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RESEARCH ARTICLE

EFFECT OF OMEGA-3 FATTY ACID ON LEAD INDUCED GENERAL AND BEHAVIORAL PROFILE IN MALE WISTAR RATS: AN EXPERIMENTAL STUDY

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ABSTRACT

Aims and objective: The present study was aimed to see the effects of omega-3 fatty acid on lead induced general and behavioral profile in wistar rats.
Materials and Methods: 32 Male wistar Rats (weight 180±20 g) were acclimatized for 1 week prior to experimental use and after that divided into four groups of 8 rats each. Group 1 served as control, treated with distilled water, p.o., daily for 90 days, Group 2 treated with lead acetate (3mg/kg body weight, p.o., daily for 90 days), Group 3 treated with omega-3 fatty acid (300mg/kg body weight, p.o., daily for 90 days) along with lead acetate (3mg/kg body weight, p.o., daily for 90 days). However, Group 4 treated with Vitamin E (100mg/ kg body weight, p.o., daily for 90 days) as standard drug along with lead acetate (3mg/kg body weight, p.o., daily for 90 days) . A set of five rats randomly selected from each treatment group was observed general and behavioral studies 24 h after the last dose of treatment. A base line body weight, food, water and lead intake was recorded after that general and behavioral studies were performed
Statistical analysis: The data was expressed as the mean±S.E. and was analyzed by one way analysis of variance (ANOVA), involving Newman-Keuls test for post-hoc comparisons. The level of significance was accepted at $p<0.05$.
Results: The results of the effect of administration of omega-3 fatty acid on lead induced general and behavioral profile in rats were seen. The body weight was significantly reduced in Pb-exposed group as compared to the other groups. In the experimental period, food intake, water intake and lead intake was significantly decreased in the Pb-exposed group as compared to the control groups and we observed that general and behavioral profile were not significant among the groups. The results obtained were compared with vitamin-E (100mg/kgb.wt.), the standard drug as antioxidant.
Conclusion: Our data suggested that omega-3 fatty acid significantly decreased the adverse effects of lead exposure on male wistar rats.

INTRODUCTION

Omega-3 fatty acids are long chain, polyunsaturated fatty acids (PUFA) of plant and marine origin. Because these essential fatty acids (EFAs) cannot be synthesized in the human body, they must be derived from dietary sources. Flaxseed, hemp, canola, and walnuts are generally rich sources of the omega-3 PUFA alpha-linolenic acid (ALA). Fish provide varying amounts of omega-3 fatty acids in the form of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). ALA can be metabolized into the longer chain EPA and DHA (Holub 2002). The results indicate that omega-3 fatty acids may be of value in the treatment of various medical conditions (Uauy 2000). The omega-3 polyunsaturated fatty acid (PUFA) is a major component of membrane phospholipids in brain, retina, and spermatozoa (Salem *et al.,* 2001; Niemoller and Bazan 2010). In the brain, has taken on a central role as a target for therapeutic intervention in Alzheimer's disease (AD) as well as other neurodegenerative disorders. DHA also keeps the membranes surrounding each synapse- the communication gap between to nerve cells in a more fluid state. This help nerve cells release chemicals in to the gap more quickly and for the detector sites (receptors) on the side of the gap. Brain cells whose membranes are rich in DHA therefore communicate more quickly with each other.

The function of omega-3 fish oil has received considerable attention in the last years because DHA and EPA are known to affect the lipid profile, vascular tone and blood coagulation

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(Thusgaard *et al.,* 2009; Marik and Varon 2009). In addition to these properties, it is apparent that they play a central role in the functioning of the brain and central nervous system. DHA is easily transferred across the placenta, presented in maternal milk and accumulated in the brain and retina during fetal and infant development. Animal studies showed that depletion of DHA from the retina and brain resulted in reduced visual function and learning deficits (Innis 2003). Epidemiological studies have shown that consumption of selected fats and antioxidants such as vitamins E and C lowers the risk of AD. In particular, growing evidence has shown that moderate fish consumption as a proxy for n-3 PUFAs is associated with a reduced risk of impaired cognitive functions (Morris *et al.,* 2005; Kalmijn *et al.,* 2004; Calon and Cole 2007). In the elderly and patients with AD, low DHA and n-3 PUFA levels have been detected in the plasma and brain showing widespread loss of neuronal synapses (Conquer *et al.,* 2000; Cunnane *et al.,* 2007).

