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Jamun, *Syzygium Cumini* Linn. Skeels, Increases The Survival Of Mice Whole Body Exposed To Different Doses Of γ -radiation

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ABSTRACT

The radioprotective effect of leaf extract of jamun, *Syzygium cumini* was studied in mice exposed to different doses of γ -radiation. Acute toxicity of jamun (*Syzygium cumini*) extract were determined initially. Animals were orally administered once daily with different doses of jamun extract for 5 consecutive days before exposure to 10 Gy γ -radiation for selection of optimum radioprotective dose. Symptoms of radiation-sickness and the survival of the animals were monitored for 30 days after the irradiation. Administration of jamun extract for five consecutive days increased the survival of animals in a dose dependent manner up to a dose of 50 mg/kg and then declined steadily up to 300 mg/kg. Further studies with 50 mg/kg extract showed that oral administration of jamun extract for five continuous days protected mice against the radiation-induced sickness and increased the survival. Whole body irradiation only of mice increased lipid peroxidation and reduced glutathione concentration in a dose dependent manner, whereas treatment of mice with 50 mg/kg jamun extract significantly increased glutathione concentration and reduced the lipid peroxidation. Free radical scavenging and increase in glutathione and reduction in lipid peroxidation may have contributed to the observed radioprotection. Jamun extract protected mice against the radiation-induced sickness and mortality. **Keywords:** Jamun, *Syzygium cumini*, radiation, radiation protection, radiation sickness, survival, mice, glutathione, lipid peroxidation

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INTRODUCTION

The increased use of ionizing radiations for medical investigative and treatment purposes in the last century, adequately stress the need to guard humans against the damaging effects of electromagnetic radiation. Therefore the search for effective non-toxic radioprotectant is continuous and there has been a renewed interest in the search for potential drugs, especially from plants and herbs, which are capable of free radical scavenging, prevents cellular death by facilitating the DNA repair [1].

Jamun or *Syzygium Cumini* Linn. Skeels belonging to family Myrtaceae is a medium sized to large tree. The fruits and seeds are involved in the treatment of diabetes, pharyngitis, and splenopathy. The leaves have been widely used to treat diabetes, constipation, leucorrhoea, mucositis of stomach, dermopathy and to prevent blood lose in the faeces [2, 3]. Preliminary studies from this laboratory have revealed the radioprotective potential of jamun leaf and seed extracts [4-6]. The above studies were in vitro or using i.p administration. However, oral administration is the method of choice in humans as it does not require clinical intervention and can be carried out at a large scale if necessary. Hence, the current study was started to investigate the effects of oral administration of *Syzygium cumini* leaf extract in mice which are subjected to various doses of γ -radiation.

MATERIALS AND METHODS

Chemicals

Chemicals required for the experiments were procured from Sigma Chemical Co., St. Louis, USA. Few compounds were delivered by the Ranbaxy Fine Chemicals, New Delhi, India.

Animal care and handling

Male Swiss albino mice were selected for the whole work and are maintained under controlled conditions of temperature ($23\pm 2^\circ\text{C}$), humidity ($50\pm 5\%$), and 12 h light-dark cycle. The mice were 8 to 10 week old, weighing 30 ± 2 g and were given with standard laboratory food and water *ad libitum*. The Institutional Animal Ethical Committee approval was taken for study. The rules and the principles fixed by the World Health Organization (WHO) and the Indian National Science Academy, New Delhi, India, were strictly followed.

Preparation of leaf extract

The leaves of the Jamun (i.e. *Syzygium cumini*) were collected, dried, powdered first. By using soxhlet instrument the powdered leaves were extracted with petroleum ether, chloroform and with other organic solvents [7]. The extract was allowed to cool and concentrated by using rotary evaporator. Then the dried extract was kept in desiccator for the experiment. Hereafter the extract of *Syzygium cumini* will be named as SCE.

Drug preparation and its mode of application

The essential amount of SCE was dissolved in 1% carboxymethyl cellulose (CMC) in sterile normal physiological saline. The mice were administered with SCE or CMC consecutively for 5 days through the oral route. The drug was prepared freshly before the experiment [6].

