Ionizing radiation is an established carcinogen, having both initiating and promoting effects. The classic view of two stage carcinogenesis states that tumor initiation by mutation is followed by epigenetic changes which cause tumor promotion. Many factors including exposure to viruses, xenobiotic chemicals and radiation contribute to tumor initiation (Yuspa, 2000). Radiation induces the production of reactive oxygen species (ROS), which include superoxide anion (O$_2^-$, a free radical), hydroxyl radical (•OH), and hydrogen peroxide (H$_2$O$_2$). These reactive species may contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein oxidation, and muscle injury), mutations and chromosome instability for many generations (Morgan et al, 2002). The carcinogenic risks of radiation exposure in people have been derived from many sources, including occupational exposures (e.g., radiologists and uranium miners), therapeutic exposures (radiotherapy, or treatment of ankylosing spondylitis), and accidental exposures (Bhatia and Sklar 2002).

Radiotherapy is one of the most effective treatments for cancer (Steel 2002). Eighty percent of cancer patients depend on radiotherapy either for curative or palliative purposes (Nair et al, 2001). The dose of radiation is determined by the intent of the therapy (i.e., curative or palliative), the volume of the tumor, the relative radiosensitivity of the tumor cells and expected toxicity to surrounding normal tissues. Most curative radiotherapy regimens consist of daily treatments or fractions in the range of 1.8 to 3 Gy per day over a period of 5 to 8 weeks. The use of ionizing radiation in cancer therapy may lead to transient and/or permanent injury to normal tissues within the treatment field. The magnitude of damage depends both on the volume of tissue irradiated and the dose of radiation delivered (Brizel et al, 2000). The toxicity of high-dose ionizing radiation (IR) is associated with induction of acute radiation syndromes (ARS) involving the haemopoietic system (HP) and gastrointestinal tract (GI) (Waselenko et al, 2004).

Acute radiation responses occur mainly in renewal tissues and are related to death of critical cell populations such as stem cells in the crypts of small intestine, in the bone marrow or in the basal layer of skin. The extreme sensitivity of HP and GI
cells to genotoxic stress largely determines the adverse side effects of anticancer radiation therapy and chemotherapy (Dodd 2001). Further, these side effects limit the maximum radiotherapy dose that can be given to the patient. In this scenario, there is continued interest and need for the identification and development of effective and nontoxic radioprotective compounds. The potential application of radioprotective chemicals in the event of planned exposures or radiation accidents/incidents has been investigated from the beginning of the nuclear era (Weiss and Simic 1988; Bump and Malaker 1998). Radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue.

Mushroom is one of the useful, delicious and mysterious member of the biosphere (Verma et al, 1987a, b). Because of their taste and fleshy construction, they have been paid the attention by mankind for ages. Macrofungi was commonly used as a nutrition supplements to a variety of diseases in Asia (Jong and Birmingham 1992; Chen et al, 2006a). They are recognized as functional foods and as a source of physiologically beneficial components (Wasser and Weis 1999). Mushrooms have been shown to boost heart health; lower the risk of cancer; promote immune function; ward off viruses, bacteria, and fungi; reduce inflammation; combat allergies; and help balance blood sugar levels and support the body’s detoxification mechanism (Ada et al, 2005). The genus *Phellinus* is classified in the family Hymenochaetaceae, Donk under the class, Basidiomycetes and it has been used as an herb in traditional medicine for many years in Asian countries. (Kim et al, 2004). *Phellinus* has been used to treat abdominal pain, stomach problems, lymphatic tumor, and menses disorders.

Polysaccharides from *Phellinus* have been reported to possess several beneficial effects. Polysaccharides isolated from *Phellinus linteus* have been reported to possess significant antioxidant and immunostimulatory activity. (Samchai et al, 2009; Gi-Su-Oh et al, 2006). The active polysaccharide from *Phellinus linteus* stimulated humoral and cell-mediated immunity (Kim et al, 1996; Song et al, 1995). Polysaccharides isolated from *P. linteus* have been reported to possess antitumor and immunomodulating activities (Moradali et al, 2007; Zhang et al, 2008).
Phellinus linteus polysaccharides had been reported to increase production of immune mediators like IL-1 in mice (Kim et al, 2003). These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans (Wasser, 2002). β-glucans are powerful immune stimulants. They can activate macrophages, enhance phagocytosis of the pathogen and can facilitate the release of proinflammatory cytokines (Sato et al, 2006).