Lead is the 5th most abundant metal in the world after iron, copper, zinc and aluminum (Wong *et al.,* 1992). Lead has many uses—for production of ammunition, bearing metals, brass and bronze, cable covering, extruded products, sheet lead, and solder, used in ceramics, type metal, ballast or weights, tubes or containers, oxides, and gasoline additives. Human exposure to lead occurs primarily through diet, air, drinking water, dust, and paint chips. Lead-based paint remains the most common high-dose source of lead exposure for preschool children. Children may be exposed to high lead levels when workers take home lead on their clothing or when they bring scrap or waste material home from work (Borschein *et al.,* 1985). Absorption may vary depending on dietary factors and the chemical form of the lead. Lead is absorbed into the body following ingestion and inhalation exposure. 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). Previous studies have proved chronic lead exposure during early postnatal development would cause cognitive and neurobehavioral dysfunction (Bellinger 2008). It has been shown that hippocampus, in which long-term potentiation (LTP) and long-term depression (LTD) underlie the neurobiological basis of learning and memory, is the major target of lead in brain (Widzowski and Cory-Slechta 1994). Epidemiologic studies have shown an association of lead exposure with brain tumours, however with conflicting results, which may be attributed to various factors including the use of crude exposure assessment techniques for lead (Cocco *et al.,* 1998; Wong and Harris 2000). Many occupational workers with lead-exposure have a significantly higher frequency of infertility, stillbirths, miscarriages and spontaneous abortion, reduced sperm counts and sperm motility, increased rates of teratospermia, and decreased libido (Levin and Goldberg 2000).

MATERIALS AND METHODS

Animals

Rats (weight 180±20 g) of adult male wistar strain (n=32) were purchased from Industrial Toxicology Research Institute (IITR), Lucknow (UP) India and acclimatized for 1 week prior to experimental use. The animals were separately housed in polypropylene cages in a room, which was maintain at a 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 Institutional Animal Ethics Committee approved the study prior to the initiation of the experiment and also approved all experimental protocols.

Treatment

Animals were divided into four groups of 8 rats each. Group 1 served as control treated with distilled water, Group 2 treated with 3mg/kg body weight of lead acetate, Group 3 treated with omega-3fatty acid (300mg/kg body weight) along with 3mg/kg body weight lead acetate for 90 days. However, Group 4 treated with Vitamin E (100mg/ kg body weight) was also given as standard drug along with 3mg/kg body weight lead acetate for 90 days.

Dose

Each solution was prepared with 2% tween-20, this aqueous suspension was directly introduced into the rat pharynx via a feeding cannula (The sharp edge of the tip of a hypodermic needle no. 16 was blunted by grinding on a stone and thereafter bent to 120^0 so that the curved needle could easily be introduced into the rat pharynx via oral cavity without the pointed tip lacerating the passage) to experimental groups and an equivalent volume of distilled water was given to control groups for 90 days.

Experimental Procedure

After 90 days rats were allowed to move for assessment of general clinical observation as per described previously (Nath *et al.,* 1988; Irwin 1968).

General clinical study

The following studies were made at least once a day at the same time each day. These studies were made outside the home cage in a standard arena and at similar times on each occasion.

(A) General condition

1. **Salivation:** Secretion of saliva was observed as dripping of saliva from mouth (+: present or -: absent).
2. **Lacrymation:** Secretion and discharge of tears observed as shedding of tears around the lower eyelid (+: present or -: absent).
3. **Skin condition:** Erection of body hairs and changes in skin color etc (+: present or -: absent).
4. **Respiration Rate:** Rate of high/,low (+: present or -: absent).
5. **Diarrhoea:** Loose and frequent excretion of faeces (+: present or -: absent).

