1.1. Introduction

The complexities and mysteries of the human body have never ceased to baffle mankind, particularly, the way in which drugs have been acting against various ailments. Illness has always been man’s heritage from the beginning of his existence and consequently, the search for remedies to combat them has been of foremost importance in his endeavor. Diseases such as cancer still challenge the prodigious human mind. Currently, cancer is one among the most prevalent diseases, which causes about 13% of all human deaths worldwide and its treatment remains an enigma even though medical science has made some spectacular advances this century [1].

Radiotherapy is used as a primary treatment modality in the cure of cancer. At present, more than 80 percent cancer patients receive radiation therapy as a part of their treatment [2, 3]. The number of patients cured by radiotherapy alone is second to those cured by surgery [4]. But in the process, the adjacent normal cells fail to bypass the radiation exposure. Therefore, unanticipated normal tissue damage leads to several co-morbid conditions [5].

The current radioprotection research focuses on both the protection of normal tissue as well as the destruction of cancer cells [5, 6]. The therapeutic outcome can be improved either by [a] increasing the radioresistance of normal tissues by using radioprotectors so that higher doses of radiation is required for effective cancer cells destruction will be tolerated, or [b] by increasing the radiosensitivity of cancer cells using radiosensitizers so that more cancer cells killing is achieved at conventional doses of radiation [7]. Therefore, radioprotectors can enhance the patient tolerance to radiotherapy.
On a broader note, radioprotectors are also required to shield human beings from dangers of radiation received through nuclear weapons and accidental radiation exposures.

1.2. Exposure to ionizing radiation

Cells that are immature, undifferentiated and actively dividing are more radiosensitive than cells that are mature, differentiated and not actively dividing [8]. Therefore, radiosensitive cells are more likely to get affected after exposure to radiation than a radioresistant cell [8]. In vivo studies indicate that the effects of radiation can cause various biological changes, which is dependent on the exposure rate and dose of radiation. Exposure to a large dose of radiation given over a shorter duration of time is more harmful than the same dose given over a longer duration of time [9]. Interaction of ionization radiation (IR) with living tissue does not necessarily result in permanent damage to the cell, because of the presence of inherent cellular repair mechanism, even though energy deposition to a cell occurs rapidly, in a time-frame of $10^{-18}$ seconds [10]. Radiation-induced damage does not appear immediately after radiation exposure but requires time to become clinically apparent (latent period). The latent period could be immediate (minutes or hours) for high radiation exposure but it takes years for low radiation doses. [11, 12].

1.3. Interaction of radiation with matter

Interaction of IR with matter leads to high biological damage. The degree and nature of such ionization depends on the energy of the individual particles. However, due to different rates of energy deposition per unit distance (Linear Energy Transfer, LET)
along the trajectories of the radiation, different concentrations of reactive species are formed in different proportions.

When IR interacts with living cells, water, which constitutes 70% of the cell, undergoes radiolysis and produces highly reactive free radical species, viz. hydrogen radicals, hydroxyl radicals, hydroperoxyl radicals and superoxide radicals [13]. In addition to this, it also produces other molecular species like hydronium ions (H$_3$O$^+$) and hydrogen peroxide. The reaction for radiolysis of water is described below.

\[
\text{IR} + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{OH}^-
\]

The relative yields of these species vary with the LET of radiation. The excessive generation of free radicals within cells after exposure to radiation leads to oxidative stress that initiates cellular damages. Among the various radical species, hydroxyl radicals have been considered primarily responsible for most of the radiation-induced cellular damage [13]. These hydroxyl radicals have been found to cause acute radiation syndrome (ARS) and mortality, which depends on the type and dose of radiation exposure. These events normally occur within hours to weeks. Sometimes delayed effects may appear months to years after irradiation. This occurs as a series of events that is depicted in the Fig 1.1.
Fig. 1.1. Major events in radiation damage and cell death [12].

Interaction of ionizing radiations with matter can be classified into:

- Direct interactions
- Indirect interactions
1.3.1. Direct interactions

Radiation can directly interact with the critical target molecules in the cell, causing ionization and excitation that lead to biochemical lesions [14]. Ionization can cause breakage of covalent bonds in DNA molecules, leading to a range of effects that include strand breaks, base loss and cross linking [15]. The fate of cell repair by itself is dependent on the type of damage caused by the different types of direct hits. Usually, when a direct hit results in complete break in the DNA, the cell either dies instantly or eventually [16]. However, humans have an abundant number of cells and the cells that die get replaced by new cells through the process of somatic cellular reproduction (mitosis). When these new cells get exposed to high doses of radiation, the system of replacing cells weakens and deleterious effects of radiation are seen. Actively dividing cells are more radiosensitive than non-dividing cells [8]. There are 4 phases of cell cycle: G\(_1\) Phase, in which cell grows and prepare for DNA replication; S Phase, in which cell replicates it’s DNA; G\(_2\) Phase, in which cells prepare for mitosis and M Phase (Mitotic phase), in which cells divide into 2 [17]. Among all these phases, cells are most sensitive in the M phase as DNA concentration is maximum due to chromosome condensation and pairing at this stage [18].

1.3.2. Indirect interactions

Indirect interactions occur when radiation interacts with water instead of the macromolecules inside the cell. This results in radiolysis of the water molecule, generating a hydrogen radical and hydroxyl radical. Two hydroxyl molecules join together to form hydrogen peroxide (H\(_2\)O\(_2\)), which is highly unstable in the cell. This may result in cell death due to depletion of endogenous antioxidant enzymes [16]. Antioxidants block the joining of hydroxyl radicals into hydrogen peroxide,
preventing the formation of stable hydrogen peroxide compounds. Due to this, antioxidants have received a lot of attention as radioprotective agents [7, 19].

