

Anticlastogenic, radiation antagonistic, and anti-inflammatory activities of *Persea americana* in albino Wistar rat model

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Abstract

The present study was undertaken to evaluate the anti-inflammatory activity and antigenotoxic effect of hydroalcoholic leaf extract of *Persea americana* (*P. americana*) in albino Wistar rats against whole body X-ray irradiation. Rats were orally administered with (25, 50, 100, 200, and 400 mg/kg body weight) of *P. americana* leaf extract for five days. On the fifth day after last administration, animals were exposed to whole body X-rays of 8 Gy. Based on Kaplan Meier's survival analysis, 100 mg/kg body weight was selected as an optimum dose for radioprotection. The radioprotective potential was analysed by bone marrow micronucleus test and comet assay in peripheral blood lymphocytes. Biochemical estimations were performed in liver tissue homogenates. DNA damage indicators analysed through comet assay displayed significant reduction in olive tail movement ($P < 0.01$), percentage DNA in tail ($P < 0.05$) and tail length ($P < 0.001$) in pretreated group when compared to radiation group. *P. americana* leaf extract restored the levels of reduced glutathione, catalase, and reduced the levels of lipid peroxidation, protein carbonyls, and cyclooxygenase-2 levels in liver homogenates of pre-treated group. Decrease in micronucleated polychromatic erythrocytes ($P < 0.05$) was witnessed in *P. americana* pretreated group when compared to radiation control. Pretreatment also resulted in the increase of animal survival with dose reduction factor of 1.28. Our findings for the first time demonstrated that *P. americana* offers significant protection to rats from whole body exposure to X-rays and helps in antagonising the radiation effects, thereby combating acute radiation induced damage in living systems.

Keywords: *Persea americana*; Albino Wistar rat; Ionizing radiation; DNA damage; Free radicals; Antioxidants

INTRODUCTION

It is a widely proven fact that majority of radiation induced damages is caused by interaction of free radicals with bio molecules. Exposure to ionizing radiation is unavoidable in nature. Ionizing radiation generates reactive oxygen species such as hydroxyl radical ($\bullet\text{OH}$), hydrogen peroxide (H_2O_2), superoxide anion ($\bullet\text{O}_2^-$), and reactive nitrogen species such as peroxynitrite (ONOO^-) and nitric oxide (1). Endogenous antioxidant enzymes such as glutathione peroxidase, catalase, manganese superoxide dismutase, and copper-zinc superoxide dismutase provide protection from ionizing radiation exposure (2). As hazardous effects of radiation have been confirmed through experimental evidences, there is a

requirement for the protection of humans against its deleterious effect through chemical and physical means. The Majority of chemical compounds were screened for the ability to protect against radiation induced damage, but due to their severe toxicity at optimum dosage the usage in clinical practice was restricted. Various natural and synthetic agents have been investigated in the past to evaluate radio protective activity. Yet, there is no ideal radio protector that could be used in clinical practice (3). Therefore, plants being rich sources of antioxidants are studied extensively in the recent years to evaluate their radioprotective potential.

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Persea americana (*P. americana*) also called as avocado or alligator pear, a tree native to Central America, cultivated in tropical and subtropical regions around the world, belongs to family Lauraceae. The leaf, bark, and fruits are used in traditional medicines to cure various ailments. Aqueous leaf extract of *P. americana* exhibited significant reduction in carrageen induced rat paw edema, displaying anti-inflammatory activity (4). Leaf and seed extracts of *P. americana* scavenged free radicals and also presented high total phenolic content *in vitro* (5). Hepatoprotective activity of leaf extract was documented against CCL₄ (carbon tetrachloride) induced rat liver damage (6). Some studies done on rats have demonstrated the antidiabetic activity (7) of the leaves confirming its traditional use in folk medicine. The aqueous leaf extract displayed significant vasorelaxant activity in isolated rat aorta (8). Antiulcer activity was documented against indomethacin and alcohol-induced ulcers (9). Antidiarrhoeal activity was also established in chloroform-methanol leaf extract of *P. americana* (10). The ethanol extract of dried leaves strongly suppressed the herpes simplex virus type 1 (HSV-1), adenovirus type 3 (ADV-3) and Aujeszky's disease virus (ADV) in cell culture (11). The methanolic and aqueous leaf extract of *P. americana* displayed antihypercholesterolemic activity by lowering blood plasma glucose and LDL levels and also restored HDL-CHOL (12). The diverse antioxidant, medicinal properties, and its widespread usage attributed to *P. americana* stimulated the authors to focus on the radioprotective effects of the plant by evaluating the antigenotoxic effect, which has not been reported so far and also readdresses the anti-inflammatory activities.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin, maygrunwald's stain, ethidium bromide, giemsa, high melting agarose, triton X 100, low melting agarose, histopaque 1077, dimethyl sulphoxide (DMSO), Qurecetin, trizma base, and EDTA were supplied from Sigma Aldrich (St Louis, USA). Rat cyclooxygenase-2 ELISA kit was obtained from Genxbio health sciences (Pvt

Ltd, India). NaOH, Na₂HPO₄, NaH₂PO₄, 5,5'-dithiobis (2 nitro benzoic acid) (DTNB), glacial meta-phosphoric acid, reduced glutathione, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA), hydrochloric acid, malonaldehyde, 2,4- dinitrophenyl hydrazine (DNPH), ethanol, ethyl acetate, guanidine hydrochloride, and H₂O₂ procured from Sisco research lab (Pvt Ltd, India). All other chemicals used were of analytical grade.

