Cancer is a multistep process in which multiple genetic alterations occur, usually over a span of years to have a cumulative effect on the control of cell differentiation, cell division, and growth control. A carcinogen may be any substance such as radiation or chemicals that are directly involved in causing cancer. Ionizing radiation is an established carcinogen, having both cancer initiating and promoting effects. 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). The specific types of cancers associated with radiation exposure include leukemia, multiple myeloma, breast cancer, lung cancer, and skin cancer. Ionizing radiation has been found to induce serious damage at the molecular level to nucleotide sequences on the chromosomal DNA leading to different types of chromosomal aberrations. Low linear energy transfer (LET) radiation such as X-rays and Gamma Rays further increases the frequency of chromosomal aberrations proportional to the radiation dose. Cells undergoing frequent mitotic cell divisions are the most sensitive to the damaging effects of radiation. For example Zygotes, gastrointestinal epithelium and haemopoietic stem cells in bone marrow are more sensitive than lung and basal skin tissues which are in turn more sensitive than parenchyma cells of the liver.

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, these drugs were was found to have some undesirable side effects which include hypotension, nausea, vomiting, sneezing, hot flashes, mild somnolence and hypocalcemia. These side effects were severe enough to limit the amount of the drug required to levels lower than necessary to achieve maximal
radioprotection (Yuhas et al., 1980; Glover et al., 1983; Kligerman et al., 1984; Schuchter and Glick, 1993). Thus, there is continued interest 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).

Mushroom is one of the useful, delicious and mysterious member of the biosphere (Verma et al., 1987a, b). A number of bioactive molecules have been identified in many mushroom species including polysachharides, 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. 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). Proteoglycans from *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* stimulate immune system and enhance the production of interleukins (Kim et al., 2006). Further, 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. A related species *Phellinus linteus* is known to be extensively used in Chinese medicine (Ying et al., 1987). *Phellinus rimosus* is a parasitic host specific polypore macrofungus often found growing on jackfruit trees (*Atrocarpus heterophyllatus*) trunks (Leelavathy 2000). Many kinds of *Phellinus* (e.g. *P. linteus, P. rimosus, P. baumii, P. igniarius*, and *P. pini*, etc.) are known to have different medicinal effects (Jung et al., 1992; Cho et al., 2002; Park et al., 2003;
Ajith and Janardhanan 2002). It is usually used in traditional oriental medicine, and has been reported to have many pharmaceutical attributes, including anti-mutagenicity, anti-cytotoxicity (Ji et al, 2000), anti-cancer as well as enhancement of immunity (Kim et al 1996; Han et al, 1999) and antioxidant properties (Song et al, 2002). Earlier investigations showed that ethyl acetate and methanol extracts of \textit{P. rimosus} possessed antioxidant, antitumor and hepatoprotective activities (Ajith and Janardhanan 200; 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 \textit{P. rimosus} (Meera et al, 2009a; Meera et al, 2009b). However radioprotective properties of this mushroom has not been identified yet. Aim of this study is to investigate the radioprotective activities of \textit{P.rimosus} derived components.

The aqueous extract and the PPC-Pr Complex were isolated from the fruiting bodies of mushroom \textit{P.rimosus}. Both the aqueous extract and the PPC-Pr Complex were found to contain polysaccharides and proteins as the major bioactive constituents. The carbohydrate content and protein content of PPC-Pr complex was found to be 50% and 40% by Dubois method and Lowry’s method respectively. The only monosaccharide unit present was found to be D-glucose. The amino acid analysis by the TLC method showed that the major amino acids present in the isolated protein-bound polysaccharide were aspartic acid, glycine and serine.

