

Radiomodulatory Role of *Psidium guajava* Leaf Extracts against X-ray Induced Genotoxicity, Oxidative stress and Apoptosis in albino Wistar Rat Model

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ABSTRACT

In the present study we investigated the radioprotective activity of hydroalcoholic leaf extracts of *Psidium guajava* (P.G) against rats exposed to X rays. Exposure to ionizing radiation like X rays induces oxidative damage in normal tissues leading to their death or transforming them into cancerous tissues. To study the optimum dose of radioprotection, rats were administered with different doses (50,100,200,400 mg/kg body weight) of *Psidium guajava* leaf extracts daily for five consecutive days. One hour after last administration of leaf extracts the rats were treated with 6.6 Gy of x rays. 200 mg/kg body weight was selected as an optimum dose for radioprotection based on survival analysis. Pretreatment of 200 mg/kg body weight of P.G leaf extract increased the levels of antioxidant enzymes such as superoxide dismutase, catalase, reduced glutathione and lowered the levels of lipid peroxidation, protein carbonyls and nitric oxide when compared to irradiated group in liver tissue homogenates. DNA damage indicators analyzed through comet assay showed a reduction in Olive tail movement and percentage DNA in tail significantly in P.G pretreated group when compared to irradiated group alone. Pretreatment of P.G leaf extract at a dose of 200mg/kg body weight protected cells from apoptosis which was analyzed microscopically by Ethidium bromide /Acridine orange staining. Our findings demonstrate the role of P.G leaf extract as a radio modulator in vivo, consequent to its powerful antioxidant activity in vitro and could be beneficial in combating radiation induced damage in living systems.

INTRODUCTION

Ionizing radiation has direct and indirect effect on biomolecules leading to cell death and/or carcinogenesis. The direct effect is by attacking the single and double bonds present in double stranded DNA while the indirect effect is by reacting with nearby water molecules forming free radicals which in turn damage DNA. It is important to protect biological system against accidental and unavoidable exposure to radiation. Ionizing radiations are considered to be a major threat to living organisms due to its deleterious effects. The situation is alarming today due to rapid advancement in technology. The reasons could vary

from treatment strategies as in cancer, to polluted atmosphere. The need to protect oneself from the hazards of radiation is extremely essential. Plants and herb extracts have served a great deal in this regard (Jagetia, 2007). Some of the chemical components that contribute to the protective mechanisms are flavonoids, phenolic acids, xanthenes, flavones etc. These polyphenolic compounds can behave both as pro oxidants and antioxidants depending on their concentration and cytosolic redox status (Girdhani *et al.*, 2005) and can hence act as radiosensitisers and or radio protectors. Recently there is an increased interest towards plants derived drugs as radioprotectors. The studies done on certain plants like Zingerone (Rao *et al.*, 2009), *Saraca indica* (Rao *et al.*, 2010), *Ocimum sanctum* (Uma devi *et al.*, 1999), *Cynodon dactylon* (Rao, 2006 & 2008), *Cymbopogon citratus* (Rao *et al.*, 2009) & *Ficus racemosa* (Veerapur *et al.*, 2009) displayed reduction in the radiation induced oxidative stress and genotoxicity. P.G (guava) is an important food crop and medicinal plant in the tropical and subtropical countries.

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Phytochemicals reported in this plant are mainly flavonoids like quercetin, guajavarin, isoquercetin, Morin etc. (Lozoya *et al.*, 1994). Guava leaf extract is capable of scavenging peroxy radicals (Hui-Yenchen, 2007). Antihypertensive and antidiarrhoeal actions of the plant is mediated by inhibiting intracellular calcium level. Aqueous extract of fruit peel showed significant hypolipidaemic and hepatoprotective effects in diabetic mice (Belemtougri *et al.*, 2006; Rai and Mehta, 2010) and in experimentally induced liver damage in animals (Roy and Das, 2011). Chemical constituents present in P.G. displayed antibacterial, antimalarial, antimycobacterial and antioxidant activity. Since, evidences to substantiate the radio modulating effects of the crude extract of these plants are scanty, as of today, we propose to highlight the protective effects of these herbal extracts in albino wistar rat model exposed to X-ray irradiation, which could probably aid in designing treatment strategies for acute effects of radiation in general and radiotherapy for cancers in particular.

