Original Paper

Radioprotective activity of *Polyalthia longifolia* standardized extract against X-ray radiation injury in mice

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**A B S T R A C T**

The radioprotective effect of *Polyalthia longifolia* was studied in mice. *P. longifolia* treatment showed improvement in mice survival compared to 100% mortality in the irradiated mice. Significant increases in hemoglobin concentration, and red blood cell, white blood cell and platelet counts were observed in the animals pretreated with leaf extract. Pre-irradiation administration of *P. longifolia* leaf extract also increased the CFU counts of the spleen colony and increased the relative spleen size. A dose-dependent decrease in lipid peroxidation levels was observed in the animals pretreated with *P. longifolia*. However, although the animals pretreated with *P. longifolia* exhibited a significant increase in superoxide dismutase and catalase activity, the values remained below normal in both liver and the intestine. Pre-irradiation administration of *P. longifolia* also resulted in the regeneration of the mucosal crypts and villi of the intestine. Moreover, pretreatment with *P. longifolia* leaf extract also showed restoration of the normal liver cell structure and a significant reduction in the elevated levels of ALT, AST and bilirubin. These results suggested the radioprotective ability of *P. longifolia* leaf extract, which is significant for future investigation for human applications in developing efficient, economically viable, non-toxic natural and clinically acceptable novel radioprotectors.

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**Introduction**

The use of radiation for therapeutics, scientific research and energy production among others always raises the problem of radiation hazards to living beings. Ionizing radiation generates various reactive oxygen species in a biological system, by the radiolysis of water, which can damage several cellular components and biomolecules. Hence, there is a great need to protect humans against the deleterious effects of ionizing radiation by pharmacological intervention. The search for new radioprotectors to combat irradiation is ongoing and medicinal plants have often been targeted as part of this search because they contain an abundance of potentially active secondary metabolites with good antioxidant activity. Moreover, natural radioprotective agents, such as cystine, cysteamine, 5-hydroxytryptophan, 5-hydroxytrytamine, glutathione, and vitamins like A, C, and E, have been extensively studied [1]. In addition, a few important synthetic molecules have been used as radioprotective agents. However, the inherent toxicity of these agents at the radioprotective concentration warrants further search for new safer and effective radioprotector agents with a new mode of action. However, the fact remains that there is not a single radioprotective drug available that meets all the prerequisites of an ideal radioprotector, i.e., produces no cumulative or irreversible toxicity, provides effective long-term protection, remains stable for a number of years without losing shelf life, and can be easily administered [2]. In view of this, the search for less toxic and more potent radioprotector drugs continues.

Hence, the current study was designed to evaluate the *Polyalthia longifolia* var. angustifolia Thw. (Annonaceae) leaf methanolic extract as a radioprotector in *vivo*. A recent study by Jothy et al. [3] reported the antioxidant activity and hepatoprotective potential of *P. longifolia*. The results of this study revealed that *P. longifolia* leaf extract could protect the liver against paracetamol-induced oxidative damage. They also reported that the observed hepatoprotective
activity of *P. longifolia* in their study might be due to its antioxid-
dant activity, resulting from the presence of phenolic compounds in
the extracts. Jothy et al. [4] also tested the genotoxic potential
against H2O2-radical-mediated DNA damage and acute oral toxicity
of *P. longifolia* leaf. The results of the *in vitro* tests in their study
demonstrated that *P. longifolia* leaf was devoid of a significant

genotoxic effect under experimental conditions. Their *Allium cepa*
assay results showed that, applied in lower concentrations, the meth-

anol extract of *P. longifolia* leaf could be important for maintaining
the genetic stability of the organism. An acute oral toxicity study
revealed that *P. longifolia* leaf extract was safe after oral administra-
tion as a single dose to female albino Wistar rats with up to
5000 mg/kg body weight. Hence, we propose that any possible ra-
diation as a single dose to female albino Wistar rats with up to
5000 mg/kg by oral route are regarded as being safe or practically
non-toxic. Hence, different doses of the extract were adminis-
trated to mice through the oral (p.o.) route. The control animals
received Tween 20 (10% v/v) alone.

Materials and methods

**Plant sample collection**

Fresh leaves of *P. longifolia* were collected from various areas in
Universiti Sains Malaysia, Penang, in March 2013, and authenti-
cated at the Herbarium of the School of Biological Sciences, Universiti
Sains Malaysia, Pulau Pinang, Malaysia, where a voucher speci-
men was deposited (Voucher specimen: USM/HERBARIUM/11306)
for future reference.

