Global Journal of Environmental Research 3 (1): 52-60, 2009
ISSN 1990-925X

Lead in Water: Neurotoxicityand Stressful Effect on Wistar Rat

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Abstract: The fauna and flora are severely threatened by discharges of industrial pollutants such as heavy metals (lead zinc cadmium arsenic…) which are highly dangerous. The study covers the identification of sites fixing mechanism lead to the brain, in rats Wistar according to several approaches: phenotypic, behavioural, immunohistochemical and histological. The rats are permanently acetate lead the dose of 250mg / l and 500mg/l diluted in distilled water for a period of 12 weeks compared to witnesses who receive only distilled water. On the phenotypic we found that in rats treated, lead causes exophthalmoses, a decrease in muscle tone, body weight, the intake and falling hair all over his body: anorectic effect and a decrease in water intake. The administration of the lead acetate oral causes a very significant decrease in the conduct of exploration (locomotors activity) and the stereotypical behaviour like (grooming, sniffing bite) compared to control group rats with rats. The experience of swimming forced and the passage of two compartments (darkness-light), were observed that lead causes a stressful effect in rats, which is in favour of the involvement of serotonin transmission systems. The immuno-histochemical study using an antibody anti-GFAP (Glial fibrillary acidic protein) demonstrated an increase on GFAP immunostaining in the cortex, the cerebellum and the corpus callosum. For more understanding these involvements of various channels, we had involved our study to compare the effects between two groups by histological different brain structure. An alteration in Purkinje cells and the layer of grains were observed with important disorganization tissues. These results, clearly, show that lead causes a decrease in nerve transmissions (catecholamineric, glutamatergic and serotonin) and a structural alteration of different brain tissues, which are the cause of behavioural and functional disorders.

Keys words: work · Behaviour · Lead acetates · Wistar rat · NCS · GFAP (Glial fibllary acidic protein)

INTRODUCTION

In the modern Algeria, some large cities by installing anarchic slums where networks for the supply of drinking water and sanitation wastewater are literally absent. The tank lost multiply and polluting the ground and setting up an ecological disaster painful. Wastewater in the open air is sowing unrest and health namely water-borne diseases (meningitis, thyroid…etc). A quantity of such polluted water is absorbed by the infiltration of surface water tables which are sources of drawing the population.

Heavy metals pollute the waters of rivers, oceans whose lead has entered human life since ancient times, which its uses are manifold with a huge toxicity and is the cause of illnesses since more A century. The metal contaminates the environment; it can be airborne by the combustion of petrol lead. Numerous studies have shown that chronic lead poisoning causes a number of disturbances neurological and behavioural among people exposed [1-3]. In entering the middle blood lead is quickly mobilized more than 95% by erythrocytes and then distributed in all organs soft. Thus, it accumulates in the brain, liver; kidneys, heart and only 3% remain in the blood plasma. In the absence of track excretion, it is deposited at a rate of 90% in the bones in the form insoluble. The nervous system is the target of lead that readily crosses the blood brain barrier and is concentrated in the nerve causing irreversible damages in the child development in the first place and some professionals and smokers. Several studies have shown that birth, maternal and foetal blood leads are strongly correlated [4-7].

To this end our study will focus on the effect of a diet rich in water lead on the brain function under different approaches physiological, biochemical and behavioural of Wistar rats. These tests namely locomotors activity, stereotypical behaviour and the taking of water that

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involve many neural pathways by the interaction of multiple neurotransmitters and the participation of receivers, primarily the ways of dopamine and serotonin [6, 7]. The chronic stress leads to a deterioration of decrease 5-HT1a). The lack of serotonin may adversely affect our moral state and as such, it creates stress. To this end lead conveyed through food by the digestive tract, is an inhibitor of serotonin reuptake, so that prevents its recapture by the nerve fibbers pre synoptic thus increasing its concentration in the synapse and strengthening its effect at the post synaptic fibbers. Stress depletes serotonin; therefore, it becomes more difficult to manage without this neurotransmitter [8-11].

MATERIAL AND METHODS

A staff of forty-eight (48) male and female rats, Wistar strain used in our experiments, their body weights means (82±4.3 g). The animals are grouped by 5 in polyester cages. An artificial cycle (day and night) of exposure is respected 12h light and 12h of darkness. The temperature of the animal is kept between 20°C ± 2°C. The study focused on the effect of the administration of the lead acetate (oral the Wistar rats) at doses of 250mg / l and 500mg / l diluted in distilled water for a period of 90 days.

