Chapter 1

Review of Literature
## Table of Contents

1.1. Carcinogenesis
1.2. Radiation Carcinogenesis
1.3. Ionizing Radiations
1.4. Radiotherapy
   1.4.1. Acute Tissue Response
   1.4.2. Late Tissue Responses
   1.4.3. Whole Body Irradiation
1.5. Radiation Interaction with cells
   1.5.1. Membrane Damage
   1.5.1.1. Lipid Peroxidation
   1.5.1.2. Protein Damage
   1.5.1.3. DNA Damage
   1.5.1.4. Chromosomal Aberrations
   1.5.1.5. Micronuclei
1.6. Reactive Free Radicals
   1.6.1. Biologically Important Radicals
   1.6.1.1. Hydroxyl Radical (•OH)
   1.6.1.2. Superoxide Radical (•O$_2^-$)
   1.6.1.3. Nitric Oxide Radical (NO’)
   1.6.1.4. Peroxyl and Alkoxyl Radicals (RO$_2^-$ And RO’)
   1.6.2. Biologically Important Non-Radicals
   1.6.2.1. Hydrogen Peroxide (H$_2$O$_2$)
   1.6.2.2. Hypochlorous Acid (Hocl)
   1.6.2.3. Singlet Oxygen (¹O$_2$)
   1.6.2.4. Peroxynitrite (ONNO$^-$)
1.7. Antioxidants
1.7.1. Enzymatic Antioxidants
1.7.1.1. Superoxide Dismutase (SOD)
1.7.1.2. Catalase (CAT)
1.7.1.3. Glutathione Peroxidase (Gshpx)
1.7.2. Non Enzymatic Antioxidants
1.7.2.1. Glutathione (GSH)
1.7.2.2. Vitamin C
1.7.2.3. Vitamin E
1.7.2.4. Carotenoids
1.8. Radioprotectors
1.8.1. Amifostine
1.9. Mushrooms
1.10. Phellinus
1.11. Phellinus Rimosus
1.12. Mushroom Polysaccharides
1.12.1. Medicinal Value of Mushrooms Polysaccharides
1.13. β-Glucans
1.13.1. Mechanism of Action
1.13.2. β-Glucan as Adjuvant to Cancer Chemo- And Radiotherapy
1.14. Scope of the Study
1.1. Carcinogenesis

Cancer is a group of more than 100 different diseases, which manifest itself in uncontrolled cellular reproduction, tissue invasion and distant metastasis (Levi et al, 2001). It is one of the leading causes of death in the world. The classic view of two stage carcinogenesis states that tumor initiation by mutation is followed by epigenetic changes which cause tumor promotion. Tumor initiation begins in cells through mutations from exposure to carcinogens. Many factors including exposure to viruses, xenobiotic chemicals and radiation contribute to tumor initiation (Yuspa 2000). The tumor initiated cells have decreased responsiveness to the intercellular and intracellular signals which maintain their architecture and homeostatic growth. Tumor promotion occurs by selective clonal expansion of the initiated cells by physical perturbation of the normal microenvironment (e.g. wounding of mouse skin or partial hepatectomy in rodents), chemical agents (Phorbol esters and phenobarbital) microbial agents (Influenza virus and hepatitis virus) or by other inflammatory process. These processes results in further selective clonal expansion and proliferation of the initiated cells, thereby enhancing the probability of additional genetic damage through endogenous mutations. These mutated cells have an altered responsiveness to their microenvironment and a selective growth advantage when compared with surrounding normal cells which then produces malignant neoplasm.

Fig 1.1: Multi stage carcinogenesis.
1.2. Radiation Carcinogenesis

Radiation injury to living cells is, to large extent, due to oxidative stress (Freeman and Crapo 1982; Epperly et al, 1998). Ionizing radiation is known to produce various types of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), in biological systems such as superoxide anion (O$_2^-$, a free radical), hydroxyl radical (•OH), and hydrogen peroxide (H$_2$O$_2$). These free radicals contribute to radiation-induced cytotoxicity (e.g., chromosome aberrations, protein oxidation, and muscle injury) mutations and chromosome instability for many generations due to successive chain reactions (Morgan et al, 2002; Devi et al, 2000). Genomic instability has been detected following both low- and high-LET (Linear Energy Transfer) radiation exposure (Limoli et al, 2000) and appears to be dependent on species, strain, and tissue type. Genomic instability leads to increased rate of alterations to the genome, including gene mutations, chromosomal abnormalities, micronuclei formation, reduced plating efficiency, and cellular transformation.

The mechanisms by which radiation induces genetic instability include (1) Mutations in genes involved in the control of DNA synthesis or DNA repair (2) The induction of chromosome instability (3) Persisting aberrant production of oxygen radicals which can damage DNA (Little, 2000; Morgan et al, 2002). However, the ultimate mechanism by which radiation induces malignant transformation is the activation of protooncogenes or inactivation of tumor suppressor genes (Cox, 1994). The degree of radiation-induced cell damage depends on several factors including the radiation dose, stage of the cell within the cell cycle, levels of cellular antioxidant defense, time of administration and the availability of oxygen in tissues during irradiation (Weichselbaum et al, 1997). Radiation-induced cancers may take 10 - 15 years or more to appear. Tumors induced by radiation have relatively long latencies, which vary in different species as a more or less constant function (UNSCEAR 1993). Within a given species the latency varies also with age at the time of irradiation and with the type of neoplasm induced. The major types of cancer inducing radiations are ionizing radiation and UV radiation.
1.3. Ionizing Radiations

Ionizing radiation is an established carcinogen, having both initiating and promoting effects. The specific types of cancers associated with ionizing radiation include leukemia, multiple myeloma, breast cancer, lung cancer, and skin cancer. The carcinogenic risks of radiation exposure in people have been derived from many sources, including occupational exposures (radiologists and uranium miners), therapeutic exposures (radiotherapy, or treatment of ankylosing spondylitis), and accidental exposures (Bhatia and Sklar 2002). However, most information was from studies of the atom-bomb survivors in Hiroshima and Nagasaki and from studies of exposures during medical X-ray examinations, particularly of pregnant women, which resulted in fetal exposure to irradiation. A positive correlation between ionizing radiation and carcinogenesis has been established from these studies (Knox et al, 1987).

The first evidence of a carcinogenic effect of ionizing radiation was an increase in the incidence of leukemia’s among the atom bomb survivors of Hiroshima and Nagasaki. The rapid increase in leukemia rates was due to short latent period for this malignancy. Acute leukemia’s, predominantly myeloid in adults and lymphocytic in children, were the most common forms among the irradiated population. In addition to leukemia, solid cancers such as thyroid carcinomas and breast cancers were also detected in a relative low rate after ionizing radiation exposure (Preston et al, 2003). Among the other forms of cancer detected are lung cancer, stomach cancer, colon cancer and multiple myeloma.

Other examples of cancer incidence from radioactivity exposures are nuclear power plant accidents (Chernobyl 1986) and weapon tests which were reported to induce both benign and malignant thyroid neoplasms particularly due to radioactive iodine. (Conard 1977). Further, significant increase in tumor incidence such as papillary thyroid tumors were reported in young children (Williams 2002) from the ingestion of contaminated milk with radioactive iodine. X-rays, a useful diagnostic tool are also a source of Ionizing radiation (Glasser 1945). The harmful effect of large doses was unknown in the beginning, and physicians using X rays in the early days received excessive doses. Several studies of children irradiated in utero with medical X-rays from about 1940 to 1975 demonstrated an increased risk
of cancer induction, especially for leukemia and solid tumors (Doll 1995; Doll and Wakeford 1997).

Specific malignant conditions have also been found to follow occupational exposures to radioisotopes, particularly those of radium series. Studies of uranium miners have shown that exposure to radon gas causes an increased risk of lung cancer, which is roughly proportional to dose (Darby et al, 1995; Lubin et al, 1995). One tragic example of excessive doses of occupational irradiation was the occurrence of estrogenic sarcomas in a group of women who had been engaged in the application of radium salts to the numbers of hands of watches to make them fluorescent (Martland 1931). Therapeutic administration of radium nucleotides for tuberculosis and ankylosing spondylitis patients had also reported to induce osteogenic sarcoma (Looney 1958). In addition to radium, radioactive thorium dioxide used for diagnostic radiography, resulted in malignant hepatic tumors (McMahon et al, 1947).

Radiation-related increase in breast cancer had been reported in tuberculosis patients who were repeatedly exposed to high dose fluoroscopic examination and in women who received radiotherapy for postpartum mastitis (MacKenzie 1965; Boice and Monson, 1977; Shore et al, 1977). Gamma rays, used in cancer patients are also a source of ionizing radiation and adversely affect the nearby normal cells also. There are reports of cancer induction by radiotherapy of cancer patients. Among them are thyroid cancers as well as lymphocytic leukemia which were reported in children after the radiotherapy of the thymus or thyroid gland (Simpson 1957; Hempelmann et al, 1975; Ron and Modan 1980).