MATERIALS AND METHODS

Animals

Adult (8 – 10 weeks old), albino wistar rats of either sex, weighing 160-180g, was used in the present study. The animals were procured from the breeding colony of KMC, Mangalore. Prior approval of Institutional ethical committee was taken. Animals were housed in well ventilated polypropylene cages with paddy husk bedding maintained at $25 \pm 2^\circ\text{C}$ temperature and $50 \pm 5\%$ humidity & light (12 hours of light and dark) for the durations of the experiment. Rats were given *ad libitum* access to laboratory food (commercial rat pellets from Gold Mohur, Lipton India Ltd.) and water. Animal care and handling was done according to the guidelines issued by World Health Organization, Geneva, Switzerland and the Indian national science academy New Delhi India.

Chemicals

Meta-phosphoric acid, Sodium chloride (NaCl), NaOH, Ethylene diamine tetra acetate (EDTA), Disodium hydrogen phosphate (Na_2HPO_4), 5,5'-Dithiobis 2nitro benzoic acid (DTNB), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Hydrochloric acid (HCl), 2,4- dinitrophenyl hydrazine, Ethanol, Ethyl-acetate, Guanidine hydrochloride, Hydrogen peroxide, Sodium dihydrogen phosphate (NaH_2PO_4), Nitro blue tetrazolium chloride, procured from SRL private limited, India. Malonaldehyde bis, Sulphanilamide, N-(1-naphthyl)-ethylene diamine dihydrochloride (NED), Sodium nitrite, Riboflavin, Methionine, Ethidium bromide, Acridine orange, Triton x, Low melting agarose, Normal melting agarose & Histopaque 1077 procured from Sigma Aldrich (St Louis, MO). All other reagents used are of analytical grade.

Plant material and Extraction

The tender leaves of *Psidium guajava* were collected during the months August to December 2013 from Soans nursery,

Alangar, Moodbidri, Mangalore, Karnataka, India. The plant specimen was authenticated by Mr. Surendranath Joshi, Botanist, St Aloysius College, Mangalore, Karnataka, India. The collected tender leaves were washed with running tap water. The tender leaves were shade dried at room temperature. After drying the leaves were powdered by an electrical mixer. 100g of dried powdered leaves were suspended in 500 ml of 50% methanol in water and refluxed at 50°C in a soxhlet apparatus for 72 hours. The crude extract was concentrated by Rotary vacuum flash evaporator. The concentrated crude extract was stored at 4°C for further usage.

Irradiation source and procedure

The animals were restrained in well ventilated acrylic box and exposed to whole body X-ray irradiation at a distance of 100 cm from the beam exit point of the Linac accelerator (LINAC) at Radiotherapy Department, KMC Hospital, Attavar, Mangalore, India. The field size was $40 \times 40 \text{ cm}^2$ with dose rate of 3.5 Gy/min.

Acute drug toxicity

Hydroalcoholic leaf extract of *Psidium guajava* was dissolved in double distilled water. Acute toxicity study was determined according to OECD guidelines. Animals were divided into four groups and in each group there were six animals. Animals from group first were administered with 0.01 ml/kg body weight double distilled water, while animals from group II-IV received a single dose of 500, 1000, 2000 mg /kg body weight of P.G leaf extract orally. After treatment animals were given *ad libitum* food and water and observed for 14 days for any mortality.

Optimum dose selection of *Psidium guajava* leaf extract for radioprotection

To study the optimum dose of plant for radioprotection, animals were divided into 6 groups. In each group there were 12 animals. First group received 0.01 ml /kg body weight double distilled water which served as sham control group. The experimental groups from II- V received different concentration of P.G leaf extract (50, 100, 200, 400 mg/kg body weight) orally once, for five consecutive days respectively and radiation control group was maintained. One hour after last administration of extract, animals were exposed to 6.6 Gy whole body irradiation of X rays & survival was recorded 30 days post irradiation.

Biochemical estimations

To explore the mechanism of radioprotection, biochemical parameters such as reduced glutathione, lipid peroxidation, nitric oxide, protein carbonyls, superoxide dismutase and catalase were analyzed in the liver tissue homogenates. Animals were divided into four groups with six animals in each group.

Group I (sham control group). Animals of this group received 0.1 ml/kg body weight of double distilled water orally.

Group II (Irradiated group). The animals were exposed to the sub lethal dose i.e., 4 Gy of X rays.

