Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 09, pp.20936-20944, September, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BEHAVIORAL AND NEUROLOGICAL EFFECTS OF OMEGA-3 FATTY ACID AGAINST LEAD ACETATE EXPOSURE IN MALE WISTAR RATS: AN EXPERIMENTAL STUDY

[1]Pramod Kumar Singh, [3]Akash Rawat, [1]Rakesh Kumar Dixit, [2]Pradeep Kumar and *[1]Rajendra Nath

[1]Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, U.P. India-226003
[2]Department of Physiology, King George's Medical University, Lucknow, U.P., India-226003
[3]Department of Pharmacology, Hygia Institute of Pharmaceutical education and Research, Lucknow, U.P., India-226020

ARTICLE INFO

Article History:
Received 25th June, 2015
Received in revised form
23rd July, 2015
Accepted 09th August, 2015
Published online 30th September, 2015

Key words:
Lead acetate,
Omega-3 fatty acid,
Neurotransmitters,
Neurobehavioral,
Wistar rats.

ABSTRACT

Omega-3 fatty acids are potent antioxidants and exhibit a biological activities including neuroprotective, anti-oxidant, anti-apoptotic and anti-inflammatory properties, and have been suggested to be useful in treatment of several diseases. The present study has been undertaken to investigate the protective effect of omega-3 fatty acid (750mg/kg bwt.) against lead acetate (7.5mg/kg bwt.) induced impairment in neurotransmitters and neurobehavioral in wistar rats. The levels of dopamine (DA), nor-epinephrine (NE) and serotonin (5-HT) were evaluated in brain regions (cerebral cortex and cerebellum) of adult male wistar rats. The result showed that the administration of acute dose of lead acetate (7.5 mg/kg bwt.) induced a significant ($P<0.05$) decrease levels of DA, NE, and 5-HT in the brain region. Treatment of rats with omega-3 fatty acid produced an improvement in most of the studied parameters as well as the neurobehavioral features. In conclusion our data showed that dietary omega-3 fatty acid provide protection on lead-induced behavioral and neurological effects.

Citation: Pramod Kumar Singh, Akash Rawat, Rakesh Kumar Dixit, Pradeep Kumar and Rajendra Nath, 2015. "Behavioral and Neurological effects of omega-3 fatty acid against lead acetate exposure in male wistar rats: An experimental study", *International Journal of Current Research*, 7, (9), 20936-20944.

INTRODUCTION

Omega-3 fatty acids (Omega-3FAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential poly unsaturated fatty acids (PUFAs) that are found in abundance in fish, as well as in some herbs, nuts and plants. Long-chain omega-3 fatty acids can be synthesized from pre-cursors, such as α-linolenic acid, but the rate of this conversion is very low in humans (Das UN, 2006). PUFAs are important building blocks for neuronal cell membranes, and they have potential roles in brain development, neuro-transmission and modulation of ion channels, as well as possessing neuroprotective effects in a number of neurodegenerative conditions including Alzheimer disease (Dyall and Michael-Titus, 2008; Grossfield *et al.,* 2006; Stillwell *et al.,* 2005). Furthermore, recent evidence suggests that the beneficial effects of omega-3 PUFA on reducing the risk of dementia and Alzheimer disease (AD) may be reduced by the presence of the apolipoprotein E ε4 (*APOE* ε4) allele (Huang *et al.,* 2005; Plourde *et al.,* 2009; Whalley *et al.,* 2008). Omega-3 PUFA have been shown to regulate signal transduction pathways and also influence brain function by affecting production and function of neurotransmitters, inhibition of phospholipase A2 and inhibition of protein kinase-C (Mirnikjoo *et al.,* 2001; du Bois *et al.,* 2006; Bennett and Horrobin, 2000; Seung *et al.,* 2001) Although specific mechanisms of action through which omega-3 PUFAs act remain unknown, but some pathways have been suggested and studied on anti-inflammatory, anti-oxidant and anti-apoptotic properties in vitro as well as in vivo (Bazan NG, 2005; Marcheselli *et al.,* 2003; Calon *et al.,* 2005; Hashimoto *et al.,* 2002).

The brain is particularly susceptible to oxidative stress, because it has a high content of easily peroxidizable long-chain PUFAs such as DHA and AA, and mitochondrial consumption of a large quantity of glucose to fuel the brain's normal energy requirements results in relatively high production of free radicals (Floyd and Hensley, 2002).

Corresponding author: Rajendra Nath,
Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, U.P., India-226003.

A variety of plant extracts and pharmacological agents have been used to investigate the beneficial effects on health against lead neurotoxicity, and their relative safeness and accumulated evidence of physiological properties in animals and human (Shenoy *et al.,* 2007; Abeer M. Waggas 2012; Bishnoi *et al.,* 2008). Lead (Pb) is a highly neurotoxic agent that induces a wide range of behavioral, biochemical and physiological dysfunctions in humans and experimental animals (Pokras and Kneeland, 2009). Both occupational and environmental exposures remain a serious public health problem in many developing and industrialized countries (Yucebilgic *et al.,* 2003). The developing brain is highly vulnerable and more susceptible to neurotoxic insults than is the adult brain (Vancassel *et al.,* 2001). Lead-exposure occurs through the respiratory and gastrointestinal systems and which is absorbed in soft tissues and bone (Mudipalli, A. 2007).

