Podophyllum hexandrum and its active constituents: Novel radioprotectants

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Radiation is a widely utilized tool in medicine, with applications including diagnostic radiography, nuclear medicine, and radiation therapy [1]. Notably, it is one of the most commonly used treatments to extend the lives of cancer patients, particularly in advanced stages.

1. Introduction

Radiation is a widely utilized tool in medicine, with applications including diagnostic radiography, nuclear medicine, and radiation therapy [1]. Notably, it is one of the most commonly used treatments to extend the lives of cancer patients, particularly in advanced stages.
either as a standalone treatment or as an adjuvant to chemotherapy [2]. Although controlled doses of radiation in the medical field are routine and regarded as safe, the high doses of radiation required in radiotherapy to effectively target cancer cells also irradiate and damage the surrounding healthy tissues. High doses of radiation generate free radicals that oxidize cellular bio-macromolecules such as DNA, proteins, and lipids, causing a variety of cellular dysfunctions, which consequently trigger cell death and tissue damage [3,4]. Radiation-induced free radicals also cause oxidative damage to bone marrow precursor cells, in addition to damaging their microenvironment, resulting in hematopoietic suppression. This process is thought to be the prime factor instigating mortality following radiological treatment [5]. DNA damage after radiation administration occurs mainly by initiating inter-strand cross-linkage, base damage and single or double-strand breaks. Ionizing radiation such as X-rays with low linear energy transfer (LET) and γ-rays, cause harm to biological systems mainly by the formation of reactive oxygen species (ROS) such as superoxide radicals (O2−), hydrogen peroxide (H2O2), singlet oxygen (O1^2), hydroxyl radicals (OH) and reactive nitrogen species (RNS), like nitric oxide (NO) and peroxynitrite (ONO0−), ROS/RNS interact with various macromolecules such as DNA, proteins, and lipids leading to DNA lesions/caspases activation at one axis, while membrane damage and lipid peroxidation (LPO) at other axes, where later resulting in the removal of sulphhydryl groups from cellular proteins, causing leaky membranes, resulting in protein fragmentation and denaturation [9,10]. These oxidative damages are known to be the prime factors that instigate pathological conditions including Alzheimer’s disease (AD), Parkinson’s disease (PD), arthritis, aging, gliculated diabetes mellitus (DM), low-density lipoprotein (LDL) oxidation in atherosclerosis, hemolysis of red blood cells (RBC) caused by glucose-6-phosphate dehydrogenase deficiency [11,12].

To protect against pathology, cells possess a series of defense pathways to repair DNA damage caused by oxidation. DNA inter-strand cross-link repair and DNA mismatch repair, homologous and non-homologous end-joining, direct adduct repair, base-excision repair, and nucleotide-excision repair are the most notable mechanisms [13]. Researchers have extensively evaluated different types of radioprotectors such as cytoprotective agents, immunomodulators, antioxidants, vitamins, and DNA binding molecules using in vitro cell and animal models [14]. However, a potent synthetic radioprotector, which remains stable, provides effective long-term protection, is easily available, cost-effective and has minimal toxicity remains to be identified. It is thus important to develop such effective radioprotectors and radiation recovery drugs given their potential to work in cases of both expected radiation exposure (e.g., radiotherapy) and incidental background radiation (e.g., irradiation from the nuclear industry, natural radiation emanating from the earth or other sources). A number of radioprotective agents, including cysteamine, aminothiols, amifostine, polyamines and DNA-binding ligands like Hoechst, protect DNA from radiation damage [15–17]. Along with synthetic compounds, other effective and sustainable radioprotective agents of biological origin are currently being developed [15]. Namely, immuno-modulators such as granulocyte macrophage-colony stimulating factor (GM-CSF), stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF) can be utilized as protective compounds against the deleterious effects of radiation [18]. Further, mitochondria-targeted antioxidants like gramicidin-S-derived nitroxide (JP4-039) and triphenylphosphonium 19 (SKQ1), have also been demonstrated to have radioprotective properties [19,20].

There is currently limited data from studies focused on gene therapy approaches, however, manganese-superoxide-dismutase-plasmid-liposomes (MnSOD-PL) have been shown to provide radioprotection in esophageal, lung and bone marrow tissue [21]. Therapeutic applications of most of these agents are hindered, however, due to their severe side effects, which include nausea, vomiting, hypotension and neurotoxicity, which limits the potential clinical use of these drugs [22]. Following the limited success in deriving a synthetic radio-protective drug suitable for clinical use, there has been an unprecedented increase in the screening of plant resources for radioprotective properties in recent years, in the hope that a plant natural compound may prove effective [23,24].

**Podophyllum hexandrum** is a recognized medicinal plant frequently used in traditional Indian medicine as a treatment for cancers, warts, and condylomas. Recent studies have validated the radioprotective potential of *P. hexandrum* extracts in mice and human cells. These results suggest that *P. hexandrum* and its constituents, protect the lungs, gastrointestinal tissues, hemopoietic system, and testis by modulating DNA repair pathways, apoptosis inhibition, free radical scavenging, metal chelation, anti-oxidation, and anti-inflammation properties (Fig. 1). In this review, we summarize, (1) the current knowledge and developments with regard to ionizing radiations and their impacts on biological systems, and (2) describe the mechanistic and radioprotective roles of the medicinal plant, *P. hexandrum* and its constituents.

