TREATMENT OF MICE WITH LEAF EXTRACT OF JAMUN (*SYZYGIUM CUMINI* LINN. SKEELS) PROTECTS AGAINST THE RADIATION-INDUCED DAMGE IN THE INTESTINAL MUCOSA OF MICE EXPOSED TO DIFFERENT DOSES OF γ-RADIATION

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Summary

Effect of 50 mg/kg body weight of leaf extract of jamun (Syzygium Cumini Linn. Skeels) was studied on the radiation-induced changes in the jejunum of mice exposed to 7, 10 or 15 Gy γ-radiation on day 1, 3 and 7 days post-irradiation. Histological examination of mouse jejunum on day 1, 3 or 7 revealed a dose dependent increase in the radiation-induced damage after exposure to 7, 10 or 15 Gy. Irradiation of mice to different doses of γ-radiation caused a significant reduction in the villus height and number of crypts per circumference accompanied by an increase in goblet and dead cells. The maximum lesions were observed on day 1 post-irradiation indicating a severe intestinal damage however, the signs of recovery were discernible at day 7 post-irradiation, where the radiation-induced lesions were lesser than day 1 post-irradiation. Treatment of mice with jamun extract before irradiation had a conducive effect as it elevated the villus height and the number of crypts and also reduced the goblet and dead cells when compared with the irradiation control. The recovery and regeneration was faster in jamun pretreated animals than the irradiation alone. The greatest damage was observed in the animals exposed to 15 Gy, where animals did not survive up to 7 days post-irradiation, whereas jamun pretreatment caused early recovery and reduced the symptoms of radiation-induced damage even at 3 days post-irradiation. Our study demonstrates that jamun extract protects the radiation-induced damage in the small intestine of mice and may be a useful to protect against the radiation-induced gastrointestinal damage.

Key words: Radiation, jamun, jejunum, mice, villi, crypt and goblet cells

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Introduction

Ionizing radiations have been successfully used to treat malignant tumors of different histological origin and stages, for several decades since the discovery of x-rays by Roentgen[1]. The success of radiation treatment totally depends on its ability to selectively kill tumor cells at the same time, spare normal tissues that are in the vicinity of tumors from the deleterious effects of radiotherapy in clinical situations. The cellular and molecular responses of mammalian cells against ionizing radiations are complex and irreversible depending on both the radiation dose and the tissue-weighting factor[2]. Gamma radiation is one of the most commonly used sources of ionizing radiations to treat neoplastic disorders in clinics. It is an electromagnetic, low linear energy transfer (LET) source that induce lesser direct damage to DNA, in comparison with high LET radiation like alpha particles, protons and fast neutrons[2]. The indirect effect by low LET radiation is the result of oxidative stress caused by reactive oxygen species produced due to radiolysis of water. This induces deleterious effects in important biomolecules that may be lethal to both quiescent and proliferating cells[2].

Acute whole body exposure to lethal doses of ionizing irradiation produces symptoms, collectively known as radiation sickness or prodromal syndrome. The intensity of radiation sickness, survival time and mode of death is directly proportional to the magnitude of the irradiation dose[2-5]. At very high doses, death occurs in a matter of hours and is the result of neurological and cardiovascular breakdown, and is known as cerebrovascular syndrome. At intermediate dose level, death occurs in a matter of days and is associated with extensive bloody diarrhea and destruction of the gastrointestinal mucosa, and it is termed gastrointestinal syndrome. At low dose level, it takes several weeks for radiation to cause death due negative alterations in the blood-forming organs, and this is called as hematopoietic syndrome [2, 4-8].

Normally, during radiotherapy of cancer, sub-lethal doses of radiations are used clinically that primarily affect the hematopoietic and gastrointestinal organs[2]. Sub-lethal doses of radiation cause impairment of bone marrow hematopoietic function leading to leucopenia, erythrocytopenia and thrombocytopenia, which ultimately predispose to infection, hemorrhage and death[6,7,2]. Despite the fact that use of radiation in treating localized gastrointestinal (GI) tumors is rare or when used is done with great caution, the GI is invariably exposed to radiation while treating colon, rectal, prostate and other closely linked sites.

The frequent use of radiotherapy results in an increased risk of radiation enteritis, the most feared and potentially life-threatening complication[9]. Radiation causes cell attrition of the gastrointestinal epithelium and progressively leads to ulceration. These cellular changes get manifested physiologically and result in nausea, anorexia, loss of electrolyte balance, diarrhea, bacterial infection and poor quality of the life for patients[10].

The gastrointestinal syndrome progression and consequent death are related to the degenerative changes induced in the intestinal mucosa ensuing irradiation[11,12,13]. Radiation induced death of an animal result due to GI injury characteristically within 3-10 days after irradiation[3, 14,8,4,12]. The gastrointestinal syndrome occurs mainly due to a failure of the crypts of Lieberkuhn, which maintain the cellular composition of the mucosal lining. Gastrointestinal syndrome may be manifested by four phases that include i) cessation of production of normal cells, ii) subsequent reduction in cell population of the mucosa, iii) destruction of the barrier between the intestinal lumen and the organism, and 4) finally death of animal[15]. The radiation-induced damage to gastrointestinal tract was first demonstrated by Walsh as early as in 1897, shortly after Roentgen's discovery of X-rays; He concluded that radiation `caused a direct inflammation of the gastrointestinal mucous membranes. However, the first systematic report of the effects of radiation on intestinal structure and function appeared 25 years later on dogs, where the effect of large doses of radiation was characterized[16,17,18].