Free radicals were a major interest for early physicists and radiologists and much later were found to be a product of normal metabolism. Today, it is well know that radicals cause molecular transformations and gene mutations in many types of organisms. 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 types of Reactive Oxygen Species (ROS) have the potential to interact with cellular components including DNA bases or the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks (Ward et al, 1988). Oxygen radicals can also oxidize lipids or proteins thus generating intermediates that react with DNA by forming adducts (Esterbauer et al, 1990). The antioxidants
present in mushrooms are of great interest as possible protective agents to reduce the oxidative damage in the human body without any interference (Adams and Wermuth 1999). The ethanolic extract of *Phellinus rimosus* (Berk) Pilat was reported to possess antioxidant and antihepatotoxic activities (Ajith and Janardhanan, 2002). In Taiwan, several different species of *Phellinus* were widely applied for antioxidant, anticancer purposes and hepatoprotective effects. In view of its traditional usage, reported activities and chemical composition, aqueous extract and PPC-Pr complex were determined for its *in vitro* antioxidant activities. 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 DPPH radical scavenging assay, ABTS+ radical scavenging assay, Ferric reducing antioxidant power (FRAP) assay, Superoxide (SOD) radical scavenging activity assay, Hydroxyl radical (OH•) scavenging assay, Inhibition of lipid peroxidation and Nitric oxide radical (NO•) scavenging assay. Further, the effect of aqueous extract PPC-Pr Complex were also evaluated in AAPH (2,2′ azobis (2-amidopropane) dihydrochloride) induced lipid peroxidation in mitochondria and microsomes.

This study revealed that both the aqueous extract and PPC-Pr Complex have significant antioxidant as well as reducing property. Aqueous extract demonstrated potent antioxidant activity in Nitric oxide radical scavenging assay, Superoxide radical scavenging assay, Hydroxyl radical scavenging assay and lipid peroxidation assay. The half inhibition concentration of the aqueous extract for Nitric oxide radical scavenging assay was (IC50) found to be 92.02 ± 4.54µg/ml. The aqueous extract also demonstrated significant reducing property in terms of FRAP, DPPH and ABTS assay. For FRAP and DPPH assay the IC50 value was found to be 2.99 ± 0.34µg/ml and 18.06 ± 0.89µg/ml respectively. The PPC-Pr Complex also demonstrated potent antioxidant as well as reducing property. The IC50 value for lipid peroxidation and hydroxyl radical scavenging assay was found to be 109.25 ± 2.11µg/ml and 509.39 ± 10.17µg/ml respectively. The reducing property was higher for FRAP assay compared to DPPH and ABTS assay. The IC50 value for FRAP, ABTS and DPPH and assay was found to be 2.99 ± 0.34µg/ml, 9.40 ± 0.97 µg/ml and 18.06 ± 0.89µg/ml respectively. Exposure to free radical generating chemical, 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. The PPC-Pr complex offered significant protection from the formation of Thiobarbituric Acid Reacting Substances (TBARS) and the percentage inhibition was found to be 76% and 89% when compared to control. The percentage inhibition of microsomal and mitochondrial Lipid hydroperoxide (LOOH) formation by the PPC-Pr complex at a concentration of 100 μg/ was found to be 69% and 71% respectively. The aqueous extract at a concentration of 100 μg/ml also offered significant protection from TBARS and LOOH formation in rat liver microsomes and mitochondria. The significant antioxidant activities 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.

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 are membranes and DNA. The major damage to membranes is due to the oxidation of lipids present in them. One of the major form of membrane damage induced by stress factors such as radiation is lipid peroxidation (LPO). The most commonly studied biologically relevant lipid peroxidation parameters are Thio Barbituric Acid Reacting Substances (TBARS) and Lipid Hydroperoxides (LOOH) respectively. 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. However, in the presence of polysaccharides, there was significant reduction of LPO parameters. Mitochondria and microsomes when exposed to 450 Gy of γ rays showed significant increase in LPO parameters like TBARS and LOOH. However, in the presence of polysaccharides, there was significant inhibition of LPO. Thio Barbituric Acid Reacting Substances (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, PPC-Pr Complex at 100 µg decreased TBARS to 20.41 nmol/mg protein and 26.42 nmol/mg protein in mitochondria and microsome respectively. Lipid Hydroperoxides (LOOH), another index of lipid peroxidation also increased significantly in the irradiated control sample. There was 2.5 and 3.3 fold increase in LOOH formation in mitochondria and microsomes respectively in radiation alone treated group when compared to untreated group. Treatment with PPC-Pr complex was effective to reduce LOOH levels to normal range. Aqueous extract in the concentration range 10-100µg was also found to be effective to reduce lipid peroxidation parameters.

