Protective effect of Wormwood extract on lead induced neurotoxicity and cognitive disorder

O. Kharoubi, M. Slimani, N. Ait Hamadouche, D. Krouf[1], A. Aoues
Department of Biology, Faculty of Science, Laboratory of Biochemistry, [1]Laboratory of Clinical and Metabolic Nutrition, University of Es-Senia Oran, Algeria

Lead is a ubiquitous and a potent neurotoxicant causes several neurophysiological and behavioural alterations. Considering the vulnerability of the developing brain to Pb neurotoxicity, this study was carried out to investigate the effects of Pb exposure on brain regions acetylcholinesterase (AchE) and monoamino oxidase (MAO) enzymes activities and on behavioural changes. Wister rat were exposed to 750 ppm of lead acetate in the drinking water for 11-weeks after weaning, and treated by Artemisia Absinthium L. (wormwood) extract (200 mg.kg-1 body weight) for 4 weeks. The activities of AchE and MAO were determined in the hypothalamus, hippocampus, cortex and striatum of male rat; and general/ Locomotors activity was evaluated in the open-field test. Results indicated a significant decrease in AchE activity in intoxicated group (Pb) compared to untreated group (as contral) (hypothalamus: -12%, hippocampus: -57%, cerebral cortex: -18% and striatum: -43%) and in MAO activity (hypothalamus: -29%, hippocampus: -41%, cerebral cortex: -28% and striatum: -51%) respectively, with decrease crossing test score and increase sniffing test score. After, wormwood extract administration, the activity of AchE and MAO were significantly increased in all brain region compared to Pb group, but were significantly lower than control. The locomotors activity was reduced compared to Pb group. These data suggest that administration of wormwood extract for 4 weeks protect against the lead acetate-induced change in behavioural and neurobiochemical parameters changes.

Key words: Acetylcholinesterase activity, behavioural, Brain region, Lead acetate, monoamine oxidase activity

INTRODUCTION

Lead (Pb) poisoning exerts its most severe consequences in the developing brain[1,2] and its deleterious effects on central nervous system (CNS) are known as lead encephalopathy or lead neuropathy.[3] Experimental evidence suggests that cellular damage mediated by free radicals can be involved in the pathology associated with Pb intoxication. Exposure to Pb has been associated with behavioural abnormalities, learning impairment, decreased hearing and impaired cognitive functions in experimental animals.[4,5] Various studies have shown that lead exposure can cause changes in catecholaminergic functions.[6] In fact, the cerebral damage induced by lead occurs preferentially in cerebral cortex, cerebellum and hippocampus. The cognitive functions are localised in the cerebral cortex, while the cerebellum regulates the execution of driving movements; whereas hippocampus area is the site of memory storage and was implicated in behavioural comportment. Consequently, these anatomical sites are crucial by modulating the emotive answer, memory, behaviour and the exposure of young brain under development to lead can compromise a variety of neurotransmitter systems.[4,7,8]

Although, no general hypothesis is known for the mechanism to explain what cellular events underlie the behavioural and cognitive dysfunction in primates and non-primates, the detrimental effects of lead have warranted interest in this area. One of the reasons for the deleterious effects of lead is its ability to strongly bind to sulfhydryl groups of proteins and to mimic or compete with calcium.[3]

Recently, the clinical importance of herbal drugs has received considerable attention. As many synthetic antioxidants have been shown to have one or other side effects,[9] there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury.[10,11] Numerous plant products have been shown to have antioxidant activity, and the antioxidant vitamins, flavonoids, and polyphenolic compounds of plant origin have been extensively investigated as scavengers of free radicals and inhibitors of lipid peroxidation.[12]

The prophylactic effectiveness of plant extract was being allotted to his antioxidant action and/or to his chelating capacity due primarily to the presence of sulfhydryl groups, which can probably reduce the deleterious effect of lead.

