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# MUSHROOM POLYSACCHARIDE PROTECTS RADIATION INDUCED INTESTINAL DAMAGE IN MICE

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## ABSTRACT

*The in vivo radioprotective effect of a polysaccharide (PS) isolated from the mushroom Ganoderma lucidum, against radiation (RT) induced intestinal damage was investigated. Young adult swiss albino mice were whole body exposed to gamma radiation. PS was administered orally (10 mg/kg body wt and 20 mg/kg body wt) 5 min after 4 Gy exposure. The radioprotective effect of PS was compared with that of clinically used radioprotective drug amifostine (WR - 2721), at 300 mg/kg body wt administered intraperitoneally, 30 min before irradiation. PS is not toxic at the radioprotective dose. Damage to the jejunum was observed in histopathological studies after radiation exposure. Administration of PS and amifostine offered protection against the intestinal damage. The level of GSH (reduced glutathione) in the jejunal mucosa was decreased after irradiation exposure. The depleted level of GSH in jejuna mucosa was restored significantly by polysaccharide and amifostine administration. Similarly serum MDA level was maintained normal by PS and Amifostine administration compared to radiation alone treated group. PS seems to have potential for use in protection against planned and unplanned radiation exposures. PS can be developed to an effective natural radioprotector without any side effects, after further studies.*

## KEY WORDS

*Radioprotection, Ganoderma lucidum, Polysaccharide, gamma radiation*

## INTRODUCTION

*Ganoderma lucidum,* commonly known as Reishi in Japan and Ling Zhi in China, is well known for its medicinal properties. *G.lucidum*contains a number of compounds among which the polysaccharides and triterpenoids have been identified as the major active components. Crude orpartially purified polysaccharides of *G.lucidum*have been reported to inhibit tumor metastasis in mice [1]. The immunomodulating property of this mushroom provides a promising approach for cancer prevention and its administration is found useful alone or in combination with chemotherapy and radiotherapy [1]. Our earlier studies suggest that the aqueous extract of this mushroom has significant radioprotective activity *ex vivo* [2]. Polysaccharides are among the major source of pharmacologically active constituents of the aqueous extract. Polysaccharides from *G.lucidum*was reported to markedly restore the mitotic activity of bone marrow cells that has been suppressed by anti-neoplastic drugs [3]. The present study was undertaken to examine the protection offered by a polysaccharide from *G.lucidum*against radiation induced intestinal damage in mice.

## MATERIALS AND METHODS

### Chemicals

Trichloroacetic acid, Sodium hydroxide, Disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate were purchased from sigma chemicals. All other chemicals used in the study were of analytical grade obtained from reputed local manufactures.

### Animals

Swiss albino mice, 6-8 weeks of age and weighing 26 ± 2 g, were used for the study. They were maintained in air-conditioned animal house and fed on standard mouse food and water ad libitum. Animal handling and experiments were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by Institutional Animal Ethics Committee.

### Isolation of Polysaccharide

The fruiting bodies of *G.lucidum*were collected from the outskirts of Thrissur district, Kerala,South India. The type specimen was deposited in the herbarium of Centre for Advanced Studiesin Botany, University of Madras, Chennai, India (HERB. MUBL. 3175). PS was isolated by themethod of Mizuno [4] with slight modification [5]. The confirmation of PS was done by Anthrone [6] and phenol sulphuric acid test [7]. Structural confirmation of PS was done by IR and NMR spectrum which were recorded at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Mumbai, India. The HNMR spectrum suggested that component sugars have beta configuration [5] (Pillai, 2009).The molecular wt of PS was determined by Gel filtration chromatography and was found to be 1.5 x $10^6$ Daltons. The Polysaccharide was subjected to acid hydrolysis with Tri fluoro acetic acid and monosaccharide analysis was done using paper chromatography. They monosaccharide units were found to be glucose, rhamnose and mannose. The powder was dissolved in double distilled water and administered orally in the experiments.

### Irradiation

The cobalt therapy unit with Gamma Cell 220 (AECL, Canada) facility of Amala Cancer Hospital, Thrissur was used for irradiation. Whole body irradiation to mice was given to anaesthetized animals, which were kept in well-ventilated Perspex boxes and was exposed at a dose rate of 1 Gy/min.