The γ irradiation induced damage to DNA was assessed by comet assay. 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. When cells were exposed to gamma radiation (4 Gy) , tail length was increased from 27.30 ± 2.73 to 38.56 ± 3.54, % DNA in tail was increased from 49.77 ± 3.06 to 71.88 ± 2.53, tail moment was increased from 8.23 ± 1.64 to 13.49 ± 2.70 and olive tail moment was increased from 8.70 ± 1.74 to 17.08 ± 3.42 in blood cells. However PPC-Pr Complex at a concentration of 100µg brought down tail length , % DNA in tail, tail moment and olive tail moment to 28.96 ± 2.5, 50.80 ± 3.8, 10.08 ± 2.01, 8.88 ± 1.27 respectively at 4 Gy of irradiation. Thus administration of PPC-Pr Complex inhibited the increase of these parameters significantly indicating its DNA protective effects. The decrease in comet parameters after the administration of aqueous extract at concentrations of 100µg was found to be 13.5 ± 1.21, 40.11 ± 3.65, 10.35 ± 1.6, 7.91 ±0.67 for tail length, % DNA in tail, tail moment and olive tail moment respectively. It was also found that both the PPC-Pr Complex and aqueous extract was effective for protecting the genomic DNA from radiation induced oxidative stress. In vitro DNA protective effects of these drugs were also assessed by the protection offered to plasmid DNA, PBR 322 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 preseving the native supercoiled form at a concentration as low as 100 µg. Thus our results suggest that both the PPC-Pr Complex and aqueous extract are potent agents to reduce the radiation induced damages to membranes and DNA. 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.

Exposure to the artificial (man-made) radiation comes from various sources like diagnostic radiotherapy and nuclear medicine apart from natural background radiation. In radiotherapy, total body irradiation is used for systemic treatment of lymphoma, leukemia and as a method of immunosuppression before bone marrow transplantation. Further, many human beings are exposed to whole body irradiations like survivors of Hiroshima and Nagasaki and military personnel exposed to fall out from nuclear tests. Whole body irradiation causes haematological, gastrointestinal and neurovascular syndromes in the body. However, at doses used in clinical radiotherapy, haemopoietic and gastrointestinal syndromes are more pronounced (Hill and Bristow, 2004). The haemopoietic syndrome occurs at doses in the range of 2 to 8 Gy in humans (3 to 10 Gy in rodents). Damage to haemopoietic system is considered to be the major hazard in mammals exposed to ionizing radiation in low dose range. Radiation attenuates the endogenous antioxidant enzyme system, which is considered as first line defense mechanism to maintain redox balance and normal biochemical processes. So administration of immunomodulating drugs in combination with conventional radiotherapy strengthens patient’s tolerance to treatment. Further, radiotherapy causes immunosuppression in the host that can significantly increase patients risk for infection and inflammation. Therefore, biological response modifiers (BRM) are used as nontoxic immune modulators or mediators of an effective immune response in the field of cancer prevention and therapy. Mushroom derived polysaccharides had been reported to boost immune cell production and ameliorate chemotherapy symptoms (Chu et al, 2002).

The *in vivo* radioprotective effect of PPC-Pr Complex was evaluated in the haemopoietic system, tissue antioxidant system and gastrointestinal system of γ irradiated 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 when 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), where 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. The PPC-Pr Complex 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. The 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 complex 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 other antioxidant enzymes such as SOD and CAT. Pre administration of PPC-Pr Complex resulted in significant elevation in the activities of these enzymes. The PPC-Pr (5 and10 mg/Kg bwt) Complex showed 1.7 and 2.2 times increase in SOD activity than control (p < 0.001) on 7th day. The CAT activity was also decreased significantly by irradiation in the control group compared to normal (p < 0.001). However, activity was restored significantly by both PPC-Pr and amifostine treatment. Thus significant restoration of antioxidant enzyme activity was seen in all of the treated groups. Lipid peroxidation in the serum was increased significantly from 1st day onwards upto the 7th day as a result of γ irradiation. Administration of PPC-Pr Complex significantly decreased serum lipid peroxidation. There was 2.2 and 3.1 times decrease in serum LPO levels in 5 mg/Kg bwt and 10mg/Kg bwt treated group respectively on 7th day when compared to control. The drug was more effective than amifostine, which provided
1.8 times decrease in serum LPO when compared to control on 7th day. Thus the results of the present study 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 serum lipid peroxidation was also significantly reduced by treatment with PPC-Pr Complex.