Lead enters the brain and selectively deposited in the hippocampus and cortex, as well as in non-neuronal elements that are important in the maintenance of the blood brain barrier function. Studies have reported that exposure to low levels of lead has been associated with behavioral abnormalities, learning impairment, decreased hearing, neuromuscular weakness, and impaired cognitive functions in humans and in experimental animals (Verina *et al.,* 2007). A high level of Pb causes damage to almost all organs and most importantly to the central nervous system, liver, kidneys, and blood component, culminating in death, all of them related to the dose and time of lead exposure (Bellinger, D.C., 2008; Abdel Moneim *et al.,* 2011; Canfield *et al.,* 2003). Lead exposure during development impairs neuronal and astroglial changes (Stoltenburg-Didinger *et al.,* 1996; Struzynska *et al.,* 2001), which leads to a reduction of brain ability to use serotonin and dopamine, neurotransmitters directly associated with a cognitive disorders and aggressiveness in mice (Liliana *et al.,* 2010).

Studies have also reported a correlation between lead and various aspects of human behavior such as violent and criminal behavior, homicide, delinquent, antisocial behavior and aggression (Pokras and Kneeland, 2009). Experimental evidence suggests that cellular damage mediated by free radicals can be involved in the pathology associated with Pb intoxication (Flora *et al.,* 2007). In fact, the cerebral damage induced by lead occurs preferentially in the cerebral cortex, cerebellum and hippocampus (Devi *et al.,* 2005). In brain, CNS is the main target for lead toxicity; symptoms of lead poisoning include dullness, forgetfulness, irritability, poor attention span, headache, fatigue, impotence, dizziness, and depression. Lead encephalopathy, a progressive and potentially fatal degeneration of the brain, is the most severe neurological effect of lead poisoning (ATSDR, 1999; Yun *et al.,* 2011). Therefore our interest to study the protective effects of omega-3 fatty acid on lead induced neurotoxicity through the measurement of neurotransmitters: dopamine, norepinepherine, serotonin and neurobehavioral activities in control, omega-3-protected and lead intoxicated rats.

MATERIALS AND METHODS

Animals and treatment

Male rats (weight 180±20 g) of wistar strain (n=24) were purchased from Indian Institute of Toxicology Research (IITR), Lucknow, (UP) and acclimatized for 1 week prior to experimental use. The animals were separately housed in polypropylene cages at room temperature of 22±2^{0}C, relative humidity of 50±10 % and 12h light dark cycles. They have free access to pellet diet (Dayal Industries, Barabanki, UP, India) and water ad libitum. The experimental protocol was approved by an Institutional Animal Ethics Committee (wide letter no-34/IAH/Pharma-12) of King George's Medical University (KGMU), Lucknow and all experiments were carried out in accordance with the guidelines by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India. Rats were randomly divided into four groups for simultaneous treatment with six animals in each group as per following details:

Group I treated with 2% tween-20 dissolved in distilled water per oral for the duration of the treatment to serve as controls for 14 days.

Group II treated with lead acetate (7.5 mg/kg b.wt./oral) dissolved in distilled water for 14 days.

Group III treated with omega-3 fatty acid (750 mg/kg b.wt. /oral) dissolved in 2% tween-20 for 14 days.

Group IV treated with lead and omega-3 fatty acid in combination identically as in groups II and III for 14 days

Selection of Dose: The dose for lead acetate was selected on the basis of previous studies (Dabrowska-Bouta *et al.,* 1996) and for omega-3 fatty acid based on studies carried out by Abdel Moneim *et al.,* 2011.

At the end of 14 days, rats were fasted for 24 hrs before being anaesthetized by injection (i.p.) of sodium pentothal solution. Neurobehavioral studies were carried out as per plan after the last dose of treatment. A set of five rats randomly selected from each treatment group was used to assess spontaneous locomotor activity, grip strength, rota- rod performance. The same set of rats was used to measure elevated plus maze and morris water maze test, after 1h interval. There after rats were sacrificed by decapitation immediately and brains were taken out quickly, washed in ice cold saline and dissected in to cerebral cortex and cerebellum following the standard procedure (Glowinski and Iversen, 1966). Both cerebral cortex and cerebellum were processed for the assay of biogenic amines: dopamine, nor-epinephrine and serotonin.

Neurobehavioral studies

Morris water maze test

The ability of omega-3 fatty acid to improve memory and learning on lead induced neuro- toxicity in rats were tested by Morris water maze according to the method of Varvel and Lichtman, 2002. The automated tracking system recorded the path length, the escape latency, and swimming speed in the control and treated groups and data were analyzed by the computer as the index of learning and memory.

Elevated plus-maze test

The elevated plus-maze test in rats were assessed according to the method of Broad Hurst (1960).The time spent in the open arms was recorded. Between each trial, the maze was carefully clean with a 10% ethanol solution. The experiment was done with both control and treated rats.

Spontaneous Locomotor activity: Acto- photometer investigation

Spontaneous locomotor activities in rats were investigated by Acto-photometer cage equipment. The movement of animals/min on the grills of the cage across was recorded following the standard procedure as mentioned by Ali *et al.,* 1990. Effect of lead as well as lead plus omega-3 fatty acid on total distance travelled, was studied in the control and treated rats.

Motor Coordination: Rota-rod performance

The performance index of rats on rota-rod equipment was assessed by standard procedure described by Rogers *et. al.,* 1997. Effects of lead as well as lead plus omega-3 fatty acid on motor coordination was studied in rats using Rotomex-rota-rod equipment(Columbus Instruments, USA) and the time of fall from the rotating rod was monitored.

Grip strength

Grip strength in rats was assessed by the standard procedure described by Terry *et al.,* 2003. A computerized grip strength meter (TSE, Germany) was used to measure the forelimb grip strength in the control and treated rats.

Neurochemical studies

Assay of biogenic amines in brain regions

Dopamine (DA), Norepinephrine (NE) and Serotonin (5-HT) were extracted and estimated in brain regions (cerebral cortex and cerebellum) which were carried out by reversed phase high performance liquid chromatography (HPLC) with electrochemical detector according to the method of Kim *et al.,* 1987 with minor modifications.