Moreover, ultraviolet (UV) rays are ionizing radiations with skin carcinogenic potential. Skin cancer is prevalent in people with high levels of sun exposure. The first report of carcinogenic effect of UV radiation was by Unna in 1894 (Unna, 1896). He reported a high incidence of skin cancer in people exposed to excessive sunlight for long periods of time. Further several studies with mice exposed to ultraviolet light for long periods confirmed the carcinogenic potential of UV rays (Findlay 1928; Rusch, 1941).
1.4. Radiotherapy

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). Radiotherapy involves both external beam radiotherapy and brachytherapy: treatment choice depends on the type of tumor and location within the body. The dose of radiation is determined by the intent of the therapy (i.e. curative or palliative), the volume of the tumor, the relative radio sensitivity 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 acute and chronic side effects that occur following local radiotherapy are directly linked to the normal structures and tissues within the irradiated volume (Table.1.1). The effects of radiation treatment on normal tissues have been divided, based on functional and histopathological endpoints into early (acute) responses and late responses. Early responses occur within a few weeks of radiation treatment, and late responses may take many months or years to develop. Acute responses occur primarily in tissues with rapid cell renewal where cell division is required to maintain the function of organ. Late responses occur in organs whose parenchyma cells divide infrequently or rarely under normal conditions. Damage to connective tissue and vasculature of the organ leads to progressive impairment of the circulation. If the damage is severe, secondary parenchymal cell death also occur due to nutrient deprivation. Ultimately, radiation exposure may cause functional failure of the organ involved.

1.4.1. Acute tissue response

The toxicity of high-dose ionizing radiation (IR) is associated with induction of abnormalities collectively known as acute radiation syndromes (ARS) involving the hematopoietic 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. Hematopoietic syndrome occurs in bone marrow. Radiation exposure causes decrease in red, white and platelet cells which cause decrease in blood clotting capacity and death within 6-8 weeks. Gastrointestinal syndrome occurs at a dose of 600 rads and death occurs within 3
<table>
<thead>
<tr>
<th>Irradiation Site</th>
<th>Tissues At Risk</th>
<th>Acute Effect</th>
<th>Chronic Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Brain, Neural Structures</td>
<td>Hair Loss</td>
<td>Cognitive dysfunction and decreased visual acuity</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>Oral Mucosa, Salivary Glands, Skin</td>
<td>Oral inflammation (mucositis), Xerostomia, Erythema</td>
<td>Permanent Xerostomia, Decreased ability to open mouth (Trimus), Dental Caries, Skin Fibrosis</td>
</tr>
<tr>
<td>Thorax</td>
<td>Esophageal mucosa, Lung, Skin</td>
<td>Esophagitis, pneumonitis</td>
<td>Lung Fibrosis, Esophageal</td>
</tr>
<tr>
<td>Abdomen</td>
<td>Intestine, Pancreas, Liver, Spleen, Kidneys</td>
<td>Nausea, hepatitis, diarrhea</td>
<td>Renal compromise, liver fibrosis,</td>
</tr>
<tr>
<td>Pelvis</td>
<td>Bladder, Rectum, Prostrate</td>
<td>Increased urinary</td>
<td>Bladder or rectal bleeding</td>
</tr>
</tbody>
</table>

Table. 1.1. Acute and chronic side effects of radiotherapy
to 10 days. 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). Acute Radiation syndromes (ARS) can be summarized in the table (Table.1.2).

Acute Radiation syndromes (ARS) include symptoms which appear within minutes, hours, days or weeks. These include nausea, fatigue, erythema (redness of skin), epilation (loss of hair), blood disorders, fever, dry and moist desquamation (shedding of skin), sterility etc. Early erythema occurs following irradiation of the skin and is related to the release of 5-hydroxytryptamine by mast cells. Erythema in human skin occurs at single doses greater than about 7 Gy, while moist desquamation and ulceration occur after single doses of 20 to 25 Gy. Hair loss (epilation) is similar to skin effects and can occur after acute doses of about 500 rad. Sterility can be temporary or permanent in males, depending upon the dose. In females, it is usually permanent, but it requires higher doses such as 400 rad to the reproductive cells. Cataracts (a clouding of the lens of the eye) appear to have a threshold of about 200 rad before they begin forming. Expression of further acute reactions (moist desquamation and ulceration) depends on the relative rates of cell loss and cell proliferation of the basal cells. The extent of these reactions and the length of time for recovery depend on the dose received and the volume of the tissue irradiated, because early recovery depends on the number of surviving basal cells that are needed to repopulate the tissue.

1.4.2. Late tissue responses
Late tissue responses occur in organs whose parenchymal cells normally divide infrequently and hence do not express mitosis linked death. The nature and timing of late reactions depend on the tissue involved and can be expressed as diminished organ function. One common late reaction is the slow development of tissue fibrosis that occurs in many tissues such as subcutaneous tissue, muscle, lung, gastrointestinal tract, after a number of years after radiation treatment. Radiation induced fibrosis appears to be associated with aberrant and prolonged expression of growth factor TGF-β following irradiation (Hakenjos et al, 2000: Martin et al, 2000). This growth factor can stimulate proliferation of fibroblasts and their differentiation to fibrocysts that produce collagen. Transforming growth factor β
also plays a major role in wound healing and the development of late radiation reactions (Denham and Hauer-Jensen 2002).

Apoptosis had been observed within hours after irradiation of a number of late responding normal tissues in rodents, such as salivary glands (Stephens et al, 1991), pulmonary and brain endothelial cells (Fuks et al, 1994) and spinal cord (Li et al, 1996). These responses usually occur within three months of start of radiotherapy but are not usually limiting for fractionated radiotherapy because of the ability of the tissue to undergo rapid repopulation to generate the parenchymal cell population. Radiation induced apoptosis has also been detected in many other cells and tissues such as lymphoid, thymic and spermatogonia. The lung is an important site of late radiation damage. There are two types of reactions in the lung tissue: pneumonitis that occurs within 2 to 6 months after irradiation and fibrosis that occur after more than one year of irradiation. If severe these reactions increase tissue density breathing rate in the lung tissue. Studies in lung cancer patients have revealed prolonged increases in TGF-β levels in plasma following radiotherapy to the likelihood of developing lung fibrosis (Anscher et al, 1998; Marks et al, 2003). The development of late radiation induced normal tissue injuries is driven in part by chronic oxidative stress.

1.4.3. Whole body irradiation

Patients are exposed to whole body irradiation before bone marrow transplantation. The response of animals to single dose whole body irradiation can be divided into three separate syndromes as hematological, gastrointestinal, and neurovascular (Mettler and Voelz 2002; Dainiak et al, 2003). The haematopoietic syndrome occurs at doses in the range of 2 to 8 Gy in humans (3 to 10 Gy in rodents) and is caused by severe loss of blood elements due to killing of precursor elements in bone marrow which leads to loss of proliferative capacity of stem cells. Various side effects including leucopenia, thrombocytopenia, loss of electrolyte and fluid balance have also been observed in patients undergoing radiotherapy. The intestine is an important dose-limiting organ during radiation therapy of tumors in the pelvis or abdomen. Radiation responses in intestine are manifested by changes in cellular function and alterations in morphology (Denham and Hauer-Jensen 2001: Denham
et al, 2002). Severity of intestinal radiation toxicity depends directly on cell death in intestinal crypts. The gastrointestinal syndrome occurs after doses greater than about 5 to 12 Gy. It causes a loss of the protective mucosal barrier with consequent infection, loss of electrolytes and fluid imbalance. This syndrome causes death in rodents (at the higher dose levels) between about 12 to 30 days after irradiation and somewhat later in larger animals, including humans. The neurovascular syndrome occurs following large doses of radiation (> 20 Gy) and usually results in rapid death due to cardiovascular and neurological dysfunction.

Although radiotherapy is an important and effective tool for the treatment of cancer, the radiosensitivity of normal tissues adjacent to tumor limits its therapeutic gain. The use of ionizing radiation in cancer therapy leads 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 responses of normal tissues to therapeutic radiation exposure range from those that cause mild discomfort to others that are life threatening. The speed at which a response develops varies widely from one tissue to another and often depends on the dose of radiation that the tissue receives (Steel 2002).

1.5. Radiation interaction with cells

Damaging effects of ionizing radiation are brought about by both direct and indirect mechanisms. The direct action produces disruption of sensitive molecules in the cells whereas the indirect actions of ionizing radiation occur when it interacts with water molecules in the cell, resulting in the production of highly reactive free radicals such as \( \cdot \text{OH} \), \( \cdot \text{H} \) and \( \cdot \text{aq} \), \( \cdot \text{O}_2 \). Further in chemical phase these free radical react with oxygen and nitrogen to produce reactive oxygen species and reactive nitrogen species such as hydrogen peroxide, organic hydroperoxides, NO (nitric oxide), NO\(_2\) (nitrogen dioxide), N\(_2\)O\(_3\) (dinitrogen trioxide) etc. (O’Neill and Fielden 1993; Evans and Halliwell 1999). Free radicals are highly unstable species and in the last phase of damage and they can react with variety of small as well as macromolecules available in cellular microenvironment and initiate chain of reactions in a small volume of cell. The half lives of these free radicals are extremely short, \(10^{-6} - 10^{-10}\) seconds. However, they immediately react with any
biomolecule in the vicinity and produce highly site-specific oxidative damage. Reactive Oxygen Species (ROS) can also induce cellular antioxidant enzymes such as superoxide dismutase and glutathione peroxidase (Zhang et al, 2005). Free radicals mediated damage to critical cellular structures, such as lipids in the membrane, proteins and nucleic acids is believed to be the principal cause of ionizing radiation induced biological effects (Natarajan et al, 1994). These changes can disrupt cell function and may kill the cells. The destructive effects of ionizing radiation can be categorized into membrane damage and DNA damage.

