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1.1. INTRODUCTION

Living systems are constantly exposed to a variety of physical and chemical agents which cause cellular damages including genetic alterations leading to mutations and cell death. Among the physical agents, ionizing and non-ionizing types of radiations are the major ones which cause damage to living organisms. In general, two things can happen when radiation is absorbed by matter: excitation or ionization.

- **Excitation** occurs when the radiation excites the motion of the atoms or molecules, or excites an electron from an occupied orbital into an empty, higher-energy orbital.

- **Ionization** occurs when the radiation carries enough energy to remove an electron from an atom or molecule.

Because living tissue is 70-90% water by weight, the dividing line between radiation that excites electrons and radiation that forms ions is often assumed to be equal to the ionization of water: 1216 kJ/mol. Radiation that carries less energy can only excite the water molecule. It is therefore called *non-ionizing radiation*. Radiation that carries more energy than 1216 kJ/mol can remove an electron from a water molecule to cause ionization, and is therefore called *ionizing radiation*. Radio waves, microwaves, infrared radiation, and visible light are all forms of non-ionizing radiation.

1.2. IONIZING RADIATIONS

Ionizing radiations include high-energy electromagnetic radiations such as X-rays and γ-rays, and particulate radiations such as alpha rays, beta rays, neutrons, protons and other less well-known particles. These when passing through matter, produce direct or indirect ionizations. Ionization involves the removal of orbital electrons and since these electrons produce the binding forces of atoms in the molecules, ionization or removal of electrons causes disruption of the integrity of the system. In addition to the process of ionization, energy transfer also occurs by the process of excitation as in the case of UV radiation. Ionizing radiation can be defined as any type of electromagnetic (such as X- or γ-rays) or particle radiation (such as neutron or alpha particles) with sufficient energy to ionize atoms or molecules; that is, to eject electrons from their outer orbitals. Ionizing radiation...
passing through living tissues generates reactive free radicals. These free radicals can interact with critical macromolecules, such as DNA, proteins or membranes, and can induce cell damage and potentially, cell dysfunction and death. Damage to DNA include a range of lesions of which DNA double strand breaks (DSBs) have a pivotal role in determining whether cells survive radiation exposure (Karbownik & Reiter, 2000; Brown et al., 1982). If DNA damage is not correctly repaired, two direct consequences can occur. Residual or unrepaired damage leads directly to chromosomal aberrations, loss of genetic material and cell death. Also, unrepaired or incorrectly repaired damage can lead to mutations that might result in carcinogenesis or cell death. When DNA is damaged, it is followed by altered cell division, cell death, depletion of stem cell pools, organ system dysfunction and, if the radiation dose is sufficiently high, the organism will die. Although cells and tissues are equipped with endogenous enzymes (e.g. superoxide dismutase) capable of the detoxification and removal of the products of water radiolysis, when these reactive oxygen species increase in the biological system following exposure to irradiation, the endogenous system is incapable of protecting cells from the hazardous effects of free radicals.

1.2.1. Ionization in Living Tissue (Cell Damage)

Ionization of living tissue causes molecules in the cells to be broken apart. This interaction can kill the cell or cause them to reproduce abnormally. Damage to a cell can come from direct action or indirect action of the radiation. Cell damage due to direct action occurs when the radiation interacts directly with a cell’s essential molecules (DNA). The radiation energy may damage vital cellular components such as the genomic DNA or membranes.

Cell damage due to indirect action occurs when radiation interacts with the water molecules, which are roughly 80% of cell composition. The energy absorbed by the water molecule can result in the formation of free radicals. Free radicals are molecules that are highly reactive due to the presence of unpaired electrons, which result when water molecules are split. Free radicals initiate harmful chemical reactions within the cells. As a result of these chemical changes, cells may undergo a variety of structural changes which lead to altered function or cell death.

Various possibilities exist for the fate of cells damaged by radiation. Damaged cells can:
• Completely and perfectly repair themselves with the body's inherent repair mechanisms.

• Die during their attempt to reproduce. Thus, tissues and organs in which there is substantial cell loss may become functionally impaired. There is a "threshold" dose for each organ and tissue above which functional impairment will manifest as a clinically observable adverse outcome. Exceeding the threshold dose increases the level of harm. Such outcomes are called deterministic effects and occur at high doses.

• Repair themselves imperfectly and replicate this imperfect structure. These cells, with the progression of time, may be transformed by external agents (e.g., chemicals, diet, radiation exposure, lifestyle habits, etc.). After a latency period of years, they may develop into leukemia or a solid tumor (cancer). Such latent effects are called stochastic (or random).

![Figure 1.1](image-url)

**Figure 1.1**

**Direct DNA damage radiation model.** The schematic shows the standard model of DNA damage responses to radiation in biological systems, with direct DNA damage having a central role and the production of DNA double strand breaks (DSBs) leading to downstream biological consequences. Cells have complex pathways for sensing DNA damage and correctly repairing the DNA damage to survive the radiation exposure. If the DNA damage is not repaired, there is a high probability of cell death. DNA damage that is misrepaired can lead to mutations, increasing the probability of transformation and carcinogenesis.
1.2.2. Ionizing radiation induced damages in DNA

Ionizing radiations can affect DNA structure by causing one or more alterations: strand scissions, damage to sugar moiety, damage to bases, alteration or elimination of bases, cross links of intra and inter strand types and cross links with proteins (Kapp & Smith, 1970). Among these, single and double strand breaks are the most important since these are caused at a much lower dose range (Setlow & Setlow, 1972). These arise from cleavages at the sugar-phosphate backbone—either at the diester linkages or at sugar moiety with the formation of malondialdehyde like products (Freidberg & Freeman, 1985). Ionization radiation exposure also causes damages in DNA bases resulting in production of a variety of modified bases.

1.2.3. Effects of ionizing radiation upon humans

The occurrence of particular health effects from exposure to ionizing radiation is a complicated function of numerous factors including:

- **Type of radiation involved**- All kinds of ionizing radiation can produce health effects. The main difference in the ability of alpha and beta particles and gamma and X-rays to cause health effects is the amount of energy they have. Their energy determines how far they can penetrate into tissue and how much energy they are able to transmit directly or indirectly to tissues.

- **Size of dose received**- The higher the dose of radiation received, the higher the likelihood of health effects.

- **Rate the dose is received**- Tissue can receive larger dosages over a period of time. If the dosage occurs over a number of days or weeks, the results are often not as serious if a similar dose was received in a matter of minutes.

- **Part of the body exposed**- Extremities such as the hands or feet are able to receive a greater amount of radiation with less resulting damage than blood forming organs housed in the torso.

- **The age of the individual**- As a person ages, cell division slows and the body is less sensitive to the effects of ionizing radiation. Once cell division has slowed, the effects of radiation are somewhat less damaging than when cells were rapidly dividing.
• **Biological differences**- Some individuals are more sensitive to the effects of radiation than others. Studies have not been able to conclusively determine the differences.

1.2.4. **Biological Effects of ionizing radiation:**

The effects of ionizing radiation on living organisms are often broadly classified as being either stochastic or non-stochastic.

- **Stochastic effects** are those that occur by chance and consist primarily of cancer and genetic effects. Stochastic effects often show up years after exposure. As the dose to an individual increases, the probability that cancer or a genetic effect will occur also increases. For stochastic effects, there is no threshold dose below which it is relatively certain that an adverse effect cannot occur. In addition, because stochastic effects can occur in individuals that have not been exposed to radiation above background levels, it can never be determined for certain that an occurrence of cancer or genetic damage was due to a specific exposure.

- Unlike stochastic effects, **non-stochastic effects** are characterized by a threshold dose below which they do not occur. Non-stochastic effects typically result when very large dosages of radiation are received in a short amount of time. These effects will often be evident within hours or days. Examples of non-stochastic effects include erythema (skin reddening), skin and tissue burns, cataract formation, sterility, radiation sickness and death. Each of these effects differs from the others in that both its threshold dose and the time over which the dose was received, cause the effect (i.e. acute vs. chronic exposure).

Exposure to high amounts of ionizing radiation results in damage to the haematopoietic, gastrointestinal or central nervous systems, depending on radiation dose (Prasad, 1995). Because the haematopoietic system has a high level of cell turnover, it is among the most radiosensitive tissues in the body. The cells are affected and suppressed at relatively low doses of acute irradiation. Exposure to ionizing radiation induces a dose dependent decline in circulating haematopoietic cells, not only through reducing bone marrow cell production, but also by redistribution and apoptosis of mature cells (Whitnall, 2000; Hosseinimehr et al., 2006; Zhou and Mi, 2005) In general, the decline in lymphocytes and granulocytes occurs over a period of hours or days after irradiation. The primary cause of mortality during the early phases of radiation-induced haematopoietic
syndrome is sepsis, resulting from opportunistic infection, due to low numbers of neutrophils and increased translocation of bacteria across the gastrointestinal mucosa. This is complicated by thrombocytopenia and concomitant haemorrhage and defects in the adaptive immune system resulting from apoptosis of lymphocytes and deficient lymphopoiesis (Whitnall, 2000). Gastrointestinal syndrome is induced by a higher irradiation dose compared to haematopoietic syndrome. In this syndrome, the gastrointestinal barrier is damaged and high amounts of water and electrolytes are lost from the body, resulting in dehydration and bacteremia. Although exposure to high doses of radiation causes mortality, it has long been known that radiation can induce a broad spectrum of DNA lesions, including damage to nucleotide bases, cross-linkage, and DNA single- and double-strand breaks. It is now accepted, however, that inappropriately repaired DNA breaks are the principle lesions of importance in the induction of both chromosomal abnormalities and gene mutations and cancer (Little, 2000).

1.2.4.1. Haematopoietic Syndrome

The haematopoietic syndrome encompasses the medical conditions that affect the blood. Haematopoietic syndrome conditions appear after a $\gamma$-dose of about 200 rads (2 Gy) (Wald, 1982). This disease is characterized by depression or ablation of the bone marrow, and the physiological consequences of this damage. The onset of the disease is rather sudden, and is heralded by nausea and vomiting within several hours after the overexposure occurred. Loss of hair (epilation), which is almost always seen, appears between the second and third week after the exposure. Death may occur within one to two months after exposure. The chief effects to be noted, of course, are in the bone marrow and in the blood. Marrow depression is seen at 200 rads and at about 400 to 600 rads (4 to 6 Gy) complete ablation of the marrow occurs. In this case, however, spontaneous regrowth of the marrow is possible if the victim survives the physiological effects of the denuding of the marrow. An exposure of about 700 rads (7 Gy) or greater leads to irreversible ablation of the bone marrow (Fliedner et al., 1996).

