



## PREVENTION OF RADIATION - INDUCED BIOCHEMICAL CHANGES IN MOUSE BRAIN BY *PUNICA GRANATUM*

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### Abstract

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Ionizing radiations cause damage to living tissues and biomolecules. Radiation exposure damages the nervous system also. In the present study *Punica granatum* fruit rind extract (PGFRE) was tested to protect radiation induced changes in brain biochemistry. For experimental purpose, healthy adult male *Swiss albino mouse* (25 ±2g) were selected and divided into four groups. Group-I was kept without any treatment (normal). Group-II was irradiated with 8Gy Co<sup>60</sup> gamma radiation only (control). Group-III was fed PGFRE (acetone) (10 mg/kg body weight) one hour before irradiation (experimental) and Group IV was given with PGFRE only. Mice were sacrificed at different intervals and brain was removed, weighed and analysed for estimation of its glutathione (GSH), lipid peroxidation (LPO), protein, DNA and RNA content. Brain weight of the control and experimental groups decreased till 3<sup>rd</sup> day post irradiation but remained higher than the control. Protein and RNA contents increased up till the 3<sup>rd</sup> post irradiation day and decreased till 28<sup>th</sup> day in control but were at higher level in the experimental group. GSH and DNA content decreased in control and experimental group but found to be higher than the corresponding control group at all the intervals. Thus, results suggest that pretreatment of PGFRE protects mouse brain against the radiation induced biochemical changes.

## INTRODUCTION

Radiation is beneficial for therapeutic purposes but high dose or continuous exposure can cause disastrous consequences by damaging cell membrane and causing radiation syndromes. Damage to the biological systems by ionizing radiation is caused primarily due to macromolecular lesions by direct interaction of radiation with macromolecules as well as indirectly through reactive Oxygen and Nitrogen species generated during the radiolysis of water and amplified by cellular Oxygen. Radiolysis of water forms a variety of free radicals that are hydroxyl radicals (OH<sup>•</sup>), superoxide radical (O<sub>2</sub><sup>-•</sup>) and organic radicals (R<sup>•</sup>). They are highly unstable species and damage critical cellular structures, including nucleic acids, membrane lipids and proteins.

Protection of biological systems against harmful effects of radiation is of paramount importance during accidental, occupational and unavoidable exposure to radiation. In spite of extensive efforts made in the last five decades to the development of effective radioprotectors. Patt *et al.*,<sup>1</sup>

demonstrated that Cysteine protects mice and rats against radiation induced sickness and mortality. Pretreatment of certain chemical compounds like 2-MPG<sup>2</sup>, WR-2721<sup>3</sup> offer some protection against the toxicity associated with exposure to ionizing radiation, but their use in clinical field is limited due to their inherent toxicity generated by them at protective dose level. Therefore, the interest is generated in development of potential drug of plant origin for the modification of radiation effect. Use of herbs exhibiting less toxicity/no side effects, low cost and easy access to common people, may offer a suitable strategy to reduce undesirable consequences. Several polyherbal formulations, single plant extracts and purified phytochemicals, such as Curcumin, Quercetin, Rutin, Ellagic acid, Gallic acid and several other polyphenols and flavonoids were studied for their radioprotective property<sup>4</sup>. Plant extracts eliciting radioprotective efficacy contain plethora of compounds including antioxidants, immunostimulants, cell proliferation stimulators, anti-inflammatory and antimicrobial agents, some of which may

act in isolation as well as in combination with other constituents of the same plant.

*Punica granatum* L. commonly called Pomegranate, is a member of the Punicaceae family. Juice and peels of pomegranate possess antioxidant and anticancer activities. It also interferes with tumor cell proliferation, cell cycle, invasion and angiogenesis<sup>5,6</sup>. Pomegranate peel extract (PPE) with an abundance of flavonoids and tannins has been shown to have a high antioxidant activity<sup>7</sup>. Pomegranate fruit rind contains tannins, anthocynins, flavonoids, pectins, ellagitannins (Punicalin, Punicalagin, Granatin, Gallagyldilactone, Casurinin), Pedunculagin, Tellimagrandin, Corilagin<sup>8</sup>, Gallic acid, Ellagic acids, Ursolic acid<sup>9</sup> and Catechin<sup>10</sup>. It is thought to provide natural antiviral<sup>11</sup>, antifungal<sup>12</sup>, antioxidant<sup>13</sup> and antibacterial activity<sup>14</sup>.

