Review
of
Literature
Exposure to ionizing radiation causes deleterious effects in living organisms. These effects are produced in a temporal manner across different levels of organization starting from the induction of primary lesions in the biological molecules and structures, eliciting repair processes and it leads to cell mortality or changes responsible for morbidity and cancer (Upadhyay et al., 2005). The damage to the biological systems by radiation takes place in two ways either directly by radiation energy absorbed by molecules or indirectly via generation of highly reactive free radicals and reactive oxygen species generated primarily from radiolysis of water. The direct effect observed during exposure to ionizing radiation is very small and the indirect action contributes to a large extent to produce radiation damage (Zaider et al., 1994). These free radicals and reactive oxygen species attack on all cellular components including macromolecules and also impair the indigenous antioxidant defense mechanism (Gracy et al., 1999). The net effect is the disruption of molecular structure and function which leads altered cell metabolism, cell death and if the dose is sufficiently high, mortality of the organism (Joshi et al., 2010).

2.1 Effects of radiations on biological system

The radiation effects on biological system depend on various factors such as physical factors (e.g. quality of radiation, exposure time and amount of absorbed radiation etc.), chemical factors (these can either protects from radiation effects or potentiate the same) and biological factors (such as actions of repair mechanism and indigenous antioxidant defense mechanism) (Soni, 2007). The effects of radiation on a tissue are proportionate to the amount absorbed by the tissue. The radiant energy may traverse without absorption; it may be completely expended, if collides with an electron
(photoelectric effect); or by removing an electron from an atom it may expend only part of its energy and continue in a altered direction and the wavelength will become longer (Compton effect) (Warren, 1980).

Because mammalian cells are composed of almost 80% water, indirect effects of ionizing radiation involve in the generation of radiolytic products of water and related reactive species collectively known as reactive oxygen species (ROS) such as hydroxyl radicals (OH\(^\cdot\)), hydrated electrons (e\(_{aq}\^-\)), hydroperoxy radicals (HO\(_2\)^\cdot\)), hydrogen radicals (H\(^\cdot\)), singlet oxygen (\(^1\)O\(_2\)), superoxide (O\(_2\)^\cdot\)), peroxyl radical (ROO\(^\cdot\)) etc. (Hosseinimehr, 2007). During the radiation exposure most oxidizing species produced in biological systems are hydroxyl radicals and singlet oxygen. These ROS deplete cellular antioxidants are capable of breaking chemical bonds, initiate lipid peroxidation, induce hazardous and undesirable changes in bio-molecules and it causes cell dysfunction or even death (Maurya et al., 2006; Paul et al., 2011).

DNA and membranes are major cellular targets of radiation damage. Radiation induced changes in DNA include altered bases, single and double strand breaks, removal of bases, inter/intra strand DNA linkage and cross linkage of DNA with adjacent protein molecules. These alterations affect the cell structure and function depending on their type and extent (Maurya et al., 2006; You et al., 2009). Lipid peroxidation products such as malondialdehyde forms adduct with cellular DNA. The double strand breaks are considered to be the most important ionizing radiation-induced lesions in DNA leading to cell death. (Sandeep and Nair, 2012).

Radiation induced free radicals can produce a variety of changes in membrane lipids and proteins. The damage in membrane lipids is resulted as lipid peroxidation which alters integrity of cell membrane structure
leading to inactivation of membrane bound enzymes, loss of permeability and decrease in membrane fluidity and impaired biological defense (Parihar et al., 2006; Paul et al., 2011).

Cells and tissues have comprehensive and integrated enzymatic as well as non-enzymatic antioxidant systems (e.g. super oxide dismutase, glutathione peroxidase, catalase, glutathione S transferase, glutathione, vitamin-E, β-carotene etc.) capable of the detoxification and removal of aquatic radiolytic products. But when these reactive species increase in living system following irradiation this endogenous enzyme system is incapable of protecting cells and tissues from the hazardous effects of free radicals (Karbownik and Reiter, 2000; Hosseinimehr, 2007). In normal tissues radiation damage is resulted from a sequence of simultaneous events that include activation of the immune system, inflammation process, regeneration of epithelium and endothelium, tissue fibrosis and remodeling. This phenomenon occurs as a result of complex interactions of molecular signals including cytokines, chemokines and growth factors (You et al., 2009). The mechanisms of lethal damages caused by radiation, both in cancer cells and in normal tissue cells are by apoptosis or clonogenic cell death (Ross, 1999).

2.2 Radiat ion sickness and mortality

Exposure to ionizing radiation results in various types of syndromes as nausea, vomiting, diarrhea (NVD) syndrome, hematopoietic syndrome, gastrointestinal syndrome and central nervous system syndrome depending on radiation dose (Maurya et al., 2006).

Tsuchihashi and coworkers (1969) reported that rats exposed to 9 Gy died within 30 days and minimum mortality was reported in animals exposed with 6 Gy. Mortality of irradiated rats was decreased with decrease
in radiation dose. Saharan et al. (1981) reported radiation induced weight loss, development of other signs of radiation sickness and mortality in Swiss albino mice exposed with 15 Gy Co\textsuperscript{60} gamma radiations. Uma Devi et al. (1999) reported that 11 Gy gamma radiation irradiated animals showed signs of radiation sickness within 2-4 days post-irradiation and all animals were died within 15 days post-irradiation. Manda (1999) reported that normal animals did not show any mortality and sickness whereas a severe radiation sickness and mortality was observed at high dose (9 Gy) of gamma radiation.

Jagetia and Baliga (2005) observed that exposure of mice to 10 Gy induced symptoms of radiation sickness like irritability, lethargy, watering of eyes, ruffling of hairs, reduced food and water intake, diarrhea, weight loss, emaciation, epilation and facial edema and majority of animals died within 10 days post-irradiation.

Kumar et al. (2005) studied that animals exposed to 8 Gy radiation showed signs and symptoms of radiation sickness such as nausea and diarrhea and these animals became lethargic resulting in reduced food and water intake. These animals showed loss of weight up to 31.32\% from initial weight on day 25 post-irradiation and no animal survived till day 30 post-irradiation. Drastic reduction in body weight of mice irradiated with 8 Gy was reported by Kumar et al. (2007) and all mice were died within 22 days. Bala et al. (2009) also reported that all animals irradiated with 10 Gy died within 12 to 15 days after irradiation.

Adaramoye et al. (2011) reported that the body weight of 5 Gy irradiated animals decreased after 1 and 8 weeks of exposure and 50\% mortality was reported. Kalpana et al. (2011) observed that mice exposed to 10 Gy radiation showed signs of discomfort characterized by decreased
physical activity and reduced food and water uptake and also showed signs of radiation sickness such as irritability, ruffled hair, weight loss, emaciation and epilation with a median survival period of 6 days.

Begum et al. (2012) also reported that irradiated animals showed 100% mortality within 12 days and exhibited signs of radiation sickness within 2-4 days after exposure to 10 Gy of $\gamma$-radiation. The main symptoms included reduction in the food and water intake, irritability, weight loss, emaciation, lethargy, diarrhea and ruffling of hair. Dose dependent radiation sickness and mortality was also reported by many researchers (Samarth and Kumar, 2003; Duan et al., 2010; Saini and Saini, 2011).

Recently, Yamaguchi et al. (2014) observed that only 12.5% survival in mice 100 days after exposure to 7 Gy gamma radiation and gradual reduction in body weight till day 30 was also observed.

2.3 Radiation induced damage to hematopoietic system

The hematopoietic system is composed of a remarkable variety of cells which include those circulating in the blood and their ancestors in bone marrow and progeny in the tissues. It also includes cells whose function is to remove both senescent cells from the bloodstream and any foreign material. The hematopoietic cell system consists of a hierarchy, in which the progenitor stem cells are capable of unlimited self-renewal and multi-lineal differentiation, giving rise to all blood cell types via committed precursor cells (Mills and Valli, 1988).