2. **Podophyllum hexandrum: an important medicinal plant**

*Podophyllum hexandrum* (Himalayan Mayapple, a high-altitude plant) is a shade-loving, erect, glabrous, succulent herbaceous plant that is found in the Himalayan areas of India and China up to 2500–4000 m above sea level. The plant roots, rhizome, leaves and fruit are all widely used in Chinese and Ayurvedic medicine to treat a range of ailments [25], such as Hodgkin’s lymphoma, monocytoid leukemia, bacterial and viral infections, veneral warts, rheumatoid arthritis associated with limb numbness and various pyogenic infections of skin tissue [26]. Further, studies have confirmed that *P. hexandrum* can be utilized as an effective treatment against radiation-induced cell and tissue damage [27].

3. **Traditional uses**

*Podophyllum* has long been known for its medicinal importance and is described as an Aindri or Divine drug in traditional Indian, Ayurveda, Unani, Siddha, Tibetan and Chinese medicine [28] (Table 1). In Indian folklore, *Podophyllum* is used as purgative, emetic, cytotoxic and anti-tumor medication [29,30]. Within the Western medical system, it is used as a laxative, while the aqueous extract of roots and leaves is used by southern Indians as a cathartic treatment [31]. In the Jammu and Kashmir regions, people use a root and rhizome powder for wound healing, as an antiseptic and to treat various skin diseases [33,35]. Rhizome and root paste can also be taken orally for the alleviation of diarrhea, gastritis, cholera, ulcers and dyspepsia [46,47]. *Podophyllum* is also mentioned as an anthelmintic treatment by Llyod [48]. *Podophyllum* fruit with other plant parts are taken by people of Ladakh to combat renal and urinary disorders, notably in controlling urine discharge and bleeding/inflammation of the kidney [49]. It is also used in the treatment of fever and cough in China and by Indian Vaidyas [40]. In addition, whole plant or plant parts are used as a blood purifier, hepatic stimulant, and in the treatment of asthma, joint pain, jaundice, liver and heart problems [32,50,51]. Tibetan and Indians also use plant parts or eat fruits to cure gynecological and menstrual conditions [41,52]. Furthermore, it is used in cancer, tumor, and wart treatment by Indian local Vaidyas [33,40].

4. **Active constituents of Podophyllum**

Extensive chemical investigation of *Podophyllum* species has revealed the presence of a resin called podophyllin, which contains several lignans containing pharmacological properties. These include podotoxin, epipodophyllotoxin, podophyllotoxone, flavonoids such as quer cetin, quer cetin-3-glycoside 4-demethyl podophillotoxin, podophyllotoxin glucoside, 4-dimethyl podophyllotoxin glucoside, kaempferol-3-glucoside, deoxy podophyllotoxin, picropodophyllotoxin, isopicropodophyllone, 4-Methyl deoxy podophyllotoxin, α-peltatin and S- peltatin (Fig. 2). The rhizomes and roots of the plant
contain anti-tumor lignans such as podophyllotoxin and podophyllotoxin 4-O-glucoside [53]. Among these non-alkaloid lignans, podophyllotoxin is considered to be the most important, it is currently utilized in the semi-synthesis of the anti-cancer drugs etoposide, teniposide, and etoposide, which were developed as less toxic derivatives of podophyllotoxin [54]. Etoposide, a glycoside of podophyllotoxin, was for example, first synthesized in 1966 and U.S. Food and Drug Administration approval was granted in 1983. Podophyllotoxin was first isolated in 1880 by Valerian Podwyssotzki. In addition to its anti-cancer activity, this molecule also possesses a large number of other medical applications, as it can inhibit the replication of both cellular and viral DNA by blocking the activity of enzymes integral to DNA replication. In addition, Podophyllotoxin can destabilize microtubules blocking cell division, thus it is also considered an antimitotic drug [55].

Ecogeoographical and seasonal variations have been reported in the podophyllotoxin content of different Podophyllum species. American Podophyllum has been shown to contain 4–5% podophyll resin, whereas Indian species contain 7–16% [56]. The highest percentage of resin obtained from this plant is recorded to be 20%, accrued in May-June during its flowering stage. Thus, Indian Podophyllum when collected at the peak season contains 2.5 times more resin compared to its American counterpart [57,58].

5. Survival studies

Animal survival studies are one of the most useful experiments to...
determine the radiation protection of a compound as they yield both a qualitative and quantitative measure of radioprotective ability [59]. Podophyllotoxin was partially fractionated from the Podophyllum hexandrum rhizome in order to study the acute toxicity and maximum tolerance of a single intraperitoneal dose. The maximum tolerant dose was found to be 60 mg/kg body weight, while a 90 mg/kg body weight dose produced toxicity resulted in the death of 50% of the mice, as observed for 72 h post-treatment. In the irradiated (10 Gy) group a drastic decline in survival was observed as all mice died within 12 days. A dose range of 15–20 mg/kg body weight when administered 2 h before radiation (10 Gy) resulted in a 66% survival rate and 10–15 mg/kg body weight administration yielded 90% survival [60].

Fig. 2. Chemical structures of the active constituents present in Podophyllum species.
REC-2001, a semi-purified fraction of *P. hexandrum* rhizome at a dose of 10 and 15 mg/kg body weight, exhibited more > 90% survival in 10 Gy whole-body irradiated Swiss albino strain “A” mice. However, a single dose of up to 75 mg/kg body weight did not cause death [61]. The results of this study demonstrate that low doses of podophyllotoxin provide maximum survival in lethally irradiated mice. In another experiment, a sub fractionated extract of *P. hexandrum* rhizome G-001 M, was observed to provide more than 90% survival in 10 Gy whole-body lethally irradiated mice, at a dose of 6 mg/kg body weight when administered intramuscularly [62]. In *Saccharomyces cerevisiae*, when pre-treated with a whole-plant extract of *P. hexandrum* at a dose of 2.5–5.0 µg/ml of 200–600, the Gy post irradiated cell culture showed a survival rate 2 times greater in the group pre-treated with podophyllotoxin, compared to untreated controls [63].