1.4. Present status of radioprotectors

A radioprotector is an agent that provides protection against the toxic effects of IR. The potential application of radioprotectors, in the event of planned exposures or radiation accidents, has been investigated from the beginning of the nuclear era.[20]. However, preventing damage of normal tissue during radiotherapy is as important as killing cancer cells. Therefore, the focus of radioprotection research has become more therapy oriented [21].

The efficacy of radioprotectors is studied in different animal models using distinct endpoints such as apoptosis, mutagenesis, carcinogenesis and protection against radiation-induced lethality due to hematopoietic or gastrointestinal (GIT) injury. [22, 23]. Dose modifying factor (DMF) is the most reliable method for evaluating the efficacy of a radioprotector in animal studies. This is determined by irradiating mice in the presence and absence of administered compounds at a range of radiation doses [22]. However, this parameter is dependent on various factors, which include radiation dose, dose rate, dosage of compounds, animal strain, time and schedule of treatment [24].

The first report of radioprotection indicated the protective effects of colloidal sulphur and thiourea on enzymes against X-rays [25]. In 1948, bacteriophages were effectively protected against radiation damage by thioglycolic acid, glutathione (GSH), tryptophan and cysteine [26]. In 1949, Argonne National Laboratory showed pretreatment of cysteine increased survival of irradiated rats [27]. Following this,
Bacq et al in 1951 discovered decarboxylated cysteine, cysteamine (2-mercaptoethylamine) and its disulphide (cystamine), which were found to be superior to cysteine and exhibited significant radioprotection against X-rays [28]. Unfortunately, both cysteine and cysteamine exhibited toxicity at the maximum effective dose [29].

Later, several compounds were evaluated for their radioprotective effect in different in vitro and invivo models. The most protective radioprotectors developed so far are aminothiols and their derivatives viz. cysteamine, AET (Aminoethanisothiuronium bromide hydrobromide) and WR-2721 (Amifostine). Some of these compounds have been successfully used to prevent complications of radiation therapy in patients with cancer and have also been considered as a protective against radiation hazards in accidental radiation exposure scenarios and in clinical use [28-30]. Amifostine is found to be effective in patients with head and neck squamous cell carcinoma [31], but its use in radiotherapy is limited due to drug toxicity like nausea, diarrhea, hypotension, hypocalcaemia, sleeplessness, dizziness, sneezing and hiccoughs [4]. These toxicities were observed at the maximum effective dose of WR-2721 warranting its clinical use. [32]. Systemic toxicity of amifostine at the maximum effective dose and the cumulative toxicity on repeated administration are contra-indicative [33-35]. Similarly, delayed toxicity on bone marrow chromosomes has been demonstrated at higher doses of amifostine [36, 37].

Practical applicability of majority of these synthetic compounds remained limited owing to their high toxicity [38]. An ideal protector is one which gives a high degree of protection to normal tissues, with little or no protection to tumor cells and most importantly, should be non-toxic.
Table 1.1. Present status of the radioprotector/radiosensitizers under clinical development.

<table>
<thead>
<tr>
<th>Name</th>
<th>Use</th>
<th>Current Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tirapazamine (SR-4233)</td>
<td>Radiosensitizers [40]</td>
<td>Under Phase III trials [41]</td>
</tr>
<tr>
<td>Tempol (SOD-mimetic agent)</td>
<td>Prevent radiation-induced hair-loss [42]</td>
<td>Under Phase II trails (Mitos Pharma, USA)</td>
</tr>
<tr>
<td>Calcipotriol (synthetic derivative of calcitriol)</td>
<td>Prevents radiation-induced skin damage [43]</td>
<td>Under Phase II trails</td>
</tr>
<tr>
<td>BIO 300 (inhibitor of protein tyrosine kinase)</td>
<td>Acute radiation syndrome [44]</td>
<td>Under Phase I trials (Humanetics corp. USA)</td>
</tr>
<tr>
<td>Ex-Rad (4-carboxystyryl-4-chlorobenzylsulfone)</td>
<td>Acute radiation syndrome [45]</td>
<td>Under Phase I trials (Onconova Therapeutics)</td>
</tr>
<tr>
<td>5-Androstenediol (NEUMUNE)</td>
<td>Acute radiation syndrome [47]</td>
<td>Under development [48]. [Hollis-Eden Pharmaceuticals, USA]</td>
</tr>
<tr>
<td>EUK-189 (superoxide dismutase/catalase-mimetic agent)</td>
<td>Acute radiation syndrome [49]</td>
<td>Phase I trials (Evaluate Pharma, Australia)</td>
</tr>
<tr>
<td>SB-415286 (GSK-3 inhibitor)</td>
<td>Prevents radiation-induced intestine damage [51].</td>
<td>Under preclinical development</td>
</tr>
</tbody>
</table>
1.4.1. Requirements for the development of radioprotectors

The failure to obtain more effective and less toxic radioprotectors prompted researchers to focus towards evaluating the radioprotective potential of natural products [6, 7].

An ideal radioprotective agent should satisfy several conditions:

- It should provide high level of protection to normal tissues, with little or no protection to cancer cells.
- It should be efficient in providing multifaceted protection against undesired effects of radiation.
- It should have a protective effect on the majority of organs.
- It should have good oral bioavailability as well as stability profile.
- It should be non-toxic and have protective time-window effect.
- It should be compatible with the wide range of other drugs available to patients.
- It should be inexpensive, have no toxic implications and a reasonably good DMF [7].

In fact, there is no radioprotector that satisfies all the criteria till today. As previously described, the initial development of radioprotective agents exhibited various adverse effects. This demanded the search of new drugs which are more effective, less toxic and orally bioavailable [7]. After these conjectures, investigators in this field diverted their attention towards natural products. In recent years, natural plant products have been examined for their ability to ameliorate radiation-induced damage [5, 6]. The benefit of using natural products is their extensive use in indigenous systems of medicine. They are usually considered safe and are also widely used by humans [6]. However, their use as radioprotectors needs scientific assessment and validation.
1.4.2. Natural products as radioprotector

In spite of more than six decades of research on the development of radioprotectors, there is no safe and effective nontoxic radioprotector available for human use [52]. This has enthused extensive search to find effective and nontoxic radioprotectors. Hence the interest has shifted towards the use of natural products in radioprotection [6].