- Group T: lot of rats treated with only distilled water.
- Group A lot of rats treated with 250mg acetate lead per litre of water.
- Group B: lot of rats treated with 500mg of acetates of lead per litre.

In this work, the mortality rate and the clinical condition are noted and exploration of behaviour is done in several stages:

l'évolution Growth of the Weight and Water Intake: All animals are weighed at the end of each week for 12 months of treatment, as the water intake is measured every day.

Test the Behaviour of Stress: For tests of behaviour and stress, each animal is observed and its stereotypical behaviour is observed every five minutes for 30 minutes for each rat. Other tests of stereotypical behaviour are offered: the sniffing and grooming. It attributes the score 1 where the presence of stereotypical behaviour and attributed the score 0 (zero) in the absence of the same conduct.

The locomotors activity: characterized by horizontal activity of rats in a cage of experimentation.

The test-stress "swim forced" by the technique of Persolt *et al*. [12]: the rat swimming in a small space and came to the surface of the water. This immobility expresses “a behavioural despair” the time of immobility (TIM) which is calculated.

The test-stress "compartment obscure and informed": this test is to measure time spent between two compartments one informed (in daylight) and the other obscure.

Tests of Behaviour According to the Technique of Irwin [13] Have Two Phases: The first phase of direct observation with the naked eye without the intervention of the investigator; behaviours studied are: exophthalmia, trembling, body posture and locomotors activity, the reflex, grooming the reflex recovery and pinch the tail.

The second phase of manipulation of animal: ptosis, bursts and spontaneous bursts of touch and catalepsy. Depending on the intensity and degree of behavioural effect, attributed the signs (-, + + +, + +, +) for each animal witness or treated, an individual is established. The normal score is recorded in column NB (normal behaviour), in column AB (misbehaving) and a column AB * (abnormal behaviour intense).

Histological Study: In order to determine whether the weight is achieved or not any alteration of tissue architecture, rats were sacrificed and a histological study was conducted at the corpus callous cortex and cerebellum of animals used for the study of behaviour. The brains are removed for a histological study according to the conventional method of colouring by the method Hémalun-Eosine.

For immunoassaying of the same structures previously studied a sample of 12 rats divided into 03 lots (witnesses and treated to 500mg/l) was used. The animals are infused with 300ml transparent solution Para formaldehyde (PFA) 4% in saline solution (PBS) (O.1M, pH 7.4). The cuts are implemented microtome and crawled under a microscope photonics.

Statistical Analysis: The exploration of statistical results is carried out using the test 'T' Student-Fisher.

P=0.05 is considered significant, P=0.001 is considered very significant.

The results are represented in the form of average + or - SEM, with signage if the difference with witnesses is significant or not. All results of the study of histology and immunohistochemistry is given in the form of plates taken by optical microscope.

RESULTS AND DISCUSSION

The terminal lead with 250 mg/l and 500mg/l for a period of 90 days induced a significant decrease in the growth weight of rats intoxicated compared to witness rats. Table 1 show that among the witnesses, the weight increases considerably from the first week and continues until the end of treatment. At the beginning of treatment (week zero) animals poisoned to 250 mg/l had an initial weight (82 ± 4.2 g) and (82 ± 9.36 g) for treaties to 500mg/l. As the weight witnesses he was (80 ± 4.2 g). At the end of the third month of treatment, the weight has increased steadily until it reaches (310.98 ± 8.79 g) for witnesses (228.7 ± 3.13 g) and (199 ± 9.07 g) respectively lots A and B (Fig. 1). However, in rats treated there is a declining growth body which can only be explained by a decline in the consumption of food (anorexia).

This result indicates that the lead has led to a very significant stunted from these two lots of rats treated with doses of 250m/l and 500mg/l (P <0.001>).

The anorectic effect exercised by this toxic metal used to justify its involvement in the nerve transmission system (catécholaminergic, glutamatergic and serotonin [14]. These results are in agreements with the results reported by Tefas *et al*, 1998 [15] where these authors have shown in their experiments on sheep and rabbits that chronic poisoning by lead causes a loss of weight.

Table 1: The evolution of body weight (g) at the end of each week treatment during 12 weeks

Ggroups/Times	Group T	Group A	Group B
Week (0)	80±4.2	82±9.36	82±4.3
4 Weeks	139.25±9.25	131.56±8.78	114.64±5.6
8 Weeks	230.11±0.4	172.81±8.27	155.32±3.4
12 Weeks	310.98±8.79	228.7±3.13	199±9.07

In addition to the anorectic effect in rats treated water consumption decreases significantly (P<0.05) compared to the control group (Fig. 2). This decrease in water intake would be related to dose, which explains the fall of weight in the treaties.