(B) Behavioral Profile

1. **Consciousness:** Rats having full response to righting reflex, their abdomen up-right (+: present or -: absent).
2. **Seizureogenesis:** Rats were responded to any seizure (+: present or -: absent).

3. **Catalepsy**: Rats were stayed in any position in which they were placed (+: present or -: absent).
4. **Sedation**: Rats were showing alertness against sudden sound (sound reflex-using call bell) as well as pricking with needle on their pinna (pinna reflex) (+: present or -: absent).

Statistical analysis

The data were expressed as the mean±S.E. The results were analyzed for statistically significant experimental differences between control and treatment groups & were analyzed using one way analysis of variance (ANOVA) involving Newman-Keuls test for post-hoc comparisons. The level of significance was accepted at $p<0.05$.

RESULTS

Changes in Body Weight

Body weight of the rats are presented in Table 1. A significant decrease was observed for the lead exposed group (19%) in body weight as compared to the control group. Marginal significant effect on body weight was observed in rats treated with lead and omega-3 fatty acid (8%) in comparison to lead alone groups. Simultaneous treatment with lead and vitamin-E in rats caused a significant increase (14%) in body weight as compared to those treated with lead alone groups.

Table 1. Effect on body weight of rats following exposure to Lead, Lead+ Omega-3 Fatty acid and Lead + Vitamin-E for 90 days

Group	Body Weight(g)
Control	325±10.84
Lead(3mg/kg)	263±6.63**a
Lead +Omega-3 Fatty acid(300mg/kg)	285±10.37*a ,b
Lead + Vitamin-E(100mg/kg)	301±11.55*b

All values are mean ± S.E. (n=5). *a ($p<0.05$), **a ($p<0.01$)-compared to control group,*b ($p<0.05$)-compared to lead treated group.

Changes on Food, Water and Lead intake

Food, water and lead intake for 90 days in rats are presented in Table 2. A significant decrease was observed for the lead exposed group (21%) in food intake as compared to the control group and an significant effect on food intake was observed in rats treated with lead and omega-3 fatty acid (11%) in comparison to lead alone groups. Simultaneous treatment with lead and vitamin-E in rats caused a significant increase (18%) in food intake as compared to those treated with lead alone groups. The water intake of the lead-exposed group (24%) was significantly lower than the control groups and an marginal significant in water intake was observed in rats treated with lead and omega-3 fatty acid (10%) as compared to lead alone groups. Simultaneous treatment with lead and vitamin-E in rats caused a significant increase (15%) in water intake as compared to those treated with lead alone groups. But in lead intakes group there was no significant among the group.

General clinical observations

(A) General conditions: There was no salivation, lacrymation, change in skin condition, respiratory rate (high/low) & diarrhoea in rats at 10 A.M./day in the morning (Table 3).

(B) Behavioral profile: There was no any change in consciousness; seizureogenesis, catalepsy & sadation in rats/day as shown in Table 4.

DISCUSSION

Lead (Pb+2) is a one of the major heavy metal known which is toxic to mammals. Lead (Pb+2) crosses blood–brain barrier in developing animals and disrupts its main structural components by injuring the brain glial cells. Once inside the brain, Pb+2-induced damage occur primarily in the cerebral cortex, cerebellum, and hippocampus, which may result in many morphological alterations in brain (Jaya Prasanthi *et al.,* 2005). Lead poisoning is associated with physiological problems such as mental retardation, learning disabilities, low birth weight and behavioral problems (Sander *et al.,* 2009). In the present study, a clinical observation shown in the rats following exposure to lead acetate. There was no significant change in salivation, lacrymation, change in skin condition, respiratory rate, diarrhoea and in behavioral profile moreover there was no

Table 2. Effect on food, water and lead intake of rats following exposure to Lead, Lead+ Omega-3 FA and Lead + Vitamin-E for 90 days

Group	Food intake (g/day)	Water intake (ml/day)	Lead intake (mg/day)
Control	28.60±1.50	34.40±1.96	0±0.0
Lead(3mg/kg)	22.60±1.28*	26.20±1.85*	0.78±0.01ns
Lead +Omega-3 FA(300mg/kg)	25.00±1.41	28.80±1.93	0.85±0.03
Lead + Vitamin-E(100mg/kg)	26.60±1.43	30.00±1.92	0.90±0.03

All values are mean±S.E. (n=5). *$p<0.05$ (compared to control group), ns (Non-significant).