**Preparation of *P. longifolia* leaf extract**

The powdered dried (in an oven at 60°C for 7 days) sample (100 g)
was soaked in methanol (400 mL) for 7 days under room temper-
ature, 28°C. The whole extract was filtered and the methanol was
evaporated from the filtrate by a rotary evaporator (Buchi, Swit-
zterland) at 40–50°C to form a paste mass. Then the extract was dried
in the oven at 60°C to get a thick paste form. The crude extract was
sealed in a Petri plate and stored at room temperature, 28°C. The crude
extract was prepared by diluting the paste in methanol and
stored in airtight bottles at 4°C for further studies. For standard-
ization purposes, quantification of Rutin was performed using the
LC–MS/MS system.

**Quantification of rutin**

The extract produced was standardized with reference to the
amount of Rutin content using the Agilent 1200 series Rapid Res-
olution liquid chromatography (RRLC) system tandem with an Agilent
6520 Accurate-Mass quadrupole time of flight mass spectrometer
(QTOF-MS) (Agilent Technologies, USA). The amount of Rutin in
*P. longifolia* leaf methanolic extract was quantified using the peak
area calculated from the equation that was obtained from the cal-
ibration curve of the standard reference Rutin.

**Administration of plant extract**

The *P. longifolia* leaf extract was dissolved in Tween 20 (10% v/v)
and given at the dose rates of 250 and 500 mg/kg body weight for
15 consecutive days before irradiation. The dose of the extract was
selected on the basis of experiments conducted to determine the
LD50 value of *P. longifolia* leaf extract in an acute oral toxicity study
[4]. The treatment dose of *P. longifolia* was determined as 1/10 of
LD50 value, that is, 500 mg/kg. Two doses such as 250 and 500 mg/kg

body weight were used in this study to investigate whether the extract exhibits concentration dependent activity. According to the
study by Kennedy et al. [7], substances with LD50 values higher than
5000 mg/kg by oral route are regarded as being safe or practically
non-toxic. Hence, different doses of the extract were adminis-
trated to mice through the oral (p.o.) route. The control animals
received Tween 20 (10% v/v) alone.

**Animals**

The adult male Swiss albino mice used in the experiments
weighed between 30 and 35 g. All mice were acclimatized for at least
1 week prior to dosing in constant environmental conditions with a
10/14 h light/dark cycle. They were fed standard diet and given
drinking water ad libitum [8]. The Institutional Animal Ethics Com-
mittee of Universiti Sains Malaysia, Penang, Malaysia, approved
the animal study (USM/Animal Ethics Approval/2011/74 (365)) for this
project.

**Irradiation**

Unanesthetized mice were restrained in a specially designed well-
ventilated acrylic box (14.5 cm × 23.5 cm) and exposed to whole-
body radiation from 6 MV, X-ray teletherapy facility (VARIAN 3100
Hansen Way, Palo Alto, CA 94304, USA) at the Radiotherapy and Onc-
ology Department, Low Guanlye Hospital, Penang, Malaysia, at a
doze of 10 Gy with a source-to-surface distance (SSD) of 100 cm
(1.33 Gy/min of dose rate).

**Experimental design**

**Whole body survival studies**

To determine the effect of *P. longifolia* leaf extract on the sur-

vival of mice against lethal whole body irradiation, the survival rate
for 30 days was investigated. The mice were distributed into four
groups of 20 animals each and randomly selected before being
marked on the tail for individual identification, as follows:

- **Group I:** Control (administered with distilled water alone or
  Tween 20 (10% v/v).
- **Group II:** X-ray irradiation alone (10 Gy).
- **Group III:** 250 mg/kg *P. longifolia* leaf extract plus X-ray irradi-
  ation (10 Gy).
- **Group IV:** 500 mg/kg *P. longifolia* leaf extract plus X-ray irradi-
  ation (10 Gy).

**Clinical signs observations**

The surviving animals were weighed daily and observed for any
clinical signs or symptoms of radio-toxicity and for mortality for
up to 30 days post-irradiation; the data were expressed as a per-
centage of survival [9]. The clinical visual observations included signs
of illness, changes in the skin and fur, eyes and mucous mem-
branes, epilation, facial edema, irritability, lethargy, diarrhea and
behavioral pattern.
Hematological study
To study the effect of *P. longifolia* on radiation-induced alteration on peripheral hematological cell counts, blood was drawn from the heart of the mice in each group in a vial containing 0.5 M EDTA. The Hemoglobin content (Hb), Red Blood Count (RBC) and White Blood Count (WBC) were studied in peripheral blood drawn from the heart of the mice on the 1st, 4th, 7th and 20th post irradiation day for studying the hematopoietic effects of radiation. Hematological analyses were performed at Gribbles Pathology (M) Sdn. Bhd., Penang, Malaysia.