## EXPERIMENTAL DESIGN

### Histopathology studies

Five groups of 6 animals each were used

Group I – Normal (Double Distilled Water)

Group II – Radiation alone (4 Gy)

Group III – Amifostine (300 mg/Kg body wt) + Radiation 4 Gy

Group IV - Radiation 4 Gy + Polysaccharides (10 mg/ Kg body wt)

Group V - Radiation 4 Gy + Polysaccharides (20 mg/ Kg body wt)

Animals were dissected after 24 hrs after irradiation. The jejunal part of small intestine was excised and processed for routine histology. The tissue was fixed in Bouin's fixative for 24 hr, dehydrated in alcohol grades, cleared in xylene, and embedded in paraffin using a semi-automatic tissue embedding system (AO Histostat, USA). Routine 5 µm thick sections were cut, fixed on clean glass slides, stained with hematoxylin-eosin, and mounted in DPX.

### Estimation of GSH in jejunal mucosa and serum MDA

Five groups with 15 animals each were used for the study. The animals were treated as above.

3 Animals in each group were dissected in alternate days from 1 to 9 ($1^{st}$ , $3^{rd}$ , $5^{th}$ , $7^{th}$ , and $9^{th}$ ) after radiation exposure. The jejunal part of

small intestine was excised and cut opened. The mucosal part was scrapped smoothly and processed for tissue GSH estimation. Reduced glutathione (GSH) in tissue was determined by the method of Moron et al, (1979)[7]. 0.5ml of tissue homogenate was mixed with 0.1 ml of 25% TCA and kept on ice for few minutes and then subjected to centrifugation at 3000 rpm for few minutes to settle the precipitate. 0.3ml of the supernatant was mixed with 0.7ml of 0.2M sodium phosphate buffer (pH8). The yellow color obtained was measured after 10 min at 412 nm using a UV visible spectrophotometer against a blank which contained 0.1 ml of 5 % TCA in place of the supernatant. A standard graph was prepared using different concentrations of GSH in 0.3 ml of 5% TCA. The GSH content was calculated with the help of this standard graph and expressed as n mole/mg protein.

**Estimation of serum MDA**

Five groups with 15 animals each were used for the study. The animals were treated as mentioned for Histopathology.3 Animals in each group were dissected in alternate days from 1 to 9 ($1^{st}$,$3^{rd}$,$5^{th}$,$7^{th}$, and $9^{th}$) after radiation exposure. Serum lipid peroxidation was determined by Ohkawa et al after precipitating the protein according to the method of Satoh [8].To 0.5 ml serum, 2.5 ml of 0.02% TCA was added and the tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rpm for 10 min, the precipitate was washed. A 4ml reaction mixture containing 0.4 ml of serum, 1.5 ml of 0.8% TBA, 1.5 ml of acetic acid (20% pH 3.5) and distilled water was kept for 1 hr in a boiling water bath at $95^0$ C. After 1hr, the reaction mixture was removed from water bath, cooled and added 1 ml of distilled water. 5 ml of butane l: pyridine mixture (15:1) was added to the reaction tube, mixed thoroughly and centrifuged at 3000 rpm for 10 min. Absorbance of the clear supernatant was measured at 532 nm against butanol: pyridine mixture. The MDA was calculated with the help of a standard graph made by using different concentrations (1-10 nano moles) of 1'1'3'3 – tetramethoxy propane in 1 ml distilled water and is expressed as n moles/ml.

## RESULTS

Damage of jejunum was observed at 4 Gy gamma irradiation. Histopatholgy of the jejunam after 24 hrs of irradiation showed apoptosis, necrosis, cytoplasmic degrannulation and vacuolization and mitotic arrest. Villi became short and blunt with severe depopulation and flattening of cells, and their tips were ruptured (**Figure.1**). Denuded cells and cellular debris could be found in the lumen of the intestine. Administration of PS and amifostine reduced the damage of villi in the animals compared to radiation alone treated ones.

GSH in jejunum decreased from $5^{th}$ day onwards. The depleted level of GSH in jejunam was restored significantly by PS at 20mg/kg body wt on $7^{th}$ day and $9^{th}$ day after irradiation. Amifostine also restored the levels of GSH significantly at 300mg/kg body wt on 7th day (**Figure.2**). The highest value was found on $7^{th}$ and $9^{th}$ day after treatment.

