Introduction

Large populations all over the globe now live in an environment contaminated by radiations from both natural and man-made sources. The origins of various types of natural background radiation are solar emissions of nuclear particles, nuclear reactions of these particles with the earth’s atmosphere, and the radioactive decay products of radioisotopes distributed throughout the earth’s crust. Man made background radiation sources include radiation from nuclear weapon testing, nuclear power station accidents, atom bomb explosions, medical X-ray exposure, emissions from burning fossil fuels such as coal fired power plants, emissions from nuclear medicine facilities and patients, emissions from radiological imaging and therapy. Radioactivity exposures also occur in uranium miners, airline crews exposed to cosmic rays, nuclear emergency workers, military personnel and radiotherapy technicians. Medical X rays exposures over past decades can account for at least 75% of all female breast cancers (Gofman, 1996). Radiation exposure to cancer patients occurs during radiotherapy at some time or other either for curative purposes or palliative purposes (Nair et al., 2001).

Radiotherapy is the use of ionizing radiation for the treatment of cancer and it is one of the most effective treatments for cancer (Steel, 2002). 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 intent is to achieve local control of the tumor to prevent further local tissue destruction, organ failure, and the seeding of secondary metastases. Palliative radiotherapy is given in order to achieve better pain control, to control bleeding, or to prevent tissue destruction or ulceration. These radiotherapy treatments are usually of short duration and consist of 1 to 3 fractions of 5 to 8 Gy or 5 to 10 fractions of 3 to 4 Gy. However, radiotherapy has achieved limited success in eradicating cancer. One major reason for this stems from the fact that normal and cancerous tissues have similar responses to radiation exposure (Mitchell et al., 2000). Consequently, radiation-induced injury may present in the normal tissues during radiotherapy treatment or some time later after the completion of radiotherapy.

Intracellular irradiation produces severe damaging events. A deleterious effect of radiation is 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$_2$) (Fang, 1991). These reactive species may contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein
oxidation, lipid peroxidation and muscle injury) and to metabolic and morphologic changes (e.g., increased muscle proteolysis and changes in the central nervous system) in animals and humans. The risk of cell toxicity is increased with the application of more intensive radiotherapy techniques intended to increase tumor cell kill. Many effects have been attributed to ionizing radiation (IR) induced damage to nuclear DNA or that occur following irradiation of the cytoplasmic compartment of cells. These can also occur in cells that have received no direct exposure to IR. This is called ‘bystander effects’, i.e., radiation induced effects in unirradiated cells, which include cell killing, increases in intracellular reactive oxygen species, the induction of mutations, enhanced cell growth, the induction of apoptosis, the induction of genomic instability and neoplastic transformation.

Traditionally the effects of radiation treatment on normal tissues have been divided, based on functional and histopathological end points into early (or acute) responses, which occur within a few weeks of radiation treatment, and late response that may take many months or years to develop. Acute radiation effects are caused by transient suppression of cell proliferation in tissues with a high rate of cell turn-over, such as the bone marrow, epidermis and the mucosal lining of the respiratory and digestive tracts (Bloomer and Hellman, 1975). Late tissue responses occur in organs whose parenchymal cells normally divide infrequently and hence do not express mitosis-linked cell death. It has been postulated that late radiation effects occur as a result of functional or structural damage to small blood vessels (capillaries, venules and arterioles) leading to disruption of blood supply to the tissues (Mathes and Alexander, 1996).

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). It has also been considered possible that radiation therapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue. In recent years, a number of cytoprotective agents capable of protecting normal tissue against damage caused by either chemo- or radiotherapy have been investigated including amifostine (WR-2721), dexrazoxane, mesna, glutathione, and N-acetylcysteine. Among these, amifostine, dexrazoxane and mesna have FDA approval for use in cytoprotection. However only amifostine has been shown in clinical trials to reduce radiation induced toxicity. Amifostine
(WR2721) is a phosphorothioate compound that is converted into a sulphydryl-containing compound *in vivo* by the action of alkaline phosphatase. In 1996, the Food and Drug Administration (FDA) registered amifostine for use as a cytoprotective agent with cisplatin-based chemotherapy against ovarian cancer.