Hematopoietic system is among the most radiosensitive tissues of the body (Hosseinimehr, 2007). The injury to hematopoietic and lymphatic tissues produced by ionizing radiation in mammals including man is one of the major features of the biological effects of radiation (Soni, 2007).
The ionizing radiation-induced hematopoietic syndrome is characterized by defects in immune function and increased mortality due to infections and hemorrhage (Whitnall et al., 2000). Irradiation induces dose-dependent declines in circulating hematopoietic cells not only through reduced bone marrow production, but also by redistribution and apoptosis of mature formed elements of the blood (Dainiak, 2002).

Kumar et al. (2005) reported significant decrease in hematological constituents (RBC, WBC, hemoglobin and hematocrit) in Swiss albino mice exposed to 8 Gy gamma radiation, maximum decline in RBC and WBC counts were noted at 24 hours post-irradiation and minimum level of hemoglobin was noted on day 15 post-irradiation.

Reduction in various hematological constituents such as RBC, WBC, platelets, hemoglobin, hematocrit etc. after exposure with different doses of radiation was reported by several researchers (Verma et al., 2006; Abouelella et al., 2007; Acharya and Goyal, 2008; Bai et al., 2010)

Duan et al. (2010) reported that RBC, WBC, platelets counts and hemoglobin levels were markedly suppressed in the 8 Gy irradiated mice and had not returned to normal levels till day 30 post-irradiation; and the minimum of white blood cells was recorded on day 3 post-irradiation, the RBC, platelets counts, and hemoglobin were at minimum levels on day 7 post-irradiation.

Maks et al. (2011) demonstrated a dose-dependent decrease in WBC counts in mice exposed to high- and low-dose-rate proton and γ radiation. Ramachandran and Nair (2012) reported that Whole body exposure of mice to gamma radiation resulted in significant depletion of different
hematological parameters such as total erythrocyte and leukocyte counts, hemoglobin concentration.

Gharib et al. (2013) and Zhao et al. (2014) also reported radiation induced significant decrease in blood cells such as WBCs, RBCs, and platelets in the peripheral blood after irradiation at 4 and 8 Gy. Recently, reduction in white blood cells and red blood cells was reported by Yamaguchi et al. (2014) in mice exposed to 7 Gy gamma radiation.

De Freitas et al. (2014) observed that Irradiated animals showed a decrease in total white blood cell count at 48, 72 and 96 h post- irradiation (6 Gy) and also demonstrated that gamma radiation affects lymphocyte count in a similar way that whole white blood cells.

Okunewick and Kretchmar (1967) stated that exposure to an acute dose of radiation is sufficient to destroy the reproductive capacity of 50 percent erythrocytic stem cells.

It was noted that an exposure to gamma radiation resulted in a significant decline in the number of bone marrow cells such as leucoblasts, myelocytes, metamyelocytes, band/stab forms, polymorphs, pronormoblasts, normoblasts, lymphocytes and megakaryocytes (Samarth, 2007; Kumar et al., 2007). Ionizing radiation exposure increases the frequency of aberrant metaphases and different types of aberrations (breaks, fragments and chromosomal exchanges such as rings and dicentrics, polyploids etc.) and a decline in the PCE/NCE ratio (Rao et al., 2009; Duan et al., 2010).

Increase in the frequency of micro-nucleated polychromatic erythrocytes (MPCEs) and normo-chromatic erythrocytes (MNCEs) in mouse bone marrow after exposure to gamma radiation was reported by
several researchers in mice (Hosseinimehr et al., 2003, 2007; Duan et al., 2010).

2.4 Radiation induced damage in liver

For the first time Selding (1904) reported the effects of radiation on liver of guinea pig exposed to X-ray and did not observe any change as compared to normal liver. Theis (1905) was the first who reported the special changes like hyperemia, hemorrhage, necrosis, karyolysis and granular degeneration in the liver of guinea pig following administration of radioactive radium. He observed localized congestion and leukocyte infiltration on the second day, swelling and degranulation of cytoplasm, vacuolation and eventually loss of nuclei on fourth day post-treatment in adult and young rabbits.

Kolodny (1925) observed changes within the tissues after deep roentgen irradiation in rabbits and found necrosis of liver. Radio-lesions in the form of vacuolation in cytoplasm, dislocation of nuclei, enucleated cells, pyknotic nuclei, hyperemia, hemorrhage and lymphocytic infiltration have been described following internal irradiation (Bhartiya, 1970; Kumar and Mehta, 1973). Hyperemia was frequently observed at all radiation doses in the initial autopsy interval. This accumulation of red blood cells in the central vein and mild congestion of portal areas are characteristic symptoms of veno-occlusive disease (Fajardo, 1982; Lawrence et al., 1995; Fajardo et al., 2001a).

Mammalian liver has been reported as highly radiosensitive organ and hepatic injury can be life threatening when whole or most of the liver is exposed to ionizing radiation (Bhatia and Jain, 2004). The principle gross symptoms of radiation exposure to liver are hepatomegaly, hyperemia, jaundice and ascites. The histopathology of liver specimens has shown
hemorrhage extravasation, parenchymal cell loss, formation of canaliculi, fibrosis and necrosis when whole or most of the liver was exposed to ionizing radiation (Geraci and Mariano, 1993; Capps et al., 1997; Jeong et al., 2007).

Radiation has also been studied to cause acute alterations in enterohepatic recirculation (HER) of bile acids 3 days after 8 Gy exposures in vivo in the rats. The results demonstrated that concomitantly with radiation induced intestinal bile acid mal-absorption, hepatic bile acid synthesis and secretion are also changed (Scanff et al., 2004).

Metabolic dysfunction of liver was also observed by Karovin et al. (2005) on repetitive nanosecond X-ray treatment. Microwaves pulses have shown to render a less significant effect on metabolic function of the rat liver as compared to X-rays. Jeong et al. (2007) reported signs of dose-dependent hepatic damage such as necrosis of hepatocytes, reduction of proliferation, and fatty change in mice exposed with fast neutron radiation.

Lin et al. (2009) studied proteomic analysis of mouse liver after irradiation. They observed that the proteins associated with antioxidant response, molecular chaperones, energy metabolism, protein, amino acid metabolism and skeletal proteins were altered by radiation. Radiation-induced late injury in liver is histologically characterized by a loss of parenchymal hepatocytes and the distortion of the lobular architecture (Chung et al., 2010).

Dixit et al. (2012) studied histological changes in liver of rats exposed to gamma irradiation at a dose of 2.0 Gy and reported subintimal edema, hemorrhage involving small hepatic veins, patchy sinusoidal congestion and focal areas of necrosis.
Irradiation of abdominal region, radiation-induced liver disease can occur in patients with normal liver function, causing anicteric hepatomegaly and mild alkaline phosphatase serum level elevation and more severe derangement of liver function occurs in patients with pre-existing liver disease, and radiation-induced liver disease can progress to fibrosis, cirrhosis, and liver failure (Shadad et al., 2013).

Severe sinusoidal congestion and hemorrhage, dilation of the central vein, degenerated hepatocytes with perinuclear vacuolization and activated Kupffer cells were observed in the irradiated rats both in the early and late phases by Özyurt et al. (2014).

2.5 **Lipid peroxidation**

Lipids are essential components of biological membranes that maintain structure and control the function of cells. Biological membranes contain significant amounts of polyunsaturated fatty acids that are esterified on phospholipids, as well as free cholesterol (Yin et al., 2011). It has been suggested that lipid peroxidation is one of the main causes of radiation-induced damage to cellular membranes (Purohit et al., 1980; Ayene et al., 1988).

Lipid peroxidation chain reaction has three stages: initiation, propagation and termination. The initial reaction of \( \cdot \)OH with polyunsaturated fatty acids generates a lipid radical (L’) which in turn combines with molecular oxygen to form a lipid peroxyl radical (LOO’). This lipid peroxyl radical can remove hydrogen from an adjacent fatty acid to produce a lipid hydroperoxide (LOOH) and a second lipid radical. The LOOH formed can undergo reductive cleavage by reduced metals such as Fe\(^{++}\), producing lipid alkoxy radical (LO’). Both alkoxy and peroxyl radicals are able to
remove hydrogen atoms from fatty acids and stimulate the chain reaction of lipid peroxidation (Yin et al., 2011; Catala, 2012).