Data were recorded and analyzed with the help of Empower 2 software (Waters, Melford, USA) and results were expressed as ng/g tissue weight.

Statistical analysis

The data have been expressed as the mean±S.E. The results have been analyzed for statistical significance between control and treatment groups by using one way analysis of variance (ANOVA) involving Newman-Keuls test for post-hoc comparisons. The level of significance has been considered at $P<0.05$

RESULTS

Neurobehavioral studies

Effect on learning and memory deficits in Morris water maze test in rats

Exposure to lead in rats caused a significant decrease in path length (82%), escape latency (2 fold) as compared to rats in the control group and no significant changes were observed in sweeming speed between the groups (Table 1). Simultaneous treatment with lead and omega-3 fatty acid in rats increased the path length (34%) and escape latency (35%) as compared to rats treated with lead alone. No significant change on any of these parameters was observed in rats treated with omega-3 fatty acid as compared to controls (Table 1).

Effect on exploratory behavior and anxiety in elevated plus maze test in rats

A significantly less time was spent in open arms (43%) of lead exposed group as compared to control group (Figure 1) Simultaneous treatment with lead and omega-3 fatty acid in rats have shown that more time was spent in the open arms (37%) as compared to rats treated with lead alone. No significant change was observed in rats treated with omega-3 fatty acid as compared to controls (Figure 1).

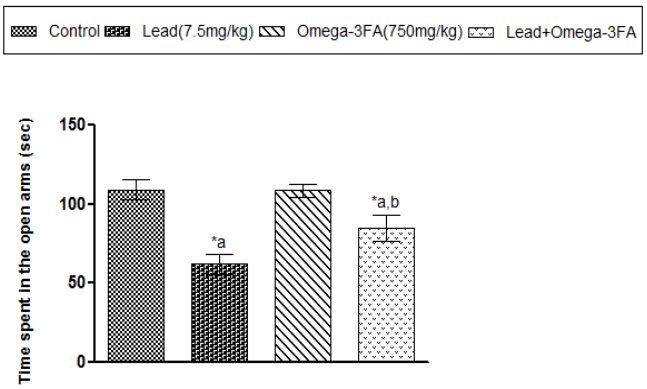

Figure 1. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the exploratory behavior and anxiety of elevated plus maze test in rats for 14 days. Values are mean ± S.E. (n=5). *a ($p<0.05$)-compared to control group, *b ($p<0.05$)-compared to lead treated group

Effect on spontaneous locomotor activity

Exposure to lead in rats caused a decrease in total distance travelled (32%) per min as compared to rats in the control group (Figure 2). Simultaneous treatment with lead and omega-3 fatty acid in rats increased the total distance travelled (23%) as compared to rats treated with lead alone. Although distance travelled increased in the co-treatment group compared with lead treatment alone, these parameters remained decreased as compared to controls. No significant change on any of these parameters was observed in rats treated with omega-3 fatty acid as compared to controls (Figure 2).

Table 1. Effect on learning and memory deficits of Morris water maze test in rats following exposure to Lead, Omega-3 Fatty acid and their co-treatment for 14 days

	Control	Lead (7.5mg/kg)	Omega-3 FA (750mg/kg)	Lead + Omega-3FA
Swimming Speed (cm)	18.3±0.73	17.5±0.66	18.4±0.71	18.1±0.5
Path length (cm)	320±15.81	582±22.67 a,*	309±13.91	386±17.78 a, b,*
Escape latency (sec.)	17.7±0.93	34.6 ±1.07a,*	17.1±1.55	22.6±1.20 b,*

All values are mean ± S.E. (n=5). a,* (P<0.05)-compared to control group, b* (p<0.05)-compared to lead treated group

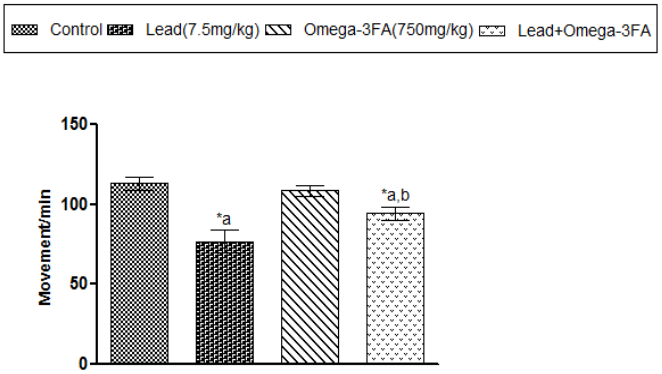

Figure 2. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the Spontaneous Locomotor activity in rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Effect on rota-rod performance

A significant impairment (50%) in motor coordination was observed in rats treated with lead since these rats fell quickly from the rotating rod on rota-rod performance test compared to controls (Figure 3). Treatment with omega-3 fatty acid in rats had no significant effect on the time of fall from the rotating rod as compared to controls. It was interesting to note that the rats simultaneously treated with lead and omega-3 fatty acid stayed on the rotating rod for a longer period of time (61%) as compared to those treated with lead alone. However, these rats fell earlier from the rotating rod as compared to controls (Figure 3).