Animal care and handling

All *in vivo* studies were carried out on adult albino Wistar rats of either sex, eight to ten weeks old weighing 160 ± 10 grams. The experimental animals were obtained from central animal house, Kasturba Medical College, Mangalore (Reg. No. 213/CPCSEA). The animals were housed in polypropylene cages with paddy husk bedding. Throughout the experiment the animals were maintained at 25 ± 2 °C temperature and 50 ± 5 °C humidity with 12 h of dark-light cycles. Rats were provided with *ad libitum* access to laboratory food (commercial rat pellets from Vinod Ramkrishna Kulkarni nutritional solutions, India) and water. The present study was approved by the institutional animal ethical committee (IAEC), Manipal University (dated 14/06/2013).

Irradiation source and procedure

The albino Wistar rats were placed in a well-ventilated rectangular perspex box restrainer (18 cm × 18 cm width and 22.2 cm length, partitioned into 6 cm × 6 cm × 11.1 cm). Six rats were placed in a single restrainer and exposed to whole body radiation of X-rays. The source of radiation was LINAC accelerator (Linear accelerator), Department of Radiology, Kasturba Medical College, Attavar, Mangalore, India. The distance between the animals and beam exit point of LINAC accelerator was 100 cm. The dose rate was set to 3.5 Gy/min with field size of 40 × 40 cm².

Plant material and extraction

The tender leaves of *P. americana* were collected during the month of December 2015 from Madikeri, India. The authentication of plant material was done by Mr. Surendranath Joshi botanist from St Aloysius College,

Mangalore, Karnataka, India with a voucher specimen number (KFP 12758) deposited in herbarium JCB, Indian institute of science Bangalore. The hydroalcoholic leaf extract was prepared according to the procedure described by Suffness and Douros (13). The tender leaves were thoroughly washed in running tap water to remove impurities and kept in shade drying for 15 days. After appropriate shade drying the leaves were powdered by electric mixer. The hydroalcoholic extract was prepared by suspending 100 g of leaf powder in 500 mL of 50% methanol in water at 50 – 60 °C in a Soxhlet apparatus for 72 h. The crude extract obtained from Soxhlet apparatus was concentrated by rotary vacuum flash evaporator and stored at 4 °C for further usage.

Acute drug toxicity

Hydroalcoholic leaf extract of *P. americana* was dissolved in double distilled water. Animals were divided into four groups, each group consisting of six animals. The First group received double distilled water, which served as normal control group. The group II-IV received a single dose of 500, 1000, and 2000 mg/kg body weight of *P. americana* leaf extract orally once. Animals were provided with *ad libitum* food and water and observed for 14 days for any mortality.

Determination of optimum dose of *P. americana* for radioprotection

To calculate the optimum dose of *P. americana* for radioprotection, animals were divided into seven groups. In each group 10 animals were maintained. First group served as normal control where animals were administered with 0.01 mL/kg body weight of double distilled water. The II-VI groups received a single dose of (25, 50, 100, 200, and 400) mg/kg body weight of *P. americana* leaf extract orally for five days, respectively. Radiation control group was also maintained. Plant extract was administered orally to all the animals for 5 consecutive days. On the fifth day, 1 h after the last administration of *P. americana*, the animals from all the groups were exposed to 8 Gy of X-rays and monitored for their survival thereafter for 30 days. Dose

optimization was carried out by Kaplan Meier's survival study.

Dose reduction factor of *P. americana*

To determine the dose reduction factor (DRF) of *P. americana*, animals were divided into six groups. 10 animals were maintained in each group. Group I served as untreated controls and the experimental group II-VI were treated with the 100 mg/kg body weight of *P. americana* leaf extract once daily for five consecutive days. On the fifth day after the administration of extract animals were exposed to whole body X-rays of different dosages 7 Gy, 8 Gy, 9 Gy, 10 Gy, and 11 Gy. Animals were provided with *ad libitum* amount of food, water, and observed for mortality and radiation sickness for 30 days. The dose reduction factor was calculated according to the method of Miller and Tainter (14).

DRF

$$= \frac{\text{Mean lethal dose (LD}_{50/30}) \text{ of } P. americana + X - \text{rays}}{\text{Mean lethal dose (LD}_{50/30}) \text{ of normal control} + X - \text{rays}}$$

Experimental protocol

To study the radioprotective effect of *P. americana*, animals were divided into four groups, with six animals in each group (15). The Group I, normal control (rats were fed with 0.01 mL/kg body weight double distilled water); Group II, drug control (preadministration of 100 mg/kg body weight of *P. americana* for five days); Group III, radiation control (animals were exposed to sub lethal dose of X-rays i.e. 4 Gy); Group IV, preadministration of *P. americana* for five days followed by exposure to 4 Gy of X-rays.

All animals were euthanized 24 h post irradiation and dissected. Blood was collected from cardiac puncture and stored in sterile heparinised vacutainers. The blood was diluted with phosphate buffer saline pH 7.4. The diluted blood was layered on the top of histopaque 1077 and centrifuged for 20 min to get intermediate layer consisting of peripheral blood lymphocytes. The extent of DNA damage was assessed in lymphocytes by single cell gel electrophoresis. Femur and tibia bones were excised and bone marrow micronucleus assay was performed. The excised liver was

used to evaluate radioprotective potential of *P. americana* by performing biochemical parameters such as reduced glutathione, catalase, lipid peroxidation, protein carbonyls, and cyclooxygenase-2.