1.5.1. Membrane damage
Cellular membranes are one of the most important targets for free radicals in the organism. The major damage to biological membranes such as mitochondria and microsomes is due to the oxidation of lipids present in them. The peroxyl radical formed from lipid peroxidation attacks membrane proteins, enzymes and reinitiates lipid peroxidation. Lipid peroxidation within the membrane has a devastating effect on the functional state of the membrane as it alters membrane fluidity, typically decreasing it and allowing ions such as Ca\(^{2+}\) to leak into the cell. Cell membrane phospholipids are very sensitive to oxidation and they are also frequent targets of radical-induced damage that enables them to participate in free radical chain reactions. The high concentration of polyunsaturated fatty acids in phospholipids enables them to participate in free radical chain reactions. Membrane proteins are also susceptible to oxidative damage by the free radicals.

1.5.1.1. Lipid peroxidation
Cell membrane phospholipids are very sensitive to oxidation and have been found to be frequent targets of radical-induced damage that enables them to participate in free radical chain reactions. Many of the fatty acids are polyunsaturated, containing a methylene group between two double bonds that makes the fatty acid more sensitive to oxidation. The high concentration of polyunsaturated fatty acids in phospholipids enables them to participate in free radical chain reactions (Porter 1986). The process of lipid peroxidation is the oxidative conversion of polyunsaturated fatty acids to products known as aldehydes or lipid peroxides, which is the most commonly studied, biologically relevant, free radical reaction. Lipid hydroperoxides are initial products of unsaturated fatty acid oxidation.
Aldehydes such as thiobarbituric acids reactive substances (TBARS) have been widely accepted as a general marker of free radical production (Clarkson 1995). The most commonly measured TBARS is the malondialdehyde (MDA) (Karlsson 1997) (Fig 1.2). Malondialdehyde (MDA) is one of the major aldehyde products of lipid peroxidation (Zollner et al, 1975). Malondialdehyde due to its high cytotoxicity and inhibitory action on protective enzymes act as tumor promoter and a co carcinogenic agent. Malondialdehyde as well as lipid peroxidation byproducts such as 4- hydroxynoneal have the ability to interact with and alter DNA and other macromolecules (Pandey et al, 2000).

1.5.1.2. Protein damage
Proteins are the most abundant organic components of living organisms and are known to react readily with biologically significant ROS (Alberts et al, 2002; Du and Gebicki 2004). Oxidative modification of proteins in vivo may affect a variety of cellular functions involving proteins such as signal transduction mechanisms, transport systems and enzymes. It could also contribute to secondary damage to other biomolecules leading to inactivation of DNA repair enzymes, loss of fidelity of DNA polymerases in replicating DNA and the development of new antigens provoking autoimmune responses (Evans et al, 1999 ). Many different types of protein oxidative modification can be induced directly by reactive oxygen species (ROS) or indirectly by reactions of secondary by-products of oxidative stress (Berlett and Stadtman 1997). The oxidation of proteins plays an essential role in the pathogenesis of an important number of degenerative diseases and in ageing, which is now widely recognized (Berlett and Stadtman 1997; Stadtman and Berlett 1998). The exposure of proteins to reactive oxygen species (ROS) can alter the physical and chemical structure of the target causing consequent oxidation of side-chain groups, protein scission, backbone fragmentation, cross-linking, unfolding, and formation of new reactive groups. The latter include oxidation of hydrophobic amino acyl residues to hydroxy and hydroperoxy (P-OOH) derivatives, protein carboxylation (PCO), oxidation of protein thiol (P-SH) groups, nitrotyrosine (NT) formation and many others. The conformational changes that result from this complex of reactions lead to the decrease or loss of protein biological function. Therefore, the maintenance of protein redox status is of fundamental importance for cell structure and function (Kaneda et al, 2002; Woods et al, 2003;
S’kvar’ilova’ et al, 2005). The oxidative damage to proteins is reflected by increase in levels of protein carbonyls (PCO) (Pansarasa et al, 2000; Mecocci et al, 1999; Niki 2000) and decrease in levels of protein thiols (P-SH) (Dubey et al, 1996). Another parameter relevant in protein oxidation is lipid hydroperoxide (LOOH, LHP). Measurement of elevated LHP, one of the stable end products of lipid peroxidation, is also an evidence for protein oxidation (Liu et al, 1996; Wolff 1994).

1.5.1.3. DNA damage

The most important target of ionizing radiation induced free radical damage is the DNA present in the nucleus and mitochondria of cells. Exposure of cells to ionizing radiation results in immediate and widespread oxidative damages to DNA by both direct and indirect mechanisms (Zhou et al, 2006). In the direct damage, DNA molecule is struck by radiation, ionized, resulting in damage. In the indirect effect, water molecule is ionized, breaks apart, and forms OH free radical (Fig1.3). This OH free radical contains an unpaired electron in the outer shell and is highly reactive which reacts with DNA. About 60% -70% of cellular damage produced by ionizing radiation is estimated to be caused by OH formed from the radiolysis of water. Different types of DNA damage produced by hydroxyl radicals include, oxidized bases, abasic sites, DNA-DNA intrastrand adducts, DNA single and double strand breaks, and DNA protein cross-links. These occur primarily by interaction of free radicals with DNA bases and to a lesser extent, with DNA sugars (Karbownik and Reiter 2000). Among the DNA bases, the most susceptible target for oxidative damage is guanine. The most mutagenic leisons formed in irradiated chromatin is formed from 8- hydroxyguanine (Gajewski et al, 1986; Kasai et al, 1990).

The interaction of free radicals with sugar moieties leads to cleavage of the sugar-phosphate backbone of DNA followed by single and double strand breaks (Karbownik and Reiter 2000) (Fig 1.4). Single strand breaks undergo repair processes relatively easily. On the other hand, double strand breaks have more serious consequences. DNA double strand breaks are converted into chromosome aberrations after a subsequent cell division. Double strand breaks are well correlated with the cytotoxic effects of ionizing radiation and are considered
Fig 1.2: Generation of MDA

Fig 1.3: Indirect and direct effects of ionizing radiation.
Fig 1.4: Effect of low and high LET radiation on DNA.
primary lesion involved in cellular death (Elia et al, 1991). If DNA repair mechanisms, which are induced after exposure to ionizing radiation, are inefficient, the damaged DNA strands that are copied during replication lead to mutagenesis and carcinogenesis (Karbownik and Reiter 2000). The types of free radicals induced DNA damage can be summarized in the table (Table 1.3). The damaging effects of ionizing radiation lead to cell death and are associated with an increased risk for numerous genetically determined diseases (Floyd RA 1990). Among the other indices of DNA damage caused by ionizing radiation are chromosome aberrations and micronuclei formation which are apparent when irradiated cells are observed microscopically.

1.5.1.4. Chromosomal aberrations

Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents (Fig 1.5). Ionizing radiation is highly efficient in producing chromosome aberrations as a consequence of misrejoining of induced DNA double-strand breaks (Savage 1998). Unstable (dicentrics, acentric fragments, and rings) and stable (translocations) aberrations are the two major classes of chromosomal alterations induced by irradiation of cells in the G0 or G1 stage of the cell cycle. Stable chromosome translocations produced by ionizing radiation persist long after exposure (Lucas et al, 1992a; 1992b; Hande et al, 1996; Hande and Natarajan 1998). This opens up the possibility of using chromosome aberrations as a biomarker of radiation damage or as a biodosimeter of radiation exposure many years after medical, accidental or occupational exposure (Tucker 2001). The initial aberrations include translocations, inversions, interstitial deletions and terminal deletions which leads to different types of alterations such as dicentrics, rings etc. Although the dicentric and ring chromosomes must constitute a large proportion of the initial aberrations, only inversions and translocations were found to survive in successive cell generations. Chromosome aberrations involving three or more breaks in two or more chromosomes are defined as complex (Savage and Simpson 1994). This preferential production of complex aberrations by densely ionizing radiation is related to the unique energy deposition patterns produced by densely ionizing radiation, which produces highly localized multiple DNA damage at the
### Table. 1.2. Acute radiation syndromes (ARS)

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Symptoms</th>
<th>Dose(rad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation sickness</td>
<td>Nausea, Vomiting</td>
<td>&gt;100 rad</td>
</tr>
<tr>
<td>Haematopoietic</td>
<td>Inability to produce blood products</td>
<td>&gt; 250 rad</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Failure of gastrointestinal tract lining, loss of fluids, infections.</td>
<td>&gt;500 rad</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>Brain death</td>
<td>&gt;2,000 rad</td>
</tr>
</tbody>
</table>

**THE TYPES OF DAMAGE TO THE DNA:**

- DNA Single Strand Breaks
- DNA Double Strand Breaks
- Sugar Damage
- Base Damage
- Local Denaturation
  (Separation of the 2 strands)
- DNA-DNA Cross-links
- DNA-Protein Cross-links

**Table 1.3: Types of damages to DNA by ionizing radiation**
chromosomal level (Brenner and Ward 1992; Prise et al, 2001; Anderson et al, 2002).