Wormwood (*Artemisia Absinthium* L.) have a high contents of nutrients and phytochemicals such as total phenolic compounds and total flavonoids, suggesting

Address for correspondence: Dr. Omar Kharoubi, Department of Biology, Faculty of Science, Laboratory of Biochemistry, University of Es-Senia - Oran, Algeria. E-mail: omarkharoubi@yahoo.fr
Received: 31-07-2009; **Accepted:** 07-12-2009; DOI: 10.4103/0973-8258.69181

that these compounds contribute to the antioxidative activity;[13] phenolic substances such as flavonols, cinnamic acids, coumarins, and caffeic acids or chlorogenic acids are believed to have antioxidant properties, which may play an important role in protecting cells and any organ from oxidative degeneration.[14,15] However, no study has reported the effects of *Artemisia Absinthium* L. on lead-induced neurotoxicity. The deficits in learning and memory in Pb-exposed rodents are accompanied by damage to neurons and changes in some neurotransmitters, such as the cholinergic and catecholamine neurotransmitter system are involved.[16,17] In this study, we used behavioural and neurochemical experiments to determine the protective effects of wormwood against the neurotoxicity induced by lead.

MATERIALS AND METHODS

Preparation of Wormwood (*Artemisia Absinthium* L.) Plant Extracts (A.Ab)

Whole plants of *Artemisia Absinthium* L. were collected from Mecheria, Algeria in the month of May. The plant was identified and authenticated at the Herbarium of Botany Directorate in Es-Senia (Oran) University. Five hundred grams of whole Wormwood plants were extracted with 1.5 l of distilled water by the method of continuous hot extraction at 60°C twice for 30 min, and the filtrate was lyophilised. The residue collected (yield 75 g) were stored at -20°C; when needed the extract was dissolved in distilled water and used in the investigation.

Animals and Tissue Preparation

In the experiment, a total of 30 male Wistar rats (18 intoxicated rats, 12 normal rats) were used. The rats were housed five per cage and had free access to food and water, except during testing. They were exposed to a 14–10 h light-dark cycle and the room temperature was controlled at 23±2°C. Animals were first exposed to Pb at the age of 2 weeks, when they weighed 40±6 g.

Experiments were performed during 11 weeks:

(a) *Untreated group*: rats that received water during 11-weeks.
(b) *Pb group*: Rats exposed to Pb (750 ppm, in the form of Pb acetate in their drinking water *ad libitum*) for 11-weeks.
(c) *Untreated+A.Ab group*: rats exposed to Pb that later receiving aqueous Artemisia Absinthium *L* . extracts (A.Ab) (200 mg/kg, in their drinking water *ad libitum*) for 4-weeks.
(d) *Pb(-) group*: Rats exposed to Pb for 11-weeks, receiving water for four additional weeks.
(e) *Pb(-)+A.Ab groups*: rats exposed Pb who later received aqueous *Artemisia absinthium* L. extract (A.Ab) (200 mg/kg, in their drinking water *ad libitum*) for 4-weeks.

Animals were sacrificed by cervical decapitation under pentobarbital sodium anesthesia (60 mg/kg). Brain was removed, washed with normal saline and all the extraneous materials were removed before weighing. Brain was kept at ice cooled conditions all the time. The brain was dissected using the method of Glowinski and Iversen[18] into four regions of interest: hypothalamus, hippocampus, cerebral cortex and striatum. Due to the small amount of tissue, tissue of three littermates was pooled.

Brain Cytosolic and Mitochondrial Fractions

The rat brain tissue was minced and homogenised in 500 µl of buffer A (20 mM HEPES, pH 7.5, 50 mM KCl, 1 mM EDTA, 2 mM $MgCl_2$, 220 mM Mannitol, 68 mM Sucrose, 1 mM leupeptin, 5 µg/ml pepstatin A, 5 µg/ml aprotonin, 0.5 mM PMSF). The homogenate subjected to differential centrifugation to collect the supernatant (cytosolic fractions) and the pellets (enriched mitochondria fractions). The cytosolic fractions were frozen at -70°C until further analysis. Pellets containing mitochondria were treated with the lysis buffer (1X PBS, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 250 mM, sucrose 20 mM, Tris HCl, pH 7.4, 1 mM DTT, and protease inhibitor) and were incubated on ice for 20 mins. The lysate was centrifuged at 10,000g at 30 mins at 4°C and the resulting supernatant was kept as the solubilised mitochondrial enriched fractions and stored at –70°C until further use.