6. Cytoprotective properties

The number of splenocyte cells (located in the spleen) decreased in the mice following 2 Gy irradiation. The rhizome of *P. hexandrum* or RP-1, a fractionated extract of *P. hexandrum* rhizome, was shown to lower this rate of decrease. Splenocytes at 72 h post-irradiation were not affected after RP-1 treatment (up to 50 µg/ml) but a higher dose of 135 µg/ml decreased survival. The study illustrates that RP-1 (1–50 µg/ml) significantly protects against the radiation-induced decrease in splenocyte survival, in a dose-dependent manner [64].

![Podophyllum mediated radiation protective pathways](image)

Fig. 3. Schematic hypothetical diagram shows *Podophyllum* mediated radiation protection pathways.

6.1. Apoptosis inhibition

Protein expression analysis revealed that intraperitoneal administration of REC-2000 in mice irradiated with 10 Gy γ radiation up-regulated the Bcl-2 expression by 2.28–2.34 times in bone marrow compared with non-treated mice. This indicates the anti-apoptotic potential of *P. hexandrum*. *Podophyllum* treatment pre-irradiation reduced the occurrence of apoptotic bodies in the mouse jejunum crypt cells in a time-dependent manner, and was very prominent at the 84 h stage; the mitotic arrest was also observed up to 24 h [83]. Further, the *Podophyllum* extract REC-2006 was found to strongly inhibit the cleavage of ATM and PARP-1, the expression of nuclear apoptosis-inducing factor, the release of cytochrome c, and restore the expression of ICAD in human hepatoma cell HepG2, when treated 2 h before irradiation. Significant inhibition of Apoptotic protease activating factor 1 (Apaf1), caspase-9 and caspase-3 were also observed, further confirming the apoptosis inhibition efficacy of *Podophyllum* [79]. In the liver of Swiss albino mice, an ethanolic extract of podophyllum was administered at a
Experimental studies establishing radioprotective roles for *Podophyllum hexandrum*.