Spleen colony-forming units (CFU-S) assay
For the endogenous spleen colony-forming units (CFU-S) assay, the animals were sacrificed by cervical dislocation on the 11th post-treatment day and spleens were removed, weighed and fixed in Bouin’s fixative for 24 h. The macroscopic nodules that appeared on the spleen surface, and visible to the naked eye, were scored individually [10].

Biochemical estimations of endogenous antioxidant
The animals in each group were sacrificed by cervical dislocation on the 1st, 4th, 7th and 20th post irradiation day. The livers were dissected and stored in ice-cold saline. The whole liver was blotted dry, weighed and a 10% homogenate was prepared with an ice-cold 150 mM potassium chloride (KCl) buffer (pH 7.4) using a homogenizer. The homogenate was used for assaying lipid peroxidation, while the remainder was centrifuged at 10,000 x g for 30 min at 4°C and the supernatant was used for analyzing the specific activities of superoxide dismutase (SOD) and catalase [11].

Lipid peroxidation (LPx) assay
The LPx was estimated using the method recommended by Pryor [12]. The reaction mixture comprised 0.2 mL of liver homogenate, 1.8 mL of Tris buffer (pH 7.4), 0.5 mL of 30% trichloroacetic acid (TCA) and 0.5 mL of 52 mM thiobarbituric acid (TBA), heated for 30 min at 95°C and was cooled in ice immediately. It was then centrifuged for 10 min at 5000 rpm and the absorbance was read at 531 nm using a UV-visible spectrophotometer. The LPx was expressed as nmol malondialdehyde (MDA) per mg of protein.

Superoxide dismutase (SOD) assay
The SOD activity was measured according to the method recommended by McCord and Fridovich [13] based on the SOD mediated inhibition of NBT formazan formation from NBT in the presence of an O2^- generator, phenazine methosulfate (PMS). The reaction was initiated by the addition of PMS to a 50 mM Tris 0.1 M NaCl buffer (pH 8.3) containing 0.20 mL of 500 mM NBT, 0.20 mL of 780 mM NADH and suitable aliquots of the supernatant to make a final volume of reaction mixture of 2 mL. The increase in absorbance was followed for 5 min at 560 nm. The activity of SOD in the samples was determined from a calibration curve drawn by taking the amount of inhibition of NBT formazan formation in the presence of aliquots of the sample versus absence of the sample (100% NBT reduction). One unit of enzyme activity was defined as the amount of SOD required to cause a 50% decrease in NBT reduction. Data were expressed as units of SOD activity per mg of protein.

Catalase assay
The catalase activity was determined spectrophotometrically according to the method recommended by Claiborne [14]. The reaction mixture (2 mL) contained 1.95 mL of 10 mM H2O2 (30%) in a 80 mM phosphate buffer (pH 7.0). The reaction was started by adding 0.05 mL of the supernatant and the reaction mixture was incubated for 5 min at 37°C. The phosphate buffer (pH 7.0) was used as a reference. The decomposition of hydrogen peroxide was monitored by recording the absorbance against a blank at 240 nm. The data were expressed as mmoles H2O2 consumed per min per mg of protein.

Macropathology and histopathology
The animals were sacrificed by cervical dislocation on the 10th post irradiation day for studying the macropathology and histopathology of the small intestine, spleen and liver. The organs were examined macroscopically for the post irradiation adverse effects before being preserved in a fixation medium of 10% solution of buffered formalin for histopathological study. The sections were embedded in paraffin, and 5 μm thick sections were cut with a rotary microtome and stained with hematoxylin and eosin. The total number of crypts, goblet cells, dead cells in the small intestine were observed and compared with the experimental and control groups. The status of the mucosal erosion, basement membrane and villus height was also assessed [7]. The number of megakaryocytes in the spleens was observed under light microscopy. The liver architecture was also observed to study the protective effects of *P. longifolia* leaf extract on the X-ray irradiation-induced liver damage in mice.

Statistical analysis
All values are expressed as mean ± SD. Comparisons between groups were performed using one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests using SPSS statistical software. A p-value of <0.05 was considered significant. The whole body survival data were analyzed by Kaplan–Meier survival curves.

Results
Standardization of *P. longifolia* leaf methanolic extract
Quantification of rutin
Rutin in *P. longifolia* leaf methanolic extract was identified and confirmed by matching of retention time and mass spectra with the Metlin database and purchased marker standard (Rutin). Rutin gives a well-distinguished peak at mean retention times of 13.168 and 13.191 minutes in *P. longifolia* leaf methanolic extract and the standard respectively (Fig. 1a and b). As for the standardization, the quantitation of rutin was based on the peak area calculated from the calibration curve ($y = 275885x, r^2 = 0.9977$). The amount of rutin present was found to be 8.96 μg (0.896%) in 1000 μg of *P. longifolia* leaf methanolic extract.