Although, amifostine can reduce radiation side effects but does not remove them completely. Further, these synthetic radioprotective agents have got several side effects within the body. Common side effects of amifostine include hypocalcaemia, diarrhea, nausea, vomiting, sneezing, somnolence, and hiccoughs (Hensley, 1999). Serious side effects include: hypotension (found in 62% of patients), erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis, immune hypersensitivity syndrome, erythoderma, anaphylaxis, and loss of consciousness (rare). Most patients receiving amifostine with radiotherapy require antiemetic. These side effects are enough to limit the use of amifostine to doses lower than required to achieve maximal radioprotection. In this scenario, there is continued interest and need for the identification and development of effective and nontoxic radioprotective compounds. Compounds with radioprotective activity from natural sources have attracted considerable attention due to their potential use and lack of toxicity (Arora et al., 2005; Kang et al., 2006).

Many natural antioxidants, whether consumed before or after radiation exposure, are able to confer some level of radioprotection (Weiss and Landauer, 2000). There is evidence that “natural” antioxidants (superoxide dismutase, *Gingko biloba* extract, and mixtures of antioxidant plant phenols, vitamins and minerals) may protect against long-term effects of radiation exposure occurring in human populations exposed to radiation. Besides these extracts, a large number of plants contain antioxidant phytochemical compounds that have been reported to be radioprotective in various model systems. These include green tea (polyphenols), dithiolthiones, compounds in *Gingko biloba* extract such as flavone glycosides and terpene lactones, milk thistle (silymarin), curcumin, allicin in garlic, and lycopene. Recent studies on the flavonoids orientin and vincristin extracted from *Ocimum sanctum* revealed significant protection against chromosome aberrations and lethality when administered to mice at nontoxic doses before radiation exposure (Uma Devi et al., 1998: Uma Devi et al., 1999).
Mushroom is one of the useful, delicious and mysterious members of the biosphere (Verma et al., 1987a; Verma et al., 1987b). The antioxidants present in dietary mushrooms are of great interest as possible protective agents which help the human body to reduce oxidative damage without any interference (Adams and Wermuth, 1999). A number of bioactive molecules have been identified in many mushroom species including polysaccharides, terpenes, polyphenols, alkaloids, lectins, AHCC (Active Hexose Correlated Compound), Psilocybins etc. Among these, polysaccharides are the best known and most potent mushroom derived substances with immunomodulating as well as antitumor properties. Polysaccharides are polymers of sugars (monosaccharides) joined to each other by glycosidic linkages which results in the formation of highly branched macromolecules. Wasser (2002) reported that mushroom polysaccharides can be regarded as Biological Response Modifiers (BRM). This basically means that they cause no harm and place no additional stress on the body, but help the body to adapt to various environmental and biological stresses.

Recently, polysaccharides isolated from several mushroom spp. have received special attention due to their potent pharmacological properties such as anti-tumor (Bae et al., 2004; Han et al., 1999) and anti-inflammatory activities (Kim et al., 2003). Protein bound polysaccharide complex isolated from mushroom Lentinus lepideus (PG 101) has been reported to recover radiation induced bone marrow suppression very efficiently (Jin et al., 2003). Polysaccharides isolated from the Phellinus gilvus (PG) have various biological activities related to inflammation, including inhibition of pulmonary inflammation, prevention of intraperitoneal adhesion under infectious circumstances (Bae et al., 2004a; Bae et al., 2004b; Bae et al., 2004c) and promotion of dermal wound healing in normal host (Bae et al, 2004d). Proteoglycans from a closely related species P.linteus had been reported to stimulate host defense immune system by boosting both humoral and cellular immune response (Kim et al., 2003). Polysaccharide protein complex from a P.linteus stimulates immune system and enhance the production of interleukins (Kim et al., 2006). Polysaccharides isolated from medicinal mushrooms act as immunopotentiator and enhance immune status in the body by a variety of mechanisms including production of immune mediators like cytokines (Gi-Su-Oh et al., 2006). Hence they are excellent agents against radiation induced immunosuppression.
Phellinus species are mostly tropical mushrooms and 18 species are known from Kerala. Phellinus linteus is known to be extensively used in Chinese medicine (Ying et al., 1987). Phellinus rimosus is a parasitic host specific polypore macro fungus often found growing on jackfruit trees (Atrocarpus heterophyllatus) trunks (Leelavathy, 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). Recent investigations have also demonstrated the profound anti-inflammatory and antiarthritic activities of polysaccharide protein complex (PPC-Pr) isolated from the aqueous extract of P. rimosus (Meera et al., 2009a; Meera et al., 2009b). However radioprotective properties of this mushroom have not been investigated till now. Aim of this study is to evaluate the radioprotective activities of Primosus derived chemical components.