Natural plant products are non-toxic with proven therapeutic benefits and have been utilized since ancient times for curing various ailments. About 60% of the 1184 new drugs developed over the past 25 years owe their origin to natural sources [53, 54]. Till today, nearly 74 plant products have been screened for their radioprotective potential in various in vitro and in vivo studies (Table. 1.2). Plants are rich sources of polyphenols which include anthocyanins, flavonoids, stilbenes, tannins, lignins, etc. [55]. Among these, several flavonoids have been reported as potent antioxidants with radioprotective abilities [56-59]. Flavonoids scavenge free radicals, thereby sparing endogenous antioxidant enzyme systems and prevent their depletion [60].

Due to the similarity between oxidative stress and radiation injury, free radical scavenging is one of the most important criteria required for a compound to be a good radioprotector and hence any agent that reduces free radical formation could act as radioprotectors. In the last two decades, extensive work has been done on the radioprotective effects of several plant extracts viz. Acorus calamus, Ocimum sanctum, Coronopus didymus, Phyllanthus amarus [61-64], which have been found to increase mouse survival, chromosome protection and spermatogonial cell survival. In addition, a large number of phytochemicals obtained from plant sources have been reported to be radioprotective in various animal models. Chlorogenic acid, caffeic
acid, dehydrozingerone, hesperidin, morin, sesamol, zingerone, etc. has been shown to enhance the survival, protects hematopoietic and GIT system in irradiated mice [65-70]. Similarly, flavonoids orientin and vicenin isolated from Ocimum sanctum protected against chromosome aberrations, hematopoietic syndrome and enhanced survival in irradiated mice [71]. As described above, several plant products have been evaluated for their radioprotective activity in animals but ethanolic extract of Gingko biloba leaves (100 mg/i.v. infusion/person) was found to be effective in treating vasogenic edema of the patients undergoing radiotherapy of brain [72] and also protect against the clastogenic factors in human plasma exposed to irradiation [73]. Gingko biloba at the dose of 40 mg/kg/p.o was found to be effective in treating workers from the Chernobyl accident site [74].