Serum MDA was found to increase on $1^{st}$ day onwards in RT alone treated animals. Significant decrease in the MDA levels was observed after PS administration from $5^{th}$ day onwards compared to RT alone treated group. Amifostine also reduced the MDA levels significantly on $5^{th}$ day compared to RT alone treated group (**Figure.3**).

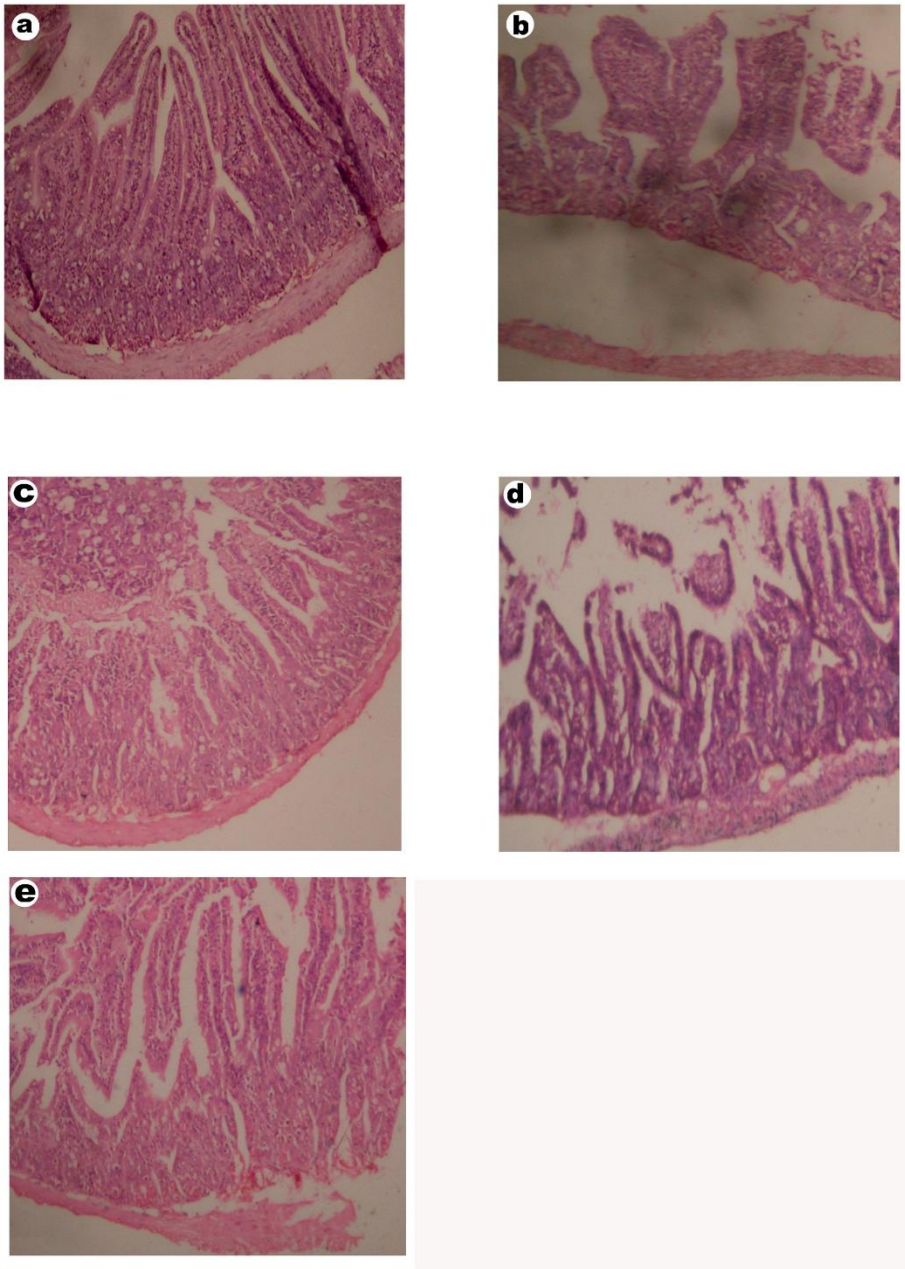


Figure.1. Effect of PS and amifostine on the damage of jejunam in mice exposed to 4 Gy gamma radiation.
a. Normal b.Radiation alone c. Radiation + amifostine
d. RT + PS (low dose) e. RT + PS (High dose)