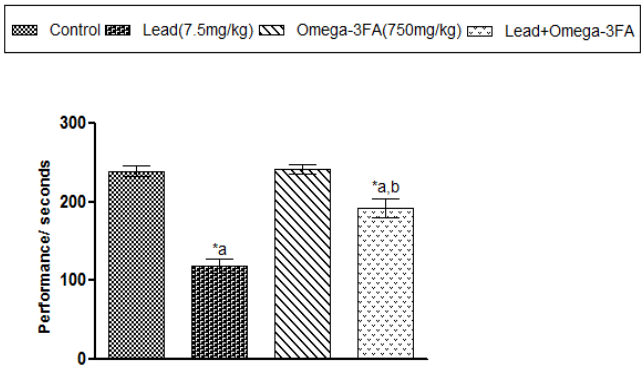

Figure 3. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the Rota-rod perfor mance in rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Effect on grip strength

The grip strength was found to be significantly decreased in rats treated with lead (34%) compared to controls (Figure 4). An improvement in grip strength (30%) was observed in rats simultaneously treated with lead and omega-3 fatty acid in comparison to those treated with lead alone. No significant change on the grip strength was observed in rats treated with omega-3 fatty acid alone compared with control rats (Figure 4).

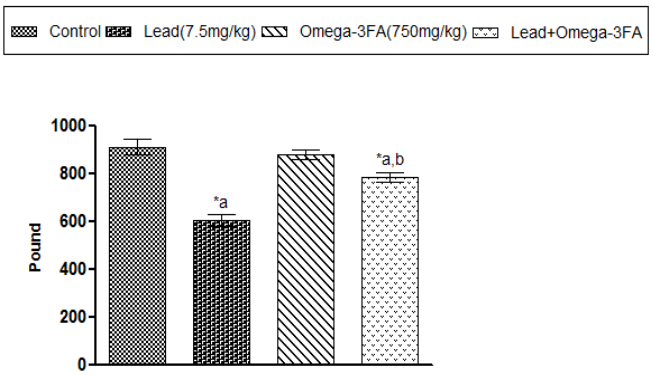

Figure 4. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the fore limb Grip strength in rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Neurochemical studies

Effect on dopamine (DA) level

The lead treated rats exhibited a significant decrease level of DA in cerebral cortex (18%) and in cerebellum (23%) as compared to control (Figure 5). Simultaneous co-treatment with lead and omega-3 fatty acid in rats caused an increase level of DA in cerebral cortex (14%) and in cerebellum (10%) as compared to lead alone. A significant increase level of DA in cerebral cortex (23%) and no significant change in cerebellum in brain regions of rats treated with omega-3 fatty acid alone as compared to controls (Figure 5).

Effect on norepinepherine (NE) level

A significant decrease NE level in cerebral cortex (31%) and in cerebellum (19%) as compared to control (Figure 6).

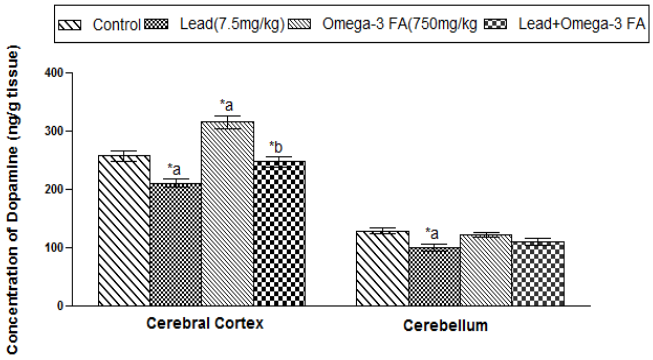

Figure 5. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the level of Dopamine in brain regions of rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Interestingly, co-treatment with lead and omega-3 fatty acid in rats caused an increase NE level in cerebral cortex (23%) and in cerebellum (17%) as compared to lead alone. A significant increase NE level in cerebral cortex (26%) and in cerebellum (19%) of rats treated with omega-3 fatty acid alone was exhibited as compared to controls (Figure 6).

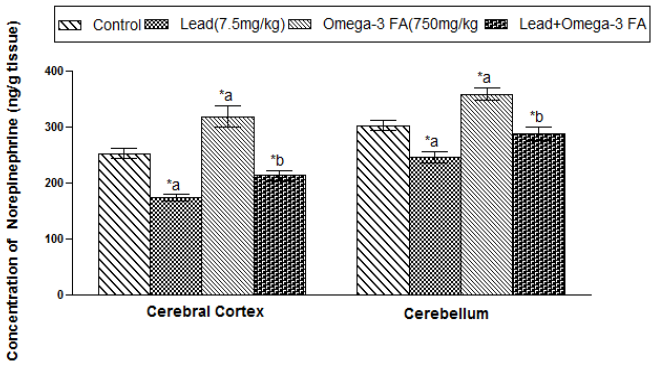

Figure 6. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the level of Norepinephrine in brain regions of rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Effect on Serotonin (5-HT) level

Exposure to lead in rats caused a significant decrease level of 5-HT in cerebral cortex (9 %) and in cerebellum (12%) as compared to control (Figure 7). Simultaneous treatment with lead and omega-3 fatty acid in rats caused an increase level of 5-HT in cerebellum (26%) and no significant change in cerebral cortex as compared to lead alone. A significant increase level of 5-HT in cerebellum (41%) and no significant change in cerebral cortex in brain regions of rats treated with omega-3 fatty acid alone was exhibited as compared to controls (Figure 7).

DISCUSSION

Lead is widespread non essential toxic heavy metal known to be toxic to mammals. The lead poisoning is most commonly seen with both environmental contaminants and potential neurotoxicological hazards (Brender *et al.,* 2006; Fowler *et al.,* 2004; Jadhav *et al.,* 2007).