Group III (P.G leaf extract pretreated group). Animals were administered with 200 mg/kg body weight of leaf extract orally for five consecutive days.

Group IV (Combination group). Animals were pretreated with 200 mg/kg body weight of P.G leaf extract for five consecutive days, and last dose of extract was given orally 1 hour before exposure to 4 Gy of X rays.

Preparation of liver tissue homogenate

All the animals were euthanized at 16 hours post irradiation and livers were perfused with ice cold saline. 10 % homogenate was prepared with ice cold phosphate buffer saline with pH 7.4 using a homogenizer (RO-1727A Remi Motors). Tissue homogenization was done under controlled chilled condition. Catalase activity was measured in terms of decomposition of hydrogen peroxide (H₂O₂) into water molecule, which was measured as decrease in absorbance at 240nm, as described by (Beers *et al.*, 1952). Superoxide dismutase (SOD) activity was assayed by the method described by (Beauchamp and Fridovich, 1971) based on NBT reduction. Lipid peroxidation was determined by analyzing TBA-reactive substances according to the method described by Buege and Aust (1978). The total GSH content was measured by the method of Ellman, (1959). Protein carbonyls was estimated in the tissue homogenate by the method described by Levine *et al.*, (1994). NO production in tissue homogenates was measured by the Griess method (Green *et al.*, 1982).

Apoptosis assay in peripheral blood lymphocytes

In this experiment, blood was collected from the experimental animals by cardiac puncture and stored in sterile heparinized vacutainers. Blood was diluted with phosphate buffer saline pH 7 in equal ratio and layered carefully on the density gradient Histopaque 1077 in 2:1 ratio and centrifuged at 3000 rpm for 20 minutes. Lymphocytes were removed from the interface carefully and washed with phosphate buffer saline. The suspension was centrifuged to yield lymphocytes pellet. Ethidium bromide and acridine orange staining was done according to the procedure described by (Renvoize *et al.*, 1998). The lymphocyte pellet was suspended in 1:1 ratio of ethidium bromide (10 µg/ml)/AO (50 µg/ml) staining for one minute and observed immediately under fluorescent microscope. Total 300 cells were counted per samples and apoptotic index was calculated.

$$\text{Apoptotic Index} = \frac{\text{Total number of apoptotic cells}}{\text{Total number of cells}}$$

Comet assay (Single cell gel electrophoresis)

Comet assay is a technique used to measure DNA damage. The assay was performed according to the method described by Singh *et al.*, (1988). The negatively charged DNA molecules migrate towards anode in the process of electrophoresis. Frosted microscopic slide was layered with 1.5 % normal melting agarose. Peripheral blood lymphocytes from various groups were mixed with 0.75 % low melting agarose and layered upon normal

melting agarose. The slides are incubated at 4 °C for one hour in ice lysis solution comprising of 100 mM EDTA, 2.5 M NaCl, 0.2 M NaOH, 1 % triton x & 10 % DMSO. After lysing the slides were placed in electrophoresis buffer (300 mM NaOH, 1 mm EDTA, pH ≥ 13). The run time was 20 minutes with 300 mA voltage. The cells were neutralized with neutralizing buffer (0.4 M Tris, pH 7.4) and stained with ethidium bromide. The observation of DNA damage was done under fluorescent microscope. Komet 5 image analysis software developed by kinetic imaging (Liverpool UK) was used to assess DNA damage.

Statistical Analysis

Analysis of data was done by one way analysis of variance (ANOVA) followed by Post Hoc test, using IBM SPSS Statistics 20. P ≤ 0.05 was considered significant. Kaplan Meir survival analysis was used to determine the optimum dose of the leaf extract.

Results

Acute drug toxicity: Oral administration of *Psidium guajava* leaf extract at concentration 500, 1000 & 2000 mg/kg body weight did not induce any mortality and behavioral changes up to 14 days observation period. Hence the extract has no lethal effect up to 2000 mg/kg body weight. Since there was no toxicity at 2000 mg/kg body weight, 1/10th, 1/5th, 1/20th, & 1/40th of the dose was taken for further experiments.

Optimum dose of *Psidium guajava* leaf extract for radiation protection

The administration of *Psidium guajava* leaf extracts at 50, 100, 200, 400 mg/kg body weight before 6.6 Gy of X ray irradiation resulted in 41.7, 66.7, 75.0, 58.3 % animal survival respectively. Because maximum survival was observed in 200 mg/kg body weight, it was considered as an optimum dose for radioprotection and further experiments were carried out with this dose [Figure 1].

