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IN VIVO RADIO PROTECTIVE PROPERTIES OF FUNGAL POLYSACCHARIDES

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ABSTRACT

In vivo radio protective properties of polysaccharides isolated from Ganoderma lucidum, a macro fungi was examined. Swiss albino mice were exposed to 4 Gy gamma irradiation. Serum lipid peroxidation and tissue GSH were taken end points on 1st, 3rd, 5th, 7th and 9th day after irradiation. Polysaccharides were administered just after irradiation at 10 and 20 mg/kg body wt. Administration of polysaccharides reduced the Serum MDA levels compared to the irradiated group. Tissue GSH was maintained at normal levels after administration of polysaccharides. The polysaccharides possess radio protective property.

KEY WORDS

Ganoderma lucidum, polysaccharides, radioprotection, radiotherapy.

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 or partially 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 the polysaccharides from the macro fungi *G.lucidum* against radiation induced damage.

MATERIALS AND METHODS

Chemicals

All 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 28 ± 2 g, were selected 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 polysaccharides

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 Studies in Botany, University of Madras, Chennai, India (HERB. MUBL. 3175). Polysacchrides were isolated by the method of Mizuno [4] with slight modification [5] The confirmation of polysacchrides were done by Anthrone [6] and phenol sulphuric acid test [7] Structural confirmation was done by IR and NMR spectrum which were recorded at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Mumbai, India. From the Gel filtration analysis the molecular weight of Polysaccharides were found to be 1.5 x 10^6 Daltons. 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. Anaesthetized animals, were exposed to 4 Gy γ-irradiation at a dose rate of 1 Gy/min.

Experimental Design

Five groups with 15 animals each were used for the study.

Group I – Normal Control (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 sacrificed in alternate days from 1 to 9 (1, 3, 5, 7, and 9)

Polysaccharides were administered orally just after irradiation.

Tissue protein and serum protein

Tissue and serum protein were determined by Bradford's [8] method. The protein determination was done according to the procedure given in the kit purchased from Banglore Genei. BSA was used as standard and diluted to get 1mg/ml.Different concentrations of BSA and unknown samples were pipetted into different test tubes. 2 ml of Bradford's reagent was added, mixed and kept at room temperature for 10 mins. OD was recorded at 595 nm using Varian DMS 200 UV-visible spectrophotometer. The value of unknown sample was recorded from the standard graph.

Serum lipid peroxidation

Serum lipid peroxidation was determined by Ohkawa et al [9] after precipitating the protein according to the method of Satoh [10] 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 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 hour in a boiling water bath at 95^0 C. After 1hour, the reaction mixture was removed from water bath, cooled and added 1 ml of distilled water. 5 ml of butanol: 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 nanomoles) of 1'1'3'3 – tetramethoxy propane in 1 ml distilled water and is expressed as nmol of MDA/mg protein.

Determination of tissue GSH

Reduced glutathione (GSH) in tissue was determined by the method of Moron et al,[9] 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 3000g for few minutes to settle the precipitate. 0.3ml of the supernatant was mixed with 0.7ml of 0.2M sodium phosphate buffer (pH[8]). The yellow colour obtained was measured after 10 min at 412 nm 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.

RESULTS AND DISCUSSION

Tissue GSH was found to be reduced from 3rd to 7th day in a radiation alone group. Administration of polysaccharides at 20 mg/kg body wt restored GSH on the 7th and 9th day (Figure1). Serum MDA was increased at 4 Gy gamma radiation (Figure.2). Administration of polysaccharides at 20 mg/kg body wt reduced significantly on 5th, 7th and 9th day. The presence of amifostine at 300mg/kg body wt also reduced MDA significantly on 5th day after radiation exposure.

Antioxidant enzymes are among the endogenous system that are available for the removal or detoxification of these free radicals and their products formed by ionizing radiation. The GSH/GST 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. GST enzymes also possess peroxidase activity and can directly attack the peroxides that may be generated via oxidative reduction recycling, resulting in decreased cytotoxicity. The present study demonstrates that a significant reduction in GSH in radiation treated group. 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 [11]. 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 antioxidant property of the polysaccharides scavenge free radicals and neutralize it, thus reducing its capacity to damage. The balance between the production of free radicals and the antioxidant defences in the body has important health implications. This antioxidant property may be a contributing factor for the radioprotective properties offered by the polysaccharides.

The interaction of ionizing radiation with biological system results in generations of free radicals, H and OH radicals, H_2 and H_2O_2. Radiations induced free radicals in turn impair the antioxidant defense mechanism leading to increased membrane lipid peroxidation, which results in the damage of membrane bound enzymes [12-13] .The increased lipid peroxidation is due to the low concentration of GSH. The membrane damage due to lipid peroxidation is confirmed by the activity of hepatic enzyme GPT. Antioxidant enzymes are among the endogenous system that are available for the removal or detoxification of these free radicals and their products formed by ionizing radiation. Polysaccharides reduced chromosomal aberrations in mice exposed to 4 Gy gamma radiation [14] 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 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 [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. In conventional radiotherapy, the use of a radioprotector, which can be administered orally is of significant advantage.

CONCLUSION

The present finding that polysaccharides gives significant radioprotection when given after irradiation points to its advantage over the other pre-administered radioprotectors and potential for use both in medical non-medical exposures.