Tissues differ significantly in their sensitivity to ionizing radiation. Brain is a soft, spongy mass of tissue. It is protected by bones of the skull and three thin membranes called meninges. Brain is highly susceptible to oxidative damage due to its high utilization of oxygen that accounts for the increased

generation of free radicals and reactive oxygen substances. It is enriched with the more easily oxidizable polyunsaturated fatty acid (PUFA) such as Docosahexaenoic acid and Eicosapentaenoic acid as it has a limited ability to perform aerobic glycolysis; it is usually vulnerable to hypoxia<sup>15</sup>. Therefore, present study is an attempt to find out the efficacy of *Punica granatum* fruit rind extract in modulating the radiation induced biochemical alterations in the brain of *Swiss albino mouse*.

## MATERIALS & METHODS

### Animals

Adult male *Swiss albino mouse* (*mus musculus norvegicus*) 6-8 weeks old, weighing 25±2 g each from an inbred colony, were selected. They were maintained under controlled conditions of temperature 37±5° C and kept at 12 hrs natural day light and dark night cycles. They were provided standard mice feed and water *ad-libitum*. Animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. Animal care and handling were performed according to guidelines issued by the World Health Organization (Genava, Switzerland ) and the

Indian National Science Academy (New Delhi, India). The experimental protocols were approved by the Institutional Animal Ethics Committee.

### Source of Irradiation

The Cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, was used for irradiation. Unanaesthetized animals were restrained in well-ventilated perspex boxes and whole body exposed to a single lethal dose of gamma radiation (8Gy).

### Preparation of Plant Extract

Rind of *Punica granatum* fruits cut into pieces and shade dried. The extract was prepared by soxhlet apparatus in Acetone by boiling it for 36 hours. The extract was dried and stored in a refrigerator. For experiments dried extract was weighed and dissolved in double distilled water to obtain the desired concentration of plant extract. It was fed orally one hour prior to irradiation.

### Selection of optimum dose

To select the most effective dose of *Punica granatum* various doses of *Punica granatum* fruit rind extract

(5,10,15,20,30,40 and 50mg/kg body weight) were tested against a lethal dose of gamma irradiation (8Gy). Experimental animals were observed for 30 days for any sign of radiation sickness, mortality, behavioral toxicity or morbidity. The dose which provides maximum protection (10mg/kg body weight) obtained was used for the experimental design.

### Experimental Design

The animals were divided into four groups keeping 36 animals in each group.

**Group I (Normal)** – Mice of this group were kept as such without any plant extract treatment and were sham irradiated. Before this they were given equal amount of distilled water that was given along with the plant extract to the group II and IV animals.

**Group II (Control)** – Mice of this group were irradiated with 8 Gy Co<sup>60</sup> gamma radiations only.

**Group III (Experimental)** – Mice of this group were given *Punica granatum* extract one hour before irradiation.

**Group IV (Extract only)** - Mice of this group were administered with *Punica granatum*

(fruit rind) extract (10 mg/kg body weight) only at the same dose rate.

The animals from each group were sacrificed by cervical dislocation at 3hrs, 1, 3, 7, 14 and 28 days after treatment. Six animals were sacrificed at each interval from every group.

### Biochemistry

The brain of the mouse was removed, weighed and analysed biochemically for its Glutathione content<sup>16</sup>, lipid peroxidation<sup>17</sup>, total protein<sup>18</sup>, DNA<sup>19</sup> and RNA content<sup>20</sup> quantitatively .

### Statistical Analysis

The results obtained in the present study were expressed as mean  $\pm$  standard error. The statistical difference between various groups were analysed by Students t- test and significance was observed at  $P < 0.05$ ,  $P < 0.01$  and  $P < 0.001$  levels.