All these reports support the argument that plant products and their isolates have great potential to be developed as radioprotectors. It is also worth mentioning that phytochemicals have the advantage of low toxicity, therefore, they might be more easily and safely used in patients undergoing radiotherapy than other radioprotective chemicals. Based on the present status of herbal radioprotectors (Table 1.2), the future holds promise for revealing the potential of natural products in radioprotector drug discovery [3-5].
Table 1.2. List of herbal plants used as radioprotectors.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Family</th>
<th>Use in radioprotection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos</em> (L.)</td>
<td>Rutaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [75].</td>
</tr>
<tr>
<td><em>Acanthopanax senticosus</em> Harms (Shigoka)</td>
<td>Araliaceae</td>
<td>Provided radioprotective effects on hematopoietic system in irradiated mice [76].</td>
</tr>
<tr>
<td><em>Acorus calamus</em> Linn.</td>
<td>Acoraceae</td>
<td>Protected radiation-induced strand breaks of DNA and enhanced the DNA repair process [64, 77].</td>
</tr>
<tr>
<td><em>Adhatoda vasica</em> Nees</td>
<td>Acanthaceae</td>
<td>Provided protection against radiation-induced alteration in hematological parameters in mice [78].</td>
</tr>
<tr>
<td><em>Ageratum conyzoides</em> Linn.</td>
<td>Asteraceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [79].</td>
</tr>
<tr>
<td><em>Allium cepa</em></td>
<td>Amaryllidaceae</td>
<td>Provided protection against X-ray induced chromosome aberrations [80].</td>
</tr>
<tr>
<td><em>Allium sativum</em> L.</td>
<td>Alliaceae</td>
<td>Prevented increase in hepatic total lipids and decrease in free fatty acids induced by irradiation [81].</td>
</tr>
<tr>
<td><em>Aloe arborescens</em></td>
<td>Liliaceae</td>
<td>Protected the mouse skin injury induced by soft X-irradiation [82].</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
<td>Xanthorrhoeaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [83].</td>
</tr>
<tr>
<td><em>Alstonia scholaris</em> L.</td>
<td>Apocynaceae</td>
<td>Provided protection against radiation-induced clastogenic and biochemical alterations in mice [84].</td>
</tr>
<tr>
<td><em>Angelica sinensis</em> (Oliv.) Diels</td>
<td>Apiaceae</td>
<td>Provided protection against radiation-induced pulmonary fibrosis in mice [85].</td>
</tr>
<tr>
<td><em>Archangelica officinalis</em> Hoffm.</td>
<td>Umbellifera</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [86].</td>
</tr>
<tr>
<td><em>Amaranthus paniculatus</em> Linn.</td>
<td>Amaranthaceae</td>
<td>Improved learning and also augmented endogenous antioxidant enzymes in the liver of irradiated mice [87-89].</td>
</tr>
<tr>
<td><em>Aphanamixis polystachya</em> (Wall.)</td>
<td>Meliaceae</td>
<td>Reduced radiation-induced chromosome damage [90].</td>
</tr>
<tr>
<td><em>Azadirachta indica</em> (L.) Adelb.</td>
<td>Meliaceae</td>
<td>Exhibited radiosensitizing effect by activating pro-apoptotic signals in neuroblastoma xenografts exposed to radiation [91].</td>
</tr>
<tr>
<td><em>Biophytum sensitivum</em> (L.)</td>
<td>Oxalidaceae</td>
<td>Stimulated the production of cytokines such as IL-1beta, IFN-gamma and GM-CSF in irradiated mice [92].</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em> L.</td>
<td>Nyctaginaceae</td>
<td>Prevented γ-radiation-induced DNA damage in mice bone marrow [93].</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Protection Effect</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Brassica oleracea (cabbage)</td>
<td>Brassicaceae</td>
<td>Provided protection against X-ray-induced mortality [94].</td>
</tr>
<tr>
<td>Brassica oleracea (wild cabbage)</td>
<td>Brassicaceae</td>
<td>Provided protection against UV radiation-induced skin carcinogenesis in SKH-1 hairless mice [95].</td>
</tr>
<tr>
<td>Caesalpinia digyna</td>
<td>Fabaceae</td>
<td>Protected radiation-induced lipid peroxidation, protein carbonylation and DNA damage in <em>in vitro</em> studies (Singh et al., 2009).</td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>Apiaceae</td>
<td>Protected radiation-induced damage to DNA and membranes both <em>in vitro</em> and <em>in vivo</em> [96].</td>
</tr>
<tr>
<td>Chelidonium majus</td>
<td>Papaveraceae</td>
<td>Increased the number of bone marrow cells, spleen cells, GM-CFC, and platelets in irradiated mice [97].</td>
</tr>
<tr>
<td>Chlorococcal algae (Ivastimul)</td>
<td>Chlorophyceae</td>
<td>Provided protection against radiation-induced hemopoiesis in mice [98].</td>
</tr>
<tr>
<td>Coronopus didymus (L.)</td>
<td>Brassicaceae</td>
<td>Provided protection against acute radiation effects on hematopoietic, GIT system and also augmented the endogenous antioxidant enzyme levels in the liver of mice [62].</td>
</tr>
<tr>
<td>Curcuma longa Linn.</td>
<td>Zingiberaceae</td>
<td>Provided protection against acute radiation effects on different organs of mice [99-101].</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>Euphorbiaceae</td>
<td>Provided protection against acute radiation effects on GIT system and also augmented the endogenous antioxidant enzyme levels in the intestine of mice [102].</td>
</tr>
<tr>
<td>Elaeocarpus sylvestris</td>
<td>Elaeocarpaceae</td>
<td>Provided protection against radiation-induced damage to hematopoietic system in mice [103]</td>
</tr>
<tr>
<td>Ficus racemosa</td>
<td>Moraceae</td>
<td>Decreased the percentage of micronucleated binuclear in irradiated V79 cells assessed by micronucleus assay [104].</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>Ganodermataceae</td>
<td>Prevented radiation-induced DNA damage and apoptosis in splenic lymphocytes [105].</td>
</tr>
<tr>
<td>Grewia asiatica</td>
<td>Malvaceae</td>
<td>Augmented the endogenous antioxidant enzyme levels in cerebellum and liver of irradiated mice [106, 107].</td>
</tr>
<tr>
<td>Genista sessilifolia DC. and Genista tinctoria L.</td>
<td>Leguminosae</td>
<td>Both plant extract inhibited UV light and nitric oxide-induced DNA damage on pBR322 [108]</td>
</tr>
<tr>
<td>Ginkgo biloba Linn.</td>
<td>Cycadaceae</td>
<td>Protected against the clastogenic factors in human plasma exposed to irradiation [73].</td>
</tr>
<tr>
<td>Glycyrrhiza glabra L.</td>
<td>Fabaceae</td>