The treatments with acetate lead for rats for a period of twelve weeks was observed also a significant decline in exploration activity during an experiment of 30 minutes (Fig. 3). The doses of acetate 250mg/l and 500mg/l causes a decrease in locomotors activity of a dose-dependent compared to the control group animals. This locomotors activity is accompanied by a decline in the demonstrations stereotypical grooming and sniffing (Figs. 4 and 5).

The Figure 6 shows the results of stress test and show how much stress poisoned rats is staying much more light than darkness, unlike the rats that remain much longer time in the dark.

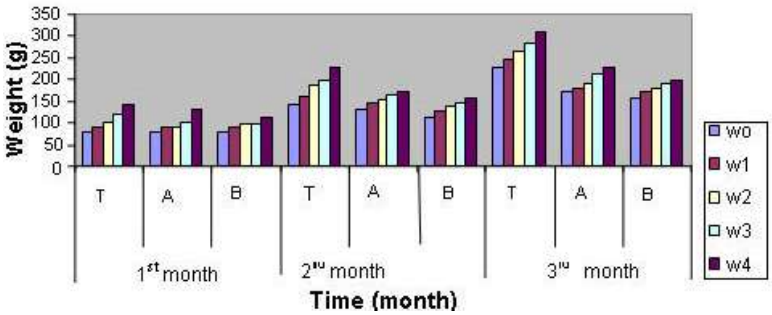


Fig. 1: Effect of lead on growth body poisoned by rats witnesses report. W1, W2, W3 and W4: weeks for each monthT (Control), A treated 250mg/l, B (treated 500mg/l) the values expressed in Means ± ES

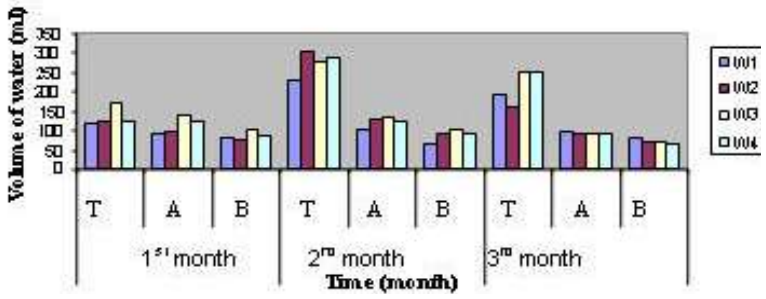


Fig. 2: The consumption water volume in ml through 12 weeks T (Control), A treated 250mg/l, B (treated 500mg/l). The time in the graphs is estimate by month

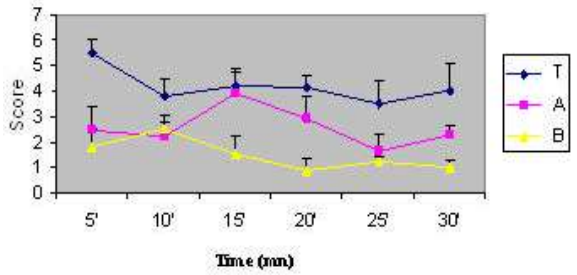


Fig. 3: The effect of lead on the locomotors activity of rats a function of time. Scores expressed in M ± ES T (Control), A treated 250mg/l, B (treated 500mg/l) the difference is significant P <0.001

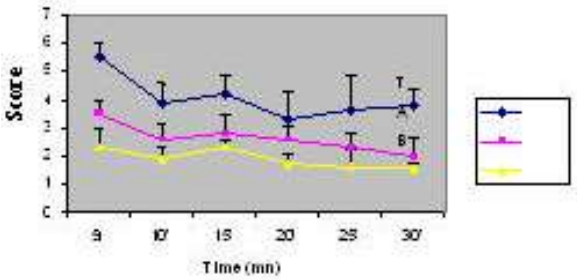


Fig. 4: The effect of lead on stereotypic activity of cleaning For each time the scores are expressed as mean± ES T (Control), A (treated 250mg/l), B (treated 500mg/l)