## RESULTS AND DISCUSSION

**Brain Weight-** Weight of brain of the control group decreased in comparison to normal till 3rd day post irradiation. Then it started to increase till the last interval in comparison to normal. Brain weight of the experimental group was significantly higher

than their corresponding control group at almost all the intervals. (Figure 1)

**Lipid peroxidation (LPO)** - At all the post irradiation intervals lipid peroxidation remained significantly higher in the control group than normal one. The maximum lipid peroxidation level was observed at 7th day post irradiation. Then it decreased till the last interval. Lipid peroxidation level remained significantly lower in *Punica granatum* pretreated irradiated groups as compared to their respective controls at all the intervals. (Figure 2)

**Reduced glutathione (GSH)** – Glutathione content decreased up till 7th day post irradiation. Glutathione content of the experimental group was found higher than the corresponding control group at all the intervals. (Figure 3)

**Protein content** – Protein content of irradiated group slightly increased up to 3rd day and then continued to decrease until the end of the experiment. Treatment of *Punica granatum* extract prior to irradiation maintains a higher level of protein in comparison to control until the end of the experiment. (Figure 4)

**DNA content-** DNA content remained significantly lower in control group as compared to the normal. After one day it decreased in *Punica granatum* pretreated irradiated group but it remained higher in comparison to control till 28th day. (Figure 5)

**RNA content** – RNA content increased significantly in the control group which was maximum on 3rd day. Then it decreased towards normal. Increase in the amount of RNA was recorded in the experimental group also till 3rd day post irradiation. Then it came down to the normal in the experimental group. (Figure 6)

## DISCUSSION

Low level of ionizing radiation may kill or damage small numbers of body cells, which will be replaced through normal growth cycles but higher radiation dosages kill large portions of cells in the body, in either a localized or generalized pattern. Radiation has been reported to induce cell death and reduce size of the brain if given a high and acute radiation dose.

In the brain its topographical regions have variable susceptibility to ionizing radiation. Radiation induced lesions tend to occur

more frequently in the white matter of brain. Brain of an adult is generally considered insensitive to ionizing radiation because neurons do not die after radiation exposure<sup>21</sup>, but a small population of neuronal precursor cells are relatively sensitive<sup>22,23</sup>. Acute effects occur during and / or shortly after the radiation exposure and are characterized by symptoms of fatigue, dizziness and sign of increased intracranial pressure. The acute effects are considered to be secondary to edema and disruption of the blood- brain barrier (BBB). Glial cells and neurons contain relatively low levels of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase etc.<sup>24</sup>. Necrosis and cerebral atrophy are considered long term complications of radio therapy which take months to decades after radiation treatment<sup>25,26,27</sup>.

Most toxic effects from acute ionizing radiation are due to an increased flux of free radicals. Oxidation is important in many brain pathologies<sup>28</sup>. Brain has a poorly developed antioxidative defense mechanism. Concentration of antioxidant enzymes is low in brain. GSH concentration is also very much reduced in the brain when compared to other organs in the body<sup>29</sup>.

Lee *et al.*<sup>30</sup> demonstrated that whole brain irradiation induces regionally specific pro-inflammatory environments through activation of AP-1, NF-KB and CAEB and overexpression of TNF $\alpha$ , IL-1B and MCP-1 in rat brain and may contribute to unique pathways for the radiation induced impairments in its functioning. Overexpression of pro inflammatory mediators may be responsible for radiation induced normal tissue injury<sup>31</sup>. Radiation injury is associated with irreversible damage to the neural stem cell compartment and induced apoptosis and depletion of oligodendrocytes may cause vacuolation and demyelination in brain<sup>32</sup>

Irradiation increases protein and lipid peroxidation which is reported to be reduced by N-Acetylcysteine<sup>33</sup>. Yoshimuna *et al.*<sup>34</sup> found protective role of vitamin E in reducing radiation induced increase in oxidative damage in rats and Chan *et al.*<sup>35</sup> observed that vitamin E prevents radiation induced cerebral necrosis also. Administration of melatonin protects brain against the increase in Malondialdehyde (MDA) levels, edema, neuronal degeneration and necrosis<sup>36</sup> and decrease in DNA damage and lipid peroxidation in

irradiated brain cells<sup>37</sup>. Pretreatment with methanolic extract of *Vernonia amygdalina* (M)(250 and 500 mg/kg/day) and alpha-tocopherol (TOCO) before gamma irradiation reduced significantly radiation induced gross morphometrical changes in rat cerebellum<sup>38</sup>.

Brain weight decreased after irradiation in the control group then it started to increase till the last interval in comparison to normal. Initial decrease appears to be due to loss of water from the cell due to increased cell permeability. Increase in brain weight at later intervals appears to be due to perivasculitis and oedema. The antioxidant activity of *Punica granatum* prevented the loss of brain weight in experimental animals.