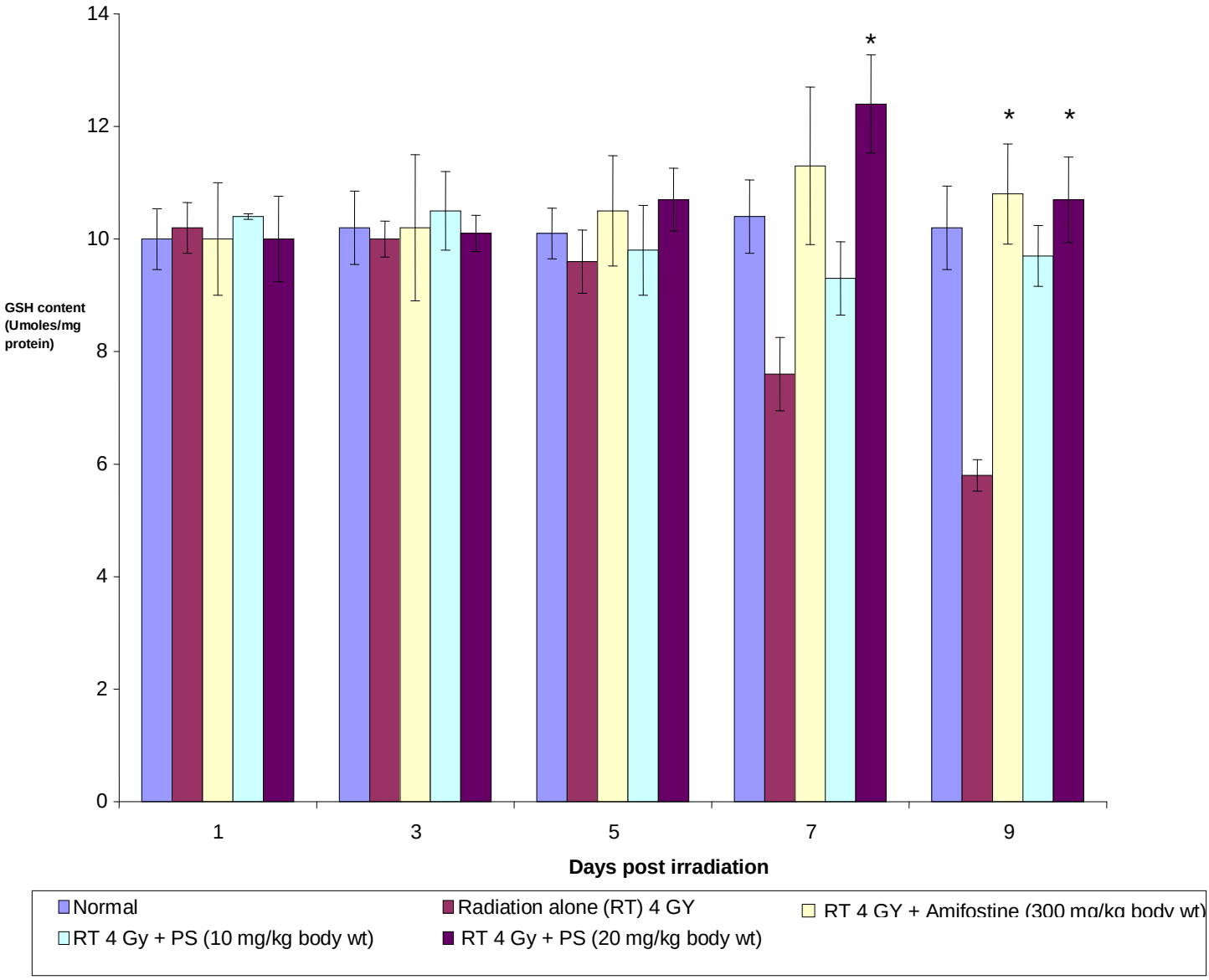


**Figure 2: Effect of polysaccharides on GSH in jejuna mucosa of mice exposed to 4 Gy gamma irradiation**

***p < 0.01 compared to radiation alone**

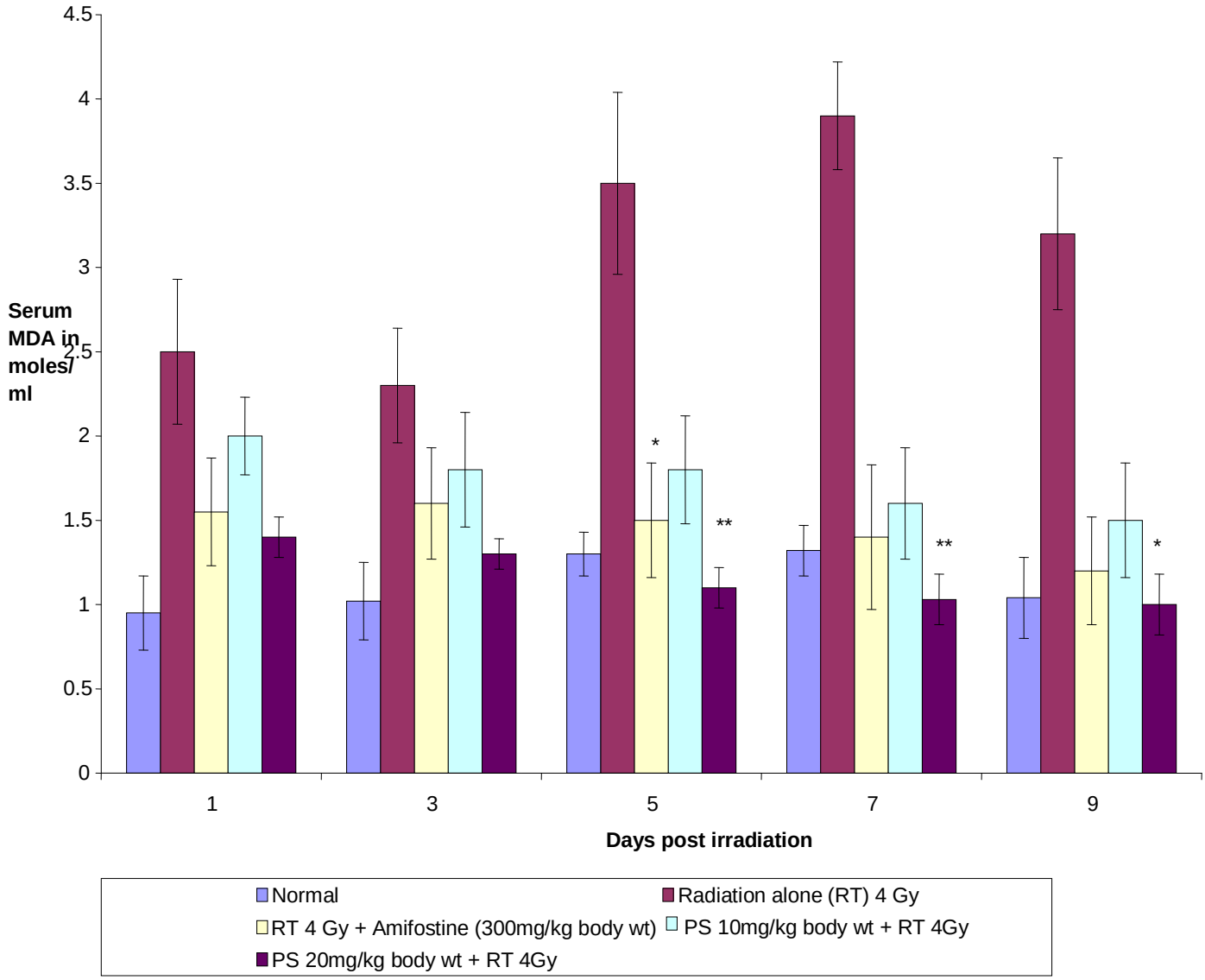


**Figure 3: Effect of polysaccharides on serum MDA of mice exposed to 4 Gy gamma Irradiation.**

***p < 0.01 compared to radiation alone**

**** p< 0.05 compared to control**

## DISCUSSION

Severe damage of the jejunum was observed in animals after radiation exposure in the present study. Administration of PS and amifostine reduced the damage of jejunum. The small intestine represents one of the major dose limiting normal tissues in radiotherapy because of its high sensitivity to radiation. Radiation kills the proliferating cells and inhibits mitosis, leading to depopulation of the crypt which in turn leads to severe loss of water and electrolytes. Damage of villi will lead to intestinal bleeding. This in conjunction with reduced water absorption will result in bloody diarrhea. Unless the crypt mucosa regenerates and supplies healthy cells to the villus, it will lead to

gastrointestinal death. The old cells at the tip of villi are continuously sloughed off and new cells produced by the crypts take their place. Crypt cells by virtue of their active division have a high capacity for repair. Our earlier studies have demonstrated that PS has the capacity to enhance DNA repair in human lymphocytes [5].

A significant reduction in GSH in radiation exposed animals was observed. This could be due to the enhanced utilization of antioxidant defense system in an attempt to detoxify the radicals generated by radiation. In the intact and healthy cells the enzymes are restored immediately after each interaction and GSH is also restored by synthesis [10]. But in the irradiated animals, the normal synthesis/repair will be disrupted