Acute toxicity

The drug tolerance of the SCE was conducted and studied by the standard method [8, 9]. At first, mice were kept for fasting for 18 h. Later the mice were separated into 5 groups of 10 each. Freshly prepared SCE was given orally to the animals of each group, with a single dose of 500, 750, 1000, 2000 or 3000 mg/kg body weight. Immediately after oral administration, the animals were given with food and water. Animals were carefully observed for 14 days after the drug administration.

Selection of optimum radioprotective dose of SCE

To decide the best dose of SCE mice were grouped as follows:

CMC + irradiation

Oral administration of 0.01 ml/g body weight of CMC was done to the animals of the above group before subjection to 10 Gy irradiation.

SCE + irradiation

Different doses of SCE, like 10, 25, 50, 75, 100, 200 or 300 mg/kg body weight was administered orally to the animals of the above group. Different doses of SCE was given daily continuously for 5 days before subjection to 10 Gy of irradiation.

From the above preliminary study, it was observed that 50 mg/kg body weight of SCE was the best protective dose and accordingly, the further evaluation of jamun were executed using 50 mg dose of SCE. A total of 240 mice were used for the above study and 30 animals were taken for each drug dose group.

Radioprotective effect

Another study was planned for the further confirmation of the radioprotective ability of SCE, where the grouping and other settings remained same to that described above, except that the CMC group was treated with 7, 8, 9, 10, 11 or 12 Gy of γ -radiation, whereas the other group was given once daily with 50 mg/kg b.w. of SCE orally, for 5 successive days before subjection to 7-12 Gy of radiation. The survival of the animals was observed and noted down up to 30 days. For each radiation dose 24 mice were used and a total of 288 animals were taken for this study considering both the groups.

Radiation Exposure

Sixty minute after the last oral dose of CMC or SCE on 5th day, animals were kept in a specially designed well-ventilated acrylic box and treated with whole body radiation of 7, 8, 9, 10, 11 or 12 Gy of ⁶⁰Co gamma radiation at a dose rate of 1.66 Gy/min at a source to animal distance. The animals of both groups in the above study were scrutinized carefully and daily for the radiation sickness indications, morbidity, behavioral changes and the rate of mortality for 30 days after the irradiation. Regression analysis was done to obtain LD 50/30 values and to determine the dose reduction factor [10].

$$\text{DRF} = \frac{\text{LD}_{50/30} \text{ of the SCE + irradiation group (experimental animals)}}{\text{LD}_{50/30} \text{ of CMC + irradiation group (control animals)}}$$

Biochemical estimations

On the day 31st after irradiation livers of the survived animals were removed through transcatheter perfusion with ice cold saline. 10% liver homogenate was prepared using a homogenizer along with ice-cold 0.2 M sodium phosphate buffer (pH 8.0). GSH content [11] and LPx concentrations [12] were measured with the help of 10% liver homogenate. Four surviving animals were taken from each group as well as from each radiation dose for the above estimations. A standard curve was plotted to calculate both GSH and LPx concentration. GSH has been expressed as $\mu\text{mol/g}$ tissue and LPx has been shown as MDA in nM per mg protein.

Statistical analysis

The data obtained from the current study are highlighted as mean \pm standard error of the mean. To do the statistical comparison Student's 't'-test was used for biochemical estimations. For the survival experiment, "Z" test [13] was conducted using the following method:

$$z = \frac{p_1 - p_2}{\sqrt{p(1-p)(1/n_1 + 1/n_2)}}$$

Where p = (number of survivors) / total sample size.

RESULTS

Acute toxicity

There was no drug induced mortality, change in behaviour, breathing pattern and also no change in the neuromuscular co-ordination in the animals. The administration of 500 to 3000 mg/kg of SCE did not cause any drug-related toxicity in the mice, which is confirmed with the 100% survival of treated animals. Therefore it was concluded that, even at the higher dosage of 3000 mg/kg b.w, SCE did not produce any toxic symptoms in the animals. Still higher doses of SCE could not be verified owing to the difficulties in drug dissolution and administration.