1.2.4.2. Gastrointestinal Syndrome

The gastrointestinal syndrome encompasses the medical conditions that affect the stomach and the intestines. This medical condition follows a total body $\gamma$-dose of about 1000 rads (10 Gy) or greater, and is a consequence of the desquamation of the intestinal epithelium (Dubois & Walker, 1988). All the signs and symptoms of
haemtopoietic syndrome are seen, with the addition of severe nausea, vomiting, and diarrhea which begin very soon after exposure. Death within one to two weeks after exposure is the most likely outcome (Goans, 2001).

1.2.4.3. **Central Nervous System**

A total body $\gamma$-dose in excess of about 2000 rads (20 Gy) damages the central nervous system, as well as all the other organ systems in the body. Unconsciousness follows within minutes after exposure and death can result in a matter of hours to several days. The rapidity of the onset of unconsciousness is directly related to the dose received (Fliedner, 2001).

1.2.4.4. **Other Acute Effects**

Radiation dermatitis of the hands and face was a relatively common occupational disease among radiologists who practiced during the early years of the twentieth century (Schmuth, 2002). The reproductive organs are particularly radiosensitive. A single dose of only 30 rads (300 mGy) to the testes results in temporary sterility among men. For women, a 300 rad (3 Gy) dose to the ovaries produces temporary sterility (Kuroda et al., 2000). Higher doses increase the period of temporary sterility. The eyes too, are relatively radiosensitive. A local dose of several hundred rads can result in acute conjunctivitis (Midena, 1999).

1.2.5. **Cellular response to radiation**

Since the basic structure of living matter is the cell, the interaction of ionizing radiation with living matter is basically an interaction with the cell or more specifically with structures and molecules in the different cell components. Damage to DNA can lead to irreversible cell damage, such as inhibition of its ability to divide, or structural changes with altered cell function. If the repair mechanism fails, the result will be biochemical changes in the cell leading to cell modification or cell killing. The cell modification process can lead to a transformation of the cell into a tumor cell or if the modification appears in a germ cell, to mutations observable as hereditary effects.

Irradiation damages absolutely all the intracellular structures. The response of a cell to radiation injury include a delay in cell division, suppression of DNA synthesis, membrane damage etc. The extent of these responses depends on the phase of the cell cycle in which irradiation was performed.
Radiation damage is primarily manifested by the loss of cellular reproductive integrity. Lethally irradiated cells are, thus, said to undergo a reproductive death. Consequently, most cell types do not show morphologic evidence of radiation damage until they attempt to divide. Alternatively, some cell types are killed via the induction of apoptosis (Hellman et al., 1995). A cell that has sustained lethal damage following radiation exposure may undergo one or two divisions prior to metabolic death.

1.2.6. Biological effects of radiation at the molecular level

The vital cellular targets of radiation damage are DNA and the membranes. Besides DNA, lipids and proteins are also attacked by free radicals induced by ionizing radiation (Edwards et al., 1984; Verma & Sonwalker, 1991). Radiation may induce an increase in the permeability for K+ and Na+ ions, reduction in amino acid uptake, auto oxidation of the fatty acid components and loss of sulfhydryl functions as well as disturbances in lipid metabolism (Nair et al., 2000).

1.2.6.1. DNA: The primary cellular target

Genomic DNA is most important cellular target for radiation inactivation leading to loss of viability and hereditary changes. 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). About 60%-70% of cellular DNA damage produced by ionizing radiation is estimated to be caused by •OH, formed from the radiolysis of water.

Ionizing radiation can affect DNA structure by causing one or more alterations; single strand breaks (SSB), double strand breaks (DSB), base damage, base loss, sugar damage, denatured zones, intra-molecular cross-links, DNA-protein cross-links, hydrogen bond breakage between chains, etc. These occur primarily by interaction of free radicals with DNA bases and, to a lesser extent, with DNA sugars (Karbownik and Reiter, 2000). DNA damage caused by radiation exhibits multiple damaged sites and clustered lesions.

Among bases and generally among nucleic acid components, •OH is non selective in its reaction while guanine is the most susceptible DNA target for oxidative reactions mediated by singlet oxygen (¹O₂), and it exhibits the lowest ionization potential. Thus, one of the most mutagenic lesions, and the most abundant lesion formed in irradiated chromatin is 8-hydroxyguanine (Kasai et al., 1986; Gajewski et al., 1990). This is reported to be a key biomarker related to carcinogenesis (Floyd,
Once formed, this product can be repaired by several mechanisms (Tchou & Grollman, 1993). The interaction of free radicals with sugar moieties leads to the cleavage of the sugar–phosphate backbone of DNA followed by single-strand breaks that undergo repair processes relatively easily (Karbownik & Reiter, 2000). On the other hand, double-strand breaks have more serious consequences. Double-strand breaks are well correlated with the cytotoxic effects of ionizing radiation and are considered the primary lesion involved in cellular death (Elia et al., 1991). 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. The damaging effects of ionizing radiation lead to cell death and are associated with an increased risk for numerous genetically determined diseases (Floyd, 1990). Among other indices of DNA damage caused by ionizing radiation are chromosome aberrations and micronuclei formation; these are apparent when irradiated cells are observed microscopically (Elia et al., 1991; Vijayalaxmi et al., 1998).

1.2.6.2. Biological membranes-the alternative target

Peroxidation of the lipids and oxidation of proteins constitute the major lesions in the membranes. In proteins, radiation exposure can lead to the formation of protein carbonyls and loss of protein thiols besides loss of activity of membrane bound enzymes (Nair et al., 2001). Membrane lipids are highly susceptible for radiation damage mainly due to the presence of polyunsaturated fatty acids. Unchecked peroxidative decomposition of membrane lipids has severe consequences for the cell and the organism. Since many cellular reactions are membrane based, they are affected by lipid peroxidation. The products formed during lipid peroxidation, such as peroxy radicals and malonaldehyde, also have effects at other targets away from the site of generation (Devasagayam and Kesavan, 2003).

Lipid peroxidation usually proceeds by the following free-radical chain reaction:

\[ \text{LH} \rightarrow \text{L} \cdot \text{ (initiation reaction)} \]
\[ \text{L} \cdot + \text{O}_2 \rightarrow \text{LOO} \cdot \text{ (propagation reaction)} \]
\[ \text{LOO} \cdot + \text{LH} \rightarrow \text{LOOH} + \text{L} \cdot \text{ (propagation reaction)} \]

where \( \text{LOO} \cdot \) is the lipid peroxy radical and \( \text{LOOH} \) is the lipid hydroperoxide.
Lipid peroxidation takes place after irradiation or free-radical attack (Edwards et al., 1984). This leads to the production of short chain fatty acyl derivatives, lipid-lipid cross-linking as well as protein-protein and lipid-protein cross-linking, oxidation of accessible amino acids, protein denaturation, and scission of disulphide bonds in proteins. Functionally, these changes can be expressed as altered membrane fluidity and permeability, which could trigger the release of potent physiological mediators. Activity of enzymes associated with these membranes may be altered by the disruption of lipid microenvironment and protein structure. The radiation-induced damages occur as a sequence of events traversing a time scale from picoseconds to few hours.

The initiation process of LPO begins when any species that can attack the lipid with sufficient energy to abstract a hydrogen atom from methylene (CH₂) group. Pure lipid peroxides are fairly stable molecules at physiological temperature, but the presence of transition metal complexes catalyses the decomposition of hydroperoxides. Products of lipid peroxidation may cause DNA damage (Halliwell & Gutteridge, 1999). Malondialdehyde is the major reactive aldehyde resulting from peroxidation of biological membrane polyunsaturated fatty acids (PUFA). MDA is a secondary product of LPO and is used as an indicator of tissue damage by a series of chain reactions (Ohkawa et al., 1979). It reacts with thiobarbituric acid to produce red colored product known as thiobarbituric acid reactive substance (TBARS).

1.3. Mechanism of radiation induced malignancy

Radiation can induce a broad spectrum of lesions and principle lesions of importance is in DNA which include damage to nucleotide bases, single and double strand breaks in DNA and unreparable double strand breaks (Jackson and Bartek, 2009). Damage to DNA may cause mutations that potentially lead to cancer (Ferguson, 2001; Ferguson et al., 2004). Radiation can induce a wide variety of stable chromosomal aberrations and reciprocal translocations (Edwards, 1997). Amplification and rearrangement of c-myc was found in a small percentage of radiation induced murine sarcomas. Mutations were also found in ras family proteins, p53 and MDM2 (Little, 1997; 2000).
1.4. ULTRAVIOLET RADIATION

UV radiation forms a part of the electromagnetic spectrum with wavelengths between 200 nm and 400 nm. It is divided into three categories dependent on wavelength, long wave UVA (320–400 nm), medium wave UVB (280–320 nm), and short wave UVC (200–280 nm) Epidemiological, clinical and laboratory studies have implicated solar ultraviolet (UV) radiation as a tumor initiator, tumor promoter and complete carcinogen, and excessive exposure of mammalian skin to UV radiation induces a number of biological responses, including development of erythema, oedema, sunburn cell formation, hyperplasia, immune suppression, DNA damage, photo aging and melanogenesis. These alterations are directly or indirectly involved in the development of keratinocyte-derived skin cancers and cutaneous malignant melanoma (Baliga & Katiyar, 2006).

1.4.1. Ultraviolet radiation induced damages in DNA

Exposure to ultraviolet and ionizing radiations result in damage to DNA though the mechanisms by which energy is deposited by the two types of radiations are fundamentally different. Ultraviolet light deposits energy in the molecule at centers where it can be easily absorbed (Jaggar, 1965). The absorption of UV light by the molecules alters the arrangement of electrons within their orbits leading to the formation of very reactive excited molecules without producing free radical or ion. Number of investigations suggest that UV radiations cause one or more photochemical alterations in the DNA: hydration of pyridines at 5,6 positions, formation of intra strand cross links, deamination of bases and formation of derivatives of pyrimidines (Smith & Hanawalt, 1967).