<table>
<thead>
<tr>
<th>Plant extract/ fraction</th>
<th>Plant part used</th>
<th>Standard drug</th>
<th>Active dose</th>
<th>Radiation dose</th>
<th>Model cell line /animal</th>
<th>Increase</th>
<th>Decrease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydro-alcoholic extract</td>
<td>Rhizome REC- 2000</td>
<td>200 mg/kg b. wt.</td>
<td>10 Gy at dose rate 0.54 rad/sec</td>
<td>Swiss albino strain A mice</td>
<td>Hemoglobin content 21.25%, total leukocyte content 83.33 times</td>
<td>–</td>
<td>[65]</td>
<td></td>
</tr>
<tr>
<td>1:9 DMSO: distilled water dissolved</td>
<td>Rhizome G-002M</td>
<td>200 µl/mice</td>
<td>7.0 Gy dose rate 0.925–0.828 cGy/sec</td>
<td>Strain ‘A’ bronchoalveolar lavage fluid (BALF)</td>
<td>Macrophage, lymphocyte, neutrophil count</td>
<td>DNA damage</td>
<td>[66]</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Rhizome REC 2006</td>
<td>15 mg/kg b. wt.</td>
<td>10 Gy at 0.312 Gy/m</td>
<td>Swiss albino A</td>
<td>β globin gene expression</td>
<td>DNA damage</td>
<td>[67]</td>
<td></td>
</tr>
<tr>
<td>Aqueous-ethanolic extract</td>
<td>Rhizome</td>
<td>500 µg/ml</td>
<td>0.25 kGy at dose rate 3.40 kGy/h</td>
<td>Swiss albino strain ‘A’ mice</td>
<td>Protection of nural and renal system</td>
<td>Lipid peroxidation</td>
<td>[68]</td>
<td></td>
</tr>
<tr>
<td>DMSO dissolved</td>
<td>Rhizome G-002M</td>
<td>0.2–0.4 µg/ 5 ml culture</td>
<td>1.5 Gy at 0.977–0.767 Gy/min</td>
<td>Human peripheral blood lymphocytic</td>
<td>DNA damage</td>
<td>DNA damage</td>
<td>[69]</td>
<td></td>
</tr>
<tr>
<td>Fractionated extract</td>
<td>Rhizome G-002M</td>
<td>2.5 mg/kg b. wt.</td>
<td>9 Gy at 1.08 Gy/min–0.955 Gy/min</td>
<td>Female C57BL/6 mice</td>
<td>85% survival, haematopoietic &amp; gastrointestinal organs protection</td>
<td>Free radical generation, lipid peroxidation, protein carbonylation</td>
<td>[70]</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome RP-1</td>
<td>0.2 ml/mice</td>
<td>2 Gy at 1.03–0.97 Gy/min</td>
<td>Swiss albino strain ‘A’ mice</td>
<td>Apoptosis frequency in bone marrow</td>
<td>Micronuclei frequency</td>
<td>[64]</td>
<td></td>
</tr>
<tr>
<td>Whole aqueous extract</td>
<td>Rhizome RP-1</td>
<td>200 mg in 0.8 ml/kg b. wt.</td>
<td>2 Gy at dose rate 0.977 cGy/s</td>
<td>Sprague-Dawley rats in utero</td>
<td>Protection against damage</td>
<td>Post-natal physiological alteration</td>
<td>[71]</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome RP-1</td>
<td>200 mg/kg b. w.</td>
<td>10 Gy at 0.8 Gy/min</td>
<td>BALB/c mice</td>
<td>82% survival CD4+ and CD8+ T cells, titer of interleukin (IL) – 1, IL-3 and IgG’s in the serum</td>
<td>NO free radical generation</td>
<td>[72]</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome RP-1</td>
<td>0.1 and 1 µg/ m; 5 and 10 µg/ml 0.2 ml 200 mg/kg b. w.</td>
<td>1.5 Gy at 0.516 Gy/min</td>
<td>Human HepG2 cell</td>
<td>–</td>
<td>Leakage of electrons from ETS, lipid peroxidation</td>
<td>[73]</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome Drug G-003M</td>
<td>200 µl/ animal 6.5 mg/kg b. wt</td>
<td>10 Gy at 0.42–0.36 Gy/m 9 Gy at 1.08 ± 0.955 Gy/min</td>
<td>Inbred Swiss Albino strain ‘A’ MaleC57BL/6J</td>
<td>89% survival, (G- CSF), (IL-6) levels</td>
<td>ROS, NO generation, prostaglandin E2, intestinal apoptosis, inflammation</td>
<td>[75]</td>
<td></td>
</tr>
<tr>
<td>Alcoholic fraction</td>
<td>Rhizome REC- 2001</td>
<td>200 mg/kg b. w.</td>
<td>10 Gy at 0.099 Gy/s, 1.78 Gy/s</td>
<td>Swiss Albino strain ‘A’</td>
<td>–</td>
<td>DNA fragmentation and lipid peroxidation</td>
<td>[108]</td>
<td></td>
</tr>
<tr>
<td>Podophyllotoxin: rutin – 1:2</td>
<td>Rhizome REC- 2001</td>
<td>1 and 15 mg/ kg bwt</td>
<td>10 Gy at 0.56 cGy/s</td>
<td>Swiss Albino strain ‘A’</td>
<td>&gt; 90% survival</td>
<td>Lipid damage</td>
<td>[61]</td>
<td></td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome –</td>
<td>200 mg/kg b. w.</td>
<td>10 Gy at 2 cGy/s</td>
<td>Swiss Albino</td>
<td>Glutathione S-transferase, superoxide dismutase</td>
<td>–</td>
<td>[76]</td>
<td></td>
</tr>
<tr>
<td>Fractionated extract</td>
<td>Rhizome G-002M</td>
<td>1 µg/ml of blood 1–10 µg/ml</td>
<td>1–10 Gy at 0.897–1.0 Gy/min dose rate 43.8 cGy/s</td>
<td>Human blood lymphocytes</td>
<td>–</td>
<td>Double stand break</td>
<td>[77]</td>
<td></td>
</tr>
<tr>
<td>Chloroform extract</td>
<td>Rhizome REC- 2006</td>
<td>1–107 µg/ ml</td>
<td>10 Gy at 3.7 Gy at 43.8 cGy/m</td>
<td>Human hepatoma cell line-Hep-G2 (p53’), Hep3B (P53’)</td>
<td>Cell cycle arrest at G1 phase, encouraging cell proliferation and DNA repair</td>
<td>P53 expression</td>
<td>[78]</td>
<td></td>
</tr>
<tr>
<td>Fractionated extract</td>
<td>Rhizome REC- 2006</td>
<td>107 µg/ml</td>
<td>10 Gy and 3.7 Gy at 43.8 cGy/m</td>
<td>Human hepatoma cell line-Hep G2 (p53’), Hep3B (P53’)</td>
<td>Increase survival</td>
<td>Inhibit apoptosis proteins</td>
<td>[79]</td>
<td></td>
</tr>
<tr>
<td>Podophyllotoxin: rutin – 1:2</td>
<td>Rhizome G-003M</td>
<td>150 µl per mouse at 6.5 mg/kg b. wt.</td>
<td>9 Gy at 0.9864 Gy/m</td>
<td>Female strain ‘A’</td>
<td>&gt; 85% survival</td>
<td>Apoptosis in bone marrow &amp; spleen</td>
<td>[80]</td>
<td></td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>Rhizome REC- 7005</td>
<td>200 mg/kg b. wt.</td>
<td>10 Gy at 0.73 cGy/s</td>
<td>Swiss albino strain ‘A’</td>
<td>Cell proliferation proteins</td>
<td>Apoptosis inducing factor, DNA damage</td>
<td>[81]</td>
<td></td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>Rhizome INM- 2001</td>
<td>200 mg/kg b. wt.</td>
<td>10 Gy at 43.8 cGy/s</td>
<td>Swiss albino strain ‘A’ liver</td>
<td>Apoptosis protein</td>
<td>Hemolysis, free radical scavenging, lipid oxidation</td>
<td>[82]</td>
<td></td>
</tr>
</tbody>
</table>
| Fractionated extract | Rhizome REC- 2001 | 20 mg/kg b. w. | 10 Gy ay 0.336 Gy/m | Swiss albino strain ‘A’ | Crypt cell survival | Apoptotic body | [83] | (continued on next page)
dose rate of 200 mg/kg 2 h before 10 Gy irradiation. Modulation of protein expression associated with apoptosis by the induction of heat shock factor-1 (HSF-1) was observed, which up-regulated heat shock protein-70 (HSP-70), thus inhibiting NF-κB translocation, resulted in an increase in proliferating cell nuclear antigen (PCNA) along with Bcl-2. The abundance of apoptotic inducing factor (AIF), p53, and caspase-3 were also found to be reduced [81].

