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Original Article

Neuroprotective Actions of Clinoptilolite and Ethylenediaminetetraacetic Acid Against Lead-induced Toxicity in Mice *Mus musculus*

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ABSTRACT

Objectives: Oxidative stress is considered as a possible molecular mechanism involved in lead (Pb^{2+}) neurotoxicity. Very few studies have been investigated on the occurrence of oxidative stress in developing animals due to Pb^{2+} exposure. Considering the vulnerability of the developing brain to Pb^{2+}, this study was carried out to investigate the effects of Pb^{2+} exposure in brain regions especially on antioxidant enzyme activities along with ameliorative effects of ethylenediaminetetraacetic acid (EDTA) and clinoptilolite. **Methods:** Three-week old developing Swiss mice *Mus musculus* were intraperitoneally administered with Pb^{2+} acetate in water (w/v) (100 mg/kg body weight/day) for 21 days and control group was given distilled water. Further Pb^{2+}-toxicated mice were made into two subgroups and separately supplemented with EDTA and clinoptilolite (100 mg/kg body weight) for 2 weeks. **Results:** In Pb^{2+}-exposed mice, in addition to increased lipid peroxidation, the activity levels of catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH) found to decrease in all regions of brain indicating, existence of severe oxidative stress due to decreased antioxidant function. Treatment of Pb^{2+}-exposed mice with EDTA and clinoptilolite lowered the lipid peroxidation (LPO) levels revealing their antioxidant potential to prevent oxidative stress. Similarly their administration led to recover the level of catalase, SOD, and GPx enzymes affected during Pb^{2+} toxicity in different regions of brain. **Conclusions:** The protection of brain tissue against Pb^{2+}-induced toxicity by clinoptilolite and EDTA in the present experiment might be due to their ability to react faster with peroxyl radicals there by reducing the severity of biochemical variable indicative of oxidative damage. Thus, the results of present study indicate the neuroprotective potential of clinoptilolite and EDTA against Pb^{2+} toxicity.

Key words: Clinoptilolite, EDTA, lead neurotoxicity, oxidative stress

INTRODUCTION

Lead (Pb^{2+}), a heavy metal is one of the most potential toxic elements in the environment and is known to be toxic to the central nervous system.[1,2] From low to high doses of Pb^{2+} exposure, there are different responses of Pb^{2+}-induced oxidative stress in various target sites including brain.[3] Studies of Khan *et al*., (2008)[4] have shown that excessive generation of reactive oxygen species (ROS) *in vivo* upon Pb^{2+} intoxication, resulted in systemic mobilization and depletion of intrinsic antioxidant defenses, destabilizing calcium homeostasis by damaging electron transport, adenosine triphosphate (ATP) depletion, and membrane ion channel(s) disruption ultimately leading to apoptosis.

Nutritional factors though indicated as effective for reversing Pb^{2+} neurotoxicity, available literature is

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inconclusive. Very few studies have revealed the influence of dietary factors on the neuronal function and synaptic plasticity.[5] Vitamin C has earlier been reported as a possible chelator of Pb^{2+} toxicity with similar potency as that of ethylenediaminetetraacetic acid (EDTA).[6] However, no change was found in blood Pb^{2+} level in workers occupationally exposed to Pb^{2+}, and supplemented with zinc and vitamin C. Studies of Ahmad *et al*., (2013)[7] have reported the protective role of microporous natural clinoptilolite on Pb^{2+}-induced (0.2%) learning and memory deficits in rats. Similarly Mikirova *et al*.,(2011)[8] have suggested EDTA chelation therapy for the treatment of metal toxicity. Although, studies have been initiated with EDTA and zeolite-clinoptilolite, their protective efficacy against oxidative stress indices in discrete regions of mice are not addressed till date. The adsorption and ion exchange properties of natural zeolite represented the hypothesis of a possible easing of the biological uptake of Pb^{2+} and its subsequent effects on antioxidant enzymes. Similarly, chelation therapy has been proposed as a treatment for heavy metal poisoning in which the removal of heavy metal ions is hypothesized to reduce the oxidative damage caused by the production of hydroxyl radicals. Therefore, the aim of this study was to assess the hazardous effect of Pb^{2+} and the protective effects of clinoptilolite and EDTA on Pb^{2+}-induced oxidative changes on biochemical variables in rat brain regions.