1.5.1.5. Micronuclei
Micronuclei are small, extranuclear bodies that arise from acentric chromosome fragments or from whole chromosomes that are excluded from the nucleus during mitotic cellular division (Fig 1.6). The micronucleus test is an in vivo and in vitro short-time screening method and is widely used to detect genotoxic effects (Villarini et al, 1998; Schmid 1975; Heddle 1973). It is one of the simplest, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage and formation of acentric fragments) and aneugenic (chromosome lagging and effects on spindle) effects (Heddle et al, 1983, Orhan et al, 1993). Micronuclei (MN) can be the result of small acentric chromosome fragments that are not incorporated into the daughter nuclei during cell division. In anaphase, any of the chromosome fragments or whole chromosomes which lack a centromere may not be integrated in the nucleus because of lack of an indispensable element for orientation in the spindle apparatus. After telophase, the fragments or whole chromosomes give rise to one or several secondary nuclei which are smaller than the main daughter nucleus and are therefore called micronuclei (Heddle 1973; Schmid 1975). The advantage of the micronucleus test for mutagenicity screening has been well established in several systems i.e. ovary, bone marrow, epithelial tissues, peripheral blood, liver, exfoliated buccal cells and fetus cells of several laboratory animals or human (Agrawal 1999; Heddle 1990; Konopacka et al, 1998; Krishna et al, 1991; Saleh and Zeytinoglu 2001). Micronuclei formation can occur in any of the dividing cells of tissues of any species (Heddle et al, 1983) as shown by the values of the spontaneous micronucleated erythrocytes (MNE) or in some laboratory animals and mammals (Zuniga et al, 1996).

1.6. Reactive free radicals
Free radicals are atoms, molecules or ions with unpaired electrons (Fig 1.7). These unpaired electrons are usually highly reactive and take part in chemical reactions. Highly reactive free radicals are produced in the body during the normal metabolic functions as well as from environment under conditions of oxidative stress.
Fig 1.5: Types of chromosomal aberrations

Fig 1.6: Induction of micronucleus
Radicals can be formed when a covalent bond is broken and if one electron from each of the pair shared remains with each atom, a process known as homolytic fission (Sonntag CV 1987). The energy required to dissociate the covalent bond can be provided by heat, electromagnetic radiation etc. They can also be formed when oxygen interacts with certain molecules. They are inherently unstable and once formed these highly reactive radicals can start chain reaction. Their chief danger comes from the damage they cause when they react with important cellular components such as DNA or cell membrane or proteins. (Ames 1989).

1.6.1. Biologically Important Radicals

1.6.1.1. Hydroxyl radical (•OH)

Since the major constituent of living cells is water, exposure to high energy radiation such as γ rays will result in •OH production from radiolysis of water (Fig1.8). The hydroxyl radical is an extremely reactive oxidizing radical that will react to most biomolecules at diffusion controlled rates (Cheeseman and Slater 1993) which means that reactions will occur immediately with biomolecules. The hydroxyl free radical is important in radiobiological damage and is several orders of magnitude more reactive towards cellular constituents than superoxide radicals. Hydroxyl radicals are responsible for a large part of the damage done to cellular DNA, proteins and lipids by ionizing radiation. Among them DNA damage, especially double strand breaks, is considered to be an important damaging event because double strand breaks cannot easily be repaired by the cell.

Reactions of •OH can be classified into three main types: hydrogen abstraction, addition and electron transfer (Fig1.9). An important biologically relevant example of H-abstraction by •OH is its ability to initiate lipid peroxidation (Fig1.10). The reaction of •OH with aromatic compounds proceeds via the addition mechanism. For example •OH adds to the purine base guanine in DNA to form 8-hydroxyguanine radical. Similarly, •OH can add on across a double bond in the pyrimidine base thymine to form thymine radical (Fig1.11). The thymine radical then undergoes a series of reactions, including reaction with O₂ to give a thymine peroxyl radical. Similarly •OH can take part in electron transfer reactions with nitrite ion to form NO₂⁻ (Halliwell and Gutteridge 1999).
**Fig 1.7: Types of Free radicals**

Oxygen, Superoxide anion, Peroxide, Hydrogen Peroxide, Hydroxyl radical, Hydroxyl ion.

**Fig 1.8. Generation of \( \cdot \text{OH} \) radical**

\[
\begin{align*}
\text{H} + \text{H}_2\text{O} & \rightarrow \text{H}_2 + \text{HO} \\
\text{H} + \text{O}_2 & \rightarrow \text{HO} + \text{O} \\
\text{O} + \text{E}_2\text{O} & \rightarrow 2 \text{HO}
\end{align*}
\]
HYDROGEN ABSTRACTION

\[ \text{CH}_3\text{CH}_2\text{-OH} + \cdot \text{OH} \rightarrow \text{CH}_3\text{CH}_2\text{-OH} + \text{H}_2\text{O} \]

ADDITION TO AROMATIC RINGS

ELECTRON TRANSFER WITH IONS

\[ \text{Cl}^- + \cdot \text{OH} \rightarrow \cdot \text{Cl} + \text{OH}^- \]

Fig 1.9. Reactions of \( \cdot \text{OH} \)

Fig 1.10: Lipid peroxidation by \( \cdot \text{OH} \)
Fig 1.11: Thymine dimer

Fig 1.12: Biochemical Impact of superoxide generation
1.6.1.2. Superoxide radical (‘O$_2^-$’)

The superoxide free radical anion is formed when oxygen is reduced by the transfer of a single electron to its outer shells (Cheeseman and Slater 1993). The major source of superoxide in-vivo is the electron leakage to O$_2$ that results from the electron transfer chain of the mitochondria (Beal 1997; Fridovich 1989; Hansford 1997). They can also be formed by activated phagocytic cells by the action of peroxidases. Superoxide in comparison with ‘OH, is far less reactive. However, it does react quickly with some other radicals, such as NO’, certain iron-sulphur clusters in enzymes and certain phenoxy radicals (Fig 1.12). The superoxide radical anion appears to play a central role as other reactive intermediates are formed from it. Its main significance lies in its being a main source for the generation of hydrogen peroxide and as a reductant of transition metals, which are precursors to the formation of the lethal hydroxyl radical (Fig 1.12). Some of the important O$_2^-$ mediated reactions are depicted below:

\[
\begin{align*}
(\text{Ascorbate } ) \text{AH}_2 + 'O_2^- & \rightarrow A^- + H_2O_2 \\
H_2O_2 + 'O_2^- & \xrightarrow{\text{Fe catalyst}} 'OH + 'OH + O_2 \\
\end{align*}
\]

1.6.1.3. Nitric oxide radical (NO’)

It is a common gaseous free radical. It is now recognized to play a role in vascular physiology and is also known as endothelium derived relaxing factor. Vascular endothelium produces nitric oxide, as do neutrophils and macrophages from arginine using the enzyme nitric oxide synthetase. This event can be stimulated by cytokines, tumour necrosis factor, or interleukins (Beckman et al, 1993). Inhibition of production is known to reduce microbicidal and tumouricidal activities of macrophages. In addition it has multiple important physiological roles in nervous system and vascular system. These include neurotransmission, neuromodulation, control of blood pressure, inhibition of platelet aggregation etc (Halliwell and Gutteridge 1999). Nitric oxide gives rise to reactive nitroxy anion, nitrous oxide,
hydroxyl radical and peroxynitrite. Some of the important NO• mediated reactions are depicted here:

\[
\begin{align*}
\text{NO}^- + \text{NO}^\bullet & \rightarrow \text{ONNO}^\bullet^- \quad \text{(nitroxy anion)} \\
\text{ONNO}^\bullet^- + \text{NO}^\bullet & \rightarrow \text{N}_2\text{O} \quad \text{(nitrous oxide)} + \text{NO}^{2-} \\
\text{ONNO}^\bullet^- + \text{H}^+ & \rightarrow \text{N}_2\text{O} \quad + \cdot\text{OH} \\
\text{NO}^- + \text{O}_2 & \rightarrow \text{ONOO}^- \quad \text{(peroxynitrite)}
\end{align*}
\]

1.6.1.4. Peroxyl and alkoxyl radicals (RO•2 and RO’)
Peroxyl and alkoxyl radicals are good oxidising agents. They are formed from the attack \(\cdot\text{OH}\) on organic compounds as well as from the degradation of organic peroxides (RCOOH). RO•2 and RO’ radicals can abstract H• from other molecules, which is a major reaction occurring in lipid peroxidation.