### Genoprotection and DNA repair

Mutagenic ionizing radiation causes a variety of damage to the DNA of exposed cells such as single-strand breaks, double-strand breaks, DNA–DNA and DNA protein cross-links and damage to nucleotide bases [87–89] (Fig. 4). *P. hexandrum* fractionated extract REC-2006, reduced p53 expression when administered 2 h before irradiation. Induction of the potent cyclin-dependent kinase inhibitor p21 down-regulates the expression of cyclin E and cyclin-dependent kinase 2 (CDK2), leading to a delay in the G1 phase of liver-derived HepG2 cells, which allows time

![Diagram](https://example.com/diagram.png)

**Fig. 4.** Schematic diagram showing possible radiation-induced damage following ionizing radiation.

<table>
<thead>
<tr>
<th>Plant extract/fraction</th>
<th>Plant part used</th>
<th>Standard drug</th>
<th>Active dose</th>
<th>Radiation dose</th>
<th>Model cell line/animal</th>
<th>Increase</th>
<th>Decrease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>Rhizome</td>
<td>RP-1</td>
<td>200 mg/kg b. w.</td>
<td>0.5, 2.0, 5.0 and 10 Gy at 0.94 rad/s</td>
<td>Swiss albino strain 'A'</td>
<td>Testis weight, tubules, primary spermatocyte, stem cell survival, sperm counts</td>
<td>Sperm morphology abnormalities</td>
<td>[84]</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>Rhizome</td>
<td>G-001M</td>
<td>200 mg/kg b. wt.</td>
<td>10 Gy at 0.94 rad/s</td>
<td>Swiss albino strain 'A'</td>
<td>Antioxidant enzyme activity, thiol content</td>
<td>Lipid peroxidation</td>
<td>[85]</td>
</tr>
<tr>
<td>Fractionated extract</td>
<td>Rhizome</td>
<td>G-001M</td>
<td>6 mg/kg b.w.</td>
<td>10 Gy at 0.47–0.40 cGy/s</td>
<td>Swiss albino strain 'A'</td>
<td>Survival, DNA protection, immunity</td>
<td>Apoptotic activity, lipid peroxidation</td>
<td>[62]</td>
</tr>
<tr>
<td>Alcoholic extract</td>
<td>Rhizome</td>
<td>G-001M</td>
<td>6 mg/kg b.w.</td>
<td>10 Gy at 0.37–0.3 cGy/sec</td>
<td>Strain A mice Jejunum</td>
<td>Intestine mitotic index, antioxidant activity</td>
<td>[86]</td>
<td></td>
</tr>
<tr>
<td>Fractionated extract</td>
<td>Rhizome</td>
<td>G-002M</td>
<td>200 μl/mouse</td>
<td>9 Gy at 0.792 Gy/m</td>
<td>Strain A mice</td>
<td>Bone marrow and spleen recovery, DNA protection</td>
<td>Hematopoietic aplasia and chromosomal aberrations, apoptotic protein, free radical scavenging</td>
<td>[27]</td>
</tr>
<tr>
<td>Extract</td>
<td>Whole plant</td>
<td>–</td>
<td>2.5–5.0 μg/ml</td>
<td>200–600 Gy</td>
<td>Saccharomyces cerevisiae</td>
<td>Cell survival</td>
<td>–</td>
<td>[63]</td>
</tr>
</tbody>
</table>
for DNA repair. This process provides significantly higher protection against acute γ-radiation in the HepG2 cell line [78]. The DNA damage protective role was observed in irradiated human blood leukocytes pre-treated with G-002 M, a mixture of three active derivatives isolated from the rhizomes of 
P. hexandrum. A reduction in γH2AX and P53BP1 biomarker levels and elevated ligase IV levels confirm suppression in radiation-induced DNA double-strand breaks (DSBs). Samples pre-treated with G-002 M and then irradiated also showed significant upregulation of DNA-dependent protein kinase, catalytic subunit (DNA-PKcs) and Ku80 (a sensor of DNA DSBs)-the two key DNA damage repair proteins, and decreased in ATM kinase and tumor suppressor p53-binding protein 1 (53BP1) expression levels that serve as the markers for DNA damage. Thus, suggesting that G-002 M plays a protective role against DNA damage that may be attributed to its ability to scavenge free radicals or assist in DNA repair in irradiated cells [77]. Different concentrations of REC-2001, administered to peritoneal macrophages pre-exposure to γ-rays, in the concentration range of 25–200 g/ml, significantly countered radiation-induced DNA fragmentation [90]. REC-2006, a bioactive fractionated extract from the rhizome of 
P. hexandrum, induced repair of radiation-induced DNA damage in murine thymocytes of mice in vivo [67]. Administering REC-2006 at a radioprotective concentration, (15 mg kg⁻¹ body weight) 1 h before whole-body exposure of animals with γ-rays (10 Gy) irradiation, resulted in a time-dependent reduction in DNA damage, evident as a decrease in relative nuclear spreading factor (RNSF) values 6.156 ± 0.576, 1.647 ± 0.534 and 0.496 ± 0.012, and an increase in β-globin gene amplification 36%, 95% and 99%, at 0, 15 and 60 min, respectively [67]. G-002 M was also found to protect DNA in human peripheral blood lymphocytes when administered 1 h before 5 Gy irradiation in culture media containing blood. This experiment demonstrates that G-002 M has the ability to arrest cells in the G2/M stage and also has the potential to decrease dicentric chromosomes,acentric fragments,micronuclei, nucleoplasmic bridges and nuclear buds in binucleate cells. This further supports the effectiveness of G-002 M in minimizing radiation-induced DNA damage [69].