<td>Reduced the lipid peroxidation level in rat liver microsomes and also protected plasmid DNA from radiation-induced strand breaks [109].</td>
</tr>
<tr>
<td>Hemidesmus indicus</td>
<td>Apocynaceae</td>
<td>Protected the DNA from radiation-induced strand breaks</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Effect Description</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hypericum perforatum Linn.</td>
<td>Hypericaceae</td>
<td>Enhanced the levels of enzymatic and non-enzymatic enzymes in irradiated rat liver microsomes <em>in vitro</em> and <em>in vivo</em> [111].</td>
</tr>
<tr>
<td>Hippophae rhamnoides Linn.</td>
<td>Elaeagnaceae</td>
<td>Protected against radiation-induced mitochondrial and genomic DNA damage [112].</td>
</tr>
<tr>
<td>Isatis tinctoria (Indigowoad root)</td>
<td>Brassicaceae</td>
<td>Protected hematopoietic cells and modulates inflammatory cytokines in irradiated mice [113].</td>
</tr>
<tr>
<td>Lycium chinense</td>
<td>Solanaceae</td>
<td>Enhanced regeneration of the hematopoietic stem cells in irradiated mice [114].</td>
</tr>
<tr>
<td>Mentha arvensis Linn.</td>
<td>Lamiaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [115].</td>
</tr>
<tr>
<td>Mentha piperita Linn.</td>
<td>Lamiaceae</td>
<td>Protected against radiation-induced testicular and hematopoietic damage in mice [116, 117].</td>
</tr>
<tr>
<td>Mentha spicata Linn.</td>
<td>Lamiaceae</td>
<td>Offered behavioral radioprotection [118].</td>
</tr>
<tr>
<td>Moringa oleifera Lam.</td>
<td>Moringaceae</td>
<td>Prevented radiation-induced oxidative stress in mice [119].</td>
</tr>
<tr>
<td>Mangifera indica</td>
<td>Anacardiaceae</td>
<td>Magniferin, a glycosylxanthone, present in the <em>Mangifera indica</em> provided protection against radiation-induced sickness and mortality in mice [120].</td>
</tr>
<tr>
<td>Myristica fragrans houtt</td>
<td>Myristicaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [121].</td>
</tr>
<tr>
<td>Nelumbo nucifera Gaertn.</td>
<td>Nelumbonaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [122].</td>
</tr>
<tr>
<td>Ocimum sativum</td>
<td>Lamiaceae</td>
<td><em>Ocimum sativum</em> extract and its two flavonoids orientin and vicenin, provided protection against radiation-induced sickness and mortality in mice [61, 71].</td>
</tr>
<tr>
<td>Olea europaea L.</td>
<td>Oleaceae</td>
<td>Oleuropein, main component, prevented UV B radiation-induced skin damage and carcinogenesis in hairless mice [123].</td>
</tr>
<tr>
<td>Panax ginseng Linn.</td>
<td>Araliaceae</td>
<td>Provided protection against radiation-induced hematological and biochemical changes in mice [124].</td>
</tr>
<tr>
<td>Phyllanthus amarus</td>
<td>Phyllanthaceae</td>
<td>Provided protection against radiation-induced damage to chromosomes and intestine in mice [125].</td>
</tr>
<tr>
<td>Phyllanthus niruri</td>
<td>Phyllanthaceae</td>
<td>Provided protection against radiation-induced clastogenicity in mouse bone marrow [126].</td>
</tr>
<tr>
<td>Podophyllum hexandrum</td>
<td>Berberidaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [127, 128].</td>
</tr>
<tr>
<td>Pothomorphe umbellate C. DC.</td>
<td>Piperaceae</td>
<td>Inhibited UV-B-induced hyperplastic response and increased p53-positive cells in hairless mouse epidermis [129].</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Effects</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Pilea microphylla</em> (L.)</td>
<td>Urticaceae</td>
<td>Provided protection against acute radiation effects on hematopoietic, GIT system and also augmented the endogenous antioxidant enzyme levels in the liver of mice [130]</td>
</tr>
<tr>
<td><em>Pinus caribaea</em></td>
<td>Pinaceae</td>
<td>Provided protection against radiation-induced DNA damage in <em>Escherichia coli</em> cells [131].</td>
</tr>
<tr>
<td><em>Pinus maritima</em> Lam.</td>
<td>Pinaceae</td>
<td>Provided protection against chronic UVB radiation-induced skin damage and carcinogenesis in melanin-possessing hairless mice[132]</td>
</tr>
<tr>
<td><em>Piper betle</em></td>
<td>Piperaceae</td>
<td>Decreased the frequency of radiation-induced micronucleated cells [133].</td>
</tr>
<tr>
<td><em>Piper longum</em></td>
<td>Piperaceae</td>
<td>Provided protection against radiation-induced alteration in hematological parameters in mice [134]</td>
</tr>
<tr>
<td><em>Plumbago zeylanica</em> Linn.</td>
<td>Plumbaginaceae</td>
<td>Plumbugin, isolated constituent, inhibited ultraviolet radiation-induced development of squamous cell carcinomas [135].</td>
</tr>
<tr>
<td><em>Prunus avium</em></td>
<td>Rosaceae</td>
<td>Provided protection against radiation-induced alteration in metabolic markers [107].</td>
</tr>
<tr>
<td><em>Panica granatum</em> Linn.</td>
<td>Lythraceae</td>
<td>Protected against radiation-induced enteritis and leukocyte apoptosis in rats [136].</td>
</tr>
<tr>
<td><em>Rhodiola imbricata</em></td>
<td>Crassulaceae</td>
<td>Provided protection against radiation-induced alteration in hematopoietic system in mice [137].</td>
</tr>
<tr>
<td><em>Rosmarinus officinalis</em></td>
<td>Lamiaceae</td>
<td>Augmented the endogenous antioxidant enzyme levels in blood and liver of irradiated mice [138].</td>
</tr>
<tr>
<td><em>Rubus spp.</em></td>
<td>Rosaceae</td>
<td>Inhibited NF-kB dependent radioprotection in human breast cancer cells [139].</td>
</tr>
<tr>
<td><em>Santalum album</em> Linn.</td>
<td>Santalaceae</td>
<td>Prevented UV-B-induced skin cancer by increasing in apoptosis proteins in mice [140].</td>
</tr>
<tr>
<td><em>Spirulina platensis</em></td>
<td>Phormidiaceae</td>
<td>Reduced the micronucleus frequencies induced by gamma-radiation in mice [141].</td>
</tr>
<tr>
<td><em>Syzygium cumini</em> L. Skeels</td>
<td>Myrtaceae</td>
<td>Protected against the radiation-induced DNA damage in mice and inhibits radiation-induced free radical formation [142].</td>
</tr>
<tr>
<td><em>Tephrosia purpurea</em> (L.) Pers.</td>
<td>Fabaceae</td>
<td>Exhibited free radical scavenging properties and also protected mice against radiation-induced haemopoietic injury [143, 144]</td>
</tr>
<tr>
<td><em>Terminalia chebula</em></td>
<td>Combretaceae</td>
<td>Provided protection against radiation-induced damage to DNA in lymphocytes [145].</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em></td>
<td>Menispermaceae</td>
<td>It prevented against radiation-induced testicular injury in mice [146].</td>
</tr>
</tbody>
</table>
1.5. Antioxidant