Preparation of tissue homogenates

Livers were perfused with chilled 0.9% saline. For biochemical estimations 10% liver homogenates were prepared with ice cold phosphate buffer saline pH 7.4 using a homogenizer (RO-1727A Remi Motors, India). Homogenization of liver tissue was done under prechilled conditions (15).

Biochemical assays

Lipid peroxidation was determined according to the procedure described by Buege and Aust (16). Malonaldehyde, a marker for oxidative stress was estimated by reaction with thiobarbituric acid. Glutathione content in the sample was measured by Ellman's method (17). Total reduced glutathione was measured by the production of yellow colour when sulfhydryl compound present in the sample reacts with DTNB. Catalase activity in the sample was measured according to the method described by Beers, *et al* (18). Catalase activity was measured by decomposition of hydrogen peroxide into water molecules with reduction in absorbance at 240 nm. The protein carbonyl in liver tissue homogenate was evaluated by method described by Levine, *et al.* (19) by DNPH method. The levels of rat's liver cyclooxygenase-2 were determined using specific enzyme linked immunosorbent assay kit (Genxbio rat COX-2 ELISA). The results were expressed in ng/mL.

Comet assay (single cell gel electrophoresis)

The method was followed according to technique described by Singh, *et al.* (20). Comet assay is a technique used to detect DNA strand breaks. Negatively charged DNA migrates towards positive end when subjected to electrophoresis. Frosted glass slides were layered with 400 μ L of 1% normal melting agarose and kept for solidifying. About 40 μ L of lymphocytes sample and 60 μ L of low melting agarose was mixed properly and poured above solidified normal melting

agarose. The slides were placed in a freezer (2 to 8 °C) for 10 min. After proper hardening of the gel the cells were sandwiched with 100 μ L of low melting agarose and allowed to cool. After proper solidifying of agarose, the slides were placed in prechilled lysing solution which comprised of 100 mM EDTA, 10 mM trizma base, 2.5 M NaCl, 1% Triton X and 10% DMSO and incubated overnight. After overnight incubation the slides were placed in electrophoresis tank. Electrophoresis buffer consisted of 300 mM NaOH and 1 mM EDTA (pH > 13). The samples were electrophoresed for 20 min at 24 volts and 300 mA. The samples were neutralized with neutralizing buffer (0.4 M tris, pH 7.4). The cells were stained with ethidium bromide. The cells were counted under the fluorescent microscope and DNA damage was analysed. Scoring of DNA damage was done by Tritex cometscore[™] freeware V. 1.5.

Micronucleus assay

Rat's bone marrow micronucleus test was carried out according to the procedure described by Schmidt (21). The bone marrow cells from femur and tibia were flushed with 1 mL of bovine serum albumin (5%). The suspension were mixed properly and centrifuged at 90 \times g for 10 min. The supernatant was removed and resulting pellet was mixed with 0.5 mL of bovine serum albumin to get thick suspension. A drop of suspension was layered on frosted glass slide and allowed to dry. The samples were methanol fixed and stained with may-grunewald's for 3 min. May-grunewald stain differentiates polychromatic erythrocytes from normochromatic erythrocytes. The samples were stained with giemsa for 10 min. Giemsa stains micronucleus appears dark blue in colour. The slides were observed under oil immersion objective for the presence of micronucleated polychromatic and normochromatic erythrocytes. About 2000 cells were scored per animal.

Statistical analysis

All results were expressed as Mean \pm SEM. Data were analysed by one-way analysis of variance (ANOVA) following post hoc test

Tukey using IBM SPSS statistics 20. $P < 0.05$ was considered significant.

RESULTS

Acute drug toxicity

Single oral administration of *P. americana* leaf extract at concentration 500, 1000, and 2000 mg/kg body weight did not display any symptoms of behavioural changes and mortality up to 14 day observation period. The plant extract was safe up to 2000 mg/kg body weight. Since the plant was nontoxic at dose of 2000 mg/kg body weight, 1/5th, 1/10th, 1/20th, 1/40th, and 1/80th of the dose was selected for further radiation dose optimization studies.

Determination of optimum dose of *P. americana* for radioprotection

Administration of a single dose of *P. americana* leaf extract at 25, 50, 100, 200, and 400 mg/kg body weight before 8 Gy whole

body irradiation of X-rays displayed 50%, 70%, 80%, 60%, and 40% animal survival, respectively. There was 0% survival in the radiation control group, when animals were observed 30 day's post irradiation. The maximum percentage survival was seen in animals pretreated with 100 mg/kg body weight of *P. americana* and same dose was chosen for further radioprotective studies (Fig. 1).

Dose reduction factor

There was an increase in the survival of animals in pretreated group when compared to the radiation group alone. The log dose of radiation is plotted in X-axis whereas probit values on the Y-axis. In this experiment LD_{50/30} of *P. americana* + X-rays were found to be 8.51. Pretreatment of *P. americana* resulted in increase of LD_{50/30} values by 1.91 Gy with dose reduction factor of 1.28. Dose reduction factor is represented in Fig. 2.