Estimation of MAO and AchE Activity in Brain

The activity of MAO was estimated by the method of Green and Haughton.[19] The assay mixture containing of 1.0 ml of semicarbazide hydrochloride (0.05 M, pH 7.4), 1.6 ml of phosphate buffer (0.2 M, pH 7.4) and 0.4 ml of mitochondrial fraction was incubated for 20 min at 37 °C in a water bath with a shaking device. The reaction was started by adding 0.5 ml of tyramine hydrochloride (0.1 M, pH 7.4) after 30 min of incubation, the reaction was stopped by adding 1.0 ml of 0.5 N acetic acid and kept in boiling water bath for 30 min. the contents were centrifuged for 10 min at 1000×g. to 2.0 ml of supernatant, 2.0 ml of 2,4-dinitriphenylhydrazine (0.5 mg/ml in 2N HCl) was added. After keeping at room temperature for 15 min, 5 ml of benzene was added. The tubes were vortexed and the aqueous layer was discarded. The benzene layer was washed with 4 ml of distilled water followed by the addition of 4 ml of 0.1N NaOH solution and the contents of tubes were mixed thoroughly. The benzene layer was discarded and the NaOH layer was allowed to stabd at room temperature for 1 h. The absorbance of samples was measured at 425 nm. The activity of MAO was calculated using the molar extinction coefficient of 9,500 and expressed as micromoles of *p*-hydroxy phenyl acetaldehyde formed per mg of protein.

The activity of AchE was estimated by the Ellman *et al*.,[20]

method. This method is based on the measurement of the rate of thiocholine production in the hydrolysis of the substrate acetylcholine. Thiocholine, when reacting with dithiobisnitrobenzoic acid (DTNB), produces a yellow colour, which can be measured photometrically. In the reaction mixture, 0.4 ml of synaptosome fractions (average protein content 0.2-0.4 mg/ml) and 2.6 ml 0.1 M phosphate buffer pH 8.0 (containing 0.749 g KH_2PO_4, 16.820 g Na_2HPO_4 2 H_2O and 1000 ml water) was incubated for 30 min at 37°C under continuous stirring. The samples were moved into photometer cells, and 100 µl 5.5-dithiobis-2-nitrobenzoic acid (DTNB) was added. 20 µl substrate, 0.075 M acetylcholine iodide was added into the photometer cells. The absorbance were measured at 412 nm after one min and five min. the enzyme activity is expressed as µmol of substrate hydrolysed/min/mg of protein

Neurobehavioural Studies

General-Locomotors activity was evaluated in the open-field test. The open-field behaviour of rats was assessed in a box measuring 90 cm×90cm×30 cm. subdivided into 19 equal squares by black lines. Immediately after, rat was placed in the center of the open-field, the movements of the rat were scored. All variables in activity were recorded during 5 min session: number of squares crossed with all paws (crossings), standing on hind legs and placing fore limbs on the wall (wall rearings) and placing nose against floor (sniffing) were counted in all sessions.

Statistics

The mean±SEM values were calculated for each group to determine the significance of inter group difference. Each parameter was analysed separately using one way analysis of variance (ANOVA). To find the difference between the groups Student's *t*-test was used. *P* values <0.05 were considered to be significant.

RESULTS

A significant difference in blood and urinary lead concentration was noted between Pb group (PbU = 6.94±1.7 µg/day, PbB = 55.62±6.30 µg/dl) at 11-weeks compared to Pb(-) (PbU = 2.11±1.23 µg/day, PbB = 22.3±5.78 µg/dl) and Pb(-)+A.Ab groups (PbU = 1.12±1.48 µg/day, PbB = 15.29±6.21 µg/dl). The activity of AChE was significantly reduced in all brain region in intoxicated group *vs.* untreated group after 11 weeks of intoxication, and by -57% in hippocampus, -43% in striatum, -18% in cortex and -11% in hypothalamus, respectively; after 4 weeks of stopped intoxication a maximum reduced activity was noted in hippocampus of Pb(-) group (-77%) (P<0.05). Administration of wormwood extract indicates a clear significant improvement in Pb/A.Ab group compared with Pb group (hippocampus +41%; striatum +37%; cortex +10% and no difference in hypothalamus, respectively) [Figure 1].