Antioxidant can be defined as “any substance that delays or inhibits oxidative damage to a target molecule” [152-154]. Antioxidants play a pivotal role in the maintenance of good health by providing protection against oxidative stress induced by free radicals. The requirement for antioxidants becomes even more important with increased exposure to free radicals [155]. As part of a healthy lifestyle, antioxidant supplementation is now being used to maintain the health of individuals [156].

1.5.1. Classification of antioxidants

The antioxidant systems are classified into two major groups, enzymatic antioxidants and non-enzymatic antioxidants (Fig 1.3).

1.5.1.1. Enzymatic antioxidants

Enzyme antioxidants are produced in the body and they act as body’s first line of defense against free radicals. They convert reactive free radicals into less reactive or inert species (Fig. 1.2). Enzymatic antioxidants present in the body include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [157-159].

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigna radiata (L.)</td>
<td>Fabaceae</td>
<td>Vitexina, active constituent, is been used in breast cancer patients undergoing radiotherapy [147].</td>
</tr>
<tr>
<td>Viscum album L.</td>
<td>(Santalaceae)</td>
<td>Reduced the side effects of conventional radiotherapy in cancer [148].</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Solanaceae</td>
<td>1-oxo-5beta,6beta-epoxy-witha-2-enolide, isolated constituent, prevented UV radiation-induced skin carcinoma in rats [149].</td>
</tr>
<tr>
<td>Xylopia aethiopica</td>
<td>Annonaceae</td>
<td>Provided protection against gamma-radiation-induced damage in liver and kidney of Wistar rats [150].</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Zingiberaceae</td>
<td>Provided protection against radiation-induced sickness and mortality in mice [151].</td>
</tr>
</tbody>
</table>
Fig. 1.2. Defense mechanism against damage by ROS. Superoxide dismutase, catalase or glutathione peroxidase eliminates many damaging oxygen species [160].

1.5.1.1.1. Superoxide dismutase

Superoxide dismutases (SODs) are a family of metalloenzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide [157, 161]. SOD was first isolated from bovine blood by Mann and Keilin in 1938 [162] and their catalytic function was discovered by McCord and Fridovich in 1969 [163]. SOD plays a major role in the protection of cells against oxidative damage.

\[
2 \, \text{O}_2^- + 2 \, \text{H}^+ \xrightarrow{\text{SOD}} \text{O}_2 + \text{H}_2\text{O}_2
\]

There are three major families of SOD- namely, (i) Cu-Zn–SOD – that has been found in eukaryotic cells, (ii) Fe-SOD – that has been observed in prokaryotic cells and (iii) Mn-SOD – that has been found in both types of cells. [164].

SOD has multiple pharmacological activities eg: it has been highly effective in treating colonic inflammation in experimental colitis [165], it has been found to ameliorate cisplatin-induced nephrotoxicity in rodents [166], and also effective in the
treatment of urinary tract inflammatory disease in humans [167]. Recent development of novel SOD-mimetic agent (TEMPOl) prevents radiation-induced hair-loss and is currently in clinical trials [42].

1.5.1.1.2. Catalase

Catalase catalyzes the decomposition of $\text{H}_2\text{O}_2$ to water and oxygen, found in nearly all living organisms exposed to oxygen. [168]. Most of the aerobic cells have catalase activity. It was first crystallized from beef liver by Sumner and Dounce [169]. Catalase is present in all major body organs, especially in liver [170]. The presence of catalase in the peroxisomes protects the cell from the deleterious effects of $\text{H}_2\text{O}_2$ [171].

\[
\begin{align*}
2 \text{H}_2\text{O}_2 & \xrightarrow{\text{catalase}} 2 \text{H}_2\text{O} + \text{O}_2
\end{align*}
\]

Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second [172]. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long [173]. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The heme group is responsible for catalase’s enzymatic activity.

Pre-administration of Eukarion-189 (SOD-catalase mimetic) effectively reduced micronucleus formation in lung fibroblasts of irradiated rats [174].

1.5.1.1.3. Glutathione peroxidase

Glutathione peroxidase (GPx) belongs to the selenoprotein enzyme family with peroxidase activity whose main biological role is to protect the organism from
oxidative damage by catalyzing the reduction of a variety of hydroperoxides, using glutathione as the reducing substrate. The GPx enzyme catalyses the oxidation of glutathione (GSH) to glutathione disulfide (GSSG) at the expense of hydrogen peroxide [175].

\[
\begin{align*}
\text{GPx} & \quad \text{H}_2\text{O}_2 \\
& \quad \rightarrow \quad \text{H}_2\text{O} \\
& \quad \text{GSH} \quad \text{GSSG}
\end{align*}
\]

In this context, we have evaluated radioprotective effect of organoselenium compound, 3,3’-diselenodipropionic acid (DSePA) in our laboratory. The mechanism of protection is probably through the maintenance of antioxidant enzymes, prevention of DNA damage, induction of DNA repair genes and inhibition of apoptosis [176].

**1.5.1.2. Non-enzymatic antioxidants**

Endogenous non-enzymatic antioxidants play an important role in scavenging reactive oxygen species (ROS) (Fig 1.3). Several non-enzymatic antioxidants have already been established as both radiosensitizers as well as radioprotectors.
**Fig 1.3.** Classification of enzymatic and non-enzymatic antioxidants.

EGCG - Epigallocatechin gallate

GSR - Glutathione reductase

GPX- Glutathione peroxidase

G6PDH- Glucose-6-phosphate dehydrogenase

SOD – Superoxide dismutase
1.6. Flavonoids

Flavonoids are a family of natural products with polyphenolic structure that are widely distributed in plants as complex mixtures of different components [177]. They are recognized with their structure, consisting of two hydroxy substituted aromatic rings joined by a three carbon link (a C₆-C₃-C₆ configuration) [178]. The biological effects of flavonoids depend upon their structural characteristics. The position of hydroxyl groups in the catechol structure (A- or B-ring) is important for their antioxidant and free-radical-scavenging effects [179]. Studies on flavonoids have shown that they not only have antioxidant [180] and radioprotective properties but also have antidiabetic [181], antihyperlipidemic [182], antibacterial [183], anti-inflammatory [184], antidepressant [185], anticancer [186], cardioprotective [187] and antigenotoxic activities.

1.6.1. Flavonoid structure

Flavonoids have a unique structure based on a C₁₅ skeleton with a chromone ring bearing a second aromatic ring B in position 2, 3 or 4 (Fig. 1.3). The preferred glycosylation site on flavonoids is the 3rd position, and less frequently the 7th position, with glucose being the most common sugar residue [179]. The antioxidant activity of flavonoids is especially related to the hydroxy substitution of the aromatic A-, B- and C-ring of the structure (Fig.1.4) [188-190]. Five major subgroups of flavonoids are distinguished based on the structures named as flavonols, flavones, flavanones, isoflavone and dihydroflavonols described in Table 1.3 [177]. It is well known that hydroxy substituted flavonoids possess strong antioxidant activity. The most promising structural characteristic appears to be the ortho-dihydroxy substitution on the B-ring. Oxidation of flavonoids, with catechol structure on B-ring, yields a
fairly stable ortho-semiquinone radical by facilitating electron delocalization, which is involved in antioxidant mechanism [191, 192].