6.3. Radioprotection of the mitochondrial system

The γ-radiation has been shown to alter the structure and physiology of mitochondria via oxidative stress, DNA damage, and modification of lipids and proteins. This ultimately leads to an increase in the leakage of electrons and enhances the accumulation of superoxide anions [91]. Whole-body 10 Gy γ-irradiated mice when treated with RP-1 (200 mg/kg body weight) 2 h of irradiation superoxide (O²⁻) generation reduced within 4 h and thiobarbituric acid reactive substance (TBARS, an indicator of lipid peroxidation levels), protein carbonyl formation reduced up to 24 h. RP-1 γ-irradiation also increased the glutathione level, which further increases complex I activity up to 16 h, at 4 h complex I/III and up to 24 h complex II/III activity and inhibited the mitochondrial membrane potential significantly increasing radioprotection of the mitochondrial system [74].

6.4. Radioprotection in spermatogenesis

P. hexandrum aqueous rhizome extract (RP-1) administered at a dose rate of 200 mg/kg body weight in mice 2 h before exposure to lower radiation doses of 0.5, 0.2, and 5.0 Gy, significantly increased the testis weight, primary resting spermatocytes, repopulating tubules, sperm count, and stem cell survival index. A reduction in sperm morphology abnormalities was also observed in comparison to non-treated irradiated mice [84]. Further investigation unraveled the molecular and cellular mechanism of RP-1 activity on the testicular system of lethally irradiated mice. RP-1 pre-treatment increased the glutathione reductase, glutathione peroxidase and glutathione S-transferase enzymes and protein contents in testicular tissues in comparison to the non-treated group.

7. Protection of physiological, organs and humoral systems

Accumulating evidence over the years revealed the protective activities of 
P. hexandrum against the radiation-induced threats to physiological and humoral systems.

7.1. Postnatal physiological alteration

Radiation-induced retardation of neurophysiological development was observed in post-natal young mice when exposed to 2 GY γ-irradiation in utero. A significant weight loss was observed in offspring, along with a delay in pinna detachment appearance [71]. Acquisition of reflexes such as surface righting, reflex suspension, visual placing, and negative geotaxis was also found to be delayed. An extraction of 
P. hexandrum, administered at a dose rate of 200 mg/kg body weight, pre-irradiation, protected against postnatal physiological alteration induced by the radiation exposure [71].

7.2. Gastrointestinal protection

Ionizing radiation is rarely used to treat localized gastrointestinal tumors, but when deemed necessary, it is used with great caution and care. When cancer of the colon, rectum, prostate and other closely linked sites are radiated, the gastrointestinal tract can become exposed to scattered radiation [92]. Histological examination of a jejunum pre-treated with G-002 M, a combination of three active principles: Podophyllotoxin, podophyllotoxin β-D-glucoside and rutin, revealed less damage to the villi, crypts and mucosal layer compared to non-treated mice [70]. In another experiment, considerable morphological improvement of the jejunum was observed. The jejuna damages were attenuated, the mucosal and submucosal layers were found relatively intact, the crypts were viable and improved, the villi found intact and an increase in crypt-villus height was observed in G-003 M pre-treated C57BL/6 J mice irradiated with 9 Gy when compared with untreated mice [75].

7.3. Radioprotection against lung injuries

Radiation induces several histopathological changes in the lung through increased ROS/RNS. These changes are often characterized by varying degrees of inflammation leading to lung injuries such as pneumonitis and fibrosis, which can be fatal [93,94]. Bronchoalveolar lavage fluid of mice exposed to 7 Gy γ-radiation after pre-treatment with G-002 M, showed a decrease in the number of neutrophils from 16% to 9%, decreased ROS, and NO generation, in addition to decreased nitric oxide synthase activity compared to the control. Histopathological examination showed non-significant infiltration of lymphocytes into the alveoli, thus edema and pneumonitis pathologies were found minimal or nil [66].

7.4. Protection of the hemopoietic system

Hydro-alcoholic extract of 
P. hexandrum rhizome, REC-2000 has been found to restore the hemoglobin content and total leukocyte count in 10 Gy lethally irradiated mice compared to control mice on day 15 post-exposure. The hemoglobin content significantly increased by 21.25% in the Rec-2000 pre-treated irradiated mice on day 10, and the total leukocyte count increased by 83.33 times compared to the control group. Enhanced expression of heme oxygenase-1 protein further supports the hemopoietic recovery process/hypothesis in irradiated mice treated with REC-2000. The in-vitro investigations also revealed that REC-2001, a semi-purified extract of low altitude 
P. hexandrum, significantly reduced radiation-induced hemolysis by 48.18% in Strain A mice. Protection of the hemopoietic system was observed following the administration of REC-200, at a dose of 20 mg/kg body weight, 30 min before 10 Gy lethal irradiation [82]. Loss of hemopoietic
myelosuppression and regeneration of bone marrow progenitor cells was found in mice given G-002 M, a novel formulation prepared with a combination of three active principles/compounds isolated from *Podophyllum* within 30 days [27]. Similarly, the white blood cell, red blood cell and hemoglobin content were found to be increased when mice were treated with G-003 M before being radiated with 9 Gy γ-radiation. Dutta et al., 2015, showed that the white blood cell, red blood cell, lymphocyte, neutrophil and platelet count did not show any significant change after 72 h in the group of lethally irradiated mice treated with G-002 M, however, the count increased on day 21, with values rising to the level of the control group. G-00 M mediated induction of hematopoietic recovery such as an increase of G-CSF and IL-6 also exhibited in lethally irradiated mice by Kalita et al., 2016.

