Preclinical studies on the use of medicinal mushroom *Ganoderma lucidum* as an adjuvant in radiotherapy of cancer

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Our previous studies have demonstrated that an extract of *Ganoderma lucidum* occurring in South India possesses significant radioprotective property *ex vivo*. The present study describes the *in vivo* radioprotection of normal cells in tumour-bearing mice exposed to gamma radiation. Oral administration of *G. lucidum* extract (GLE) to tumour-bearing Swiss albino mice along with exposure to gamma radiation resulted in tumour regression. Single-cell gel electrophoresis (comet assay) on cells of normal and tumour tissues from tumour-bearing animals treated with GLE and radiation, revealed that there was significant reduction in radiation-induced damage to cellular DNA in normal tissues compared to the tumour, indicating preferential protection to normal tissues. The findings suggest the potential use of this mushroom extract as an adjuvant in radiotherapy, for tumour regression and prevention of radiation-induced cellular damages in normal tissues.

**Keywords:** Adjuvant, cancer, *Ganoderma lucidum*, preclinical studies, radiotherapy.

Ionizing radiation is one of the well-established and widely used therapeutic modalities either for curative or palliative treatment of tumours, but the major problem associated with cancer radiotherapy is the severe side effects and damage to normal tissues. In radiotherapy of cancer, normal tissues need to be protected whereas cancer cells are exposed to high radiation. Even though many compounds have been studied for their radioprotecting property, an agent producing differential radiation response in the tumour and normal cells would be of importance in effective treatment of cancer by radiation therapy.

*Ganoderma* (Figure 1), commonly known as reishi mushroom is highly ranked in Oriental folklore. In Chinese medicine reishi has been considered as a panacea for all types of diseases. Reishi has attracted significant attention in recent years due to its large number of pharmacological properties. The fruiting bodies of this mushroom contain a variety of chemical substances. A number of medicinal mushrooms have recently been reported to possess significant antioxidant activity. One of the major activities reported for *Ganoderma* is its antioxidant activity. *Ganoderma lucidum* extract (GLE) also contains ergosterols, complete proteins, unsaturated fatty acids, vitamins and minerals. It is the only known source of a group of triterpenes known as ganoderic acids, which have a molecular structure similar to steroid hormones and contains the most active polysaccharides among medicinal plant sources. Ganoderic acids may lower blood pressure and decrease LDL cholesterol. Anti-aging properties have also been reported for this mushroom. In addition, it is reported that the mushroom is used for the preparation of an HIV tonic. *G. lucidum* has been found to play an important role in combination with radiotherapy and chemotherapy, to render complete regression of the tumours. Since both polysaccharides and organic germanium derived from *G. lucidum* are not cytotoxic to tumour cells, the anti-tumour effect is attributable to induced immunopotentiation.

Our earlier reports suggest that the aqueous extract of this mushroom has significant radioprotective activity *ex vivo*. The present study describes the *in vivo* radioprotection of normal cells in tumour-bearing mice exposed to whole-body gamma radiation and the sensitization of its tumour to radiation (as there was enhanced radiation-induced damage to cellular DNA in the tumour) by oral administration of GLE. The work is also focussed on the effect of GLE in tumour regression in tumour-bearing Swiss albino mice when administered orally along with radiotherapy.

GLE was prepared from *G. lucidum* collected from the outskirts of Thrissur, Kerala, India. Sporocarps of the mushroom were dried at 40–50°C and powdered. Several batches of 100 g powder were extracted with 1:1 ethanol:distilled water mixture at 80°C for 8–10 h. The extracts were combined, filtered, concentrated and evaporated at low temperature. The residue thus obtained was used for the experiments. The yield was 10.6%.

Swiss albino mice about 8–10 weeks old and weighing 22–25 g were purchased from the Small Animal Breeding

![Figure 1. *Ganoderma lucidum*.](image-url)
Section (SABS), Mannuthy, Thrissur. They were maintained under standard conditions of temperature and humidity in the Animal House Facility at our Centre. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore) and water *ad libitum*. All animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted by the Animal Welfare Division, Government of India.

Na₂-EDTA was purchased from Sisco Research Laboratories Ltd, Mumbai. High melting point agarose, low melting point agarose, TritonX-100, DMSO and bovine serum albumin were purchased from Sigma Chemical Company Inc, MO, USA. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

Irradiation, i.e., exposure to gamma radiation was carried out using a 60Co-Teletron Phoenix teletherapy unit (Atomic Energy Ltd, Ottawa, Canada) at the Amala Cancer Hospital, Thrissur at a dose rate of 1.88 Gy/min.