Fig. 1.4. Basic structure of flavonoids and UV-spectrum
<table>
<thead>
<tr>
<th>Flavonoid class</th>
<th>General structure</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonol</td>
<td><img src="image" alt="Flavonol" /></td>
<td>Quercetin</td>
</tr>
<tr>
<td>Flavone</td>
<td><img src="image" alt="Flavone" /></td>
<td>Apigenin</td>
</tr>
<tr>
<td>Flavanone</td>
<td><img src="image" alt="Flavanone" /></td>
<td>Naringenin</td>
</tr>
<tr>
<td>Isoflavone</td>
<td><img src="image" alt="Isoflavone" /></td>
<td>Genistein</td>
</tr>
<tr>
<td>Dihydroflavonol</td>
<td><img src="image" alt="Dihydroflavonol" /></td>
<td>Taxifolin</td>
</tr>
</tbody>
</table>

Table 1.3. Classification, structure and example of the main classes of flavonoids [177].
Flavonoids are isolated as single compounds or in mixtures from plants and further tested for their biological activity. The isolation, identification and characterization of each isolated constituent are often difficult, due to similarities in structure and polarity. In recent years, development of high-performance liquid chromatography (HPLC) has improved the analysis and identification of flavonoids. HPLC technique is commonly used for their separation, screening, and quantitative determination in plant products [178]. Further, introduction of diode array detectors associated to HPLC (HPLC-DAD) has led to a great improvement, as not only the retention time but also the UV-visible spectrum is used for the identification of flavonoids. The identification of unknown flavonoids can be based additionally on their UV-Visible spectra and the correlation with standard compounds [193].

The characteristic UV-Visible spectra of flavonoids comprise of two absorbance bands (Band A and Band B). Band A lies in the 310-350 nm range for flavones and 350-385 nm for flavonols. Band B, found in the 250-290 nm range (Fig. 1.4), is much the same in all the above-mentioned flavonoid subgroups [178, 194].

1.6.2. Structure–activity relationships (SAR) of flavonoids

The antioxidant activities of flavonoids vary considerably depending on structures and functional groups [192, 195]. The difference in their antioxidant activity is dependent on the variation in number and arrangement of functional groups attached to the main nucleus (Fig. 1.4). There are several functional groups that contribute to increase free radical scavenging. These include the presence of ortho-dihydroxy group (catechol structure) in the B or A ring and the C2=C3 double bond in conjugation with a 4-oxo group (carbonyl group) in the C ring. Further, the presence of a 3-OH and 5-OH group with 4-oxo group (carbonyl group) on rings C and A also potentiate the efficacy
of free radical scavenging (Fig. 1.4) [179]. As described earlier, the position of hydroxyl groups plays an important role. Among these, B-ring hydroxyl configuration is the most significant in conferring antioxidant activity [192]. It is a known fact that an increase in the number of hydroxyl groups increases the free radical scavenging ability of flavonoids [177]. However, substitution of the hydroxyl group by the methoxy group in the B ring diminishes antioxidant activity [196]. Antioxidant activity of flavonoids is more related with the position than the number of hydroxyl substitutions. For example, dihydroxyl groups in the ortho position show a better antioxidant activity with respect to dihydroxyl groups in the meta position. [197]. The glycosylation of flavonoids also reduce their antioxidant activity. For example, rutin, has lower antioxidant activity than unglycosylated quercetin [192]. Quercetin is more potent than other flavonoids such as apigenin and hesperidin, which lack essential functional groups in their structures [179].

1.6.3. Mechanisms of flavonoid activity

1.6.3.1. Free radical scavenging

Flavonoids prevent free radical-induced damage by direct radical scavenging. Flavonoids are oxidized by radicals, resulting in a more stable molecule due to high reactivity of the hydroxyl group of the flavonoids. The reaction of flavonoids with the radical is as follows [60].

\[
\text{Flavonoid (OH) + R}^\cdot \rightarrow \text{Flavonoid (O') + RH}
\]

In the above reaction, \( R^\cdot \) is a free radical and \( O' \) is an oxygen free radical.

Flavonoids inhibit the formation of ROS in cells and thereby prevent further attack on DNA and other macromolecules. As ROS are mostly short-lived, they react immediately with DNA after their production by IR [177, 198]. The ortho-dihydroxy
moiety seems to be the main requisite for the scavenging activity of catechol-containing flavonoids [191]. Oxidation of the B-ring in the catechol structure of a flavonoid yields a fairly stable ortho-semiquinone radical, by facilitating electron delocalization, involved in antioxidant mechanism [192, 199]. Thus, the structural criteria contributing to high antioxidant activity of flavonoids include the ortho-dihydroxy groups (catechol structure) in the B or A-ring, 3-hydroxyl or 3-galloyl group (catechol structure) in the C-ring and the 2,3-double bond in conjugation with 4-oxo group (carbonyl group) in the C-ring [191, 200]. Therefore, flavonoids are excellent free radical scavengers. It is projected that high in vitro antioxidant activity is also closely related to high radioprotective effects for flavonoids. *Vitis vinifera* extract, which contains procyanidins (flavan-3-ols), *Coronopus didymus* extract, which contains chrysoeriol (flavone), *Ficus racemosa* extract, which contains several flavonoids (bergenin), *Caesalpinia digyna* extract, which contains gallic acid, showed free radical-scavenging activity and radioprotective effects [62, 104, 201, 202]. Therefore, it can be said that free radical scavenging abilities and radioprotective effects of flavonoids seem to be linked.

