Indian Journal of Fundamental and Applied Life Sciences ISSN: 2231-6345 (Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jls.htm
2014 Vol. 4 (3) July-September, pp.580-588/Azadmanesh et al.
Research Article

THE EFFECT OF LEAD ON BRAIN DISORDERS AND NERVOUS STRAIN IN RAT

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ABSTRACT
Since lead is one of the most important environmental pollutants that can cause nerve injury and behaviors disorders in human and laboratory animals, different studies about effects of lead have been done. There is a meaningful effect of lead acetate density (100 ppm) on decrease of seizure threshold that represents this amount of lead cause some effects on human population and it increases the seizures in the population when they confront with lead. Considering the liner connection between lead amount and seizure threshold, it seems that existence of a basic density for effects of lead on seizure threshold is sufficient and the mechanisms that are seen in effects of high density, there are in effects of low density of lead. The present study has been done by experimental methods. Animals were divided into three groups: one group that called control group used drinking water. Also, there are two experimental groups that a given density of lead was in their drinking water. For making of convulsion, slow venous infusion of PTZ has been used. Time between start infusion and appearance of each step of convulsion has been recorded on the basis of second and it was converted by equitation relating to seizure threshold doze. After doing all experiments, blood samples of animals have been collected and lead density has been evaluated by atomic absorption method. The obtained dada has been analyzed by statistical method such as Anova and t-test.

Keywords: *Lead, Nervous System, Nervous Convulsion, Rat and Brain Disorders*

INTRODUCTION
Today, in industrial world and even in small cities, lead is considered as one of the important environmental pollutants that the electrical conductivity of it is low and resistant against decay. For this reason, it is used for keeping of erosive liquids (such as sulfuric acid). Also, with increase of small quantities of Antimony or other metals to lead, makes hard it. This metal after iron, aluminum, copper and zinc has most application and it is second metal that is used extensively and probably it is oldest chemical poisonous that its harmful effects on human was known. Lead can affects any organ or system of body by mechanisms that involve other biochemical processes .these mechanisms consist of ability of lead to control of calcium and proteins mechanisms. In reactions of proteins, lead is connected to any available operations such Sulfhydryl group, phosphate and carboxyl among the harmful effects of lead on body creation of brain disorders and negative effects on nervous system is considered as the most important disadvantages of lead. Lead has various effects on hematopoiesis, nervous system, kidney, reproduction and bone. The most common chronic lead poisoning contains muscular weakness, anger, tremor, emaciation, headache and abdominal complications (Ford *et al.*, 2001).
Lead is one of the metals used greatly. Lead entered to air by chimney of factories. However, dangers and extensive source of these pollutants are related to the automobiles. In petrol, components of tetraethyl lead and tetra methyl lead is found. When the petrol is burnt, the lead is changed to lead oxide and it is left from exhaust pipe and entered to human's lungs. In refinery to produce petrol and to increase octane rating of petrol, the Tetralin lead is added to it that easily is evaporated and its steam is entered to body by skin, mucus and lack of observation of personal hygiene. This material is stored on the organs and tissue and over time it is consumedly gathered in the body and the probably of unpleasant injuries will be increased. Lead by incomplete combustion of automobiles is increased in the air and it is entered to surface waters and consumption of this water is threatened the human health (Levin, 2000).

The existing lead in the air which is concerning issue about long term resident of the lead is controllable. Lead is heaviest and poisonous element in environment especially when petrol has been used extensively; this element is found in polar ices and deep marine sediments. Non-solvent compositions of lead are absorbed in the earth sediments. Also, the aquatic plants are stored lead; biochemical oxidation of organic matters is stopped in densities above 1.0 mg/L. groundwater is affected by solvent composition of lead (nitrate, lead chloride). Drinking waters that are passed from lead pipe may contain high density of lead. In inner walls of lead pipe with carbonate water, carbonate sediments are formed. High amounts of lead enter to brook by fuel process. There are significant differences between lead densities in urban areas and rural areas. Lead compositions may is transferred to significant distances that it depends on speed and direction of wind, rainfall rate and moisture. Today it has been increased in environment because of urbanization activities (Domino, 1994).