The radiation-induces cytotoxic effects during radiotherapy, which can be reduced using certain pharmacological agents (radiosensitizers or radioprotectors), that can exploit the narrow therapeutic margin of radiation therapy, and enhance the therapeutic outcome. The radiosensitizing agents are meant to increase tumor's sensitivity to radiation without affecting the radiation response of normal tissues[19]. In contrast, radioprotectors safeguard normal tissues from the deleterious effects of ionizing radiation, while ideally affording no such protection to the tumor[2]. Due to lack of an effective protective agent, newer compounds are currently under investigation as possible adjuvants in the radiation treatment of cancer and herbal medicines have only recently begun to receive some attention as possible modifiers of the radiation response[20, 13]. It is necessary to evaluate potential pharmacological agents for radioprotection that could not only be useful during nuclear accident, space travel or nuclear terror attacks but also during radiotherapy of tumors [13].

Jamun, *Syzygium Cumini* Linn. Skeels (or Eugenia cumini Linn. Druce) families Myrtaceae has been shown to possess several medicinal properties. The bark of the jamun is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelmintic, febrifuge, constipating, stomachic, antibacterial, antioxidant, anti-inflammatory, antidiabetic and gastroprotective[21-25]. The fruits and seeds of jamun are useful in treating diabetes, pharyngitis, splenopathy, urethrorrhea and ringworm infection[26]. The leaves have been extensively utilized to treat diabetes, constipation[27], leucorrhoea, stomachache, fever, gastropathy, dermopathy and to inhibit blood discharges in the faeces[26,27]. Recent investigation from this laboratory has shown that the leaf extract of *Syzygium cumini* inhibit radiation induced micronuclei formation in the cultured human peripheral blood lymphocytes[28]. However, no attempt has been undertaken to investigate the effect of jamun treatment on the radiation-induced changes in the gastrointestinal epithelium of mice. Therefore, present study was undertaken to evaluate the effect of leaf extract of jamun on the histological alteration in the small intestine of mice exposed to different doses of gamma radiation.

Materials And Methods

Animal care and handling

The animals care and handling were carried out according to the guidelines of World Health Organization (WHO), Geneva, Switzerland, and the Indian National Science Academy, New Delhi, India (INSA), and the "Guide for the care and use of Laboratory Animals" (NIH publication #86-23, revised in 1985). Eight to ten week old male Swiss albino mice weighing 30±2 g were selected from an inbred colony maintained under controlled conditions of temperature (23±2°C), humidity (50±5%) and light (12 h of light and dark cycle). The animals were provided with sterile food and water *ad libitum*. Each polypropylene cage were housed with 5-6 animals containing sterile paddy husk (procured locally) as bedding throughout the experiment. The animals were euthanized after the termination of the experiment to avoid unnecessary suffering according to the WHO guidelines. The study was approved by the Institutional Animal Ethical Committee.

Preparation of the extract

The mature leaves of jamun i.e. *Syzygium cumini* Linn. Skeels (or *Eugenia cumini* Linn. Druce), family Myrtaceae were collected locally during the month of May, and were identified by Dr. G.K. Bhat (Department of Botany, Poorna Prajna College, Udupi, Karnataka, India). The leaves were cleaned, shade dried, and powdered and the extract was prepared as described earlier[29]. Briefly, the leaf powder was extracted in petroleum ether and chloroform and finally in 1:1 dichloromethane and methanol at 50-60°C using a Soxhlet apparatus[29]. The extract was cooled and concentrated by evaporating its liquid contents in vacuo and freeze dried. The extract was stored at -70°C until further use. Henceforth the extract of *Syzygium cumini* will be called as SCE.

Preparation of drug and mode of administration

The required amount of SCE was dissolved in 1% carboxymethyl cellulose (CMC) in sterile normal physiological saline. The animals were administered orally with SCE or CMC, consecutively for 5 days[30,5]. The drug was prepared freshly immediately before use and the animals were divided into following groups:

CMC + irradiation

The animals of this group were administered with 0.01 ml/g body weight of CMC orally before irradiation.

SCE + irradiation

The animals of this group were administered with 50 mg/kg body weight SCE orally once daily for 5 consecutive days before exposure to 7, 10 or 15 Gy of γ-radiation[30,5].

Irradiation

One hour after the last administration of CMC or SCE on 5th day, the prostrate and immobilized animals (achieved by inserting cotton plugs in the restrainer) were whole body exposed to 0, 7, 10 or 15 Gy of ^{60}Co gamma radiation (Theratron, Atomic Energy Agency, Canada) in a specially designed well-ventilated acrylic box. A batch of twelve animals was irradiated each time at a dose rate of 1.66 Gy/min.

Histological changes

The animals were euthanized by cervical dislocation at 1, 3, and 7 day post-irradiation and the jejunum was excised cleaned of intestinal contents with ice cold PBS, and was fixed in Bouin's fixative for histological studies. Five-micron thick sections were cut with a rotary microtome

(A.O. Scientific Instruments, USA) and spread in a temperature regulated tissue float. The sections were placed on to precleaned coded slides, stained with hematoxylin and eosin (H & E). Usually four to six slides were prepared from each animal. Detailed microscopic observations were made to assess the histological alterations produced in the jejunum in response to different treatments, both qualitatively and quantitatively. Qualitative studies included visual assessment of pathological changes in submucosal crypt and villus of the jejunal epithelium. The quantitative assessment was carried out by assessing the villus height, total number of crypts, number of goblet cells/villus section and the number of dead cells/jejunum section.