MATERIALS AND METHODS

Chemicals

Natural clinoptilolite, (MZ: Micronized zeolite) with empirical formula: $(Na^{+}, K^{+,} Ca^{++,} Mg^{++})^{+6}$ $[(AlO2)_6 (SiO_2)_{30}]^{-6}$ $24H_2O$ was procured from ZEO inc. 2104 Augusta, (Mc Kinney, TX 75070, USA) EDTA-Na^{+} and other AR Grade chemicals were purchased from BDH and Sigma- Aldrich. Pb^{2+} acetate trihydrate (C4H6O4Pb3H20) of molecular weight 379.33 was supplied by Merck India Ltd. Each 1 g of Pb^{2+} is found in 1.8307 g of finely powdered Pb^{2+} acetate.[9]

Animals

The protocol of this study was approved by the Institutional Animal Ethics Committee, Bangalore University, Bangalore, India Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA) registration no. 402, file no. 25/525/2009 awd dt 23.03.2011). Four sets ($n = 6$ in each set) of developing Swiss mice *Mus musculus* of 3-weeks-old (10-12 g) along with mother rat were procured from Sri Raghavendra Enterprises, Bangalore and acclimatized to laboratory conditions (25 ± 2°C with 12 h dark/light cycle) in individual standard polyethylene cages (41 × 28 × 14 cm; B.I.K Industries, Mumbai) with stainless steel top grill. During the whole experimentation, all animals had access to tap water and commercial food pellets (Amruth Feeds, India) *ad libitum*.

Experimental protocol

The developing mice were divided into six groups ($n = 6$ in each group):

- Group 1: Control; received only water and food pellets
- Group 2: Toxicated; received Pb^{2+} acetate intraperitoneally, dissolved in water (w/v) (100 mg Pb^{2+}/kg body weight/day) at a constant small volume of 0.1 mL for 21 days (selection of dose and duration of exposure was based on earlier studies)[10,11]
- Group 3-4: Pb^{2+}-toxicated mice were further subgrouped and separately received antioxidants for 2-weeks at dose of 100 mg/kg body weight/day (dissolved in water, w/v) EDTA[12] and clinoptilolite.[13]

During experimentation, all experimental animals were housed along with mother-mice separately. Growth rate, fluid, and food consumption was measured on a weekly basis. Prior to necropsy, blood samples (3 mL) were drawn from jugular vein under diethyl ether anesthesia for determining Pb^{2+} levels and mice were quickly dissected out to separate brain tissues viz., cerebral cortex, cerebellum, hippocampus, and brain stem for biochemical assessments.

In vivo biochemical assays

Estimation of Pb^{2+}

Pb^{2+} levels in blood and brain tissue was evaluated by adapting the procedure given by Berlin and Schaller (1974).[14] Briefly, 0.2 mL of heparinized blood was mixed with 1.3 mL of distilled water and incubated for 10 min at 37°C for complete hemolysis. After adding 1 mL of standard d-ALA, the tubes were incubated for 60 min at 37°C. The reaction was stopped after 1 h by adding 1 mL of trichloroacetic acid (TCA) and centrifuged. To the supernatant, an equal volume of Ehrlich reagent was added and the absorbance was recorded at 555 nm after 5 min.

Lipid peroxidation

LPO product was estimated by measurement of thiobarbituric acid reactive substances using the method of Nehius and Samuelson (1968).[15] The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde (MDA), a secondary product of lipid peroxidation, was estimated at 535 nm.

Superoxide dismutase (SOD; EC 1.15.1.1)

SOD activity was assayed by measuring the inhibition of epinephrine auto-oxidation as described by Misra and Fridovich (1972).[16] The absorbance was recorded at 480 nm for 60 s.

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Catalase (CAT; EC 1.11.1.6)

Catalase activity was measured as described by Aebi (1984).[17] The rate constant of hydrogen peroxide (H_2O_2) decomposition was monitored by measuring the decrease in absorbance at 240 nm for 60 s.

Glutathione peroxidase activity (GPx; EC 1.11.1.9)

The enzyme activity was estimated as described by Rotruck *et al*., (1973)[18] and change in absorbance was measured at 412 nm.

Reduced glutathione

GSH content was determined by using 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) as fluorescent reagent according to the method of Ellman (1959).[19] GSH levels were monitored at 412 nm.

Protein assay

Total protein content was estimated by the method of Lowry *et al.*, (1951),[20] using bovine serum albumin (BSA) as a standard.

