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Original Article

BEHAVIOR AND GLUTAMATE TRANSAMINASE CHANGES IN RAT EXPOSED TO LEAD AND TREATED BY WORMWOOD EXTRACT

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ABSTRACT

Objective: Lead poisoning induced severe behavioral abnormalities and impaired cognitive functions in experimental animals. The aim of the present study is to investigate the detrimental effects of lead exposure on the behavior of rats and its association with altered neurochemistry.

Methods: Twenty-four young male Wistar rats were divided into 4 groups: G1: a control group receiving drinking water. G2: intoxicated group (Pb) exposed to lead acetate (1000 ppm in drinking water). G3: receives Wormwood aqueous (A. Ab) extract at a dose of 300 mg/l in drinking water. G4: rats are receiving Pb+A. Ab mixture for 4 additional weeks after intoxication for 8 w. In the present study, locomotors activity in rats was assessed by open field test (OFT) while anxiety and depressive behavior were monitored by elevated plus maze (EPM) and the forced swim test (FST), the evaluation of glutamate metabolizing enzymes in whole brain and lipid peroxidation was carried out in all groups.

Results: our results showed that lead acetate intoxication increased the level of lipid peroxidation in brain, decreased brain glutamate oxaloacetate transaminase activities and increased glutamate pyruvate transaminase. Also, lead (pb) exposure resulted in increased anxiety and fear-related behavior in both elevated plus maze and light dark box tests, showed hyperactivity in open field test presented by increased horizontal locomotion. However, A. Ab extract reduced the TBARS level by preventing oxidative stress induced by lead and increased glutamate pyruvate transaminase activity.

Conclusion: The wormwood extract administration reduced anxiety, fear and locomotion and improved learning ability and memories. Therefore, these results indicated that wormwood is ameliorating the deleterious effects of lead and it appeared to be a protective agent against lead-induced toxicity.

Keywords: Lead, Whole brain, Glutamate enzymes, Lipid peroxidation, Behavior changes.

INTRODUCTION

Lead (Pb) is a non-essential toxic heavy metal widely distributed in the environment and chronic exposure to low levels of Pb has been a matter of public health concern in many countries. Some works suggest a link between Pb exposure and memory impairment [1].

Rats are sensitive to variations in environmental complexity. Moreover, the behavioral responses can be quite varied depending on characteristics of both the stressor and to the individual subjected to it. Although animals may initially display signs of acute stress, they often adapt or learn to cope with many conditions [2]. Gestational exposure to Pb strongly impairs spatial learning in male offspring without affecting motor performance and visual function, whereas in female offspring the impairment is less evident [3, 4] demonstrated that Pb exposure during pregnancy and lactation increases emotionality reactivity in male rats measured in the open-field test and depressive-like behavior in females in the forced swimming test. On the other hand, Kharoubi *et al.*, [5] showed that oral administration of lead acetate to young rats for 90 d caused a large variation in sniffing, licking, biting and grooming behavior during a 20 minute testing period. Behavioral inhibition and anxiety, when exposed to novelty, are typical results which may underline the effects of metallic stress on learning and various behavioral responses on the young rat, this seems to be related to increased or prolonged activity in the hypothalamic-pituitary-adrenal axis produced by impaired negative feedback of glucocorticoids in the hippocampus, although other neuroendocrine pathways may be involved. Since behavioral and neuroendocrine effects of metallic stress in rodents are quite similar to those found in depressed humans and for increased fearfulness and frustration is implicated farm animals subjected to lead may be predicted to show a reduced ability to cope with a different environment and have an increased propensity for developing behavioral disturbances and reduced welfare [6]. Moreover, it has been reported that numerous cognitive abilities are of importance for learning maze so poor performance is usually interpreted as an impairment of spatial memory formation [7]. The effect of different periods of lead exposure on the deficits of learning and memory is still unclear.

The clinical importance of herbal drugs has received considerable attention. There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury [8, 9]. 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 [10, 11]. The use of *Artemisia absinthium*, commonly known as wormwood, as an antiseptic, antispasmodic, febrifuge, cardiac stimulant, for the restoration of declining mental function and inflammation of the liver, and to improve memory [12-14].