1.6.2. Biologically Important Non-radicals

1.6.2.1. Hydrogen peroxide (H2O2)
Hydrogen peroxide is not a free radical but falls in the category of reactive oxygen species. It is an oxidizing agent that is not particularly reactive but its main significance lies in that it is the main source of hydroxyl radicals in the presence of transition metal ions or ultraviolet light (Cheeseman and Slater 1993). It is also involved in the production of HOCl in the neutrophils by the enzyme myeloperoxidase.

\[
\begin{align*}
\text{H}_2\text{O}_2 & \rightarrow 2\text{OH} \\
\text{H}_2\text{O}_2 + \text{Cl}^- & \rightarrow \text{HOCl (Hypochlorous acid)} + \text{OH}^-
\end{align*}
\]
1.6.2.2. Hypochlorous acid (HOCl)
Activated polymorphonuclear cells and neutrophils produce HOCl as a major bactericidal agent. It is generated by the action of myeloperoxidase on chloride ions in the presence of H$_2$O$_2$. Hypochlorous acid has attracted much attention because of its high reactivity and ability to damage biomolecules, both directly and by decomposing to form chlorine. The addition of HOCl can oxidize thiols, ascorbate, NADPH and lead to chlorination of DNA bases and tyrosine residues in proteins (Halliwell and Gutteridge 1999).

1.6.2.3. Singlet oxygen (¹O$_2$)
Singlet oxygen (¹O$_2$) is an electronically excited and mutagenic form of oxygen. It is generated by input of energy, radiation, but can also be generated enzymatically by the action of peroxidases, lipoxygenases or by the reaction of hydrogen peroxide with hypochlorite/ peroxynitrite, or during the respiratory burst of phagocytes. They are also generated in biological systems in a number of pigment reactions of chlorophylls, retinal and flavins when they are illuminated in the presence of oxygen. The best studied chemical reactions of singlet oxygen are those involving with carbon-carbon double bonds which are present in many biological molecules, including carotenoids, chlorophyll and fatty acids. Addition of singlet oxygen to such molecules can damage them severely. Further addition of ¹O$_2$ to compounds with conjugated double bonds leads to the formation of endoperoxides (Halliwell and Gutteridge 1999).

1.6.2.4. Peroxynitrite (ONOO$^-$)
It is produced by the reaction of nitric oxide with superoxide. The resulting ONOO$^-$ decomposes to give OH radical (Cuzzocrea et al., 2001). Addition of ONOO$^-$ to aromatic compounds leads to both hydroxylation and nitration. Peroxynitrite undergoes homolytic fission to generate OH and nitrogen dioxide radicals (Halliwell and Gutteridge, 1999d).

\[
\text{NO}^- + \text{O}_2 \rightarrow \text{ONOO}^- \text{ (peroxynitrite)}
\]

\[
\text{ONOOOH} \rightarrow \text{NO}_2^- + \text{OH}
\]
1.7. Antioxidants

Protection of biological systems against the harmful effects of radiation is of paramount importance during accidental, occupational and unavoidable exposure to radiation. In spite of extensive efforts made in the last decades, the development of radioprotectors as well as antioxidant agents suitable for human applications is still a great challenge for the biomedical scientists. A large number of plants contain antioxidant phytochemicals that have been reported to be radioprotective in various model systems. These include green tea (polyphenols), Chinese herbal medicines, Ayurvedic preparations, cruciferous vegetables (e.g. cabbage and broccoli), dithiolthiones, Panax ginseng, Eleutherococcus senticosus or Shigoka extract, Gingko Biloba extract (flavone glycosides and terpene lactones), milk thistle (silymarin), curcumin, garlic (allicin), and lycopene. Polysaccharides are nonenzymatic free radical scavengers and was found be effective in enhancing the cellular defense mechanism against endogenous and exogenous oxidants (Nardini et al, 1997). “Natural” antioxidants may protect against long-term effects of radiation exposure occurring in human populations exposed to radiation (Emerit et al, 1995; Emerit et al, 1997a; Emerit et al, 1997b).

To counteract the effects radiation induced oxidative stress, mammalian cells have elaborate antioxidant defense mechanism including enzymatic scavenging systems such as superoxide dismutases, catalase, glutathione peroxidases as well as thiol-reducing buffers consisting of small protein thiols with redox-active sulfhydryl moieties (i.e. glutathione). Antioxidant enzymes and non-enzymatic antioxidants such as vitamin E and selenium offer protection against ionizing radiation induced oxidants (El-Nahas et al, 1993; Carroll et al, 1995). Dietary antioxidants such as vitamins E, C and beta-carotene have been reported to be effective in protecting normal tissue during radiation therapy (Prasad et al, 2002; El-Nahas et al, 1993). These antioxidant systems convert oxidants to nontoxic molecules, thus protecting the organism from the deleterious effects of oxidative stress.
1.7.1. Enzymatic Antioxidants

1.7.1.1. Superoxide dismutase (SOD)

Superoxide dismutases (SODs) are a class of closely related enzymes that catalyse the breakdown of the superoxide anion into oxygen and hydrogen peroxide. (Zelko et al, 2002; Bannister et al, 1987).

\[ 2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \]

Superoxide radicals, the product of one electron reduction of molecular oxygen are produced in mitochondria as a result of the imperfect flow of electrons through the electron transport chain. At least two sites in electron transport chain, complex I and ubisemiquinone, have been identified as primary sources of superoxide production in mitochondria (Halliwell and Gutteridge 1989). Four classes of SOD have been identified, containing a dinuclear copper Cu, Zn, mononuclear Fe, Mn or Ni cofactors. (Bannister et al, 1987). In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion (Bannister et al, 1987). Mn-SOD is a nuclear encoded primary antioxidant enzyme that functions to remove the superoxide radicals generated in the mitochondria (Mates and Sanchez- Jimenez 1999).

1.7.1.2. Catalase (CAT)

Catalase is the second enzyme which acts in cellular detoxification. It is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphrin group per subunit, and has a molecular mass of about 240 kDa (Aebi 1980). CAT reacts very efficiently with H donors (methanol, ethanol, formic acid or phenols) with peroxide activity as well as with \( H_2O_2 \) to form water and molecular oxygen and with catalase has a double function because it catalyses the following reactions

1) Decomposition of \( H_2O_2 \) to give \( H_2O \) and \( O_2 \)

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]
2) Oxidation of H donors with consumption of 1 mole of peroxide

\[ \text{ROOH} + \text{AH}_2 \rightarrow \text{H}_2\text{O} + \text{ROH} + \text{A} \]

Most aerobic cells contain catalase activity. In animals catalase is present in all major body organs, being especially concentrated in liver. Catalase in erythrocytes help protect the animals against \( \text{H}_2\text{O}_2 \) generated by dismutation of \( \text{O}_2^- \) generated by hemoglobin autooxidation. The catalase activity of plant and animal tissues is largely or completely located in subcellular organelles bounded by a single membrane known as peroxisomes.

### 1.7.1.3. Glutathione Peroxidase (GSHPx)

Glutathione Peroxidase converts \( \text{H}_2\text{O}_2 \) via the oxidation of reduced glutathione (GSH) to oxidised glutathione (GSSG).

\[ \text{H}_2\text{O}_2 + \text{GSH} \rightarrow \text{GSSG} + 2\text{H}_2\text{O} \]

GPx is an 80 kDa protein that is composed of four identical subunits. Five distinct GPx isozymes have been characterized in mammals (Brigelius-Flohe 1999). Glutathione Peroxidase exists in soluble form associated with the membrane and which acts on lipid hydroperoxide (Deleve and Kaplowitz 1991). Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides (Table 1.4).

The glutathione S-transferases (GST) are a family of Phase II detoxification enzymes that have co-evolved with GSH and are abundant throughout most life forms. GSTs catalyze the conjugation of GSH to a wide variety of endogenous and exogenous electrophilic compounds. Many xenobiotics supplied to living organisms are metabolized by conjugation with GSH, catalysed by glutathione S-transferase enzymes. These groups of enzymes show high activity with lipid peroxides and are at particularly high levels in the liver. (Sharma, 2004; Hayes, 2005).
There also exist glutathione associated enzymes known as **Glutathione Reductase (GR)** involved in the metabolism of oxidized glutathione (GSSG) to GSH via the oxidation of NADPH to NADP⁺. The reaction is essential for the bioavailability of GSH *in vivo*.

\[
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+ 
\]

### 1.7.2. Non enzymatic antioxidants

#### 1.7.2.1. Glutathione (GSH)

The tripeptide glutathione (g-L-glutamyl-L-cysteinyl-glycine, GSH, Fig 1.17), which is widely distributed in most living cells, is a principal antioxidant and a low-molecular weight non-proteinous thiol compound. Glutathione plays an important role in maintaining the intracellular thiol redox state and protecting cells against oxidative damage, xenobiotic organic chemicals, and heavy metals (Meister et al, 1989). It also act as a co factor for enzymes involved in the detoxification of xenobiotics (Meister and Anderson 1984). Glutathione participates directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintains exogenous antioxidants such as vitamins C and E in their reduced (active) form. It is not required in the diet and is instead synthesized in cells from its constituent amino acids (Meister 1988). The tripeptide can exist intracellularly in either an oxidized (GSSG) or reduced (GSH) state (Fig 1.13). Maintaining optimal GSH: GSSG ratios in the cell is critical to survival of the organism. A deficiency of GSH puts the cell at risk for oxidative damage and leads to a wide range of pathologies, including, cancer, neurodegenerative disorders, cystic fibrosis (CF), HIV and aging. Thus glutathione is one of the most important cellular antioxidants.
1.7.2.2. Vitamin C
Vitamin C is also a very important and powerful antioxidant that works in aqueous environments of the body, such as the lungs and lens of the eye (Shang et al, 2003). Vitamin C’s major role is to make collagen, the main protein substance of the human body that holds connective tissues together in skin, bone, teeth and other parts of the body. Vitamin C is also critical for the proper function of our immune system, for manufacturing certain nerve transmitting substances and hormones, and for the absorption and utilization of other nutrients, such as vitamin E and iron (Fang et al, 2002; Griffiths and Lunec 2001).