Whole body survival studies
Exposure to X-ray irradiation resulted in death in 100% of mice in Group II within 14 days (Fig. 2). However, the pretreatment of mice with *P. longifolia* leaf extract (250 mg/kg and 500 mg/kg in Group III and Group IV, respectively) caused a remarkable improvement in their survival and body weight. In the treated groups, the mice survived for a longer time (20 days and 27 days in Group III and Group IV, respectively) compared with the untreated Group II. Moreover, according to the findings, *P. longifolia* leaf extract exhibited a dose-dependent manner of radioprotective activity (Fig. 2).

All mice survived until the end of the experimental period in the control Group I (Fig. 2a).

Clinical signs observations
Epilation, hair loss, loss of appetite, facial edema, irritability, lethargy and diarrhea were observed in the mice in Group II (Fig. 3). The food and water consumption of the X-ray irradiated mice, which were measured throughout the study, were also decreased compared to the control mice. In contrast, treatment with *P. longifolia* leaf extract provided protection against the toxic effect of X-ray irradiation in the pretreated mice with *P. longifolia* leaf extract (250 mg/kg and 500 mg/kg in Group III and Group IV, respectively)
in a dose-dependent manner. A significant reduction in the X-ray irradiation toxic effect on the appearance and clinical signs was observed when the concentration of *P. longifolia* leaf extract increased from 250 mg/kg to 500 mg/kg compared to Group II (Fig. 3). The consumption of food and water of the X-ray irradiated mice pretreated with *P. longifolia* leaf extract was improved amazingly compared to Group II. No toxic symptoms or mortality was observed in control Group I.

**Hematological study**

**Hemoglobin (Hb)**

In the irradiated group II (X-ray irradiation alone) mice, the Hb level decreased continuously up to the 7th post-irradiation day, and the data for later periods could not be collected because all mice had died by that time in this group. *P. longifolia* leaf extract treatment depressed the Hb levels during the first 4 days of treatment, but, later, it gradually increased up to the 20th post-irradiation day in a dose-dependent manner. In summary, the amount of Hb obtained was in the following order: Group I > Group IV > Group III > Group II. Hence, control Group I exhibited the highest level of Hb compared to other groups (Fig. 4a).

**Red blood cell (RBC)**

Irradiation decreased the RBC count sharply up to 7 days in group II (X-ray irradiation alone) and the data for the 20th day could not be obtained because of the death of all animals by that time in this group. *P. longifolia* leaf extract treatment decreased the RBC count during the first 4 days of treatment, but, later, it gradually increased up to the 20th post-irradiation day in a dose-dependent manner.

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**Figure 1.** LC–MS/MS chromatograms of (a) *P. longifolia* leaf methanolic extract and (b) standard rutin.
manner. In synopsis, the RBC count obtained was in the following order: Group I > Group IV > Group III > Group II. Therefore, control Group I showed the highest RBC count compared to other groups (Fig. 4b).

White blood cell (WBC)
Irradiation decreased the WBC count sharply up to 7 days in group II (X-ray irradiation alone) and the data for the 20th day could not be obtained because of the death of all animals by that time in this group. *P. longifolia* leaf extract treatment decreased the WBC count during the first 4 days of treatment, but, later, it gradually increased up to the 20th post-irradiation day in a dose-dependent manner. In synopsis, the WBC count obtained was in the following order: Group I > Group IV > Group III > Group II. Therefore, control Group I showed the highest WBC count compared to other groups (Fig. 4c).

Platelets
Irradiation decreased the platelet count sharply up to 7 days in group II (X-ray irradiation alone) and the data for the 20th day could not be obtained because of the death of all animals by that time in this group. Treatment with *P. longifolia* leaf extract decreased the platelet count during the first 4 days of treatment, but, later, it gradually increased up to the 20th post-irradiation day in a dose-dependent manner. In synopsis, the platelet count obtained was in the following order: Group I > Group IV > Group III > Group II. Therefore, control Group I showed the highest platelet count compared to other groups (Fig. 4d).

Spleen colony-forming units (CFU-S) assay
Exposure to X-ray irradiation did not result in the formation of any spleen colony. Conversely, pre-X-ray irradiation treatment with *P. longifolia* leaf extract rendered significantly higher (*p* < 0.05) CFU-S counts in comparison with the irradiated and control groups. The CFU-S count in the spleen increased with increasing *P. longifolia* leaf extract concentration from 250 mg/kg to 500 mg/kg (Fig. 5i). No CFU-S were observed in control Group I.