Objectives

1. Evaluation of In vitro antioxidant activities of aqueous extract and Polysaccharide Protein Complex (PPC-Pr) isolated from the mushroom, Phellinus rimosus.

2. Evaluation of in vitro radioprotective activities of aqueous extract and PPC-Pr Complex.

3. Evaluation of In vivo radioprotective activities of PPC-Pr Complex.
   a) Effect of PPC-Pr Complex on haematopoietic system consequent to radiation.
   b) Effect of PPC-Pr Complex on tissue antioxidant system consequent to radiation.
   c) Effect of PPC-Pr Complex on radiation induced damages on gastrointestinal system.

4. Evaluation of In vivo genoprotective activities of PPC-Pr Complex.

5. Toxicity studies of PPC-Pr Complex.

METHODS

Isolation of PPC-Pr Complex:

Fruiting bodies of P. rimosus were collected from the outskirts of Thrissur, Kerala, India. Fruiting bodies were dried at 45 to 50°C for 48 h and powdered. The powdered material was
defatted with petroleum ether using a Soxhlet apparatus for 8 to 10 h. The defatted material was extracted with double distilled water at 95°C for 8-9 hours and concentrated to obtain the aqueous extract. Polysaccharide protein complex was isolated from the aqueous extract. Briefly, aqueous extract was precipitated with ethanol, deproteinised with Sevag’s method, dialyzed and finally lyophilized to obtain PPC-Pr complex (Meera et al., 2009a; Meera et al., 2009b). The yield of the preparation was 0.9% of total dry weight. Standard phytochemical investigations were carried out to determine the active components present both in the aqueous extract and the PPC-Pr complex. The carbohydrate content of the PPC-Pr complex was determined by the phenol-sulphuric acid method and the protein content was determined by the Bradford method.

**In vitro antioxidant activity:**

The *In vitro* antioxidant activities 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 by nitric oxide radical (NO´) scavenging assay. Further, the effect of aqueous extract PPC-Pr Complex was also studied in AAPH (2, 2′ azobis (2-amidopropane) dihydrochloride) induced lipid peroxidation in mitochondria and microsomes.

**In vitro radioprotective activities:**

*In vitro* radioprotective effects were evaluated by the protection offered to membrane and DNA by the aqueous extract and PPC-Pr Complex. The membrane damage was induced both in the mitochondrial and microsomal membrane by 450 Gy of γ radiation. The membrane damage induced was evaluated in terms of lipid peroxidation markers such as TBARS (thiobarbituric acids reactive substances) and LOOH (Lipid hydroperoxide). *In vitro* DNA protective effects of aqueous extract and PPC-Pr Complex were evaluated in terms of protection offered to plasmid DNA, PBR 322 and by comet assay. Plasmid DNA, PBR 322 was exposed to 25 Gy of γ-radiations in the presence and absence of different concentrations of aqueous extract and PPC-Pr Complex. Radiation-induced damage was determined by the agarose gel electrophoresis of plasmid DNA. Comet assay was performed to evaluate radiation-induced damage to DNA in the human blood lymphocytes. Human blood lymphocytes were exposed to 4 Gy of γ radiation in the
presence or absence of different concentrations of aqueous extract and PPC-Pr Complex. Alkaline single-cell gel electrophoresis was then performed and the slides were stained to get the images. Quantification of DNA strand breaks of the stored images was done by the imaging software CASP, by which the percentage DNA in tail, tail length, tail moment and olive tail moment of the comet could be obtained directly.