The combined effect of 4 Gy gamma radiation and GLE on tumour growth in vivo was analysed as follows. Solid tumour was produced by injecting Dalton’s Lymphoma Ascites (DLA) cells (1 × 10⁶ cells/animal) subcutaneously into the right hind limb of Swiss albino mice weighing 22–25 g. The animals were divided into four groups, each consisting of six animals. The first group was kept as untreated control, the second received 16 doses of GLE in 15 days (200 mg/kg body wt/dose), the third was exposed to single dose of 4 Gy gamma radiation and fourth group was administered with 200 mg/kg body wt of GLE 1 h prior to and immediately after exposure to 4 Gy gamma radiation and also daily for the next 14 days. The treatments were started on the 7th day after transplanting tumour cells (when the tumour reached a size of 1.0 cm³) and continued for 15 consecutive days. The thickness of the hind leg was measured using a vernier calliper once in three days from the 7th day of tumour transplantation. The tumour volume was calculated as follows: Tumour thickness = Thickness of tumour-induced leg – Thickness of normal leg.

Tumour volume = 4/3πr³, where r is the tumour radius, the average of r₁ and r₂ which are the tumour thicknesses.

The effect of GLE on radiation-induced damage in cellular DNA of cells of normal tissues and tumour in tumour-bearing mice exposed to whole-body gamma radiation was studied as follows. Swiss albino mice were divided into four groups: Group I – 0.2 ml distilled water (oral) + Sham irradiation; Group II – 0.2 ml distilled water (oral) + 4 Gy 60Co-γ-rays; Group III – 200 mg/kg GLE (oral) + Sham irradiation and Group IV – 200 mg/kg GLE (oral) + 4 Gy 60Co-γ-rays.

Animals in groups I and II were orally administered with distilled water, and groups III and IV with 200 mg/kg GLE. The animals in groups II and IV were exposed to 4 Gy whole-body gamma radiation, 1 h after administration of distilled water or GLE. Immediately after irradiation the animals were sacrificed and blood, brain, bone marrow and tumour tissues were collected for performing alkaline single-cell gel electrophoresis (comet assay)³⁰,³¹.

The DNA strand breaks in various tissues of mice were estimated using alkaline single-cell gel electrophoresis which was performed using the method given by Singh¹⁰, with minor modifications¹¹. Microscopic slides were coated with normal melting point agarose (1% in PBS containing 0.8% NaCl, 0.02% KCl, 0.14% Na₂HPO₄, 0.02% NaH₂PO₄), the coverslip was placed immediately and kept at 4°C for 10 min to solidify the agarose. After removal of the coverslip, 200 μl of 0.8% low melting point agarose containing 50 μl of treated cells was added.
**Figure 3.** Effect of GLE and gamma radiation on solid tumour in mice. Swiss albino mice bearing solid tumour (Dalton’s Lymphoma Ascites) in hind limb were administered with GLE and exposed to 4 Gy gamma radiation. After radiation exposure, GLE administration was continued for 14 days and the tumour volume monitored on alternate days, as described in the text.

**Figure 4.** Photographs of silver-stained comets from cells of different tissues of tumour-bearing Swiss albino mice orally administered with GLE and exposed to 4 Gy gamma radiation.
to the slides, the coverglasses were placed immediately and the slides were kept at 4°C. After solidification, the coverglasses were removed and the slides were immersed in pre-chilled lysis solution containing 2.5 M NaCl; 100 mM Na₂EDTA, 10 mM Tris-HCl (pH-10), 1% DMSO and 1% TritonX, and kept for 1 h at 4°C. After lysis, the slides were drained properly and placed in a horizontal electrophoretic apparatus filled with freshly prepared electrophoresis buffer containing 300 mM NaOH, 1 mM EDTA and 0.2% DMSO (pH ≥ 13). The slides were equilibrated in the buffer for 20 min and electrophoresis was carried out for 30 min at 25 V and 300 mA. After electrophoresis the slides were washed gently with 0.4 mM Tris-HCl buffer (pH 7.4) to remove the alkali. The slides were again washed with distilled water and kept at 37°C for 2 h to dry the gel; then silver staining was carried out. The comets were visualized using Olympus BX-41 microscope and the images were captured and analysed using the software ‘CASP’, which directly gave the comet parameters such as %DNA in tail, tail length, tail moment (TM) and Olive tail moment (OTM). TM is the product of tail length and %DNA in tail, and OTM is the product of the distance between the centre of gravity of the head and centre of gravity of the tail and %DNA in the tail. Data of the comet parameters were presented as mean ± standard deviation.

The results of the study on the effect of gamma radiation (4 Gy) and GLE administration are presented in Figures 2 and 3. Figure 3 presents data on tumour volume, following the treatments and Figure 2 shows representative photographs of the animal and its tumour-bearing limb. The growth of the tumour was found to be inhibited in animals exposed to 4 Gy gamma radiation compared to untreated animals. The GLE itself reduced tumour growth to some extent. The tumour growth was found substantially inhibited in the group of animals administered with GLE and exposed to 4 Gy gamma radiation.