Statistics

The results are expressed as mean ± standard deviation (SD) of six observations ($n = 6$) in each group. Differences between treatment groups were assessed by one-way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) software package for windows version 15.0 program. Post-hoc testing was performed for intergroup comparisons using Bonferroni test at probability $P < 0.05$ level of significance. To assess the interactions, three-way ANOVA was carried out in addition to Duncan's test for multiple comparisons to define the nature of the effect. In addition, the average rate of feed and water consumption (per day), body weight (per week) was recorded. The organ somatic index (OSI) was calculated by using the formula:

$$\text{OSI} = \frac{\text{Weight of organ (g)}}{\text{Total body weight (g)}} \times 100$$

RESULTS

A significant ($P < 0.05$) reduction in the body weight was recorded in mice upon Pb^{2+} exposure, while EDTA and clinoptilolite administration has considerably favored the animals in gaining weights [Table 1]. Pb^{2+} levels measured in blood and brain tissues upon 4 weeks of Pb^{2+} exposure raised the blood Pb^{2+} levels to approximately 34.89 mg/dL, while clinoptilolite and EDTA treatments reduced Pb^{2+} levels to 18.39 and 14.59 mg/dL, respectively [Table 2]. In comparison, cerebellum and cerebral cortex regions found to be more sensitive and showed higher accumulation of Pb^{2+}. Further, EDTA supplementation offered better protection in eliminating Pb^{2+} than clinoptilolite. Clinoptilolite supplementation caused −119.44, −109.09, −89.18, and −90.32% reductions in tissue Pb^{2+} levels, while EDTA supplementation produced −16.12, −9.09, −24.32, and −16.12 reductions in cortex, brain stem, cerebellum, and hippocampus regions, respectively [Table 2].

Antioxidant enzymes

Pb^{2+}-intoxication in mice caused +156.57, +185.50, +87.62, and +55.63% increase in MDA level(s) in cortex, brain stem, cerebellum, and hippocampus regions, respectively; while treatment with both EDTA and clinoptilolite significantly ($P < 0.05$) lowered the MDA level indicating therapeutic efficacy of antioxidants in reversing the toxicity induced by Pb^{2+} exposure. A significant ($P < 0.05$) decrease in CAT, SOD, and GPx activities along with GSH levels were observed in all the regions of brain studied as a function of Pb^{2+} exposure [Table 3]. Results showed −60.90, −65.01, −47.04, and −36.82% reductions in CAT; −57.06, −46.60, −38.95, and −53.25% reductions in SOD; −19.03, −53.38, −45.66, and −46.11% reductions in GSH; −38.86, −41.33, −22.61, and −40.74% reductions in GPx activities in cortex, brain stem, cerebellum, and hippocampus regions, respectively. Exogenous supplementation of antioxidants caused a significant ($P < 0.05$) amelioration by augmenting the

Table 1: Weekly body weight (g) changes upon 4 weeks of lead exposure in mice and amelioration by clinoptilolite and EDTA

Groups	Week 1	Week 2	Week 3	Week 4	Week 5
Control	14.24	16.46	18.98	24.37	28.89
Lead exposure	13.39	14.37	15.89	18.32	22.19
Lead+EDTA	-	-	-	21.49	24.25
Lead+zeolite	-	-	-	23.72	25.98

Values are mean of six animals in each group. EDTA = Ethylenediaminetetraacetic acid

Table 2: Lead concentrations (mg/g wet tissue) in blood and discreet brain tissues upon 4-weeks of lead exposure and amelioration by clinoptilolite and EDTA

	Control	Lead	Lead+clinoptilolite	Lead+EDTA
Blood	1.09±0.01	34.89±4.1^b (+202.77%)	18.39±2.9^c (+119.44%)	14.59±1.0^a (−16.12%)
Cerebral cortex	0.36±0.03^a	1.09±0.09^{NS} (+ 202.77%)	0.79±0.06^{NS} (+119.44%)	0.31±0.05^a (−16.12%)
Brain stem	0.33±0.03^a	1.33±0.08^b (+303.03%)	0.69±0.04^c (+109.09%)	0.30±0.08^a (-9.09%)
Cerebellum	0.37±0.05^a	0.92±0.06^b (+148.64%)	0.70±0.03^c (+89.18%)	0.28±0.09^a (−24.32%)
Hippocampus	0.31±0.03^a	0.84±0.03^b (+170.09%)	0.59±0.04^c (+90.32%)	0.26±0.04^c (−16.12%)