We observed a significant decreased (P<0.05) in MAO activity in different cerebral area at Pb group compared to untreated group, respectively, in hippocampus -41%; in striatum -51%; in cortex -28% and in hypothalamus -29%. After treatment with wormwood extract, Pb/A.Ab group indicate a significant increase in MAO activity in all brain

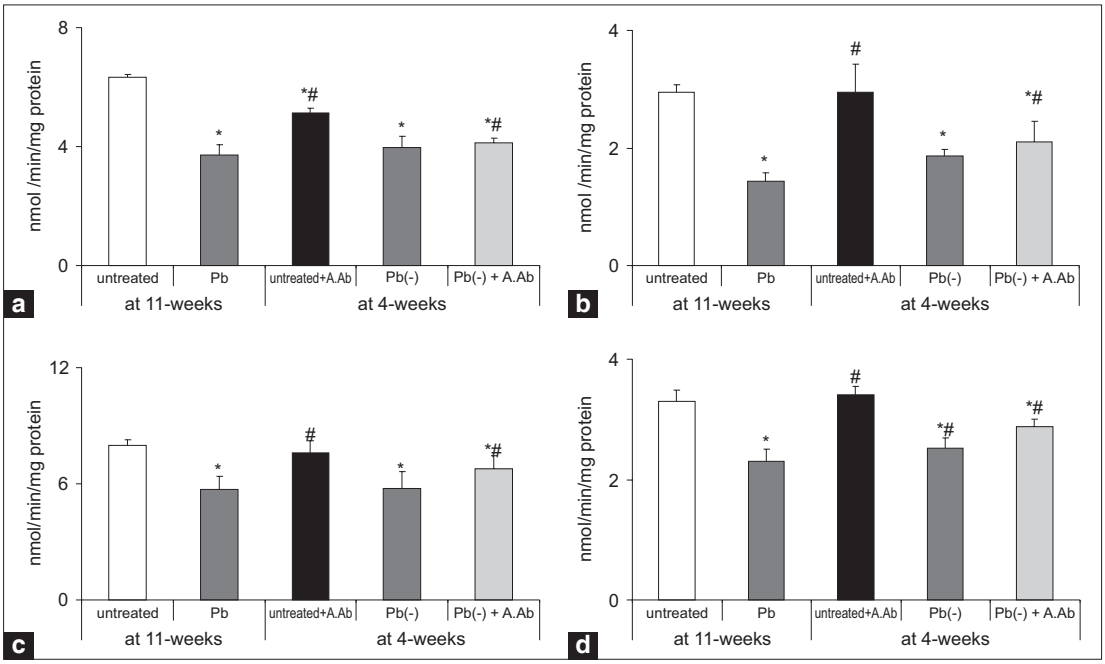

Figure 1: Brain region Monoamine oxidase activity before and afterwards treatment by wormwood extract in rat exposed to lead. (a) hippocampus, (b) striatum, (c) cortex and (d) hypothalamus. Values are mean ± SE (n = 6). *P < 0.05, Pb group, Untreated+A.Ab group, Pb(-) group and Pb(-) +A.Ab group were compared vs. Untreated group (as Control). #P < 0.05, Untreated+A.Ab group, Pb(-) group and Pb+A.Ab group are compared vs. Pb group (Student's 't' test)

region compared to Pb group, by +10%, +47%, +18%, +24%, in hippocampus, striatum, cortex and hypothalamus, respectively [Figure 2].

The general behaviour, crossing was assessed through open-field test [Figure 3]. Chronic exposures of lead increase significantly the total general behaviour which included, crossing (untreated 15±1.8 *vs* Pb group 21±1.34), wall rearing (untreated 2.3±0.8 *vs* Pb group 1.8±0.6) and sniffing (untreated 0.3±0.2 *vs* Pb group 0.7±0.1). Administration of wormwood extract during 4-weeks had a significant difference score ($P<0.05$) between Pb(-)+A.Ab and Pb group in wall rearing and sniffing test, by -62% and -73%, respectively; but no difference are noted between Pb(-)+A.Ab and untreated+A.Ab group [Figure 4].