1.4.2. Mechanism of Ultraviolet radiation induced malignancy

Direct UVB absorption by DNA leads to dimers of nucleic acid bases including cyclobutane pyrimidine species and pyrimidine (6-4) pyrimidone compounds. These classes of dimers are implicated in the mutagenicity of UV radiation, which is typified by a high level of CC-->TT and C-->T transversions (Pattison and Davies, 2006). UV exposure to the skin results in generation of reactive oxygen species. The majority of UV-induced protein damage appears to be mediated by ¹O₂, which reacts preferentially with Trp, His, Tyr, Met, Cys and side chains of cystine. Excess of free radicals results in a a cascade of events mediating
progressive deterioration of cellular structure and function, and this can lead to a loss of cellular integrity by modification of DNA and abnormal expression of cellular genes. Mutations are frequently observed in the ras proto-oncogene and p53 tumor suppressor gene in human skin cancers of sun-exposed area and in UV-induced mouse skin cancers (Nishigori, 2006). UV-generated ROS affect mitogen-activated protein kinase (MAPK) signalling cascades. These events have been shown to activate NF-κB as well as c-Jun N-terminal and p38 MAP kinases followed by activation of transcription factor AP-1. Persistent oxidative stress in cancer may also cause activation of transcription factors and protooncogenes such as c-fos and c-jun as well as genetic instability (Nishigori, 2006).

1.5. CELLULAR RADIOSENSIVITY

Living organisms are open dynamic systems and the effect of radiation exposures occur at a wide temporal scale as the effects manifest from femtoseconds to microseconds to milliseconds for the physical and chemical changes, to minutes, hours and years in case of biological changes and several extrinsic and intrinsic factors contribute to the cellular radiosensitivity. Radiosensitivity is the relative susceptibility of cells, tissues, organs, organisms, or other substances to the injurious action of radiation. The factors which govern cellular radiosensitivity include:

i) physiological status of the cells,

ii) difference in the cellular DNA content,

iii) efficiency of the cellular DNA repair systems,

iv) conditions during irradiations as well as after irradiation such as temperature, pH and presence of various chemicals (Hall & Giaccia, 2006).

Cellular radiosensitivity is an important determinant in radiotherapy which is the most common modality of the treatment of human cancers. One of the major problems encountered in radiotherapy of cancers is the radiation damage to normal cells surrounding the tumor. One of the approaches to circumvent this problem is the use of hypoxic cell radiosensitizers to enhance the radiation damage to tumor cells and the use of radioprotectors to preferentially reduce the deleterious effects of radiation and thereby protect normal cells (Masunaga et al., 2006). In general, it has been found that cell radiosensitivity is directly
proportional to the rate of cell division and inversely proportional to the degree of cell differentiation. In short, this means that actively dividing cells or those not fully mature are most at risk from radiation (Lehnertin, 1975). The most radio-sensitive cells are those which:

- have a high division rate
- have a high metabolic rate
- are of a non-specialized type
- are well nourished

Tissues having highest radiosensitivity are lymphoid organs, bone marrow, blood, testes, ovaries and intestines (Rubin & Casarett, 1968).

Examples of various tissues and their relative radiosensitivities are listed below.

<table>
<thead>
<tr>
<th>High Radiosensitivity</th>
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Lymphoid organs, bone marrow, blood, testes, ovaries, intestines

<table>
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<tr>
<th>Fairly High Radiosensitivity</th>
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</thead>
</table>
Skin and other organs with epithelial cell lining (cornea, oral cavity, esophagus, rectum, bladder, vagina, uterine cervix, ureters)

<table>
<thead>
<tr>
<th>Moderate Radiosensitivity</th>
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</thead>
</table>
Optic lens, stomach, growing cartilage, fine vasculature, growing bone

<table>
<thead>
<tr>
<th>Fairly Low Radiosensitivity</th>
</tr>
</thead>
</table>
Mature cartilage or bones, salivary glands, respiratory organs, kidneys, liver, pancreas, thyroid, adrenal and pituitary glands

<table>
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<tr>
<th>Low Radiosensitivity</th>
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Muscle, brain, spinal cord
1.5.1. Natural Products as Potential Radiosensitizers

A large number of natural compounds have shown potential of cytotoxic effects in a variety of pathological situations including cancer either alone or together with radiation (Nobili et al., 2009). The compounds exhibiting radiosensitizing effects are believed to work at different levels of cellular phases and one of the suggested mechanisms of treating cells with the particular radiosensitizer before irradiation probably consists in synchronizing them in sensitive cell cycle phase (Biaglow, 1981). Moreover, presence of compounds during irradiation amplify their effects by multi-factorial mechanisms including toxic reactions of free radicals. Those compounds that are given after radiation show the effect by inhibiting repair of the radiation induced lethal and sub-lethal damage apart from down regulation of numerous pro-survival factors (Girdhani et al., 2005).

1.6. RADIOTHERAPY

Radiotherapy is the most common modality for treating human cancers. Eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purpose. To obtain optimum results, a judicious balance between the total dose of radiotherapy delivered and the threshold limit of the surrounding normal critical tissues is required. In order to obtain better tumor control with a higher dose, the normal tissues should be protected against radiation injury. Thus, the role of radioprotective compounds is very important in clinical radiotherapy (Nair et al., 2001). The aim of radiotherapy is to destroy cancer cells with as little damage as possible to normal cells. Radiation causes damage to the DNA of cells; the basic principle of radiotherapy is to cause enough damage to kill cancer cells. If the cells cannot repair their DNA, they cannot grow or reproduce (Toda et al., 2009). However, radiation causes damage to normal cells and, hence, can result in adverse side effects. The nature and degree of such unwanted side effects depends upon the dose of ionizing radiation and the sensitivity of the organs that are irradiated. With respect to the potential application of ionizing radiation in medical practices (e.g. radiotherapy and nuclear medicine) and also potential accidental exposure to radiation (e.g. industrial nuclear accident), the development of effective radiomodifiers is of great medical importance (Kiseleva et al., 1986).
1.6.1. Importance of radiotherapy

Radiotherapy is the most important nonsurgical modality for the curative treatment of cancer. In 2004 in the United States, nearly 1 million of the ~1.4 million people who developed cancer were treated with radiation. Of the 10.9 million people diagnosed with cancer worldwide each year (International Agency for Research on Cancer), around 50% require radiotherapy, 60% of whom are treated with curative intent. Radiotherapy is also highly cost effective, accounting for only 5% of the total cost of cancer care (Ringborg et al., 2003). Substantial gains in the therapeutic ratio, that is, the balance between cure and toxicity of treatment, have been made with the development of new technologies such as image-guided radiotherapy (Dawson & Sharpe, 2006; van Herk, 2007) and intensity modulated radiotherapy (Glatstein, 2002; Hong et al., 2005; Moran et al., 2005; Ten Haken & Lawrence, 2006). Other approaches for improving therapeutic ratios include altering radiotherapy fractionation, for example the Conventional or Hypo-fractionated High Dose Intensity Modulated Radiotherapy in Prostate Cancer (CHHiP) trial and the European organization for Research and Treatment of Cancer (EoRTC) trial of hyperfractionation in head and neck cancer (Dearmaley et al., 2007; Bourhis et al., 2006). The use of concurrent chemotherapy has become standard practice for a number of cancers, and the addition of new, molecularly targeted agents in combination with radiotherapy is promising to improve cure rates further (Bonner et al., 2006). With improved cure rates, survivorship issues become increasingly important. The long-term toxicity that is associated with cancer treatment negatively affects quality of life, so strategies aimed at toxicity reduction are important. Although there are still gains that can be achieved through technical advances, altered fractionation and new drug combinations, it is ultimately the radiosensitivity of the few that will limit our ability to further maximize patients’ toxicity free survival, so understanding the genetics of radiosensitivity is crucial.

1.6.2. Adverse Effects of Radiotherapy

The clinical manifestations of either acute (occurring during or within weeks of treatment) or late (occurring 6 months to many years later) radiation toxicity are well documented, although the mechanistic basis for the separation of early and late effects has changed considerably in recent years (Bentzen, 2006). This distinction provides a useful framework with which to describe radiotherapy...
toxicity in terms that might be useful for wider genomic studies. Acute effects occur during or shortly after completion of treatment and are usually reversible and not generally considered dose-limiting. They occur in rapidly proliferating tissues, such as skin, gastrointestinal tract and the haematopoietic system. Early reactions tend to be relatively insensitive to changes in the radiation dose per fraction but are sensitive to the time over which radiation is delivered. Protracted treatment reduces acute toxicity but can compromise tumour control. Late effects manifest 6 months to several years after radiotherapy. The long time frame prevents titration of radiation dose against toxicity in individual patients, and the relationship between acute and late effects remains unclear (Burnet et al., 1996; Bentzen & Overgaard., 1991; Bentzen et al., 1993). As late side effects can be permanent, they provide the basis for dose constraints to radiation toxicity. Late effects typically occur in more slowly proliferating tissues, such as kidney, heart and central nervous system. The pathogenesis includes fibrosis, atrophy and vascular damage. Other important late normal tissue side effects include hormone deficiencies, infertility and second malignancies. Late toxicity tends to be more sensitive to changes in the radiation dose per fraction than acute reacting tissues and less sensitive to the overall treatment time. Late effects can be irreversible and limit the dose in radical radiotherapy regimens; monitoring late toxicity is therefore crucial in assessing therapeutic benefit and follow-up must be sufficiently long.
1.6.3. Pathogenesis of normal tissue damage

Although the functional and structural tolerance of normal tissue to radiotherapy is contextual, studies of cell and tissue response to ionizing radiation have led to an improved understanding of the pathogenesis of radiation toxicity. Recent radiobiology research suggests that normal tissue injury is a dynamic and progressive process. The deposition of energy results in DNA damage and changes in the microenvironment through chemokines, inflammatory cytokines, fibrotic cytokines, altered cell–cell interactions, influx of inflammatory cells and the induction of reparative and restorative processes (Bentzen, 2006). Therefore, genes involved in DNA damage recognition as well as signalling, apoptosis, proliferation and inflammatory processes may have a role in the development of normal tissue damage.
1.6.4. Agents which affect the radiation response in cells

- **Radiosensitizers**

A number of chemicals, most of them sulphydryl binding agents have been shown to enhance radiation-induced lethality of microorganisms when present in non-toxic concentrations along with the bacterial cells during irradiation. (Nair, 1971; Adams, 1972).

- **Radiomodifiers**

Response of cells and organisms to ionizing radiation can be altered by a variety of physical and chemical agents, and these agents are broadly termed as radiomodifiers. The radiomodifiers can either reduce or enhance the deleterious effects of radiation. The utilization of various radiomodifiers (hyperbaric oxygenation, metronidazole and their combination, tourniquet and total body gas hypoxia, unconventional dose fractionation) in radiation therapy of over 2000 cancer patients with tumors of the main sites made it possible to raise therapeutic efficacy using a differentiated approach to a choice of therapeutic tactics with relation to radiobiological specificities. The agents which reduce the radiation damage are called ‘radioprotectors’ and those which enhance the radiation induced damage are called ‘radiosensitizers.’