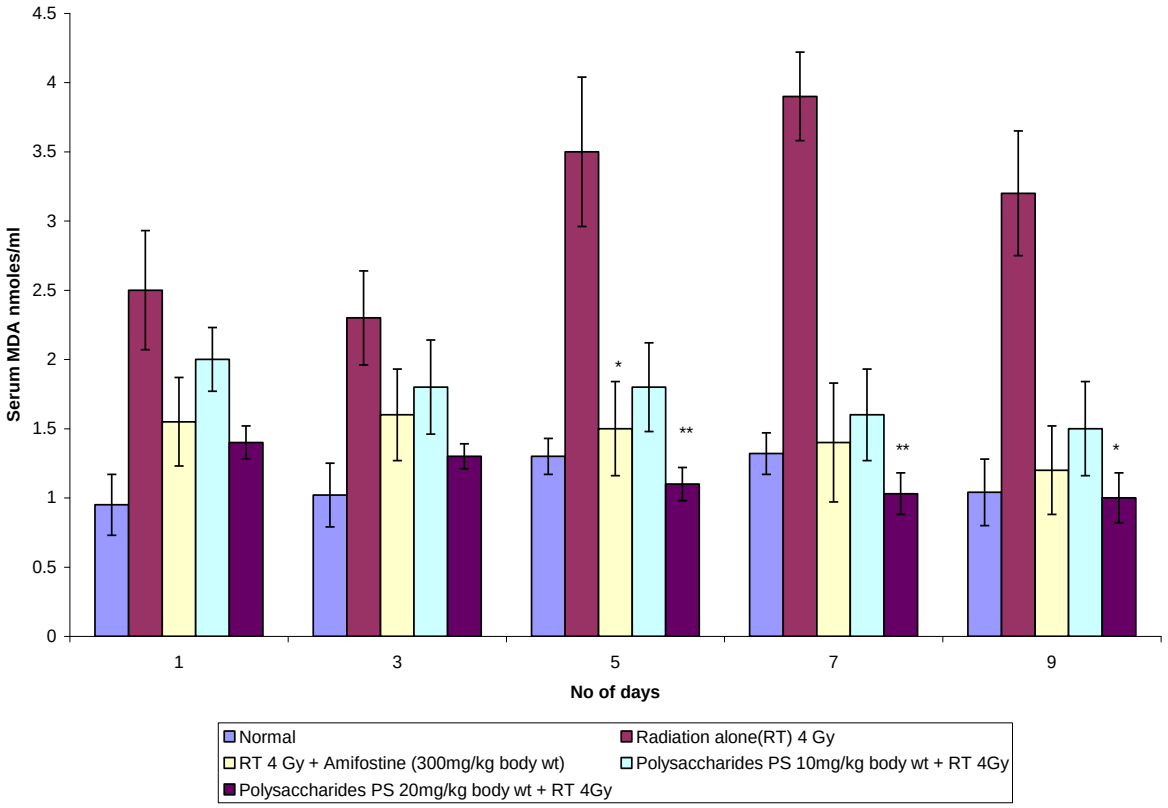

Figure .1. Effect of polysaccharides on serum MDA after gamma Irradiation (4 Gy).

*p < 0.01 compared to radiation alone.

** p< 0.05 compared to normal control.

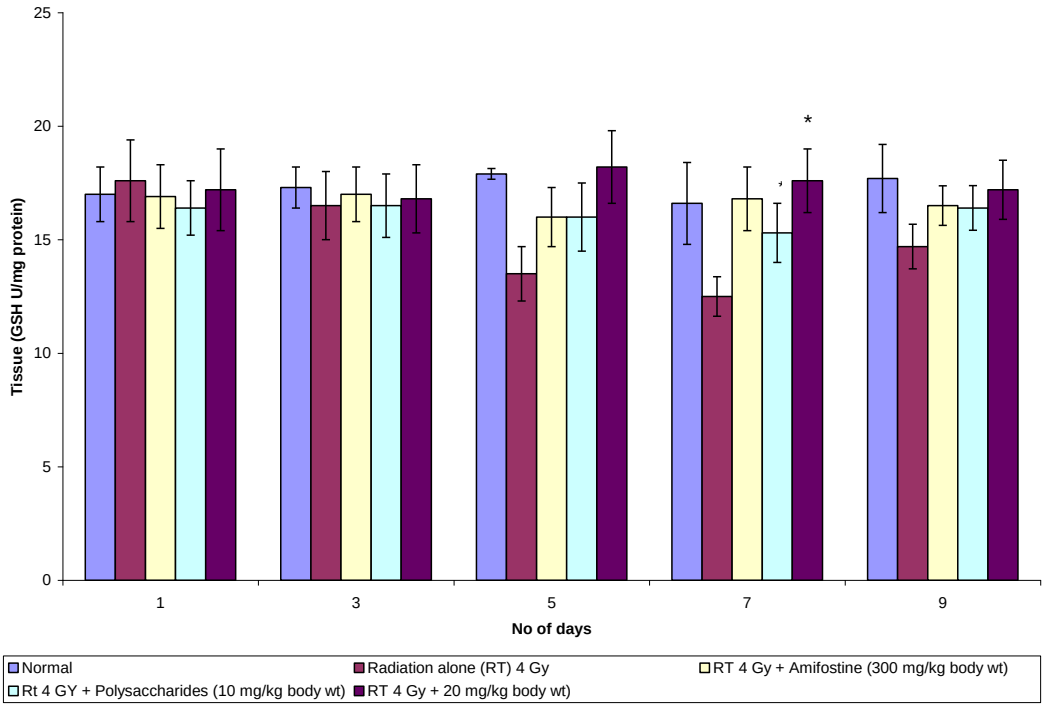

Figure .2. Effect of polysaccharides on tissue GSH after 4 Gy gamma irradiation.

*p < 0.01 compared to radiation alone.

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