7.5. Protection of Immune system/cells

Treating mice with the aqueous extract RP-1, from *P. hexandrum* (200 mg/kg body weight) 2 h before 10 Gy irradiation, was shown to increase the population of CD4+ and CD8+ T-cells and bone marrow GM-CFU compared to solely irradiated mice. RP-1 recovered the reduction of the titer of IL-1, IL-3 and various IgG’s isotypes in the serum of mice, which indicates the immunostimulatory potential of *Podophyllum* [72].

7.6. Anti-inflammation properties

The NF-κB and its effector proteins cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), play a vital role in radiation-induced inflammation. G-003 M restricted the nuclear translocation of inducible nitric oxide synthase (iNOS), play a vital role in radiation. Dutta et al., 2015, showed that the white blood cell, red blood cell, lymphocyte, neutrophil, and platelet count did not show any significant change after 72 h in the group of lethally irradiated mice treated with G-002 M, however, the count increased on day 21, with values rising to the level of the control group. G-00 M mediated induction of hematopoietic recovery such as an increase of G-CSF and IL-6 also exhibited in lethally irradiated mice by Kalita et al., 2016.

8. Mode of radioprotective action

8.1. Free radical scavenging

Methanolic and chloroformic fractions of *Podophyllum* with varying aryl-tetralin lignins were examined for their peroxide scavenging properties. At the 48 h time interval, the maximal peroxyl ion scavenging potential of methanolic extract observed was 45.88% at 1000 mg/ml and for chloroformic extract was found to be 41% at 1000 mg/ml. In the non-site-specific assay, the chloroformic extract exhibited ROS scavenging potential of 73.12% inhibition at 500 mg/ml. NO scavenging occurs in a dose-dependent manner and shows maximum inhibition of 58.40 ± 0.8% at 0.0125 mg/ml [95]. RP-1 application 4–8 h before 2 Gy radiation in HepG2 cells reduced radiation-induced ROS at a dose of 5 µg/ml and also reduced the NO level in a dose-independent manner [73]. Further, an aqueous extract of *P. hexandrum* at a concentration of 300 µg/ml inhibited peroxide generation by 81.16% and HZO₂ accumulation by 67.51%, supporting a role for RP-1 as a strong ROS scavenger [96].

The rhizome extract of *P. hexandrum* showed significant 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging, reduced reactive hydroxyl radical-mediated DNA damage and lipid peroxidation, and increased antioxidant enzyme activities in rat liver microsomes [96]. There is evidence that the rhizome aqueous extract of *P. hexandrum* could reduce the chemokine CCL4-induced hepatotoxicity, lipid peroxidation and suppression of alanine transaminase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities. However, the aqueous extract exhibited cytotoxic effects on some human leukemia cell lines [97].

8.2. Metal ion chelating

The radioprotection potential of *Podophyllum* by iron chelation was investigated by Kumar et al. using chelating agent 2, 2’-bipyridyl and potassium thiocyanate [98]. By the formation of chromogen, the chelation of iron in Fe²⁺ and Fe³⁺ was observed. Fe²⁺ was found to be more efficiently chelated, 36.5% compared to Fe³⁺ which was 30%, relative to control mice when Podophyllum extract was applied at a dose rate 40 µg/ml.

8.3. Lipid peroxidation inhibition

Lipid peroxidation was shown to be significantly lowered following pre-treatment of RP-1 in radiated HepG2 cells in a dose-dependent manner but in a time-independent manner (1, 5 and 10 µg/ml, –2 h) [73]. In 10 Gy whole-body irradiated mice, the lipid peroxidation was measured in terms of the malondialdehyde (MDA) content as µM/mg protein, and it was found to be decreased between 2 h and 24 h after pre-administration of G-001 M [86]. Aqueous ethanolic extract of *Podophyllum* contains 3-O-β-D-galactoside of querectin, found to protect linoleic acid degradation induced by Fe²⁺ and Cu²⁺ and therefore protects against lipid peroxidation in the presence of 250 Gy super lethal γ-radiation. The protection against lipid peroxidation was found to be higher in neural tissues than kidney in mice [95]. *P. hexandrum* was also found to inhibit FeSO₄ induced lipid peroxidation in a dose-dependent manner and obtained the highest inhibition, 92%, with a concentration of 1000 µg/ml in liver homogenate in terms of TBARS, an indicator of malondialdehyde (MDA) content, a key marker of lipid peroxidation [98]. Ganje et al. also found significant decreases in lipid peroxidation levels in the lung and kidney homogenate of mice treated with an aqueous extract of *Podophyllum* for 15 days post-exposure [96].

