Effect of Dietary Poly Unsaturated Fatty Acids on Electron Beam radiation induced oxidative stress 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 Lipid Peroxidation, enzymatic non-enzymatic 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. This study suggests that the dietary intake of PUFAs may help in prevention and recovery of the oxidative stress caused by radiation.

Keywords: Electron Beam Radiation, Learning Ability, Memory, Anxiety, PUFA.

INTRODUCTION

Acute whole body exposure to lethal dose of ionizing radiation produces a set of symptoms, collectively known as radiation sickness or prodromal syndrome. The major symptoms in rodents include reduced food and water intake, weight loss, diarrhea, leathargy, ruffling of hair, facial edema, disorientation, epilation and tail necrosis. The number of symptoms and their severity increases with radiation doses. But the effects of sub lethal and low doses of radiations are not clearly evident from the currently existing studies. Radiation can cause metabolic disturbances and cell injury in many ways¹. The killing action of ionizing radiation is mainly mediated through the free radicals generated from the radiolytic decomposition of cellular water². Radiation is known to produce various reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical in the biological systems and in turn various types of tissue damage due to free radical chain reactions³. Lipid peroxidation is one of the important effects on biological membranes and the investigations on lipid peroxidation can provide important information about detrimental effects of ionizing radiation. Lipid peroxidation (L•) was believed to be formed by the reaction of •OH generated by ionizing radiation with (LH). It can readily react with an oxygen molecule forming lipid peroxyl radical (LOO•). These radicals can attract hydrogen atoms from other polyunsaturated fatty acids to become LOOH. The major alterations were known to occur upon exposure of DNA to lipid hydroperoxides (LOOH). It was also reported that lipid peroxidation product such as malondialdehyde forms adduct with cellular DNA. Apart from the lipid peroxidation, ROS can also alter the balance of endogenous protective systems such as reduced glutathione and enzymatic antioxidants (SOD, and CAT defense systems.) The endogenous antioxidant defenses are inadequate to reduce the radiation-induced free radicals. Appropriate antioxidant intervention seems to inhibit or reduce free radical toxicity and thus offer protection against radiation. A number of dietary antioxidants have been reported to decrease free radical attack on biomolecules⁴.

MATERIALS AND METHODS

Animal care and handling

Animal care and handling was carried out according to the guidelines set by CPCSEA. The institutional animal ethical committee has approved this study. 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 (300mg/Kg of body weight) orally everyday (30 days before and after 6 Gy irradiation) and Group IV animals were fed with 0.5ml 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. The animals were restrained in well-ventilated perspex boxes and exposed to whole-body electron beam at distance of 30cm from the beam exit point of the Microtron accelerator at a dose rate of 72Gy/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 peroxidation was measured by the method of Beuege and Aust, 1978. Total antioxidant capacity was determined by the phosphomolybdenum method as described by Prieto et al, 1999. SOD activity was measured according to method described by Sun et al., UV-Visible spectrophotometer (UV-1601 Pc, Systronics, India) was used for these analysis.

**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).

**RESULTS AND DISCUSSION**

In actively metabolizing cells, there was considerable amount of water apart from the target macromolecules. EB (Electron Beam) generates ROS (Reactive Oxygen Species) as a result of radiolysis of water. ROS can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins and membranes. Thus, radiation-induced damage might result in adverse health effects within hours to weeks or delayed effects observable many months after exposure.

Lipid peroxidation (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. In the present study, exposure to EB resulted in a significant increase (p<0.001) of malondialdehyde (MDA) levels (Fig. 1). The increase in lipid peroxidation was shown to be the principal damage induced by radiation in biological membranes.

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 rats showed significantly low (p<0.001) MDA levels suggesting that n-3 PUFA's in diet might enhance the recovery process in oxidative stress.

Superoxide dismutase (SOD) is dimeric antioxidant enzyme responsible for the quenching of superoxide radicals which are released during the chemical reactions of the various metabolic pathways. The EBR exposed mice showed significant decrease (p<0.027) in the SOD level (Fig. 1). The decreased level of SOD might be inhibiting the superoxide dismutase transcription and utilization for the scavenging of superoxide radical produced during the exposure of EB. This data is in accordance with the other reports, where gamma radiation damages biological antioxidant systems by means of inhibiting superoxide dismutase transcription. But, our study demonstrated that the significant restoration (p<0.027) of SOD level by dietary supplementation of n-3 PUFA (Fig. 2), suggesting that n-3 PUFA's in diet might enhance the recovery process in oxidative stress.

The irradiated mice showed significant decrease (p<0.001) in the levels of Total Antioxidant Capacity (TAC) in comparison with control group (Fig. 3). This decrease of TAC might be due to...
its utilization for the scavenging of lipid peroxides produced during the exposure to EB. 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: Levels of Lipid peroxidation, Total antioxidant and SOD in the whole Brain homogenate

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Peroxidation (Concentration of MDA $\mu$M/g tissue)</td>
<td>1.0180± 0.5335</td>
<td>24.3100± 5.774</td>
<td>7.0290± .96200</td>
<td>11.87±1.806</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>Total Antioxidant Capacity ($\mu$g/mL)</td>
<td>95.9300±22.25</td>
<td>30.1800±3.301</td>
<td>52.8000±5.5980</td>
<td>39.18±2.287</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Super Oxide Dismutase Activity (Units/mg Tissue)</td>
<td>1.6500±0.8018</td>
<td>0.5510±0.3206</td>
<td>0.6040±0.05574</td>
<td>1.1330±0.1796</td>
<td>P=0.0272</td>
</tr>
</tbody>
</table>

$p<0.05$ is significant


**Fig. 1: Effects of radiation and dietary PUFA on Lipid peroxidation levels in the brain of mice**


**Fig. 2: Effects of radiation and dietary PUFA on SOD activity in the brain of mice**

CONCLUSION
Supplementation of dietary PUFA has been illustrated to having a protective effect against the oxidative stress caused by exposure to ionizing radiation. 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. The present study evaluates the antioxidative effect of flax seed and fish oil as whole but, further explorations are needed to claim the radioprotective effect of individual poly unsaturated fatty acids that are present in flax seed and fish oil. This study suggests that the dietary intake of PUFAs may help in prevention and recovery of the oxidative stress caused by radiation.

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