1.6.4.1. Factors which affect the radiation response in cells

- **Hyperthermia**

One of the well known physical agents which modify the radiation response of cells and organisms is thermal shock or hyperthermia. It has been reported that prior exposure of mice to mild whole body hyperthermia (40 °C for 1 hour, 20-48 hour before total body irradiation offered radiation protection as evidenced from increased survival and biochemical parameters (Patil et al., 1996). The mechanism has been attributed to the effect of hyperthermia on haematopoietic tissues mediated through certain cytokines (Zaidi et al., 2001). Exposure of cells to elevated temperature (hyperthermia) has been shown to enhance radiation damage. Local hyperthermia has been used along with radiation in cancer therapy for better tumor control where hyperthermia sensitzes tumor cells. The mechanism has been thought to be due to changes in the blood flow, vascular...
permeability, metabolism and oxygenation with respect to tumor tissue (Vujaskovic & Song, 2004).

Oxygen effect

The best example of a tissue nonspecific radiosensitizer is Oxygen. It is one of the most important factors which affect the cell’s response to radiation stress. It has been known that the cells irradiated under nitrogen (anoxic) or in presence of very little oxygen (hypoxic) are less sensitive to ionizing radiation, than if irradiated in the presence of air or oxygen. This is known as ‘Oxygen effect” (Karam et al., 2001). Involvement of oxygen in cellular response to radiation hold great importance since presence of hypoxic region in the core of solid tumors is one of the most difficult problems faced by radiation oncologists.

DNA Repair

DNA repair mechanisms have been studied mainly in cells injured by radiations that cause loss of viability by damage to cellular DNA. The mode of DNA repair is determined by the nature of the lesions in DNA, and by the external environment. Type of DNA repair is specified by the nature of DNA lesions. The nature of radiation induced lesions in DNA depends on the type of radiation to which the cells are exposed (Hellday et al., 2007).

The repair processes, which have been studied in great detail initially, are the ones operating on pyrimidine dimers in DNA induced by UV radiation. Photoreactivation is one of the earliest known repair mechanisms in DNA are monomerized in presence of light of 330-450 nm wavelength by an enzyme photolyase (Pradhan et al., 1973).

1.6.5. Repair of ionizing radiation induced lesions

The chemical nature of various lesions in DNA produced by ionizing radiations are very complex (Gajewski et al., 1990). All of these DNA lesions could lead to cell death, mutagenecity or altered function, or may be repaired and cells may recover from radiation-induced effects (Orr, 1984; Friedberg, 1985). Endogenous radioprotective substances have been investigated with respect to their role in cellular recovery from radiation and chemical onslaughts. Some of the repair enzymes act on more than one type of lesion apparently recognizing general features of the damage, in addition to the specific lesions (Price, 1993). In general,
the repair of most of the damaged bases and single strand breaks follow the nucleotide excision repair pathway as in the case of UV lesions while the double strand breaks repair takes place by a recombinational pathway (Van Buul et al., 1999).

1.6.5. DNA repair deficiency and human disorders

Deficiency of DNA repair has been correlated with higher incidence of neoplasia in certain human genetic diseases such as Xeroderma pigmentosum[XP] Ataxia telangiectasia[AT] (Fanconi’s anemia [FA] etc) (Nelson et al., 2005).

1.6.7. Concept of radioprotectors and chemoprotectors as adjuvant in cancer therapy

Radiotherapy is one among three main strategies used in cancer therapy. Radiotherapy is a regional therapy and is mostly toxic to proliferating cells. Mammalian cells are more sensitive to radiation induced damage during the late G2 and M phase of the cell cycle (Tanaka et al., 2000). Chemotherapeutic regime uses either a single drug or a combination of different drugs and is frequently used in several types of advanced solid tumors and haematological malignancies.

Both radiotherapy as well as chemotherapy possesses several side effects of its own. The damage to normal cells along with the tumor cells is the major toxicity associated with both the types of therapies. In both radiotherapy and chemotherapy, protection of normal tissues is as important as the destruction of cancer cells. A judicious balance between total radiation delivered and threshold limit of the surrounding normal tissue is required for getting optimum results. Therefore the role of radioprotectors is very important in clinical radiotherapy. Several compounds have been shown to act as radioprotectors (Nair et al., 2001; Arora et al., 2005). In clinic [S-2-(3-aminopropyl-amino) ethyl phosphorothioic acid] which is commonly known as WR-2721 or amifostine is the only approved drug. It is highly expensive and associated with side effects of its own.

1.6.8. Radiation induced resistance

An initial exposure to a very small dose of ionizing radiation (conditioning dose) has been found to offer protection against subsequent larger radiation dose in several organisms. Termed as ‘radiation hormesis” (Calabrese, 2002), this
phenomenon of induced radiation resistance has attracted much attention in recent years owing to the implications in radiation therapy employing fractionated doses of radiation. The increased radiation resistance could be attributed to induced synthesis of DNA repair enzymes or modulation of the cell cycle.

1.7. RADIOPROTECTORS

Ionizing radiations produce deleterious effects in the living organisms and the rapid technological advancement has increased human exposure to ionizing radiations enormously. There is a need to protect human against such effects of ionizing radiation. Attempts to protect against the deleterious effects of ionizing radiations by pharmacological intervention were made as early as 1949 and efforts are continued to search radioprotectors, which may be of great help for human application (Jagetia, 2007). The research on development of radioprotectors commenced with the Manhattan project in the US and Walter Reed Army Research Institute synthesized and screened about 4500 compounds for this purpose. Among these, except one compound, ‘Amifostine” which finds applications in radiotherapy of cancer to protect normal tissues during radiation exposure, none was found suitable for human applications due to acute toxicities. This is the present scenario in spite of more than six decades of research on the development of radioprotectors or anti-radiation drugs and the spectacular advances made over the last few decades in the areas of cell and molecular biology, synthetic chemistry and biochemistry. The development of a safe and effective nontoxic radioprotector for human use has remained elusive till today. Several compounds, which have been found very effective in the laboratory studies, have failed in human applications due to toxicity problems or lack of significant protective effects (Koukourakis, 2000). The protection of healthy tissue during radiotherapy for cancer has been one of the strong motivations for continuing research on exogenous radioprotectors.

Since exposure to irradiation in radiotherapy, or accidental exposure to radiation, can produce significant unwanted side effects, it is important to ameliorate such effects by the use of radioprotective drugs (Maisin, 1998).

Radioprotective agents are synthetic compounds or natural products that are immediately administrated before irradiation to reduce injuries caused by ionizing radiation. Over the past 60 years, as a result of the great clinical need for effective
radioprotectant agents, many have been prepared and tested to find more effective, less toxic, drugs (Margulies et al., 2008). Initial attempts were focused on synthetic thiol compounds. These agents are highly effective at reducing lethality induced by irradiation. Of this class, amifostine is the only radioprotector that has been clinically approved by the Food and Drug Administration (FDA) for mitigating side effects (xerostomia) in patients undergoing radiotherapy (Brown et al., 1982; Cassatt et al., 2002). This drug offers good protection, but is relatively toxic (nausea, vomiting and hypotension being some of the most common adverse effects (Koukourakis, 2000). In view of this, the search continues for less toxic, more effective radioprotectors that can be easily self-administered. Immunomodulators and cytokines represent the bulk of agents in this category. Naturally occurring compounds that function as antioxidants and immunostimulants are another strategy for the development of radioprotective agents with low toxicity. Therapeutic agents that can be administered following irradiation are another strategy for reducing side effects induced by ionizing radiation; cytokines and immunomodulators, through induction of bone marrow recovery and extra haematological tissue regeneration can represent such a class of agent (Joshi et al., 2010).

A radioprotector is an agent that when applied before or during irradiation evokes a significant reduction in the radiation injury.

The ideal radioprotective agent should fulfil several criteria:

(a) It must provide significant protection against the effects of radiation.

(b) It must have a general protective effect on the majority of organs.

(c) It must have an acceptable route of administration (preferably oral, or alternatively intramuscular).

(d) It must have an acceptable toxicity profile and protective time-window effect.

(e) It must have an acceptable stability profile (both of bulk active product and formulated compound).

(f) Have compatibility with the wide range of other drugs that will be available to patients or personnel.
Radioprotecting agents can be broadly classified into three groups; i) Radioprotectors, ii) adaptogens and iii) absorbants (Nair et al., 2001). The first group i.e. the radioprotectors generally comprises of sulphydryl compounds and antioxidants. These include several myelo-, entero and cerebro protectors. Mainly they act by scavenging the multitude of free radicals generated by radiolysis and the direct reactions of with the chemical substances present in the cellular milieu. Consequently these compounds create a kind of shielding effect, thereby preventing the interaction of highly damaging free radicals with the important biomolecules such as DNA and membrane as much as possible. Adaptogens act as stimulators of radioresistance. These are natural protectors, which offer chemical protection under low levels of ionizing radiation. These are generally extracted from the cells of plants and animals and have least toxicity. They can influence the regulatory system of exposed organisms, mobilize the endogenous background of radioresistance, immunity and intensify the overall nonspecific resistance of an organism. Absorbants protect organisms from internal radiation and chemicals. These include drugs which prevent the incorporation of radioiodine by the thyroid gland and the absorption of radionuclides 137cs, 90Sr, 239 Pu, etc (Nair et al., 2001).