8.4. Effect on antioxidant molecules

RP-1 *P. hexandrum* aqueous extract treatment at a low concentration of 1 µg/ml, elicited a maximum accumulation of the key tripeptide antioxidant, glutathione (GSH) at 2 h and 5 µg/ml, after 4 h in 2 Gy post-irradiated HepG2 cells. When treated at a concentration of 10 µg/ml, at 8 h the GSH level decreased significantly by 1.298 ± 0.016 µg/mg, compared to the control groups [73]. Further, GSH level depletion by the chemokine CCL4 was found to be ameliorated by the aqueous extract of *Podophyllum* in lung and kidney homogenates of mice [96].

8.5. Antioxidant enzyme modulation

The activities of antioxidant enzymes including catalase (CAT), glutathione S-transferase (GST) and superoxide dismutase (SOD) were investigated in mice liver, jejunum and ileum cells following treatment with the aqueous radioprotector, *Podophyllum*. CAT activity in the liver did not yield a significant result but in the jejunum and ileum, depressed activity in comparison to the control groups was demonstrated. GST activity was found to be enhanced by *Podophyllum* in pre-treated (200 mg/kg body weight) lethally irradiated mice live at 12 h post-irradiation and SOD activity was enhanced in the liver and jejunum of mice [76]. Similarly, Verma et al. also measured the CAT, glutathione reductase (GR) and GST expression in mice liver, lungs and serum and found significant regression in G-002 M pre-treated mice following irradiation [99]. An aqueous extract of *Podophyllum* at higher concentrations, 50 mg/kg dose regime, increased the glutathione peroxidase (GPX) level by 49.30%. GR and SOD activity also increased by a significant margin [96].

9. Conclusions and future perspectives

Humans are now more frequently being exposed to ionizing radiation and its damaging effects as a consequence of developments in
The root, rhizome, leaves, and fruit are the main sources of podophyllotoxin and research as a potential radio-protective treatment. Fusarium solani, Phialocephala fortinii and reduced production costs. Several medicinal plants have been utilized due to their effectiveness in protecting against radiation-induced damage such as Ginkgo biloba, Mentha arvensis, Podophyllum hexandrum, Emblica officinalis, Centella asiatica, Aloe barbadensis and Morinda oleifera [100].

**Podophyllum** in particular, has enormous medicinal importance. The healing properties of this plant have long been exploited by traditional medicine and more recently **Podophyllum** has become the subject of intense interest and research as a potential radio-protective treatment. The root, rhizome, leaves, and fruit are the main sources of podophyllotoxin and other active compounds, which have extensive pharmacological properties. In addition, an abundance of endophytes is beginning to gain attention. Researchers have isolated the novel potential of preventing the deleterious effects of ionizing radiation in clinical settings and other radiative environments [63,68,72,76,78,81,85,90,95,101]. These findings have been validated in both mice and human cell lines, which provides further evidence of the clinical suitability of **Podophyllum** as an effective drug for human use. These experiments also provide information regarding the mode of action, physicochemical properties and effective doses/dosing of this radioprotectant.

However, **Podophyllum** is marked as an endangered plant species in the Red data book, as a result of over-exploitation, as this plant is highly sought-after, being valued for its medicinal properties. At present two species are available: the American mayapple (**Podophyllum peltatum**) and Himalayan mayapple (**Podophyllum hexandrum**). The Indian species is very rare and scarcely populated in the western part of the Himalayas, disappearing completely from some areas. The long juvenile phase and poor fruit setting ability of **Podophyllum** also result in the limited availability of this plant, thus mass cultivation has been negatively affected. Environmental conditions such as low temperature and high altitude are major factors impacting the podophyllotoxin content. For these reasons, researchers are aiming to propagate this plant in vitro and break the seed dormancy artificially. As somaclonal variation is a major problem within in vitro culture, this may result in wide variations in the content of podophyllotoxin in the generated plants. Therefore, efforts must be made to both maintain germplasm and generate genetically stable lines.

As with the generation of **Podophyllum**, obtaining sufficient Podophyllotoxin by natural harvest from **Podophyllum** species is also problematic, due to their limited populations. Further, the molecular complexity of natural products such as Podophyllotoxin, with multiple chiral centers typically precludes chemical synthesis or make this approach economically unviable. In addition, little is known to date regarding the details of the biosynthetic pathway of podophyllotoxin, precluding both the engineering of this pathway in **Podophyllum** species and also synthetic biology approaches in heterologous organisms such as Saccharomyces cerevisiae or Escherichia coli. Therefore, alternative approaches are rapidly required to provide a robust source of Podophyllotoxin for the growing list of applications in biomedicine for this key natural product, including its utility in protecting from ionizing radiation. For industrial podophyllotoxin production, biosynthesis by endophytes is beginning to gain attention. Researchers have isolated Fusarium solani, Phialocephala fortinii and Trametes hirsuta and Mucor fragilis like fungi which can produce 29–49.3 µg/gm dry weight of podophyllotoxin [102–105]. In addition, cultured plant cells, with production being scaled as required, may provide an effective solution [94,106]. This strategy has already been successfully applied to the production of paclitaxel, a blockbuster anticancer drug [107].

In sum, extracts from **Podophyllum** species have been shown to convey significant protection against ionizing radiation in animals, cultured cells, and humans [100]. To date, these organisms and their associated molecules provide an underexploited resource for plant-based radio-protective compounds against ionizing radiation in both medical and environmental settings, with lower side effect profiles and reduced production costs relative to alternative chemical interventions.

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**Conflict of interest statement**

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