DISCUSSION

In the present work, we investigated the effects of wormwood extract on lead-induced locomotors, and stereotypic impairment and changes in some enzyme activities in different regions of brain. The prevention of lead-induced neurotoxic injury by wormwood extract is reported here for the first time. Exposure to lead during early development has been implicated in lasting behavioural abnormalities and cognitive deficits in experimental animals.[5,21] We performed three behavioural tests (crossing, wall rearing and sniffing test), in which the deficit induced by lead were restored by the wormwood extract. Besides, the present study showed that the administration of lead to rat, during 11 weeks, induced crossing hyperactivity. Other studies realised during the postnatal period found the some results in rat treated by lead.[22,23]

The hyperactivity found in present study could be explained by lead effect on dopaminergic system and glutamatergic transmission on the level of NMDA receptor (N-métyl-D-aspartate).[16] To elucidate the cognition-enhancing and neuroprotective mechanisms of wormwood extract, we undertook to study the effect of lead exposed on the activity of acetylcholinesterase (AchE), implied in the hydrolysis of acetylcholine, and monoamines oxidases (MAO), responsible for catecholamine and serotonin degradation, are regarded as being markers of neurotoxicity of heavy metals.[23,24]

Pb-exposed are consistent with dysfunction of cholinergic innervations.[25] The involvement of the cholinergic system has been implicated by the observations that early lead exposure results in a significant reduction in high-affinity choline uptake in mouse forebrain synaptosomes[26] and a depressed acetylcholine turnover in rat brain.[27] However, these changes in the cholinergic ways could be associated with the peroxydatives (peroxidaives) damage caused on the neuronal membrane.[28] Although, the underlying mechanisms of these effects has not been resolved. We found that the activity of AchE was decreased by lead, which are increased by wormwood extract. Administrations of wormwood extract can enhance activity of AchE compared to Pb group; according to these results, wormwood extract can maintain acetylcholine levels in the brain and improve cognitive ability. Various studies have shown that lead exposure can cause changes in catecholaminergic functions.[17,29]

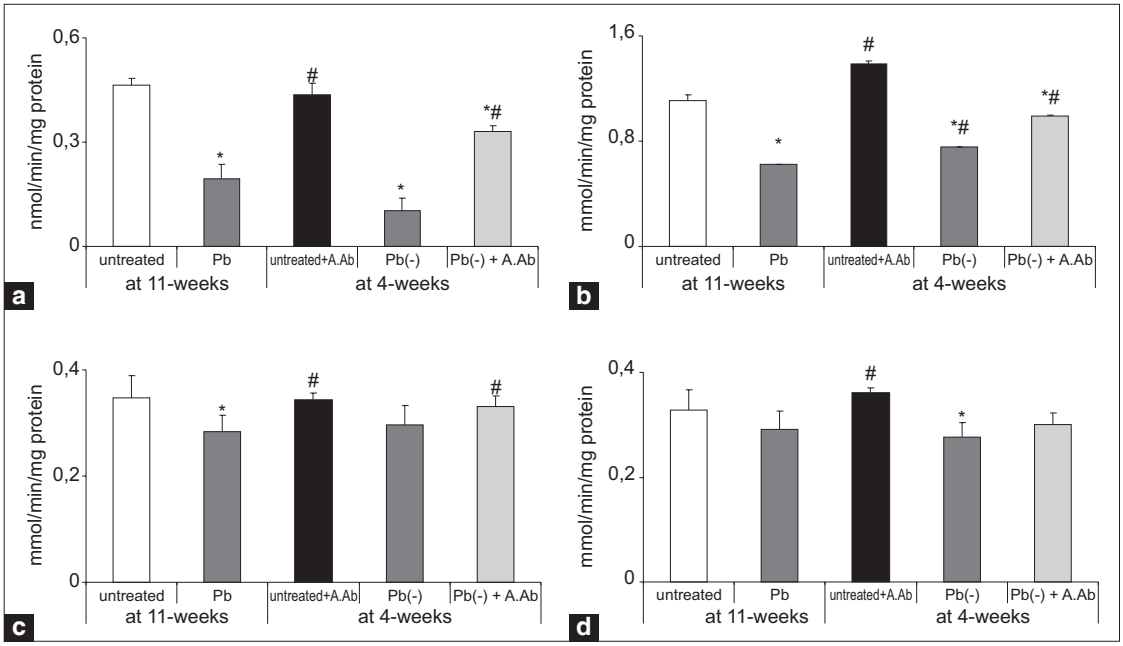

Figure 2: Brain region acetylcholinesterase activity before and afterwards treatment by wormwood extract in rat exposed to lead. (A) hippocampus, (B) striatum, (C) cortex and (D) hypothalamus. Values are mean ± SE (n = 6). *P < 0.05, Pb group, Untreated+A.Ab group, Pb(-) group and Pb(-) +A.Ab group were compared vs. Untreated group (as Control). #P < 0.05, Untreated+A.Ab group, Pb(-) and Pb(-)+A.Ab group are compared vs. Pb group (Student's 't' test).