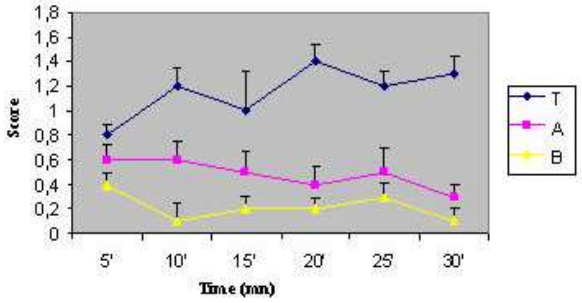


Fig. 5: The effect of lead on the behaviour of sniffing. For each time the scores are expressed in M ± ES the difference is quite significant P <0.001

Fig. 6: The effect of lead on stress (darkness, light) in rats a function of time. Values are expressed as Mean±ES

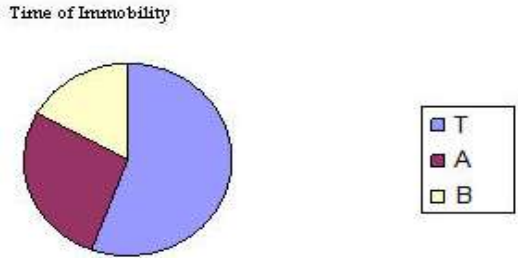


Fig. 7: The effect of lead on the stress of forced swim for each time the scores are expressed in Mean ± ES

Table 2: Different Irwin tests (simplified) and evaluation of experimental results

Irwin Test Simplified	Groups		
Animals Group:	Group T	Group A	Group B
Symptomalogy			
Observation	NB	AB	AB*
Posture body	+	+±	±
Locomotors Activity	++	++	±
Abnormal Behaviour	--	++	+++
Exophthalmia	--	++	+++
Tremor	--	±	++
spontaneous jump	±	±	--
Jump in the touch	++	±	±
Catalepsy	--	--	++
Reflex of to climb	++	+	±
Traction	+++	++	+
Reflex recovery	--	±	++
Tonus abdominal	+	±	±
Tearful	--	±	+
Toe angle of the tail	+++	+	±
Cray	±	+	++
Mortality	±	+	++

AB: Normal Behaviour
CA: Abnormal Behaviour
CA*: Abnormal Behaviour intense

The test results of the forced swim show that the time of immobility (TIM) decreases in rats treated with 250mg / l 500mg / l confirming a state of stress (Fig. 7). Compared with rats behaviour and poisoned rats is affected after the injury probably caused the level of cells responsible for production of Dopamine. According Djebli [21] decrease in the neurotransmitter causes stress and depression and this explains their stereotypical behaviour such as sniffing and bite.

The study by direct observation by the method of Irwin is summarised in Table 2 and the Fig. 8a where there is a way of example that the body posture is normal in rats witnesses (Fig. 8b) against among some anomalies appear

a: Control Group rats climbed the box bares (NB)

b: Exophthalmia in rat from poisoned parents rats with lead

c: Sag legs Rear in rats treated with lead (500mg/l)

d: fall of hair all over his body in rats treated (500mg/l)

Fig. 8: The effect of lead on the stress and phentypical aspect

intoxicated; animals take very little about the legs (Fig. 8d), they are disoriented in space and tripping more often. We note also that locomotors activity and space among animals is moderate compared to treaties; they present a greater spatial disorientation. The animals are recovering immediately, while the recovery is slower in the treaties. The muscle tone is completely flaccid at the group B where the poisoning is more important, so that witnesses show a slight resistance. The latter pose risks incurable disease that can cause death. We have also noticed the phenomenon of cannibalism in rats treated with lead acetate.

The Figure 8c shows that rats are probably suffering from a motor apraxia resulting in an inability to properly dispose of its legs to walk; the photo represents 4 rats suffering from falling hair at the entire body. Table 2 shows, for example, that body posture is normal in control group rats (Fig 8a) against, among some anomalies appear intoxicated; animals take very little about the legs. We note that the effect increases in rat from the second generation of parents intoxicated, where they are completely inability to properly dispose of its legs to walk on hind legs (Fig 8 b, c and d).