Free radicals generated by radiation can react with unsaturated lipids and thus generate hydroperoxides, which in turn can induce peroxidation in the lipid bilayer thereby altering the membrane permeability<sup>39</sup>. In the present study lipid peroxidation in the irradiated group was found significantly higher than the normal. It was found maximum on 7<sup>th</sup> day post irradiation. In *Punica granatum* pretreated

and than irradiated group it remained significantly lower than the group which was irradiated without *Punica granatum* at all the intervals. The products of lipid peroxidation such as malonaldehyde and 4-hydroxynonal are toxic to the cell<sup>40,41</sup>. Presence of antioxidants in plant extracts suppresses formation of free lipid radicals and thus prevents the formation of peroxidation. The measurement of thiobarbituric acid reactive substances (TBARS) gives an index of free radical activity. Radical scavenging by protectors results in inhibition of TBARS. Lipid peroxidation can be initiated by hydrogen abstraction from lipid molecules by lipid radiolytic products, including hydroxyl and hydroperoxyl radicals<sup>42</sup>. Pomegranate fruit rind extract exhibit antioxidant effect which could be due to the available constituents. All the compounds including the isomers of Punicalagin, Tannin derivatives and Anthocyanins (Delphinidin, Cyanidin and Pelargonidin, 3-Glucoside and 3,5-Diglucosides) have free radical scavenging and antilipid peroxidation activity<sup>43</sup>. Punicalagin [2,3-(S)-Hexa hydroxydiphenyl-4,6-(S,S)-galloyl-D-glucose] inhibit lipid peroxidation due to its ability to provide

electrons to eliminate the free radicals resulting from lipid peroxidation<sup>5,44</sup>.

Under normal conditions, the inherent defense system including Glutathione and antioxidant enzymes protect against the oxidative damage. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation<sup>45</sup>. A significant decrease in GSH content in brain was observed following gamma irradiation (8Gy). This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the radicals generated by radiation. Decreased GSH levels have been considered as an index of increased formation of reactive oxygen species (ROS) in many tissues, and thus subsequent GSH depletion may cause oxidative stress induced cellular damage<sup>46</sup>. Pretreatment of Pomegranate rind extract reduced the depletion of GSH levels and provide protection to the brain. Punicalagin originating from the peels of pomegranate is one of the major polyphenol contributing to the total antioxidant capacity whilst anthocyanins play only a minor role in this activity<sup>47</sup>. Ellagic acid and Punicalagin play an

important role in the antioxidant activity of pomegranate peel<sup>48</sup>.

In irradiated group RNA and protein content increased till 3<sup>rd</sup> day and then decreased till the 28<sup>th</sup> day. Pretreatment of *Punica granatum* fruit rind extract maintained a higher level of protein content in comparison to group irradiated without *Punica granatum* till 28<sup>th</sup> day. Lesser decrease in protein content is apparent which seems to be due to decreased brain weight as protein content is estimated in terms of per gram tissue weight. Proteins are required to repair radiation induced damage in the cell. Protein content may be reimbursed due to increased RNA activity and rate of protein synthesis. Protein content decreases due to cell lysis induced by gamma radiation and their subsequent removal. Radiation induced apoptosis in the external granular cell layers results in transient decrease in the expression of synaptic proteins in cerebellum of developing rat<sup>49</sup>. The decrease observed in protein content may be due to its denaturation by irradiation or may be at the synthesis level or by inhibition of release of synthesized polypeptides from polysomes<sup>50</sup>. Protein content also depletes after

irradiation due to increased requirement for repair process.

In the present study decrease in the amount of DNA in irradiated control group was observed 24 hours after irradiation. Pretreatment with *Punica granatum* fruit rind extract reduces this decrease in the DNA level in the experimental group. Ionizing radiations induce damage to cellular DNA, which is of prime biological significance. The types of damage include strand breaks, base damage, elimination of bases and sugar damage<sup>51</sup>. Due to induction of DNA double strand breaks, the ionizing radiations are extremely effective in producing chromosomal aberrations leading to genomic instability<sup>52</sup>. The oxidation of guanine by the hydroxyl radical (OH<sup>•</sup>) to 8-Hydroxyl-2-deoxyguanosine (8-OHdG) alters DNA<sup>53</sup> and leads to mutagenesis<sup>54</sup>. Ionizing radiation induce a variety of DNA lesions by direct and indirect interactions including DNA base alterations, DNA – DNA and DNA-protein cross-links, single and double strand breaks, out of which DNA double strand breaks are lethal to the cell.

