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Ameliorative effect of docosahexaenoic acid and gamma-linolenic acid on lead induced histopathological changes in olfactory bulb of adult swiss albino mice

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ABSTRACT

It has been known since ancient times that lead is virtually toxic to every organ of body including central nervous system where it may manifest as encephalopathy and hyposmia yet the exact mechanism of these clinical manifestations remains inconclusive. The present study was aimed to see the microscopic changes in the olfactory bulb of mice induced by oral administration of a lead compound in adult albino mice. A total number of 36 adult albino mice of either sex were included in the present study consisting of equal numbers in both control and experimental groups. Experimental group received 4.5% and 5% lead nitrate and lead acetate trihydrate orally and with dietary supplement for a period of 3 weeks then animals of all groups were euthanized with overdose of general anaesthesia and perfused with 10% formalin. Olfactory bulbs were dissected out and processed for paraffin embedding. Sections of 10μ thick were stained with H&E and observed under light microscope. On gross examination brains from the experimental group revealed generalized edema and petechial haemorrhages. Histopathology of the olfactory bulbs revealed edema and congestion with vacuoles of variable sizes almost throughout. Distortion of glomeruli, clumping of periglomerular cells and increasing number of pyknotic cells were also noticed. It was concluded that lead has toxic effects on the central nervous system including olfactory bulb in the form of edema, microscopic hemorrhages and neuronal loss which may explain the clinical manifestations of lead toxicity.

Key Words: Albino mice, Olfactory bulb, Lead nitrate and lead acetate trihydrate, Neurotoxicity, Edema, Haemorrhage , DHA, GLA.

INTRODUCTION

It has been known since ancient times that lead may cause poisoning in man [1]. Exposure of lead can take place either through inhalation of dust, fumes, vapors or ingestion of contaminated

foods or drinks. Because of its cumulative property it is capable of exerting toxic effects at any level of exposure. Toxic effect of lead on the body is known as Plumbism and it is now well recognized that inorganic lead produces not only clinically defined encephalopathies and neuropathies, but also various behavioral changes indicative of cerebral dysfunction. However, only within the past fifty years attention has been called to its effects in children [2, 3] in whom toxicity can easily be overlooked until clinically recognizable encephalopathy occurs. The brain is exceptionally sensitive to the effects of lead poisoning [4], and it is the young-from birth to about 7 years of age who show the most serious brain damage following lead poisoning. The clinical manifestations of lead poisoning are well defined and include headache, incoordination, tremor, twitching, convulsion, paralysis, coma and death [5]. In the brain, cerebellum was found to be most severely affected [6]. Significant decrease in spine density [7] and reduction in the maximum width of the hippocampus [8] have also been reported. Bilaterally symmetrical spongiform changes in the roof nuclei of cerebellum [9] was also reported in dogs exposed to orally fed lead while bilaterally symmetrical areas of vacuole formation were observed at the tips of cortical gyri [10]. Other heavy metals like Cadmium dust induced anosmia [11] and in another study it was reported that inhalation of cadmium affected olfactory bulb and contributed to olfactory dysfunction [12]. Zinc gluconate trihydrate induced cellular and tissue damages to olfactory neuroepithelium and to mitral cells in rat olfactory bulb [13]. Exposure to high levels of mercury (a heavy metal) has also been thought to cause olfactory loss [14]. The present study was aimed to see the effect of lead on the histology of the olfactory bulb which may explain the olfactory dysfunction in the individuals exposed to lead.

MATERIALS AND METHODS

Drugs and Chemical

Lead nitrate (Merck Specialities Pvt Ltd, Mumbai), Lead acetate trihydrate (Central Drug House Pvt Ltd, New Delhi), PRO-PL (British Biologicals, Bangalore). All the chemicals and solvents were of analytical grade.

Animals

Swiss Albino mice of either sex (young, age 10-12 weeks, 25-30g) were used for the study. Animals were housed in polypropylene cages and maintained under standard laboratory environmental conditions; temperature 25 ± 2ºC, 12h dark cycle and 50±15 relative humidity with free access to food and water ad libitum. Animals were acclimatized to laboratory condition before the test. Each group consisted of six (n=6) animals. All the experiments were carried out during light period (08:00-16:00). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional Animal Ethical Committee of Banathali University, Rajasthan approved the protocol of the study (Ref. No.IAEC/257).

Experimental method

A total number of 36 adult albino mice (18 male & 18 female) weighing 25-30g were used in the present study. Mice with equal number of either sex 6 (3 male and 3 female) were treated with 4.5% and 5% lead nitrate and lead acetate trihydrate respectively, while the other 4 (2 male and 2 female) served as control did not receive any active compound. The concentration of lead nitrate and lead acetate trihydrate was ascertained after a careful trial in order to find maximum survival

of 15 to 20 days. After this period, mice were anaesthetized with ether and perfused with buffered 10% formalin. Both olfactory bulbs were dissected out from superior aspect and separated from the brain. Olfactory bulbs were cut transversely into two parts and processed for paraffin embedding. From each blocks 10µ thick sections were cut with rotary microtome. Haematoxylin and Eosin stained sections were used for light microscopic observations.

