

Glucan from Macro fungi : A Promising Radioprotector

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Received: February 13, 2026; Accepted: February 27, 2026; Published: March 06, 2026

ABSTRACT

The in vivo radioprotective effect of a beta-glucan isolated from the macro fungi *Ganoderma lucidum*, against radiation (RT) induced damage was investigated taking the in vivo antioxidant parameters - serum lipid peroxidation, GSH in blood and tissue catalase as end points at alternate time intervals for 9 days. Young adult swiss albino mice were whole body exposed to 4 Gy gamma irradiation. Beta glucan (BG) was administered orally (10 mg and 20 mg/kg body wt) just after radiation exposure. The efficacy of BG was compared with that of the standard drug amifostine at 300mg/kg body wt. Serum MDA, which was increased by irradiation was reduced by BG at 20 mg/kg body wt significantly on 5th, 7th and 9th day after radiation exposure. Blood GSH and tissue catalase was found to increase by the administration of BG at 20 mg/kg body wt.

Keywords: *Ganoderma Lucidum*, Beta Glucan, Radiation, Radioprotection, Serum MDA, Tissue Catalase, Blood GSH.

Introduction

Radiations are the inevitable accompaniment of life and it is difficult to create an environment totally free from radiations. At low doses of radiation, the hazards from these scenarios may not be apparent immediately, but may result in the late arising disease syndromes. A safe compound that would prevent the radiation – induced tissue damage would provide an effective medical countermeasure against radiation effects arising from nuclear attack. Radiotherapy is an inevitable modality in cancer treatment. Cellular damage produced by the radiation therapy is an indirect result of ionizing chemicals in the cell to very reactive compounds. Cytotoxicity is primarily caused by oxygen-derived free radicals such as Hydrogen Peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radical ($\cdot OH$). In modern clinical practice, radiotherapy is frequently combined with surgery and chemotherapy to provide the most effective tumor control with least damage to normal tissue [1].

Despite over four decades research and development work in this area, no compound has been identified and fielded that has broad-spectrum radioprotective attributes necessary to protect populations from the unwanted radiation exposures and also to minimize the side effects caused by radiotherapy. The most effective in vivo radioprotectors like plant flavanoids and thiol compounds studied so far and even the FDA approved drug, amifostine are effective when administered before irradiation.

A radioprotective drug which can give protection after the radiation exposure is more important. The present study was aimed to find out such a radioprotector. *G. lucidum* contains a number of components among which the beta glucan have been isolated and identified as one of the major active components. The radioprotective potential of the fungi is unexplored. In the present study, the in vivo studies were carried out to evaluate the radioprotective potential of the beta glucan.

Materials and Methods

Swiss albino mice 6-8 weeks of age and weighing 25 ± 2 g, were selected from our mouse colony. They were maintained in environmentally controlled conditions with free access to standard food (Sai Durga Foods, Bangalore.) and water.

All the experiments were conducted strictly according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of the Government of India and by the Institutional Animal Ethics Committee of Amala Cancer Research Centre, Thrissur.

Chemicals

Acetic acid, methanol, anhydrous sodium sulphate, sulphuric acid, ferrous sulphate (F_eSO_4), hydrochloric acid, potassium chloride (KCl), ferrous ammonium sulphate ($F_eSO_4(NH_2)_2SO_4 \cdot 7H_2O$), sodium nitroprusside, sulphanilamide, O-phosphoric acid and naphthyl ethylenediamine dihydrochloride, sodium dodecyl sulphate (SDS), Thio barbituric acid (TBA), pyridine, butanol,

ethylene diamine tetra acetic acid (EDTA), hydrogen peroxide (H_2O_2), potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), sodium chloride, acetic anhydride, colchicine, Giemsa were purchased from Sigma Chemical Co. USA.

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 unanesthetized animals, which were kept in well-ventilated Perspex boxes and was exposed at a dose rate of 1 Gy/min.

Isolation and characterization of Beta Glucan

The fruiting bodies of *Ganoderma lucidum* were collected from the outskirts of Thrissur, Kerala, India. The type specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB. MUBL. 3175). The polysaccharides were isolated from the fruiting bodies by the method of Mizuno, with slight modifications [2,3]. Structural confirmation of BG was done by infrared radiation (IR) and nuclear magnetic resonance (NMR) spectrum which were recorded at Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Bombay, India. The molecular wt of BG was determined by gel filtration chromatography.

Free Radical Scavenging and Antioxidant Studies

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 + BG (10 mg/ Kg body wt)

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

Animals were sacrificed in alternate days from 1 to 9 (1, 3, 5, 7, and 9)

Determination of Tissue Protein and Serum Protein

Tissue and serum protein were determined by Bradford's method [4]. The protein determination was done according to the procedure given in the kit purchased from Bangalore Genei. Absorbance was recorded and detected at 595 nm. The value of unknown sample was recorded from the standard graph.

Determination of Serum Lipid Peroxidation

Serum lipid peroxidation was determined by Ohkawa et al after precipitating the protein according to the method of Satoh. 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 [5,6].

Determination of GSH in Blood

Reduced GSH in blood was determined according to the method of Moron, 1979 after preparing a hemolysate in water. A 20% hemolysate of heparinised blood was prepared in distilled water and 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 (pH8). 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 standard graph and expressed as in micromoles/ml of blood [7].