1.7.2.3. Vitamin E
Vitamin E is a fat-soluble vitamin that exists in eight different forms. Each form has its own biological activity, the measure of potency or functional use in the body. α-Tocopherol (Fig1.14) is the most active form of vitamin E in humans, and is a powerful biological antioxidant (Buettner 1993). α-Tocopherol is considered to be the major membrane bound antioxidant employed by the cell (Van Acker et al, 1993) and its main antioxidant activity is protection against lipid peroxidation (Richter 1987; Gutteridge 1995).

1.7.2.4. Carotenoids
Carotenoids are pigments that are found in plants and microorganisms but are not synthesized by animals (Edge et al, 1997). They are responsible for the red, yellow, and orange colour of fruits and vegetables. There are over 600 carotenoids occurring in nature, which can be grouped into carotenes, xanthophylls (containing oxygen) and lycopene. The antioxidant activity of carotenoids arises as a result of the ability of the conjugated double bond structure (Fig 1.15) to delocalise any unpaired electrons. This is primarily responsible for the excellent ability of β-carotene to physically quench singlet oxygen without degradation and for the chemical reactivity of β-carotene with free radicals such as peroxyl radical (ROO•), hydroxyl radical (•OH), and superoxide radical (O•– 2 ) (Van Lieshout et al, 2003; Burton and Ingold 1984). Carotenoids prevent or inhibit certain types of cancer, atherosclerosis, age-related muscular degeneration and other diseases (Torun et al, 1995).
Fig 1.13: Reduced and oxidized forms of glutathione

Fig 1.14: Antioxidant action of vitamin E and vitamin C
1.8. Radioprotectors

When cancer patients undergo radiotherapy, a clear dose-response relationship usually exists between radiation dose and tumor response. Normal tissue reactions limit the total dose that can be given and may cause lasting discomfort and disability for the patient. Thus, additional strategies are needed if antitumor efficacy of radiotherapy is to be increased further without causing unacceptable toxicity. The use of chemical radioprotectors to selectively protect normal tissues represents an obvious strategy. A large number of substances have shown variable degrees of radioprotective properties (Weiss and Landauer 2000) (Table 1.5). However, the vast majority of these are either too weak in terms of radioprotection, too toxic, or without any apparent mechanisms to ensure selective normal tissue protection. Therefore, only a very limited number of substances are presently of clinical relevance. The involvement of free radical scavengers in protecting the tissue against radiation damage was highlighted when scientists found out that whole body irradiation decreases the total antioxidant capacity of the organism and the levels of known antioxidants such as ascorbic acid and uric acid was depleted (Karbownik and Reiter 2000). Several different strategies for radio-protection have been proposed including (1) direct scavenging of reactive oxygen species (ROS), (2) inducing/altering the endogenous levels of ROS detoxifying enzymes such as MnSOD and (3) enhancing DNA damage signaling and repair (Grdina et al., 2005). The ability of certain substances to provide protection against the damaging effects of ionizing radiation was first published in 1949 (Dale et al, 1949). The best known radioprotectors were the sulfhydryl compounds, such as cysteine and cysteamine (Patt et al, 1949; Becq et al, 1951). However, these compounds produce serious side effects, such as nausea and vomiting, and are considered to be toxic at the doses required for radioprotection. With the memory of devastating effects of nuclear bomb attack in Nagasaki and Hiroshima following World War II, a development program was initiated in the late 1950s by the United States Army. The Walter Reed Army Research Institute synthesized and tested over 4,000 compounds in an attempt to find a useful radioprotector, without toxic side effects. The most effective compound of this type, originally tested against lethal doses of X rays and gamma rays in mice was WR-2721, with the common name amifostine (Bump and Malakar 1997).
**RADIO PROTECTORS**

<table>
<thead>
<tr>
<th>A.</th>
<th>Sulphydryl compounds</th>
<th>Free radicals scavenging.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cystein, Cysteamine, Glutathione</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B</th>
<th>Antioxidants</th>
<th>Free radical scavenging.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vitamin A, E &amp; C, TMG, Melatonin etc.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C</th>
<th>ACE inhibitors</th>
<th>Protease inhibition (through renin – angiotesin system), collagen synthesis inhibition.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Captopril, Elanopril penicillamine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>Cryoprotective agents</th>
<th>Reduced toxicity of chemotherapeutic drugs, Protection from urotheletic toxicity and nephrotoxicity.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mesna, Dexrazoxane, Amifostin (WR 2127)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E</th>
<th>Immunomodulators</th>
<th>Immuno stimulation, increased production of cytokines.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gamma-interferon, polysaccharides, AM5, AM218, Heat killed Lacto bacillus cells</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F</th>
<th>Lipopoly saccharides &amp; Prostaglandins</th>
<th>Prostaglandin synthesis, elevated levels of cAMP, DNA repair</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>G</th>
<th>Plants extract and isolated compounds: Orientin, Vicimin</th>
<th>Free radicals scavenging and antioxidant property</th>
</tr>
</thead>
</table>

**Table 1.5: List of radioprotectors**

<table>
<thead>
<tr>
<th>Allelic variation in GPx and corresponding tissue distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allele Tissue distribution</td>
</tr>
<tr>
<td>GPX1 Erythrocytes, kidney, liver</td>
</tr>
<tr>
<td>GPX2 GI tract</td>
</tr>
<tr>
<td>GPX3 Kidney</td>
</tr>
<tr>
<td>GPX4 Ubiquitous, highest in renal epithelium and testis</td>
</tr>
<tr>
<td>GPX5 Ubiquitous</td>
</tr>
</tbody>
</table>

**Table 1.4: Allelic variation of GPx**
1.8.1. Amifostine

Amifostine is the prototype pharmacologic radioprotector that functions via free radical scavenging. It is a hydrophilic compound that does not readily cross cell membranes (Yuhas 1982). After its intravenous administration, amifostine is rapidly dephosphorylated to its active metabolite WR-1065 and cleared from the plasma with a half-life ranging from 1 to 3 minutes (Shaw et al, 1999). Dephosphorylation of amifostine is either caused by spontaneous nonenzymatic hydrolysis or by a catalyzed process involving alkaline phosphatase with a pH optimum at 8 to 9 (Yuhas 1980). Uptake of WR-1065 varies considerably between different tissues. Extensive uptake is seen in salivary glands, kidneys, and intestinal mucosa, whereas markedly lower uptake generally is seen in tumor tissues (Yuhas 1980; Shaw et al, 1999; Utley et al, 1984). Inside the cell, WR-1065 is further metabolized to the disulfide, WR-33278 (Fig 1.16), that may also cause radioprotection, although to a much lesser extent (Savoye et al, 1997; Peters et al, 1995. The mechanism of cytoprotection against ionizing radiation is complicated and not entirely understood: WR-1065 and to a lesser extent, WR 33278 act as free radical scavenger that protect sub cellular components like membranes and DNA from damage. (Hall 2000; Travis 1984; Denekamp et al, 1982).

Amifostine was reported to be tolerated well in radiotherapeutic clinical trials. However, it was found to have some undesirable side effects which include hypotension, nausea, vomiting, sneezing, hot flashes, mild somnolence and hypocalcaemia. 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). Briefly, it has at least two inherent properties that do not make it an ideal radio-protector. First, at doses necessary for optimal radio-protection there are toxic side effects in humans including hypotension and nausea. Second, because it primarily works as a free radical scavenger it must be present during radiation exposure. Despite its drawbacks, amifostine (Ethyol®) is the only radioprotector that has been approved by the Food and Drug Administration (FDA), USA (FDA 1999) today. Currently, a number of trials are evaluating whether amifostine could be used for clinical radioprotection in broader terms. (Komaki et al, 2002; Jatoi
**Fig 1.15: Types of carotenoids**

I. All-trans-beta-carotene

II. Lycopene

III. Lutein

IV. Canthaxanthin

V. Astaxanthin

**Fig 1.16: Mechanism of action of amifostine**

\[
\text{H}_2\text{N}-\left(\text{CH}_2\right)_3\text{NH}-\left(\text{CH}_2\right)_2\text{S}-\text{PO}_3\text{H}_2
\]

WR-2721 (amifostine)

\[
\downarrow
\]

\[
\text{H}_2\text{N}-\left(\text{CH}_2\right)_2\text{NH}-\left(\text{CH}_2\right)_2\text{SH}
\]

WR-1065

\[
\downarrow \quad \uparrow
\]

\[
\text{H}_2\text{N}-\left(\text{CH}_2\right)_3\text{NH}-\left(\text{CH}_2\right)_2\text{S}-\left(\text{CH}_2\right)_2\text{S}-\left(\text{CH}_2\right)_2\text{NH}-\left(\text{CH}_2\right)_3\text{NH}_2
\]

WR-33278
However, agents which are modulating biological response to radiation may represent an alternative pharmacologic approach for the reduction of normal tissue damage (Trotti 1998).