Artemisia absinthium has been reported to enhance the cognitive ability as evidenced by it's nicotinic and muscarinic receptor activity in cerebral cortical membranes [12]. Wormwood has a high content of phytochemical such as total phenolic compounds and total flavonoids, suggesting that these compounds contribute to the antioxidative activity[15]; Phenolic substances such as flavonols, cinnamic acids, coumarins and caffeic acids or chlorogenic acids are believed to have antioxidant properties that may play an important role in protecting cells and any organ from oxidative degeneration [16] and was used on the case of hepatic cell damage [17]. However, 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, glutamatergic and catecholaminergic neurotransmitter system are involved [18]. In this study, we used behavioral and neurochemical experiments to determine the regulator and protective effects of wormwood against the neurotoxicity induced by lead.

MATERIALS AND METHODS

Preparation of wormwood plant extracts (A. Ab)

Whole plants of *Artemisia absinthium* L. were collected from Boussefer (Oran), Algeria, in the month of June. 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 lyophilized. The residue collected (yield 75 g) was 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 32 male Wistar 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 exposed to Pb when they weighed 36.82±6.16 g.

Experiments were performed during 12 w. The 32 Wistar rats were divided into five groups according to:

-G 1: Rats (n=8) received water during 12 w.

-G 2: Rats exposed to Pb (1000 ppm, in the form of Pb acetate in their drinking water ad libitum) for 12 w.

-G 3: After 8 w of intoxication, this group received A. Ab extracts at the dose of 300 mg/kg in drinking water ad libitum for additional 4 w.

-G 4: (+A. Ab+Pb): Rats exposed to a mixture of Pb at a dose of 1000 ppm and 300 mg/Kg of wormwood extract for 12 w.

Animals were sacrificed by cervical decapitation under sodium pentobarbital anesthesia (60 mg/kg). The brain was removed, washed with normal saline and all the extraneous materials were removed before weighing. The brain was kept at ice-cooled conditions all the time. The brain was removed and quickly excised, minced with ice-cold saline, blotted on filter paper and homogenized in 50 mmol/l phosphate buffer (pH7.4). The supernatant was frozen at-20 C for further determination of enzymes activities and lipid peroxidation level.

The present work was in strict respect of the ethics regarding the use, the handling and preservation of the animals as specified by the Ethics Committee of the scientific committee of our university (University of Oran 1 Ahmed benbella) (02/2010).

Biochemical estimation

The activities of glutamate oxaloacetate transaminase and glutamate pyruvate transaminase were measured through spectrophotometric determination of 2,4 dinitrophenyl hydrazone of pyruvate [19]. Lipid peroxidation in the brain was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) by the method of Niehius and Samuelsson [20].

Behavioral observations

Post-weaning behavioral tests

At 12 w, the behavioral tests were performed in the first half of the light phase of light/dark cycle. All behaviors were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stopwatches were used to score animals' behavior.

Motor activity (Open field test, (OFT))

The open field test provides simultaneous measures of locomotion, and anxiety [21]. The open field used was a square wooden arena measured (90 x 90 x 25 cm). The floor was divided by white lines into 36 smaller squares (15 x 15 cm). The open field maze was cleaned between each rat to avoid odor cues. The rats were carried to the test room in their home cages and tested once at a time for 10 min each. Other elements of exploratory activity such rearing, grooming and sniffing were carefully observed and time spent performing each behavior was recorded. These parameters were defined as follows: rearing (standing on hind legs with paws pressed against the wall of the arena); sniffing (continuous placing nose against the floor for at least 2s); grooming (using paws or tongue to clean/scratch body) [22].

Elevated plus maze test

The elevated plus-maze (EPM) was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze has been considered as a measure of the strength of fear drive [23]. The apparatus consists of 4 crossed arms, two open arms (50 x 10 x 30 cm) and two closed arms (50 x 10 x 30 cm). The maze was elevated 65 cm above the floor. The rat was placed in the center of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 min test session was recorded during 30 min [24].