Most of the existing lead in the atmosphere is deposited or exited by raining or snow. Lead sticks to dust particles and they are settled on the plants and soils. More nutritional absorption of lead is done by drinking. Led in unclean areas is considered as an important issue which from 30 to 50 percent of lead remain in lungs. The jobs that lead is found consist of: mining, ceramics, automotive repair, glass manufactures, and automotive manufactures. Today, amount of existing lead in oceans is 50 times higher than normal amounts. Signs of diseases related to lead are different and in first step are Indistinguishable. The signs of poisoning by lead are: impatience, anorexia, confusion, delirium, I. Q decrease, high mobility and activities and movement weakness in children (Garretson *et al.*, 1990).

Decrease of weight and blood are other signs of lead poisoning. Also the general changes is occurs in body such as deformation of red blood cells or gray teeth and gum. This gray color remains even after elimination of poisoning. Other effects of poisoning arising from lead may consist of: hypertension, pulse rate, rapture or sever muscle pain, nervous diseases with severe headache and epilepsy. The first signs of poising with lead are nervous signs, increase of brain disorders in children and hypertension in adult. 30 micrograms of lead per deciliter or less than this amount makes disorder in nervous system, red blood cells and hypertension in adult. High amount of lead in blood cause some problems in central nervous system, kidney, anemia, sexual disability. Among the disorders, behavior-nervous disorders is considered as main important poisoning arising from lead since brain is main source of lead activity (Goyer,1996).

In addition, so far many diseases such as nervous diseases, paralysis, joint disease and anemia have been seen. Also, genetic changes are occurred that cause some pedagogic effects on central nervous system (Hsiang, 2011).

Intense poisoning by lead is common between persons who continually are exposure to lead. They contain workers of manufactures who are exposure to lead. Increase of crime in teenagers, behavioral rudeness in children and teenagers, academic failures are the effects of lead poisoning to next generations that is created in childhood because of high sensitiveness (Omaye, 2004) or by mothers due to pass lead from placenta and fatal blood-brain membrane (Garg, 2000). This issue in our country that has low standards of environments is so important. Lead is stored in body tissues, so there are danger of entering lead to human body by animals that their meets is used (Radostits, 2000).

Lead is not spread evenly throughout the body when it is absorbed. Absorption is rapid in soft tissues than other parts of the body. Then, absorption extensively is done in bones. Lead has a tendency toward link with Sulfhydryl group especially with metallo–enzyme. Also the lead affects production of nerve mediations. This effect may occur because of disability of lead to prevent Calmodulin and Pyravate kinase action and other existing Enzymes in nervous cells. Some of lead Neurotoxic effects is appeared because of prevention of cell actions of zinc and calcium. Ability of lead to link to proteins that is activated by calcium is ten thousand times more than calcium. Central and peripheral nervous system, skeleton system, urinary system, digestion system, hematopoietic system, reproductive system and cardiovascular system are some places in body that lead affect them more than other system (Jones and King, 1997).

Protection of brain health during the life is one of the main aims of human health. High sensitivity of nervous system toward lead causes some concerns about quality of human life. Nervous diseases like

Alzheimer and Parkinson are considered as these issues and generally lead and cadmium are known as more important polluters in the world. Many studies have been done about the metals by international organization chemical control. Done studies were about effects of lead on human and laboratory animals. Human studies have been done on industrial workers and children in polluted cities. In both groups, some disorders have been seen such as IQ decrease, behavioral disorders, weakness of learning and depression (Hass, 2003).