Biochemical estimation of endogenous antioxidant
Lipid peroxidation (LPx)
Figure 6 depicts a steady increase in LPx up to 7 days both in liver and intestine in the X-ray irradiated Group II; data for the 20th day could not be obtained because of the death of all animals by that time. Conversely, pre-X-ray irradiation treatment with *P. longifolia* leaf extract (Group III and Group IV) gradually increased the LPx during the first 4 days of treatment; however, later, it decreased the LPx up to the 20th post-irradiation day in a dose-dependent manner.
in both the liver and intestine. Control Group I showed the lowest level of LPx compared to other groups.

**Superoxide dismutase (SOD)**

Whole body X-ray irradiation of mice resulted in declined SOD in the X-ray irradiated Group II up to 7 days; data for the 20th day could not be obtained because of the death of all animals by that time (Fig. 6). However, although in the beginning it showed a decrease in the SOD activity up to four days, the administration of *P. longifolia* leaf extract (Group III and Group IV) 30 days prior to X-ray irradiation resulted in a significant increase in SOD activity in a dose-dependent manner in both the liver and intestine at 20 days of post-treatment. Control Group I showed the highest level of SOD activity compared to other groups.

**Catalase activity**

A significant decrease in catalase activity was observed in whole body X-ray irradiated animals up to 7 days; data for the 20th day could not be obtained because of the death of all animals by that time (Fig. 6). The treatment of mice with 250 and 500 mg/kg body weight with *P. longifolia* leaf extract (Group III and Group IV, respectively) for 30 days before exposure to X-ray irradiation resulted in a significant increase in catalase activity in a dose-dependent manner both in the liver and intestine at 20 days of post-treatment. Control Group I showed the highest level of SOD activity compared to other groups.

**Macropathology and histopathology**

**Spleen**

Exposure to X-ray irradiation resulted in remarkable changes in the spleens of mice at the 10th day post-irradiation, as manifested by a 60% decrease in relative spleen size compared to the control mice (Fig. 5ii). These results are also associated with a 75% decrease in the number of megakaryocytes compared to the control mice. The spleens of mice exposed to X-ray irradiation revealed the absence of melanin pigments, hyperplasia in the melanomacrophage cells, and an increased amount of vacuolation (Fig. 5ii). *P. longifolia* leaf extract treatment prior to X-ray irradiation provided full protection of the relative size, as well as the number of megakaryocytes in the spleen in a dose-dependent manner (Fig. 7). When mice were treated with *P. longifolia* leaf extract prior to exposure to X-ray irradiation, the histopathological lesions, such as hyperplasia in the melanomacrophage cells, and an increased amount of vacuolation, were not observed in the splenic tissues and the spleens appeared similar to the control group, showing an increase in melanin (Fig. 7).

**Small intestine (ileum)**

Exposure to X-ray irradiation resulted in severe dilation of the ileum compared to the control mice (Fig. 5iii). On autopsy, we also observed an abnormal accumulation of feces in the ileum tract of the X-ray irradiated mice without *P. longifolia* leaf extract treatment (Fig. 5iii). The incidence of this severe dilation of the ileum was significantly reduced by the administration of *P. longifolia* leaf extract prior to exposure to X-ray irradiation in a dose-dependent manner. Figure 7A,B shows normal Group I mouse ileum villous architecture in which the mucus cells are seen in the full length of the villi. Figure 7 shows that the X-ray irradiated Group II mouse crypts are not uniform, distorted, and depopulated in cells. The villi are shortened, fused, and eroded. Inflammatory infiltration and focal hemorrhage are noted in the lamina propria. However, treatment of mice with 250 and 500 mg/kg body weight with *P. longifolia* leaf extract (Group III and Group IV, respectively) for 30 days before exposure to X-ray irradiation resulted in regeneration of the mucosal crypts and villi. The crypts are increased in number and the villi are longer and less distorted compared to those in the radiation alone group (Group I). In the 500 mg/kg body weight with *P. longifolia* leaf extract treatment group, there is nearly complete regeneration of the crypts and villi.