Unfortunately, to date, there is no radioprotector that fulfils all of these criteria. Although the initial development of radioprotective agents led to the discovery of effective, synthetic thiol compounds, as previously mentioned, the side effect profile of these agents necessitated the search for second-generation drugs that are more effective, less toxic and with more acceptable properties with respect to route and frequency of administration. Many researches appear to use the intraperitoneal (i.p.) route of administration in their studies, since it is technically easy and optimises exposure of the compound (the i.p. route having similar pharmacokinetics to i.v.). In reality, however, such drugs will never be administered i.p. to humans. The only likely acceptable routes for human dosage would be oral, subcutaneous (s.c.) and intramuscular (i.m.). In the mouse model, s.c. is often preferred because of the lack of i.m. injection sites, while in the non-human primate the i.m. route is more readily accessible. In recent years, an array of immunomodulatory agents, haemopoietic growth and stimulating factors, synthetic chelating agents and natural antioxidants have been examined for their ability to ameliorate radiation induced damage (Furuse et al., 1997)
1.7.1. Types of radioprotectors

1.7.1.1. Thiol and synthetic radioprotectors

The radioprotective property was first demonstrated in the case of sulfhydryl compounds. Thiols are molecules containing free or potential sulfhydryl (SH) groups in their structure; they received a great deal of attention, from 1950 to 1985, as radioprotectants of mammalian cells. They were the first generation of radioprotectors. Aminothiols, and their phosphothioate derivatives, have been investigated as tissue radioprotectors over the past four decades (Brown et al., 1992). Several mechanisms were proposed for this group, including free radical scavenging, hydrogen transfer, inducing hypoxia and stabilizing DNA through direct binding (Cassatt et al., 2002; Maisin, 1998; Held & Biaglow, 1994). Approximately, 4400 compounds had been developed and tested by 1973. One of the most effective drugs developed was WR-2721 or amifostine [s-2(3-aminopropylamino) ethyl phosphorothioic acid] which is a prodrug, in which the thio-ester bond is cleaved by membrane-bound alkaline phosphatase, yielding a free active thiol, the active metabolite WR-1065. Amifostine has been used in clinical trials and it protects normal tissue from the acute and long-term effects of radiation and chemotherapy. It has been approved by FDA as a radioprotector and chemoprotector (Cassatt et al., 2002). This drug is more effective in reducing radiation-induced cellular injury of normal tissues than in tumour cells. Alkaline phosphatase is needed to convert WR-2721 to its active metabolite which produces higher concentrations in normal cells (Weiss et al., 1990; Holland, 2001; Mazur, 2000). WR-2721 has been reported to reduce the effect of a radiation dose by a factor of up to 2.7 in mice taking this drug intraperitoneally 30 min before exposure to gamma irradiation. This is the highest dose reduction factor (DRF) seen in a mouse 30-day lethality model (Brown et al., 1992).

Radioprotectant combination therapy has been tested in an attempt to increase protection against radiation damage and/or to decrease the toxicity through the use of mixtures of chemical and biological protectants. The combination most frequently studied was immunomodulators and thiol compounds (Maisin et al., 1993; Weiss et al., 1990). An additive effect was obtained upon combination of 16, 16- dimethyl PGE2 and WR-2721. When PGE2 was combined with a dose of WR-2721 (200 mg/kg), the protection increased synergistically to a maximal DRF of 2.5 (Maisin et al., 1993).
Despite amifostine’s current clinical applications, it has not been approved for use in any clinical nuclear/radiological exposure setting. The disadvantages of amifostine are as follows: toxicity; limited routes of administration; narrow time windows; cost and limited protection of the central nervous system (Tannehill & Mehta, 1996).

1.7.1.2. Nitroxides

A series of free, stable nitroxides have been prepared and tested as radioprotectors (Hahn et al., 1999). The main mechanisms are thought to be a free radical scavenging, superoxide dismutase-like activity. Tempol [4- hydroxy-2, 3, 6, 6-tetramethyl piperidine-1-oxyl] is the lead compound in this group (Hahn et al., 1998). Nitroxide had a differential protection for normal tissue (bone marrow) compared to tumour tissue. Tempol-H provided protection against the lethality of whole-body radiation in mice with a dose modification factor of 1.3, which was similar to tempol (Hahn et al., 2000). Administration of tempol, before irradiation, significantly reduced radiation-induced salivary hypofunction in mice (Vitolo et al., 2004; Cortrim, 2005). Tempol had a significant effect on producing hypotension and increasing heart rate at the doses required to produce radioprotection. It also has a short time-window of effect (Hahn et al., 2000). These negative attributes of nitroxides clearly limit their usefulness for clinical applications.

1.7.1.3. Bis-benzimidazol

The bis-benzimidazol family has two benzimidazole groups and one phenol group, conferring minor DNA groove-binding properties. These compounds fluoresce strongly upon binding to dsDNA and have been marketed by Hoechst as reagents for the in vitro estimation of DNA concentration and for histological applications. Hoechst 33342 is the most well-known compound from this family; it binds strongly and selectively to double-stranded DNA but not to double-stranded RNA (Martin et al., 2004). The mechanism of radioprotective activity of Hoechst 33342 is to donate an electron from the ligand to damaged DNA (Martin et al., 2004; Celaries et al., 2003). Intravenous administration of Hoechst 33342 (70 mg/kg) 30 min before irradiation results in a significant radioprotective effect with DMF of 1.2 in a mouse lung model (Martin, 1996).
1.7.1.4. Superoxide dismutase and metal complexes

Superoxide dismutase (SOD) enzymes are naturally occurring intracellular enzymes which scavenge O$_2^-$ by catalyzing its conversion to hydrogen peroxide and oxygen. It has become clear that these enzymes provide an essential defense against the superoxide radical. The copper-, zinc- and manganese-containing SODs (Cu, Zn, Mn and SOD) are the most common type of SOD (Epperly et al., 2000; Vujaskovic et al., 2002). A pharmaceutical version of a copper–zinc-containing SOD has been marketed under the name of Orgotein, which has been used for ameliorating radiation side effects in patients (Guo et al., 2003; Epperly et al., 2001 a, b). Intraoral administration of a manganese superoxide dismutase-plasmid/ liposome (Mn SOD-PL) 24 hours before a single-fraction 30 Gy irradiation prevented oral cavity mucositis in mice (Guo et al., 2003). More studies are needed to evaluate the efficacy and toxicity of SOD-PL in animal by systemic administration. In addition, more studies will be required to find SOD related therapies with a greater half-life of action. The limitations for these agents are their short half-life, large molecular weight and potential immunogenicity (Vujaskovic, 2002; Somack et al., 1991). To overcome these limitations, there has been a considerable interest in developing synthetic SOD mimics that have long half-life, low molecular weight, reduced toxicity and cost-effectiveness.

A group of synthetic SOD mimetic compounds has been developed to be used for ameliorating radiation-induced tissue injury. These agents have a metal ion (Cu, Fe, Mn and Zn) at their active centres to form chelates, which behave like the metal centre of native SOD. Daily administration of the SOD mimic, AEOL 10113 [manganese( III) mesotetramis (N-ethyl pyridinium-2-yl)] porphyrin for five days at a dose of 6 mg/kg, which began 15 min before irradiation, increased the tolerance of lung to ionizing radiation and it significantly reduced the severity of radiation -induced lung injury (Vujaskovic., 2002 ). Because of the short half-life of protection of the aminothiols and native biological compounds, these complexes have the advantage of an extended time-window of radioprotection (24 hours). It is clear that further research is needed in order to assess the toxicity and pharmacokinetics of these agents in animals.
1.7.1.5. Cytokines

Ionizing radiation affects haematopoietic tissues and reduces the neutrophil and platelet numbers (Kalechman et al., 1995). Reduction in these circulating blood cells can result in septicaemia, haemorrhage, anaemia and death. One of the strategies for novel radioprotective agents is the stimulation, maintenance and proliferation of progenitor cells from bone marrow (Weiss & Simic, 1998). Cytokines can stimulate haematopoietic stem cells. Combination treatment with stem cell factor (SCF) and thrombopoietin (TPO) synergistically protected CD34+ CFU megakaryocytes against X-ray-induced death (Kashiwakura et al., 2003). Haematopoietic recovery depends on the percentage of residual haematopoietic stem cells. The higher the radiation dose is, the weaker the efficacy of haematopoietic growth factors is. Thus, the use of haematopoietic growth factors should be restricted to the range of intermediate radiation doses (Herodin & Drouet, 2005). Granulocytes, lymphocytes and platelets are the most important cells to be reconstituted, because these cells have a short half life in circulating blood and are reduced rapidly after exposure to irradiation. As a result of this, there will be a rapid onset of infection and thrombocytopenia following exposure to radiation (Herodin & Drouet, 2005). Treatment of human peripheral mononuclear cells with IL-3 and SCF prevents apoptosis induced by gamma irradiation in vitro (Kim et al., 2005). Administration of a single dose of FLT-3 ligand (SCF) resulted in a significant survival rate (65%) in irradiated mice (Hudak et al., 1998). The effectiveness of cytokines as regeneration agents is increased when combined with other cytokines. Combination protocols with different cytokines have been shown to enhance neutrophil and platelet recovery after irradiation and enhance the survival rate in irradiated animals (Drouet et al., 2004; Whitnall et al., 2002). Unfortunately, some cytokines have disadvantages that limit their use in clinical practice. Also, some of them have adverse side effects, such as proinflammatory activity or immunogenicity (Neta, 1998). These agents have been proved to be infective when administrated systemically.

1.7.1.6. Immunomodulators

Some agents are able to induce haematopoietic cytokines; they are referred to as immunomodulators (Pujol et al., 1993). Immunomodulators are noncytokine drugs that have been proposed as an alternative to stimulate haematopoietic stem cells (Landauer, 1997). The release of cytokines through the effect of
immunomodulators can stimulate growth, differentiation and proliferation of haematopoietic progenitor and stem cells. In this way, this agent may protect and repair through enhanced production of bone marrow cells, circulating granulocytes, lymphocytes and platelets (Maisin, 1998). Beta Glucans are water-soluble polysaccharides that are reported to have immunopharmacological activity; they seem to act particularly as biological response modifiers, regulating host immune response. Good protection with oxymetholone, as an anabolic-androgenic steroid, was observed when it was administrated orally 24 hours before 8 Gy gamma irradiation in mice. The survival rate, 30 days after irradiation, in the group treated with 640 mg/kg of oxymetholone was 75%, with a DRF of 1.14, versus 15% in the control group. Oral administration of oxymetholone ameliorated the radiation-induced decrease in circulating platelets and erythrocytes, but had less of an effect on the number of white blood cells (WBC). This drug has advantages, such as: it can be administered orally; it has an extended window of effect and is less toxic (Hosseinimehr et al., 2006). Further studies are needed to establish the exact mode of action of oxymetholone. Recently Whitnall et al. developed 5-androstenediol (AED) as a new radioprotector (Stickney et al., 2007; Safeukui et al., 2004). AED is a natural hormone (a dehydroepiandrosterone derivative) produced in the reticularis of the adrenal cortex. It stimulates cytokines, such as IL-1, IL-3, and IL-6 facilitates recovery from radiation-induced haematopoietic injury.