Physical and Chemical Features of Lead

Lead is a soft metal with grayish color and atomic number 8. Also its standard atomic weigh is 207.2, with density 11.34; melting point 237.5 ^{0}c and boiling point 1740 ^{0}c. Lead takes place naturally on Earth exclusively in the form of four isotopes: lead-204, -206, -207, and -208. Lead is radioactive element. Also lead compounds exist mainly in +2 and +4 main oxidation state sand. Main mineral components of lead are lead sulfide, lead carbonate and lead sulfate. Some of its mineral salts like oxide and lead sulfide are solved in water. Nitrate, chlorate and chloride are soluble in water. Some of salts formatted by organic acid such as lead oxalate are dissolved in water. Lead is a divalent metal that mostly competes with other divalent ions like iron, calcium from the viewpoint of physiologic and biochemical process. Also lead may supersedes other ions and plays the role of same ion (Ramamorthy and Moore, 1988).

The Main Components of Lead Consist of

a) Organic components: The main important organic components are lead tetraethyl and tetra methyl that is added to gas in order to prevent gas explosion at low pressure; however, today Dichloroethylene and Dibromo-ethylene are used instead of these components.

b) Mineral components: main important mineral components of lead are lead oxides such as PbO, Pb_3O_4, and PbO_2which is used for preparation of stainless and battery platelets. Colored salts of lead are white lead, lead Antimoniate and lead Oxychloro (Sanaii, 1992).

Literature Review

The studies showed when amount of lead in blood is about 10 microgram per 100 milliliter, behavioral disorders and delay on behavioral growth is seen in children (El-Sokkary, 2003). Various studies about animals and human depict that lead has more effects on central nervous system especially during genesis because of cell reproduction, difference and Synaptogenesis that is occurred during this period. Lake of good performance of blood- brain barriers and lack of proteins complex which is able to separates the lead from brain increase brain vulnerability against the lead (Goyer, 1996). Lead passes from brain-blood barrier and is gathered in brain (Marchetti, 1989).

In many done studies on the laboratory animals, lead toxic trouble has been reported which decrease of neuronal density in vision cortex of monkey (Reuhl *et al.,* 1989), decrease of acetylcholine in the rat newborn (Bielarczyk, 1994) and creation of injury in Astrocyte and endothelial cell of blood- brain barriers have been seen in the studies (Costa, 2004; Kagi, 2004).

In this way, Garavan *et al.,* (2000) and Vait *et al.,* (2000) reported that low density of lead in blood about 10-15 microgram per milliliter make decrease of behavioral- cognitive ability. In addition, recent studies report lead ability to stimulate oxidation pressure. Also the finding shows that there are affective role of oxidation pressure on pathophysiology of lead toxicity (Candsn and Tuzmen, 2008).

The studies have been done by electron microscope that showed damaging effects of lead on Astrocyte of rat (Holtzman, 1987; Tang, 1996). Astrocyte protects natural physiology of brain during evolution of living organisms. Therefore, any changes in mentioned cells activity frustrate the natural function of nervous system. So far, effects of lead on growth of Astrocyte cells of fetus have not been reported. Effects of pollutants on behavior have not been studied while behavioral changes have many important because of the effects that apply on animals and human.

MATERIALS AND METHODS

Methodology

In the present study, density and type of effect of lead on nervous system of rat has been examined. In this regard, hypothesis of the study are as follows:

- A certain amount of lead is affective on creation and increase of nervous strain.
- Blood lead level in animals that are exposure to lead changes in comparison with control group.
- Some of the nervous strain signs such as convulsion are seen more among the experiment group of rat.
- With increase of blood lead level in rats that are exposure to lead; nervous impulse rate is enhanced significantly.

For testing these hypotheses, 36 male Wistar rats have been bought from Pastuer institute of Iran and they were preserved in common standard condition. Animals have been divided in 3 groups randomly. Each group has 12 rats. 2 groups of them used drinking water with 50 and 100 ppm of lead during 4 weeks (28 days). One group used drinking water without lead. Behavior of animals in Elevated plus Maze (EPM) have been examined in last day. For this reason, animals individually were set free to central part of Maze and they have been examined by IP camera during 5 minutes. The parameters that have been examined contained: the period of presence in close arms, the period of presence in open arms, and the period of presence in a third terminal of open arms.