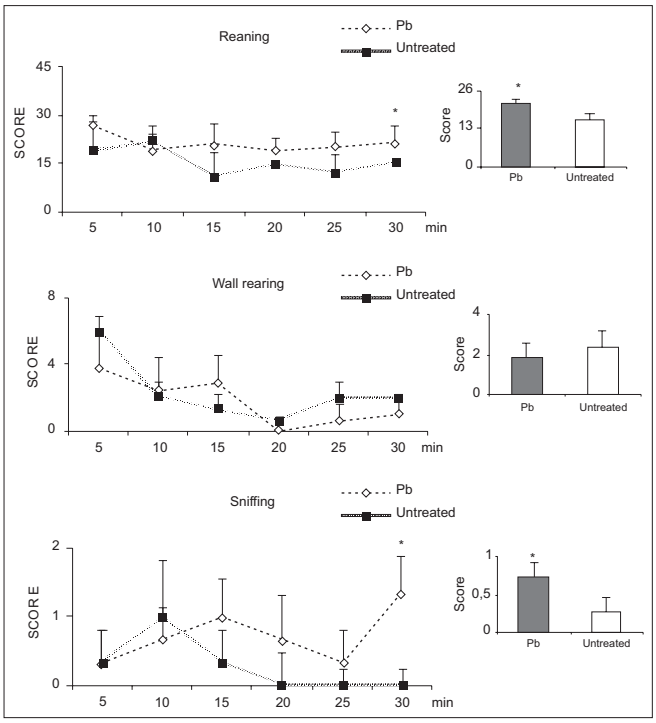

Figure 3: locomotors, wall rearings and sniffing test in rat exposed to lead for 11 weeks. *P < 0.05, Pb group, Untreated+A.Ab group, Pb(-) group and Pb(-)+A.Ab group were compared vs. Untreated group (as Control) (Student's t-test)

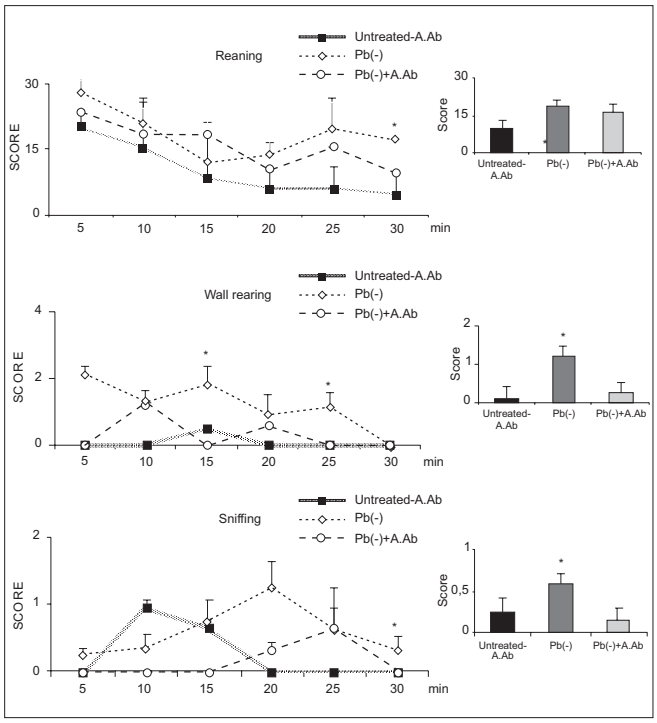

Figure 4: Locomotors, wall rearings and sniffing test in rat treated during 4 weeks by wormwood extract after lead-exposed. * P < 0.05, Untreated+A.Ab group, Pb(-) and Pb(-)+A.Ab group are compared vs. Pb group (Student's 't' test). (Student's t-test)