1.7.1.7. Natural antioxidants

Natural compounds in the diet provide functional antioxidants, such as vitamins, minerals and enzymes. Reduction of oxidation damage by such natural antioxidants provides a degree of protection against ionizing radiation injury (Kumar et al., 1996; Hsu et al., 1999; Uma Devi & Ganasoundari, 1999; Umadevi et al., 1999; Chaudhary et al., 1999; Shinoda, 1995; Kamat et al., 2000; Zhang et al., 1997). Although thiol synthetic compounds such as WR-2721 showed good radioprotection, their toxicity at optimum protective doses promoted the search for alternatives to synthetic compounds that would be less toxic and highly effective. In general, natural radioprotectors have a lower degree of protection compared to synthetic thiol agents. Generally, DRFs lower than 1.3 for 30-day survival is reported (Weiss & Landauer, 2000). Vitamin E (alpha tocopherol) and related analogues are nutraceuticals that can scavenge singlet oxygen and superoxide- anion radicals. Vitamin E, administrated at a dose of 400 IU/ kg s.c.
before irradiation in mice, showed an increase in survival rate of up to 79% versus 4% in the vehicle-treated control group (Kumar et al., 2002). Vitamin E significantly enhanced 30-day survival of treated mice at a dose of 400 IU/kg with a DRF of 1.23 (Seed et al., 2002). Oral administration of vitamin E did not increase the survival rate in mice treated with gamma irradiation (Kumar et al., 2002). A water-soluble derivative of vitamin E called tocopherol monoglucoside (TMG) showed radioprotective activity; the LD50 for 24 and 72 hours at 30-day survival were found to be 1120 and 1000 mg/kg, respectively (Satayamitra et al., 2001). Although reduction of oxidative damage by vitamin E and related analogues is generally thought to be the mechanism of these compounds, other mechanisms may play a significant role in their overall biological effect. Improved survival was related to enhanced regeneration of haematopoietic stem cells and accelerated neutrophil and platelet recovery (Davis et al., 2007).

1.7.2. Mechanisms of action of radioprotective agents

The radioprotectors can elicit their action by various mechanisms, such as i), by suppressing the formation of reactive species, ii), detoxification of radiation induced species, iii), target stabilization and iv), enhancing the repair and recovery processes. Ionizing radiation interacting with water in cells can produce reactive free radicals, such as hydroxyl radicals, hydrogen radicals and the toxic substance, hydrogen peroxide, all of which can damage critical macromolecules (Halliwell & Gutteridge, 1997, Yuhas, 1982, Kollmann et al., 1973). The elimination of the free radical species from the cell environment can inhibit the side effects induced by irradiation. The presence of sulphhydryl groups or other molecules capable of scavenging the radiolysis radicals arising from irradiation of water molecules can confer protection to radiation. Due to very short life of the radicals, such protective agents need to be present in the cell environment before the production of free radicals in order to neutralize their destructive properties.

Increasing the partial pressure of oxygen in the cell environment sensitizes tissues to radiation. Radioprotective agents induce hypoxia and consumption of oxygen in the cells to decrease the levels of reactive oxygen species (ROS) and hydrogen peroxide. The aminothiols can decrease oxygen levels in the cells. Thiol radioprotectors consume oxygen by forming by products, such as disulfide and hydrogen peroxide (Held & Biaglow, 1994).
The mode of action (MOA) of another class of radioprotectant agents involves the stimulation, proliferation and modification of the function of haematopoietic and immunopoietic stem cells. This class of agents is commonly referred to as immunomodulators. These agents make the stimulated cells release a variety of cytokines that act on pluripotent bone marrow stem cell to stimulate their production and differentiation. These agents mitigate radiation-induced haematopoietic injury and reduce mortality (Whitnall, 2000). Cytokines activate cellular signalling transduction pathways by binding to high affinity membrane receptors. Activation of cytokine cascades results in the release of intracellular protein messenger. Cytokine activation affects many cell functions, including growth, proliferation, differentiation, death caused by apoptosis and growth inhibition. The consequence of these effects depends on the type of released cytokines. Primarily, cytokines affecting the proliferation and differentiation of haematopoietic cells include GM-CSF, CSF, EPO, G-CSF, TPO and interleukins (Drouet et al., 2004; Herodin et al., 2003; Whitnall et al., 2002).

### 1.7.3. The role of Antioxidants as radioprotectors

Antioxidants are substances, when present in small quantities prevent the oxidation of cellular organelles by minimizing the damaging effects of ROS and RNS or oxidative stress. Under normal healthy conditions, a balance is maintained between oxidative stress and antioxidant requirements. The endogenous antioxidant defense comes mainly from three different types of systems, viz., antioxidant enzymes e.g. catalase, superoxide dismutase (SOD), metal sequestering proteins e.g. ferritin and low molecular weight molecules like vitamin C, vitamin E etc. However under pathological conditions or during radiation injury, stress, and pollution etc. the balance is lost and excessive supplementation of antioxidants is necessary. It has been found that fruits and vegetables, rich in antioxidants, decrease the risk of oxidative stress. Exposure of ionizing radiation to cells also causes similar effect as oxidative stress. Interaction of ionizing radiation is non-selective and in cells, water being the major constituent undergoes radiolysis producing hydroxyl radicals, which can react with cellular organelles, similar to those produced by oxidative stress. Due to this similarity between oxidative stress and radiation injury, an antioxidant can also act as a radioprotector in principle.
Antioxidants, depending on the chemical structures, have diverse mechanism of action. Preventive antioxidants, like deferrioximine or desferal, are compounds which form chelates with metals, thereby help in preventing the free radical production. The second type of antioxidants known as, chain-breaking antioxidants are the most important class of antioxidants, which can scavenge chain propagating free radicals like peroxyl radicals and converting the reactive free radicals to inactive products, e.g. vitamin-E or α-tocopherol, curcumin. The approach to the development of antioxidants has in general been based on macroscopic biochemical changes by both in vitro and in vivo studies and from such studies several phytochemicals have been reported as potent antioxidants. However, in order to manipulate the phytochemicals for therapeutic gains, it is necessary to understand the antioxidant action on molecular level. Therefore it is important to understand the molecular mechanisms responsible for the antioxidant action of phytochemicals. In vitro biochemical experiments on inhibition of free radical induced damage to cellular organelles like inhibition of lipid peroxidation in membrane lipids, inhibition of strand breaks in DNA and enzyme inhibitory studies have been performed to estimate in vitro antioxidant status of the compound.

The plant extracts and formulations with good antioxidant efficacy can be promising radioprotectors of the future. Development of such class of radioprotectors is beneficial, as they are potential alternatives to expensive and toxic drugs. Also, they are commercially viable and marketed as nutritional supplements (Weiss & Landauer, 2003).

Several natural compounds are now known as radioprotectors which include antioxidants like vitamin A, E and C, cytoprotective agents like MESNA, lipopolysaccharides and prostaglandins (Nair et al., 2001). The plants which reported to have radioprotective activity include Aegle marmelos, Allium sativum, Glycyrrhiza glabra, Mentha arvensis, Syzygium cumini, Centella asiatica, Mentha piperita, Zingiber officinale, Ocimum sanctum, Terminalia chebula, Podophyllum hexandrum, Emblica officinalis etc (Arora et al., 2005; Maurya et al., 2006). Some of the herbal formulations like Brahma rasayana, Chyvanaprasha and Triphala also possess radioprotective potential (Praveen et al., 1996; Jeena & Kuttan, 1998; Rekha et al., 2000).
1.8. HERBAL RADIOPROTECTORS

Plant products have various pharmacological properties and have been used for the treatment of various diseases long ago. Therefore, screening herbal drugs offers a major focus for new drug discovery. In this way, attention over the past 15 years has shifted towards the evaluation of plant products as radioprotectors, due to their efficacy and low toxicity. The proposed radioprotective efficacy of plant extracts is as a result of their containing a large number of active constituents, such as antioxidants, immunostimulants and compounds with antimicrobial activity. Most efficacy studies on plants have been on total extracts for their ability to protect against radiation-induced chromosomal aberrations and micronuclei formation; they were assessed by genotoxic tests, such as micronucleus and metaphase analysis. The radioprotective effect seems to be largely due to the high levels of phenolic and flavonoid compounds with strong antioxidant activities (Hosseinimehr et al., 2003; Hosseinimehr et al., 2007). A number of plants have been found to be effective in providing protection against radiation-induced lethality in mice. Several studies have been done on the radioprotecting activity of the plant Ocimum sanctum. The aqueous extract of the leaves of Indain holy basil, Ocimum sanctum, was found to protect bone marrow against clastogenesis (Ganasoundari et al, 1997) and stem cell lethality in the mouse. The two flavonoids, orientin (Ot) and vicenin (Vc) obtained from the leaves of Ocimum sanctum have been found to protect mouse bone marrow chromosomes against radiation induced challenges (Uma Devi et al, 1998). Another important plant Podophyllum hexandrum has been reported to protect against radiation induced mortality, gastrointestinal damage and embryonic nervous system of developing mice (Goel et al, 2002; Salin et al, 2001). Oral administration of an aqueous extract of guduchi, Tinospora cordifolia has been reported to increase the survival of mice exposed to radiation (Paliadiya & Sharma, 2003). Treatment of mice with hydroalcoholic extract of T.cordifolia, has been found to protect the radiation induced micronuclei formation, oxidative stress and decline in the mouse survival (Goel et al, 2004). Oral administration of Hippophae rhamnoides fruit juice concentrate to rats before or after irradiation increased life span, restored the 11-oxytocicosteroid level in the blood and weight of isolated adrenals, and also normalized their basal activity and response to ACTH under in vitro conditions (Goel et al, 2002). Several botanicals such as Gingko biloba, Panax ginseng, Amaranthus paniculatus, Emblica officinalis, Phyllanthus amarus, Piper longum, Mentha
piperita, Syzygium cumini, Zingiber officinale, Aegle marmelos etc were found to protect against radiation induced lethality, lipid peroxidation and DNA damage (Jagetia, 2007).

The doses of herbal preparations that were effective in radioprotection was significantly lower than the toxic dose and this is one of the major advantages of these preparations, compared to synthetic compounds. There are disadvantages to using plants as radioprotective agents, such as: low to mild efficacy (with a DRF <1.3) and a short protective time-window (in most cases 30 min to 2 hours before irradiation). Further studies are necessary to identify the bioactive compounds responsible for radioprotective efficacy and to extend time-window (e.g. 24 hours prior irradiation). Although there have been many plants evaluated for their ability to reduce radiation-induced sickness in animals, there is insufficient evidence at present to support their potential use in patients during radiotherapy.