Statistical analysis

The significance between the treatments was carried out using student-'t' test within groups and ANOVA test was used for multiple comparisons.

Results

Qualitative changes

SCE treatment alone did not show any alteration/s in the histological architecture of jejunum when compared to the sham-irradiation group. A marked edema in the sub-mucosa with mild surface erosion was observable at all post-irradiation days in the animals exposed to 7, 10, and 15 Gy. The degree of damage to jejunal mucosa was dose dependent and the greatest damage was observed on day 1 post-irradiation after 15 Gy exposure. The villus and crypt architecture was distorted as is evident by depopulated and degenerating crypts (Fig 1). The tips of villi were also ruptured and goblet cells as well as dead cells increased in a dose dependent manner. Irradiation of mice to different doses of γ-radiation caused a reduction in the villus height and mitotic activity. The intestine showed a greater damage on day 1 when compared with day 7 post-irradiation after exposure to 7 and 10 Gy, whereas the degree of radiation-induced damage was more on day 1 when compared with day 3 in the animals exposed to 15 Gy. The administration of 50 mg/kg body weight of SCE increased the villus height and also maintained the crypt architecture when compared to CMC + irradiation group (Fig 2-4). SCE treatment resulted in a significant elevation in the mitotic activity, which was accompanied by a reduction in dead and goblet cells. The animals receiving SCE before irradiation exhibited recovery of radiation damage in jejunum on day 7 after exposure to 7 and 10 Gy and on day 3 after exposure

to 15 Gy as evidenced by increased number of crypts and villus height when compared with day 1 post-irradiation (Fig 2-4).

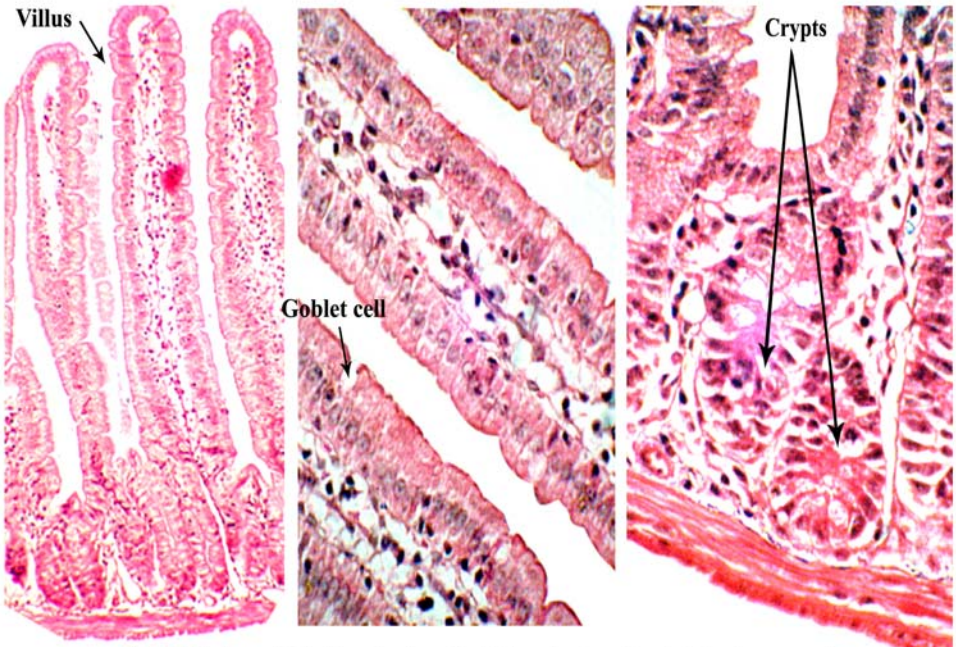

Fig 1. Histology of Intestine of mice with SCE pre-treatment and without exposure to gamma-irradiation (Sham - irradiation)