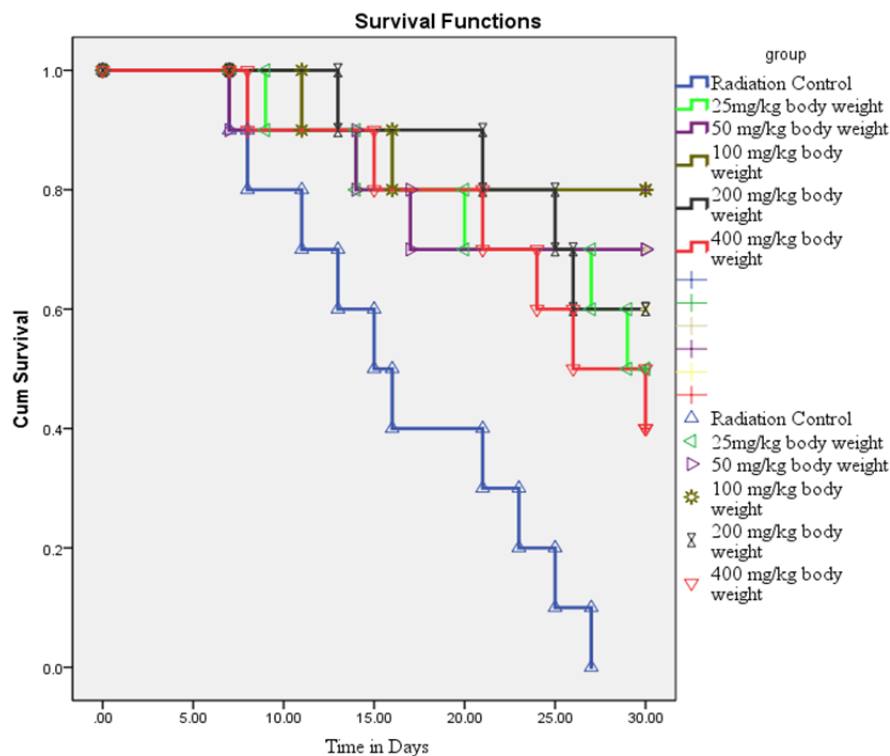


Fig. 1. Kaplan Meier's estimate of survival for rats treated with the different doses of *P. americana*. Different concentration of *P. americana* leaf extracts (25 - 400 mg/kg body weight) administered orally once for five days. One hour after last administration, on the fifth day, animals were exposed to 8 Gy of X-rays. Survival was recorded 30 days post irradiation.

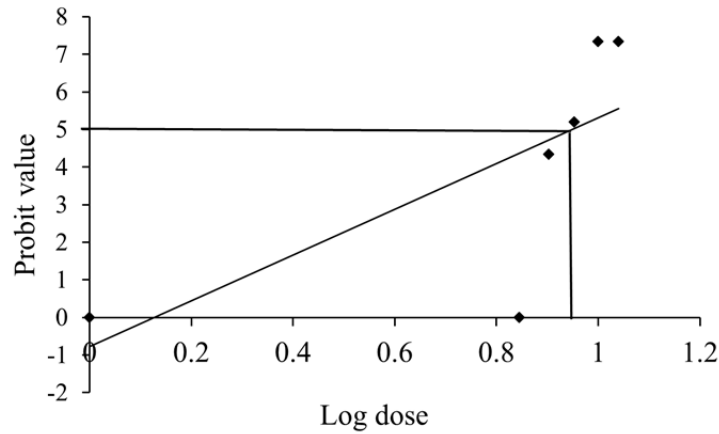


Fig. 2. Radiation dose response curve for the 30 day survival of rats after whole body X-ray irradiation (7 Gy, 8 Gy, 9 Gy, 10 Gy, 11 Gy) with pretreatment of *P. americana*. Radiation dose is represented in log doses. Percentage mortalities are represented as probit values in the Y-axis.

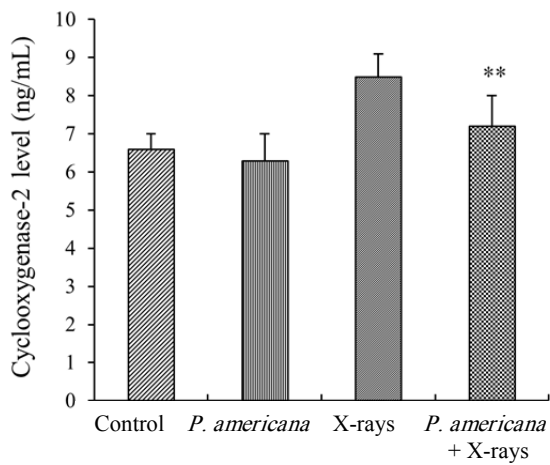


Fig. 3. Changes in rat's liver cyclooxygenase-2 level after exposure to 4 Gy with or without 100 mg/kg body weight *P. americana* leaf extracts given orally for five consecutive days. Values are mean \pm SEM (n = 6). The significant levels ** $P < 0.01$ when compared to radiation group alone.