The end products of the chain reaction are a variety of hydroperoxides and cyclicperoxides. Reactive species that can remove the first hydrogen atom include the hydroxyl (·OH) radical, alkoxyl radical (RO·), peroxyl radical (ROO·) and possibly HO2· but not H2O2 or O2·. The consequence of peroxidation of unsaturated fatty acids is severe: damage of membranes function, enzymatic inactivation, toxic effects on the cellular division, etc or the damage caused by LPO is highly detrimental to the functioning of the cell (Gutteridge, 1995; Devasagayam et al., 2004; Catala, 2009).

Peroxidation brings alterations in structure, fluidity and permeability of a membrane (Nakazawa and Nagatsuka, 1980; Varshney and Kale, 1996; Srivastava et al., 1998), inactivates a number of membrane bound enzymes and protein receptors (Yukawa and Nakazawa, 1980; Jones et al., 1983; Maridonneau et al., 1983), mediates DNA damage (Inouye, 1984), and alters RNA transport from nucleus to cytoplasm (Yannarelli and Award, 1982).

Lipid peroxidation of both model and intact membranes has been studied by various authors from different points of view with special emphasis on the radiation chemistry of lipids (Stone et al., 1978; Helszer et al., 1980). Lipid peroxidation products interact with proteins and enzymes leading to the inhibition of the metabolic pathways. Membrane lipid peroxidation occurs much earlier than DNA fragmentation and loss of cell viability and it might contribute to the apoptotic signaling (Hockenbery et al., 1993). The increase in the degree of lipid peroxidation is a well known mechanism of liver damage (Tribble et al., 1987).
Radiation induced free radicals cause lipid peroxidation and several studies have been reported the increased level of lipid peroxidation in different experimental systems (Bhatia et al., 2008; Gaur, 2010; Azab et al., 2011; Nada et al., 2013).

2.6 **Endogenous antioxidant mechanisms**

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed and degraded by all aerobic organisms. ROS/RNS generation and removal are in balance in the presence of effective antioxidant defense mechanisms. Any increase in the ratio of ROS/RNS generation to antioxidant defense can create oxidative stress in cells (Coleman et al., 2003). Antioxidant defense mechanisms include antioxidants enzymes and non-enzyme antioxidants.

Radiation at doses used in radiotherapy depletes cellular alpha-tocopherol in normal cells, thereby increasing their risk of damage and studies in animals show that whole-body exposure to X-ray irradiation decreases tissue concentrations of vitamins C and E (Umegaku et al., 1995; Borek, 2004)

**Glutathione** (GSH), a non-enzymatic antioxidant, is a tri-peptide containing glycine, glutamic acid and cysteine. GSH is highly abundant in the cytosol, nuclei, and mitochondria. It is considered to be the major thiol-disulphide redox buffer of the cell and plays a crucial role in the detoxification of xenobiotics (Masella et al., 2005; Valko et al., 2007; Demirel et al., 2009). Its antioxidant function is provided by the sulphhydryl group of cysteine (Rennenberg, 1982). It exists both in reduced and oxidized states, mostly in reduced form (approximately 90%) in normal healthy cells.
Reduced glutathione directly participate in the neutralization of reactive species and on oxidation its sulphur forms a radical which reacts with another oxidized glutathione and forms glutathione disulphide (GSSG). GSH also acts as substrate or cofactor for the antioxidant enzymes (e.g. glutathione peroxidase, glutathione S transferase). GSH exerts its multiple functions mainly by two means i.e. enzymatic and non-enzymatic reactions (Sies, 1999; Dickinson and Forman, 2002; Chatterjee, 2013). GSH has a role in DNA damage and repair, redox regulation, signal transduction, gene expression and in apoptosis (Chatterjee, 2013).

Chapman and Cronkite (1950) found that glutathione protects mice from lethal radiation injury. Saunders et al. (1991) reported the role of glutathione in modifying survival after gamma irradiation. It has been shown that an exogenous addition of GSH could effectively reduce radiation-induced micronuclei and chromosome aberrations in different systems (Chatterjee and Jacob–Raman, 1986; Mazur, 2000). Glutathione peroxidase (GPx) has peroxidase activity whose important biological function is to protect the organism from oxidative damage by catalyzing the reduction of a variety of hydro-peroxides using glutathione as the reducing substrate (Weydert et al., 2006). It has been reported that exposure to ionizing radiation decreased the GSH level (Enginar et al., 2007; Bhatia et al., 2008; Gaur, 2010; El shahat, 2013).

The superoxide dismutases (SODs) are the first and most important line of antioxidant enzyme defense systems against ROS and particularly superoxide anion radicals (Zelko et al., 2002). SOD was first isolated from bovine blood by Mann and Keilin (1939) and its catalytic function was discovered by McCord and Fridovich (1969). Three distinct iso-forms of SOD have been found in mammals. Two isoforms of SOD have Cu and Zn
in their catalytic centre (CuZn-SOD or SOD1 and EC-SOD or SOD3) and third isoform (Mn-SOD or SOD2) has manganese (Mn) as a cofactor. SOD1 is present in cytosol, SOD2 in matrix of mitochondria and SOD3 found in extracellular elements (Marklund, 1984; Zelko et al., 2002). SOD is present in all oxygen metabolizing cells and it provides them an endogenous defense against reactions of \( \text{O}_2^\bullet \) produced in aerobic biological systems (Pajovi et al., 2001). SOD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide.

\[
\text{SOD} \quad \text{O}_2^\bullet^- + \text{O}_2^\bullet^- + 2 \text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2
\]

Superoxide dismutase (SOD) helps ameliorate radiation injury by converting radiation-induced superoxide radicals to hydrogen peroxide, which is disposed of by catalase and glutathione peroxidase (Srinivasan et al., 2008). The efficacy of SOD to function as a radio-protective agent has been investigated in a number of experimental systems (Das, 1998). Rabbani et al. (2005) reported that administration of external superoxide dismutase has radioprotective effect in mice.

**Catalase** an enzyme which catalyzes the decomposition of \( \text{H}_2\text{O}_2 \) into water and oxygen, presents in most of the aerobic cells (Aebi, 1984). It is one of the three main families of enzymes in mammalian cells critical for removing peroxide (Focea et al., 2012). It was first crystallized from beef liver (Sumner and Dounce, 1937). Even though catalase is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Oral et al., 2000).

\[
\text{CAT} \quad 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2 \text{H}_2\text{O}
\]
Numerous studies have shown that several synthetic mimetics of SOD and/or catalase has protective potential against radiation induced damages (Srinivasan et al., 2008; Vorotnikova et al., 2010). Decrease in SOD and catalase activity was reported after irradiation by several authors (Dokmeci et al., 2006; Begum et al., 2012; Ran et al., 2014).

2.7 Radioprotection and protective agents

Normal tissue response and injury after exposure to ionizing radiation are of great importance to patients with cancer, populations potentially subjected to military, accidental or intentional exposure including terrorist activities, and workers in the nuclear power industry (Coleman et al., 2003). Lead shielding and other physical measures are cumbersome to use in such situations and therefore pharmacological intervention could be the most prudent strategy to protect humans against the deleterious effects of ionizing radiation (Jagetia, 2007).

World wide research in the development of effective radioprotective agents for use in a variety of radiation scenarios has continued for more than six decades (Weiss and Landauer, 2009). Research using rodents first indicated protection by anoxia (Lacassagne, 1942) and chemicals causing hypoxia, such as cyanide (Herve and Bacq, 1949), were investigated (Weiss and Landauer, 2009).

A number of compounds of both synthetic and natural origin e.g. sulphhydryl compounds (Ramnath et al., 1997; Weiss, 1997), metallo-elements (Miko et al., 1998), immuno-modulators (Real et al., 1992; Furuse et al., 1997), cyto-protective agents (Links and Lewis, 1999), lipopolysaccharides and prostaglandins (Hanson et al., 1988; Riehl et al., 2000), cytokines (Legue et al., 2001; Kim et al., 2005), antioxidant nutrients (Weiss and Landauer, 2003), vitamins (Martin and Anderson,
1999; Singh et al., 2013a), bioflavonoids (Vasin, 2014) and hydrogen (Qian et al., 2013) have been investigated in both in vivo and in vitro models for their efficacy to protect against radiation induced damage in the sublethal to supralethal range of exposure.