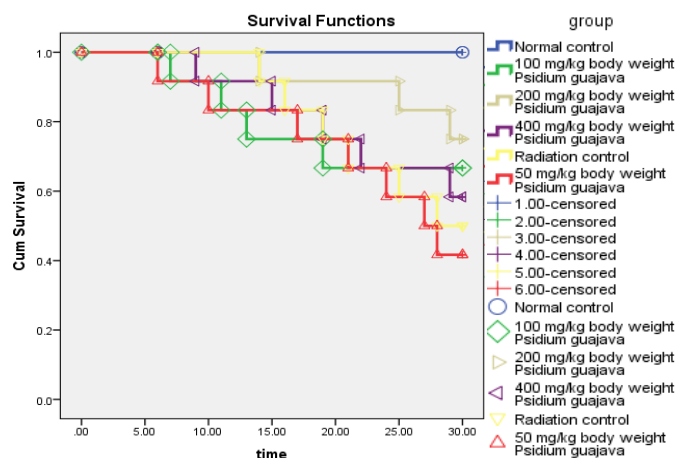


Fig. 1: Kaplan Meir survival analysis of rats administered with different dose of *Psidium guajava* leaf extract (50, 100, 200, 400 mg/kg body weight) orally for five consecutive days. One Hour after last administration rats were exposed to whole body X ray irradiation of 6.6 Gy. In each group 12 rats were maintained.

Table 1 a :Induction of DNA damage assessed by alkaline Comet assay (% DNA in tail) in peripheral blood lymphocytes of the experimental animal treated with 200 mg/kg body weight of *Psidium guajava* leaf extract & in the groups exposed to 4 Gy of sub lethal dose of X rays.

| Groups | % Tail DNA \pm S.E.M |
|------------------------------------|-----------------------------|
| Sham control | 1.6 \pm 0.4 |
| Radiation control | 4.7 \pm 1 |
| <i>Psidium guajava</i> | 1.1 \pm 0.3 |
| <i>Psidium guajava</i> + Radiation | 1.7 \pm 0.09 ^b |

The significant levels a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ when compared to radiation group alone.

Table 1 b:Induction of DNA damage assessed by alkaline Comet assay (olive tail movement) in experimental animal treated with 200 mg/kg body weight of *Psidium guajava* leaf extract & in the groups exposed to 4 Gy of sub lethal dose of X-rays.

| Groups | Olive Tail Movement \pm S.E.M |
|------------------------------------|---------------------------------|
| Sham control | 0.4 \pm 0.02 |
| Radiation control | 5 \pm 1.3 |
| <i>Psidium guajava</i> | 0.2 \pm 0.09 |
| <i>Psidium guajava</i> + Radiation | 1.1 \pm 0.3 ^c |

The significant levels a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ when compared to radiation group alone.

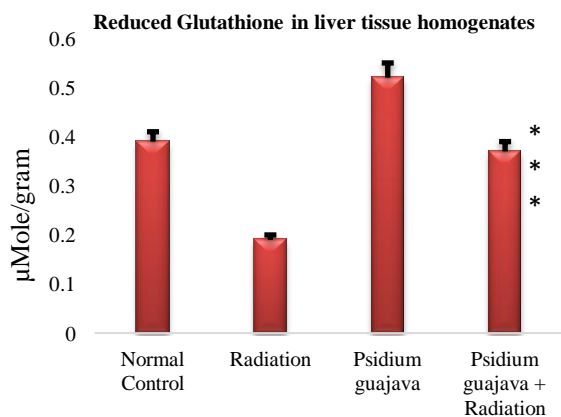


Fig. 2: Changes in reduced glutathione level after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6).The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol =not significant when compared to radiation group alone.

Biochemical analysis

Reduced Glutathione: Animals treated with whole body irradiation of 4 Gy displayed significant ($p < 0.001$) reduction in the level of reduced glutathione when compared to normal controls. Pretreatment of *Psidium guajava* leaf extract at a dose of 200 mg/kg body weight, one hour before irradiation displayed significant increase ($p < 0.001$) in the level of reduced glutathione when compared to irradiated group [Figure 2& Table 2].