due to damage to DNA and membranes. As a result, restoration will be delayed till the cells are recovered. This could explain the slow recovery in the levels of GSH and antioxidant enzymes after radiation treatment. The GSH detoxification system is an important part of cellular defense against a large array of endogenously or exogenously formed injurious agents. GSH offers protection against oxygen-derived free radicals and cellular lethality following exposure to ionizing radiation. In the present study post irradiation administration of PS conserved the levels of GSH significantly compared to RT alone treated group. Stimulation of the cellular antioxidants like GSH may contribute to observable radioprotective effect as GSH has been suggested to aid in biochemical repair processes, especially those associated with DNA strand break rejoining, probably by acting as a cofactor in enzymatic reactions [11-12].

Increase in the serum MDA levels was observed in animals exposed to 4 Gy gamma radiations. MDA levels indicate the oxidant stress in the system as it is produced by the indirect action of radiation. Free radicals which are produced by radiation exposure react with other molecules in the cells and form a chain reaction producing malondialdehyde, which is harmful to the cells.MDA levels in the PS and amifostine administered animals were low compared to radiation alone treated animals. PS was found to reduce TBARS formation in mouse liver microsomes exposed to 350 Gy gamma irradiation [13].

Amifostine is an FDA approved radioprotector used clinically. Amifostine was used as a standard drug to compare the activity of *Ganoderma* polysaccharide. The protection offered by amifostine at 300mg/kg body wt, a dose which provided maximum protection with minimum toxicity and by the polysaccharides at 20mg/kg body wt was comparable. Thus the dose at which the