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EFFECT OF DIETARY POLY UNSATURATED FATTY ACIDS ON TOTAL LIPID CONCENTRATION AND ELECTRON BEAM RADIATION INDUCED LIPID PEROXIDATION IN MICE BRAIN

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ABSTRACT
The whole brain irradiation causes injury to the nervous system at various levels. Omega-3 Poly Unsaturated Fatty Acids are very much essential for the growth and development of nervous system. Dietary supplementation of these nutrients will promote the development of injured neuronal cells. Therefore this study was undertaken to establish the role of Omega-3 Poly Unsaturated Fatty Acids on oxidative stress in the brain of irradiated mice. The effect of Electron Beam Radiation (EBR) on total brain lipid concentration, Lipid Peroxidation and total antioxidants level were investigated in male Swiss albino mice. The study groups were subjected to a sub-lethal dose of EBR and also the Flax seed extract and Fish oil were given orally to the irradiated mice. In the present study, exposure to EB resulted in a significant increase ($p < 0.001$) of malondialdehyde (MDA) levels. Elevated LPO by radiation exposure could be attributed to formation of free radicals and involvement of free radical induced oxidative cell damage. The observation of total lipids in brain show a significant decrease in concentration in the irradiated groups, the differences in the variables follow the similar patterns as of that the MDA levels. The oral supplementation of natural PUFA sources like Flax seed and Fish oil has led to the increase in total antioxidant levels when compared to the radiation control groups. This suggests that the PUFA supplementation in irradiated group helps in recovery of total lipid concentration by decreasing the lipid per oxidation in the brain. This study suggests that the dietary intake of PUFAs may help in prevention and recovery of the oxidative stress caused by radiation in brain.
Keywords: Electron Beam Radiation, Lipid per oxidation, PUFA.

INTRODUCTION

A living cell is a dynamic biological system composed primarily of nucleic acids, carbohydrates, lipids, and proteins that structurally and functionally interact with many other molecules; organic and inorganic to carry out normal cell metabolism. Exposure of a cell to radiation can both directly and indirectly alter molecules within the cell to affect cell viability. Radiation energy absorbed by tissues and fluids is dissipated by the radiolysis of water molecules and bio molecules[1-3]. These reactions result in redox-reactive products such as hydroxyl radical (HO*), hydrogen peroxide (H_2O_2), hydrated electron (e-aq), and an array of bio molecule-derived carbon-, oxygen-, sulfur-, and nitrogen-centered radicals (i.e., RC*, RO*, RS*, and RN*) that can in turn lead to the formation of organic peroxides and superoxide anion radicals (O2*-) in the presence of molecular oxygen[3]. Understanding the behavioral and neurophysiological consequences of radiation exposure is of great importance. Radiogenic damage to the brain, in the forms of altered performance and neuropathology, may occur after an exposure of less than 15 Gy and is a well accepted finding at higher doses. LC-PUFA, particularly AA (Arachidonic Acid) and DHA (docosahexaenoic acid), are integral components of neuronal membranes. Alterations in membrane lipid components can influence crucial intra- and intercellular signaling pathways in various ways. Deficiencies of EFA influence specific neurotransmitter systems in animals, particularly the dopamine systems of the frontal cortex, as well as associated behaviors[4].

MATERIALS AND METHODS

Animal care and handling: Animal care and handling was carried out according to the guidelines set by CPCSEA. The animal ethical committee of K.S. Hegde Medical Academy has approved this study (Institutional Ethical Clearance no: KSHEMA/AEC/19/2010). Swiss albino mice aged 6-8 weeks and weighing 25 ± 5 g, taken from an inbred colony, was used for this study. The mice were maintained under controlled conditions of temperature and light (light: 10 h; dark: 14 h). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed and water *ad libitum.*

Experimental Design

30 male Swiss Albino mice were used and were randomly divided into 4 groups of 6 animals each. Group I served as control. Group II animals were exposed to 6 Gy (sub-lethal dose) Electron Beam Radiation. Group III animals were given powdered Flax seed (300 mg/Kg of body weight) orally everyday (30 days before and after 6 Gy irradiation) and Group IV animals were fed with 0.5 ml of Fish oil daily (30 days before and after 6 Gy irradiation).