Table 2: Changes in GSH, CAT, SOD, LPO, NO and protein carbonyls levels after exposure to 4 Gy with or without *Psidium guajava* given orally for five consecutive days.

| Groups | Catalase U/gram protein | SOD activity U/mg protein | Protein carbonyls nm/mg protein | GSH μ mole/gram tissue | μ mole MDA/gram tissue | Nitric Oxide μ Mole/gram tissue |
|---|-------------------------|------------------------------|---------------------------------|-------------------------------|-------------------------------|-------------------------------------|
| Distilled water alone | 21.79 \pm 1.5 | 1.02 \pm 0.4 | 8.79 \pm 0.55 ^c | 0.397 \pm 0.02 ^c | 19.02 \pm 2.77 ^c | 74.19 \pm 2.0 ^e |
| Distilled water + 4 Gy X- rays | 11.81 \pm 2.6 | 0.754 \pm 0.2 | 15.70 \pm 4.02 | 0.19 \pm 0.01 | 27.54 \pm 1.07 | 106.7 \pm 3.6 |
| <i>Psidium guajava</i> leaf extract alone | 28.92 \pm 2.9 | 1.53 \pm 0.18 | 8.04 \pm 1.14 | 0.52 \pm 0.03 | 19.76 \pm 1.26 | 77.61 \pm 6.6 |
| <i>Psidium guajava</i> + 4 GY X rays | 15 \pm 5.4 | 1.14 \pm 0.26 ^a | 10.04 \pm 1.44 ^b | 0.37 \pm 0.02 ^c | 21.31 \pm 1.68 ^c | 75.8 \pm 2.4 ^e |

Values are mean \pm SD (n=6). The significant levels a = $p < 0.05$, b = $p < 0.01$, c = $p < 0.001$ and no symbol = non-significant when compared to group 2 against group 1 and group 4. GSH: glutathione, CAT: Catalase, SOD: Superoxide dismutase, LPO: Lipid peroxidation (MDA), NO: Nitric oxide.

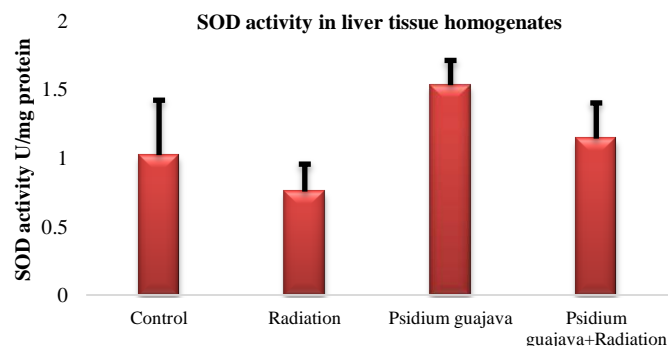


Fig. 3: Changes in Superoxide dismutase enzyme level after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6).The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol =not significant when compared to radiation group alone.

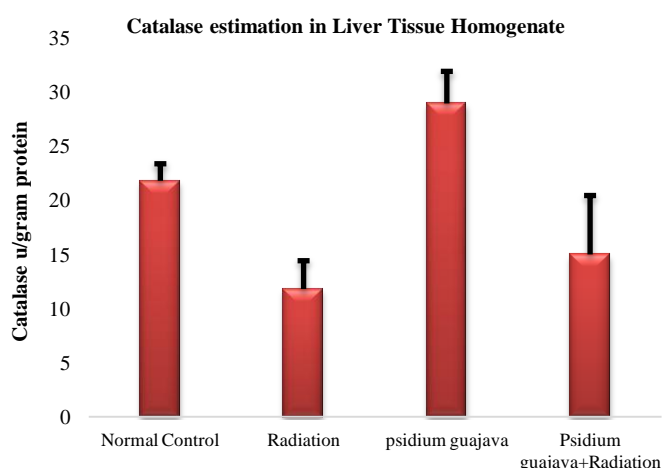


Fig. 4: Changes in Catalase level after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6).The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol =not significant when compared to radiation group alone.