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Protective effects of \textit{P. longifolia} leaf extract on X-ray irradiation-induced liver damage in mice

Histopathologic liver sections of control Group I showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein (Fig. 7A,B). Figure 7C,D illustrates the liver of X-ray irradiated mice (Group II) showing wide necrosis across the cells. The liver sections of these mice showed cytoplasm disintegration, and vacuolization, pyknosis, area of necrosis, hemorrhage, fatty change and inflammation could be seen. The hepatocytes are disrupted and sinusoids are also damaged. Figure 7E,H show the histological architecture of the livers of X-ray irradiated mice pretreated with \textit{P. longifolia} leaf extract (250 mg/kg (Fig. 7E,F) and 500 mg/kg (Fig. 7G,H). The examined sections from Group III, which were pretreated with 250 mg/kg of \textit{P. longifolia} leaf extract, showed no remarkable pathological changes and the hepatocytes were at a recovery stage (Fig. 7E,F). However, the examined section from group IV, which was pretreated with 500 mg/kg of \textit{P. longifolia} leaf extract as radioprotectors, showed a trend toward lowering the incidence of hepatic pathological changes induced by X-ray irradiation, a remarkable restoration of normal cell structure. The cells retained their regularity and size, with normal homogeneous cytoplasm and rounded nucleus (Fig. 7G,H). A significant reduction in the X-ray irradiation toxic effect on the liver architecture was observed when the concentrations of \textit{P. longifolia} leaf extract increased from 250 mg/kg to 500 mg/kg compared to Group II (Fig. 7D). The findings of the liver histopathology analysis corroborated the results of the biochemical analysis. In the same manner, data on ALT, AST and bilirubin level revealed that exposure to X-ray irradiation resulted in a significant elevation of ALT, AST and bilirubin level in the serum compared to control Group I, while pretreatment with varying doses (250 mg/kg and 500 mg/kg) of \textit{P. longifolia} leaf extract before radiation (Groups III–VI, respectively) led to a significant reduction in the elevated levels of ALT, AST and bilirubin compared to the X-ray irradiated mice (Group II) (Table 1).

Discussion

The trend in the use of alternative and complementary healthcare has prompted scientists to investigate the various biological activities of medicinal plants. Many such medicinal plants have hepatoprotective, neuroprotective, radioprotective, anti-inflammatory, genoprotective and also antioxidant or radical-scavenging properties [15]. Thus, in recent years in the field of radioprotection, major emphasis has been placed on the use of medicinal plants with various therapeutic values. Therefore, the present study was conducted to elucidate in vivo the radioprotective potential of the \textit{P. longifolia} leaf extract.

For the standardization, rutin is one of the phenolic compounds that was chosen as a marker compound since many studies suggested that rutin is the active chemical constituent present in many plants. Rutin (quercetin-3-rhamnosyl glucoside), a natural flavone derivative, was first discovered in buckwheat in the 19th century. It is...
a low molecular weight polyphenolic compound that is widely distributed in medicinal plants. Rutin has been widely used in treating disease; its several pharmacological activities include antiallergic, anti-inflammatory and vasoactive, antitumour, antibacterial, antiviral and antiprotozoal properties [16]. It has also been found to prevent gastric mucosal ulceration in animal models including restraint stress [17]. It was used as a marker compound for the standardization of *Fumaria vaillantii* Loisel by Rajopadhye and Upadhye [18].

The results of the current study revealed that *P. longifolia* leaf extract could reduce the symptoms of radiation sickness compared to the irradiated controls. Exposure to radiation leads to hair loss, loss of appetite, facial edema [19], diarrhea, nausea, vomiting and inflammation. The abdomen is one of the body parts most vulnerable to radiation injury, and the gastrointestinal syndrome after a large dose of total-body irradiation is one of the major causes of acute radiation lethality [20]. Hence, this explained the final cause of death for the mice, as depicted in Fig. 2A in this study. Plants and their active ingredients that have anti-emetic and anti-inflammatory activity could provide good radioprotection under such circumstances [21]. Recently, Mandal et al. [22] reported that the ethanolic and aqueous extracts of *P. longifolia* leaf produced anti-inflammatory activity due to the presence of flavonoids and phenolic compounds at various time intervals, which was evaluated using the Carrageenan-induced paw edema model. They found that the ethanolic and aqueous extracts of *P. longifolia* reduced the edema induced by Carrageenan. They also reported that *P. longifolia* might produce the anti-inflammatory activity at all three phases of inflammation by inhibiting more than one mediator. This could explain the radioprotective ability of the *P. longifolia* leaf extract to protect against X-ray irradiation-induced sickness and increase the survival of mice administrated with *P. longifolia* leaf extract prior to exposure to X-ray irradiation in this study.
The results of the present study indicate that pretreatment with *P. longifolia* leaf extract protects the hematopoietic tissues in mice from the lethal effects of ionizing X-ray radiation. Animals pretreated with *P. longifolia* leaf extract and X-ray irradiated exhibited less relative spleen size reduction compared to their respective irradiation alone groups, which further confirmed the protection of hematopoietic tissue in the mice by the extract. The mature blood cells (monocytes, platelets, RBCs and granulocytes) are functionally radioresistant to moderate doses of radiation. In contrast to other elements in the blood, lymphocytes are extremely radiosensitive and have been suggested as a biological dosimeter [23]. In addition to the loss from the circulation, morphological changes rapidly appear in the lymphoid tissues, such as the spleen and thymus, which quickly decrease in size. Therefore, the changes in the size of spleen seen in this investigation may be due to alterations induced in the lymphocyte production centers by radiation. It was evident that *P. longifolia* leaf extract pretreatment elevated the number of radiation induced endogenous spleen colonies (S-CFU) and increased the relative spleen size. The increment of the S-CFU count in the spleen of the *P. longifolia* leaf extract treated irradiated mice in comparison to irradiated control indicates the role of the *P. longifolia* leaf extract in protecting the hematopoietic stem cells and/or stimulating the proliferation of the surviving cells. *P. longifolia* leaf extract, which enhances stem cell proliferation, can therefore yield an appreciable recovery of the damaged tissue following radiation exposure and, thereby, contribute to the survival of the mice pretreated with *P. longifolia* leaf extract. Similar results were observed during the histopathological study of the spleen as *P. longifolia* leaf extract with irradiation showed an early recovery compared to their respective irradiation alone groups. The megakaryocytes are bone marrow cells that are responsible for the production of blood platelets, which are necessary for normal blood clotting [24]. X-ray irradiation decreased the number of megakaryocytes in the spleen. However, treatment with *P. longifolia* leaf extract protected the blood platelet levels at 1 week post-irradiation, which was associated with the increase in the normalization of the number of megakaryocytes.