The radioprotective activity of plants and herbs may be mediated through several

mechanisms, since they are complex mixture of many chemicals. The majority of plants and herbs contain polyphenols which could be effective in scavenging of radiation induced free radicals and elevation of cellular antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and thereby counteract the deleterious consequences of ionization radiation<sup>55,56</sup>.

The antioxidant constituents of pomegranate are compounds with phenolic hydroxyl groups and double bonds including tannins, flavonoids and unsaturated fatty acids. Pomegranate extract or pomegranate derived compounds have therapeutic use for the treatment of inflammatory diseases. It is able to decrease the production of pro-inflammatory cytokine interleukin IL-6 and IL-8 and suppresses the activation of NF- $\kappa$ B in activated human mast cells and basophils<sup>57</sup>. According to Rasheed *et al.*<sup>58</sup> pomegranate extract or its derived compounds may be useful in blocking the activation of Mitogen activated protein kinase kinase 3 (MKK3) and provide the benefit of P<sup>38</sup>-Mitogen activated protein kinase (MAPK) inhibition. It may develop as Mitogen activated protein kinase kinase

(MKK) inhibitor. Pomegranate peel aqueous extract involve in modulation of cell signaling molecule in the cell cycle machinery (eg. WAF1/P21).

According to Li *et al.*<sup>59</sup> peel of pomegranate have higher antioxidant activity than its pulp and seed. Ricci *et al.*,<sup>60</sup> observed antioxidant capacities of some extracts from *Punica granatum* arils, juice and rind which were in correlation with their polyphenol content. Okonogi *et al.*<sup>61</sup> reported that the extract of pomegranate peel have highest antioxidant activity with an IC<sub>50</sub> of 0.003 mg/ml in a DPPH assay and highest TEAC value of 4.59mM/mg. *Punica granatum* peel extract decreased lipid peroxidation in hepatic, cardiac and renal tissues<sup>62</sup>. Toklu *et al.*<sup>7</sup> also reported that chronic pomegranate peel extract supplementation alleviated oxidative injury of the liver and improved the hepatic structure and function in rats exposed to bile duct ligation.