Selection of optimum SCE dose

The signs of radiation sickness appeared within 2-4 days for the animals of CMC + irradiation group after subjection to 10 Gy of gamma radiation. The irradiated mice revealed lethargy, watering of eyes, decreased appetite and body weight, ruffling and loss of hairs, thinness and weakness. A few mice also showed facial swelling after 1st week of exposure. Paralysis and difficulty in movement was also observed in few animals during 2nd week. On the 5th day first death of the mice was reported and all the animals perished within 17 days after the irradiation (Fig.1).

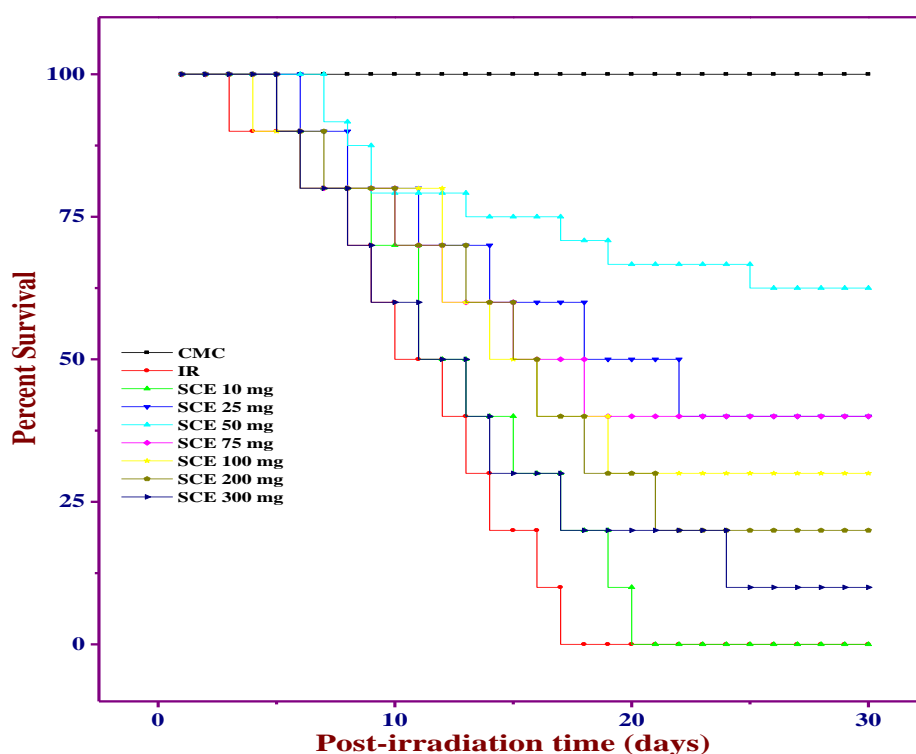
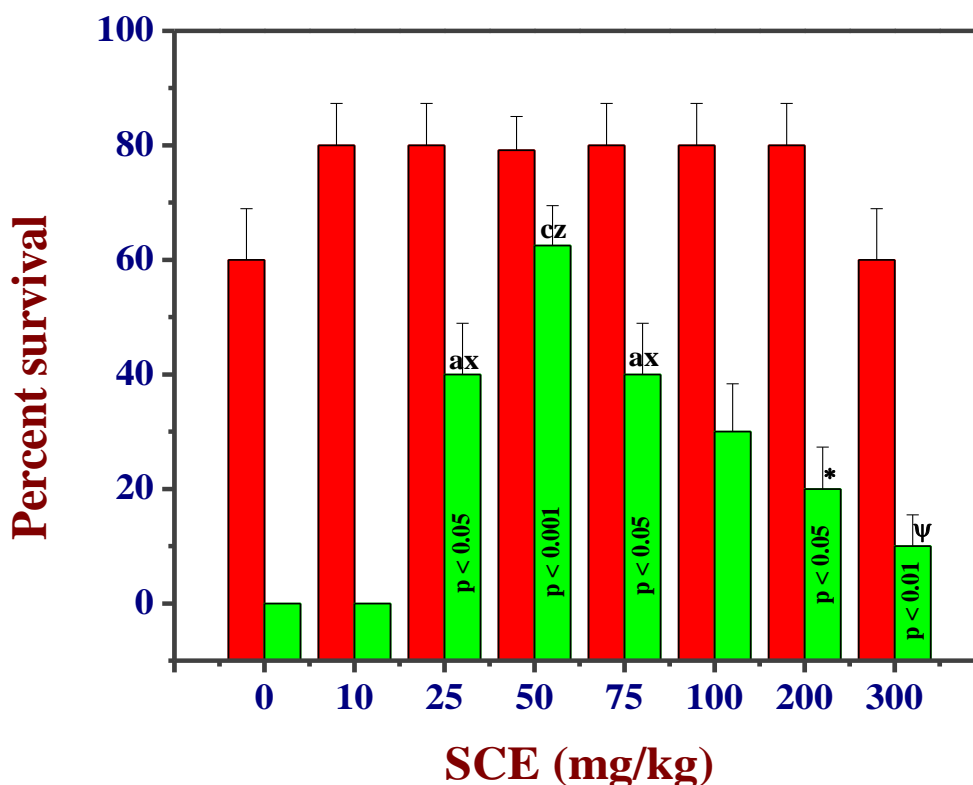