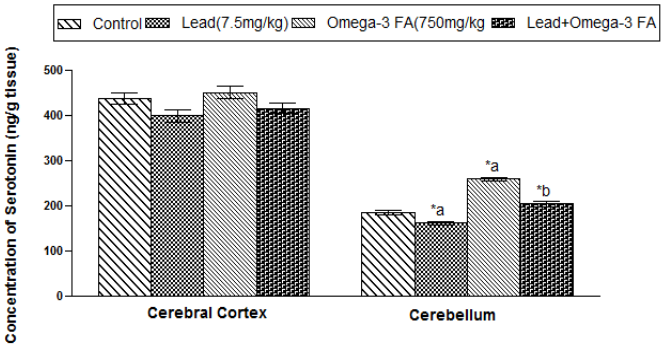

Figure 7. Effect of Lead, Omega-3 Fatty acid and their co-treatment on the level of Serotonin in brain regions of rats for 14 days. Values are mean ± S.E. (n=5). *a (p<0.05)-compared to control group, *b (p<0.05)-compared to lead treated group

Toxic effect of lead absorption occurs primarily in the brain cells of cerebellum, cortex, and hippocampus. This is accountable for the enhanced central nervous system (CNS) damage in vitro and vivo studies (Gu *et al.,* 2009). After entering blood, lead is quickly mobilized by erythrocytes and distributed in all organs. Ultimately, it accumulates in the brain, liver; kidneys, heart and only 3% remain in the blood plasma. In the absence of excretion, it is deposited at a rate of 90% in the bones in the insoluble form. Absorption may vary depending on dietary factors and the chemical form of the lead. Adult humans absorb 10–15% of ingested lead; however, children absorb up to 50% of ingested lead. Absorption is also increased in children suffering from iron or calcium deficiencies (Barton *et al.,* 1978). It is known that Pb induces growth retardation and reduced food consumption through the contact of lead with appetite-depressant receptors in the gastrointestinal tract (Hammond, 1989; Sharifi, 2002).

Lead induces oxidative damage through interference with glutathione (GSH) utilization and thus it may be a possible contributor to the pathogenesis of Pb poisoning (Lawton and Donaldson, 1991). It was stated that Pb^{2+}/Ca^{2+} interactions may play an important role in the toxicity of Pb in a neurotransmission process and also that inhibitory effects of Pb on voltage-sensitive calcium channels, the inhibition of Na^{+}–K^{+}–ATPase by Pb, and the interaction of Pb with proteins may represent mechanisms underlying lead toxicity (Dąbrowska-Bouta *et al.,* 1996). Pb has different effects on biogenic amines levels in brain regions depending on the dose. In the present study, lead exposure produced decreased levels of norepinephrine, dopamine, and serotonin as compred to controls. Simultaneous co-treatment with lead plus omega-3 fatty acid was shown to increase the level of NE, DA and 5-HT as compared to lead treated rats. These data are in accordance with previous studies (Sidhu and Nehru, 2003; Abeer MW, 2012)

Omega-3 fatty acids are essential component of CNS membrane phospholipid-acyl chains and are critical to the dynamic structure of neuronal membranes, where omega-3 fatty acids may influence brain function by affecting production and function of neurotransmitters such as serotonin and dopamine, inhibition of phospholipase A2, and inhibition of protein kinase C (Bourre *et al.,* 1991; du Bois *et al.,* 2006;

Logan AC, 2003). In addition, low levels of the omega-3 fatty acids may produce low levels of 5-hydroxyindolacetic acid present in cerebrospinal fluid, which is the major metabolite of serotonin and in which known to be protective against depression. Omega-3 fatty acid deficiency is also associated with decrements in serotonergic, dopaminergic and noradrenergic neurotransmitters release (Yehuda S, Rabinovitz and Mostofsky, 2006).

Exposure to lead in humans has been reported to cause both CNS and peripheral neuropathy (Flora *et al.,* 2006).Which results in alterations of brain structure, neurobehavioral impairments, hyperactivity and learning and cognitive deficits in children (Needleman, 1993). Both an increase and decrease in motor activity have been observed by lead exposure in rats and mice (Moreira *et al.,* 2001; Correa M, *et al.,* 2004). It was suggested that spontaneous locomotor activities are important determinant in altered response to dose of lead and duration of treatment (Correa M, *et al.,* 2004). In our study, we used acute lead acetate exposure in wistar strains of male rats for the assessment of the deleterious effects of this metal on neurobehavioral. Several of the previous neurobehavioral studies in this area have focused on the effects of lead (Bressler and Goldstein, 1991).

A decrease in locomotor activity and forelimb grip strength was observed in lead exposed groups as compared to control in the present study which is consistent with previous studies (Saritha S, *et al.,* 2014). Interestingly, co-treatment with lead plus omega-3 fatty acid increased improvement in the locomotor activity and forelimb grip strength as compared to lead exposed groups. Rota-rod is a preferred test to evaluate the performance of motor coordination in experimental studies. Exposure to lead also affected the rota-rod performance of rats as compared to those of controls; these are in agreement with previous studies (Roger's *et al.,* 1997). Simultaneous, co-treatment with lead plus omega-3 fatty acid protected the lead induced alterations in rota-rod performance.

Furthermore, experiments on elevated plus maze have shown that lead exposed rats spent shortest time in the open arm as compared to control rats. Interestingly, co-treatment with lead plus omega-3 fatty acid spent longest times in the open arms than the lead treated groups. Our data suggested that rats with acute exposure to Pb have less exploratory activity and high state of anxiety. These data are in agreement with previous studies (Kahloula, *et al.,* 2013). In our study, using a morris water maze, a significant impairment in path length and escape latency (which an indicator of learning and memory deficit) was exhibited in lead treated rats as compared to controls, which is supported by previous studies (Kamel *et al.,* 2010). Simultaneous, co-treatment with lead plus omega-3 fatty acid improved learning and memory as compared to lead treated rats. Animal studies showed that depletion of DHA from the retina and brain caused reduced visual function and learning deficits (Innis SM, 2003). Now DHA has been shown to be involved in many important physiological functions, such as promoting neuronal growth in hippocampal neurons and ameliorating the impairment of learning ability in Alzheimer's disease and traumatic brain injury (Calderon and Kim, 2004; Hashimoto *et al.,* 2005).