Irradiation

The radiation work was carried out at Microtron Centre, Mangalore University, Mangalore, Karnataka, India. The animals were restrained in well ventilated perspex boxes and exposed to whole body electron beam at distance of 30 cm from the beam exit point of the Microtron accelerator at a dose rate of 72 Gy/min.

Determination of changes in oxidative stress markers

After 15 days of study, blood was collected from the animals by cardiac puncture and biochemical assays were carried out. Lipid per oxidation was measured by the method of Beuege and Aust[5]. Total antioxidant capacity was determined by the phosphomolybdenum method as described by Prieto *et al*[6]. UV-Visible spectrophotometer (UV-1601 Pc, Systronics, India) was used for these analyses.

Estimation of Total lipids from Mice Brain

Total lipids were estimated according to the method described by Folch *et al*[7]. 1 g of tissue blot, dried oven at 100°C overnight. Then dried tissue is homogenized with 2:1 chloroform-methanol mixture. The crude extract is mixed thoroughly with adequate solution, allowed to stand for 5-10 minutes in a separating funnel. The mixture was separate into two phases. Upper phase contain methanol with water. Lower phase contain chloroform with lipids. The lower phase was collected in pre-weighed (w3) tubes. Then it was kept in oven at 100°C overnight. Weighed (w4), w3-w4 gives total lipid content of the brain tissue[7].

Statistical Analysis

Results were expressed as Mean ± Standard Deviation (S.D). Statistical significance was determined by one-way analysis of variance (ANOVA). P values < 0.05 were considered as significant. All statistical analysis was carried out using the instant statistical package (Graph Pad Prism version 3.0 software).

Table 1: Showing the levels of Lipid per oxidation, Total antioxidant and Total Lipid concentration in the whole Brain homogenate

	Group 1	Group 2	Group 3	Group 4	P Value
Lipid Per oxidation (Concentration of MDA µM/g tissue)	1.0180 ± 0.5335	24.3100 ± 5.774	7.0290 ± .96200	11.87 ± 1.806	P < 0.0001
Total Antioxidant Capacity (µg/mL)	95.9300 ± 22.25	30.1800 ± 3.301	52.8000 ± 5.5980	39.18 ± 2.287	P < 0.001
Total Lipids Concentration (µM/L)	0.26 ± 0.13	5.19 ± 0.38	2.53 ± 0.31	2.32 ± 0.28	P < 0.001

P < 0.05 is significant, Group I: control. Group II: Radiation. Group III: powdered Flax seed and Group IV: Fish oil