1.9. Mushrooms
Mushrooms are delicious and mysterious members of the biosphere (Verma et al., 1987a, b). Because of their taste and fleshy construction, they have attracted the 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). Today mushrooms are considered as alternative food source to provide adequate nutrition to world's increasing population. The antioxidants present in dietary mushrooms are of great interest as possible protective agents to reduce oxidative damage in the human body (Adams and Wermuth 1999). 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). They produce various classes of secondary metabolites with interesting biological activities and have the potential to be used as valuable chemical resources for drug discovery (Zjawiony 2004). Furthermore, several companies are developing drugs from different mushrooms and these capsules, although expensive, have been shown to be health beneficial, including fight against cancer (Mau et al, 2005). Some of the medicinal effects of mushrooms are listed in the table below (Table 1.6).

1.10. *Phellinus*
The genus *Phellinus* is classified in the family Hymenochaetaeaceae, 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. There are approximately 220 known species of *Phellinus* mushrooms in the world, and they were found mainly in tropical areas of America Africa and Asia (Kim et al,
<table>
<thead>
<tr>
<th>No</th>
<th>Fungi Source</th>
<th>Main bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Ganoderma lucidum</em></td>
<td>Hyperglycemic, immunomodulating, antitumor, antioxidative, antibacterial, antifungal, anti-viral, anti-cancer, hepatoprotective, anti-decrepitude.</td>
</tr>
<tr>
<td>2</td>
<td><em>Pleurotus species</em></td>
<td>Hepatoprotective, anti-breast cancer, antitumor</td>
</tr>
<tr>
<td>3</td>
<td><em>tuber-regium</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Schizophyllum commune</em></td>
<td>Antitumor, antibacterial, hepatoprotective, immunomodulating</td>
</tr>
<tr>
<td>5</td>
<td><em>Lentinus edodes</em></td>
<td>Immunosuppressant, antitumor, Antiviral, anti-inflammatory, hepatoprotective, kidney tonic, immunomodulating</td>
</tr>
<tr>
<td>6</td>
<td><em>Grifola frondosa</em></td>
<td>Immunomodulating, antitumor, antiviral, hepatoprotective</td>
</tr>
<tr>
<td>7</td>
<td><em>Agaricus blazei</em></td>
<td>Antitumor</td>
</tr>
<tr>
<td>8</td>
<td><em>Inonotus obliquus</em></td>
<td>Antitumor, immunomodulating</td>
</tr>
<tr>
<td>9</td>
<td><em>Polyporus umbellatus</em></td>
<td>Antitumor, immunomodulating</td>
</tr>
<tr>
<td>10</td>
<td><em>Auricularia auricula</em></td>
<td>Hyperglycemia, immunomodulating, antitumor, anti-inflammatory</td>
</tr>
<tr>
<td>11</td>
<td><em>Phellinus linteus</em></td>
<td>Antitumor, immunomodulating</td>
</tr>
<tr>
<td>12</td>
<td><em>Polystictus versicolor</em></td>
<td>Immunomodulating, antitumor, antiradivative, hyperglycemia, antiflammatory</td>
</tr>
<tr>
<td>13</td>
<td><em>Trametes versicolor</em></td>
<td>Antibacterial, antiviral, hepatoprotective, kidney tonic</td>
</tr>
<tr>
<td>14</td>
<td><em>Flammulina velutipes</em></td>
<td>Antitumor, antiflammatory, antiviral, immunomodulating</td>
</tr>
</tbody>
</table>

Table 1.6: Medicinal properties of mushroom species
Many kinds of *Phellinus* (e.g. *P. linteus, P. baumii, P. igniarius, P. tremulæ, P. robustus, P. robustus, P. Gilvus, P. weirii*, *Phellinus durissimus* (Lloyd) Roy 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; Lahiri et al, 2010). It is usually used in traditional oriental medicine and has been reported to have many pharmaceutical attributes, including anti-mutagenicity and anti-cytotoxicity, anti-cancer, immune enhancement (Ji et al, 2000; Kim et al 1996; Han et al, 1999; Song et al, 2002) and antioxidant properties. In Taiwan, several different species of *Phellinus* were widely applied for anticancer, antioxidant purposes and hepatoprotective effects. *Phellinus linteus*, also known as Sang-Hwang, Mesima or Meshimakobu has long been used in traditional medicine. It a yellow-orange colored mushroom that grows well on mulberry trees. *P. linteus* demonstrated anti-cancer effect by activating cytotoxic cells and macrophages thus increasing potential immune response potential (Lee et al, 2001). *Phellinus linteus* demonstrated anti-tumor activity in several studies (Lin et al, 2003; Kim et al, 2003b; Li et al, 2004; Bae et al, 2005). The methanolic extract of the basidiocarps of *Phellinus linteus* demonstrated antioxidative effect (Chung et al, 1998) and antimutagenic activities (Sohn and Nam, 2001). Studies indicated that *Phellinus linteus* could protect primary cultured rat hepatocytes against hepatotoxins (Kim et al, 2004). Several other *Phellinus* species such as *Phellinus linteus, Phellinus ribis* and *Phellinus igniarius* have been used as traditional medicines for the treatment of gastrointestinal cancer, cardiovascular disease, tuberculosis, liver or heart diseases, fester, bellyache, bloody gonorrhea, stomach ailments, and diabetes (Nakamura et al, 2004).

Polysaccharide from *Phellinus* has 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* stimulates humoral and cell-mediated immunity (Kim et al, 1996; Song et al, 1995). Acidic polysaccharides and proteoglycans from *P. linteus* activate protein tyrosine kinase and protein kinase C (Kim et al, 2003). Polysaccharides isolated from *P. linteus* have been reported to possess antitumor and immunomodulating activities (Moradali et al, 2007; Zhang et al, 2007; Kim et al, 2006; D Sliva et al, 2008).
Phellinus linteus polysaccharides had been reported to increase production of immune mediators like IL-1 in mice (Kim et al, 2003c). Protein bound polysaccharide isolated from P.linteus had been reported to possess antiproliferative effect for SW480 human colon cancer cells (Li et al, 2004).

1.11. Phellinus rimosus

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 heterophyllus Lam.; Moraceae) trunks (Fig1.17). 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). Further, chemopreventive as well as antimutagenic activities of P.rimosus has also reported recently (Ajith and Janardhanan 2006; Ajith and Janardhanan, 2011). Ethyl acetate extract of P.rimosus showed significant radiation induced lipid peroxidation inhibiting activity (Lakshmi et al, 2005). Our recent investigations have also demonstrated profound anti-inflammatory , antiarthritic as well as radioprotective activities of a polysaccharide protein complex (PPC-Pr) isolated from the aqueous extract of P. rimosus. (Meera et al, 2009 a, b).

1.12. Mushroom polysaccharides

Recently, the biological activities of polysaccharides or polysaccharide-protein complexes derived from mushrooms have received much attention in biomedical sciences. One of the most promising activities of these polysaccharides is their immunomodulating and anti-cancer effects. Medicinal mushroom research has now focused on discovery of compounds that can modulate positively or negatively the biologic response of immune cells. In this regard, mushroom polysaccharides offer a lot of hope for cancer patients and sufferers of many devastating diseases. They stimulate the human immune response and are being sought for the treatment of
Fig. 1.17. Phellinus rimosus
cancer, immunodeficiency disease or for generalized immunosuppression following drug treatment. They are also sought for combination therapy with antibiotics and as adjuncts for vaccines (Jang et al, 1997). Wasser (2002) reported that mushroom polysaccharides are 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. Mushroom polysaccharides support some or all of the major systems of the body, including nervous, hormonal and immune systems as well as regulatory functions. The polysaccharides from mushrooms do not attack cancer cells directly, but produce their beneficial effects by activating different immune response in the host (Wasser 2002). Polysaccharides or polysaccharide-protein complexes are considered as multi-cytokine inducers and are able to induce gene expression of various cytokines and cytokine receptors (Liu 1996; Kato et al, 1995). A variety of polysaccharides from a number of mushroom varieties have been demonstrated to enhance the immune system. The mushroom polysaccharides appear to be well tolerated and compatible with chemotherapy and radiation therapy.

Polysaccharides are a structurally diverse class of macromolecules able to offer the highest capacity for carrying biological information due to a high potential for structural variability (Wasser 2002). The polysaccharides of mushrooms occur mostly as glucans. Polysaccharides especially β-glucan, are considered to be responsible for their biological activity. The β-glucan chains are linked by β-(1-3), (1-6) glycosidic bonds and α-(1-3) glycosidic bonds but many are true heteroglycans. Most often there is a main chain, which is either β (1-3), β (1-4) or mixed β(1-3), β(1-4) with β(1-6) side chains. Hetero-β-D-glucans, which are linear polymers of glucose with other D-monosaccharides, can have anticancer activity but α-D-glucans from mushroom usually lack anticancer activity (Wasser 2002).