In this study, a significant deficit in the hematological constituents of peripheral blood in mice of the X-ray irradiation alone group was observed. This might be due to the direct destruction of mature circulating cells, loss of cells from the circulation by hemorrhage, or leakage through capillary walls and a loss of the production of cells [25]. Hence, the hematological parameter levels were decreased during the first 4 days but, later, it gradually increased up to the 20th post-irradiation day in a dose-dependent manner.

**Figure 6.** Effect of *Polyalthia longifolia* administration on lipid peroxidation (LPx), superoxide dismutase (SOD) and catalase activity in Swiss albino mice (A) small intestine and (B) liver. Control; 10 Gy whole body irradiation; 500 mg/kg of *Polyalthia longifolia* b.w. + 10 Gy whole body irradiation; 250 mg/kg of *Polyalthia longifolia* b.w. + 10 Gy whole body irradiation. Data expressed as mean ± SD.
Figure 7. Histopathological demonstration of the protective effect of *Polyalthia longifolia* in the spleen, small intestine (Ileum) and liver of irradiated mice. Photomicrographs of spleen section stained with H&E staining (Magnification ×20 to ×40). A, B: Control; C, D: 10 Gy whole body irradiation; E, F: 250 mg/kg of *Polyalthia longifolia* b.w. + 10 Gy whole body irradiation; G, H: 500 mg/kg of *Polyalthia longifolia* b.w. + 10 Gy whole body irradiation. Megakaryocytes; ER, Erythrocyte; →Macrophages pro-erythroblasts; CV, central vein; SS, Sinusoid; HC, hepatocyte; HR, hemorrhage; PK, cytoplasmic disintegration and pyknotic nuclei; NC, necrosis of the hepatocytes; N, nucleus; I, infiltration of the inflammatory cells.

Table 1
Effect of *P. longifolia* leaf extract on liver marker enzymes and serum bilirubin content.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (Group I)</th>
<th>10 Gy whole body irradiation (Group II)</th>
<th>250 mg/kg of <em>P. longifolia</em> b.w. + 10 Gy whole body irradiation (Group III)</th>
<th>500 mg/kg of <em>P. longifolia</em> b.w. + 10 Gy whole body irradiation (Group IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/L)</td>
<td>35.31 ± 2.16</td>
<td>108.75 ± 8.25**</td>
<td>51.87 ± 2.17*</td>
<td>41.21 ± 3.21*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>34.82 ± 4.17</td>
<td>98.24 ± 7.92**</td>
<td>42.15 ± 3.87*</td>
<td>38.72 ± 2.46*</td>
</tr>
<tr>
<td>Bilirubin (mg/L)</td>
<td>1.58 ± 0.7</td>
<td>9.21 ± 2.31**</td>
<td>4.10 ± 2.11*</td>
<td>3.12 ± 2.34*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Statistically significant compared to irradiated alone animals (P < 0.05).