Phellinus species are mostly tropical mushrooms and 18 species are known from Kerala. Phellinus rimosus is a parasitic host specific polypore mushroom often found growing on jackfruit trees (Artocarpus heterophyllatus Lam.; Moraceae) trunks. In Kerala, Phellinus rimosus is quite common on living Moraceae members. It causes white pocket rot initially but later, the heartwood is transformed into a white spongy mass. The basidiocarp is locally used in treating mumps (Leelavathy and Ganesh, 2000). Earlier investigations showed that ethyl acetate and methanol extracts of P. rimosus possessed antioxidant, antitumor and hepatoprotective activities. (Ajith and Janardhanan 2001; Ajith and Janardhanan 2002; Ajith and Janardhanan 2003). Ethyl acetate extract of P. rimosus showed significant radiation induced lipid peroxidation inhibiting activity (Lakshmi et al, 2005). Our aim is to investigate radioprotective effects of PPC-Pr complex isolated from mushroom Phellinus rimosus.

The aqueous extract and the PPC-Pr Complex were isolated from the fruiting bodies of mushroom P. rimosus. Both the aqueous extract and the PPC-Pr Complex was found to contain polysaccharides and proteins as the major bioactive constituents. The total carbohydrate content was found to be 55% and 50% by anthrone and phenol sulphuric acid method respectively. The amount of protein was estimated as 40% by Lowry’s method. The paper and thin layer chromatographic analysis of the isolated protein-bound polysaccharide showed that the monosaccharide present was only D-glucose. The amino acid analysis of protein-bound polysaccharide by the TLC method showed that the major amino
acids present in the isolated protein-bound polysaccharide were aspartic acid,
glycine and serine. Thus, the polysaccharide isolated was found to be a protein-
bound polysaccharide or polysaccharide-protein complex.

Radiation breaks down water in living cells into dangerously reactive free radicals
in the tissue environment; these include hydroxyl radicals (the most damaging),
superoxide anion radicals and other oxidants such as hydrogen peroxide. All ROS
have the potential to interact with cellular components including DNA bases or the
deoxyribose backbone of DNA to produce damaged bases or strand breaks (Ward
et al, 1988). The antioxidants present in mushrooms are of great interest as possible
protective agents against oxidative stress (Adams and Wermuth, 1999). The In
vitro antioxidant activity of aqueous extract and Polysaccharide Protein Complex
(PPC-Pr) Complex isolated from the mushroom Phellinus rimosus were evaluated
using various in vitro antioxidant assays, including the DPPH radical scavenging
assay, the ABTS\(^+\) radical scavenging assay, the ferric reducing antioxidant power
(FRAP) assay, the superoxide radical scavenging activity assay, hydroxyl radical
(OH\(^-\)) scavenging assay, the inhibition of lipid peroxidation and nitric oxide
radical (NO\(^-\)) scavenging assay. Further, the effect of aqueous extract and PPC-Pr
Complex was also evaluated in AAPH (2, 2' azobis (2-aminopropane)
dihydrochloride) induced lipid peroxidation in mitochondria and microsomes.

Aqueous extract demonstrated potent nitric oxide radical scavenging activity,
superoxide radical scavenging activity, hydroxyl radical scavenging activity, and
inhibition of lipid peroxidation. The aqueous extract also demonstrated significant
reducing property in terms of FRAP, ABTS and DPPH assay. For FRAP and
ABTS assay the half inhibitory concentration (IC\(_{50}\)) value was found to be 2.99 ±
0.34µg/ml and 9.40 ± 0.97 µg/ml respectively. The PPC-Pr Complex also
demonstrated significant antioxidant and reducing property. The IC\(_{50}\) values for
lipid peroxidation and hydroxyl radical scavenging assay were found to be 109.25
± 2.11 µg/ml and 509.39 ± 10.17 µg/ml respectively. The IC\(_{50}\) values for FRAP,
ABTS and DPPH and assay were found to be 3.67 ± 0.23 µg/ml, 25.06 ± 2.34
µg/ml and 118.91 ± 11.30 µg/ml respectively. Exposure to peroxyl radical
generator, AAPH resulted in significant increase in TBARS and LOOH level in both mitochondria and microsomes. The membrane damage was more pronounced in microsomes than mitochondria. At a concentration of 100µg/ml, both the PPC-Pr complex and aqueous extract offered significant protection from the formation of Thiobarbituric Acid Reacting Substances (TBARS) and Lipid hydroperoxide (LOOH) level in rat liver microsomes and mitochondria.