SOD: Exposure of rats to the sub lethal dose of x rays i.e., 4 Gy leads to the decline in its level when compared to control groups. Since radiation disturbs the antioxidant status of a cell it leads to depletion of SOD levels. Pretreatment of the leaf extract one hour prior to radiation significantly increased the level of superoxide dismutase enzyme ($p < 0.05$) [Figure 3 & Table 2]

Catalase: The levels of catalase decreased in the liver tissue homogenate when rats were exposed to sub lethal dose of X rays i.e., 4 Gy. Pretreatment of *Psidium guajava* leaf extract displayed slight increase in the enzyme level when compared to irradiation alone [Figure 4 & Table 2].

Lipid peroxidation: Rats exposed to sub lethal dose of X rays, displayed increase in the level of lipid peroxidation compared to that of normal controls ($p < 0.001$). Pretreatment of *Psidium guajava* leaf extract displayed significant decrease in the level of lipid peroxidation when compared to irradiated group alone ($p < 0.001$) [Figure 5 & Table 2].

Oxidation of proteins leads to carbonyl formation. Reactive oxygen species such as hydroxyl radical, superoxide radical, peroxy radical, alkoxy and hypochlorous chloride are responsible for the oxidation of proteins. There is increase in the protein carbonyl content in liver when rats are exposed to whole body radiation of sub lethal dose (4Gy) when compared to normal control ($p < 0.001$). Pretreatment of *Psidium guajava* leaf extract one hour before radiation displayed reduction in the protein carbonyl level which is statistically significant ($p < 0.01$) [Figure 6 & Table 2].

In this experiment animals exposed to sub lethal dose exhibited an increase in the nitric oxide level when compared to normal controls which is significant. Five days pretreatment of *Psidium guajava* leaf extracts one hour before exposure to radiation displayed decline in the nitric oxide levels in liver tissue homogenates when compared to the irradiated group alone ($p < 0.001$) [Figure 7 & Table 2]

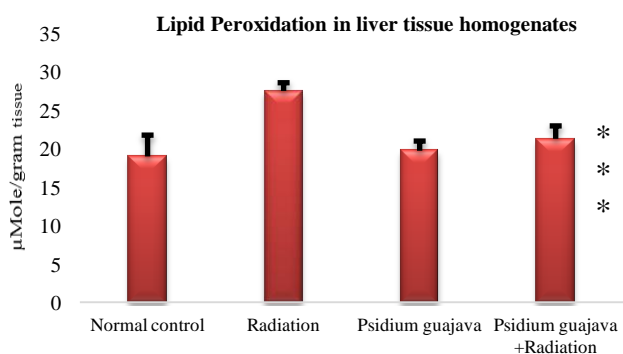


Fig. 5: Changes in Lipid peroxidation level after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6). The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol = not significant when compared to radiation group alone.

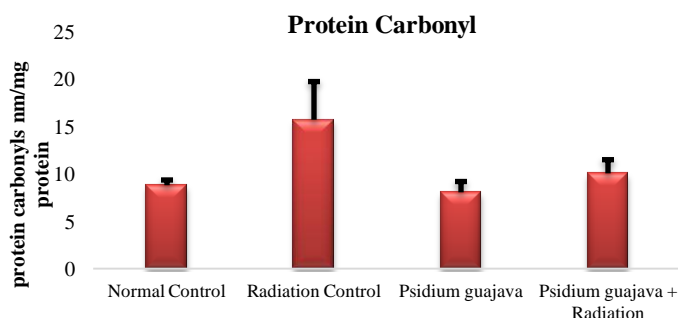


Fig. 6: Changes in Protein carbonyl content after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6). The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol = not significant when compared to radiation group alone.

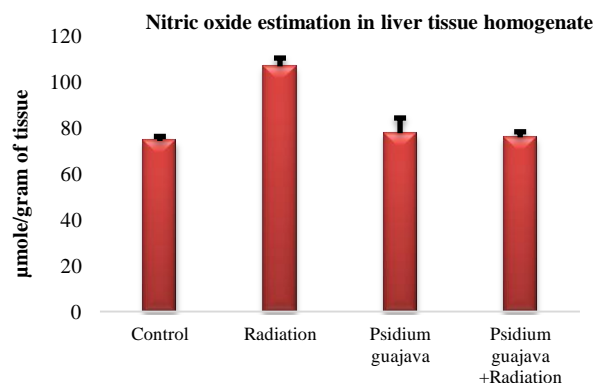


Fig. 7: Changes in Nitric oxide content after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6). The significant levels * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and no symbol = not significant when compared to radiation group alone.

Apoptosis assay

Cytomorphological analysis of peripheral blood lymphocytes by ethidium bromide/ acridine orange staining.