**In vivo radioprotective activities:**

*In vivo* radioprotective activities were evaluated in terms of protection offered by the PPC-Pr complex to haematopoietic, tissue antioxidant and gastrointestinal system of Swiss albino mice.

**a) Effect of PPC-Pr Complex on haematopoietic system.**

Damage to haemopoietic system is considered to be the major hazard in mammals exposed to ionizing radiation in low dose range. For the determination of *in vivo* haematopoietic parameters, male Swiss albino mice were exposed to whole body irradiation of 4 Gy in the untreated control group. Animals were treated with PPC-Pr Complex (5 and 10mg/ kg bwt, i.p) daily for five consecutive days and irradiated with 4Gy, one hour after the last dose to determine the protective effects of PPC-Pr Complex against irradiation. Amifostine (300mg/kg bwt, i.p), which is considered as a gold standard drug for radioprotection was used as standard in the experiment. The animals were sacrificed 1, 3, 5, 7 and 14 days after irradiation and different haematological parameters were assessed. Haematological parameters evaluated were changes in total count, bone marrow cellularity, blood antioxidant parameters such as glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and serum lipid peroxidation in terms of MDA equivalent (malondialdehyde).

**b) Effect of PPC-Pr Complex on tissue antioxidant status**

For the determination of *in vivo* tissue antioxidant parameters, male Swiss albino mice were exposed to whole body irradiation of 4 Gy in the untreated control group. Animals were administered with PPC-Pr Complex (5 and 10mg/ kg bwt, i.p) daily for five consecutive days and irradiated with 4Gy. The animals were sacrificed 1, 3, 5, 7 and 14 days after irradiation. Tissue antioxidant parameters such as glutathione (GSH), glutathione peroxidase (GPX), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation in terms of MDA equivalents (malondialdehyde) were evaluated in both liver and brain homogenate. The effect of
Complex on survival of organisms was evaluated with 9 Gy irradiated animals. Amifostine (300mg/kg bwt, i.p), was used as standard for the experiments. The effect of PPC-Pr Complex on intracellular ROS level was evaluated in mitochondria of treated animals to ascertain the radioprotective mechanisms.

c) Effect of PPC-Pr Complex on gastrointestinal damage.

For the determination of protection offered to gastrointestinal system, animals were administered with 5 and 10 mg/kg bwt, i.p five days consecutively and irradiated with 4Gy. Animals were sacrificed 5th, 7th, 14th days after irradiation. Intestinal radioprotection was assessed in terms of antioxidant enzyme activities of intestinal mucosal cells and compared to standard amifostine. Further, histopathologic examination of mucosal cells was also performed to assess radioprotective effects.

In vivo genoprotective activity:

In vivo genoprotective effects of *P. rimosus* against γ radiation was evaluated by scoring of micronucleus and by analysis of chromosomal aberrations. For the determination of micronucleus index, animals were administered with 5 and 10 mg/kg bwt (i.p) five days consecutively and irradiated with 4Gy. The bone marrow cells of treated mice 24h prior to exposure to 4Gy radiation were taken out. The number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) and the frequency of micronucleated polychromatic erythrocytes (MPCE) and micronucleated normochromatic erythrocytes (MNCE) were recorded. The data were expressed as number of MPCE or MNCE per 1000 PCE or NCE respectively. The ratio of PCE to NCE (P/N ratio) was also calculated. For the determination of chromosomal aberrations animals were administered with PPC-Pr Complex 5 and 10 mg/kg bwt, (i.p) five days consecutively and irradiated with 4Gy, one hour after the last dose. They were injected (i.p.) with colchicine (4mg/kg bwt) 1.5 h prior to sacrifice. Animals were sacrificed by cervical dislocation 24 hours after the exposure. Bone marrow cells were collected from both the femur bones. Different types of radiation induced aberrations such as chromosome breaks, chromatid breaks, rings, dicentrics, polyploidy and severely damaged cells were counted. The metaphase plates containing 40 ± 2 chromosomes were examined to score different types of aberrations.
Toxicity studies of PPC-P<sub>r</sub> Complex:

Acute and subacute toxicity studies of PPC-P<sub>r</sub> Complex were carried out using Swiss albino mice. In acute study, mortality after the administration of a single high dose of drugs was noted. In subacute toxicity study haematological parameters, liver and renal function enzymes were evaluated after the administration of drugs intraperitoneally for a period of 15 days. Small portions of the selected tissues of liver and kidney of treated animals were also examined for histopathological changes.

RESULTS
The aqueous extract of mushroom <i>Phellinus rimosus</i> showed significant in vitro antioxidant activity. The aqueous extract efficiently scavenged DPPH radical, (IC<sub>50</sub> 18.06 ± 0.89 µg/ml) ABTS radicals, (IC<sub>50</sub> 9.40 ± 0.97 µg/ml), nitric oxide radicals, (IC<sub>50</sub> 92.02 ± 4.54 µg/ml) super oxide radicals (IC<sub>50</sub> 124.41 ± 12.65 µg/ml) hydroxyl radical, (IC<sub>50</sub> 166.54 ± 16.68 µg/ml), showed significant ferric reducing activity (IC<sub>50</sub> 2.99 ± 0.34 µg/ml) suggesting its potent free radical scavenging activity. The aqueous extract effectively inhibited the lipid peroxidation induced by Fe<sup>2+</sup>-ascorbate system in rat liver homogenate (IC<sub>50</sub> 274.07 ± 2.21 µg/ml). In vitro membrane damage was induced both in the mitochondrial membrane and microsomal membrane by the peroxyl radicals generated from AAPH. The membrane damage was more pronounced in microsomes than mitochondria. The protection offered by the extract was assessed by the level of lipid peroxidation markers such as TBARS and LOOH. The aqueous extract at a concentration of 100 µg/ml demonstrated 69% and 80% protection from AAPH induced TBARS formation in rat liver microsomes and mitochondria respectively. Similarly, the inhibition of LOOH formation in microsomes and mitochondria was found to be 67% and 68% at a concentration of 100 µg/ml when compared to control. The PPC-P<sub>r</sub> complex exhibited significant ferric reducing activity (IC<sub>50</sub> 3.67 ± 0.23 µg/ml) ABTS radical scavenging activity (IC<sub>50</sub> 25.06 ± 2.34 µg/ml), DPPH radical scavenging activity (IC<sub>50</sub> 118.91 ± 11.30 µg/ml), hydroxyl radical scavenging activity (IC<sub>50</sub> 509.39 ± 10.17 µg/ml), nitric oxide radical scavenging activity (IC<sub>50</sub> 562.78 ± 52.56 µg/ml) and efficiently inhibited in vitro lipid peroxidation (IC<sub>50</sub> 109.25 ± 2.11 µg/ml). The antioxidant activities were comparable to standard antioxidants such as Butylated Hydroxy Anisole (BHA) and ascorbic acid. At a concentration of 100µg/ml, the
PPC-Pr complex showed 76% and 89% protection from AAPH induced TBARS formation in rat liver microsomes and mitochondria respectively when compared to control. The percent inhibition of microsomal and mitochondrial Lipid hydroperoxide LOOH induced by AAPH at 100 µg/ml concentration was found to be 69% and 71% respectively. The antioxidant activities of these mushroom derivatives can be attributed to various mechanisms, such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging (Diplock, 1997). The antioxidant property could be exerted by the protein fraction (especially the sulfhydryl or -SH- group of amino acids) present in the PPC-Pr Complex.