EPA has similar effects on learning and memory as DHA (Song and Horrobin, 2004). Dietary DHA has shown to improve neuronal development, restoring and enhancing cognitive functions (Greiner *et al.,* 1999; Gamoh *et al.,* 2001). It is critical for cellular functioning, normal brain development, and memory and cognitive processes in animals and humans (Calderon and Kim, 2004; Wainwright *et al.,* 1994).

Conclusion

Here, this study clearly indicated the ameliorating effects of omega-3 fatty acid administered in combination with lead acetate to minimize the effects of neurobehavioral changes and impairment in neurotransmitters caused by lead exposure. So our result suggested that omega-3 fatty acids may play a protective role on lead induced neurotoxicity and associated human health risk.

Acknowledgements

The authors thank Dr Abbas Ali Mahdi (Prof. & Head, Department of Biochemistry, King George's Medical University, Lucknow) for helpful suggestions and insightful comments. We also extend my thank to Late Dr. Ramesh Chander for solving the puzzle of statistics and for giving his valuable time in this study. This study was financially supported by Indian Council of Medical Research (ICMR), New Delhi.

REFERENCES

Abdel Moneim, A.E., Dkhil, M.A. and Al-Quraishy, S. 2011. The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. *J. Hazard Mater*, 194, 250–5.

Abeer M. Waggas, 2012. Grape seed extract (vitisvinifera) alleviate neurotoxicity and hepatotoxicity induced by lead acetate in male albino rats. *Journal of Behavioral and Brain Science*, 2, 176-84.

Agency for Toxic Substances and Disease Registry (ATSDR), 1999. Toxicological Profile for Lead Atlanta," GA: US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.

Ali, M.M., Mathur, N. and Chandra, S.V. 1990. Effect of chronic cadmium exposure on locomotor activity of rats. *Indian J. Exp. Biol.,* 28, 653–6.

Barton, J.C., Conrad, M.E., Harrison, L. and Nuby, S. 1978. Effects of calcium on the absorption and retention of lead. *J Lab. Clin. Med*., 91(3), 366–76.

Bazan, N.G. 2005. Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. *Brain Pathol*., 15, 159-66.

Bellinger, D.C. 2008. Very Low Lead Exposure and Children's Neurodevelopment. *Current Opinion in Pediat- rics*, 20 (2), 172-7.

Bennett, C.N. and Horrobin, D.F. 2000. Gene targets related to phospholipid and fatty acid metabolism in schizophrenia and other psychiatric disorders: An update. *Prostaglandins Leukot Essent Fatty Acids*, 63(1–2), 47–59.

Bishnoi, M., Chopra, K. and Kulkarni, S.K. 2008. Protective effect of curcumin, the active principle of turmeric

(Curcuma longa) in haloperidol-induced orofacial dyskinesia and associated behavioral, biochemical and neurochemical changes in rat brain. *Pharmacol. Biochem. Behav*., 88, 511–22.

Bourre, J.M., Dumont, O., Piciotti, M., *et al.,* 1991. Essentiality of omega 3 fatty acids for brain structure and function. *World Rev. Nutr. Diet*, 66, 103-17.

Brender, J.D., Suarez, L., Felkner, M., Gilani, Z., Stinchcomb, D., Moody, K., *et al.,* 2006. Maternal exposure to arsenic, cadmium, lead, and mercury and neural tube defects in offspring. *Environ. Res*, 101, 132–9.

Bressler, J.P. and Goldstein, G.W. 1991. Mechanisms of lead neurotoxicity. *Biochemical Pharmacology*, 41, 479–84.

Broad Hurst, P.L. 1960. The place of animal psychology in the development of psychosomatic research. *Fortschr. Psychosom., Med*, 1, 63-9.

Calderon, F. and Kim, H.Y. 2004. Docosahexaenoic acid promotes neurite growth in hippocampal neurons. *J. Neurochem*., 90, 979-88.

Calon, F., Lim, G.P., Morihara, T., Yang, F., Ubeda, O., Salem, N., *et al.,* 2005. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. *Eur. J. Neurosci*., 22, 617-26.

Canfield, R., Henderson, C., Cory-Slechta, D., Cox, C., Jusko, T. and Lanphear, B. 2003. Intellectual Impairment in Children with Blood lead concentration below 10 microgram per deciliter. *N. Engl. J. Med*, 348 (16), 1517-26.

Dabrowska-Bouta, B., Struzynska, L. and Rafalowska, U. 1996. Effect of acute and chronic lead exposure on the level of sulfhydryl groups in rat brain. *Acta Neurobiol. Exp. Wars*, 56, 233–6.

Das, U.N. 2006. Essential fatty acids—a review. *Curr. Pharm. Biotechnol*., 7, 467–82.

Devi, C.B., Reddy, G.H., Prasanthi, R.P., Chetty, C.S. and Reddy, G.R., 2005. Developmental lead exposure alters mitochondrial monoamine oxidase and synaptosomal catecholamine levels in rat brain. *Int. J. Dev. Neurosci*., 23(4), 375–81.

Du Bois, T.M., Deng, C., Bell, W. and Huang, X.F. 2006. Fatty acids differentially affect serotonin receptor and transporter binding in the rat brain. *Neuroscience*, 139(4), 1397–403.

Dyall, S.C. and Michael-Titus, A.T. 2008. Neurological benefits of omega-3 fatty acids. *Neuromolecula.r Medicine*, 10(10), 219–35.

Flora, S.J., Saxena, G. and Mehta, A. 2007. Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: Role of reactive oxygen species and intracellular Ca $^{(2+)}$. *J. Pharmacol. Exp. Ther.,* 322 (1), 108–16.