We observed that administration of lead acetate decrease significantly the MAOs activity in the various cerebral areas. The work undertaken by Devi *et al*,[8] showed that lead administration modifies the aminergic system by the reduction the activity of mitochondrial monoamines oxidase and tyrosin hydroxylase. The effects observed during the exposure to the high Pb levels on MAO and catecholamines at the cerebral level are not a direct consequence of the intoxication by lead, but a resultant of the inhibiting effect of the cholinergic system.[30] The reduction in the activity of MAOs in the various cerebral areas, during the exposure to lead, can be due to the cellular damage[31] and with the high affinity of Pb to sulfhydryl group of these enzymes.[32]

In addition, we observed that the administration of aqueous extract of Wormwood made it possible to record a clear improvement in the various behavioural tests compared to the rats exposed to lead. In the same way, we recorded that administration of plant extract beforehand poisoned rats induced a re-establishment of the enzymatic activities (AchE and MAO) in the various cerebral structures, compared with Pb groups. The prophylactic effectiveness of this extract can be allotted to its antioxidant action and/or its chelating capacity due primarily to the action of sulfhydryl groups. These results agree to the fact that the natural compounds rich in antioxidants (garlic, olive oil) involve a considerable improvement in the activity of AchE;[33] whereas, the effectiveness of the antioxidant compounds on the activity of MAOs was shown by using selenium and zinc, which play a significant role in the toxicity inversion of lead by forming inert complexes and inhibiting its toxicity on the dopaminergique neurons.[34]

CONCLUSION

In conclusion, administration of lead for 11 weeks induced a significant disorder in locomotors and stereotypic behaviour and reduce AchE and MAO activities. Moreover, *Artemisia Absinthium* L. has protective mechanisms which prevent the neurotoxicity induced by lead; and can modify the behaviour by restoring the enzymes activities to near normal.

REFERENCES

1. Burdette LJ, Goldstein R. Long-term behavioural and electrophysiological changes associated with lead exposure at different stages of brain development in the rat. Brain Res 1986;394:101-10.
2. Guilarte TR, McGlothan JL, Nihei MK. Hippocampal expression of N-methyl-D-aspartate receptor (NMDAR1) subunit splice variant mRNA is altered by developmental exposure to Pb(2+). Brain Res Mol Brain Res 2000;76:299-305.
3. Cloues RK, Cibulsky SM, Sather WA. Ion interactions in the high-affinity binding locus of a voltage-gated Ca2+ channel. J Gen Physiol 2000;116:569-86.
4. Verina T, Rohde CA, Guilarte TR. Environmental lead exposure during early life alters granule cell neurogenesis and morphology