** Statistically significant to control animals (P < 0.05).
Nevertheless, *P. longifolia* leaf extract pretreatment showed a gradual recovery of the hematological constituents of the peripheral blood of animals against X-ray irradiation exposure. This increase in the hematological constituents of peripheral blood in the mice of the pre-X-ray irradiation treated with *P. longifolia* leaf extract groups (Group III and Group IV) may possibly be due to accelerated hematopoietic regeneration, as discussed previously. The decrease in the levels of hematological parameters might due to the irradiation and the gradual increase in the levels of this parameter in the later days might be contributed by the hematopoietic tissues’ protective effects of *P. longifolia* leaf extract treatment which warrants further investigation. Moreover, *P. longifolia* leaf extract is a good radical scavenging antioxidant that interrupts the chain reaction of lipid per-oxidation by reacting with lipid peroxy radicals resulting in a highly significant increase in the WBC, RBC and Hb contents [3]. Jothy et al. [3] recently reported the antihyperlipidemic effect of methanolic extract of *P. longifolia* leaf on chicken liver homogenate by measuring the levels of malondialdehyde (MDA) that were produced, based on the acid-catalyzed decomposition of lipid peroxides. They confirmed that the antioxidant activity was due to X-ray irradiation. However, *P. longifolia* leaf extract pretreatment prior to X-ray irradiation inhibited the decline in the intracellular antioxidant enzymes in the liver and intestine. It is commonly accepted that X-ray irradiation induces free radical injury by converting superoxide anion (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$), thus preventing the formation of the hydroxyl radical (OH$^-$); furthermore, H$_2$O$_2$ can be removed by catalase by converting H$_2$O$_2$ into water and oxygen. Due to the inhibition of SOD, superoxide anion radicals are left to combine with nitric oxide to form peroxynitrite anion (ONOO$^-$), which initiates LPx. The decrease in the activities of antioxidant enzymes is in close relationship with the induction of LPx [26], in which the activities of SOD and catalase decline with the increase in LPx (Fig. 6). An increase in the endogenous antioxidant level by *P. longifolia* leaf extract may be responsible for the scavenging of radiation-induced free radicals including LPx, thereby protecting against radiation-induced mortality. Our previous study, which showed that *P. longifolia* leaf extract possessed significant antioxidant (free-radical scavenging) and hepatoprotective activity against various free radicals with a high content of polyphenol and flavonoid compounds, supports the above statement [3]. Such free radical scavengers have a key role in radioprotection because radiation induced sickness is mainly mediated through the generation of free radicals. Accordingly, free-radical scavenging appears to be a likely mechanism of protection by *P. longifolia* leaf extract for the observed radioprotective activity. Moreover, the earlier report on the genoprotective effect of *P. longifolia* leaf extract by Jothy et al. [4] on DNA damage induced by hydroxyl radicals indicated that the extract exerted a significant geno or mitodepressive protection effect. It is well established that ionizing radiation induces different types of lesion in the DNA. Thus, in this study, Jothy et al. [3,4] predicted that the antioxidant molecules in *P. longifolia* leaf extract might also play an important role in the prevention of genotoxic damage. Therefore, the genoprotective effect of *P. longifolia* leaf extract may have also rendered a radioprotective effect as observed in the present study.

After the hematopoietic system, the gastrointestinal tract is the most sensitive organ to the effects of irradiation and is the major site of injury during radiation therapy. Injury to the small intestinal tissues may be direct, as this tissue is particularly sensitive to radiation, as well as indirect as a result of radiation burns and bone marrow aplasia [27]. Our data indicate that the mouse intestines of the X-ray irradiation alone groups are significantly affected by acute radiation reaction. This may induce diarrhea in the radiation alone groups, which was observed in this study. In the present study’s histopathological observation, radiation exposure caused severe degenerative changes. The microvilli of the epithelial cells also showed changes in the length and frequency and alteration of the bordering membrane. These data are corroborated by previous studies reported by other investigators on radiation-induced intestinal injury in animals [28]. In our study, the administration of *P. longifolia* leaf extract before irradiation resulted in a decrease in the severity of the degenerative changes in the small intestine cells of mice than that observed in the irradiation only treated groups. Several researchers have confirmed that the natural antioxidants from medicinal plants scavenge the free radicals and protect the cellular organelle against the indirect effects of ionizing radiation [29]. Being a good antioxidant, it is quite possible that *P. longifolia* leaf extract might have scavenged various free radicals and inactivated them by electron donation/hydrogen transfer, as reported previously [3].

Conclusions

In summary, the present study shows the radio-protective effects of the *P. longifolia* leaf extract on X-ray irradiation-induced damage in mice. The mechanism of radioprotection by *P. longifolia* leaf extract on X-ray irradiation-induced damage which may involve free radical inhibition warrants further studies aimed at identifying the major bioactive ingredients in the extract.

Conflict of interest

The authors have no conflicts of interest concerning this article to declare.
Acknowledgments

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References