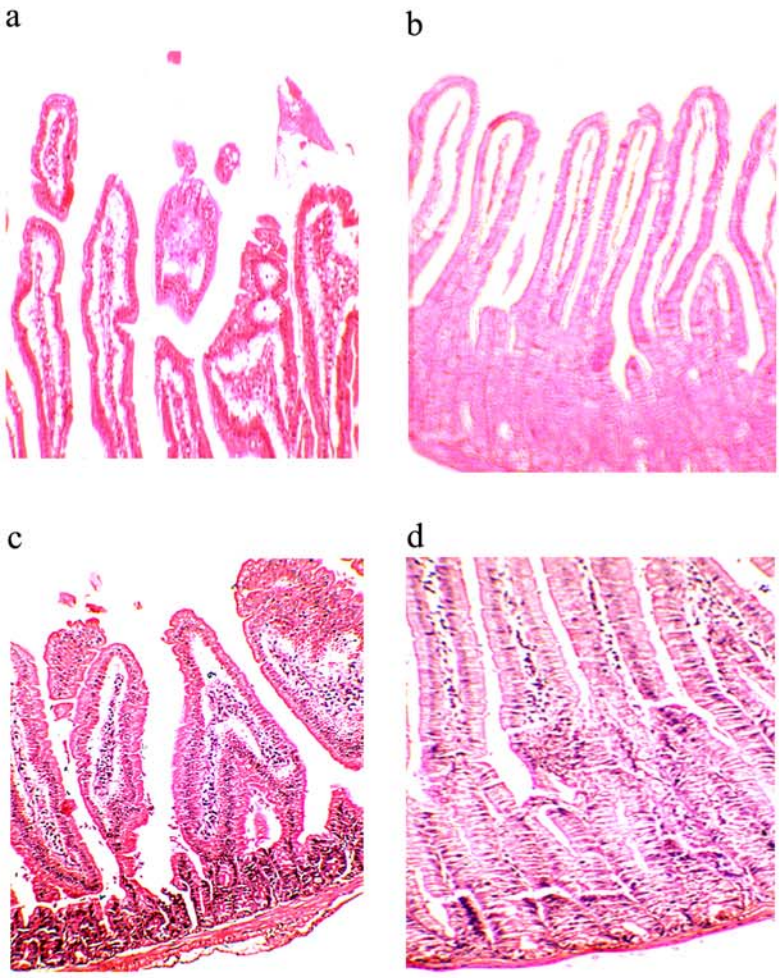

Fig 2. Histology of Intestine of mice treated with or without SCE before exposure to 7Gy gamma-irradiation.
a:CMC+ irradiation day 1, b:SCE + irradiation day 1,
c:CMC+ irradiation day 7, d:SCE + irradiation day 7.

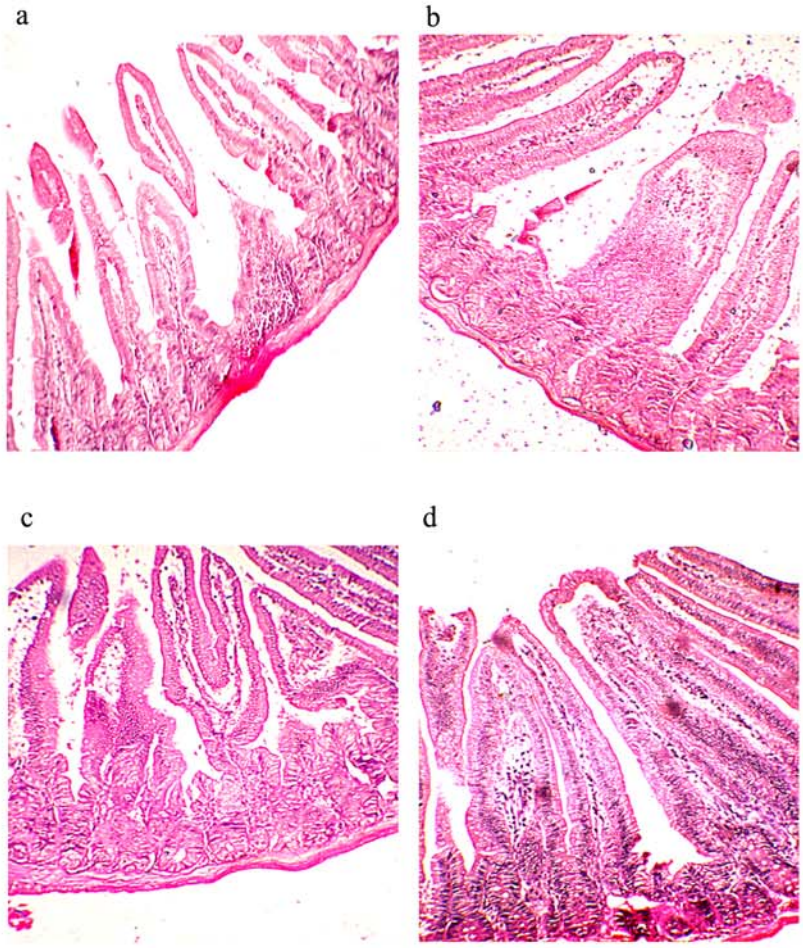

Fig 3. Histology of Intestine of mice treated with or without SCE before exposure to 10Gy gamma-irradiation.
a:CMC+ irradiation day 1, b:SCE + irradiation day 1,
c:CMC+ irradiation day 7, d:SCE + irradiation day 7.