Flora, S.J.S, Flora, G. and Saxena, G. 2006. Environmental occurrence, health effects and management of lead poisoning, in Lead: Chemistry, Analytical Aspects, Environmental Impacts and Health Effects (Cascas SB and Sordo J eds), 158–228.

Floyd, R.A. and Hensley, K. 2002. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging*, 23, 795–807.

Fowler, B.A., Whittaker, M.H., Lipsky, M., Wang, G. and Chen, X.Q. 2004. Oxidative stress induced by lead, cadmium and arsenic mixtures: 30-day, 90-day, and 180-day drinking water studies in rats: an overview. *Biometals.,* 17, 567–8.

Gamoh, S., Hashimoto, M., Hossain, S. and Masumura, S. 2001. Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats. *Clin. Exp. Pharmacol. Physiol*., 28, 266–70.

Glowinski, J. and Iversen, L.L. 1966. Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. *J. Neurochem*., 13(8), 655–9.

Greiner, R.S., Moriguchi, T., Hutton, A., Slotnick, B.M. and Salem, N. Jr, 1999. Rats with low levels of brain docosahexaenoic acid show impaired performance in olfactory-based and spatial learning tasks. *Lipids*, 34, S239–43.

Grossfield, A., *et al., 2006.* A role for direct interactions in the modulation of rhodopsin by omega-3 polyunsaturated lipids. *Proc. Natl. Acad. Sci*., 103, 4888–93.

Gu, C., Chen, S., Xu, X., Zheng, L., Li, Y., Wu, K., Liu, J., Qi, Z., Han, D., Chen, G., *et al.,* 2009. Lead and cadmium synergistically enhance the expression of divalent metal transporter 1 protein in central nervous system of developing rats. *Neurochem. Res*., 34, 1150.

Hammond, P.B., Chernausek, S.D., Succop, P.A., Shukla, R. and Bornschein, R.L. 1989. Mechanisms by which lead depresses linear and ponderal growth in weanling rats. *Toxicol. Appl. Pharmacol*., 99, 474-86.

Hashimoto, M., Tanabe, Y., Fujii, Y., Kikuta, T., Shibata, H. and Shido, O. 2005. Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid betainfused rats. *J. Nutr*., 135, 549-55.

Hashimoto, S., Hossain, T., Shimad, K., Sugioka, H., Yamasaki, Y., Fujii, Y., Ishibashi, Y., Oka, J. and Shido, O. 2002. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. *J. Neurochem*., 81, 1084-91.

Huang, T.L., Zandi, P.P., Tucker, *et al.,* 2005. Benefits of fatty fish on dementia risk are stronger for those without APOE epsilon4. *Neurology*, 65(9), 1409–14.

Innis, S.M. 2003. Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. *J. Pediatr*., 143(4), 1-8.

Jadhav, S.H., Sarkar, S.N., Patil, R.D. and Tripathi, H.C. 2007. Effects of subchronic exposure via drinking water to a mixture of eight water contaminating metals: a biochemical and histopathological study in male rats. *Arch. Environ. Contam. Toxicol*., 53, 667–77.

Kahloula, K., Slimani, M., Adli, D.H., Rachdi, S. and Boumediene, D. 2013. Neuro beneficial effects of Pimpinella anisum against lead exposure. *Int. J. Green Pharm*., 7, 18-24.

Kamel, M.M., El-lethey, H.S., Shaheed, I.B. and Kamel, G.M. 2010. Black Tea Forestalls Sodium Fluoride-Induced Neurobehavioral Toxicity in Laboratory Rats. *J. American Sci*., 6 (12),1655- 73.

Kim, C., Speisky, M.B. and Klarevla, S.N. 1987. Rapid and sensitive method for measuring norepinephrine, dopamine, 5HT and their major metabolites in rat brain by high

performance liquid chromatography. *J. Chromatogr*., 386, 25–35.

Lawton, L.J. and Donaldson, W.E. 1991. Lead-induced tissue fatty acid alterations and lipid peroxidation. *Biol. Trace Elem. Res*., 28(2), 83–97.

Liliana, N.F., Nora, M.R., Norma, S., Maria, J.G., Patricia, M.V. and Nora Riera de Martinez Villa, 2010. Behavior Alterations of Contaminated Lead Mice Exposed to Ansiogenic Stimulus. *Revis. Neuropsychol. Neurosci*., 10, 23-33.

Logan, A.C. 2003. Neurobehavioral aspects of omega-3 fatty acids: possible mechanisms and therapeutic value in major depression. *Altern. Med Rev*., 8(4), 410–25.

Marcheselli, V.L., Hong, S., Lukiw, W.J., Tian, X.H., Gronert, K., Musto, A., Hardy, M., Gimenez, J.M., Chiang, N., Serhan, C.N. and Bazan, N.G., 2003. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and proinflammatory gene expression. *J Biol Chem*., 278(44), 43807-17.

Merce Correa, Antoni, F., Roig-Navarrob and Carlos, M.G.A. 2004. Motor behavior and brain enzymatic changes after acute lead intoxication on different strains of mice. *Life Sciences*, 74, 2009–21.

Mirnikjoo, B., Brown ,S.E., Kim, F.S., Marangell, L.B., Sweatt, D.J. and Weeber, E.J. 2001. Protein kinase inhibition by omega-3 fatty acids. *Journal of Biological Chemistry*, 276(14), 10888–96.

Moreira, E.G., Vassilieff, I. and Vassilieff, V.S. 2001. Developmental lead exposure: behavioral alterations in the short and long term. *Neurotoxicology and Teratology*, 23, 489–95.

Mudipalli, A. 2007. Lead hepatotoxicity and potential health effects. *Indian J. Med. Res*., 126, 518-27.