The animals are disoriented in space and tripping more often. We note also that locomotors activity and space among animals is moderate compared to treaties, where they present a greater spatial disorientation. We note that the effect of lead on the means of transmission increases nerve in rats of the second generation from parents intoxicated, where they are not able to walk completely (Fig 8 c). After mating Inter group a significant decrease in the number of staves with a high mortality are observed. For six (6) witnesses impregnated females, 48 were born small forward, unlike the number of pups born to parents intoxicated (500mg/ l) where there are 17 pups which 4 died at birth, or 27% births compared to untreated. The lead would also lead to a reduction in the number of newborns and their birth weight by 17%. Similar work has shown that lead causes of premature births among pregnant and impaired growth and foetal development [16-18]. The results are consistent with those of other researchers [19] who conducted a study on more than 4000 subjects occupationally exposed to lead. The results showed a significant decrease in the number of births compared to a control group (5000 persons) in all age groups studied. In the same context, Wilson [20]

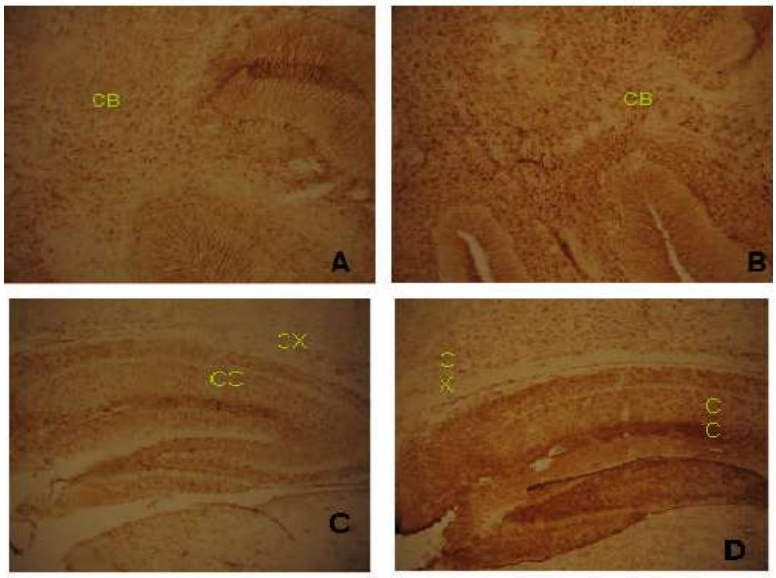


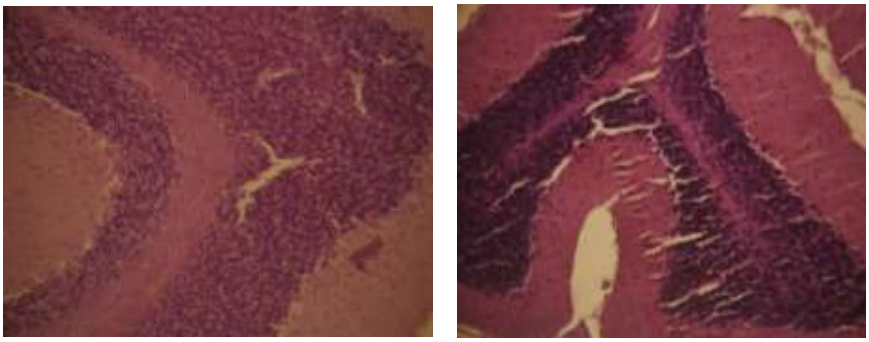
Fig. 9: Immunoassaying astrocytes rat cerebellum (GX40):
A Histological cup cerebellum witnesses,
B Histological Cup at the cerebellum treated
C: Cup cortex, hippocampus and the corpus callosum (control)
D: Histological Cup at cortex the hippocampus) (GX10) and corpus callous (treated)
The nerve structure are dealt with by GFAP shows that exposure to lead (cut B) the dose of 500mg/ls' accompanied by a severe violation of astrocytes, which is manifested by an increase in their volume.
(GX10). Immunoassaying astrocytes cortex (CX), the corpus callosum (CC) of rat control (C) and processed (D) The cut represents D structures nerve after chronic poisoning with lead acetate dose 500mg/ l. The astrocytes are more dense compared to the witness C

reported that the central nervous system is particularly at risk of subtle failures during the gestation period, leading to abnormal foetal development, where the foetal death, malformation, stunting and functional deficit. Lead disrupts the secretion of sex hormones, which is the likely disruption to direct any local nerve caused by this metal. It is probably for this reason that a decline in fertility and the number of birth could be observed.