### CONCLUSION

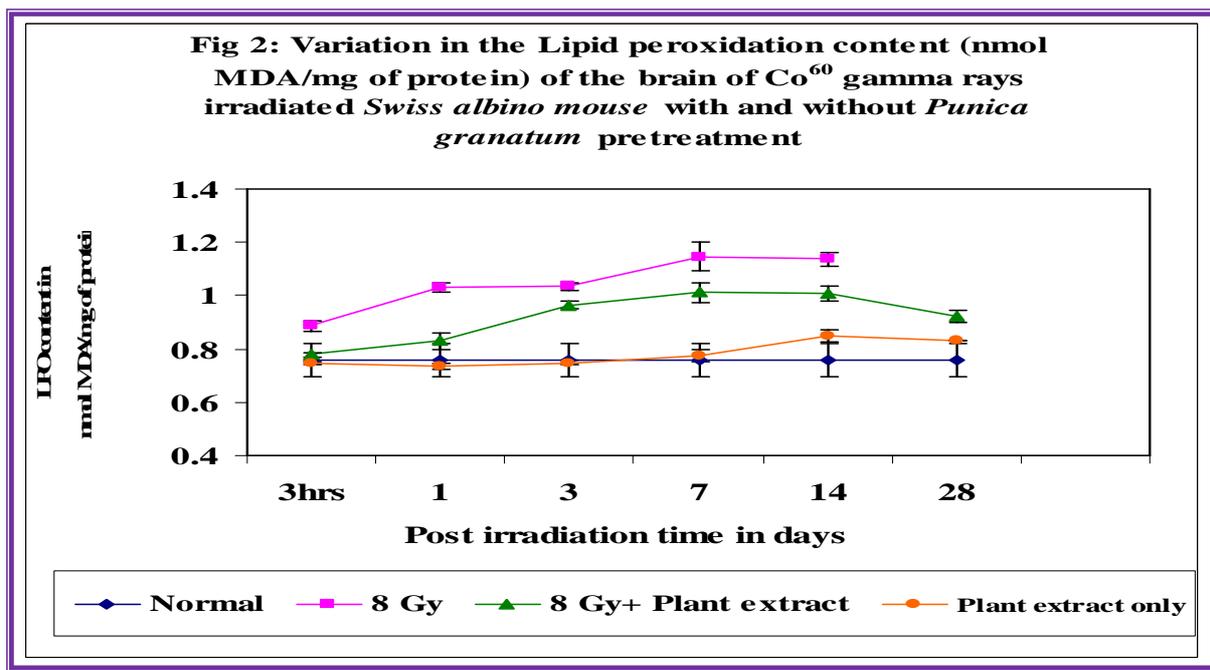
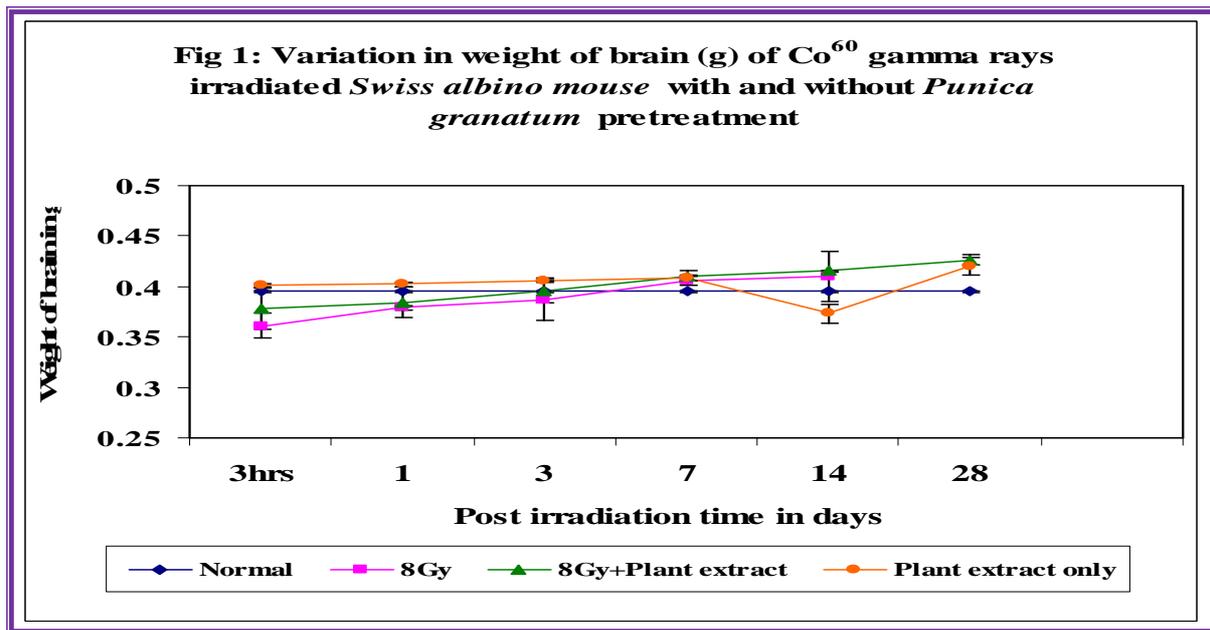
Results from the present study suggest that oral pretreatment of *Punica granatum* fruit rind extract protect mouse brain against the radiation induced damages. It works at a

very low dose rate without causing side effects.

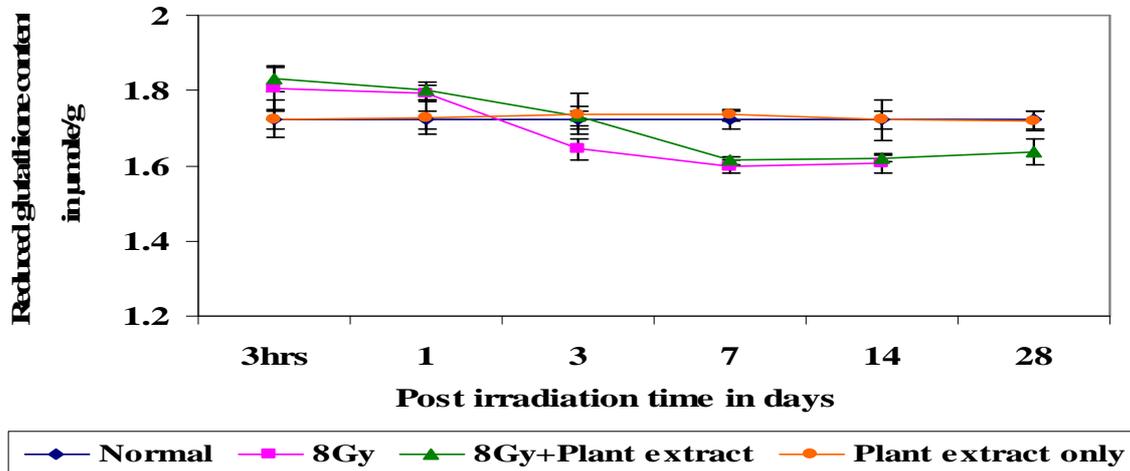
Hospital, Jaipur, for providing radiation facilities.