*In vitro* radioprotection was assessed by the protection offered to membrane and DNA. Mitochondria and microsomes when exposed to 450 Gy of γ rays showed significant increase in lipid peroxidation parameters like TBARS and LOOH. TBARS were increased in mitochondria and microsome to 64.70 ± 0.04 nmol/mg protein and 72.19 ± 0.03 nmol/mg protein from 20.50 ± 0.01 nmol/mg protein and 25.32 ± 0.01 nmol/mg protein respectively in the irradiated samples. However both the aqueous extract and PPC-Pr Complex at 100 µg significantly inhibited TBARS in mitochondrial and microsomal membrane. Another index of lipid peroxidation, LOOH, was also increased significantly in the irradiated control sample. There was 2.5 and 3.3 fold increase in LOOH formation in the irradiated mitochondria and microsomes when compared to normal. Both the aqueous extract and PPC-Pr Complex in the concentration ranging from 10 to 100 µg significantly inhibited LOOH levels in the irradiated sample. Gamma irradiation (4 Gy) resulted in increase of comet parameters such as (tail length, % DNA in tail, tail moment and olive tail moment) of blood cells due to damage to cellular DNA. The aqueous extract and PPC-Pr Complex showed a significant protection against the radiation induced DNA strand breaks, as revealed by comet assay. This was evident from the reduction of comet parameters such as % tail DNA, tail length, tail moment and olive tail moment that were considered as the indices of DNA damage. The *in vitro* radioprotection was also assessed by the protection offered to irradiated plasmid DNA, PBR 322. The reduction of the quantity of the super coiled form (CCC) of plasmid DNA is directly related to the radiation induced strand breaks. Both the aqueous extract and PPC-Pr complex at a concentration of 100 µg offered significant protection from radiation induced plasmid DNA breakage.
In vivo radioprotective activities were evaluated in terms of protection offered by the PPC-Pr complex to haematopoietic, tissue antioxidant and gastrointestinal system of Swiss albino mice.

Gamma irradiation (4 Gy) induced a significant decrease in both total WBC count and bone marrow cellularity. Total WBC count was found to be decreased significantly from 1st day onwards after irradiation in the control group compared to normal (p < 0.001). The PPC-Pr complex at doses of 5 and 10 mg/Kg bwt showed 3.7 and 4.2 fold increase in total W.B.C count than control on 7th day (p < 0.001), when maximum radiation toxicity was observed. The protection offered was further supported by the improved bone marrow cellularity found in treated group than that of control. PPC-Pr at both doses (5 and 10 mg/ Kg bwt) as well as amifostine showed significant improvement in bone marrow cellularity from 5th day onwards when compared to control (p < 0.001). On 14th day both bone marrow cellularity and total leukocyte count was restored significantly in the treated group when compared to irradiated control (p < 0.001). The level of blood GSH, a major cellular antioxidant was brought down significantly after irradiation up to 14th day with respect to that of normal (p < 0.001). However, treatment with PPC-Pr complex was able to ameliorate the effects of radiation to a great extent. GSH level in PPC-Pr complex treated group (5 and 10 mg/ Kg bwt) was found to be increased 1.6 and 1.9 fold higher than control on 7th day (p < 0.001). Activity of GPx in blood of control animals was found to be decreased significantly after whole body irradiation when compared to normal (p < 0.001). Administration of PPC-Pr prevented initial fall in GPx activity. Increase in GPX activity on 7th day in the treated group (5 and 10 mg/ Kg bwt) was found to be 2.9 and 3.8 times higher than control (p < 0.001). Gamma irradiation induced a significant decrease in the activity of SOD and CAT. Pre administration of PPC-Pr complex resulted in significant elevation in the activities of these enzymes. However, activity was restored significantly by both PPC-Pr complex and amifostine treatment (p < 0.001). Lipid peroxidation in the serum was increased significantly from 1st day onwards upto the 7th day. However on 14th day, serum lipid peroxidation was found to decrease when compared to 7th day. Administration of PPC-Pr Complex significantly decreased serum lipid peroxidation. There was 2.2 and 3.1 times decrease in serum lipid peroxidation levels in PPC-Pr complex 5 mg/Kg bwt and 10mg/Kg bwt treated group respectively on 7th day when compared to control. The PPC-Pr complex was more effective than amifostine, which provided 1.8 times decrease in serum lipid peroxidation levels
when compared to control on 7th day.