Values are mean±standard deviation (SD) of six observations, Values in parenthesis indicate percentage change found, '+' sign indicate increase and '−'sign indicate decrease over controls, Value $^aP<0.001$, $^bP<0.005$, $^cP<0.01$, NS = Not significant, EDTA = Ethylenediaminetetraacetic acid

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Table 3: Regional changes in brain oxidative stress indices upon lead exposure and amelioration by EDTA and clinoptilolite supplementation

Regions	Control	Lead	Lead+EDTA	Lead+clinoptilolite
LPO (*n* moles of MDA formed/g wet tissue)				
Cortex	267.34±13.48	685.93±29.40[a] (156.57%)	357.19±22.72[a] (33.60%)	222.4±29.79[a] (−16.81%)
Cerebellum	280.50±15.64	800.84±18.18[a] (185.50%)	275.87±46.31[b] (1.65%)	198.53±56.96[NS] (29.22%)
Brainstem	260.77±24.92	489.28±12.02[a] (87.62%)	337.21±28.74[a] (29.31%)	278.43±35.22[a] (6.79%)
Hippocampus	264.71±8.37	411.97±19.30[a] (55.63%)	346.23±20.35[NS] (30.79%)	264.64±29.96[NS] (−0.02%)
Catalase (*n* moles of H_2O_2 consumed/min/mg protein)				
Cortex	69.09±2.9	27.01±1.3[a] (−60.90%)	35.85±2.3[b] (−48.11%)	32.4±2.8[a] (−53.10%)
Cerebellum	80.50±2.0	28.16±1.4[a] (−65.01%)	28.03±4.7[c] (−65.18%)	31.36±3.8[c] (−61.04%)
Brain stem	49.61±1.9	26.27±2.7[b] (−47.04%)	35.54±3.0[b] (−28.36%)	29.26±0.8[a] (−41.01%)
Hippocampus	42.80±2.7	27.04±1.0[a] (−36.82%)	35.22±2.1[b] (−17.71%)	27.30±1.5[a] (−36.21%)
SOD (U/mg protein/min)				
Cortex	14.16±0.20	6.08±0.83[a] (−57.06%)	10.68±0.56[a] (−24.57%)	12.99±0.32[NS] (−8.26%)
Cerebellum	16.20±0.11	8.65±0.07[a] (−46.60%)	10.86±1.68[a] (−32.96%)	11.63±0.88[a] (−28.20%)
Brainstem	12.22±0.13	7.46±0.38[a] (−38.95%)	10.41±0.36[a] (−14.81%)	10.56±0.37[a] (−13.58%)
Hippocampus	15.23±0.16	7.12±0.54[a] (−53.25%)	09.78±0.41[a] (−35.78%)	12.09±0.82[a] (−20.61%)
GPx (µg of GSH consumed/min/mg protein)				
Cortex	60.44±0.87	36.95±2.42[a] (−38.86%)	53.20±2.50[a] (−11.97%)	48.98±0.91[a] (−18.96%)
Cerebellum	56.42±1.22	33.10±1.83[b] (−41.33%)	49.38±1.36[b] (−12.47%)	55.32±1.41[b] (−1.94%)
Brainstem	60.18±1.89	46.57±0.68[a] (−22.61%)	58.85±2.59[a] (−2.21%)	60.61±2.00[NS] (0.71%)
Hippocampus	58.91±0.61	34.91±2.74[a] (−40.47%)	56.64±1.17[a] (−3.85%)	56.64±1.17[b] (−3.85%)
GSH (mg of GSH/g tissue)				
Cortex	2.68±0.67	2.17±0.20[NS] (−19.03)	1.71±0.09[a] (−36.19)	2.90±0.85[NS] (+08.21)
Cerebellum	5.92±0.31	2.76±0.52[a] (−53.38)	1.56±0.47[a] (−73.65)	2.91±0.16[a] (−50.84)
Brainstem	3.92±0.33	2.13±0.65[a] (−45.66)	1.96±0.64[a] (−50.00)	1.62±0.11[a] (−58.67)
Hippocampus	4.88±0.45	2.63±0.43[a] (−46.11)	2.23±0.65[a] (−54.30)	1.81±0.11[a] (−62.91)