A discrete classification of radioprotective agents is very difficult. Koukourakis (2012) identified five distinct types of radiation damage [(i) cellular depletion, (ii) reactive gene activation, (iii) tissue disorganization, (iv) stochastic effects and (v) bystander effects] in his review and classified the radioprotective agents into five relevant categories-

1. protectants against all types of radiation effects
2. death pathway modulators
3. blockers of inflammation, chemotaxis and autocrine/paracrine pathways
4. antimutagenic keepers of genomic integrity
5. agents that block bystander effects.

2.7.1 Chemical / synthetic radioprotectors

Radioprotection against ionizing radiation using chemicals began more than 65 years ago. The initial studies were mainly focused on sulfhydryl compounds. In 1942, Dale reported that sulfhydryl compounds could protect the enzymes, amino acid oxidase and carboxypeptidase against inactivation induced by X-rays. Latarjet and Ephrati (1948) and Chapman et al., 1950 showed protection of bacteriophages from radiation damages by thioglycolic acid, glutathione (GSH), tryptophan cysteine and cystine. Thereafter, a large number of chemical compounds, alone or in combination have been examined for their radioprotective efficacy.

Cysteine (a sulfur-containing amino acid and a precursor of glutathione) has been screened for its radioprotective potential. Patt et al.
(1949) reported that cysteine could protect rats from a lethal dose of X-rays. Pretreatment of cysteine diminished radiation effects like splenic atrophy and anemia (Patt et al., 1950; Smith et al., 1950). Sakaibara et al. (1965) showed that cysteine protects mice from enhanced infection after X-irradiation.

Bacq et al. (1951) reported that cysteamine, the decarboxylated form of cysteine (2-mercaptopthylamine - MEA) was radioprotective. Derivatives of cysteine and cysteamine of increasing complexity were studied extensively in mice for their radioprotective and toxic properties (Brown et al., 1988). Protection against radiation-induced lethality was greater after cysteamine treatment compared with the results for N-acetylcysteine treatment, but the lethal toxicity and behavioral toxicity of cysteamine is much greater (Landauer et al., 1988). Cysteine and cysteamine together play a major role in determining cellular radiosensitivity (Koch, 1998).

Cystamine, the oxidized form of cysteamine afforded 60 percent survival in lethally irradiated rats (Beaumariage, 1957). It has been reported to be as effective as cysteamine with low toxicity when administered orally to mice and rats (Bacq and Alexander, 1961). This compound have shown the protective effects against radiation induced hematopoietic damage in mice (Vacek et al., 1992).

Radioprotective effect of AET [S-(2-aminoethyl)] isothiouronium dihydrobromide] was first described by Doherty and Burnett (1955). It was demonstrated that a combination of a large dose of AET with a small dose of 5-hydroxytryptamin (5-HT) protected the bone marrow of mice irradiated with supra-lethal doses of X-rays more efficiently than either compound given separately (Maisin and Doherty, 1960). It was reported that AET given prior to the second radiation dose provide protection to hematopoietic
system and increases survival of mice (Vittorio et al., 1969). Garriott and Crowe (1983) reported that AET reduced the frequency of micronuclei in bone marrow cells of mice exposed to gamma radiation. Treatment with 2-Aminoethylisothiouronium bromide hydrobromide (AET) + 5-hydroxy-L-tryptophan (HT) 30 minutes prior to a single whole body exposure of 8 and 12 Gy Co\textsuperscript{60} gamma rays showed protection to bone marrow cells of Swiss albino mice (Gupta and Ghose, 1993).

WR-2721 or amifostine is one and most important aminothiol compound out of 4400 compounds synthesized, and screened \textit{in vivo} for their radioprotective potential under an Anti-radiation Drug Development Program at the Walter Reed Army Institute of Research, USA. WR-2721 or amifostine [S-2-(3-aminopropylamino) ethylphosphorothioic acid] is a prodrug in which the thioester bond is cleaved by membrane bound enzyme alkaline phosphatase yielding a free active thiol the active metabolite WR-1065. It has been used in clinical trials and it shows protection of normal tissues from the acute and long term effects of radiation and chemotherapy (Hosseinimehr, 2007).

Akerfeldt (1963) reported that pretreatment of WR-2721 provided maximum protection against radiation induced death in mouse. WR-2721 has also been exhibited to protect preferentially normal tissues rather than tumor tissue in a number of experimental systems (Yuhas and Strorer, 1969; Yuhas, 1972; Yuhas et al., 1980). Topical administration of amifostine to the oral mucosa was found effective treatment of acute radiation-induced mucositis (Li et al., 2014b). Amifostine has been approved by U.S. Food and Drug Administration (FDA) as a radioprotector and chemo-protector (Cassatt et al., 2002). However, Amifostine administration in patients has dose-limiting side effects, for example, hypotension, nausea, and vomiting (Rades et al., 2004).
There has been extensive research on **mercaptopropionylglycine** (MPG) for its radioprotective potential (Sugahara et al., 1970; Tanaka, 1972; Nagata et al., 1972; Uma Devi and Saharan, 1978; Ayene et al., 1988). Saini et al. (1978) reported that administration of MPG prior radiation exposure increased the radio-resistance of lymphocytes and it helped in enhancing recovery. The protective effect of WR-2721 against radiation induced chromosome damage, lethality and gastro-intestinal damage in mice is improved by the addition of low doses of MPG (Uma Devi and Prasanna, 1990; Prasanna and Uma Devi, 1993).

Nicaraven a chemically synthesized hydroxyl radical-specific scavenger significantly improved the survival of mice that suffered a lethal dose of $\gamma$-ray (Mori et al., 1993a). It has also been found to reduce radiation-induced cell death and effectively diminished the effects of radiation-induced injury in hematopoietic stem/progenitor cells (Mori et al., 1993b; Watanabe et al., 2006; Kawakatsu et al., 2013).

The 30-day survival rate of mice irradiated at the dose of 8 Gy was substantially increased to 91% by **Oltipraz** pretreatment (100 mg/kg/day for 2 days) compared with 48% in animals irradiated alone (Kim et al., 1998). Oltipraz provide protection to both DNA and RNA against radiation induced testicular damage (Johari et al., 2011).

Satyamitra et al. (2001) studied the *in vivo* radioprotective effects of **alpha-TMG** ($\alpha$-Tocopherol Mono Glucoside), a novel water-soluble derivative of vitamin E.

**Diltiazem** is a calcium channel blocker which is used in cardiovascular therapy. Diltiazem pre-treatment protects the hematopoietic system of mice against radiation-induced damage (Nunia and Goyal, 2004). Studies
conducted by Nunia et al. (2007) demonstrated that diltiazem provides protection against radiation-induced hematological and biochemical alterations in Swiss albino mice.

**Edaravone** is a brain protecting drug used clinically to treat acute ischemic stroke. Anzai et al. (2004) reported that edaravone shows radioprotective properties *in vivo* when administered before irradiation in a dose dependent and injection time dependent manner.

**Sulfasalazine** (SAZ) a prescribed drug for inflammatory bowel disease protects mice against radiation-induced chromosomal damage and cell cycle progression delay (Mantena et al., 2008).

**Dendro\[C_{60}\]fullerene** (DF-1) a C\(_{60}\) fullerene derivative, provides powerful protection against several deleterious cellular consequences of irradiation in mammalian systems including oxidative stress, DNA damage, and cell death (Theriot et al., 2010).

**Sodium orthovanadate** (vanadate) an inorganic vanadium compound, reported as a potent mitigator suppressing the acute lethality of hematopoietic syndrome and minimizing the detrimental effects such as anhematopoiesis and delayed genotoxic effects induced by total body irradiation in mice (Wang et al., 2013).