Gamma radiation is known to damage vital cellular components through the free radicals generated in the aqueous milieu in the cell. The most important targets of free radicals in vitro are DNA and membranes. The major damage to membranes is due to the oxidation of lipids present in them. The exposure to γ irradiation produced significant increase in membrane lipid peroxidation parameters such as TBARS and LOOH in mitochondrial as well as microsomal tissue. Our results suggest that even at low concentrations (10-100 µg) both the PPC-Pr Complex and aqueous extract could effectively prevent the formation of TBARS and LOOH. Similarly γ irradiation induced damage to DNA was assessed by comet assay. When blood lymphocytes, were exposed to 4 GY of γ irradiation, there was significant increase in comet parameters indicating the extent of damage. It was found that both the PPC-Pr Complex and aqueous extract were almost equally effective in reducing DNA damage induced by γ irradiation. In vitro DNA protective effect of these drugs were also assessed by the protection offered to PBR 322 DNA at a dose of 25 Gy. The damage to PBR 322 DNA is indicated by its conversion to open circular or linear form its native supercoiled form. Both the drugs were effective in preserving the native supercoiled form at a concentration as low as 100 µg. Thus our result suggest that both the P. rimosus derivatives are potent agents to reduce the radiation induced damages. The observed significant in vitro genoprotective nature of P. rimosus against oxidative stress has been assumed to be due to its significant antioxidant or free radical scavenging activity.

The in vivo radioprotective effects were evaluated by protection offered to haemopoietic system, tissue antioxidant system and intestinal mucosal system. The results concluded that polysaccharide protein complex (PPC-Pr) isolated from
the aqueous extracts of mushroom, *Phellinus rimosus* imparted significant protection against radiation induced hematological toxicities as evident from increased bone marrow cellularity as well as W.B.C count in the treated groups. They also restored antioxidant status in a dose dependent manner in radiosensitive tissue such as blood. Further radiation induced lipid peroxidation was also significantly reduced by treatment with PPC-Pr Complex. The PPC-Pr complex at a concentration of 5 and 10 mg/kg bwt significantly protected against radiation induced suppression of antioxidant status in both liver and brain tissues. Improved antioxidant statuses in the treated animals were further supported by decreased ROS level in the mitochondrial tissues of treated animals. Increased survival rate of radiation exposed animals after PPC-Pr treatment further confirms radioprotective effect of PPC-Pr complex. The intestine is a dose limiting organ during radiotherapy of pelvic or abdominal region. It also limits effective dose used in radiotherapy. In this study, intestinal radioprotective effects of PPC-Pr Complex were evaluated in terms of antioxidant assays of intestinal mucosal cells as well as histopathological analysis of jejunal cells. The present study concluded that PPC-Pr Complex effectively protected intestinal tissue, one of the most radiosensitive tissues from radiation induced damages. It also provided significant protection to intestinal mucosa as evident from histopathological analysis of jejuna mucosal cells. Thus the results of the present study concluded that PPC-Pr Complex could be effectively used as protective agents during intestinal radiotherapy.

The indices of DNA damage caused by ionizing radiation are chromosome aberrations and micronulei formation, which are apparent when irradiated cells are observed microscopically (Elia et al, 1991; Sankaranarayanan, 1999; Olivieri et al, 1984; Vijayalaxmi et al, 1995a, Vijayalaxmi etal, 1995b, Vijayalaxmi et al, 1996; Vijayalaxmi et al, 1998). For the whole-body exposure to sparsely ionizing radiation, chromosomal aberrations provide a reliable estimate of the average absorbed dose (Edwards 1997). DNA strand breaks of the double strand type also lead to the formation of micronuclei. Damage to the chromosome manifested as breaks and fragments, appear as micronuclei in the rapidly proliferating cells. Pretreatment with PPC-pr Complex 5mg/kg and 10 mg/kg decreased the radiation
induced micronuclei, which supports its radioprotective activity. The present study demonstrates that PPC-Pr at doses of 5 and 10mg/kg bwt when given before irradiation protected the bone marrow chromosomes from genotoxicity effects of whole body irradiation. The polysaccharide protein complex with profound antioxidant activity and immunostimulatory activity may be responsible for this effect.

The mushroom *P.rimosus* is a medicinally valuable mushroom and therefore, has a possibility to be developed as a potent medicine or nutraceutical in near future. But before developing any pharmaceutical or dietary supplement it is essential to evaluate the toxicity of the compound. The animals administered with PPC-Pr Complex of *P.rimosus* did not produce any external symptoms of toxicity or mortality up to the dose of 100mg/kg body weight intraperitoneally. In subacute toxicity studies, treatment with two different concentration of the extract did not produce any statistically significant change in the hematological or biochemical parameters when compared to the normal group of animals. No clinical signs of any adverse or toxic symptoms were noticed throughout the period of sub acute toxicity study. The study also revealed the potential use of this mushroom for the production of safe and non toxic agents with profound therapeutic and nutraceutical properties.