Historically plants have provided a source of inspiration for novel drug compounds and have shown great promise in the treatment of diseases. The great variety of secondary metabolites from plants has been sources of commercially important pharmaceutical compounds. Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Or say, traditional medicine is the synthesis of therapeutic experience of generations of practising physicians of indigenous systems of medicine. The traditional preparations comprise medicinal plants, minerals, organic matter, etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita. The herbal medicines/traditional medicaments have, therefore, been derived from rich traditions of ancient civilizations and scientific heritage. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis,
immune disorders, etc. for which no modern medicine is available. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a decimal share of the world market due to export of crude extracts and drugs.

1.8.1. Herbal medicine standardization

In indigenous/traditional systems of medicine, the drugs are primarily dispensed as water decoction or ethanolic extract. Fresh plant parts, juice or crude powder are a rarity rather than a rule. Thus medicinal plant parts should be authentic and free from harmful materials like pesticides, heavy metals, microbial or radioactive contamination, etc. The medicinal plant is subjected to a single solvent extraction once or repeatedly, or water decoction or as described in ancient texts. The extract should then be checked for indicated biological activity in an experimental animal model(s). The bioactive extract should be standardized on the basis of active principle or major compound(s) along with fingerprints. The next important step is stabilization of the bioactive extract with a minimum shelf-life of over a year. The stabilized bioactive extract should undergo regulatory or limited safety studies in animals. Determination of the probable mode of action will explain the therapeutic profile.

1.8.2. Prospective aspects of herbal radioprotectors

Plants are naturally gifted with the ability to withstand the harmful radiations from the sun. Therefore, it can be said that they are equipped with several defensive machineries to protect themselves from the radiation stimulated injuries and oxidative stress. The use of phytochemicals in radioprotection has received much attention in the last decade owing to certain discoveries with special properties as antioxidants. Generally, they are popular because the phytochemicals are lower in toxicity in human beings, easy availability, inexpensive and good radioprotection exhibited in preclinical studies. The radioprotective activity of phytochemicals may be mediated through several mechanisms such as free radical scavenging, improvement in the antioxidant status and anti-lipid peroxidation potential, conferred due to the presence of variety of phenolic hydroxyl groups attached to the ring structure.
1.8. ROLE OF PLANTS AND PHYTOCHEMICALS IN CANCER THERAPY AND PREVENTION

Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to bring cancer under control. The plants have been selected as an excellent source for new phytochemicals. Plants and plants based medicines have been used since the dawn of the civilization to maintain health and to treat a variety of diseases. Even though we enter the new century with its exciting prospect of gene therapy, herbal medicines remains one of the common forms of therapy available to much of the world population. The field of chemoprevention is an active area of research and several molecules are identified as cancer chemopreventive agents. They include curcumin, resveratrol, sulfur compounds, epigallocatechin gallate, silibinin, gingerol, capsaicin etc (Surh, 2003; Kundu & Surh, 2005; Aggarwal et al., 2006).

1.9.1. Mechanism of action of plants and herbs

Ionizing radiations induce reactive oxygen species in the form of ‘OH, ·H, singlet oxygen and peroxyl radicals that follows a cascade of events leading to DNA damage such as single-or double-strand breaks (DSB), base damage, and DNA-DNA or DNA-protein cross-links, and these lesions cluster as complex damaged sites. The DNA-DSBs are considered the most lethal events following ionizing radiation and has been found to be the main target of cell killing by radiation.

The putative mechanism of radioprotection by plant and herbal radioprotectors are shown in figure 1.3.

The radioprotective activity of plants and herbs may be mediated through several mechanisms, since they are complex mixtures of many chemicals. The majority of plants and herbs contain poly phenols, scavenging of radiation-induced free radicals and elevation of cellular antioxidants by plants and herbs in irradiated systems could be the leading mechanism of radioprotection. The poly phenols present in the plants and herbs may up regulate mRNAs of antioxidant enzymes such as catalase, glutathione transferase, glutathione peroxidase, super oxide dismutase and this may counteract the oxidative stress induced by ionizing radiation (Jagetia, 2007).
1.9.2. Assessment of radioprotective potential of plants and herbs

The most pragmatic approach to select the possible candidate to evaluate radioprotective effect is to look into the available properties of the substance. Whether a substance has anti-inflammatory, antioxidant, anti-microbial, immunomodulatory, free radical scavenging or anti-stress properties, if so, it may act as a potential radioprotector and could be the right candidate for evaluation of its radioprotective activity.

Short term in vitro tests can provide a basis for detailed evaluation of radioprotective activity. The simplest tests could be the evaluation of lipid peroxidation in vitro. Assay of free radicals and antioxidant status of a pharmacological agent can also provide some leads regarding the radioprotective potential of such agents. If a plant or natural product is found to inhibit lipid peroxidation and scavenge free radicals, it may act as a possible radioprotector.
The next step is to evaluate its radioprotective potential in vitro using cell survival and micronucleus assays. Micronuclei originate from chromosome fragments or whole chromosomes that are not included in the main daughter nuclei during nuclear division. Thus, MN provide a measure of both chromosome breakage and chromosome loss and it has been shown to be at least as sensitive an indicator of chromosome damage as classical metaphase chromosome analysis (Fenech & Morley, 1986; Miller et al., 1997; Evans, 1997). The key advantage of the MN assay is the relative ease of scoring and the statistical power obtained from scoring larger numbers of cells than are typically used for metaphase analysis. If it is found to elevate cell survival and reduce radiation-induced micronuclei formation, it certainly has a potential as a radioprotector.

There are other short term tests like DNA strand breaks, apoptosis and estimation of glutathione(GSH) and enzymes like catalase, glutathione peroxidise etc. that can also provide an inkling of the radioprotective activity of any pharmacological agent. The comet assay has been utilized as a sensitive, rapid, and simple technique for the evaluation of DNA damage and repair since in this assay, a small number of cells are required to evaluate the DNA damage and it is possible to measure the DNA damage in individual cells within the cell population in terms of various comet parameters such as % DNA in tail, tail length, tail moment, olive tail moment. However the gold standard for radioprotective activity is the evaluation of 30-day survival in rodents, since the animal studies with death as the endpoint are the most confirmatory, because the 30-day survival after lethal whole body irradiation clearly indicate the capacity of the pharmacological agent in test to modulate the recovery and regeneration of the gastrointestinal epithelium and the haematopoietic progenitor cells in the bone marrow, the two most radiosensitive organs that are essential for sustenance of the life (Jagetia & Baliga, 2003).

With sufficient loss of haematopoietic stem cells, death follows sue to infection, haemorrhage and anaemia. The GI syndrome in mice can be assessed by determining survival up to 10 days (measure of GI death) after exposure to comparatively high doses of whole body radiation, where as haematopoietic syndrome can be assessed by monitoring the survival of irradiated animals up to 30 days posy-irradiation (Brown et al., 1988; Jagetia et al., 2002).
1.8.3. Challenges and Future Directions

Treatment of various solid tumors such as renal cell carcinoma (RCC), Prostate cancer, Head and Neck cancers and Breast cancers is a challenge. Approximately, one third of RCC presented clinically are in metastatic stage at the time of diagnosis and is usually followed by poor prognosis with only 0% to 18% of stage IV patients surviving the 5-year period (Mejean et al., 2003). Prostate cancer, which is of second largest incidence, amongst the male populations, is only modestly responsive or non-responsive to radiotherapy or chemotherapy. Postoperative radiotherapy can be effective in achieving local control in these tumors. The limitation of this approach in prolonging survival appears to be caused by the intrinsic radioresistance of these tumor cells. The success of radiotherapy, therefore, depends on increasing the sensitivity of the malignant cells to radiation induced cell kill coupled with a reduction in metastasis phenotypes of these cells. Various dietary modulators and phytochemicals can work as excellent adjuvants to radiation therapy in a variety of cancers. These phytochemicals work at increasing oxidative damage or by synchronizing the cells to a radiosensitive phase of cell cycle thus causing enhanced killing. The future perspectives lie in identifying more such compounds and elucidating the mechanism through which they act for developing effective protocol for cancer radiotherapy.

1.9. SOME HERBAL RADIOPROTECTORS- AN ALTERNATIVE AND SAFE APPROACH TOWARDS DEVELOPING RADIOPROTECTORS

Recently, many of the investigators have focused the radioprotective research towards the phytochemicals and plant extracts. A review by Arora et al on the present status of herbal radioprotectors and future prospective emphasize the potential in the area of natural product based radioprotector drug discovery (Arora et al., 2002). Plants have been utilized since time immemorial for curing diseases. Even today, nearly 70 % of the world’s healthcare is dependent on plants. A number of medicinal plants evaluated for their radioprotective efficacy have shown protective effects against the damaging effects of ionizing radiation (Jagetia & Baliga 2002; Uma Devi et al., 2000). Plant extracts eliciting radioprotective efficacy contain a plethora of compounds including antioxidants, immunostimulants, cell proliferation stimulators, anti-inflammatory and
antimicrobial agent etc. Most studies using plant products have focused on evaluation of radioprotective efficacy of whole extracts or poly herbal formulations, and in some cases, fractionated extracts and isolated constituents (Arora & Goel, 2000).

The medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing disease has been documented in the history of all civilizations with the onset of research, it was concluded that plants contain active principles, which are responsible for curative action of the herbs. Ayurvedic drugs are used in crude forms like expressed juice, powder or infusion. In Ayurveda, the traditional Indian system of medicine, several plants have been used to treat free radical mediated ailments and therefore it is logical to expect and such plants may also render some protection against radiation damage. A systematic screening approach leads to identifying potential new candidate drugs from plant source for mitigation of radiation injury (Arora et al., 2005).

Here our studies are undertaken on the following plants.