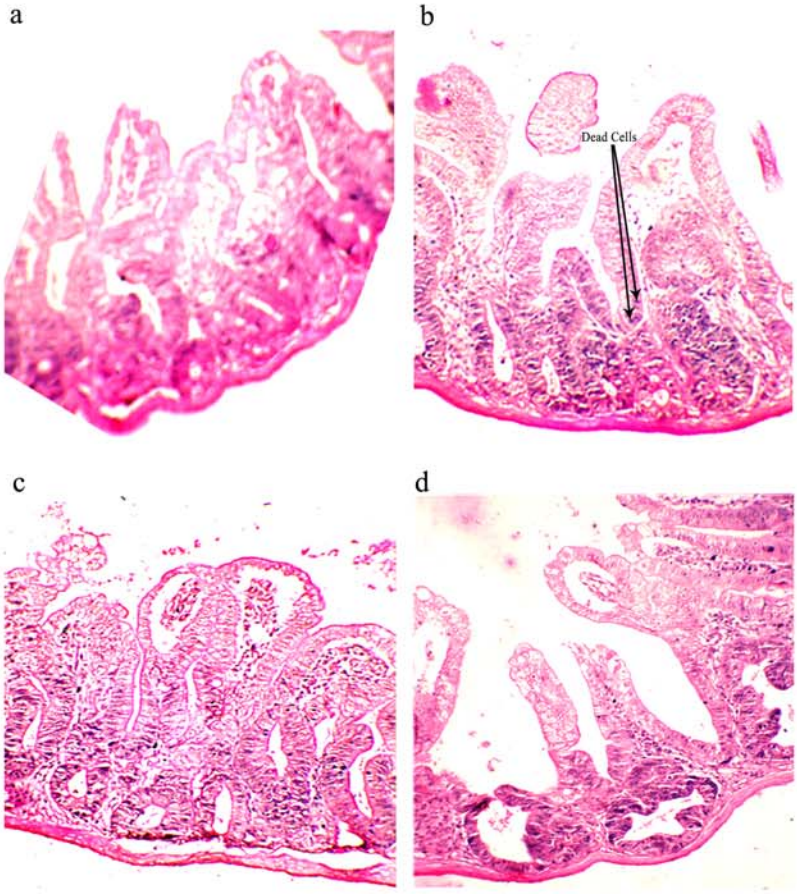

Fig 4. Histology of Intestine of mice treated with or without SCE before exposure to 15Gy gamma-irradiation.
a:CMC+ irradiation day 1, b:SCE + irradiation day 1,
c:CMC+ irradiation day 7, d:SCE + irradiation day 7.

Quantitative changes

Villus length

Irradiation resulted in a significant ($p<0.001$) and dose dependent decline in the villus height on day 1 and 7 post-irradiation when compared to sham-irradiation group, except for the 15 Gy, where complete degeneration of villi was observed on day 1 post-irradiation. Analysis of villus height on day 7 post-irradiation showed signs of recovery as the villus height increased significantly when compared to day 1 in the jejunum of mice exposed to 7 and 10 Gy (Table 1). Treatment of mice with SCE alone did not significantly (Table 1) alter the villus height (489±10.69-498±15.37) when compared to the CMC + sham-irradiation group (483±10.81-491±18.35). Administration of mice with 50 mg/kg body weight of SCE elevated the villus height significantly ($p<0.05$, 0.01 or 0.001) after 7, and 10 Gy irradiation on day 1 and 7 post-irradiation when compared with CMC + irradiation group. Likewise, SCE pretreatment also increased the villus height after 15 Gy irradiation on day 1 and 3 post-irradiation in comparison to concurrent CMC+irradiation group. The basal villus height could not be restored completely in SCE + irradiation group even at day 7 post-irradiation after exposure to 7 and 10 Gy (Table 1).

Table 1. The changes in the villus height of the intestinal mucosa of mouse treated with 50mg/kg SCE before exposure to 7, 10, & 15 Gy γ-radiations.

Exposure dose (Gy)	**Villus height (μm)** (Mean ± SEM)			
	Day 1		Day 7	
	CMC+IR	SCE+IR	CMC+IR	SCE+IR
0	483 ± 10. 81	489 ± 10. 69	491 ± 18.35	498 ± 15.37
7	332 ± 10. 40^{c*}	372 ± 4.58^{a*}	352 ± 10.53^{a*}	387 ± 11.50^{ar*}
10	281 ± 12. 09$^{c@*}$	314 ± 7.00$^{a\$*}$	330 ± 7.93^{cr*}	334 ± 7.90$^{ar@*}$
***15	0	18.33 ± 4.10	0	40 ± 5.13^{r}

***Since animals do not survive up to 7 days in 15 Gy exposure dose, the data shown are for 1 and 3 days only.**

a: $p<0.05$, **b:** $p<0.01$, **c:** $p<0.001$ No **s**ymbol **:** Non-**s**ignificant, when compared with control **r:** $p<0.05$, **s:** $p<0.01$, No **s**ymbol **:**Non **s**ignificant, when compared with day 1 group

@: $p<0.05$, **$:** $p<0.01$, ***p<0.001** No **s**ymbol**:** Non-**s**ignificant when compared it by ANOVA.

Crypt number

SCE treatment alone did not alter total number of crypts per jejunum section in SCE + sham-irradiation group when compared to CMC + sham-irradiation group (Table 2). Irradiation of mice to 7, 10 and 15 Gy irradiation caused a drastic but dose dependent decline in the number of crypts per jejunum section when compared with CMC + sham-irradiated group ($p<0.001$). This decline in crypt number was approximately two fold for 7 and 10 Gy, whereas 7 folds after 15 Gy exposure on day 1 post-irradiation (Table 2). A regeneration of crypts was evident on day 7 post-irradiation when compared to day 1 post-irradiation as the number of crypts per section increased by 1.7 and 1.2 fold after exposure to 7 and 10 Gy, respectively. Administration of 50 mg/kg body weight of SCE resulted in a significant elevation in the crypt number on day 1 ($p<0.01$) and 7 ($p<0.01$) post-irradiation, when compared with the CMC+irradiation group. Similarly, SCE also increased crypt number significantly on day 7, when compared to day 1 post-irradiation after exposure to 7 or 10 Gy. However, this effect was not discernible for 15 Gy in SCE+irradiation group on day 1 and 3 post-irradiation (Table 2).

Table 2. The changes in the number of crypts of the intestinal mucosa of mouse treated with 50mg/kg SCE before exposure to 7, 10, & 15 Gy γ-radiations.