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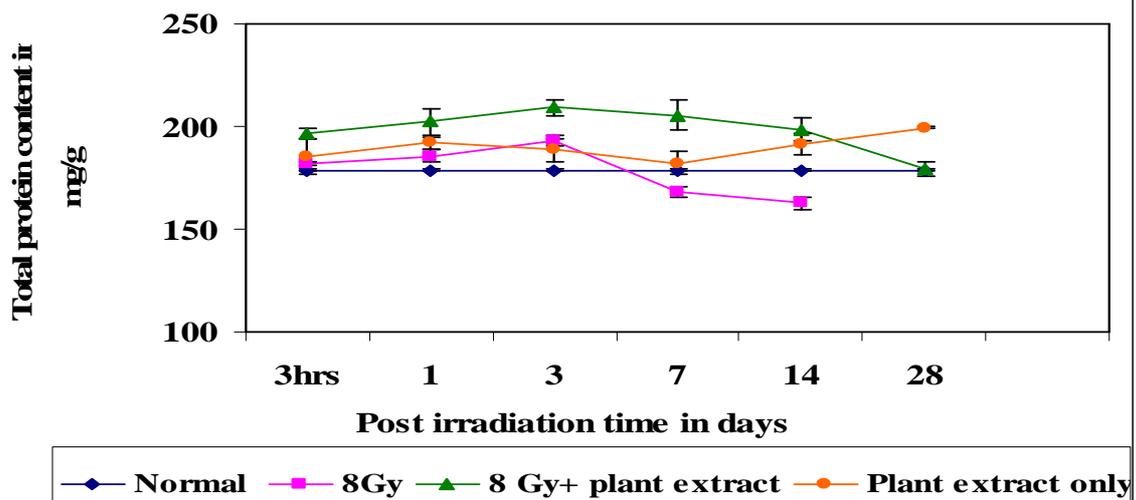
We are thankful to the Radiotherapy Department SMS Medical College and