Alkaline comet assay was performed to analyse the effect of administration of GLE on radiation-induced cellular DNA damage in normal and tumour tissues. Figure 4 shows representative photographs of the comets from the cells of these tissues. It can be seen from Figures 5–8 that the cellular DNA from various tissues such as blood, bone marrow, brain and tumour of the tumour-bearing animals exposed to whole-body 4 Gy gamma radiation shows increased comet parameters such as %DNA in tail, tail length, TM and OTM. However, except in the tumour tissues, these parameters were found to be lower in the normal tissues of tumour-bearing animals administered with GLE 1 h prior to radiation exposure (Figures 5–8). This would suggest that the administration of GLE offered protection against radiation-induced damages to
Figure 6. Effect of oral administration of GLE on radiation-induced cellular DNA damage as assessed by comet assay in bone marrow cells of mice-bearing tumour on hind limbs, exposed to 4 Gy whole-body gamma radiation. Mean comet parameters like %DNA in tail, tail length, tail moment and Olive tail moment of single cells subjected to alkaline single-cell gel electrophoresis are presented with ± SD. ns, Not significant; ***p < 0.001 when compared with respective control.

Figure 7. Effect of oral administration of GLE on radiation-induced cellular DNA damage as assessed by comet assay in blood leucocytes of mice-bearing tumour on hind limbs, exposed to 4 Gy whole-body gamma radiation. Mean comet parameters like %DNA in tail, tail length, tail moment and Olive tail moment of single cells subjected to alkaline single-cell gel electrophoresis are presented with ± SD. ns, Not significant; ***p < 0.001 when compared with respective control.
cellular DNA in normal tissues, and in the tumour tissues the extract offered no protection but helped enhance the radiation-induced cellular DNA damage, as can be evidenced from the data in Figures 4–8.

The present study is focused on the radioprotective and anti-cancer properties of *G. lucidum* under *in vivo* conditions using mouse as the model system. Radioprotective agents offer a possible solution to counteract the radiation damage to living systems. The extracts of *G. lucidum*, certain medicinal plants, vitamin derivatives and dietary supplement formulations with good anti-oxidant activity can be considered as safe radioprotectors, whereas many of the synthetic drugs and chemicals prepared for radiation protection have limited application in the living systems due to their toxicity and side effects. Our previous studies have revealed that aqueous extract of *G. lucidum* possesses radioprotective activity.

GLE has been reported to exhibit anti-tumour activity and this has been attributed to immune-related mechanisms or cytotoxicity. Some of the active ingredients in the extract have been identified as polysaccharides and triterpenes. The extract has also been shown to prevent proliferation of cancer cells, mediated through inhibition of DNA synthesis. Inhibition of DNA synthesis following irradiation could bring about enhanced apoptosis in the cells. This could be a possible mechanism by which GLE enhances the anti-tumour activity of gamma radiation.

The tumour regression study carried out with solid tumour-bearing animals revealed that oral administration of GLE (200 mg/kg body wt) to the animals resulted in significant reduction in tumour volume, and this anti-tumour effect was more prominent in conjunction with gamma-radiation treatment (4 Gy). Studies on *in vivo* radioprotection efficiency by comet analysis demonstrated the efficiency of GLE to offer protection to normal tissues against gamma radiation-induced DNA damage, whereas sparing tumour tissues where the extract offered no protection against radiation-induced cellular DNA damage.

The mushroom *G. lucidum* could be effectively used as a radioprotector for normal tissues in radiotherapy. It is to be noted that there was an increased extent of cellular DNA damage in cells of the tumour tissues of animals administered with GLE prior to radiation exposure which could be due to induction of apoptosis in these cells. Further studies are needed to support this possibility. The present results suggest the possibility of using this medicinal mushroom extract as an adjuvant in cancer radiotherapy to protect normal tissues from radiation damage and also to enhance the anti-tumour activity of gamma radiation.

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DNA microarray analysis targeting pmoA gene reveals diverse community of methanotrophs in the rhizosphere of tropical rice soils

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The diversity of the methanotrophs community of two different rice fields of a typical tropical rice agroecosystem was assessed using microarray targeting pmoA gene-based approach. The presence of types I and II methanotrophs was observed with the dominance of Methylocystis in both the fields. The study revealed that the Barkachha rice field harbours more diverse groups of methanotrophs than the Ghazipur rice field. It was also observed that in some members of types I and II methanotrophs, even the peat-associated group was present in the enriched culture of the soils. The Ghazipur soil and its enriched mixed methanotrophic culture showed higher methane oxidation potential than the Barkachha soil. These results suggest that the methanotrophs community and its potential for methane oxidation vary with change in soil type within the same ecosystem.

Keywords. Methane oxidation, methanotrophs, microarray, pmoA gene, rice soil.

Due to their significant role in global CH4 cycling, methane-oxidizing bacteria (MOB; methanotrophs) have been the focus of several scientific researchers. Methanotrophs, abundantly found in the aerobic layer of the soil, the rhizosphere1,2, oxidize significant amount of CH4 generated by methanogens. It is expected that methanotrophic bacteria utilizing CH4 as substrate in the rhizosphere will vary in the population composition and density within the rice rhizosphere3. The methanotrophic community is complex and diverse, containing 10 genera which belong to type I (Gammaproteobacteria), and four genera to group

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