Forced swim test

The forced swim test (FST) involved two exposures to a cylindrical tank of water (23±1 °C) where rats cannot touch the bottom of the tank or escape. For the first exposure, the rats were placed in the water for 10 min. Twenty-four hours later; the rats were placed in the water again for a 5-min session [25]. Behavior was videotaped for later analysis and the periods of immobility were scored from video tapes.

Light-dark transition task

The light and dark exploration tasks represent a natural conflict between the tendencies of mice to explore a novel environment versus the tendency of mice to avoid a brightly lit open field. The light-dark box apparatus consists of a rectangular box (44 cm × 8.5 cm × 25 cm) divided equally into a light, open compartment, connected by a door (17 cm in height) leading to a dark closed compartment in which the animal is placed. Each animal was placed facing the side away from the door and then released. During three min [26], the time spent in dark and light compartments, respectively, was measured to determine degrees of anxiety.

Statistics

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

RESULTS

Body weights

The body weights of the rats after 12 w of treatment are presented in fig. 1 A. A significant decreased gain in body weight was observed in all treated group compared to control group by-33.6% and-44.4% respectively in G2 and G4 group. During the course of the treatment, a decrement in water drinking intake was observed in the lead-treated rats of both treated groups (fig. 1 B).

Motor activity

Locomotors activity

Male rats exposed to Pb displayed significantly increased locomotors activity counts (94±7) compared to control rats (34±5, $P<0.05$). There was no significant difference in locomotors activity counts between G3 groups (44±6) as compared to control (fig. 2A).

Grooming

Male rats exposed to Pb displayed a lightly increased grooming activity counts (15±1) compared to control rats. There was no significant difference in grooming activity counts between all treated groups as compared to the corresponding control (fig. 2B).

Sniffing activity

No significant effect of Pb exposure was observed in sniffing activity compared to all groups (control 12±3; G2: 15±3; G3: 14±2 and G4: 14±3) (fig. 2C).

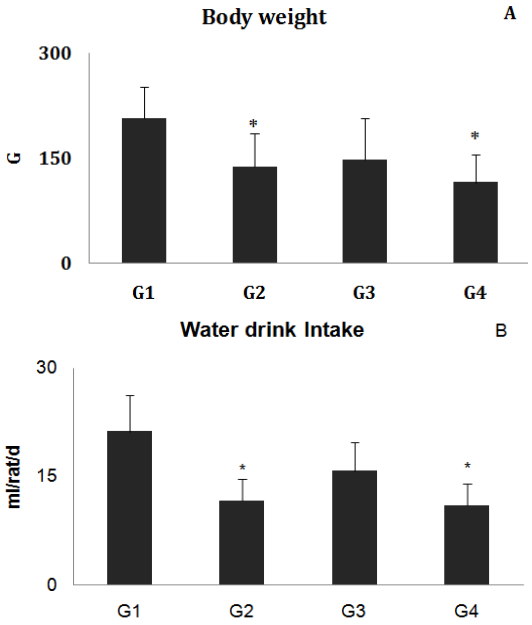

Fig. 1: Body weight and water drink intake in all groups treated by lead and wormwood extract. Values are mean±SE (*n* = 6).*P<0.05, All group were compared vs. control. (Student's "*t*"-test)