These results indicate that lead is gifted with a power hypo locomotive, which suggests an involvement of this metal on dopamine pathways by low release of dopamine caused a competition with the Ca^{2+} essential for the process of liberation, a strong MAO activity of enzymes involved in the degradation of catecholamine and a post-receptor inhibition of synaptic dopamine. In addition, the state of chronic stress animals would lead to a deterioration of certain serotonin receptors (increase of 5-HT2a receptor and decrease 5-HT1a). The decrease in the transmission of dopamine and serotonin leads to a decrease in different behavioural tests studied. The lack of serotonin can have a negative impact on the behaviour promote the emergence of stress.

To confirm the effect of lead in the brain, we use the technique of immunoassaying GFAP (Glial fibllary acidic protein) glial cells, astrocytes lining neurons. They are activated and transmit the signal to other astrocytes that spread slowly in turn entrance of Ca^{2+} (calcium wave) to other astrocytes until the end of another arm astrocytes, which will affect communication of neurons that surrounds it. The astrocytes, "control" of neurons and their alteration prevents them from working properly, because they facilitate the spread of nervous message, by cleaning the synaptic space, provide their survival and protect them. The glial cells give a good indication of the integrity of the SNC, because the latter have the ability to inflammatory reaction or multiply in the case of a neuronal death (gliosis).

The application of an antibody anti-GFAP on corpus callosum, cortex and cerebellum showed an intense immunostaining on astrocytes. An increase in the



TM: Histological cup of the cerebellum (control)

TR: Histological cup of the cerebellum (treated)

Fig. 10: Histological cups at the cerebellum at the Wistar Rat witness and intoxicated to 500 mg / l (G20): lead tends to accumulate in the white substance rich in glial cells, large spaces between cells Purkinje and the granular layer is observed.

astrocytes size in different studied structures was also observed in Lead acetate treated rats (500 mg/l) (Fig. 1), comparing to witness (Fig. 2).

This astrogliose reaction seen after lead poisoning in structures studied may be due to an accumulation astrocytes lead can be used initially in the protection of neurons. Similar observations were observed by Lindhal *et al*. [22] and O'Callaghan and Little [23]. Christian and Tryphonas [24] reported that in cattle chronic poisoning by lead resulting in swelling astrocytes, a development of a focused and spongiosis necrosis neurons. Under certain conditions of the goat and deer, a demyelination of the nerve sheath was observed by other work. The argument could be that diets rich in protein sulphur mobilize some toxic metals contained in the amount of land significant that the animals eat grazing on contaminated soil.

In addition, histological analysis of the cerebellum (Fig. 9C) reveals a spectacular attack since there are alterations and a dissociation of the layer of cells Purkinje layer of the grain in rats treated with the report rats. There is a cell destruction more pronounced with spaces vascular more expanded and the differences in size and shape of cells. It seems, however, that its local disturbances are caused by the accumulation of lead in the cerebellum [25].

These histological results are consistent with the work Sidhu and Nehru [26] who observed a disruption cell at the cortex and a separation of Purkinje cells compared to the layer of grains. Other authors accept the idea that the cerebellum plays a crucial role in learning and locomotor's control of the posture [27, 28]. Moreover, many have suggested the existence of a more elaborate role of the cerebellum in the control of behaviour or learning capabilities and guidance. By altering these nerve structures, lead causes of unrest not only in controlling the march, but also in the behaviour in general of rats treated compared to witnesses [29-32].

The exposition of lead entrained incontestable of alterations in level of all nervous structures that cause all these risks so for example the new givens about human orient to think that cerebellum is neither implicated neither only in the mechanism of "stimulus-answer" nor also in behaviour cognitive moor elaborated.

These results are in agreements with other work [33-35] on injuries at cerebellar structures, including those caused by heavy metals. The latter not only disrupt the mechanisms release of neurotransmitters regulated by calcium, but also induced damage to mechanisms of DNA repairs [36, 37]. Its role inhibitor could come from its interaction with the sites that could be occupied by calcium and release too much or too prolonged glutamate causes neuronal death.

CONCLUSION

The neurotoxcity caused by lead present in food is a function of different doses administered. The sprawl of treatment beyond twelve weeks seems an aggravating effect. Improved protection system is needed to help optimize monitoring for lead content in drinking water

(25µg/l) recommended by who is respected and ensure consumer health. The studies known so far require all measures appropriate protection by the occupational medicine and public authorities [38-40]. It is for this reason that the entire human community must agree to protect her health in dealing with technological threats and natural, manageable. Now the whole planet is suffering from pollution, since the dangers threatening the fauna and flora.

KNOWLEDGMENT

The authors thank Miss K. Behilez for the translation of the paper, Dr. C. Mesenge and Pr. M. Kihel for their help.

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