Used lead acetate in this study is from production of Germen Company. The data were recorded secondly and the results were obtained by mean and standard deviation. Animals after receipt the lead acetate and distilled water were preserved in laboratory condition in order to examine hormonal and enzymatic studies. From all animals at the end of 15 days, phlebotomy was done from ventricle of the heart to evaluate changes rate of blood level of lead. Measurement of lead rate in blood because of ease of work was the main important method of examination of blood lead level and poisoning with it. The blood lead level shows recent contact with lead and after contact cutting, this situation changes to natural level. Lead is active in blood about 30 days and after that it is gathered in bone tissue and especially in tooth. Therefore, blood lead level is the most important index to measure total dose of lead. After phlebotomy of each sample during 15 minutes they were centrifuged with 3000 cycle in order to separate serum from coagulum. After separation of blood serum by sampler, the samples were preserved in -20 ^{0}c until completion of hormonal and enzymatic studies.

RESULTS AND DISCUSSION

Findings

Performing of study process in each branch of science in order to reach a series of results and research finding is based on the finding of each study that contains description and determination of work according differences and relations. So this section based on the gathered data have been examined the research hypotheses and analysis of them by descriptive and inferential statistics. Descriptive statistics used in this study consist of central and distribution indexes and interventional statistics contains ANOVA and etc.

Mean comparison of received total strain between control and experimental groups shows that mean of received total strain in experimental group is more than control group ($p > 0.05$).

Table 1: Estimation of mean and standard deviation of rats' weight (g)in each group

Variable	Number	Mean	Standard deviation
Control group	12	22.58	2.02
50 ppm group	12	22.33	1.92
100 ppm group	12	23.25	1.71

In table 1, mean and standard deviation of rats' weight has been estimated. Therefore, as the table represents mean of rats' weight in control group is 22.58 ±2.02. Weight of rats group that are exposure to lead acetate (50 ppm) is 22.33 ± 1.92 and the weight of rats' group exposure to lead acetate (100ppm) is 23.25 ± 1.71.

Table 2: Estimation of mean and standard deviation of rats' blood level (microgram per deciliter) in each group

Variable	Number	Mean	Standard deviation
Control group	12	0.019	0.001
50 ppm group	12	6.35	0.918
100 ppm group	12	10.58	1.009

In table 2, mean and standard deviation of rats' blood level has been estimated. In this regard, as the table represents mean of rats' blood level in control group is 0.019± 0.001. Blood level of rats group that are exposure to lead acetate (50 ppm) is 6.35 ±0.918 and the blood level of rats group exposure to lead acetate (100ppm) is 10.58± 1.009 microgram per deciliter.

Table 3: Estimation of mean and standard deviation of PTZ density for start of Myoclonic movement step in each group

Variable	Number	Mean	Standard deviation
Control group	12	39.72	1.047
50 ppm group	12	40.028	2.013
100 ppm group	12	32.800	1.128

In above table, mean and standard deviation of PTZ density for each group has been estimated. In this regard, as the table shows mean of Ptz density in control group is 39.72± 1.074.PTZ density of rats group that are exposure to lead acetate (50 ppm) is 40.28 ± 2.013 and the PTZ density of rat's group exposure to the lead acetate (100ppm) is 32.80 ± 1.28.

Table 4: Estimation of mean and standard deviation of PTZ density for start convulsion step in front limbs of each group

Variable	Number	Mean	Standard deviation
Control group	12	51.77	2.05
50 ppm group	12	49.19	1.15
100 ppm group	12	46.43	1.42

In table 4, mean and standard deviation of PTZ density for start convulsion step in front limbs of each group has been estimated. In this regard, as the table shows mean of Ptz density in control group is51.77 ± 2.05. PTZ density of rats group that are exposure to lead acetate (50 ppm) is 49.19 ± 1.15 and the PTZ density of rats group exposure to lead acetate (100ppm) is 46.43 ± 1.42.