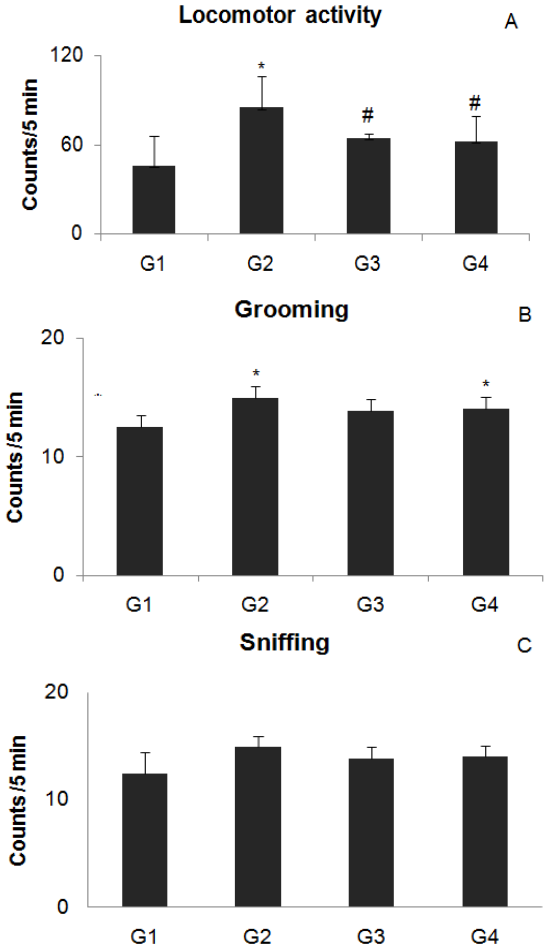

Fig. 2: Effect of exposure to Pb in the drinking water in (A) locomotor activity; (B) frequency of grooming response; and (C) frequency of sniffing response. Values are mean±SE (*n* = 6).*P<0.05, All group were compared vs. control. #P<0.05, All group are compared vs. Pb group (G2) (Student's "*t*"-test)

Elevated plus maze test (EPM)

The effect of lead on the measurement of elevated plus maze was demonstrated in Fig.3. Animals under lead effects significantly diminished the numbers of entries in the open arms of the maze, accompanied by a significant increase of this measure in the closed arms compared to control group (G1). A significant increased of time spent in the open arms in G3 and G4 groups it be noted compared to G2 group.

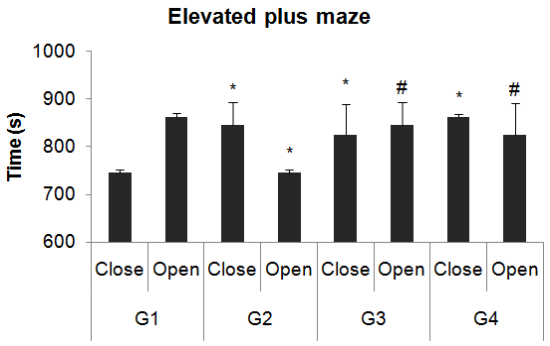

Fig. 3: Effect of exposure to Pb in the drinking water on time passed in the open and closed arm. Values are mean±SE (*n* = 6).*P<0.05, All group were compared vs. control. #P<0.05, All group are compared vs. Pb group (G2) (Student's "*t*"-test)

Forced swim test

Rats treated with lead demonstrated significantly (p<0.05) reduced immobility time compared to control in the FST, suggesting an antidepressant-like action (fig. 4). All groups (G2, G3, and G4) showed a significantly decreased value compared to control by-36%,-33%,-52%, respectively.

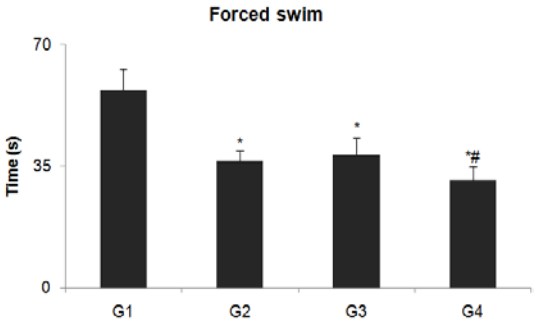

Fig. 4: The effect of lead on forced swimming after 12 w. Values are mean±SE (*n* = 6).*P<0.05, All group were compared vs. control. #P<0.05, All group are compared vs. Pb group (G2) (Student's "*t*"-test)

Dark-light box test

Results from dark-light box test (fig. 4), showed that time spent in the dark box was significantly increased in the lead-treated rats (G2) and wormwood-treated group 4 (G4) compared with control groups (p<0.05). Conversely, G3 group showed a significant reduction compared to other groups (p<0.05).

Lipid peroxidation and enzymes activity in brain

Lead exposure caused a marked lipid peroxidation accumulation in G1 and G4 group compared to control, by+177% and+237%, respectively. no difference was noted between G3 groups and control (table 1). The glutamate oxaloacetate transaminase (GOT) activities of the whole brain in all groups of rats are presented in table 1.