Exposure dose (Gy)	**No. of Crypts** (Mean ± SEM)			
	Day 1		Day 7	
	CMC+IR	SCE+IR	CMC+IR	SCE+IR
0	95.66 ± 6.50	99.66 ± 7.76	91.33 ± 11.01	94.33 ± 6.80
7	$47.33 \pm 4.04^{c*}$	$69.33 \pm 1.52^{b@}$	71.33 ± 9.71^{r}	81 ± 2.00^{s}
10	$40.00 \pm 1.00^{c*}$	$54.00 \pm 6.20^{a*}$	$48.00 \pm 2.00^{br@}$	$64.00 \pm 7.02^{a@}$
✱15	$14.00 \pm 1.00^{c*}$	13.89 ± 6.20	$15.34 \pm 2.00^{c*}$	14.42 ± 7.02

✱Since animals do not survive up to 7 days in 15 Gy exposure dose, the data shown are for 1 and 3 days only.

a: $p<0.05$, **b:** $p<0.01$, **c:** $p<0.001$ No **s**ymbol **:** Non-**s**ignificant, when compared with control

r: $p<0.05$, **s:** $p<0.01$, No **s**ymbol **:**Non **s**ignificant, when compared with day 1 group

@: $p<0.05$, **$:** $p<0.01$, *****$p<0.001$ No **s**ymbol**:** Non-**s**ignificant when compared it by ANOVA.

Goblet cell

Administration of SCE alone did not significantly modulate the number of goblet cells when compared with the CMC + irradiation group. Exposure of animals to different doses of γ-radiation resulted in a dose dependent increase ($p<0.01$) in the number of goblet cells on day 1, which declined on day 7 post-irradiation in the CMC + irradiation group (Table 3). Administration of 50 mg/kg of SCE to mice before irradiation caused a marginal but non-significant decline in the goblet cell number on day 1 ($p<0.05$), whereas this reduction in goblet cells was non-significant on day 7 post-irradiation, when compared to CMC+irradiation group (Table 3). SCE administration before 15 Gy reduced the number of goblet cells significantly ($p<0.05$) on day 1 as well as day 3 post-irradiation. Despite this decline the number of goblet cells could not restored completely to baseline level by day 3 and 7 post-irradiation in SCE+irradiation group (Table 3).

Table 3. The changes in the number of goblet cells of the intestinal mucosa of mouse treated with 50mg/kg SCE before exposure to 7, 10, & 15 Gy γ-radiations.

Exposure dose (Gy)	**No. Of Goblet Cells** (Mean ± SEM)			
	Day 1		Day 7	
	CMC+IR	SCE+IR	CMC+IR	SCE+IR
0	5.33 ± 3.00	6.66 ± 1.52	4.66 ± 1.52	6.33 ± 2.51
7	13.98 ± 0.98^{c}	9.66 ± 1.01^{a}	7.66 ± 1.52	7.33 ± 2.30
10	$18.33 \pm 1.76^{b\$}$	$14.00 \pm 1.15^{a@}$	$15.00 \pm 1.53^{b@\$}$	12.67 ± 1.20
✱15	22.19 ± 1.20^{b}	17.67 ± 1.20^{a}	20.64 ± 0.22^{c}	13.33 ± 1.76^{br}

✱Since animals do not survive up to 7 days in 15 Gy exposure dose, the data shown are for 1 and 3 days only.

a: $p<0.05$, **b:** $p<0.01$, **c:** $p<0.001$ No **s**ymbol **:** Non-**s**ignificant, when compared with control **r:** $p<0.05$, **s:** $p<0.01$, No **s**ymbol **:**Non **s**ignificant, when compared with day 1 group

@: $p<0.05$, **$:** $p<0.01$, **✱**$p<0.001$ No **s**ymbol**:** Non-**s**ignificant when compared it by ANOVA.

Dead (Apoptic) cells

Apoptosis in the jejunal crypt cells was scored on the basis of pycnotic nuclei, marginal condensation of the chromatin, fragmentation of the nuclear material and fragments extruding into the crypt lumen (Potten *et al.,* 1994; Meritt *et al.,* 1995). Dead cells were completely absent in the CMC+sham-irradiation and SCE+sham-irradiation groups (Table 4). Irradiation induced a significant increase in the number of dead cells on day 1 ($p<0.01$) and 7 ($p<0.05$) post-irradiation after 7, 10 Gy exposure. Similarly, 15 Gy exposure caused an almost 13- and 11 fold increase in number of dead cells on day 1 ($p<0.01$) and 3 ($p<0.001$) post-irradiation, respectively. Oral administration of SCE for five consecutive days before irradiation significantly decreased the dead cells on day 1 ($p<0.01$), in the jejunum of mice exposed to 7, 10 or 15 Gy, whereas this decline was significant on day 7 ($p<0.05$) post-irradiation after 10 Gy exposure (Table 4). The dead cells continued to exist in SCE + irradiation group even up to day 7 (day 3 for 15 Gy) post-irradiation in the jejunum of mice exposed to 7 and 10 Gy (Table 4).

Table 4. The changes in the number of dead cells of the intestinal mucosa of mouse treated with 50mg/kg SCE before exposure to 7, 10, & 15 Gy γ-radiations.