polysaccharide renders protection is much lower than that of amifostine. Moreover, the polysaccharide is effective by oral administration, which is the most convenient mode of administration in treatment of human diseases. The polysaccharides from *Ganoderma* administered to mice (5g/kg p.o for 30 days) produced no changes in body wt, organ wt or hematological parameters and produced no adverse effect [14]. This indicated that the mushroom is free from toxicity and is absolutely safe. In conventional radiotherapy, the use of a radioprotector, which can be administered orally, is of significant advantage. The intestinal cells are very sensitive to radiation as they are actively proliferating. Drug which can minimise the side effects of radiotherapy are very important in the present context.

Protection against ionising radiation is of paramount importance during accidental and unavoidable exposures to radiation [15] and development of novel and effective approaches to combat radiation damage using non-toxic radioprotectors are of considerable interest for defense, nuclear industries, radiation accidents,

space travels, etc., besides protection of normal tissues during radiotherapy of tumors and other medical exposures [15]. The most effective *in vivo* radioprotectors like plant flavonoids and thiol compounds studied so far are effective when administered before irradiation, as they must be present in the system at the time of irradiation [16]. Radioprotectors for use in a post-irradiation scenario are very few [16] and information about these is scarce. The present study demonstrates that PSis effective in protecting mouse intestine against radiation-induced damage. PS can be developed into a effective natural radioprotector having application both in medical and non-medical radiation exposures.

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