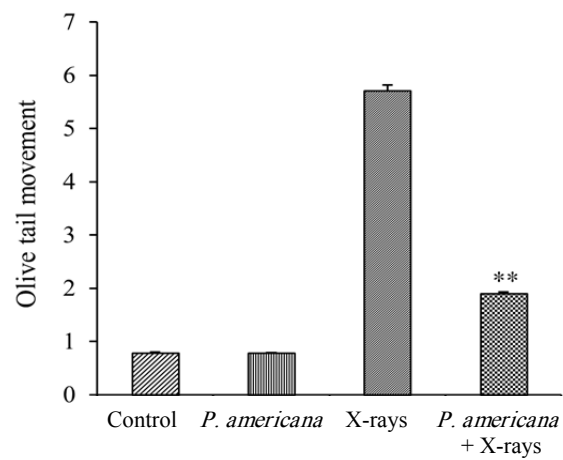


Fig. 4. Induction of DNA damage assessed by alkaline comet assay (olive tail moment) in peripheral blood lymphocytes of the experimental animals with and without treatment. The significant levels ** $P < 0.01$ when compared to radiation group alone.

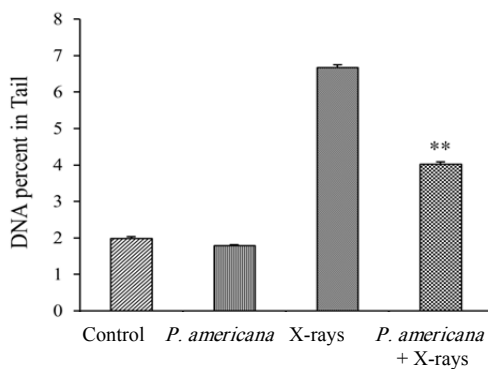


Fig. 5. Induction of DNA damage assessed by alkaline comet assay (DNA% in tail) in peripheral blood lymphocytes of the experimental animal with and without treatment. The significant levels ** $P < 0.01$ when compared to radiation group alone.

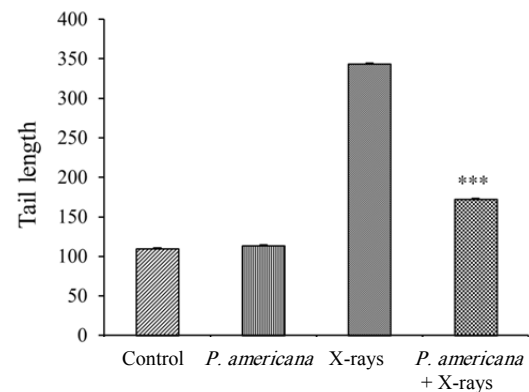


Fig. 6. Induction of DNA damage assessed by alkaline comet assay (tail length) in peripheral blood lymphocytes of the experimental animal with and without treatment. The significant levels, *** $P < 0.001$ when compared to radiation group alone.

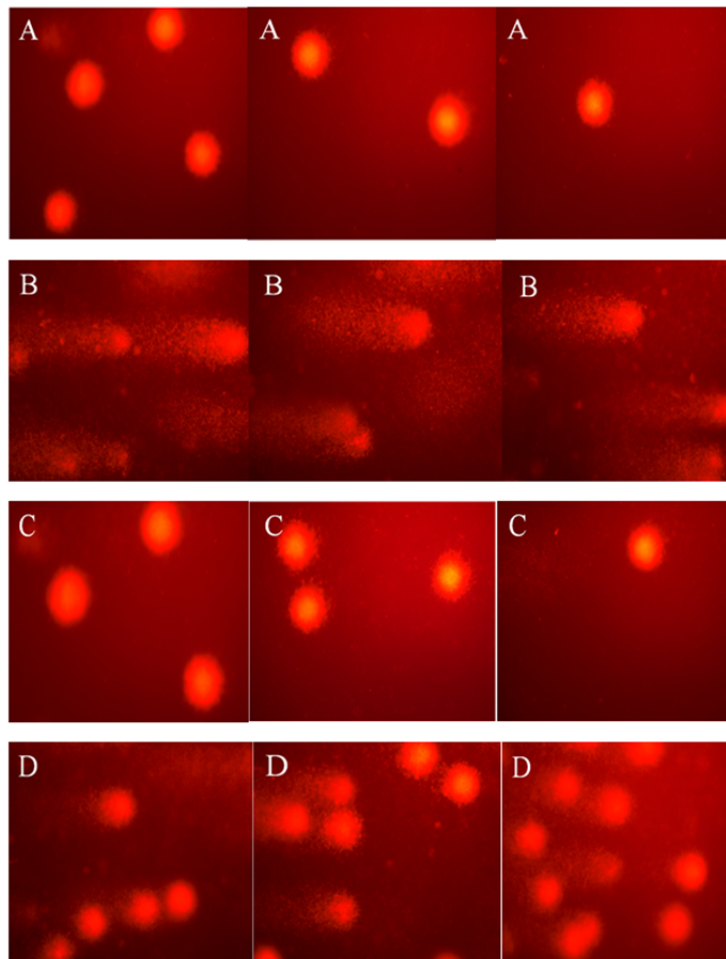


Fig. 7. Changes in the levels of DNA damage in normal, X-rays treated and *P. americana* pretreated group. (A) Normal control, (B) X-ray irradiated group, (C) drug control group, and (D) *P. americana* + X-rays.

Effect of P. americana on radiation-induced inflammation

The results of anti-inflammatory marker cyclooxygenase-2 in liver with or without treatment are given in Fig. 3. Animals exposed to whole body irradiation of X-rays (4 Gy) resulted an increase in the level of hepatic cyclooxygenase-2 in radiation control group when compared to normal controls ($P < 0.001$) and drug control group ($P < 0.01$). Pretreatment of *P. americana* 100 mg/kg body weight for five consecutive days resulted in a decrease in hepatic cyclooxygenase-2 level ($P < 0.01$) when compared to the irradiated group alone. In the present study, there was no significant difference observed between plant pretreated groups as well as a normal control group.