Table 5: Estimation of mean and standard deviation of PTZ density for start convulsion step during to jump in each group

Variable	Number	Mean	Standard deviation
Control group	12	70.59	2.91
50 ppm group	12	69.47	1.66
100 ppm group	12	55.78	2.15

In table 5, mean and standard deviation of PTZ density for start convulsion step during to jump in each group has been estimated. In this regard, as the table shows mean of Ptz density in control group is 70.59±

2.91. PTZ density of rats group that are exposure to lead acetate (50 ppm) is 69.47 ±1.66 and the PTZ density of rats group exposure to lead acetate (100ppm) is 55.78 ± 2.83.

Table 6: Estimation of mean and standard deviation of PTZ density for start convulsion step in hind limbs of each group

Variable	Number	Mean	Standard deviation
Control group	12	76.43	2.42
50 ppm group	12	75.53	2.83
100 ppm group	12	65.24	2.60

In table 6, mean and standard deviation of PTZ density for start convulsion step in hind limbs of each group has been estimated. In this regard, as the table shows mean of Ptz density in control group is 46.43 ± 2.42. PTZ density of rats group that are exposure to lead acetate (50 ppm) is 75.53 ± 2.83 and the PTZ density of rats group exposure to lead acetate (100ppm) is 65.24 ± 2.60.
Second section: inferential statistics.

Table 7: Examination of blood lead level in animal exposure to lead in comparison with control groups

Variable	Mean	Standard deviation	F - statistics	p-value
Control group	0.019	0.001	546.166	0.000
50 ppm group	6.35	0.918		
100 ppm group	10.58	1.009		

Table 8: Mean of blood level of groups

Groups			Mean difference	p- value
Control group	50	ppm group	-6.339	0.000
	100	ppm group	-10.560	0.000
50 ppm group	100	ppm group	-4.221	0.000

Table 9: Examination of convulsion in animals that are exposure to lead in comparison with control group

		Mean	Standard deviation	f- statistics	p- value
Front limbs	Control group	51.77	2.05	34.014	0.000
	50 ppm group	49.19	1.15		
	100 ppm group	46.43	1.42		
to jump	Control group	70.59	2.91	154.059	0.000
	50 ppm group	69.47	1.66		
	100ppm group	55.78	2.15		
Hind limbs	Control group	76.43	2.42	67.287	0.000
	50 ppm group	75.53	2.83		
	100 ppm group	65.24	2.60		

In above table, means of blood lead level changes in animals that are exposure to lead were compared toward control group. In this regard, based on the error level less than 0.01 (p-value<0.01) it can be concluded that there is a significant differences between group based on the blood lead level changes

(confidence level= 99%). Also, in order to compare the groups in term of blood lead level, Tukey test has been used as follows:

In above table, mean of blood level of groups was compared separately. According to the mean differences of groups and also rate of error level that is less than 0.01 (p-value< 0.01) it is concluded that there is a significant differences between groups.

In above table, the mean of seizure threshold dose in 3 situations were compared among the animals that are exposure to lead in comparison with control group. According to the mean differences of groups and also rate of error level that is less than 0.01 (p-value< 0.01). It is concluded that there is a significant differences between groups (confidence level= 99%). In addition, in order to compare the groups in term of seizure threshold dose in 3 situations, Turkey test has been used as follows:

Table 10

	Groups		**Mean differences**	**p- value**
Front limbs	Control group	50 ppm group	2.58	0.001
		100 ppm group	5.34	0.000
	50 ppm group	100 ppm group	2.76	0.000
to jump	Control group	50 ppm group	1.11	0.470
		100 ppm group	14.80	0.000
	50 ppm group	100 ppm group	13.68	0.000
Hind limbs	Control group	50 ppm group	0.891	0.686
		100 ppm group	11.19	0.000
	50 ppm group	100 ppm group	10.29	0.000

In above table, mean of seizure threshold dose changes in 3 situations were compared separately. According to the mean differences of groups and also rate of error level that is less than 0.01 (p-value< 0.01) it is concluded that there is a significant differences between 3groups in seizure threshold of front limb. Also there are significant differences in seizure threshold during to jump between control group and group that were exposure to lead with 100ppm lead density and seizure threshold between confrontation group with 50 ppm lead density and confrontation group with 100ppm 1ead density. It is true in hind limbs of seizure threshold. So, probability of seizure in population under confrontation with lead is increased when seizure threshold is decreased. According to the Pearson-statics value (0.862) and error level less than 0.01 it can be concluded that relationship between mentioned variables is significant. So, increase of blood lead level about 0.86 affect decrease or increase of nervous impulses.