The influence of PPC-Pr Complex on tissue antioxidant enzyme system was evaluated in the liver and brain homogenate of irradiated mice. Significant decrease (p < 0.05) in tissue antioxidant enzymes such as GPx, SOD, CAT, were observed in the irradiated control group from 24 h onwards after irradiation when compared to normal. It was observed that the PPC-Pr Complex treatment (10 mg/kg bwt, i.p) significantly elevated (p < 0.001) antioxidant enzymes GPx, SOD, CAT, when compared to control. Comparable effects were obtained for both PPC-Pr Complex (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. Lipid peroxidation was significantly increased in the irradiated control animals. Lipid peroxidation marker, MDA level was increased significantly (p < 0.001) in the irradiated control mice when compared to normal. There was 2.5 and 4.3 fold increase in MDA level in liver and brain homogenate respectively on 7th day after irradiation. The PPC-Pr Complex at both the concentration (5 and 10 mg/kg bwt) offered significant improvement in the inhibition of MDA level. The effect of PPC-Pr Complex on survival rate of organisms was studied in 9 GY irradiated mice. Animals were observed upto 30 post irradiation days. The survival rate in the irradiated control group was found to be 30% on 15th post irradiation day while, PPC-Pr Complex at 5 and 10 mg/kg bwt offered 50% and 60% survival. The survival rate of animals after 30th post irradiation day was found to be 20% in the untreated control group. PPC-Pr Complex at 10 mg/kg bwt offered 40 % survival on 30th post irradiation day. The survival of animals by PPC-Pr Complex treatment reflect its wholesome effect of scavenging of radiation induced free
radicals by the enhanced antioxidant status in the treated group. Reactive Oxygen Species (ROS) level can be taken as general indicator of oxidative damage. Intracellular ROS production was measured using the dye 2, 7 dichlorodihydrofluorescein diacetate (DCFDA). The extent of the reactive oxygen species (ROS) formation in the mitochondria from irradiated mice (4Gy) was determined compared to unirradiated control. Mitochondria from both liver and brain of irradiated animals showed significant increase in fluorescence suggesting high ROS accumulation. Significant reduction in ROS level was observed in both the PPC-Pr treated group. Thus Improved antioxidant status in the treated animals is 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 an important dose-limiting organ during radiation therapy of tumors in the pelvis or abdomen. Radiation-induced injury to the GI tract is a critical dose-limiting factor during treatment of abdominal and pelvic tumors. Radiation responses in intestine are manifested by changes in cellular function and alterations in morphology (Denham et al, 2001; Denham and Hauer-Jensen, 2002). Severity of intestinal radiation toxicity depends directly on cell death in intestinal crypts. Radiation treatment damages the mucosal lining of gastrointestinal tract which result in diarrhoea, vomiting and ultimately leads to loss of electrolyte and fluid balance. Intestinal damage limits the efficacy of radiotherapy for cancer and can result in death if the whole body is exposed to too high dose. Intestinal radioprotection was assessed in terms of antioxidant enzyme activities of intestinal mucosal cells of PPC-Pr Complex treated groups and was compared with standard amifostine. Further, histopathologic examination of mucosal cells was also performed to assess radioprotective properties. The activities of antioxidant enzymes such as GSH and GPx in intestinal mucosa were significantly decreased by whole body irradiation in the control group when compared to normal (p < 0.001). The 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. The SOD activity was restored significantly by PPC-Pr Complex treatment when 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. Significant restoration of mucosal antioxidant enzyme activity was observed in the treated group than control on 14th day after irradiation. 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 Complex and amifostine, these changes were less pronounced. The present study concluded that PPC-Pr Complex effectively protected the intestinal tissue, one of the most radiosensitive tissues from radiation induced damages. It also provided significant protection to intestinal mucosal cells as evident from histopathological analysis of intestinal jejunum. Thus the results of the present study concluded that the PPC-Pr Complex could be effectively used as protective agents during intestinal radiotherapy.

The interaction of free radicals with sugar moieties leads to cleavage of the sugar-phosphate backbone of DNA followed by single-strand breaks that undergo processes relatively easily. However, irradiation also exhibits multiple damaged sites and clustered regions which leads to double strand breaks. (Karbownick and Reiter, 2000). Double strand breaks are well correlated with the cytotoxic effects of ionizing radiation and are considered the primary lesion involved in cellular death (Elia et al, 1991). The indices of DNA damage caused by ionizing radiation are chromosome aberrations and micronuclei 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 et al, 1995b, Vijayalaxmi et al, 1996; Vijayalaxmi et al, 1998). Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. Chromosome aberrations are widely used as a biomarker of radiation damage or as a biodosimeter of radiation exposure many years after medical, accidental, or occupational exposure (Tucker, 2001). DNA
strand breaks of the double strand type also lead to the formation of micronuclei. Damage to the chromosomes manifested as breaks and fragments, usually appear as micronuclei in the rapidly proliferating cells. Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or whole chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damages (Fagr et al, 2008).