Comet assay (single cell gel electrophoresis)

The evaluation of genotoxicity by comet

assay is shown in Figs 4, 5, 6, and 7 with respect to olive tail moment, percentage DNA in tail and comet length, respectively. There was a significant increase ($P < 0.001$) in percentage DNA damage in tail, olive tail moment ($P < 0.05$) and comet length ($P < 0.001$) in X-ray irradiated animals when compared to normal controls. Furthermore, there was a significant decrease in the comet parameters in drug control group when compared to irradiated group alone. Pretreatment of *P. americana* for five consecutive days before X-rays treatment displayed significant reduction in the comet length ($P < 0.001$), olive tail moment ($P < 0.01$) and percentage DNA in tail ($P < 0.05$) when compared to X-rays irradiated group. *P. americana* alone at 100 mg/kg body weight did not show any changes in olive tail moment, percentage DNA in tail and comet length when compared to normal controls.

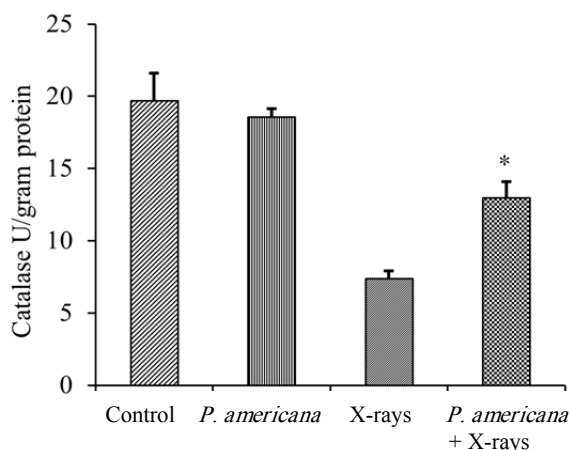


Fig. 8. Effect of *P. americana* on liver antioxidant enzyme catalase after treatment with 4 Gy of X-rays with and without treatment of 100 mg/kg body weight of *P. americana*. Values are mean \pm SEM (n = 6). The significant levels * $P < 0.05$, when compared to radiation group alone.

Biochemical estimations

The endogenous cellular antioxidant enzymes were analysed in radiation treated and *P. americana* pretreated groups. Animals irradiated with X-rays showed decrease in the endogenous antioxidant enzymes such as reduced glutathione and catalase in liver tissue homogenates. Irradiated animals also displayed elevated lipid peroxidation and protein carbonyl levels.

The liver catalase and glutathione levels were significantly ($P < 0.001$) decreased in radiation treated group when compared to normal controls as well as drug control group ($P < 0.001$) at 24 h post irradiation. Animals pretreated with *P. americana* one h before irradiation significantly increased cellular endogenous antioxidant enzyme catalase ($P < 0.05$) shown in Fig. 8.

The drug control group displayed significant increase ($P < 0.001$) in the catalase levels when compared to irradiated group alone. There was no statistically significance found in catalase levels in drug control and normal control group. Pretreatment of *P. americana* five days prior to irradiation significantly increased ($P < 0.05$) the reduced glutathione levels as shown in Fig. 9, respectively, when compared to irradiated group alone. There was no significant difference found between the reduced glutathione levels in drug control group as

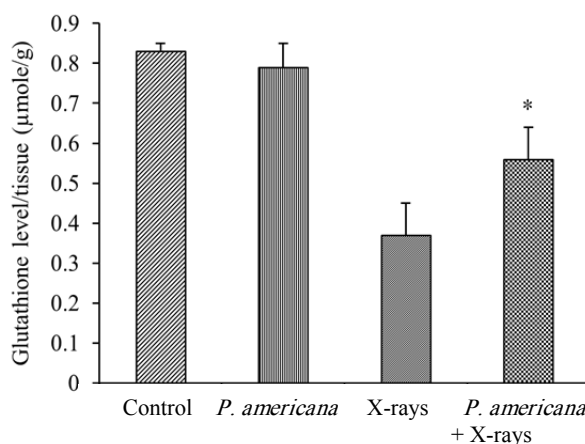


Fig. 9. Changes in reduced glutathione level after exposure to 4 Gy with or without 100 mg/kg body weight *P. americana* leaf extracts given orally for five days. Values are Mean \pm SEM (n = 6). The significant levels * $P < 0.05$ when compared to the radiation group alone.

well as a normal control group. Animals exposed to X-rays, displayed elevated levels of lipid peroxidation and protein carbonyls when compared to normal controls ($P < 0.01$) and drug control ($P < 0.01$), respectively. Pretreatment of 100 mg/kg body weight of *P. americana* one hour before radiation protected animals from lipid peroxidation and protein carbonyl formation, respectively. Pretreatment of *P. americana* significantly ($P < 0.01$) reduced the protein carbonyls level when compared to X-ray treated group as shown in Fig. 10. There was a considerable decrease in lipid peroxidation levels in pretreated group when compared to X-rays treated alone, but without any significance as shown in Fig. 11.

Micronucleus assay

The effect of X-ray irradiation with and without *P. americana* on the initiation of micronucleus in bone marrow cells are represented in Table 1.