Table 3. Effect on general conditions of rats following exposure to Lead, Lead+ Omega-3 fatty acid and Lead + Vitamin-E for 90 days

Group	Salivation	Lacrymation	Skin condition	Respiration	Diarrhoea
Control	Excess Secretion of saliva-No (normal)	Excess shedding of tears –No (normal)	Erection of hair, abnormal changes in skin color etc-No (normal)	Rate of (high/slow) changes in respiration-No (normal)	Loose and frequent excretion of faeces-No (normal)
Lead acetate	NAD	NAD	NAD	NAD	NAD
Lead + omega-3 fatty acid	NAD	NAD	NAD	NAD	NAD
Lead + Vitamin-E	NAD	NAD	NAD	NAD	NAD

All values are mean ± S.E. (n=5) NAD=No abnormality detected.

Table 4. Effect on behavioral profile of rats following exposure to Lead, Lead+ Omega-3 fatty acid and Lead + Vitamin-E for 90 days

Group	Consciousness:	Seizure genesis	Catalepsy:	Sedation:
Control	Rats had full response to righting reflex as they cannot be place their abdomen up-right.	Rats did not show any tendency of seizure.	Rats stayed in their normal position at back-right. They are not kneeling their back at the left or right side	Rat shows full alertness against sudden sound (sound reflex-using call bell) as well pricking with needle on their pinna (pinna reflex)
Lead acetate	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)
Lead + Omega-3 fatty acid	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)
Lead+ Vitamin-E	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)	NAD (Animals behaved as in control)

All values are mean ± S.E. (n=5) NAD=No abnormality detected.

significant change in consciousness; seizureogenesis, catalepsy and sadation as well in rats. A decrease in body weight has been reported in rats following exposure to lead acetate at different doses and time indicates the general toxic effect of the chemical and has been associated with decreased food, water and lead intake (Benlahcen *et al.,* 2009, Seddik *et al.,* 2010 and HyeJun *et al.,* 2011). In the present study, a decrease in the body weight, food, water and lead intake in lead exposed rats as observed which is consistent with above mentioned studies. Marginal significant effect on body weight was observed in rats treated with lead and omega-3 fatty acid in comparison to lead alone groups. Simultaneous treatment with lead and vitamin-E in rats caused a significant increase in body weight as compared to those treated with lead alone groups. The body weight in these rats however remained decreased as compared to control.

The protective function of fish oil has received considerable attention in the past, because DHA and EPA are known to affect the lipid profile, vascular tone and blood coagulation (Thusgaard *et al*., 2009; Marik and Varon 2009). In addition to these properties, it is apparent that they play a central role in the functioning of the brain and central nervous system. In addition, omega-3 fatty acids participate in numerous cellular functions, including membrane fluidity, membrane enzyme activities, and eicosanoid synthesis which are essential for brain development in infants and is also required for maintaining normal brain function (Mazza *et al.,* 2007). Although the results of the present study exhibit protective effect of omega-3 fatty acid, further studies are required to understand the complete mechanism of lead toxicity.

Conclusion

The results of the present study conclude that omega-3 fatty acid significantly decreased the adverse harmful effects of lead acetate exposure on physical changes in male wistar rats. These non invasive predictive markers have shown lead induced toxicity in animals and they could be used as clinical indices in human study as well. The omega-3 fatty acid reduces the risk of Lead induced toxicity. Omega-3 fatty acid in 300mg dose were appropriate to ameliorate Pb toxicity. Consequently, the exposure to Pb should be reduced and precautions should be taken towards the sources of lead e.g. lead based paint, leaded gasoline, ceramic products, foods, water, air and soil in human beings. Moreover, further studies are required for gaining the knowledge regarding the mechanism of action of omega-3 fatty acid protection over Pb toxicity.

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