Ethidium bromide & acridine orange staining is a differential stain used to analyze the morphological changes in a cell. Live cells having intact plasma membrane allows acridine orange to stain the cytoplasm. The cells which take up acridine orange fluoresce green under fluorescent microscope. If the membrane integrity is lost ethidium bromide permeates into the cell making its nucleus fluoresce red under fluorescent microscope. Animals pretreated with *Psidium guajava* leaf extract alone (200 mg/kg body weight) displayed green stained unfragmented nuclei, indicating non apoptotic live cells. The rats exposed to sub lethal dose of x rays i.e., 4Gy exhibited increase in the red stained fragmented nuclei.

There was significant increase in the apoptotic cells in radiation group alone when compared to control group ($p < 0.001$). Pretreatment of leaf extract 200 mg/kg body weight one hour prior to radiation displayed decrease in the apoptotic index although not statistically significant [Figure 8].

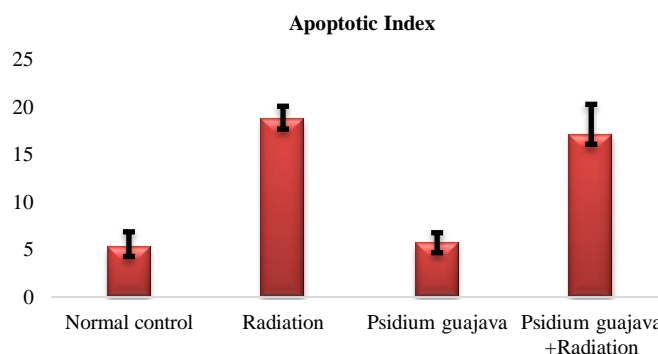


Fig. 8: Changes in Apoptotic index in peripheral blood lymphocytes after exposure to 4 Gy with or without 200mg/kg body weight *Psidium guajava* leaf extracts given orally for five consecutive days. Values are mean \pm SD (n=6).

Comet assay (Single cell gel electrophoresis)

The results of alkaline single cell gel electrophoresis is shown in table 1a & 1b. In our study we found an increase in the comet parameters like percentage tail DNA and olive tail movement in rats exposed to 4 Gy of x ray irradiation. Pretreatment of PG continuously five days prior to radiation displayed significant reduction in the comet parameters ($p < 0.01$) when compared to radiation group alone.

DISCUSSION

Ionizing radiation targeted to a cell causes radiolysis of water, generating free radicals, initiating biological damage to the cell. Hydroxyl radical thus produced causes DNA strand breaks which have been proved by in vitro studies (Rhease, 1968) producing oxidative stress in the tissues. Unsaturated fatty acids of membranes are particularly vulnerable to attack by free radicals generating lipoperoxides which have a damaging effect on cellular components (Barber, 1967). Free radicals promote oxidation of amino acid residue side chains, formation of protein-protein cross linkages and oxidation of protein back bone resulting in protein fragmentation (Berlett, 1997). Nitric oxide is reported to form peroxy nitrite which causes DNA damage by deamination of bases in DNA (Rojas, 1995). The extent of tissue damage is based on the balance between the levels of oxidants and antioxidants within the tissue (Lichtenstein, 1987). Antioxidants like glutathione (reduced) scavenge hydroxyl radicals and singlet oxygen while SOD and catalase detoxify the effects of superoxide and hydrogen peroxide respectively. Our results are well in agreement with the data reported earlier. We have observed a highly significant increase in the levels of lipid peroxides, protein carbonyls, nitric oxide and DNA damage as assessed by comet assay and apoptotic index, in the animal group exposed to a sub lethal dose of 4 Gy of X-rays. This particular group has also shown a significant decrease in glutathione, SOD and catalase compared to sham control. Natural herbs have a profound influence on the free radical generating and scavenging system that operates within the body, net effect being alleviation of toxic effects induced by free radicals (Rao *et al.*, 2008). Therefore extracts of natural herbs are advocated as supplements in free radical mediated diseases. They are reported to produce therapeutic effects on radiation induced normal tissue damage in experimental animals (Rao *et al.*, 2009). A wide variety of natural herbs have been screened for their antioxidant and radio protective properties (Rao *et al.*, 2010).

