CHAPTER -6-

PRECLINICAL EVALUATION OF
MEDICINAL MUSHROOM *GANODERMA LUCIDUM*
AS AN ADJUVANT IN
RADIOTherAPY OF CANCER
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6.1. INTRODUCTION

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 (Nair et al., 2001). In radiotherapy of cancer, normal tissues need to be protected while 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 great importance in effective treatment of cancer by radiation therapy (Shueng et al., 1998). Though silver nanoparticle complexes of some of the compounds exhibited good therapeutic potential for tumour control in murine system, due to various reports on systemic toxicity limit their application in human situations. There is a long way for their acceptance in human situations. Hence a study on tumour control was conducted using medicinal mushroom *Ganoderma lucidum*, which is used for treatment of several human ailments.

*Ganoderma* (Figure 6.1.), commonly known as Reishi, is highly ranked in oriental folklore. In Chinese medicine it 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 (Tim et al., 2004). 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.

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

![Figure 6.1. Ganoderma lucidum](image-url)
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 (Maruyama and Murofushi, 1989). Anti-aging properties have also been reported for this mushroom, *Ganoderma lucidum*. In addition, it is reported that this mushroom is used for the preparation of an HIV tonic (Chang, 1993). *Ganoderma lucidum* has been found to play an important role in combination with radio- and chemo-therapy, to render complete regression of the tumours (Wang and Weng, 2006; Jiang et al., 2004; Stanley et al., 2005). Since both polysaccharides and organic germanium derived from *Ganoderma lucidum* are not cytotoxic to tumour cells, the anti tumour effect is attributable to induced immunopotentiation.

Earlier reports suggest that the aqueous extract of this mushroom have significant radioprotective activity *ex vivo* (Pillai et al., 2006, 2008, 2010; Pillai and Devi, 2013). 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 in 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.

6.2. MATERIALS AND METHODS

6.2.1. Preparation of alcoholic extract of *Ganoderma lucidum*

The GLE was prepared from the *Ganoderma lucidum* collected from the outskirts of Thrissur, Kerala, India. Sporocarps of these mushrooms were dried at 40 to 50°C and powdered. Several batches of 100g powder was extracted with 1:1 ethanol distilled water mixture at 80°C for 8-10 hrs. 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%.

6.2.2. Animals

Swiss albino mice of 4-6 weeks old, weighing 22-26 g, were purchased from the Small Animal Breeding Section (SABS), Mannuthy, Thrissur, Kerala. They were maintained under standard conditions of temperature and humidity in the Centre’s Animal House Facility. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) 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 of Government of India.
6.2.3. **Exposure to gamma-radiation**
Irradiation was carried out using a $^{60}$Co- Theratron Phoenix teletherapy unit (Atomic energy Ltd, Ottawa, Canada) at the Amala Cancer Hospital, Thrissur, Kerala, India, at a dose rate of 1.88 Gy per minute.

6.2.4. **Combined effect of 4 Gy gamma radiation and *Ganoderma lucidum* extract on tumour growth *in vivo***
Solid tumour was produced by injecting DLA (Dalton’s Lymphoma Ascites) cells ($1 \times 10^6$ cells/animal) subcutaneously into the right hind limb of Swiss albino mice weighing 22-25g. The animals were divided into 4 groups each consisting of 6 animals. The first group was kept as untreated control, the second group received 16 doses of GLE in 15 days (200mg/kg body weight/dose), third group was exposed to single dose of 4 Gy gamma-radiation and fourth group was administered with 200mg/kg body weight of GLE one hour prior to and immediately after the exposure to 4 Gy gamma-radiation and also daily for next 14 days. The treatments were started on the $7^{th}$ day after transplanting tumour cells (when the tumour reached a size of 1.0 cm$^3$) and continued for 15 consecutive days. The hind leg thicknesses were measured using a vernier calliper once in three days from $7^{th}$ day of tumour transplantation. The tumour volume was calculated as follows:

\[
\text{Tumour thickness} = \text{Thickness of tumour induced leg} - \text{Thickness of normal leg}
\]

\[
\text{Tumour volume} = 4/3 \pi r^3, \text{ where } r \text{ is the tumour radius.}
\]

6.2.5. **Effect of *Ganoderma lucidum* extract on cellular DNA of normal and tumour tissues in DLA solid tumour-bearing mice exposed to whole body gamma-radiation**
Swiss albino mice were divided into four groups, as detailed below.

- **Group I** - 0.2 ml distilled water (oral) + Sham irradiation
- **Group II** - 0.2 ml distilled water (oral) + 4 Gy $^{60}$Co-γ-rays
- **Group III** - 200 mg/kg GLE (oral) + Sham irradiation
- **Group IV** - 200 mg/kg GLE (oral) + 4 Gy $^{60}$Co-γ-rays

Animals in Group I and II were orally administered with distilled water, Group III and IV with 200 mg/kg GLE. The animals in Group II and IV were exposed to 4 Gy whole body gamma-radiation, one hour after distilled water or GLE administration. Immediately after irradiation the animals were sacrificed and blood, brain, bone marrow and tumour tissues were collected for performing alkaline single cell gel electrophoresis or comet assay (Singh, 2000; Chandrasekharan et al., 2009).
6.2.6. Statistical analysis
The data are expressed as mean ± standard deviation (SD). The significance levels for comparison of differences were analyzed using ANOVA with Tukey-Kramer multiple comparisons test. The treated groups were compared with the respective control groups. The differences between means were considered statistically significant at P <0.05.