Fig. 1. A Kaplan Meir's estimate of the survival of mice treated with different doses of jamun (*Syzygium cumini*; SCE) p.o. before whole-body exposure to 10 Gy of γ -radiation.

Administration of 10, 25, 50, 75, 100, 200 or 300 mg/kg b.w. of SCE for 5 successive days did not induce any death. After giving above doses of SCE, mice were subjected to 10 Gy of gamma radiation. SCE treatment hindered or minimized the signs of radiation illness and also postponed the beginning of radiation-induced death depending on the dose of SCE. The extended delay in the mortality was noticed for 25 and 50 mg/kg SCE, where the 1st death was reported by day 7 and 8 post-irradiation (Fig.1). For the dose of 100 mg/kg, we could notice the shortest delay in the mortality which was recorded on day 5 after irradiation. There was a remarkable reduction in the 10th day mortality after the administration of 25, 50, 75, 100, 200 or 300 mg/kg body weight of SCE when compared with the radiation treated (control) group. Except for the 10 and 300 mg/kg of SCE, rest of the doses of SCE witnessed approximately 80% of survival of mice by day 10 after the irradiation, whereas it was 70 % and 60% of survival for 10 and 300 mg/kg of SCE respectively (Fig. 2).



a:p<0.05; c:p<0.001 V/s CMC+10Gy; x:p<0.05; z:p<0.001 V/s 10mg/kg+10Gy; *:p<0.05; Ψ:p<0.01 v/s 50mg/kg+10Gy

Fig. 2: Effect of different doses of jamun (*Syzygium cumini*; SCE) extract (p.o) on the survival of mice exposed to 10 Gy of gamma-radiation. Red bars, 10-day survival; and green bars, 30 day survival. Error bars are 95% confidence limit.

The experiment of 30 day survival exposed that 50 mg/kg of SCE showed highest survival of 62.5%, which declined with increasing dose (Fig. 1 and 2). The survival rate of the mice decreased with the increase of SCE dose up to 75, 100, 200 or 300 mg, when compared with 50 mg/kg SCE. As a result the best protective dose of SCE was confirmed as 50 mg/kg, where a maximum number of survivors has been noted when compared with CMC + irradiation group, where no survivors could be witnessed after 17 days post-irradiation (Fig. 1).

Radioprotective effect

An additional evaluation of radioprotective effect of SCE was done by treating the mice with 50 mg/kg SCE continuously for 5 days before subjection to 7-12 Gy of γ -radiation (SCE + irradiation group). The mice group which are exposed only to 7 to 12 Gy of radiation showed dose-related symptoms and infection within 2-5 days after exposure. The subjection of mice to higher radiation dosages like 9 and 10 Gy resulted in an earlier arrival of deepened radiation sickness and indications like facial edema and difficulty in locomotion. The results of survival are stated as percent survival as a function of time after subjected to various doses of γ -

radiation (Fig.3). In the 7 Gy radiation dose there were no report of death for 30 days in either group. However, as the radiation dose increased, survival rate deteriorated in a dose-dependent manner reaching a lowest point after 12 Gy irradiation, where all animals succumbed to death by 13 days after the radiation (Fig.3). After plotting survival on a log scale it was found that the LD_{50/30} for the CMC + irradiation group was 8.7 Gy (Fig. 4A & B).