In our earlier studies, we have reported that *Psidium guajava* showed the presence of flavonoids, saponions, terpenoids, tannin and glycosides. *Psidium guajava* leaf extracts are potential source of natural antioxidants and has remarkably high total phenolic content (Ojan and Nihorimbere, 2004). The phenolic content such as quercetin, ferullic acid, ascorbic acid, gallic acid and guavin B reported by (Thaipong *et al.*, 2005) are responsible for antioxidant activity. Particularly the leaves of *Psidium guajava* possess several plant secondary metabolites which exhibited antioxidant, antiviral and anti-inflammatory activity (Gutierrez *et*

al. 2008; Deguchi *et al.*, 1998; Ojewole, 2006; Mai *et al.*, 2007). In one of the previous in vitro study which was conducted on plasmid DNA, it is reported that flavonoids and phenolic content protected the plasmid DNA by action on hydroxyl free radicals (Anderson E F *et al.*). The antioxidant supplementation enhances the endogenous antioxidant levels and helps to block DNA adduct formation which is the product of free radical DNA damage. In our earlier studies we have reported hydroxyl radical scavenging activity of $77.92 \pm 1.8\%$ at a concentration of $10\mu\text{g}$ as compared to standard quercetin which displayed $89.84 \pm 0.89\%$ scavenging activity. A dose dependent increase in scavenging activity was observed in the crude extract. Metal chelating activity of plant extract was also observed to be increased in a dose dependent manner. At $10\mu\text{g}$ concentration *Psidium guajava* displayed $57.49 \pm 0.9\%$ iron chelating activity respectively, as compared to standard disodium EDTA that displayed $75.44 \pm 0.33\%$ iron chelating activity (Kumar *et al.*, 2014). Further total antioxidant activity of *Psidium guajava* was $242.3 \pm 0.019\mu\text{g}$ compared to $100\mu\text{g}$ equivalence of quercetin and the total phenolic content in the crude extract was found to be $32.5 \pm 4.33\text{ mg GAE/g}$ of extract. *Psidium guajava* displayed reducing property in concentration dependent manner and displayed around 50% reducing property exhibited by that of standard quercetin. However the ABTS free radical scavenging effect was very weak (28.48 ± 2.06) compared to standard ascorbic acid (77.67 ± 0.28) (Kumar *et al.*, 2015). In vivo study results presented in this work are well correlated with in vitro study results reported by us earlier. *Psidium guajava* has established its role as an antioxidant within the living system, particularly in the irradiated animals by significantly lowering the levels of lipid peroxidation, protein carbonyls and NO to control levels and increasing the levels of antioxidants like reduced glutathione and SOD significantly. An increase in catalase level was also observed although non-significant. Apoptotic index improved slightly by pretreatment with *Psidium guajava*. Apoptosis is an event that follows mitochondrial membrane damage which is a common feature in radiation. Apoptosis is initiated by various factors in which radiation is one among them. Apoptosis results in structural changes of cells like shrinkage of cells, chromatin condensation, nucleosome DNA fragmentation (Kerr *et al.*, 1972; Sgonc and Gruber, 1998). There are evidences where antioxidants and free radicals have reduced radiation induced apoptosis (Weiss and Landauer, 2003). Study done on Hesperidin and cimetidine displayed anti apoptotic activity against cultured human peripheral blood lymphocytes when exposed to radiation (Kojima *et al.*, 2002; Kalpana *et al.*, 2009). Pretreatment of zingerone leads to the suppression of apoptotic morphological changes, and also reduced apoptotic index. *Psidium guajava* does not have a role in the protection of mitochondrial damage, based on our results. Comet assay results in the different study groups have indicated that *Psidium guajava* has a profound role in minimizing radiation induced double strand breaks in DNA and consequent cell death. Since plants are rich in antioxidant activity, have a role to play in reducing apoptosis initiated by radiation as per the earlier reports *Psidium guajava* has proved to be a good antioxidant as per our

previous study (Kumar *et al.*, 2015) and therefore may be effective in reducing apoptosis induced by radiation.

CONCLUSION

Psidium guajava is a powerful antioxidant in vitro and can well be used as an antioxidant supplement in vivo, to minimize the damaging effects of radiation within living system, particularly by preventing DNA damage and apoptosis. Further studies with the lead molecule in the extract, may provide valuable tools that may aid in ameliorating the harmful effects of radiotherapy on normal tissues, in cancer patients.

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