6.3. RESULTS
6.3.1. Combined effect of 4 Gy gamma radiation and *Ganoderma lucidum* extract on tumour growth *in vivo*
The results of the study on the effect of 4 Gy whole body gamma irradiation and GLE administration on tumour growth inhibition in DLA solid tumour bearing mice are presented in figure 6.2.(a) and figure 6.2.(b). Figure 6.2.(a) presents the data on the tumour volume, following the treatments and figure 6.2.(b). gives representative photographs of the animals and their tumour bearing limbs. 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 the 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 whole body gamma radiation.

![Figure 6.2.(a). Effect of oral administration of GLE and 4 Gy whole body gamma-irradiation on tumour growth delay in DLA solid tumour bearing mice. Values are expressed as mean±SD.](image)
Figure 6.2.(b). Effect of GLE and 4 Gy whole body gamma-irradiation on solid tumour growth in mice.

1a, 1b – Untreated Control (0 Gy); 2a, 2b – GLE + 0 Gy; 3a, 3b – 4 Gy Control; 4a, 4b - GLE + 4 Gy.
6.3.2. Effect of *Ganoderma lucidum* extract on cellular DNA of normal and tumour tissues in DLA solid tumour-bearing mice exposed to whole body gamma-radiation

Alkaline comet assay was performed to analyze the effect of administration of GLE on radiation induced cellular DNA damage in normal and tumour tissues. It can be seen in figures 6.3.(a) to 6.3.(d) that the cellular DNA from various tissues such as brain, bone marrow, blood and tumour of the tumour-bearing animals exposed to whole body 4 Gy gamma-radiation showed increased comet parameters such as % DNA in tail, Tail length, Tail moment and Olive tail moment.

![Figure 6.3.(a).](image)

Figure 6.3.(a). Effect of oral administration of GLE on radiation induced cellular DNA damage in brain cells of DLA solid tumour bearing mice exposed to 4 Gy whole body gamma-radiation, assessed by comet assay. Mean comet parameters- % 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. (d- indicates ‘not significant’ and c- indicates p < 0.001 when compared with respective control).
Figure 6.3.(b). Effect of oral administration of GLE on radiation induced cellular DNA damage in bone marrow cells of DLA solid tumour bearing mice exposed to 4 Gy whole body gamma-radiation, assessed by comet assay. Mean comet parameters- % 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. (d- indicates ‘not significant’ and c- indicates p < 0.001 when compared with respective control).
Figure 6.3(c). Effect of oral administration of GLE on radiation induced cellular DNA damage in blood leukocytes of DLA solid tumour bearing mice exposed to 4 Gy whole body gamma-radiation, assessed by comet assay. Mean comet parameters- % 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. (d- indicates ‘not significant’ and c- indicates p < 0.001 when compared with respective control).
Figure 6.3.(d). Effect of oral administration of GLE on radiation induced cellular DNA damage in tumour cells of DLA solid tumour bearing mice exposed to 4 Gy whole body gamma-radiation, assessed by comet assay. Mean comet parameters- % 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. (d- indicates ‘not significant’ and c- indicates p < 0.001 when compared with respective control).

However, except in the tumour tissues, these parameters were found to be lower in the normal tissues of tumour-bearing animals administered with GLE one hour prior to radiation exposure. This would suggest that the administration of GLE offered protection against radiation induced damages to cellular DNA in normal tissues and in the tumour tissues the extract offered no protection but it helped to enhance the radiation-induced cellular DNA damage, as can be evidenced from the data. Figure 6.3.(e) presents the representative photographs of the comets from the cells of these tissues.
Figure 6.3.(e). Photographs of silver stained comets from cells of different tissues of tumour bearing Swiss albino mice, orally administered with GLE and exposed to whole body 4 Gy gamma radiation.
6.4. DISCUSSION

Present study is focussed on the radioprotective and anti-cancer properties of *Ganoderma 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 (Nair *et al.*, 2001). The extracts of *Ganoderma lucidum*, certain medicinal plants, vitamin derivatives and dietary supplement-formulations with good anti-oxidant activity can be considered as safe radioprotectors while many of the synthetic drugs and chemicals prepared for radioprotection had limited application in living systems due to their toxicity and side effects (Weiss and Landauer, 2003; Arora *et al.*, 2005; Maurya *et al.*, 2006; Nair *et al.*, 2003). Previous studies have revealed that aqueous extract of *Ganoderma lucidum* possess radioprotective activity (Pillai *et al.*, 2006).

GLE has been reported to exhibit anti-tumour activity and this has been attributed to immune related mechanisms or cytotoxicity (Lin and Zhang; 2004; Wang *et al.*, 2002; Lakshmi *et al.*, 2009). Some of the active ingredients in the extract has been identified as polysaccharides and triterpenes (Paterson, 2006; Boh, 2007). The extract has also been shown to prevent proliferation of cancer cell, mediated through inhibition of DNA synthesis (Gieni *et al.*, 1995). Inhibition of DNA synthesis following irradiation could bring about enhanced apoptosis in the cells, this could be a possible mechanism by which the GLE enhance the anti-tumour activity of gamma-radiation. The tumour regression study carried out with solid tumour-bearing animals revealed that oral administration of GLE 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 while sparing tumour tissues where the extract offered no protection against radiation induced cellular DNA damage.

The mushroom *Ganoderma lucidum* could be effectively used as a radioprotector for normal tissues in radiotherapy situations. 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 adjuvant in cancer radiotherapy to protect normal tissues from radiation damages and also to enhance the anti-tumour activity of gamma-radiation.