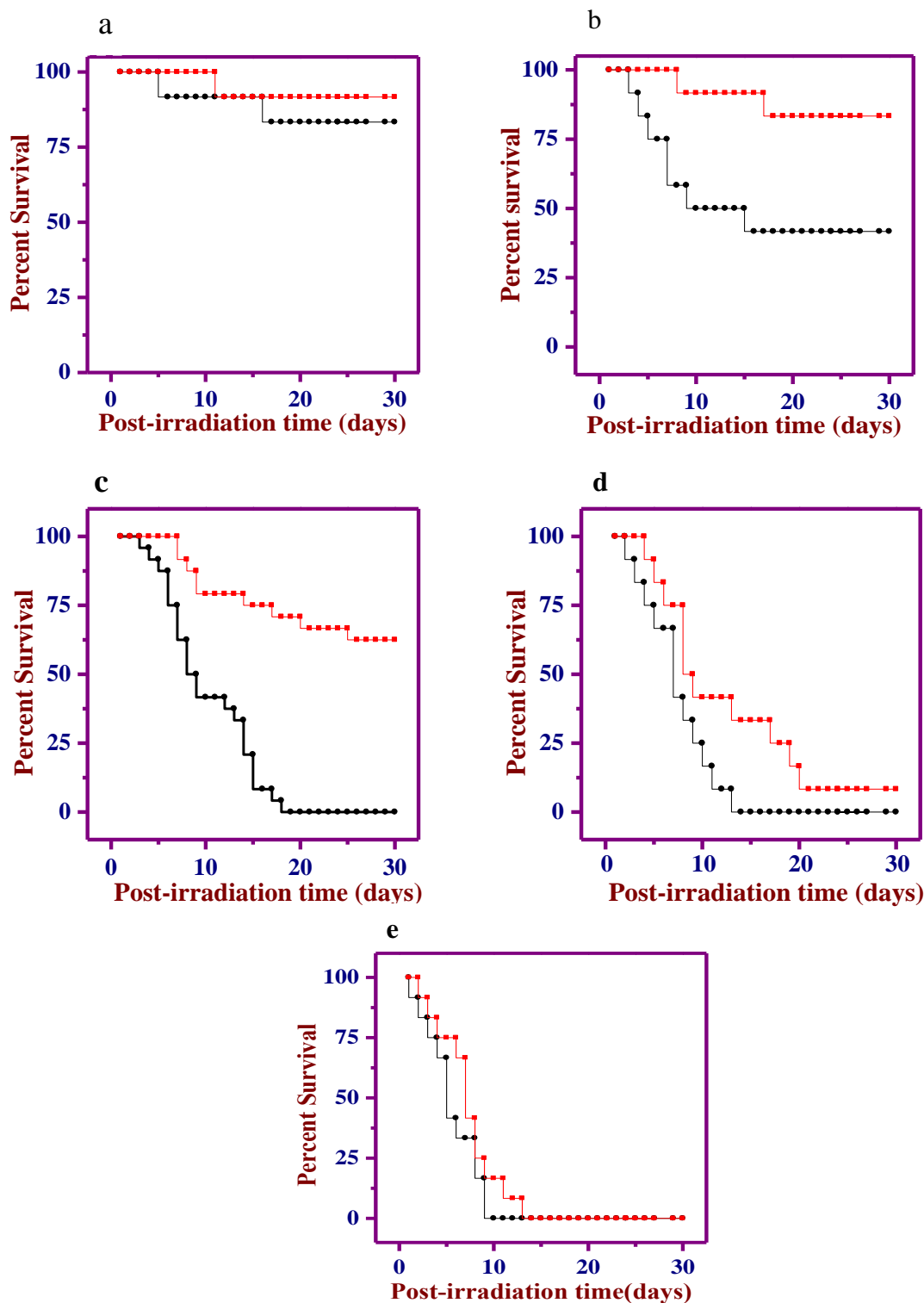


Fig. 3: Kaplan-Meier's estimate of survival of mice treated with 50 mg/kg b. wt. of jamun (*Syzygium cumini*; SCE) p.o. before exposure to different doses of γ -radiation. Circles, CMC+irradiation and Squares, SCE+irradiation. a. 8 Gy; b. 9 Gy; c. 10 Gy; d. 11 Gy and e. 12 Gy.