### 1.6.3.2. Flavonoid–DNA interaction

Several mechanisms have been proposed for the anti-genotoxic effect of flavonoids and the most important among these is their ability to intercalate into DNA double helices, which stabilizes DNA structure and condenses it into a highly compact form, inaccessible to free radical attack. Flavonoids have numerous hydroxyl groups, which easily donate hydrogen atoms to radicals formed in the DNA bases. The flavonoid–DNA complex enables the flavonoid to repair the radical base very efficiently. Flavonoids can also interact with the phosphate moiety of the DNA backbone through
hydrogen bonding that repairs the sugar radicals. They act as reducing agents owing to their low oxidation potential and react easily with electron-accepting radicals before the ROS can attack DNA components. There are also reports supporting the efficient hydrated electrons scavenging potential of flavonoids [177]. Flavonoids have several hydroxyl groups, which enhance DNA binding. For example, hydroxylation at the 7th position enhances flavone–DNA interaction, whereas methoxy substitution at the 7th position reduced the binding constant.

1.6.3.3. Endogenous antioxidant enzymes
Pre-administration of flavonoids increases the cellular antioxidant status and further decreases the attack of free radicals on biomolecules, and thereby attenuating the harmful effects of radiation on biological system. Administration of various plant products like *Ficus racemosa, Coronopus didymus* which are rich in flavonoids prevented the radiation-induced oxidative stress in the liver of mouse by increasing the activity of enzymatic and non-enzymatic antioxidants [62, 104]. Similarly, administration of quercetin, hesperidin, sesamol and morin augmented the endogenous antioxidant enzyme levels in the liver of irradiated mice, indicating that these flavonoids pretreatment restored the endogenous antioxidant status in irradiated mice [66, 67, 70, 203].
1.7. Hypothesis and Objectives of the Present Work

Since time immemorial, the plant kingdom has exhibited a diverse array of biological activities. Several plant products have also been employed for mitigating the ionizing radiation-induced damage in mammalian systems [4, 5]. This is owing to the polyphenolic constitution of plant material, among which flavonoids are the most abundant, responsible in attenuating the radiation-induced oxidative distress [7]. In the search for an effective nontoxic radioprotector, our lab has been screening several plants and plant products over the past decade e.g. *Ocimum sanctum, Coronopus didymus, Ficus racemosa*, etc.[62, 71, 104].

1.7.1. Background of *Pilea microphylla*

*Pilea microphylla* (L.), (Family: Urticaceae), (PM) is also known as artillery plant. It consists of a multitude of minute lime green leaves on short arching stem which gives the plant a fine textured fern like appearance. It grows 8 to 12 inches tall and forms spreading clumps up to two feet wide.

PM is used as folk medicine to treat several allergies/wounds in and around South Canara district of Karnataka, India. It is reported to possess antibacterial, antidiabetic and antidyslipidemic activity and treat reproductive problems [204-206]. Bioactivity-guided fractionation of the ethanolic extract of PM resulted in the most active fraction (PM1) with highly potent free radical scavenging ability. PM1 protected hematopoietic, GIT system from acute radiation effects and also augmented the endogenous antioxidant enzyme levels in the liver of irradiated mice. [130]. In continuation to the previous work on PM1, the phytoconstituents were identified and correlated with the outcomes under the following major objectives:
To carry out isolation and characterization of compounds in order to identify the biologically potent constituents responsible for conferring radioprotection to PM.

To evaluate antioxidant potential of the isolated compounds using in vitro free radical scavenging assays and their radioprotective efficacy against $\gamma$-radiation-induced damage in V79 cells.

To evaluate in vitro radioprotective mechanism of the most potent compound in V79 cells.

Based on the above findings, carry out radioprotection studies of the most potent compound in Swiss albino mice.

To study the effect of the most potent compound in preventing radiation-induced toxicities in different organs and investigate the possible mechanism of action.

To study the effect of the most active fraction in comparison with its constituents (individually and in combination) in preventing radiation-induced toxicities in V79 cells.
1.8. Structure of the Thesis

Chapter 1
This chapter describes new drug discovery in developing radioprotectors, list of plant products screened for their radioprotective activity, role of flavonoids and their mechanisms in the treatment of radiation induced sickness and objectives of the proposed work.

Chapter 2
This chapter describes the isolation and characterization of six phenolic compounds namely, quercetin-3-O-rutinoside (PMC-1), 3-O-caffeoylquinic acid (PMC-2), luteolin-7-O-glucoside (PMC-3), apigenin-7-O-rutinoside (PMC-4), apigenin-7-O-β-D-glucopyranoside (PMC-5) and quercetin (PMC-6) from the whole plant of PM by using conventional open-silica gel column chromatography and preparative HPLC. Further, these compounds were characterized by 1D, 2D NMR techniques and high-resolution LC-MS. Further, we investigated antioxidant potential of the compounds using in vitro free radical scavenging assays and their radioprotective efficacy against γ-radiation-induced damage in V79 cells.

Chapter 3
The chapter deals with the in vitro radioprotective mechanism of PMC-1, the major constituent isolated from PM. In vitro studies were carried out to establish the mechanism of PMC-1.

Chapter 4
This chapter describes the in vivo radioprotective efficacy of PMC-1. Further, we focused, for the first time, on evaluating in vivo radioprotective effect of PMC-1 in hematopoietic, gastrointestinal and endogenous antioxidant enzymes in mice. Also, the effect of PMC-1 on certain aspects of cell signaling has been elucidated by
monitoring the mRNA expression levels of the pro-apoptotic (BAX) and pro-survival (ERK) genes in the spleen. This study was aimed at identifying both cellular and molecular mechanisms of radioprotection exhibited by the flavonoid PMC-1. This chapter describes the effect of PMC-1 on animal models of acute radiation syndrome.

**Chapter 5**

This chapter describes the comparative cytoprotective and antigenotoxic activity of PM1 with that of its active polyphenolic constituents (both individually and in combination) against γ-radiation in V79 cells. It was evaluated for its free radical scavenging potential using Fenton reaction-induced DNA damage and lipid peroxidation. Further, PM1 was subjected against γ-radiation-induced cytotoxicity and genotoxicity in V79 cells.

**Chapter 6**

This chapter summarizes the outcomes of the entire work, list of publications and abstract presented in various conferences.
1.9. References


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