Treatment groups

Animals were divided into 6 groups having 6 animals each.
Group I - Control
Group II - Control + Dietary supplement
Group II - Lead nitrate (4.5%)
Group IV - Lead nitrate (4.5%) + Dietary supplement
Group V – Lead acetate trihydrate (4.5%)
Group VI - Lead acetate trihydrate (4.5%) + Dietary supplement

Observations

On histological examination of olfactory bulb of treated group, it was observed that as compared to control, control + dietary supplement, Lead nitrate (4.5%) + Dietary supplement and Lead acetate trihydrate (4.5%) + Dietary supplement (Fig. 1 A, B, C and D) there was generalized edema and congestion in almost all layers of olfactory bulb. Capillaries appeared dilated and congested. Distortion of glomerular contour was obvious. Periglomerular cells were hyperchromatic and showed clumping. Multiple vacuoles of variable sizes were noticed in the outer plexiform layer (Fig. 1 E, F, G and H). Granule cell layer showed loss of cells. Dark and pyknotic nuclei were also present. No such types of abnormalities were found in control group of mice.

DISCUSSION

The layers of the olfactory bulb which show damage mainly include the lamina glomerulosa, outer plexiform layer and the granule cell layer. Gross damage in the region of olfactory bulb was also seen in present study in the form of petechial haemorrhage which might have been due to capillary dilatation. These findings are in partial agreement with those reported by certain workers [15] who exposed adult guinea pig to Lead carbonate and reported vascular changes in addition to encephalopathic effects of lead mediated directly at the neuronal level. Some other workers [16] have demonstrated hypertrophy of vascular pericytes. Lead pellets implantation in the mice forebrain produced vascular changes in addition to parenchymal necrosis and spongiosis in the hypothalamus [17]. Histological study of many parts of brain e.g. cerebral cortex, corpus striatum, choroid plexus and cerebellum after lead exposure revealed cerebellum to be most severely damaged [6]. In addition in this study [6] hemorrhages noticed along with damage to molecular and Purkinje cell layers and edema in the granule cell layer which correlated very well with the findings of the present study.

Histopathological findings of olfactory bulb on neuron and neuropil in the present study are to a great extent in agreement with those reporting degeneration of cells in the cerebral cortex [8] and reduced number of Purkinje and granule cells [18] of cerebellum and of hippocampal neurons on lead exposure [19] as well as vacuolations after incubation of guinea pig hippocampus in a lead containing medium [20] which was more pronounced in outer plexiform layer of olfactory bulb.

The vascular changes observed in the present study are in agreement with those reported after exposure of lead in dogs [5] which indicates that irrespective of animal species, olfactory bulb is vulnerable to lead acetate toxicity.

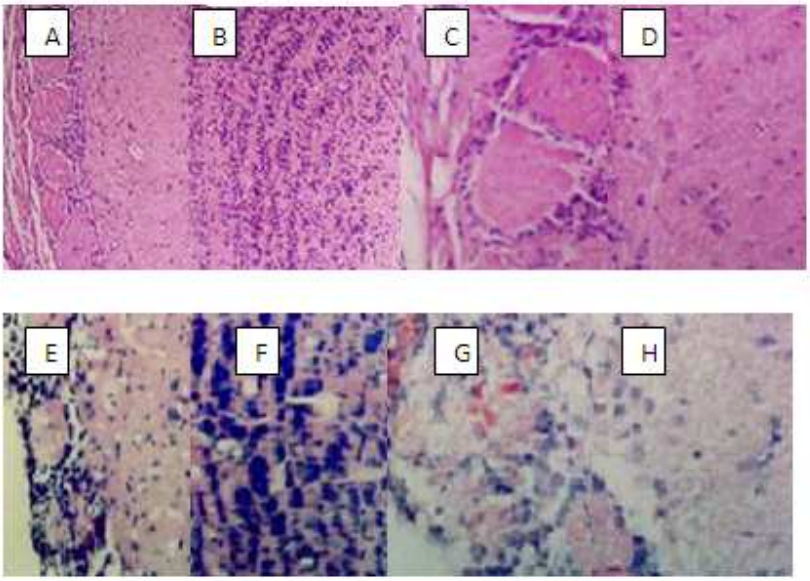

Figure 1 Photomicrographs from olfactory bulb of control mice A, control + dietary supplement B, Lead nitrate (4.5%) + Dietary supplement C and Lead acetate trihydrate (4.5%) + Dietary supplement D showing typical laminar pattern without edema, congestion or vacuolation while those from experimental group Lead nitrate (4.5%) E, F and Lead acetate trihydrate (4.5%) + Dietary supplement G, H show edema, vacuolation, congestion, loss of glomerular contour and clumping of periglomerular cells. H &E stain, X100 (A &C). X400 (B&D).

CONCLUSION

From the above study it was concluded that olfactory bulb is vulnerable to toxicity of lead similar to the other parts of brain and that histopathological changes mainly included edema, vacuolation and congestion, glomerular distortion and pyknotic periglomerular cells.

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