The ratio of polychromatic erythrocytes and normochromatic erythrocytes in a normal condition remain close to one whose imbalance leads to genotoxicity. In this study animals pretreated with 100 mg/kg body weight of *P. americana* did not produce any signs and symptoms of genotoxicity in rat bone marrow micronucleus test. Animals exposed to sub lethal dose of X-rays (4 Gy) displayed a significant increase in micronucleated

polychromatic erythrocytes ($P < 0.001$) and micronucleated normochromatic erythrocytes ($P < 0.001$) when compared to normal controls and drug control group. Pretreatment of *P. americana* for five consecutive days before exposure to whole body X-rays displayed a

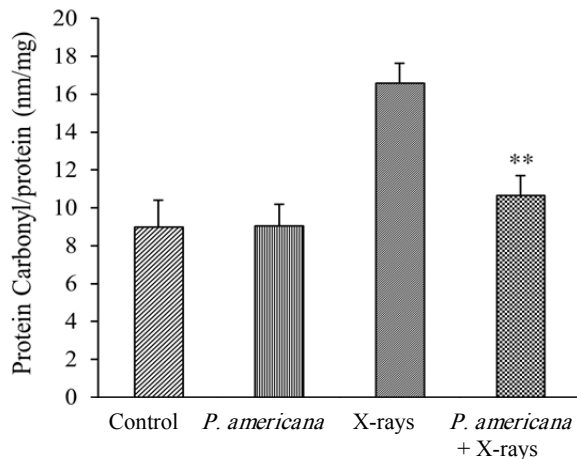


Fig. 10. Changes in protein carbonyl content after exposure to 4 Gy with or without 100 mg/kg body weight *P. americana* leaf extracts given orally for five days. Values are Mean \pm SEM (n = 6). The significant levels ** $P < 0.01$ when compared to radiation group alone.

significant reduction in micronucleated polychromatic erythrocytes ($P < 0.05$) and micronucleated normochromatic erythrocytes ($P < 0.01$), respectively. There was also increase in PCE/NCE ratio in pretreated group compared to irradiated group alone.

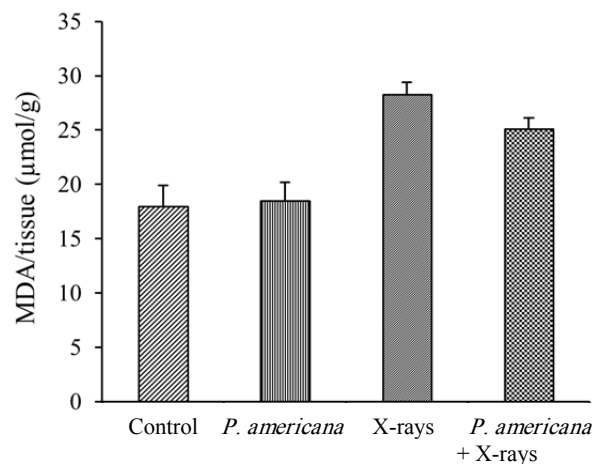


Fig. 11. Changes in lipid peroxidation levels after exposure to 4 Gy with or without 100 mg/kg body weight *P. americana* leaf extracts given orally for five days. Values are mean \pm SEM (n = 6). Results were non-significant between groups.

Table 1. Effect of treatment with *P. americana* on protection against micronucleus formation induced by X-rays.

Groups	Control	<i>P. americana</i>	X-rays	<i>P. americana</i> + X-rays
PCE	47.3 \pm 1.05	47.3 \pm 0.54	30.1 \pm 1.18	36.1 \pm 1.45
MnPCE	0	0.21 \pm 0.04	16.3 \pm 0.61	10.7 \pm 0.89 (a)
NCE	52.7 \pm 1.04	52.1 \pm 0.55	51.1 \pm 1.66	50.7 \pm 1.33
MnNCE	0	0.03 \pm 0.005	2.58 \pm 0.18	1.07 \pm 0.12 (b)
PCE/NCE	0.89	0.91	0.59	0.71

Data expressed as average percentage (%) of cells. Significant levels (a), $P < 0.05$; (b), $P < 0.01$ when compared to radiation group alone.

(PCE), polychromatic erythrocytes; (NCE), normochromatic erythrocytes; (MnPCE), micronucleated polychromatic erythrocytes; (MnNCE), micronucleated normochromatic erythrocytes.

DISCUSSION

Ionising radiations such as X-rays or gamma rays induce oxidative stress through free radicals resulting in the imbalance between antioxidant and pro-oxidant status in a cell. In the present study hydroalcoholic leaf extract of *P. americana* was evaluated for its antigenotoxic, anti-inflammatory, and radioprotective property in rats exposed to X-rays. Plants consisting of various phytoconstituents such as terpenoids, saponins, flavonoids, and glycosides reduce the intensity

of free radicals generated by oxidative stress (22). *P. americana* has a variety of health benefits in terms of traditional medicine. In our earlier studies, we found the ability of *P. americana* chelating metal ions and also scavenging hydroxyl radicals (23). In our previous studies, we observed that *P. americana* scavenged ABTS free radicals in a dose dependent manner and also displayed high total phenolic content (24). Exposure to ionizing radiation such as X-rays upregulate cyclooxygenase levels in the tissues with simultaneous increase in prostaglandin E₂