Exposure dose (Gy)	**No Of Dead Cells** (Mean ± SEM)			
	Day 1		Day 7	
	CMC+IR	SCE+IR	CMC+IR	SCE+IR
0	0	0	0	0
7	4.33 ± 0.57	2.66 ± 1.15	1.33 ± 0.57^{s}	1.14 ± 0.57
10	9.00 ± 1.00	6.66 ± 0.57$^{a\$}$	4.66 ± 1.52$^{r@}$	4.33 ± 1.52$^{@}$
✱15	13.08 ± 0.11	11.39 ± 0.19^{c}	11.08 ± 0.56^{r}	10.71 ± 0.34

✱Since animals do not survive up to 7 days in 15 Gy exposure dose, the data shown are for 1 and 3 days only.

a: $p<0.05$, **b:** $p<0.01$, **c:** $p<0.001$ No **s**ymbol **:** Non-**s**ignificant, when compared with cont**r**ol

r: $p<0.05$, **s:** $p<0.01$, No **s**ymbol **:**Non **s**ignificant, when compared with day 1 group

@: $p<0.05$, **$:** $p<0.01$, ***p<0.001** No **s**ymbol**:** Non-**s**ignificant when compared it by ANOVA.

Discussion

Ionizing radiation is one of the important modality to treat cancer and in many cases it is the only tool that is used to treat neoplastic tumors. The ionizing radiations kill cells by inducing efficient DNA-damage with a high spatial specificity. However, it does not distinguish between the neoplastic and normal cells as a result radiotherapy is always accompanied by severe side effects on the normal cells[31]. The cells with a higher rate of proliferation like gastrointestinal tract and hematopoietic system are more prone to radiation-induced damage[3] and need protection during radiotherapy. Despite great efforts that have been made to protect normal tissues during radiation treatment, some contact is unavoidable with the adjacent normal cells causing side effects. Hence, like chemotherapy, radiotherapy and their selection in clinics and persistence involves a careful monitoring of its effect on tumor and the normal cells[32,33]. Use of certain pharmacological agent may of great help to neutralize the deleterious effect of ionizing radiation in clinical conditions or even otherwise. Therefore, present study was undertaken to investigate the radioprotective effect of jamun extract in the small intestine of mouse exposed to different doses of γ-radiation.

Despite the fact that most adult mammalian tissues show resistant to ionizing radiations and do not undergo morphological or physiological alterations even after treatment with high doses, the acute radiation reactions are usually observed in proliferating “renewing” tissues accompanied by a reduction in the number of functional parenchymal cells. Death induced by lethal dose of ionizing radiations is caused by the failure to repair radiation-induced damage in a few sensitive tissues including the haematopoietic system and epithelium of the small intestine and differential sensitivity of most tissues to ionizing radiations is directly linked to their proliferation rates[2, 3].

The whole-body irradiation mainly affects rapidly proliferating germinal epithelium, gastrointestinal epithelium, and bone marrow and spleen progenitor cells. While the germinal epithelium does not have a life supporting function for the exposed individual, but the bone marrow, spleen progenitor cells and gastro-intestinal epithelium cells are crucial for the sustenance of life, and any damage to these cells will disturb the normal physiological host defense processes drastically, causing an adverse impact on survival of individual[2,3,8,4,34]. Of the two organs, the gastrointestinal epithelium is less sensitive than the bone marrow progenitor cells

but as the cell transit time is quick; the damage in the gastro intestinal tract is expressed earlier than the hematopoietic syndrome[3, 10].

The gastrointestinal tract is a cell renewal system and consisting of cells with different radiosensitivity. According to Withers and Elkind[35] crypt cells are more sensitive than villus epithelial cells as indicated by the presence of more severe pathological lesions in crypts than those of villi at early intervals. Identical observations are made in the present study, where reduction in number of crypts per section declined with increasing dose of radiation in the intestinal mucosa exposed to 7, 10, and 15 Gy of γ–rays with a maximum decline on day 1 post-irradiation. The proliferative cells in the intestine are situated at the base of the villi in crypts, where villus-cell production originates and injury to the intestine can result in the destruction of the crypt-cell population. An identical effect has been observed earlier, where a reduction in crypt cell division and cellularity has been reported in the first 24 h after irradiation[36,37,12]. The migration of cells from crypt to villus, cell death, and lack of mitosis after irradiation may be the cause of reduced cellularity[36,37]. Several authors have reported a decrease of cells in the crypt region after irradiation[38-46,12,34]. The ionizing radiation reduces DNA synthesis, mitotic activity and induces apoptosis of rapidly replicating transit cells of crypts or stops their replication that leads to the decline in the crypt cell number[47,48,49]. Further, movement of crypt cells into the villus region may also be responsible to some extent in the reduction in crypt cells. This is reflected as a reduction in the number of crypts per section in the present study with a maximum decline on day 1 post-irradiation.

The crypts are the most important part of intestine, where the epitielial cells are born migrate up the walls of the villi, and are finally sloughed from the tip of the villi into the lumen, thus maintaining a dynamic steady state in normal conditions[50-53,11,34]. However, irradiation disrupted this state leading to a marked edema in the intestinal sub mucosa with mild surface erosion, distorted architecture of villus along with depopulated and degenerating crypts. A similar effect has been observed earlier[11,54-58,35]. Degenerative changes in the mouse intestinal crypt and decline in the villus height after different doses of gamma radiations is similar to that observed earlier by various workers[59,60,56,57,43,12,34]. Radiation induced-damage to the villi may be due to the alterations in the epithelial cells and underlying stroma leading to diminished or collapsed villi[61]. This may also be one of the reasons for reduction in the villus height. The SCE pre-treatment protected against the radiation-induced damage to the crypt cell as is evident by