### 2.7.2 Natural radioprotectors

A variety of chemical or synthetic compounds have been studied for their radioprotective potential, however, toxic side effects at their optimum doses restrained their clinical use (Jagetia, 2007). FDA was approved amifostine as protective compound for the prevention of radiation induced xerostomia (Weiss and Landauer, 2009). Despite current clinical applications
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of amifostine, it has not been approved for use in any clinical nuclear/radiological exposure setting. The limits of amifostine are toxicity, limited routes of administration, narrow time windows, cost and limited protection of the central nervous system (Hosseinmehr, 2007). Such severe toxic effects associated with most of these compounds at their optimum effective doses restricted their use. Hence, search for more effective and less toxic agents for protection against radiation has shifted towards natural products.

A large number of plants and fungi contain compounds other than essential human nutrients that have been showed radioprotective potential in various model systems (Weiss and Landauer, 2009). Various natural products such as plant extracts, isolated constituents and phytochemicals and herbal formulations etc. have been extensively studied for their efficacy to render protection against radiation induced damage (Hazra et al., 2012; Aditya and Nair, 2013).

**Herbal preparations as radioprotectors**

Saini (1985) reported that Liv.52 an indigenous preparation protected the mammalian liver against radiation induced changes. Daga et al. (1995) found that prior administration of Liv.52 protects mice against radiation induced hematological changes. Recently, Waghmare et al. (2011a) reported that prior administration of Liv.52 significantly prevented the depletion of leukocytes count and initiated recovery towards normal at 28 days in mice exposed to low level gamma irradiation.

Kumar et al. (1996) reported the radioprotective effects of rasayanas, they postulated that the possible mechanisms of action of rasayanas could be increased stem cell proliferation and its effect on free radical induced injury produced by radiation. Rekha et al. (2000) studied the radioprotective effect of rasayana in mice and showed that it stimulates the hematopoietic
system. Aditya and Nair (2013) reported that administration of Brahma rasayana and chyavanaprash formulations to 4 Gy whole-body gamma irradiated mice resulted in faster cellular DNA repair as revealed from the increased cellular repair index and decrease in the formation of micronucleus.

A traditional herbal preparation abana which is used as cardio-protective agent in India has been found to protect mice bone marrow against the radiation induced micronuclei formation (Jagetia and Aruna, 1997) and it has provided protection against the radiation induced sickness and mortality having the optimum protective dose of 20 mg/kg was far lower than the LD$_{50}$ dose which was found to be 1.8 g/kg body weight (Jagetia et al., 2003a). Baliga et al. (2004) reported that the radioprotective activity of abana may be due to increased GSH level and free radical scavenging activity.

Jagetia and Baliga (2002a) found that aqueous extract of cystone (an ayurvedic herbal medicine) reduced the symptoms of radiation sickness and delayed the onset of mortality in comparison with irradiated alone animals.

Jagetia et al. (2002) reported that triphala reduced the symptoms of radiation sickness and delayed the onset of mortality. Jagetia et al. (2004) found that the administration of triphala resulted in an increase in the radiation tolerance.

Jagetia and Baliga (2004a) found that 50% ethanolic extract of septilin (a herbal preparation) provide protection against the gastrointestinal death. Septilin administration reduced the symptoms of radiation sickness and delayed the onset of mortality. Mansour et al. (2014) observed that septilin exhibited potential antioxidant activity and showed radioprotective
effect against $\gamma$-radiation by preventing oxidative stress and scavenging free radicals.

Jagetia and Baliga (2004b) reported that 50% ethanolic extract of *chyavanaprasha* (an Ayurvedic rejuvenating herbal preparation) delayed symptoms of radiation sickness, onset of mortality and provided a significant protection against gastrointestinal and bone marrow death.

El-Ghazaly et al. (2014) investigated the radioprotective effects of STW 5, a herbal preparation against radiation induced intestinal damage in rats and found that pre-treatment with STW 5 has the potential to decrease the severity of radiation-induced mucositis.

**Natural compounds and phytochemicals as radioprotectors**

Protective potential of *genistein*, an active metabolite of genistin glycoside found in soybeans, was demonstrated in gamma irradiated mice receiving a single subcutaneous injection 24 hours prior to irradiation without any toxicity (Landauer et al., 2003; Day et al., 2008). It was also found that subcutaneous administration of genistein prior to exposure of lethal dose of radiation supports multi-lineage, hematopoietic progenitor cell recovery and this recovery may be due to enhanced production of serum granulocyte colony stimulating factor and interleukin-6 (Davis et al., 2007; Singh et al., 2009). Kim et al. (2012) reported that genistein protects from testicular dysfunction induced by gamma-irradiation by an antiapoptotic effect and recovery of spermatogenesis.

*In vitro* and *in vivo* studies have shown that *gallic acid* (3, 4, 5-trihydroxybenzoic acid, a naturally occurring plant phenol) protected DNA and membranes against ionizing radiation (Gandhi and Nair, 2005).
The radioprotective effects of **mangiferin**, a glucosylxanthone isolated from *Mangifera indica*, was studied in the DBAxC57BL mice whole body exposed to gamma radiation and found to protect mice against the radiation induced sickness and mortality (Jagetia and Baliga, 2005).

**Hesperidin**, a flavonone glucoside found in *Citrus* species has powerful protective effects on the radiation induced DNA damage and on the decline in cell proliferation in mouse bone marrow (Hosseinimehr and Nemati, 2006) and radioprotective effects of hesperidin against genotoxicity induced by gamma irradiation in human lymphocytes were studied by Hosseinimehr et al. (2009). It also showed effective protection against radiation induced damage in the liver of mice (Kalpana et al., 2011).

Mansour et al. (2008a) reported that **Curcumin** exerts its antioxidant effects by decreasing LPO and improving the antioxidant status and acts as an effective radioprotector in rats. Sharaf et al. (2012) reported that curcumin treatment prior to exposure to ultraviolet rays in a small dose (5 mg/kg) led to restoration of the normal structure in most of the seminiferous tubules in albino rats.

Park et al. (2008) have investigated the radioprotective efficacy of **eckol**, a component of brown algae *Ecklonia cava* against the gamma ray induced damage in mice and reported that eckol significantly decreased the mortality of lethally irradiated mice by accelerating hematopoietic recovery.

**Propolis**, a resinous substance manufactured by honeybees from leaves, buds and sap of trees and flower blossoms was found to be protective against lethal effects of gamma radiation on white blood cells and primary DNA damage in mice (Benkovic et al., 2008). Montoro et al. (2011) reported the concentration-dependent protection by propolis against γ-ray-induced chromosome damage in cultured human blood lymphocytes.
The radioprotective potential of phenolic alkanone **Zingerone**, an active component isolated from *Zingiber officinale*, was investigated in gamma irradiated Swiss albino mice by Rao et al. (2009). They reported that Zingerone mitigates radiation induced mortality and cytogenetic damage.

Ahn et al. (2011) have demonstrated that **diplorehothydroxy-carmalol** (DPHC), a compound isolated from brown algae *Ishige okamurae* protects against cell death in the intestinal crypts and hematopoietic bone marrow as well as against the oxidative stress caused by gamma irradiation.

**α-Asarone**, an active component isolated from *Acorus calamus*, administration enhanced the endogenous spleen colony formation and reduced radiation-induced mortality and facilitated recovery from the radiation-induced loss of body weight in mice surviving after 8Gy gamma radiation exposure (Sandeep and Nair, 2011).

Patil et al. (2012) reported the radioprotective potential of **rutin** (a bioflavonoid). They observed maximum protection at dose of 10 mg/kg rutin and highest survival was reported by 30 days post-irradiation. Rutin treatment protected mice against the gastrointestinal death as well as bone marrow deaths.

**Epicatechin** (a flavanol and potent antioxidant present in the human diet predominantly in grapes, tea, apple and cocoa) pre-treatment ameliorated radiation mediated systemic oxidative stress which also prevented liver and testis from further damage (Das et al., 2013).