Needleman, H.L. 1993. The current status of childhood low-level lead toxicity. *Neurotoxicology,* 14, 161–6.

Plourde, M., Vohl, M.C., Vandal, M., Couture, P., Lemieux, S. and Cunnane, S.C. 2009. Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men. *British Journal of Nutrition*, 102, 1121–4.

Pokras, M.A. and Kneeland, M.R. 2009. Understanding lead uptake and effects across species: A conservation medicine based approach. In Watson, R. T., Fuller, M., Poras, M., Hunt, W. G. (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Funds, Boise, Idaho, USA.

Rogers, D.C., Campbell, C.A., Jennifer, L., Stretton, L. and Macky, K.B. 1997. Correlation between motor impairment and infarct volume after permanent and transient middle artery occlusion in rat. *Stroke*, 28, 2060–5.

Saritha, S., Praveen, K., Sreenivasula, P.R., Tripathy, N.K. and Rajarami, R.G. 2014. Developmental arsenic and lead exposure: Behavioral and neurochemical perturbations of albino rats. *Indo American Journal of Pharmaceutical Research*, 4(03), 1707-16.

Seung, K.H.F., Weeber, E.J., Sweatt, J.D., Stoll, A.L. and Marangell, L.B. 2001. Inhibitory effects of omega-3 fatty acids on protein kinase c activity in vitro. *Mol. Psychiatry*, 6(2), 246–8.

Sharifi, A.M., Baniasadi, S., Jorjani, M., Rahimi, F. and Bakhshayesh, M. 2002. Investigation of acute lead poisoning on apoptosis in rat hippocampus in vivo. *Neurosci. Lett.,* 329: 45-8.

Shenoy, S.F., Keen, C.L., Kalgaonkar, S. and Polagruto, T.A. 2007. Effect of Grape Seed Extract Consumption on Platelet Function in Postmenopausal Women. *Thrombosis Research*, 121(3), 431-2.

Sidhu, P. and Nehru, B. 2003. Relationship between lead-induced biochemical and behavioral changes with trace element concentrations in rat brain. *Biol. Trace Elem. Res*., 92(3), 245–56.

Song, C. and Horrobin, D. 2004. Omega-3 fatty acid ethyl-eicosapentaenoate, but not soybean oil, attenuates memory impairment induced by central IL-1beta administration. *J Lipid Res*., 45, 1112-21.

Stillwell, W. *et al.,* 2005. Docosahexaenoic acid affects cell signaling by altering lipid rafts. *Reprod Nutr Dev*, 45, 559–79.

Stoltenburg-Didinger, G., Punder, I., Peters, B., Marcinkowski, M., Herbst, H., Winneke, G., *et al.,* 1996. Glial fibrillary acidic protein and RNA expression in adult rat hippocampus following low level lead exposure during development. *Histochem Cell Biol*., 105, 431–42.

Struzynska, L, Bubko, I., Walski, M. and Rafałowska, U. 2001. Astroglial reaction during the early phase of acute lead toxicity in the adult rat brain. *Toxicology*, 165, 121–31.

Terry, A.V., Stone, Jr J.D., Buccafusco, J.J., Sickles, D.W., Sood, A. and Prendergast ,M.A. 2003. Repeated exposure to subthreshhold doses of chlorpyrifos in rats: hippocampal damage, impaired axonal transport and deficits in spatial learning. *J. Pharmacol. Exp. Ther*., 305, 375–84.

Vancassel, S., Durand, G., Barthelemy, C., Lejeune, B., Martineau, J., Guilloteau, D., Andres, C. and Chalon, S. 2001. Plasma fatty acid levels in autistic children. *Prostaglandins Leukot Essent Fatty Acids*, 65 (1), 1-7.

Varvel, S.A. and Lichtman, A.H. 2002. Evaluation of CB1 receptor knockout mice in the morris water maze. *Pharmacol Exp. Ther*., 301, 915-24.

Verina, T., Rohde, C.A. and Guilarte, T.R. 2007. Environmental lead exposure during early life alters granule cell neurogenesis and morphology in the hippocampus of young adult rats. *Neuroscience*, 145(3), 1037–47.

Wainwright, P.E., Huang, Y.S., Coscina, D.V., Levesque, S. and McCutcheon, D. 1994. Brain and behavioral effects of dietary n-3 deficiency in mice: a three generational study. *Dev Psychobiol*., 27, 467–87.

Whalley, L.J., Deary, I.J., Starr, J.M., Wahle, K.W., Rance, K.A., Bourne, V.J. and Fox, H.C. 2008. N-3 Fatty acid erythrocyte membrane content, APOE ε4, and cognitive variation: an observational follow-up study in late adulthood. *American Journal of Clinical Nutrition*, 87(2), 449–54.

Wu, A., Ying, Z. and Gomez-Pinilla, F. 2004. Dietary omega-3 fatty acids nomalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. *J. Neurotrauma*., 21: 1457-67.

Yehuda, S,, Rabinovitz, S. and Mostofsky, D.I. 2006. Nutritional deficiencies in learning and cognition. *J Pediatr Gastroenterol Nutr*., 43 (3), S22–5.

Yucebilgic, G., Bilgin, R., Tamer, L. and Tukel, S. 2003. Effects of lead on Na^{+}-K^{+} ATPase and Ca^{2+} ATPase activities and lipid peroxidation in blood of workers. *Int. J. Toxicol*., 22, 95-7.

Yun, H.J., Kim, I., Kwon, S., Kang, J. and Om, A. 2011. Protective Effects of Chlorella Vulgaris against Lead-Induced Oxidative Stress in Rat Brains. *Journal of Health Science*, 57(3), 245-54.

****