increased number of crypts per section on day 7 post-irradiation indicating that SCE pretreatment did alter the sensitivity of clonogenic stem cells favourably. This may have led to an increase in the long-term regenerative capacity of the intestinal epithelium, and thus increased the animal survival in SCE+irradiation group. These results indicate that SCE may have clonogenic stem cell modulatory effect, and may be useful in clinical situations in reducing the radiotherapy-induced damage. SCE pretreatment protected mice against radiation-induced reduction in the villus height as is evidenced by a significant increase in the villus height in the drug treated group when compared to CMC + irradiation group at day 1 and 7 post-irradiation. Several other agents like AET, WR-2721, beta-carotene, *mentha piperita*, MPG and *Aegle marmaelos* have been reported to protect small intestine of mouse exposed to irradiation[15,62-68,40,43,12]. Intestinal mucosa possess a marked capacity to recover after irradiation and the recovery of intestine is faster at lower doses (0.5-1.5 Gy) than at higher doses (>1.5 Gy), which takes longer time to restore the damage. The complete recovery of the crypt cells was not seen during the observation periods in CMC + irradiation group, whereas in SCE + irradiation group the rate of recovery in crypts on day 7 was better than the former group. An identical effect has been reported earlier with WR-2721 and *Aegle marmaelos*[40,12].

The goblet cells are quantitatively the most important cells in the small intestine epithelium after the coloumnar cells. Goblet cells secrete mucus, which protects the mucosal lining. The neutral and acidic mucopolysaccharides secreted by goblet cells protect the intestinal epithelium from the intestinal micro-flora and toxins[69]. Irradiation of mouse to different doses of γ-radiation increased the number of goblet cells on day 1 that declined on day 7 post-irradiation in CMC + irradiation group. Further, ionizing radiation also changes the morphological structure of the goblet cells[70]. The goblet cells after irradiation elicit response in three phases: an initial phase of increase in number, followed by a II phase of reduction and finally the III phase, where goblet cells attempt to return to normal levels when compared with un-irradiated subjects. The SCE pretreatment might have accelerated the regeneration process and thus helped in the restoration of normal number of goblet cells on day 7 post-irradiation. Irradiation of mouse to different doses of γ-radiation increased the number of dead cells on day 1 that declined on day 7 post-irradiation in CMC + irradiation group. The levels of radiation-induced cell death can be assessed by counting the number of cells undergoing nuclear pycnosis or karyorrhexis in histological preparations. The incidence of dead or dying cells can be expressed as the number of

pycnotic or apoptotic cells. Apoptosis has been described in detail elsewhere[71-75] and was originally used to describe processes involving programmed cell deletion from a tissue. The SCE pretreatment might have helped in the acceleration of removal of dead cells and restoration to normal level on day 7 post-irradiation.

The exact mechanism of action of SCE is not known. The radioprotective action of SCE may not be due to single mechanism but may be due to operation of multiple mechanisms. Ionizing radiation induces free radicals that eventually lead to lipid peroxidation and damage to cellular genome and cell death[76,77,78,79]. Hence scavenging of radiation-induced free radicals by SCE may be one of the important mechanisms to reduce radiation induced DNA damage to crypt cells and increasing crypt survival and reducing the damage to intestine. This contention is supported by the in vitro results (data not shown), where SCE scavenged $^{\bullet}OH$, $O_2^{\bullet -}$, DPPH and $ABTS^{\bullet +}$ free radicals and inhibited lipid peroxidation in a concentration dependent manner. Further, some of the flavonoids including quercetin, kaempferol and myricetin, which are present in the SCE, have been also reported to scavenge free radicals like OH, $O_2^{\bullet -}$, and inhibit lipid peroxidation earlier[80,81,82]. Similarly, kaempferol and quercetin have been reported to suppress the cytotoxicity of superoxide ion and hydrogen peroxide in Chinese hamster V79 cells[83,84]. The polyphenol ellagic acid, which is also present in SCE, has been reported to be antimutagenic, chemopreventive[85], antioxidant and it has also been found to inhibit the radiation-induced lipid peroxidation in the liver of mice[86]. The presence of flavonoids and ellagic acid in SCE extract might have been responsible for the observed radioprotection in mice intestine. The presence of SCE before irradiation may have also increased the antioxidant status of mice (data not shown) thus resulting in the protection of gastrointestinal mucosa. Although no attempts have been made to investigate the molecular mechanisms however, there is no reason to believe that SCE may not have acted using molecular pathways to exert its radioprotective action, since radiation has been reported to induce the transcriptional activation of NF-κB[87], COX-II, and LOX[88], which have been associated with inflammation and oxidative stress[89,90] including DNA damage. SCE pretreatment may have blocked the activation of NF-κB, COX-II at mRNA level and LOX, thus protecting against the radiation-induced DNA damage and increased the survival of crypts. Quercetin, a flavonoid present in SCE has been reported to inhibit the activation of NF-κB and COX-II mRNA[91,92]. Myricetin, another flavonoid present in SCE has been reported to increase the expression of DNA polymerase beta gene in a dose dependent manner, an enzyme

responsible for the error-free DNA repair[82], which may have helped to increase the crypt survival. The protective effect of SCE on mouse jejunum may be due to free radical scavenging, increased antioxidant status and inhibition of inflammatory response.

This study demonstrates that jamun has protected against the radiation-induced damage to intestinal mucosa and crypts. The protection may have been due the capacity of jamun extract to scavenge free radicals including lipid peroxidation and increased antioxidant status. Jamun may have also inhibited the activation of NF-κB and COX-II mRNA. It may have also upregulated DNA polymerase and efficiently repaired the lesions induced by radiation in the cellular genome and thus protected against the radiation-induced damage to intestinal crypts and eventually villi.

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