The frequency of micronuclei as well as chromosomal aberrations were tested in the PPC-Pr Complex treated groups (5 & 10 mg/ Kg bwt). 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 0.87 from 1.11 in the irradiated control group. The PPC-Pr Complex increased the P/N ratio to 0.91 and 0.94 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 and Severly Damaged Cells (SDC). Chromosome breaks and Chromatid breaks were the predominant aberrations seen in the irradiated control group. Significant reduction in the aberrant metaphases was observed in the treated groups 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 demonstrated that the PPC-Pr Complex 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 PPC-Pr Complex appeared to be compatible with radiation therapy and thus suggests its potential role as a genoprotective agent.

The mushroom *P.rimosus* is a medicinally valuable as well as a nutritious mushroom and therefore, has a possibility which can be developed as a functional food and livestock for industrial application in near future. But before developing any pharmaceutical or dietary supplement, it is essential to evaluate the toxicity of the compound. Results of the toxicity study revealed that PPC-Pr Complex of *P.rimosus* did not produce any acute toxicity. The extracts up to a dose of 100mg/kg body weight, i.p was not lethal to animals and LD$_{50}$ could not be determined. In subacute toxicity studies, treatment with two different concentration of the extract (10 & 50 mg/Kg bwt, i.p) did not produce any statistically significant change in the hematological or biochemical parameters when compared to the normal group of animals. The haemoglobin content of the drug administered animals was also found to be almost constant throughout the period of study. There was no significant change in the the total leukocyte count and R.B.C. count of animals treated with both the doses of PPC-Pr Complex. Similarly, treatment of the extract for 15 days did not produce any significant change in the liver function and kidney function tests when compared to the normal group of animals. The ALP activities in the normal and PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were found to be 128.03 ± 10.32, 116.92 ± 8.09 and 110.96 ± 2.75 IU/L respectively. The SGPT activities in the normal and PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were 37.6 ± 3.33, 33.4 ± 6.94 and 38.9 ± 5.99 IU/L respectively. The SGOT activities in the normal as well as PPC-Pr Complex treated (10 and 50 mg/kg bwt) group of animals were 149.2 ± 12.33, 116.92 ± 8.72 and 110.96 ± 7.75 IU/L respectively. The normal as well as the PPC-Pr Complex (10 and 50 mg/kg bwt) treated group of animals showed serum urea level of 46.55 ± 4.33, 48.03 ± 7.76 and 50.89 ± 2.59 mg/dl respectively. The creatinine concentration in the normal and PPC-Pr Complex treated group were found to be 7.68 ± 0.09, 6.84 ± 0.07, 8.52 ± 0.13 mg/dl respectively. Thus no significant changes were observed in any of the liver or kidney function
parameters. Further, no clinical signs of any adverse or toxic symptoms were noticed throughout the period of sub acute toxicity study. Histopathological analysis of liver and kidney also did not show any pathological lesions in the organs of animals treated with PPC-Pr Complex. The study also revealed the scope of this mushroom for the production of safe and non toxic agents with therapeutic and nutraceutical properties.

In summary, the findings of the present study revealed that both the aqueous extract and PPC-Pr Complex isolated from *P. rimosus* possessed significant *in vitro* antioxidant activity and *in vitro* radioprotective activity. The PPC-Pr Complex offered significant radioprotection to haemopoietic system, tissue antioxidant levels and gastrointestinal system of the irradiated mice. The administration of PPC-Pr Complex significantly increased survival of the animals when compared to untreated control. Radioprotective mechanisms might be due to the significant reduction of ROS level seen in mitochondria tissues of treated group 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 assays. Further, acute and sub acute toxicity tests showed that this compound is nontoxic. The findings thus suggest the therapeutic use of PPC-Pr Complex as a potential radioprotective agent.

**Key words:** PPC-Pr Complex; Mushrooms; Phellinus; Radioprotection; Antioxidants; γ radiation; Haemopoietic; Reactive Oxygen Species (ROS); Micronucleus; Chromosomal aberrations; Amifostine.