Significant decrease (p < 0.05) in antioxidant enzymes such as GPx, SOD, CAT, were observed in the irradiated control group 24 h after irradiation when compared to normal both in the liver and brain tissues. It was also observed that PPC-Pr complex treatment (10 mg/kg bwt) significantly elevated (p < 0.001) antioxidant enzymes GPx, SOD, CAT, when compared to control. Comparable effects are obtained for PPC-Pr at a concentration of 5mg/kg bwt and amifostine. The level of antioxidant moiety GSH was also decreased significantly from 1st day onwards upto 7th day both in the liver and brain tissue. The PPC-Pr complex at both the concentrations as well as amifostine was equally effective for the restoration of GSH content in the irradiated animals. Further, PPC-Pr isolated from P. rimosus exhibited dose dependent increase in the activity of antioxidant enzymes consequent to the radiation induced oxidative stress. On 14th day antioxidant enzyme activity was restored significantly in the treated group when compared to irradiated control (p < 0.001). The increase in GSH content was justified by the decrease in lipid peroxidation levels in the treated animals. Tissue TBARS level was increased significantly (2.5 and 5.3 fold in liver and brain respectively) in the irradiated control mice when compared to normal. The PPC-Pr complex at both the concentration (5 and 10 mg/kg bwt) offered significant improvement in the inhibition of TBARS level. For the survival studies, animals were observed upto 30 days post irradiation of 10 Gy of γ radiation. The survival rate of animals after 30th post irradiation day was found to be 20% in the untreated control group. The PPC-Pr complex at 10 mg/kg bwt offered 40 % survival on 30th post irradiation day. The protective effect of PPC-Pr Complex on the tissue antioxidant system as well as on the survival of the organism was further supported by Reactive Oxygen Species (ROS) level in the treated groups. ROS level can be taken as general indicator of oxidative damage. The extent of the reactive oxygen species (ROS) formation in the mitochondria in irradiated mice (4Gy) was determined compared to unirradiated control . Mitochondria from both liver and brain of animals showed significant increase in fluorescence suggesting increased ROS accumulation. Significant reduction in ROS level was observed in both the PPC-Pr treated group suggesting its radioprotective effect.

The protection offered to gastrointestinal system was evaluated by the changes in mucosal antioxidant enzyme activities. GSH and GPx activities in intestinal mucosa were significantly
decreased by whole body irradiation in the control group when compared to normal (p < 0.001). GSH and GPx activity in PPC-Pr complex treated group (10 mg/Kg bwt) was found to be 1.7 and 4.3 times higher than control on 7th day. SOD activity was restored significantly by PPC-Pr treatment compared to control. The SOD activity in PPC-Pr complex treated group (10 mg/Kg bwt) was found to be 2.4 times higher than control on 7th day (p < 0.001). Gamma irradiation induced significant decrease in the activity of mucosal CAT enzyme. The PPC-Pr complex administration showed significant increase in CAT activity from 5th day onwards in the treated groups. On 14th day significant restoration of mucosal antioxidant enzyme activity was observed in the treated group than control. Amifostine treatment also significantly increased mucosal antioxidant status when compared to control group. Microscopic examination of tissue slices after 7 days of irradiation revealed that γ irradiation led to prominent damage of small intestine, there was villi atrophy as well as mucosal erosion in the tissue, while in animals administrated with both doses of PPC-Pr and amifostine, these changes were less pronounced.