**Resveratrol** is a natural non-flavonoid polyphenol found in the skin of red grapes, inhibits ionizing irradiation induced inflammation in mesenchymal stem cells (Fu et al., 2013) and it effectively antagonized oxidation induced by irradiation in hippocampus of rats (Li et al., 2014a).
Kojic acid is a naturally available fungal metabolic product. The 30-day survival rate of mice pre-treated with kojic acid (75 or 300 mg/kg body weight) subcutaneously 27 h prior to a lethal dose (8 Gy) gamma irradiation was reported higher than that of mice irradiated alone and also found to be protective against radiation induced hematological changes in mice (Wang et al., 2014).

Rhoifolin (apigenin 7 neohesperidoside), a flavone glycoside, have radioprotective effect against radiation-induced decrease of blood platelets and cardiac biochemical lesions in whole body irradiated mice (El-Shawi and Eldahshan, 2014).

WPT-A, a type of water-soluble homogeneous lichen polysaccharide, was isolated and purified from Parmelia tinctorum attenuated radiation-induced DNA damage in mice (Xu et al., 2014).

Plant extracts as radioprotectors

The 30-day mortality due to bone marrow failure after irradiation was significantly reduced in Acanthopanax senticosus (Shigoka) extract treated mice and plant extract provide protection against radiation induced suppression of haemopoiesis (Miyanomae and Frindel, 1988). Oral administration of polysaccharides from A. senticosus dose-dependently reduced the 15 Gy X-irradiation induced injury in rats (Li and Zhou, 2007).

The radioprotective property of Ocimum sanctum was first reported by Uma Devi and Ganasoundari (1995). Ganasoundari et al. (1997) studied the effect of Ocimum sanctum on the survival of mice after whole-body lethal irradiation and compared it with WR-2721, a standard radioprotector. A combination of WR-2721 and Ocimum sanctum extract produced a significantly higher inhibition of the OH• radical activity compared with
either agent individually (Ganasoundari et al., 1998). Orientin and vicenin are compounds isolated from *Ocimum sanctum*. Vicenin provided a slightly higher protection, compared with orientin in murine model system (Uma Devi et al., 1999) and protected human lymphocyte chromosomes (Vrinda and Uma Devi, 2001).

Kuttan (1996) reported that 75% methanolic extract of *Withania somnifera* increases the total WBC count, normalized the ratio of normochromatic erythrocytes and polychromatic erythrocytes in mice after the radiation exposure.

Pande et al. (1998) showed radioprotective effect of the extract of *Aloe vera* in Swiss albino mice. Saini and Saini (2011) found that treatment of mice with *Aloe* before irradiation with different doses of gamma radiation (6-12 Gy) delayed the onset and reduced the severity of radiation sickness. Agarwal et al. (2011) reported that *Aloe vera* protected against cadmium and radiation induced hematological changes in the Swiss albino mice. *Aloe vera* extract have a protective effect against radiation-induced oxidative stress by improving in the antioxidant status of liver tissue and restoring the levels of Fe and Cu levels in liver and intestine as well as intestinal Zn when administered pre- and post-γ-irradiation (Nada et al., 2013).

The radioprotective efficacy of *ginseng* has been reported by several workers (Song et al., 2003; Kumar et al., 2003). Red ginseng showed photo-protective effect against ultraviolet radiation-induced chronic skin damage in the hairless mouse (Lee et al., 2009). Ginseng protects against gamma-irradiation induced cardio-nephrotoxicity via enhancing the antioxidant activity and inhibition of endothelial dysfunction (Mansour, 2013).
Goel et al. (1999) showed protective effects of aqueous extract of roots of *Podophyllum hexandrum* against radiation damage in Swiss albino mice. Kumar and Goel (2000) demonstrated that *Podophyllum* exhibits antioxidant properties and inhibits radiation induced lipid peroxidation in a dose dependent manner. Mittal et al. (2001) studied the influence of *Podophyllum* on endogenous antioxidant defense system and its possible role in radioprotection of mice. Verma et al. (2014) reported that the bioactive phyto-constituents of *Podophyllum hexandrum* could significantly protect lung and liver against radiation, predominantly by reduction in lipid peroxidation and elevation of EC-SOD along with a set of endogenous defense enzymes.

*Vitis vinifera* grape seed extract shows radioprotective effect against chromosomal damage in mouse bone marrow exposed to X-rays (Castillo et al., 2000) and enhances the antioxidant status and decreases the incidence of free radical induced lipid peroxidation in blood of rats acutely whole-body exposed to 6 Gy X-rays, with a higher efficiency than vitamin E (Enginar et al., 2010).

Rao et al. (2001) reported the radioprotective property of *Moringa oleifera* leaves. The pretreatment with the methanolic leaf extract of *Moringa oleifera* protects bone marrow chromosomes against radiation induced damage in mice and improved survival after lethal whole body irradiation. *Moringa oleifera* leaf extract treatment protected against γ-radiation-induced liver damage in mice through inhibiting NF-κB translocation and lipid peroxidation, and increasing SOD, CAT and GSH in liver (Sinha et al., 2011, 2012).

Jagetia and Baliga (2002b) demonstrated that leaf extract of *Syzygium cumini* (jamun) protects against the radiation-induced DNA
damage. Jagetia and Baliga (2003) reported that leaf extract of *Syzygium cumini* delayed the onset of mortality and reduced the symptoms of radiation sickness.

It has been reported that *Hippophae rhamnoides* play a vital role in radioprotection (Goel et al., 2002, 2003b; Prem kumar et al., 2002). Saini et al. (2014) reported that *Hippophae rhamnoides* leaf extract protects against radiation induced oxidative stress and tissue histological changes in kidney.

*Citrus* extract has been found to reduce the frequencies of micro-nucleated polychromatic erythrocytes and normochromatric erythrocytes of $\gamma$-irradiated mice (Hosseinimehr et al., 2003).

Maharwal et al. (2003) reported the radiomodulatory effect of *Amaranthus paniculatus* leaf extract. It has been reported that pre-treatment of *Rajgira* extract (*Amaranthus paniculatus*) protect the hematopoietic tissues in mice from the lethal effects of ionizing radiation (Krishna and Kumar, 2005).

Jagetia et al. (2003b) reported the radioprotective effect of the hydro-alcoholic extract of ginger rhizome, *Zingiber officinale*. Pre-treatment of mice reduced the severity of radiation sickness and the mortality at all doses. Du et al. (2012) reported that *Zingiber officinale* extract at 800 mg/kg body weight had radioprotection against radiation-induced antioxidant damage by scavenging the excessive free radicals and lipid peroxides caused by irradiation.

Samarth and Kumar (2003) reported that hematopoietic stem cells can be protected from radiation induced free radical damage by *Mentha piperita* extract which was evident in the increased number of radiation
induced spleen colonies and hematological constituents in peripheral blood in mice. Samarth et al. (2005) reported the radiomodulatory influence of aqueous leaf extract of *M. piperita* (Linn.) on hepatic antioxidant status and lipid peroxidation in Swiss albino mice. The possible mechanism of protection of extract against radiation induced damage was its antioxidant and free radical scavenging properties (Samarth et al., 2006).

Treatment with *M. piperita* extract prior irradiation resulted in significant increase in the number of leucoblasts, myelocytes, metamyelocytes, band/stab forms, polymorphs, pronormoblasts and normoblasts, lymphocytes and megakaryocytes in bone marrow cells in comparison with irradiated alone mice (Samarth, 2007) and also protects against radiation induced testicular damage in Swiss albino mice (Samarth and Samarth, 2009).

Alcoholic extract of *Ageratum conyzoides* effectively protected mice against 10 Gy-induced gastrointestinal and bone marrow related death (Jagetia et al., 2003c). Gupta et al. (2013) reported the radioprotective effect of *Alstonia scholaris* extract against hematological dysfunctions in mice.