Values are mean±standard deviation (SD) of six observations, Values in parenthesis with '−' sign indicate percentage decrease found over controls, Superscript symbols indicate level of significance [a]$P<0.001$, [b]$P<0.05$, [c]$P<0.01$, NS = Not significant, EDTA = Ethylenediaminetetraacetic acid, LPO = Lipid peroxidation, MDA = Malondialdehyde, SOD = Superoxide dismutase, GPx = Glutathione peroxidase, GSH = Glutathione

levels of CAT, SOD, and GPx activities in all the regions indicating recovery from Pb^{2+} induced toxic stress [Table 3]. Comparatively clinoptilolite supplementation offered good protection by bringing values to near control levels; however, a sizeable protection was noticed in SOD levels in mice treated with EDTA. Despite the fact that both supplements offered protection in decreasing the impairment caused by Pb^{2+} exposure, 2 weeks of dietary antioxidant exposure was found beneficial than 1 week.

DISCUSSION

Pb^{2+} poisoning leads to severe complications in the developing brain due to the intense cellular proliferation, differentiation, and synaptogenesis.[21] A large number of studies lend support to the claim that at low doses, Pb^{2+} produces a variety of biochemical changes in the brain and neurons of small mammals. There is no proven safe lower limit for Pb^{2+} exposure,[9] while dose dependent effects have been observed by several authors; behavioral to biochemical assessments were made in rats/mice upon exposure of 100 mg/kg body weight.[10,11] Pb^{2+} is shown to produce significant decrease in the formation of myelin, particularly during the postnatal period[22,23] and the blood-brain barrier, which does not fully develop until 3 weeks of postnatal life, is highly sensitive to Pb^{2+}.[22] The endothelial cells that form the main structural component of the blood-brain barrier are the first to be targeted to Pb^{2+} passage into the brain.[24] Hence, a dose of 100 mg/kg body weight Pb^{2+} was chosen to simulate low-dose exposures over a specific period of time (21 days), as a minimum exposure for 2-3 weeks is required to bring changes in neuronal function.[10,11] In the developing animals, a five-fold greater absorption of Pb^{2+} was reported due to poor functional blood-brain barrier,[25,26] while it readily crosses the blood-brain barrier in adults and cause deleterious effects by altering the metabolism and physiology of the brain. To date, the studies that have evaluated on oxidative stress indices following Pb^{2+} exposure were carried out either on adult animals[27,28] or in new born(s).[29] In this study, mice were exposed to Pb^{2+} for a period of 3 weeks followed by treatment with clinoptilolite and EDTA during the 4th and 5th week. A higher concentration of Pb^{2+} accumulation was observed in discrete brain regions upon Pb^{2+} exposure which is presumed to be associated with increased oxidative stress, and caused regional alterations indicating their vulnerability. The supplementation of antioxidants reduced

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the burden of Pb^{2+} accumulation thereby offered protection to brain tissues. Several studies advocated Pb^{2+} induced changes which were shown to be transient and reversible on withdrawal of Pb^{2+} exposure as well as by feeding antioxidants.[5,30] In this study, Pb^{2+} exposure was found to inhibit antioxidant enzymes such as SOD, CAT, GPx, and GSH; such an inhibition(s) would not only have increased the free radical injury but also enhance excitotoxicity, since ROS as well as nitrogen species and lipid peroxidation products can trigger this process.[31] The disruption of the pro-oxidant/antioxidant balance might cause injury to brain via oxidative damage to critical biomolecules such as lipids, proteins, and DNA. Further, these functional alterations would result in morphological changes in the brain that can remain even after Pb^{2+} levels have fallen. Although the source of pro-oxidant formation during Pb^{2+}-induced oxidative stress is not exactly known, it is suggested that auto-oxidation of excessively accumulated amino levulinic acid due to inhibition of amino levulinic acid dehydratase, resulted in the formation of superoxide and H_2O_2.[31] Several studies[30,31] documented that participation of iron in Fenton reaction *in vivo*, leading to the production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 results in increased lipid peroxidation. This might be one of the reason(s) for enhanced LPO with significant decrease in the activity of antioxidant enzymes viz., catalase, SOD, and GPx.[31]