Determination of Tissue Catalase

Tissue catalase (CAT) was determined by method of Beers and Sizer, 1952. 0.1 ml of tissue homogenate (approximately 0.1mg protein) from mouse liver was mixed with 1.9ml of Phosphate buffer (0.5M pH 7). The decrease in extinction was measured at 240 nm, 1 min interval for 3 min immediately after adding 1 ml of 10 mM H_2O_2 solution in buffer. A sample control was placed in reference cuvette containing 0.1 ml of tissue homogenate and 2.9 ml of buffer. Activity of catalase was calculated using the molar extinction coefficient of 43.6 cm^{-1} .

$U/mg \text{ Protein} = \Delta/min \times 1000 \times 3$

$43.6 \times mg \text{ protein in sample}$

Statistical Analysis

Data were analysed by student's t-test. A value of $p < 0.05$ was considered to be Significant.

Results and Discussion

In the present study, serum lipid peroxidation was increased at 4 Gy gamma radiation. Administration of BG at 20 mg/kg body wt reduced MDA levels 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 (Figure.1). Blood GSH was found to increase by the administration of BG at 20 mg/kg body wt after 4 Gy radiation exposure, though not significantly (Figure.2). Amifostine also showed similar level of protection at 300 mg/kg body wt of that of BG. Catalase in tissue was found to be restored by BG on 7th and 9th day after radiation exposure (Figure.3). Equal levels of protection was found for amifostine also.

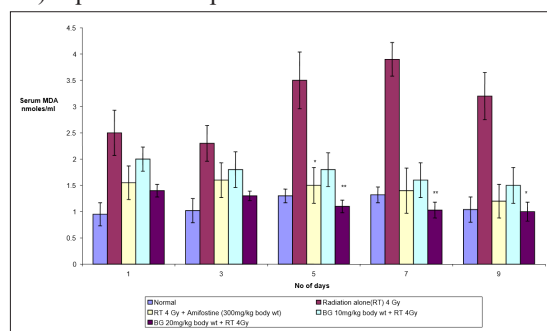


Figure 1: Effect of BG on Serum MDA After Gamma Irradiation (4 Gy).

* $p < 0.01$ compared to radiation alone.

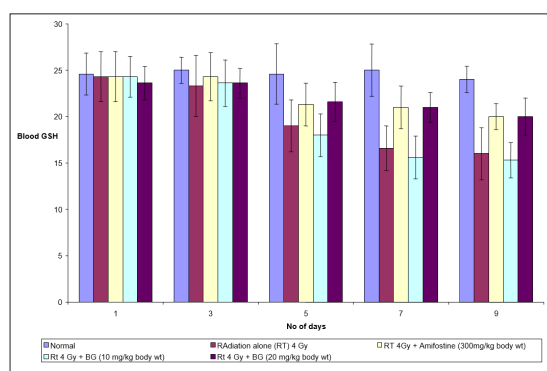


Figure 2: Effect of BG on Blood GSH After Gamma Irradiation (4 Gy).

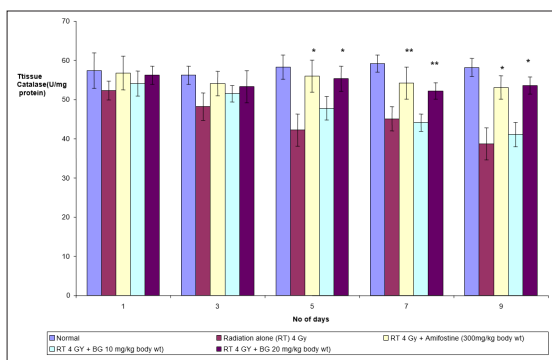


Figure 3: Effect of Polysaccharides on Tissue Catalase after Gamma Irradiation (4Gy).

* $p < 0.01$ compared to radiation alone.

** $p < 0.05$ compared to control.

** $p < 0.05$ compared to control.

Ionizing radiation is toxic to organisms since it induces deleterious structural changes in essential macromolecules. One important line of defence for our body against free radical damage is a system of enzymes, including glutathione peroxidases, superoxide dismutases and catalase, which decrease concentrations of the most harmful oxidants in the tissues. Catalase, which is concentrated in peroxisomes located next to mitochondria but formed in the rough endoplasmic reticulum and located everywhere in the cell, reacts with the hydrogen peroxide and forms water and oxygen. Glutathione peroxidase reduces hydrogen peroxide by transferring the energy of the reactive peroxides to a very small sulfur containing protein called glutathione. The selenium contained in these enzymes acts as the reactive center, carrying reactive electrons from the peroxide to the glutathione. 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 [8]. 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. 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 and the activities of all antioxidant enzymes 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 [9]. 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 BG scavenges free radicals and neutralizes 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 BG. Our previous studies have revealed that BG also have DNA repair enhancing capability [3, 10].

Amifostine is an FDA approved radioprotector used clinically. Amifostine was used as a standard drug to compare the activity of BG. The protection offered by amifostine at 300mg/kg body wt, a dose which provided maximum protection with minimum toxicity and by the BG at 20mg/kg body wt was comparable. Thus the dose at which the BG 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. Earlier reports suggest that 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. This indicated that the fungi is free from toxicity and is absolutely safe [10]. From the present study we arrive at the conclusion that the antioxidant property together with the repair enhancing capacity may be the contributing factor for the radioprotective property of BG. Thus BG is found to be a promising potential radioprotector. Further studies in the mechanism of action of the compound and clinical trials are thus worthwhile.

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