NEUROPROTECTIVE ROLE AND ANTI-AMNESIC EFFECT OF DOCOSAHEXAENOIC ACID AND GAMMA-LINOLENIC ACID IN LEAD INDUCED NEUROLOGICAL DEFICIT AND AMNESIA IN SWISS ALBINO MICE.

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ABSTRACT

Lead is virtually toxic to every organ of body including central nervous system where it may manifest as encephalopathy's and neuropathies, but also various behavioral changes indicative of cerebral dysfunction like; periodic convulsions, irritability, hyperactivity, retardation of normal development, emotional instability, behavioral disorders, low attention span, impaired motor development, and antisocial behavior. The purpose of present study was to characterize the putative neuroprotective role and anti-amnesic effect of docosahexaenoic acid and gamma-linolenic acid. PRO-PL (British Biologicals, Bangalore) dietary supplement containing docosahexaenoic acid and gamma-linolenic acid was fed in diet to study neuroprotective role and anti-amnesic effect using rota rod apparatus and elevated-plus maze model respectively. A total number of 48 adult Swiss albino mice of either sex were included in the study consisting of equal numbers (six each) in Standard, Control, Control + Dietary Supplement and Experimental, Experimental + Dietary Supplement Groups. Experimental groups received 4.5% and 5% Lead Nitrate and Lead Acetate Trihydrate orally alone and with Dietary Supplement for a period of 3 weeks. Diazepam (1 mg/kg i.p.) and Piracetam (200 mg/kg i.p.) was used as the standard drug. All the experimental work was approved by the Institutional Animal Ethics Committee (Ref. No.IAEC/257). In rota rod apparatus, there was significant increase in time spent by the animals on revolving rod whereas; in elevated-plus maze there was significant increase in time spent and number of entries into the open arms. Dietary supplement containing docosahexaenoic acid and gamma-linolenic acid shows prominent neuroprotective and anti-amnesic effect in lead induced Swiss albino mice.

Keywords: Docosahexaenoic acid, Gamma-linolenic acid, Amnesia, Neurological deficit, Lead nitrate, Lead acetate trihydrate.

INTRODUCTION

Anxiety and depression are extremely dramatic and debilitating multifaceted disorders and it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. Over the past decades, there has been intensive study of a variety of neurobiological aspects of depression and anxiety. Mice and human share more than 90% of their genes (1). Currently the most widely prescribed medications for anxiety disorders are Benzodiazepines (2), but the clinical applications of Benzodiazepines as Anxiolytics are limited by there unwanted side effects. The use of herbal medications by physicians in Europe and Asia is becoming more common and researchers are exploring the traditional remedies to find a suitable cure for these mind affecting diseases (3). Alzheimer's disease is a progressive neurodegenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and ultimately death (4). It is a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language. Nootropic agents such as piracetam (5), pramiracetam, aniracetam (6) and choline esterase inhibitors like donepezil are presently used for improving memory, mood and behavior. As the disease progresses, people with Alzheimer's became unable to care for themselves and loss of brain cells eventually leads to failure of other systems in body. However, the resulting adverse effects associated with these agents have limited their use (7). The present study explores the utility of dietary supplement in the prevention and treatment of various cognitive and anxiety disorders.

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MATERIALS AND METHODS

Drugs and Chemical

Diazepam hydrochloride (Calmpose injection, Ranbaxy Laboratories, Gurgaon) and Piracetam Injection (Nootropil, UCB India Pvt Ltd, Mumbai) was used as reference drug. It was diluted with distilled water to the required strength before use. PRO-PL (British Biologicals, Bangalore) was used as dietary supplement. Distilled water was used as solvent.

Animals

Swiss Albino mice of either sex (young, age 10-12 weeks, 20-25g) 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 Banasthali University, Rajasthan approved the protocol of the study (Ref. No.IAEC/257).

Table: I. Transfer Latency (sec) showed by different groups.

Treatment	Transfer latency (sec)
Control	18.12 ± 2.9
Control + Dietary supplement	17.95 ± 1.7
Piracetam	12.95 ± 2.3*
Lead nitrate	19.45 ± 3.5
Lead nitrate + Dietary supplement	18.70 ± 2.3
Lead acetate trihydrate	19.30 ± 2.6
Lead acetate trihydrate + Dietary supplement	18.68 ± 1.7

Values are expressed as Mean± S.E.M, n=6; ANOVA followed by Tukey's multiple comparison test, * P≤0.01, **P≤ 0.5 Vs Piracetam.

Treatment Schedule

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

Table: II. Time (sec) of animals remained without falling from revolving rod showed by different groups.

Treatment	Time (sec) of animals remained without falling from revolving rod	
	30 min	45 min
Control	218.0 ± 7.92	165.5 ± 3.68
Control + Dietary Supplement	237.0 ± 9.67	188.2 ± 4.96
Diazepam	165.3 ± 9.50[b]	93.00 ± 11.07[c]
Lead nitrate	145.8 ± 9.50[c]	116.5 ± 5.57[c]
Lead nitrate + Dietary Supplement	186.5 ± 10.12	140.7 ± 6.52

Values represents mean ±SEM (n=6); [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 vs vehicle- treated control group (one-wayANOVA followed by Dunnett"s "t" test)

Experimental Method

Rota rod apparatus consists of a base platform and an iron rod of 3cm diameter and 30cm length, with a non-slippery surface. This rod is divided into four equal sections by three disks, thus enabling four mice to walk on the rod at the same time at the speed of 32rpm. Interval between the mounting of the animal on the rod and falling off of it were recorded as the performance time (8).

Elevated Plus Maze (EPM). The elevated plus maze served as the exteroceptive behavioral model (where in the stimulus existed outside the body) to evaluate learning and memory in mice whereas, passive avoidance apparatus is a punishment based exteroceptive model used to test long-term memory (9). The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm). The arms ex-tended from a central platform (5 cm × 5 cm) and maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer Latency (TL)

Table: III. Time (sec) of animals remained without falling from revolving rod showed by different groups.

Treatment	Time (sec) of animals remained without falling from revolving rod	
	30 min	45 min
Control	188.2 ± 4.96	140.7 ± 6.52
Control + Dietary Supplement	165.5 ± 3.68	116.5 ± 5.57
Diazepam	118.5 ± 11.98[c]	84.30 ± 7.78[c]
Lead acetate trihydrate	86.00 ± 6.37[c]	56.67 ± 3.33[c]
Lead acetate trihydrate + Dietary Supplement	141.0 ± 7.74[b]	91.67 ± 5.70[c]

Values represents mean ±SEM (n=6); [a]P< 0.05, [b]P< 0.01, [c]P< 0.001 vs vehicle- treated control group (one-wayANOVA followed by Dunnett"s "t" test)

was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arms within 90 sec., it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The mouse was allowed to explore the maze for 10 sec and then re-turned to its home cage. Memory retention was examined 24h after the first day of trial on the second day (9, 10, 11, 12).

STATISTICAL ANALYSIS

Data obtained from pharmacological experiments are expressed as Mean ± SD. Difference between the control and the treatments in these experiments were tested for significance using ANOVA followed by Dunnet's t-test for neurological deficit assessment and Tukey's multiple comparison test for nootropic activity.

RESULT & DISCUSSION

In rota rod apparatus, there was significant increase in time spent by the animals on revolving rod whereas, in elevated-plus maze there was significant increase in time spent and number of entries into the open arms. Dietary supplement containing docosahexaenoic acid and gamma-linolenic acid shows prominent neuroprotective and anti-amnesic effect in lead induced Swiss albino mice.

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