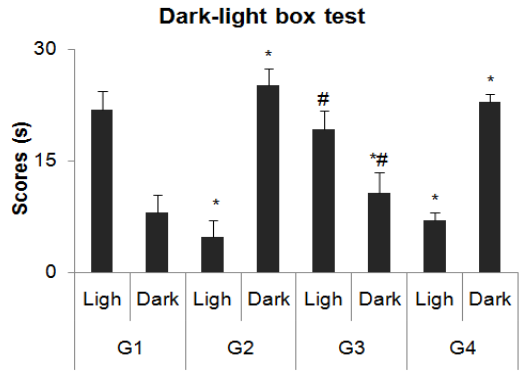

Fig. 6: Effect of lead-exposed on dark/light test in rats exposed for 12 w. Values are mean±SE (*n* = 6).P*<0.05, All group were compared vs. control. #*P*<0.05, All group are compared vs. Pb group (G2) (Student's "*t*"-test)**

The treated rats with lead showed a significantly decreased activity of GOT compared to control (p<0.05) by-13%,-18% and-27% respectively in G2, G3 and G4. However, GPT activities of a lead-expose group show a significant reducing compared to control and no difference was observed in G3 and G4 compared to control.

DISCUSSION

In the present study, we examined the effect of chronic lead intoxication, initiated before conception, with the development of different behaviors and chemical parameters in rats and to explore the effects of the aqueous wormwood extract on lead-induced locomotors, behavioral inhibition, anxiety and grooming impairment and changes in some enzyme activities in different regions of the brain and lipid peroxidation; different authors have reported that the preferred site of the action of lead in the central nervous system is the glutaminergic system [27].

The observation that glutamate oxaloacetate transaminase (GOT) is present in cerebral rat homogenate at about the same concentrations as in liver suggests its importance in the brain amino acid pool homeostasis [28]. The GOT activity is found in both mitochondrial and soluble fractions of the brain. The critical role of mitochondrial dysfunction in the pathogenesis of age-related nerve cell degeneration has been suggested on several occasions [16]. On the other hand, co-exposure to lead and glutamate is reported to produce morphological abnormalities of mitochondrial structures in pyramidal neurons of hippocampus formation, which are not observed when exposed to lead [5].

Thus, it may be suggested that lead exposure causes a specific alteration in amino acid metabolism. Hence, altered amino acid distribution pattern within the brain might be expected in response to lead exposure. The brain alterations may also be attributed to the level of accumulated lead in the whole brain. The diencephalon is reported to be the most vulnerable portion in the case of metal accumulation in the brain [5, 29].

Though glutamate pyruvate transaminase (GPT) is less active in the brain than GOT; however, Matthews *et al.* [30] reported that both the enzymes degrade glutamate; though only GPT was able to reduce toxic levels of glutamate (500 µM) into the physiologic range (<20 µM). In the present investigation, the GPT activity is increased in G4 group treated with wormwood extract compared to lead-treated. These observations support brain-specific sensitivity to lead exposure [31]. In addition to these, direct inhibition of GPT by lead may also be involved in the present observation of the reduction GPT activity. These transaminase reactions are reversible, but the equilibrium of the GOT and GPT reactions favor formation of aspartate and alanine, respectively [32]. Increased aminotransferase activities might participate in the enhanced synthesis of excitatory amino acid neurotransmitters in the nervous system [30].