Conclusion

Heavy metals in developing countries like our country have many applications in industries. One of these metals is lead that affects air pollution. So, it can apply its effects on various body organs and systems. Undesirable effects of this metal is more in children that adults. Lead makes negative effects in children such as anemia and mental retardation. The obtained result of the study shows that lead affects nervous system performance. Also, this study shows lead acetate causes some deficiencies in coordination of movement and movement activities. In this study, blood level of rat's group was compared with each others. The results show there is a significant difference between rats' group in blood lead level. Also, comparison between mean of seizure threshold dose in 3 conditions among the animals that are exposure to lead and control group shows that there is a significant differences between them. In addition, there is a significant difference between three groups in seizure threshold dose of front limbs. However, this situation is true in seizure threshold dose of hind limbs. Overall, it can be said that there is a direct relationship about 86.4% between increase of blood lead level and nervous impulses. A group of researchers examined the negative effects of lead in Wistas rat. According to their findings, the signs such as hyperactive, learning disorders, Alzheimer and decrease of activity were observed (Moreira *et al.*, 2001). Other group of researchers examined a similar test and their findings showed an increase of

anxiety symptoms in rats' newborn that used combination of lead and cadmium in drinking water (Leret *et al.*, 2003).

In addition, affects of lead ion on brain in initial steps of nervous evolution are obvious. When rat uses lead, it selectively is gathered in hippocampus. Collins *et al.,* (1982) showed that there is a significant amount of lead in brain tissue during 2 or 4 weeks after the end of lead exposure (Collins *et al.,* 1982). Also, the results showed that septum cholinergic has more vulnerability to negative effects of lead and it is assumed that disorder in appetence of Muscatinic receptors because of lead exposure is an important factor to decrease learning (Bielarczyk *et al.,* 1994).

Limitations and Suggestions

The present study in spite of simplicity and repletion in initial laboratory condition is important since it is new.

Lake of serious studies in environmental pollutants on behavior of animals' samples and human samples made negligence of health official related to this subject. Study of lead is important since it affects basic organs such as heart, liver and kidney, and probability effect of toxic in behavior of human can deranges human's life. Since, existing lead in the air rapidly settles near the production place, possibility of lead's entrance is increased by use of drinking water that was produced by polluted water arising from raining in big cities like Tehran. According to the lead removal from petrol, it seems that the main important way for entrance of lead to body is the digestive system since gradual entrance of existing lead in polluted soils arising from sediment of lead particle in recent years increased density of lead to groundwater and surface water. Also, there is an industrial development of country in recent years. Oil refinery, automotive industries and some of big factories are in Tehran. So, heavy metals such as lead penetrate into water sources and then it enters to drinking water of citizens. It is necessary to establish industrial wastewater treatment to remove the pollutants. In addition, it is requisite to refine river water, surface water and aquifer.

Other way of lead entrance into body is food industry. Lead may enter to water used in food products process or it may enter to final products. Since the water used in agriculture is from groundwater, purification of water is necessary in production process and the industries should use pure water source in production process.

Since source of many food products has plant origin, so there are some limitation for farms to use industrial wastewater with heavy metal. Output of industrial wastewater treatment should be filtered periodically. If density of pollutants exceeds standard limit, permission of their consumption is stopped in agriculture since the plants can attract and store heavy metals. Consumption of plant root directly or in shape of processed food in industrial sections causes the increase of these pollutants in body.

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