1.10.1. **CENTELLA ASIATICA LINN**

*Centella asiatica* Linn (syn. *Hydrocotyle asiatica*), a plant of the family Umbelliferae, is a weakly scented species occurring in parts of India, Sri Lanka, China, Indonesia, Malaysia, Australia and Southern and Central Africa. It has been used in traditional medicine in India for the treatment of leprosy, varicose veins, ulcers, lupus and certain eczemas, and of mental retardation since prehistoric times (Kartnig, 1988). Infusions or poultices of *C. asiatica* have been used in Europe since the eighteenth century for the treatment of lesions of leprosy (Kartnig, 1988). Clinical trials have shown that extracts of *C. asiatica* heal wounds, burns and ulcerous abnormalities of the skin, cure stomach and duodenal ulcers, and are effective in the treatment of leprosy, lupus, scleroderma and diseases of the veins (Kartnig, 1988). Many commercial drug preparations of *C. asiatica* are available in West Germany and France (Kartnig, 1988). Along with the use of *C. asiatica* in medicine, the plant is also finding acceptance as a vegetable. Several groups are in the process of domesticating and developing cultivars of this species.
Centella asiatica, a plant mentioned in Indian literature has been described to possess CNS effects such as stimulatory-nervine tonic, rejuvenant, sedative, tranquilizer and intelligence promoting property. In the Indian system of medicine Ayurveda, Centella asiatica (Umbelliferae) syn Hydrocotyl asiatica has been used in various parts of India for different ailments like headache, body ache, insanity, asthma, leprosy, ulcers, eczemas and wound healing (Shukla et al., 1999). In course of pharmacological studies, the plant showed CNS depressant activity antitumor (Qian et al., 1982; Babu et al., 1995), and an inhibitory effect on the biosynthetic activity of fibroblast cells (Veechai et al., 1984). C. asiatica mentioned as ‘Medhya Rasayana’ in Ayurvedic texts of the Indian system of medicine has been described to counteract the effect of mental stress by tranquilizing the users and improving their memory span and intelligence (Chopra et al., 1956).

Figure 1.4. Centella asiatica Linn

The extract of C. asiatica significantly increased the wound breaking strength in incision wound model compared to controls ($P < .001$). The extract-treated wounds were found to epithelize faster, and the rate of wound contraction was significantly increased as compared to control wounds (Shetty et al., 2006). Generation of reactive oxygen species and mitochondrial dysfunction has been implicated in adriamycin induced cardiotoxicity. Mitochondrial dysfunction is characterized by the accumulation of oxidized lipids, proteins and DNA, leading to disorganization of mitochondrial structure and systolic failure. Pre-co-treatment
with aqueous extract of *Centella asiatica* (200 mg/kg body wt, oral) effectively counteracted the alterations in mitochondrial enzymes and mitochondrial defense system. In addition, transmission electron microscopy study confirms the restoration of cellular normalcy and accredits the cytoprotective role of *Centella asiatica* against adriamycin induced myocardial injury (Gnanapragasam *et al.*, 2006). It was also shown that treatment during postnatal developmental stage with *C. asiatica* extract can influence the neuronal morphology and promote the higher brain function of juvenile and young adult mice (Rao *et al.*, 2005). Pretreatment with *Centella asiatica*, 1 h prior to irradiation at a dose rate of 100 mg/kg body weight was found to be effective against radiation induced damage in the liver. The number of normal hepatocytes was higher in the *Centella asiatica* pretreated group in comparison with the irradiated only group (Sharma & Sharma, 2005). Axonal regeneration is important for functional recovery following nerve damage. The studies done by Soumyanath *et al* indicated that components in *Centella* ethanolic extract may be useful for accelerating repair of damaged neurons (Soumyanath *et al.*, 2005). Free radicals have been hypothesized to play an important role in ageing process. There exists an imbalance between free radical production and antioxidant defense mechanism, which may lead to cell death during ageing. Supplementation of *C. asiatica* (300 mg/kg body weight/day p.o) was effective in reducing brain regional LPO and PCO levels and in increasing the antioxidant status. Thus, *C. asiatica* by acting as a potent antioxidant exerted significant neuroprotective effect and proved efficacious in protecting rat brain against age related oxidative damage (Subathra *et al.*, 2005). Asiatic acid, madecassic acid, asiaticoside and madecassoside are the principle terpenoids with an ursane skeleton found in *Centella asiatica* (L) Urb. ((Bonfill *et al.*, 2006).

## 1.10.2. ASIATICOSIDE

One of the major active triterpenoid glycoside present in the plant *Centella asiatica* is asiaticoside. The molecular weight of asiaticoside is 960 g. The schematic representation of asiaticoside is shown in figure 1.5.

Shukla, *et al.*, (1999a) reported the antioxidant effect of asiaticoside. They reported that topical application of 0.2% asiaticoside solution twice daily for 7 days to skin wounds shows an increased in both enzymatic and non-enzymatic antioxidant activity namely superoxide dismutase (35%), catalase (67%), glutathione peroxidise (49%), vitamin E (77%) and ascorbic acid (36%) in newly formed tissue.
It also results in several fold decrease in lipid peroxide levels (60%) as measured in terms of their thiobarbituric acid reactive substance (TBARS). Asiaticoside, a saponin component isolated from *Centella asiatica*, has been shown to induce type I collagen synthesis via the activation of the beta RI kinase-independent Smad pathway in human dermal fibroblast cells (Lee *et al.*, 2006). Asiaticoside derived from the plant *Centella asiatica* is known to possess good wound healing activity. Enhanced healing activity has been attributed to increased collagen formation and angiogenesis. asiaticosides enhanced induction of antioxidant levels at an initial stage of healing which may be an important contributory factor in the healing properties of this substance (Shukla, 1999).

![Figure 1.5. Schematic representation of asiaticoside](image)

1.10.3. **RUBIA CORDIFOLIA LINN**

*Rubia cordifolia* L. (Rubiaceae), known as *Manjista* in Sanskrit, is a very variable, scandant, perennial, climber or creeper that grows in the North-West Himalayas, Nilgiris, Mahabaleshwar, and other hilly districts of *India*. A number of anthraquinones and triterpenes have been reported from *R.cordifolia*. Traditionally it is considered useful in inflammations, ulcers and skin diseases.
Four free anthraquinones (lucidin-ethylether, pseudopurpurin, alizarin and purpurin) and one anthraquinone glycoside (ruberythric acid) were isolated from the cultured *Rubia cordifolia* cells.

In the traditional Chinese system of medicine, *Rubia cordifolia* is used for the treatment of vertigo, insomnia, rheumatism, tuberculosis, hematemesis, menstrual disorders and contusions. The plant was recently reported to possess antimicrobial activity and previous phytochemical examinations have shown that it produces triterpenoids, anthraquinones, cyclopeptides and phenolics.

*Rubia cordifolia* Linn. (RC) has been reported to possess a significant antioxidant activity in *in vitro* studies (Tripathi et al., 1998). However, it is not known whether it is equally effective *in vivo*. Moreover, one of the earlier studies has shown that RC can also prevent the polluted air induced immunosuppression (Joharapurkar et al., 2003). The alcoholic extract of *Rubia cordifolia*. Linn has been shown to have neuroprotective effect on β-amyloid induced cognitive dysfunction in mice (Chitra & Pavan Kumar, 2009).

### 1.10.4. HOLARRHENA ANTIDYSENTERICA WALL

*Holarrhena pubescens*(antidysenterica) (L.) WALL. (Apocynaceae) is a deciduous tree found throughout tropical India, Burma, Sri Lanka, Pakistan, Nepal and Africa. The stem bark, which is commonly known as ‘kurchi’ in the Indian subcontinent and as ‘conessi bark’ in Europe is used in traditional Ayurvedic medicine to treat dysentery, especially amoebic dysentery. In addition the plant has been reported...
to possess antihelminthic, appetising, antidiarrhoeal and astringent properties (Chopra et al., 1982). These properties are due to the presence of steroid alkaloids of the conaine and aminopregnane types, the principal one being conessine. *H. pubescens* is a rich source of further steroid alkaloids such as kurchine, kurchimine, conessidine, konkurchicine, holarrhimine, and regholarrhimine (Radt, 1965).

Stem bark and seeds of the plant are reported to contain a number of steroidal alkaloids, such as conanines, 3-aminoconanines, 20-aminoconanines, 3-aminopregnans, 3, 20-diaminopregnanes and their derivatives. Being the principle alkaloid, conessine is mostly studied for its antidiarrhoeal properties (Gopal & Chauhan, 2006).

![Figure 1.7. Holarrhena antidysenterica Wall](image)

Chemical investigations on the stem bark of *Holarrhena antidysenterica* resulted in the isolation of a new steroidal alkaloid designated as holadysenterine. A new steroidal alkaloid, named antidysentericine, has been isolated from the seeds of *Holarrhena antidysenterica* and characterized as 3β-dimethylaminocon-5-enin-18-one (Kumar & Ali, 2000).

### 1.11. SCOPE OF THE THESIS

Understanding the factors governing cell’s response to radiation damage will be of great advantage in management of radiation exposure cases, both intentional and accidental as well as in improving the use of radiation in diagnosis and therapy.
Radioprotectors are also required to reduce normal tissue injury during radiotherapy of cancer. Use of an ideal radioprotector and a tumour specific radiosensitizer in combination during radiotherapy, would facilitate better tumour control without recurrence of malignancy and other side effects. The work presented in the thesis is an attempt to explore modification of cellular radiosensitivity by the phytoceutical asiaticoside and the extracts of *Centella asiatica* Linn, *Rubia cordifolia* Linn and *Holarrhena antidysenterica* Wall.

### 1.12. OBJECTIVES

2. To understand the *in vitro* radioprotecting ability of the extracts and asiaticoside, the major component present in *Centella asiatica*, at the molecular level by assessing their ability to protect membrane lipids and plasmid DNA against γ-radiation induced damages.
3. To determine the ability of the extracts / phytoceutical to prevent the γ -radiation induced DNA strand breaks *in vivo* using mouse models and *ex vivo* using human peripheral blood leukocytes as measured by single cell gel electrophoresis (comet assay).
4. Evaluating the ability of the extract of *Centella asiatica* and asiaticoside to enhance the repair of radiation-induced DNA strand breaks.
5. To determine the ability of *Centella asiatica* extract and asiaticoside to prevent the radiation induced genomic instability via studying the micronuclei formation.
6. To study the effect of administration of the extracts and the phytoceutical on lethal dose of radiation induced mortality.
7. To determine the haematological parameters and antioxidant status in asiaticoside and *Centella asiatica* extract administered animals exposed to different doses of γ -radiation.
8. To study the free radical scavenging potential of the extracts of *Centella asiatica*, *Rubia cordifolia* and asiaticoside and by evaluating their anti-inflammatory activity in mouse models.
9. To assess the efficacy of the *Rubia cordifolia* extract to ameliorate the toxicity of cancer chemotherapeutic cisplatin.

The experimental studies were mainly based on monitoring radiation-induced damage to DNA and membranes under conditions of *in vitro, in vivo* and *ex vivo* radiation exposure.