Jagetia et al. (2003c) found that the radioprotective efficacy of *Aegle marmelos* may be due to scavenging of radiation-induced free radicals and increased oxidant status. Hydro-alcoholic leaf extract of *A. marmelos* reduced micro-nucleated polychromatic, normochromatic erythrocytes and polychromatic/normochromatic erythrocyte ratio in γ-irradiated mice bone marrow cells (Baliga et al., 2010). A dose of 100 mg/kg b.wt./animal/day for 5 consecutive days of *A. marmelos* fruit extract prior irradiation (6 Gy) resulted in a noticeable increase in the number of crypt cells, mitotic figures and villus length in intestine and significant rise in count of erythrocytes, hemoglobin and hematocrit in peripheral blood and showed
protection of intestinal constituents and bone marrow cells of Swiss albino mice against gamma radiation (Agarwal and Goyal, 2011, 2012).

Kumar et al. (2004) reported that pre-treatment with 2.5 g/kg b.wt. of fruit pulp of *Emblica officinalis* for 10 consecutive days prior irradiation (7 Gy) significantly increased the total leukocyte count, hematopoietic and bone marrow viability which were decreased by irradiation and enhanced the activity of various antioxidant enzymes and GST as well as glutathione system in blood.

Bhatia and Jain (2004) reported the protective effects of *Spinacia oleracea* against radiation induced oxidative stress. Verma et al. (2006) reported the protective effect of *Spirulina* on radiation induced hematological and biochemical changes in Swiss albino mice.

Oral administration of *Adhatoda vasica* leaf extract (800 mg/kg body weight) prior to whole body irradiation (8 Gy) showed a significant protection in terms of survival percentage, hematological parameters (Kumar et al., 2005) and radiation-induced changes in terms of histological alterations in testis, reduced glutathione (GSH), lipid peroxidation (LPO), acid and alkaline phosphatases levels and chromosomal alterations in Swiss albino mice (Kumar et al., 2007).

Soni et al. (2006) reported the protective effect of *Brassica compestris* seed extract on radiation induced hematological and biochemical changes in Swiss albino mice. They calculated a dose reduction factor as 1.59 for *Brassica compestris* seed extract.

Hosseinimehr et al. (2007) reported that a single intra-peritoneal administration of *hawthorn* (*Crataegus microphylla*) fruit extract at doses
25, 50, 100 and 200 mg/kg one hour prior gamma irradiation (2 Gy) reduced the frequencies of micro-nucleated polychromatic erythrocytes.

Sharma and Kumar (2007) found that deleterious effects of radiation may be reduced by *Myristica fragrans* seed extract pre-treatment in terms of increased survival, significant decrease in LPO level and ACP activity and significant increase in GSH content in comparison to irradiated alone mice.

*Phyllanthus amarus* has been found to protect the clastogenic effects of radiation as seen from decreased number of micronuclei and chromosomal aberrations percentage (Kumar and Kuttan, 2007).

Radioprotective effects of *Rosmarinus officinalis* against radiation induced haematological alterations and biochemical changes (Soyal et al., 2007a; Acharya and Goyal, 2008) were studied. It has also been reported that *R. officinalis* extract inhibit γ-radiation (3Gy) induced lipid peroxidation and elevated glutathione levels in irradiated mice (Jindal et al., 2010).

Abouelella et al. (2007) found that *Echinacea purpurea* administration significantly ameliorated detrimental reduction effects of γ-rays on peripheral blood hemoglobin and the levels of red blood cells, differential white blood cells and bone marrow cells. *E. purpurea* is shown to have a radio-protective effect against gamma irradiation by preventing oxidative stress in spleen tissues and modulation of immune responses (Ezz, 2011).

Post-treatment of fruit pulp extract of *Grewia asiatica* inhibited γ-radiation-induced glutathione depletion and ameliorated lipid peroxidation levels in mice (Sisodia et al., 2008; Sharma and Sisodia, 2009).

Joy and Nair (2009) reported that *Centella asiatica* rendered radioprotection to DNA and membranes against radiation exposure, both in
vitro and in vivo. Administration of the extract prevents a radiation-induced decline in antioxidant enzyme levels.

It was reported that Indigowood root (radix of *Isatis indigotica*) extract can reduce tissue injury caused by radiation demonstrates radioprotective effect in hematopoietic system recovery, modulation of serum inflammatory cytokines and improvement of severe enteropathy in irradiated mice (You et al., 2009).

*Olea europaea* has been found to protect against UV-B-induced skin damage in hairless mice by inhibiting the expression of matrix metalloproteinase MMP-2, MMP-9, MMP-13, vascular endothelial growth factor and cyclooxygenase-2 in the skin (Kimura and Sumiyoshi, 2009).

Andrade et al. (2009a,b, 2011) found that *ad libitum* black grape (*Vitis labrusca*) juice intake provide radioprotection over selected hematological parameters and organs with an abrogation of immediate acute radiation syndrome symptoms and was able to restore the liver primary antioxidant system against adverse effects due to whole body acute X-irradiation in rats, as supported by decrease in liver lipid peroxidation and the increase in SOD and GPx antioxidant enzyme activities in black grape juice supplemented rats.

Duan et al. (2010) found that pre-treatment with procyanidins extracted with acetone–water from lotus (*Nelumbo nucifera* Gaertn.) seedpod (LSPCs) could effectively maintain spleen index close to normal, stimulate endogenous spleen colony forming units, promote the levels of red blood cells (RBC), white blood cells (WBC), platelets and hemoglobin in peripheral blood and prevent spleen and skin damage in irradiated mice, reduce the level of radiation-induced micro-nucleated polychromatic
erythrocytes in bone marrow, maintain the polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) ratio (P/N ratio) and significantly decrease bone marrow chromosomal damage in Swiss albino mice.

Methanol extract of *Xylopia aethiopica* fruit reduced γ-radiation-induced oxidative stress in brain of adult male Wistar rats (Adaramoye et al., 2010). Adaramoye et al. (2011) reported that dried fruit extract from *X. aethiopica* could increase the antioxidant defense systems in the liver and kidney of irradiated animals, and may protect from adverse effects of whole body radiation.

Veeraraghavan et al. (2011) reported that leaf extract of *Azadirachta indica* exhibited radio-sensitizing effect by activating pro-apoptotic signals in neuroblastoma xenografts exposed to single (10 Gy) or fractionated (2Gy/day for 5 day) doses of radiation.

*Haberlea rhodopensis* extract pretreatment at the dose of 0.12 g/kg has reduced the radiation-induced oxidative stress by significantly preserving the antioxidant SOD and CAT activities and significantly reducing the MDA formation in rabbits (Popov et al., 2011). *H. rhodopensis* extract administration prior irradiation also found to be protective against radiation induced chromosomal abbreviations and DNA damage in lymphocytes of rabbit (Georgieva et al., 2012).

Oral administration of *Acorus calamus* extract (250 mg/kg body weight) to mice 1 hour prior to whole body gamma irradiation significantly increased the activities of major enzymes of the antioxidant defense system specially SOD, catalase and GPx and levels of GSH in 2, 6 and 10 Gy
irradiated mice and decreased the formation MDA and also decreased DNA strand breaks (Sandeep and Nair, 2012).

Jha et al. (2012) studied the radioprotective potential of *Pistia stratiotes* and reported that methanolic extract of *P. stratiotes* was capable of reduction of chromosomal aberration and micronuclei formation in bone marrow cells of mice.

Madhu et al. (2012) reported that treatment of mice with *Nardostachys jatamansi* extract before irradiation caused a significant depletion in lipid peroxidation followed by significant elevation in reduced glutathione, total antioxidants, glutathione peroxidase and catalase activity. It also showed a reduction in the micronucleus formation in the bone marrow cells. Gowda et al. (2013) reported that *N. jatamansi* root extract (NJE) protects against whole body electron beam radiation induced hematological damage in rats.

*Tinospora cordifolia* root extract pre-treatment significantly ameliorated radiation induced elevation in cholesterol and lipid peroxidation levels, whereas, a decline in glutathione and total proteins concentration was noted (Sharma and Goyal, 2013).

Treatment with aqueous extract of *Aframomum melegueta* at a dose of 200 and 400 mg/kg before and after irradiation significantly decreased the elevated levels of LPO restored GSH level near normal and enhanced CAT and GPx activities and protects the liver from radiation-induced damages in rats (Nwozo et al., 2013).