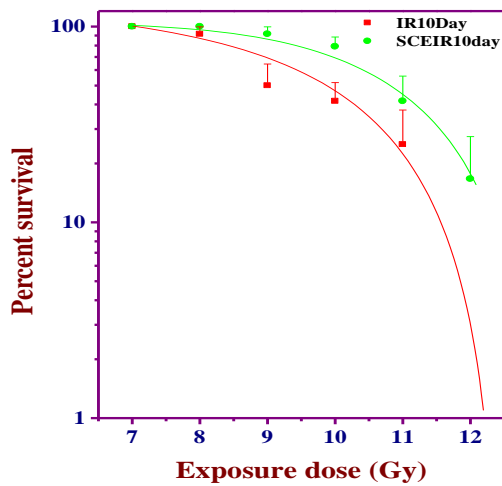


Fig 4A: 10 day survival

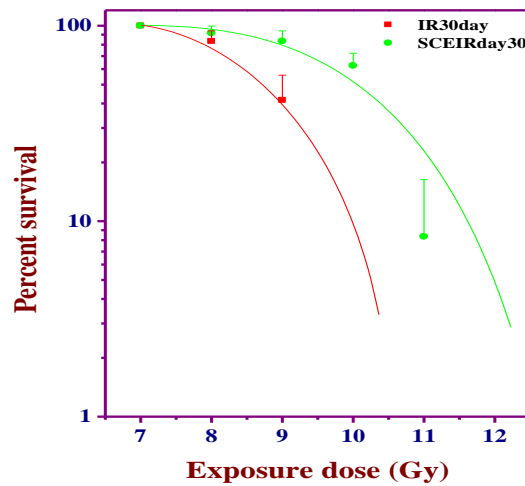


Fig 4B: 30 day survival

Fig. 4: Effect of 50 mg/kg b. wt. SCE p.o on the survival of mice exposed to different doses of gamma-radiation.

SCE treatment alone did not instigate death in the sham-irradiated mice during the observation period. Initial treatment of mice with 50 mg/kg SCE before irradiation has decreased the severity of radiation illness in comparison with the radiation treated group. SCE treatment caused greater survival of mice at day 10, where all animals survived after exposure to 8 Gy. There was a dose dependent drop in the 10 day survival that was 92 %, 79%, 42% and 17% after 9 ($p < 0.01$), 10 ($p < 0.01$), 11 and 12 Gy irradiation, respectively (Fig. 4A). Likewise, SCE pretreatment elevated 30 day survival at all exposure doses 8 (92%), 9 (83%), 10 (62.5%) and 11 (8%), except in 12 Gy, where all the animals were died at the end of 30 day post-irradiation (Fig. 4B). SCE pretreatment raised the 30 day survival significantly after 9 ($p < 0.01$) and 10 ($p < 0.001$) Gy (Fig.4B). The SCE pretreatment increased the LD_{50/30} with a DRF of 1.2. (Fig. 4B).

Biochemical estimations:

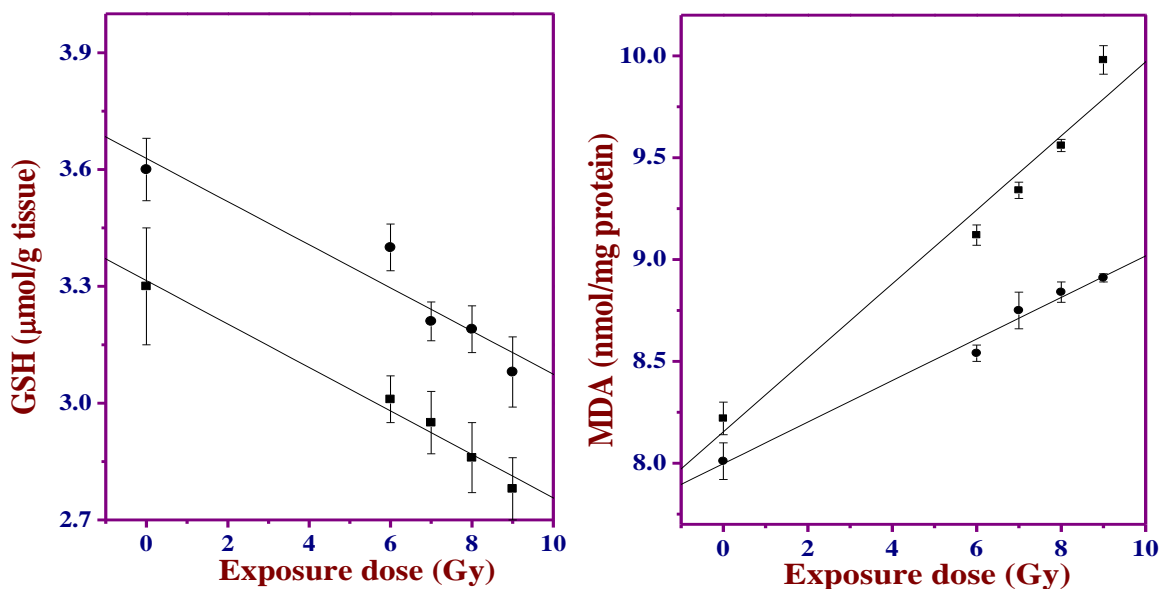


Fig. 5: Alterations in the GSH concentrations (left) and lipid peroxidation (right) in the mice treated with 50 mg/kg b. w. of the leaf extract of the jamun (*Syzygium Cumini*; SCE) before exposure to various doses of gamma-radiation. Squares: CMC+IR and Circles: SCE+IR.

Concentration of GSH and then spontaneous level of LPx did not alter in CMC + sham-irradiation and SCE + sham-irradiation groups. After treating the mice to different doses of gamma radiation, there was a remarkable dose dependent decrease in the GSH content (Fig. 5). Similarly lipid peroxidation was elevated with increased dose of radiation and a peak level was noticed at 9 Gy irradiation (Fig. 5). But the oral administration of SCE not only elevated GSH concentration significantly but also minimized the LPx in the SCE + irradiation when compared with the CMC + irradiation group. The results of GSH and LPx supports the radioprotective action of SCE on mice against radiation. In spite of significant changes done by SCE, the level of GSH was below normal and the values of LPx were higher than the control in both the groups at day 31 after the irradiation (Table. 1).

Table 1: Modulation of the radiation-induced changes in the glutathione and lipid peroxidation by *Syzygium cumini* extract in the liver of mice surviving up to day 30 after exposure to different doses of gamma radiation.

Exposure dose(Gy)	Assay type Glutathione($\mu\text{mol g}^{-1}$ tissue \pm SEM)		Assay type Malonaldehyde (nmol mg^{-1} protein \pm SEM)	
	CMC+IR	SCE+IR	CMC+IR	SCE+IR
0	3.3 \pm 0.15	3.6 \pm 0.08	8.22 \pm 0.08	8.01 \pm 0.09
6	3.01 \pm 0.06	3.4 \pm 0.06 ^b	9.12 \pm 0.05	8.54 \pm 0.04 ^c
7	2.95 \pm 0.08	3.21 \pm 0.05 ^a	9.34 \pm 0.04	8.75 \pm 0.09 ^d
8	2.86 \pm 0.09	3.19 \pm 0.06 ^a	9.56 \pm 0.03	8.84 \pm 0.05 ^e
9	2.78 \pm 0.08	3.08 \pm 0.09 ^a	9.98 \pm 0.07	8.91 \pm 0.02 ^f

SCE, *Syzygium cumini* extract; IR, irradiation; SEM, standard error of the mean; CMC, Carboxy Methyl Cellulose.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$, ^d $p < 0.005$, ^e $p < 0.0005$, ^f $p < 0.0001$, p were calculated for comparison between CMC+IR versus SCE+IR values.