Chelation therapy has been proposed as a treatment for heavy metal poisoning in which the removal of heavy metal ions is hypothesized to reduce the oxidative damage caused by the production of hydroxyl radicals.[32,33] In clinical use, EDTA is credited with an anecdotal report of relief as well as reduction of oxidative stress.[33] In this study, EDTA at a dose of 100 mg/kg body weight was selected based on the clinical application and on the results from previous experiments.[12] It is evident from results that the treatment with EDTA after cessation of Pb^{2+} exposure caused a reduction in the lipid peroxide levels in all regions of brain indicating its antioxidant potential, and ability to chelate Pb^{2+} toxicity, thereby the results strongly advocate its strong chelating actions. The results of this study corroborate with the findings of Mikirova *et al*., (2011)[34] where authors showed that meso 2, 3-dimercaptosuccinic acid (DMSA) and intravenous EDTA both chelation agents reduce cellular Pb^{2+} uptake in a concentration dependent fashion, DMSA appears to have the larger effect. The protection offered might be associated with the ion-exchange and cation binding properties of clinoptilolite, and its supplementation could help to prevent alterations in redox homeostasis of cells as it traps and encounters the free radical buildup, thereby helping the body to defend itself against a surge of ROS radicals.

Zeolites are widely used in industry, agriculture, and animal husbandry; their effects in appropriate animal models and possible medical applications await detailed studies. Clinoptilolite is the most appropriate zeolite with specific physical and chemical properties and abundant in the world, ensuring its availability at low cost.[7] Accumulating evidences indicate that zeolites play an important role in the modulation of the immune system[33] and several studies used tribomechanically activated zeolites (TMAZ). The ability of clinoptilolite (zeolite) in augmenting indigenous antioxidants which were rendered inefficient during toxic assault were reported by Madhusudhan *et al.*,(2009).[35] The protection offered might be associated with the ion-exchange and cation binding properties of clinoptilolite, and its supplementation could help to prevent alterations in redox homeostasis of cells as it traps and encounters the free radical buildup, thereby helping the body to defend itself against a surge of ROS radicals.[28,35] The protective role of clinoptilolite has also been demonstrated in many animal models. For instance, the ameliorative role of clinoptilolite upon short and long term Pb^{2+} toxicity in rats was reported by Ahmad *et al.*, (2013).[7] Dietary effects of Ca^{2+}-zeolite supplementation in blood and tibial bone characteristics of broilers was reported by Eleroglu and Yıldırım (2011).[36] Similar study by Safaeikatouli *et al.*, (2011)[37] has reported the safety and efficacy of the dietary kaolin and zeolite on broiler's blood parameters, thyroxine (T4), thyroid stimulating hormone (TSH), and growth hormones. Prvulovic *et al.*, (2007)[38] measured the beneficial effects of a clinoptilolite supplement in pig diets on performance and serum parameters. Hence, the adsorption and ion exchange properties of natural zeolite represented the hypothesis of a possible easing of the biological uptake of Pb^{2+} and its subsequent effects on antioxidant enzymes. Muck-Seler and Pivac (2003)[13] conducted experiments to assess the effect of clinoptilolite (MZ) on the serotonergic receptor function in the brain of mice with mammary carcinoma. During experimentation, adult mice were fed with standard food supplemented with 25% of zeolite for different interval periods of 14 and 28 days, and 2-3 weeks zeolite supplementation offered better efficacy and their results inferred that amelioration was achieved by the alterations in the electrolyte balance, and/or by the regulation of the immune system thereby, supplementation length restricted to 2 weeks in this study. In the present study, treatment with clinoptilolite on Pb^{2+}-exposed mice led to significant decrease in LPO level in all the discrete brain regions studied, with significant decline in Pb^{2+} concentration. In addition, it also led to recover the level of CAT, SOD, and GPx activities affected during Pb^{2+} toxicity. Unlike the classic antioxidants, clinoptilolite does not neutralize free radicals by donating an electron to stabilize them; instead its structure captures the free radicals. Once trapped inside the cage, the inactivated free radicals can then safely be eliminated from the body. The high affinity of clinoptilolite for Pb^{2+} would significantly reduce the amount of dietary Pb^{2+} available for absorption by the body. The extent of reversal observed by clinoptilolite and

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EDTA supplementation in this study was found to be significant ($P < 0.05$) and highly beneficial.

In summary, Pb^{2+} poisoning exerts severe complications in the developing brain by inducing oxidative stress and *in vivo* supplementation of EDTA and clinoptilolite offered protection by normalizing the antioxidant levels which might be due to their ability to react faster with peroxyl radicals, thereby reducing the severity of oxidative damage and hence the results support the neuroprotective potential of EDTA and clinoptilolite against Pb^{2+} toxicity.

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