Beta glucan isolated from *Ganoderma lucidum* possessed significant radioprotective activity with DNA repairing ability and antioxidant activity
and found to be protective radiation induced cellular damages (Pillai et al., 2014).

2.8 Mechanism of radioprotection

Various radioprotective mechanisms have been proposed to describe the prophylactic and therapeutic effects of a large number of agents (Weiss and Landauer, 2003; Hazra et al., 2012). It is most likely that no single mechanism can account for the protection offered by a radioprotective agent. Certain compounds may operate primarily by means of physiological effects resulting in hypoxia or hypothermia in critical tissues. Others may operate primarily by influencing the intrinsic radio-sensitivity of target molecules by causing localized radical scavenging or by donating a hydrogen atom. Metabolic effects such as biochemical shock, release of endogenous non-protein sulfhydryls, induction of structural changes in target molecules or delay in DNA synthesis and cell division are also possible mechanisms for radioprotection (Copeland, 1978).

Various proposed mechanisms of radioprotection are: free radical scavenging, hydrogen donation to target molecules, formation of mixed disulfides, release of endogenous nonprotein sulfhydryls, DNA repair and recovery process, delay of cellular division, induction of hypoxia and hypothermia in the tissues and biochemical shock, promoting the recovery of hematopoietic and immune functions, up regulating mRNAs of antioxidant enzymes (such as catalase, glutathione transferase, glutathione peroxidase and superoxide dismutase), inhibiting activation of protein kinase, nitrogen activated protein kinase, cytochrome P-450 and nitric oxide. It is well established that protective activity of radioprotective agents is accomplished through different mechanisms on three special levels of cell organization i.e. at molecular level, physiological-biochemical level and at organic level (Yamini and Gopal, 2010; Velpula et al., 2013).
Free radical scavenging mechanism of action suggests that certain agents are oxidized by free radicals and forms stable compounds which are incapable of reacting with other cellular components. This mechanism prevents the free radicals from reacting with the cell vital components. Another mechanism is the repair by hydrogen donation to target molecules. If an R-H molecule is converted into an R• (radical R) by exposure to radiation, a protective agent can donate a hydrogen atom to this radical, restoring it to its original state (Varanda and Tavares, 1998).

The mechanism of radioprotection by formation of mixed disulfides is proposed for aminothiols and involves radioprotector binding to cellular components. Sulfhydryl compounds of the aminothiols form mixed disulfides with sulfhydryl compounds of cellular proteins and when free radicals attacked one of these disulfides in which one of the sulfur atoms is reduced and the other is oxidized. If the sulfur atom of the protein is reduced and the sulfur atom of the protective agent is oxidized the protein is not damaged (Velpula et al., 2013).

2.9 Prosopis cineraria (L) Druce- radiomodifiers used in present study

Prosopis cineraria (L) Druce, commonly known as khejri, is one of the forty four species in the genus Prosopis belongs to the family legumenosae, subfamily mimosoideae. It is a moderate sized, thorny, irregularly branched, evergreen tree. P. cineraria are most important feed species providing nutrition and highly palatable green as well as dry fodder for livestock. Pods are locally called sangri used as vegetable.

P. cineraria is a small moderate sized, thorny, evergreen or nearly so irregularly branched tree forming an open crown and has thick, rough gray bark with deep fissures. Leaves are alternate, bipinnately compound with 1-3 pairs of pinnae. Each pinna has 7-14 pairs of leaflets, 4- 15 mm
long and 2-4 mm broad. The thorns are straight with a conical base and distributed sparsely along the length of the stem (Mahoney, 1990).

Many medicinal uses have been recorded for extracts from *Prosopis* plant parts from studies on the ethno botany of populations in areas of the entire native range of the genus. Three main groups of ailments are treated with leaf and bark extracts: mouth and throat infections including ulcers and bronchitis; internal diseases including general pains, parasites and urinary disorders; and skin disorders, dermatitis and parasitic infections. In South America, preparations from fresh buds of various species are used to treat conjunctivitis. Leaf preparations are used to mend broken bones, liver stones, dyspepsia and venereal disease, and are often mixed with other products (D’Antoni and Solbrig, 1977).

*P. cineraria* flower is pounded, mixed with sugar and used during pregnancy as safeguard against miscarriage. The wood ash which contains 31 percent of soluble potassium salts may be used as a source of potash. The bark of the tree is dry, acrid and bitter with a sharp taste, cooling anthelmintic; tonic, cures leprosy, dysentery, bronchitis, asthma, leucoderma, piles and tremors of the muscles. The bark is also used for tanning. Mesquite pollen serves as a dietary source for mice. The bark is used as a remedy for rheumatism, in cough colds, asthma. The plant is recommended for the treatment of snakebite. The bark is prescribed for scorpion sting (ICFRE, 1993; Khatri et al., 2010).

Leaf paste of *P. cineraria* is applied on boils and blisters including mouth ulcers in livestock and leaf infusion on open sores on the skin. The smoke of the leaves is good for eye troubles. The fruit is dry and hot with a flavor, indigestible cause biliousness and destroys the nails and the hair. The pod is considered astringent in Punjab. Recently processing composition,
nutritional evolution and utilization of mesquite (*Prosopis* sp.) pods as a raw material for food industry had been reported (Khatri et al., 2010).

Phytochemical studies by Jewers et al. (1974 and 1976) reported alkaloids namely spicegerine; steroids namely campstool, stigmasterol, sitosterol, cholesterol; alcohols namely octacosanol and triacontan-1-ol; and alkane hentriacontane in the leaves of *P. cineraria*. Malik and Kalidhar (2007) isolated methyl docosanoate and other compounds from the methanol extract of plant leaves.

Flowers contain prosogerin A, B, C, D and E (Rastogi and Mehrotra, 1995). Seeds contain polyphenolics, gallic acid, patuletin, luteolin, patulitrin, rutin and prosogerin– D and E; fatty acids such as palmitic acid, stearic acid, oleic acid & linoleic acid (Bhardwaj et al., 1980, 1981; Khatri et al., 2010). Recently, two phyto-constituents from bark namely paenol and ferulic acid were isolated from the ethanol extract of air dried bark of *P. cineraria* (Singh et al., 2013b).

Tapia et al. (2000) reported the biological activity from the extracts of aerial parts of five Argentinean *Prosopis* species and the exudate of *P. flexuosa* were assessed for DNA binding, b-glucosidase inhibition and free radical scavenging effect using the DPPH discoloration assay.

Studies have shown significant activity of plant extracts against lung carcinoma (Merzabani et al., 1979) and against lymphocytic leukemia and other carcinomas (Ahmad and Sultana, 1989). All parts of *P. juliflora* and *P. pallida* are used in the preparation of medicinal products to treat human ailments. There are many records of the use of these products from historical literature where *Prosopis* species are native and from recent descriptions where they have been introduced. In India, for example, an
Astringent decoction is made from boiling wood chips - a bark extract is used as an antiseptic on wounds and gum is used to treat eye infections (Vimal and Tyagi, 1986). Recently, antisedative and antiparasitic, indolizidine compounds isolated from *Prosopis glandulosa* var. *glandulosa* (Samoylenko et al., 2009).

Antimicrobial properties were reported by researchers from stem bark, leaflets and unripe pods of *P. cineraria* (Robertson et al., 2010; Velmurugan et al., 2010; Napar et al., 2012; Sharma et al., 2012). Ethanol and petroleum ether extracts from stem bark of the plant exhibited significant analgesic and anti-pyretic activity in albino rats (Manikandar et al., 2009). Sharma et al. (2010) reported that *P. cineraria* bark extract shows antihyperglycemic, antihyperlipidemic and antioxidative properties.

Robertson et al. (2011) reported the antitumor activity of the hydro-alcoholic extract of the leaves and stem barks of *P. cineraria* against Ehrlich ascites carcinoma-induced in mice. Purohit and Ram (2012) reported that *P. cineraria* bark extract exhibit hypolipidemic and antiatherosclerotic effect in experimentally induced hyperlipidemic rabbits.