In vivo genoprotection was assessed by micronucleus assay and chromosomal aberration test. Significant increase in the frequency of micronucleated polychromatic erythrocytes (MPCE) and micronucleated normochromatic erythrocytes (MNCE) were observed in mice exposed to 4.0 Gy whole body gamma irradiation. The MPCE, MNCE and P/N (polychromatic/normochromatic) ratio of 4 Gy irradiated animals were found to be 114.21, 11.94 and 1.11 respectively per 1000 cells. Both MNCE and MPCE were significantly reduced in 10mg/Kg bwt treated group when compared to control. The decrease in MNCE and MPCE was found to be 3 and 1.6 times than irradiated control in the treated group (10 mg/ Kg bwt). Drastic fall in P/N ratio was also observed in irradiated control animals compared to sham irradiation. The ratio was decreased to 0.57 ± 0.50 from 1.11 ± 0.15 in the irradiated control group. PPC-Pr Complex increased the P/N ratio to 0.91 ± 0.38 and 0.94 ± 0.11 respectively in the 5 and 10 mg treated group. Chromosomal aberrations were also evaluated in the PPC-Pr Complex treated groups (5 & 10 mg/ Kg bwt). Significant increase was observed in all types of aberrations counted such as chromatid breaks, chromosome breaks, Rings, Dicentrics, Polyploids, Severly Damaged Cells (SDC) etc. Chromosome breaks and Chromatid breaks were the predominant aberrations seen in the irradiated control group. There was significant reduction in the aberrant metaphses when compared to control. There was 3.8 and 1.6 times decrease in chromosome breaks and chromatid breaks when compared to control in the PPC-Pr Complex treated group (10mg/kg bwt).
Significant decrease was also seen in individual aberration types. The present study demonstrates that PPC-Pr complex at doses of 5 and 10mg/kg bwt when given before irradiation protected the bone marrow chromosomes from genotoxic effects of whole body irradiation.

Both acute and subacute toxicity studies indicated that PPC-Pr complex isolated from *P. rimosus* did not produce any symptoms of toxicity, behavioral change and mortality of animals in all the tested doses. Acute toxicity test for PPC-Pr complex was performed with a single dose of 100mg/kg bwt, i.p and no toxic effect was observed. In sub acute model, 10 and 50mg/kg bwt (i.p) PPC-Pr complex were given to the animals for 15 days and no significant change (P > 0.05) in the haematological and biochemical parameters were observed when compared to the normal. Liver marker enzymes such as GOT, GPT, ALP and kidney function test such as serum urea and creatinine did not show any significant (P > 0.05) increase in the treated group. Histopathological examination of liver and kidney of treated animals did not show any pathological manifestations in treated animals. The animals were absolutely healthy and devoid of any adverse reactions, throughout the treatment period.

In summary, the findings of the present study revealed that both the aqueous extract and PPC-Pr Complex isolated from *P. rimosus* possess significant *in vitro* antioxidant activity and *in vitro* radioprotective activity. The PPC-Pr Complex offered significant radioprotection to haematopoietic system, tissue antioxidant levels and gastrointestinal system. The administration of PPC-Pr Complex significantly increased survival of the animals when compared to untreated control. Radioprotective mechanisms may be explained by the significant reduction of ROS level seen in mitochondrial tissues of treated group when compared to control. The PPC-Pr Complex also offered significant *in vivo* genoprotection to mice irradiated with 4 Gy as revealed by the micronucleus and chromosomal aberration assay. The PPC-Pr Complex was found to contain 50% carbohydrate and 40% proteins by standard analytical methods. Further, this compound was found to be non toxic by acute and sub acute toxicity tests. Thus our findings suggest therapeutic use of PPC-Pr Complex as a potential radioprotective agent.

**The thesis has been divided into following 7 chapters:**

Chapter 1: Introduction and Review of literature
Chapter 2: Materials and methods
Chapter 3: *In vitro* antioxidant activities of aqueous extract and Polysaccharide Protein Complex (PPC-Pr) Complex isolated from the mushroom, *Phellinus rimosus*

Chapter 4: *In vitro* radioprotective activities of aqueous extract and PPC-Pr Complex.

Chapter 5: *In vivo* radioprotective activities of PPC-Pr Complex.

a) Effect of PPC-Pr Complex on haematopoietic system.

b) Effect of PPC-Pr Complex on tissue antioxidant levels.

c) Effect of PPC-Pr Complex on gastrointestinal system.

Chapter 6: *In vivo* genoprotective studies of PPC-Pr Complex.

Chapter 7: Toxicity studies of PPC-Pr Complex

**References:**