Table 1: Glutamate oxaloacetate transaminase, Glutamate pyruvate transaminase activity (µmol. g^{-1}. min^{-1}) and Lipid peroxidation level ($mmol^{-1}$. cm^{-1}) in rat brain

	GOT	GPT	TBARS
G1	3.33±0,12	1.32±0.07	2.42±0.18
G2	2.88±0.11*	0.96±0.08*	7.45±2.81*
G3	2.73±0.21*	1.06±0.04*	3.04±0.35#*
G4	2.43±0.18*#	1.14±0.04*	9.15±1.77*

Values are mean±SE (*n* = 6).**P*<0.05, All group were compared vs. control. #*P*<0.05, All group are compared vs. Pb group (G2) (Student's "*t*"-test)

It is demonstrated that metal accumulation is associated with high levels of lipid peroxidation in different regions of the brain, such as hippocampus and cerebellum [33]; the vulnerability of neuronal membrane oxidative stress and cellular peroxidation induced by lead is due to the presence of a relatively high concentration of fatty acids that are readily oxidizable. In addition, production of ROS and alteration of homeostasis *in vivo* may be a major factor in the severity of lead poisoning and affect the reduction of the transaminase activity in the brain.

Lead neurotoxicity results in a behavioral and neurochemical alteration in neurons as a result of changes and disruption of the main structural components of the blood brain barrier, through primary injury to astrocytes and to secondary damage of the endothelial microvascular [34]. There are numerous studies utilizing experimental animal models on the central nervous system; these studies have mainly been concerned with the possible effects of lead on certain performance tasks that might reflect a cognitive function (learning and memory) or sensory-motor function in the infant animal exposed to lead very early in life [35]. Lead affects primarily the inhibition of the action of calcium, as a result, lead can affect calcium-dependent processes and interact with proteins including sulfhydryl, amine, phosphate, and carboxyl groups [36, 37]. Neurotoxicity may be a consequence of alterations in cholinergic function mediated by the acetylcholinesterase (AchE) [5]. The enzyme inhibition is generally reached its significance after 10 to 20 d of lead acetate intake orally to the rabbits, such alteration in cholinergic transmission suggests that lead is able to reach the CNS and exerts its neurotoxic effect [38].

In this study, the effects of exposure to chronic lead administered and it's exposed to wormwood extract on parameters of anxiety and related fear behaviors were investigated in male rats. Our result indicated that the chronic lead exposure caused a significant increase in the anxiety levels of rats. Interestingly, no significant improvement in the indicated measurement was observed when the rat was treated with both lead and wormwood extract (G4). Our result is in disagreement with Kharoubi *et al.*,[5] who found a very significant amelioration in all behavior tests compared to groups exposed to lead and treated with wormwood extract at the dose of 200 mg. this effect was certainly due to the concentration of wormwood extract. In addition, Seddik *et al.*, [39] indicates that exposure to lead provokes an anxiogenic effect demonstrated by the elevated plus Maze. On the other hand, the data obtained was not the same as that obtained by Trombini *et al.*,[40] who reported that exposure to 750 ppm of lead acetate in drinking water during pregnancy and lactation had no effect on the behavior determined by EPM. Our results reported in the dark-light box test showed in lead-treated male rats that time spent in the light compartment is reduced significantly compared to control animals, after stopping

intoxication (G3) the rat spent significantly more time in the light chamber compared with lead-treated group, this result is in disagreement with Benammi *et al.*,[41]. The data obtained herein in EPM and dark/light box tests showed that, an obvious anxiety like the behavior of chronic exposure to Pb in the male rat. This finding indicates that Pb-treated rat showed abnormal behavior when compared to control. Wormwood extract administration has a very beneficial effect no with respect to the state of anxiety. Studies have been reported the role of oxidative stress in anxiety-like behavior in rodents, and increased anxiety has been found to be positively correlated with increases in reactive oxygen species in granulocytes [42]. Hence, increased anxiety-like behaviors in the current study in Pb-exposed rats (G2) may be attributed to oxidative stress, which indicated by significantly increased of TBARS level and changes in transaminase activity ($P<0.05$), that catalyze reversible reactions amine group transfer between glutamic acid and pyruvate[30], in brain of lead treated rats (G2).

CONCLUSION

In conclusion, lead exposure induced a significant behavioral alteration as well as neurochemical alterations in the brain in rats exposed to Pb. Moreover, *Artemisia Absinthium* L. modified neurotoxic effects of lead on enzyme activity and locomotors and anxiolytic behaviors, grooming and changing in enzyme activities involved in expression neurotransmitter.

CONFLICT OF INTERESTS

Declared none

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