role of *C. sativum* extract in maintaining copper, zinc, chromium, and iron levels in different regions of the brain.

Overall, the results of the present investigation support the hypothesis that the hydroalcoholic seed extract of *C. sativum* protects the rat brain region specifically from lead-induced oxidative stress by its antioxidant and metal-chelating properties. Tissue oxidative stress parameters and lead concentration in the brain showed an increase region specifically on exposure to lead, and the degree of restoration was not uniform on treatment with *C. sativum* indicating region-specific response in the brain. δ -ALAD enzyme activity, copper, zinc, chromium, and iron levels showed decrease on lead exposure and treatment with *C. sativum* showed protection against lead toxicity. Maximum protection from lead toxicity was seen in the CST2 group. Further studies are needed to interpret the exact mechanism by which *C. sativum* protects rat brain from lead toxicity. Overall, these results suggest that the seed extract of *C. sativum* might reduce the lead-induced oxidative stress region specifically by its antioxidant and metal-chelating activities.

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Conflict of Interest The authors declare that they have no conflict of interest.

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