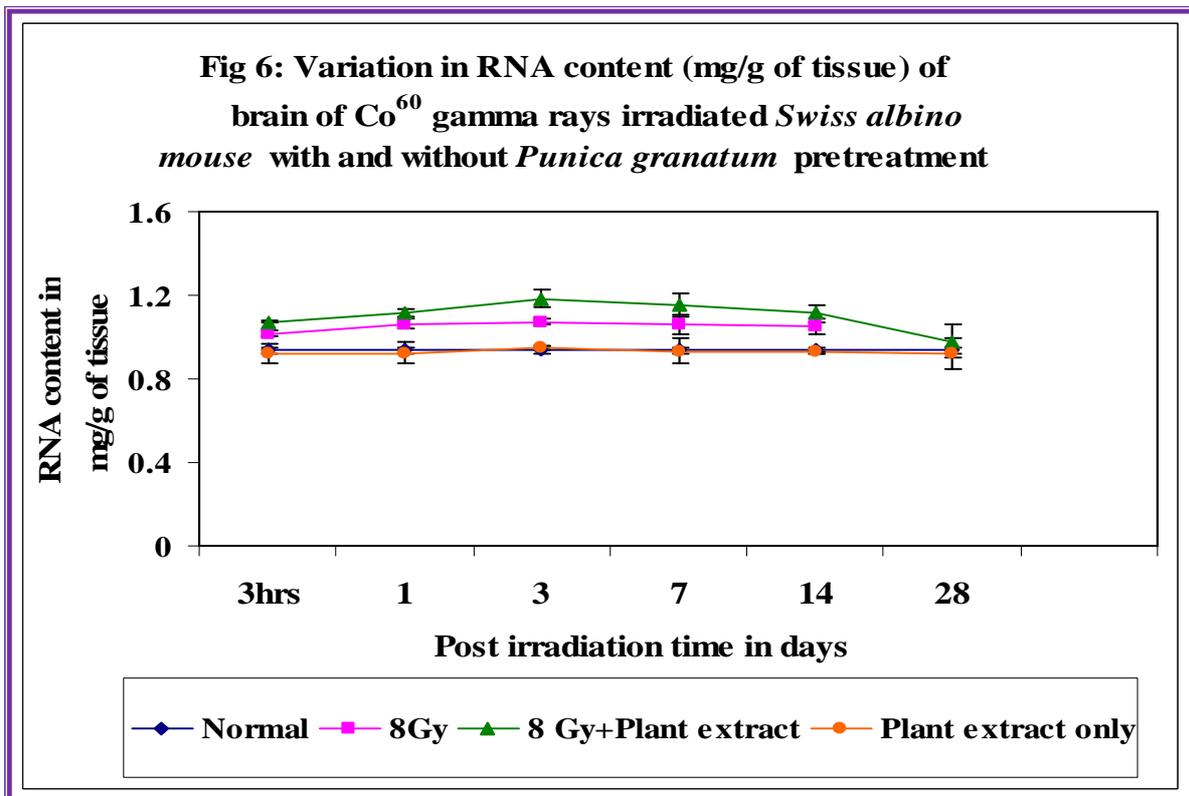
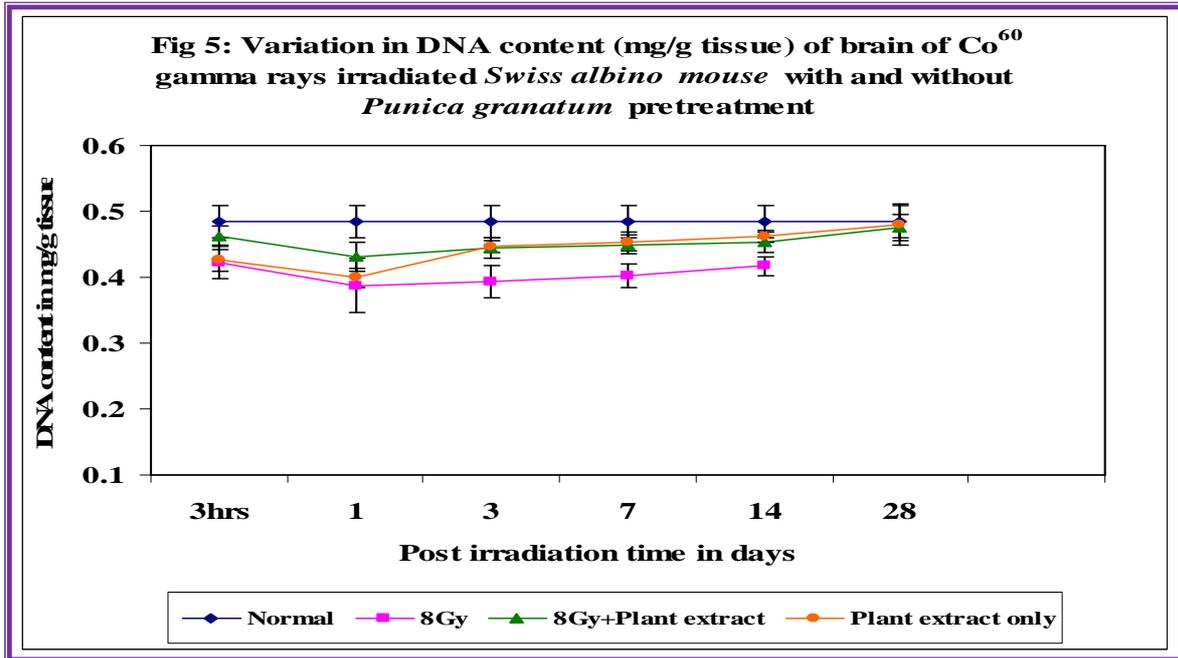


**Fig 3: Variation in reduced glutathione content ( $\mu\text{mole/g}$ ) of brain of  $\text{Co}^{60}$  gamma rays irradiated *Swiss albino mouse* with and without *Punica granatum* pretreatment**



**Fig 4: Variation in Total protein content ( $\text{mg/g}$ ) of brain of  $\text{Co}^{60}$  gamma rays irradiated *Swiss albino mouse* with and without *Punica granatum* pretreatment**





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