DISCUSSION

Oral administration of various doses of SCE to mice resulted in an increased survival up to 50 mg/kg after irradiation and it dropped subsequently with increasing dose of drug. A comparable outcome has been stated before where intraperitoneal administration of jamun leaf and seed extracts has been reported to protect the animals against gamma radiation at 30 and 80 mg/kg, respectively, and the radioprotective effect weakened after that [5, 6]. Likewise, SCE has been found to protect human lymphocytes against the gamma radiation and minimized DNA damage at 12.5 $\mu\text{g/ml}$ and this effect dwindled with increasing SCE dose [4]. Similarly, other phytochemical agents that have been reported to provide protection at a specific dose can be compared to the efficacy of the Jamun [14 – 19].

In mice death within 10 days after the radiation exposure is due to gastrointestinal syndrome whereas death between 11 to 30 days is due to the haemopoietic syndrome [20, 21]. In our experiment we found the radiation illness within 2-5 days depending on the dose of radiation after the whole body exposure of mice to different doses of γ -radiation. A similar effect has been observed earlier studies [22-26]. As the radiation dose increased there was noticeable decrease in the survival and 50% of mice subjected to 10 Gy of radiation died within 10 days because of the functional failure of the gastrointestinal tract. Remaining animals succumbed to death within 20 days after 10 Gy irradiation due to bone marrow damage.

Preliminary studies from this laboratory have shown that SCE offered protection against radiation-induced mortality and micronuclei formation [4-6]. However, no study has been done to know the effect of oral administration of SCE against several doses of γ -radiation and determine the DRF earlier. Considering the practical implications, mice were orally administered with 50 mg/kg SCE continuously for 5 days once daily before subjection to different doses of whole body radiation, where SCE protected the mice from radiation sickness and increased the survival. The animals even survived after 11 Gy (8%) irradiation in SCE pretreated group. These studies indicate protection of gastrointestinal as well as haemopoietic compartment by SCE which increased LD_{50/30} with a dose reduction factor of 1.2. An identical effect has been reported earlier with other plant extracts including *Panax ginseng*, *Hippophae rhamnoides*, *Podophyllum hexandrum*, *Tinospora cordifolia*, *Embllica officinalis*, and *Amaranthus paniculatus* [27-37]. Earlier studies from this laboratory reported a similar effect with *Ocimum sanctum*, ginger (*Zingerber officinale*), bael (*Agele marmelos*) [38-42].

SCE may protect animals against the harmful toxic effects of radiation using multiple putative mechanisms. Free radical scavenging activity by SCE might be the main action that may have played a significant role in reducing the initiation of lesions and thus protecting the proliferating compartment. In fact, SCE inhibited generation of $\cdot\text{OH}$, DPPH, $\text{ABTS}^{\cdot+}$, $\text{O}_2^{\cdot-}$ and NO radicals [43]. This is supported by an increase in GSH concentration after 30 days accompanied by a subsequent decline in lipid peroxidation. Earlier report supports that flavonoids present in the SCE, helps to scavenge the free radicals and also prevent lipid peroxidation [44 – 47]. Quercetin, one of the chemical constituent of SCE has been proved to minimize the genetic damage and TBARS significantly and a potent inhibitor of NF- κ B activation [48, 49]. Studies has been stated that quercetin can also influence its role in the radiotherapy by significantly elevating the radio-sensitization of the tumor cells by inhibiting the ATM kinase [50]. Myricetin, an important flavonoid present in SCE has been described to enhance the DNA repair by promoting the activity of DNA polymerase enzyme [47]. And also the upregulation of PPAR (peroxisome proliferator-activated receptor) could have induced proliferation of stems cells in the irradiated tissues and may allowed faster recruitment of fresh cells resulting in the increased survival. PPAR has been found to be essential for cell proliferation [51].

CONCLUSION

Present study demonstrates that oral route of 50 mg/kg of *Syzygium Cumini* extract protected mice from the gamma radiation and also from the radiation-illness. SCE also increased the survival of mice with a DRF of 1.2 and there was no toxic side effects up to a dose of 3000 mg/kg. It may protect the animals using various pathways including scavenging of free radicals, upregulation of NPSH (GSH) level, reduction in lipid peroxidation, abrogation of transcriptional activation of NF- κ B, down regulation of COX-II mRNA, upregulation of DNA repair genes and PPAR.

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