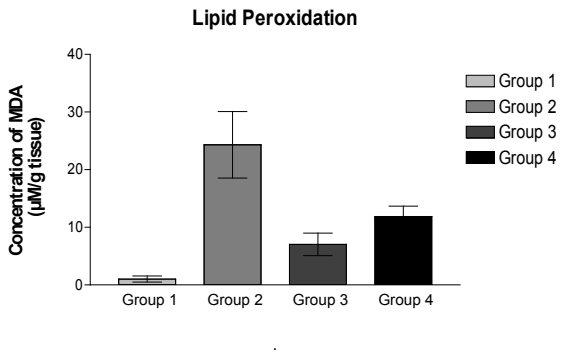

Figure 1: Showing of Lipid per oxidation levels in whole Brain homogenate

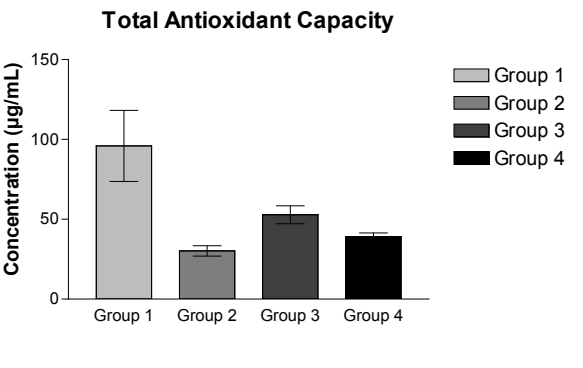

Figure 2: Showing Total antioxidant levels in the whole Brain homogenate

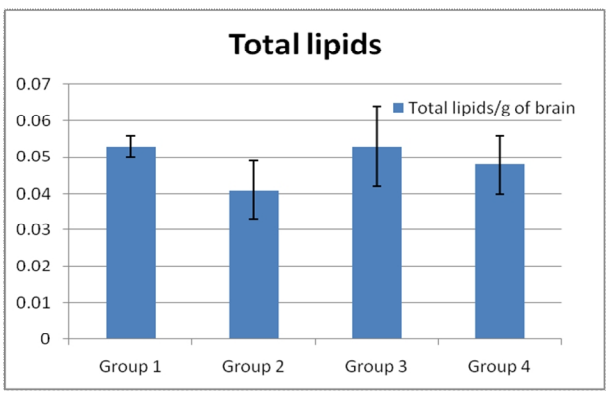

Figure 3: Showing Total Lipid concentration in mice Brain homogenate

RESULTS AND DISCUSSION

Irradiation caused a significant increase (Table 1) in MDA level compared to control group. Dietary supplementation of PUFA showed significant decrease in the brain MDA level when compared to the irradiated group ($p < 0.001$). Lipid per oxidation (LPO) is a hallmark of oxidative stress which disrupts the structural integrity of cell membrane and can lead to formation of aldehydes which in turn lead to lipid, protein and DNA damage[8]. In the present study, exposure to EB resulted in a significant increase ($p < 0.001$) of malondialdehyde (MDA) levels (Figure 1). The increase in lipid per oxidation was shown to be the principal damage induced by radiation in biological membranes[9]. Elevated LPO by radiation exposure could be attributed to formation of free radicals and involvement of free radical induced oxidative cell damage. Thus, increased LPO is suggestive of progressive increase in membrane permeability, disruption of structural and functional integrity of cell organelles. There was no significant change in the MDA levels in drug control group compared with normal control animals. Dietary supplementation with n-3 PUFA in irradiated mice showed significantly low ($p < 0.001$) MDA levels (Figure 1) suggesting that n-3 PUFA s in diet might enhance the recovery process in oxidative stress.

Total Antioxidant Capacity

The total antioxidant levels were significantly decreased in the irradiated group when compared to the control group. The dietary supplementation with flax seeds in irradiated mice showed considerable amount of increase in ($p < 0.001$) levels of TAC compared to that of irradiated groups (Table 1, Figure 2).

Brain Lipid Composition

The observation of total lipids in brain show a significant decrease ($P < 0.0001$) in concentration in the irradiated groups, the dietary supplementation of PUFAs showed significant increase in the total brain lipid concentration (Table 1, Figure 3). The Analysis of Lipid per oxidation, Total antioxidant capacity and Total lipid concentration showed a significant level of changes when compared between control and radiation groups. Gamma radiation has been shown to increase lipid per oxidation in brain in previous studies[10]. Dietary PUFA supplementation showed a significant level of decrease in the lipid per oxidation in the irradiated groups. The total antioxidants levels were found increased in the PUFA supplemented groups when compared to the irradiated groups. The observation of total lipids in brain showed a significant decrease in concentration in the irradiated groups, and the differences in the variables follow the similar patterns as that of the MDA levels. This suggests a correlation between the brain lipid per oxidation and total brain lipid concentration. This suggests that the PUFA supplementation in irradiated group helps in recovery through decreasing the lipid per oxidation and increasing the total lipid concentration in the brain. From this investigation, we observed that dietary supplementation with Flax seed were more effective when compared to the Fish oil. This might be due to presence of phytochemicals in flaxseed. Thus, supplementation with flax seed will be beneficial for safe guarding the radiation hazards and also as an ideal source for nutrition.

CONCLUSION

Supplementation of dietary PUFA has been illustrated to having a protective effect against the oxidative stress caused by exposure to ionizing radiation in brain. From this investigation, authors found that dietary supplementation with Flax seed were more effective when compared to the Fish oil. This might be due to presence of phytochemicals in flaxseed. Thus, supplementation with flax seed will be beneficial for safe guarding the radiation hazards and also as an ideal source for nutrition. PUFA supplementation in irradiated group helps in recovery through decreasing the lipid per oxidation and increasing the total lipid concentration in the brain. This study suggests that the dietary intake of PUFAs may help in prevention and recovery of the oxidative stress caused by radiation in brain.

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