(PGE₂) synthesis. In the present study, whole body exposure to X-rays resulted in elevated levels of cyclooxygenase-2 level in liver tissue homogenates. Similar results were reported in a study done on keratinocytes where UVB radiation upregulated the expression of COX-2 by using free radicals as intermediates (25). Cyclooxygenase-2 levels in normal control as well as plant pretreated group were unaltered in liver tissue homogenates. Pretreatment of *P. americana* reduced the inflammation ($P < 0.01$) by acting on the free radicals generated when exposed to sublethal dose of X-rays. Exposure to lethal dose of X-rays targets specific tissues in an animal resulting in death, the primary target being differentiating cells found in bone marrow as well as gastrointestinal tract. The low dose of ionizing radiation causes diverse alterations in sensitive tissues which include lung, kidney, gastrointestinal tract, bone marrow, skin, and liver. The animals exposed to 8 Gy of X-rays showed behavioural changes, ruffled hairs, intense diarrhoea, weight loss, irritability, and erythema in 3 to 4 days of whole body exposure. There was 100% mortality in animals exposed to 8 Gy of X-rays within 30 day's post irradiation interval. In our study Pretreatment of *P. americana* before irradiation reduced the onset of acute radiation syndrome in dose-dependent manner. The survivals of animals were merely due to protection of differentiating cells in bone marrow and gastrointestinal tract by rich antioxidant property of *P. americana*. In our earlier experiments LD_{50/30} for X-rays were found to be 6.6 Gy (26). Rats administered with 100 mg/kg body weight of *P. americana* for five consecutive days before irradiation raised the mean lethal dose up to 8.5 Gy. The animals exposed to 7, 8, 9, 10, and 11 Gy displayed 0%, 33%, 75%, 100%, and 100 % mortality, respectively. The calculated dose reduction factor is 1.28. Endogenous antioxidant enzymes such as glutathione, a key mediator protects cells from damage by oxidative stress. The sulfhydryl groups present in glutathione protects DNA and other biomolecules by combining various mechanisms which include scavenging of reactive oxygen and nitrogen species, donating

hydrogen ions and modulation of repair process (27). In our study, we found decreased levels of reduced glutathione in liver tissue homogenates when rats were exposed to sublethal dose of X-rays (4 Gy), when animals were dissected 24 h post irradiation. This result is in accordance with an earlier study; where mice exposed to γ -radiation displayed reduction in reduced glutathione levels in liver homogenates when dissected 24 h post irradiation (28). Pretreatment of *P. americana* showed significant increase in the glutathione levels when compared to irradiated group ($P < 0.05$). In the present study animals treated with X-rays showed increased levels of hepatic malonaldehyde concentration which is an end product of lipid peroxidation. Animals pretreated with *P. americana* slightly decreased the levels of hepatic MDA when compared to radiation control. The liver being a radiosensitive organ undergoes various alterations in metabolic functions when exposed to radiation. Recent findings demonstrated that apart from DNA, proteins are also a significant target for the pathological action of ionizing radiation. Free radical mediated protein oxidation includes carbonylation, nitrosylation, and fragmentation (29). Animals exposed to 4 Gy of X-rays displayed increase in the protein carbonyls levels in liver tissue homogenates. Pretreatment of *P. americana* reduced the oxidation of protein significantly ($P < 0.01$) when compared to radiation control alone. Comet assay is a sensitive method to assess the DNA damage induced by genotoxic agents *in vitro* and *in vivo* (30). Several mechanisms have been proposed where flavonoids reduce the intensity of DNA damage by intercalating itself in DNA double helices thereby stabilising DNA structure by the attack of free radicals (31). The dried leaves of *P. americana* comprised of various biologically active flavonoids such as quercetin, quercetin 3-O-b-glucopyranoside, kaempferol 3-O- α -D-arabinopyranoside, and quercetin 3-O- β -D-arabinopyranoside (11). The flavonoids such as quercetin and rutin exert radioprotective activity by scavenging free radicals generated by γ -rays (32). The phytoconstituents such as apigenin, luteolin, and isorhamnetin present in

P. americana leaves prevents the development of oxidative stress related diseases (33). The flavone apigenin triggers the endogenous antioxidant levels in liver and intestine by mitigating radiation induced haematological and biochemical alterations when exposed to whole body gamma irradiation (34). Studies done on flavones such as orientin and vicenin protected mice from chromosomal aberrations as well as lethality caused by radiation (35). Studies done on *Olea europaea* leaf extract displayed a reduction in micronucleated polychromatic erythrocytes when exposed to X-rays where flavonoids and polyphenolic compounds played a lead role in neutralizing the free radicals produced during irradiation of X-rays (36). Similar findings were observed in a study where antioxidants such as glutathione and amofistine reduced MnPCE levels when treated with gamma radiation (37). There was no genotoxicity found in plant pretreated group alone.

The frequency of MnPCE was elevated significantly ($P < 0.001$) in irradiated group when compared to normal controls. *P. americana* pretreatment before X-ray irradiation significantly reduced the frequency of MnPCE ($P < 0.05$) elucidating its anticlastogenic property.

CONCLUSION

Our findings for the first time demonstrated the protective role of *P. americana* by mitigating radiation effects *in vivo*. *P. americana* at the optimum dose of 100 mg/kg body weight protected animals from clastogenic effects caused by X-rays, and also displayed anti-inflammatory properties. This protection is attributed to free radical scavenging activity of *P. americana* by maintaining cytoplasm antioxidant levels in cells subsequently decreasing oxidative stress and inhibiting DNA damage.

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