in the hippocampus of young adult rats. Neuroscience 2007;145: 1037-47.
5. Flora SJ, Saxena G, Mehta A. Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: Role of ROS and intracellular Ca2+. J Pharmacol Exp Ther 2007;322:108-16.
6. Nation JR, Frye GD, Van Stultz J, Bratton GR. Effects of combined lead and cadmium exposure: Changes in schedule-controlled responding and in dopamine, serotonin and their metabolites. Behav Neurosci 1989;103:1108-14.
7. Leret ML, Garcia-Uceda F, Antonio MT. Effects of maternal lead administration on monoaminergic, GABAergic and glutamatergic systems. Brain Res Bull 2002;58:469-73.
8. Devi CB, Reddy GH, Prasanthi RPJ, Chetty CS, Reddy GR. Developmental lead exposure alters mitochondrial monoamine oxidase and synaptosomal catecholamine levels in rat brain. Int J Dev Neurosci 2005;23:375-81.
9. Nocentini S, Guggiari M, Rouillard D, Surgis S. Exacerbating effect of vitamin E supplementation on DNA damage induced in cultured human normal fibroblasts by UVA radiation. Photochem Photobiol 2001;73:370-7.
10. Siddique MS, Eddeb F, Mantle D, Mendelow AD. Extracts of Ginkgo biloba and Panax ginseng protect brain proteins from free radical induced oxidative damage *in vitro*. Acta Neurochir [Suppl] 2000;76:87-90.
11. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, *et al*. Dietary intake of antioxidants and risk of Alzheimer disease. JAMA 2002;287:3223-9.
12. Tapiero H, Tew KD, Ba GN, Mathé G. Polyphenols: Do they play a role in the prevention of human pathologies? Biomed Pharmacother 2002;56:200-7.
13. Čanadanović-Brunet JM, Đilas SM, Ćetković G, Tumbas VT. Free-radical scavenging activity of wormwood (Artemisia absinthium L.) extracts. J Sci Food Agric 2005;85:265-72.
14. Osawa T. Protective role of dietary polyphenols in oxidative stress. Mech Ageing Dev 1999;111:133-9.
15. Wiseman H, Okeily JD, Adlercreutz H, Mallet AJ, Bowery EA, Sanders AB. Isoflavones phytoestrogen consumed in soya decrease: F, 2, -isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr 2000;72:397-400.
16. Guilarte TR, McGlothan JL. Selective decrease in NR1 subunit splice variant mRNA in the hippocampus of Pb2+-exposed rats: Implications for synaptic targeting and cell surface expression of NMDAR complexes. Brain Res Mol Brain Res 2003;113:37-43.
17. Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin JP. 5-HT2A and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. Eur J Neurosci 2004;20:3073-84.
18. Glowinski J, Iversen LL. Regional studies of catecholamines in the rat brain: I: The disposition of [3H]norepinephrine, [3H]dopamine and [3H]dopa in various regions of the brain. J Neurochem 1966;13:655-69.
19. Green AL. Hanghton TM. A colorimetric method for the estimation of monoamine oxidase. Biochem J 1961;78:172-5.
20. Ellman GL, Courtney KD, Andres V Jr, Feather-stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
21. Azzaoui FZ, Ahami AO, Khadmaoui A. Impact of lead sub-chronic toxicity on recognition memory and motor activity of Wistar rat. Pak J Biol Sci 2009;12:173-7.
22. Trombini TV, Pedroso CG, Ponce D, Almeida AA, Godinho AF. Developmental lead exposure in rats: Is a behavioural sequel extended at F2 generation? Pharmacol Biochem Behav 2001;68: 743-51.
23. Tähti H, Engelke M, Vaalavirta L. Mechanisms and models of neurotoxicity of n-hexane and related solvents. Arch Toxicol [Suppl] 1997;19:337-45.
24. Kakko I, Toimela T, Tähti H. The synaptosomal membrane bound ATPase as a target for the neurotoxic effects of pyrethroids, permethrin and cypermethrin. Chemosphere 2003;51:475-80.
25. Petit TL. Developmental effects of lead: Its mechanism in intellectual functioning and neuralplasticity. Neurotoxicology 1986;7:483-96.
26. Silbergeld EK, Goldberg AM. Pharmacological and neurochemical investigations of lead-induced hyperactivity. Neuropharmacology 1975;14:431-44.
27. Shih TM, Hanin I. Effect of chronic lead exposure on levels of acetylcholine and choline. Psychopharmacology (Berlin) 1978;58:263-9.
28. Flora GJ, Seth PK. Alterations in some membrane properties in rat brain following exposure to lead. Cytobios 2000;103:103-9.
29. Schwartz BS, Lee BK, Lee GS, Stewart WF, Simon D, Kelsey K, *et al*. Associations of blood lead, dimercaptosuccinic acid-chelatable lead and tibial lead with polymorphisms in the vitamin D receptor and delta-aminalaevulinic acid dehydratase genes. Environ Health Perspect 2000;108:949-54.
30. Reddy GR, Basha MR, Devi CB, Suresh A, Baker JL, Shafeek A, *et al*. Lead induced effects on acetylcholinesterase activity in cerebellum and hippocampus of developing rat. Int J Dev Neurosci 2003;21:347-52.
31. Alfano DP, Petit TL, LeBoutillier JC. Development and plasticity of the hippocampal-cholinergic system in normal and early lead exposed rats. Brain Res 1983;312:117-24.
32. Bagchi D, Vuchetich PJ, Bagchi M, Hassoun EA, Tran MX, Tang L, *et al*. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. Free Radic Biol Med 1997;22:471-8.
33. Yassin Maged M. Prophylactic efficacy of crushed garlic lobes, black seed or olive oils on cholinesterase activity in central nervous system parts and serum of lead intoxicated rabbits. Turk J Biol 2005;29:173-80.
34. Satija NK, Vij AG. Preventive action of zinc against lead toxicity. Indian J Physiol Pharmacol 1995;39:377-82.

Source of Support: Nil, **Conflict of Interest:** None declared.