1.12.1. Medicinal value of mushrooms polysaccharides

In recent years, clinical trials using medicinal mushrooms or mushroom polysaccharides for the treatment of various types of cancers have achieved some success in extending survival of cancer patients and/or improving the quality of life
in patients with advanced cancer (Kidd 2000; White 2002). Mushroom derived polysaccharides had been reported to possess diverse biological activity. (Vinogradov and Wasser 2005) (Table 1.6). The mushroom *L. edodes* has been reported to produce two bioactive preparations, mycelium extract and Lentinan (Hobbs et al, 2000). These two bioactive polymers appear to act as host defence potentiators restoring and enhancing the responsiveness of host cells to lymphocytokines, hormone and other biologically active substances. The immunopotentiation has been shown to occur by stimulating the maturation, differentiation or proliferation of cells involved in host defence mechanism. Further, Lentinan increase host’s resistance against various kinds of cancer and has the potential to restore the immune function of affected subjects (Chihara et al, 1989; 1992). Mushroom polysaccharides can also exhibit direct inhibitory effects on cancer cell growth by modulating cell-cycle progression and inducing apoptosis (Wang et al, 2002).

Medicinally valuable mushroom derived polysaccharide preparations include Schizophyllan derived from the mushroom *Schizophyllum commune*, Active hexose correlated compounds (AHCC) which is a proprietary extract prepared from co-cultured mycelia of several species of basidiomycete mushrooms, including Shiitake (*Lentinus edodes*), Maitake D-fraction from *Grifola frondosa* (also known as Maitake), PSP isolated from edible mushroom *Coriolus versicolor* and Polysaccharide-K, also known as PSK or krestin which is a protein bound polysaccharide found in the polypore fungus *Trametes versicolor* (Table 1.7). PSK, is now approved as a adjuvant in cancer therapy (Fig1.18) (Daba and Ezeronye 2003). Majority of these preparations are β-glucan polysaccharides or polysaccharide peptide complexes. PSK and PSP were found to boost immune cell production, ameliorate chemotherapy symptoms and enhance tumor infiltration by immune cells (Fisher and Yang 2002). Polysaccharides isolated from mushroom *G. lucidum* (GLPS) has been reported to possess multiple pharmacological effects, such as immunomodulation, anti-oxidation, hepatoprotection, anti-proliferation, anti-angiogenesis, antitumor and prolong patients’ survival time (Xu et al, 2011).
<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Source of fungus</th>
<th>Linkages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentinan</td>
<td><em>Lentinus edodes</em></td>
<td>(1-3)βD</td>
</tr>
<tr>
<td>Schizophyllan</td>
<td><em>Schizophyllum commune</em></td>
<td>(1-6)βD</td>
</tr>
<tr>
<td>Ganoderan</td>
<td><em>Ganoderma lucidum</em></td>
<td>(1-3)βD</td>
</tr>
<tr>
<td>Auricularia glucan</td>
<td><em>Auricularia auriculata</em></td>
<td>(1-3)βD</td>
</tr>
<tr>
<td>Grigolan</td>
<td><em>Grifola frondosa</em></td>
<td>(1-3)βD</td>
</tr>
<tr>
<td>Agrocybe</td>
<td><em>Agrocybe cylindrica</em></td>
<td>(1-3)βD</td>
</tr>
</tbody>
</table>

Table 1.7. Antitumor polysaccharides from mushrooms
1.13. β-glucans

β-glucans are naturally occurring polysaccharides. These glucose polymers are constituents of the cell wall of certain pathogenic bacteria and fungi (Wasser, 2002). β-glucan is the most known powerful immune stimulant and a very powerful antagonist to both benign and malignant tumors; further, it lowers cholesterol and triglyceride level, normalizes blood sugar level, heals and rejuvenates the skin and has various other benefits (Akramienė et al, 2007). These substances increase host immune defense by activating complement system, enhancing macrophages and natural killer cell function (Akramienė et al, 2007). They are a heterogeneous group of glucose polymers, mostly consisting of backbone of β(1→3)-linked β-D-glucopyranosyl units with β(1→6)-linked side chains of varying distribution and length (Fig1.18). These polysaccharides are major cell wall structural components in fungi and are found in plants and some bacteria as well. β-glucans mostly show a triple-strand right winding helix structure. Various β-glucans have been isolated from diverse mushroom species showing different immunological activity, which could be correlated with their solubility in water, molecular weight, conformation (tertiary structure) and degree of branching. Mushrooms β-glucans have short β (1,6)-linked branches coming off of the β (1,3) backbone. β -Glucan has been isolated from some mushrooms as Lentinan (Lentinus edodes), maitake (Grifola frondosa), schizophylan (Schizophillum commune) (Mizuno et al, 1995; Brochers et al, 1999). β -Glucan extracts from Lentinus edodes and Schizophillum commune are used in traditional medicine for cancer treatment in Japan since 1980. Further, β -Glucans as adjuvant to cancer chemotherapy and radiotherapy demonstrated the positive role in the restoration of hematopiesis following by bone marrow injury. (Akramienė et al, 2007). Different types of β –Glucan preparations are lentinan, grifolan, Schizophyllan.

Lentinan is isolated from the fruiting body of Lentinus edodes (shiitake mushroom) and consists of five β(1→3)-β-D-glucopyranosyl units in a linear linkage and two β(1→6)- linked side chains (degree of branching: 0.4). The molecular weight is about 4-8 x 10⁵ g/mol (Chihara et al, 1970; Yap et al, 2001)
Fig 1.18. Antitumour polysaccharides from mushrooms
A: Krestin (PSK) from *Trametes versicolor* (mycelium); B: Lentinan from *Lentinus edodes* (fruit body); and C. Schizophyllan from *Schizophyllum commune* (medium product)

Fig 1.19: Structure of β-glucans
Grifolan has a degree of branching of 0.33 and a molecular weight of approximately $5 \times 10^6$ g/mol. The compound is isolated from the liquid-cultured mycelium of *Grifola frondosa* (maitake mushroom). Another $\beta$-glucan has also been extracted from this mushroom: Maitake D-fraction consists of a $\beta(1\rightarrow6)$ main chain with $\beta(1\rightarrow3)$ branches (Kodama et al, 2003).

Schizophyllan is another $\beta$-glucans obtained from a culture medium of *Schizophyllum commune* (split gill fungus) (Hashimoto et al., 1991). Its branching rate is 0.33 and the molecular weight is approximately $4.5 \times 10^5$ g/mol.

1.13.1. Mechanism of action

$\beta$-D-glucans has a different mode of action from the conventional chemotherapeutic agents in that it is immunotherapeutic. $\beta$-D-glucans have modulating effects on the immune system by activating of macrophages, phagocytosis of the pathogen, release of proinflammatory cytokines (Sato et al, 2006). Patients who suffer from systemic infections including those caused by *Candida*, *Aspergillus*, and *Cryptococcus* species have been described to possess high levels of circulating $\beta$-glucans in their plasma. $\beta$-glucans activate macrophage functions which play a critical role in all phases of host defense in case of an infection and increases host immune defense. Thus $\beta$-Glucans provide increased resistance to infectious challenge (Jung et al, 2004; Babineau et al, 1994). Glucans are thought to mediate their effects via interaction with membrane receptors on macrophages, neutrophils, and NK cells (Mizuno 2002; Wasser 2002). Till now, four $\beta$-glucan receptors have been identified as candidates mediating these activities. It is namely complement receptor 3 (CR3; CD11b/CD18), lactosylceramide, selected scavenger receptors, and dectin-1 (bGR) (Akramienė et al, 2007). Anticarcinogenic activities of $\beta$-glucans were demonstrated by inhibition of cell transformation and angiogenesis (Akramienė et al, 2007; Wendy Hsiao et al, 2004; Ho et al, 2004).
1.13.2. β-Glucan as adjuvant to cancer chemo- and radiotherapy

The major side effect of most chemotherapeutic drugs is neutropenia. The administration of these anticancer drugs impairs blood forming function. These functions are important to maintain defense system of the patient. As a result, chemotherapy may accelerate risk of infections that decrease the quality of life for cancer patients. It was reported that β-Glucan increases IL-6 concentration and thus help in B cells differentiation, T cells activation, induction of acute phase proteins and reduction of G₀-residence time of the hematopoietic cells (Harada et al., 2002). Similarly, radiotherapy often results in hematopoietic and immune depletion. Consequently, patients often experience anemia, lymphocytopenia, thrombocytopenia, and granulocytopenia. This leads to high risk of development of serious infections and increase the mortality and morbidity of these patients. β-glucans increase the levels of IL-1β, IL-6, and GM-CSF significantly in irradiated mice (Jin et al., 2003). Thus, glucans increase serum levels of radioprotective cytokines, while decreasing the level of radioinduced TNF-α, which is increased as a consequence of tissue injury and anemia due to radiation. Glucans are able to modulate the dysregulation of cytokine production in radiation damage. (Akramiené et al., 2007). β-glucan have a novel and significant role in the restoration of haematopoiesis following by bone marrow injury and radiation exposure (Cramer et al., 2006).

1.14. Scope of the study

In the present study we evaluated radioprotective effects of mushroom P.rimosus. Radioprotective effects were evaluated in terms of antioxidant assays, in vitro membrane and DNA damage studies, in vivo antioxidant and genoprotective studies etc. Toxicity studies were also carried out in Swiss albino mice. The results reported in the thesis give ample evidence for the radioprotective properties of P. rimosus and suggest the drug as a promising radioprotective agent even though more studies are required.