Volume 12, No 1 | International Journal of Radiation Research, January 2014

Radioprotective efficacy of *prunus avium* fruit in mice brain with reference to *in-vitro* and *in-vivo* studies

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► ***Original article***

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Submitted: Oct. 2012
Accepted: June 2013

Int. J. Radiat. Res., January 2014; 12(1): 21-31

ABSTRACT

Background: Radioprotective efficacy of *Prunus avium* fruit extract (PAE) rich in vitamin A, C, E and anthocyanin was studied against radiation induced biochemical alterations in mice brain. ***Materials and Methods:*** *In-vitro assays* were performed with PAE for its antioxidant studies; thereafter for *in-vivo* study, Swiss albino mice were divided into five groups. Group 1 (control) normal mice. Group 2 (PAE treated) PAE supplemented (450 mg/kg. b.wt/day) for 15 days. Group 3 (Irradiated) 5 Gy gamma-radiation treated. Group 4 (PAE + Irradiated) was administered PAE once daily for 15 consecutive days, thereafter exposed to 5 Gy gamma-radiation. Group 5 (Irradiated + PAE) was exposed to 5 Gy gamma-radiation than PAE was administered for 15 consecutive days. Brains were removed at various post treatment intervals for biochemical estimations. ***Results:*** The IC_{50} values of PAE for 2,2-diphenyl-1 -picrylhydrazyl ($DPPH^{\cdot}$) scavenging assays, superoxide radical scavenging assays, inhibition of *in vitro* lipid peroxidation assays and protein carbonyl formation assay were 413 µg/ml, 7.63 µg/ml, 136.18 µg/ml and 16.94 µg/ml respectively. Furthermore the total phenolic content in PAE was equivalent to 8.38 mg/ml of gallic acid. The rate of $^{\cdot}OH$ radical scavenging activity of PAE was 0.57 times slower than SCN^{-} derived from nanosecond pulse radiolysis studies. *In-vivo* study also showed that radiation (5 Gy) induced augmentation in the levels of lipid peroxidation and depletion in glutathione and protein level in mice brain were significantly ameliorated by PAE pre/post treatment. ***Conclusion:*** Results suggest that the radioprotective efficacy of PAE may be due its antioxidant property.

Keywords: *Prunus avium, in-vitro antioxidant scavenging assays, in-vivo studies, mice brain.*

INTRODUCTION

During radiotherapy, ionizing radiation particles interact with biological systems to induce excessive oxygen free radicals or reactive oxygen species (ROS), which attack various cellular components including DNA, proteins and membrane lipids, thereby leading to significant cellular damage. ROS also have negative impact on the antioxidant defense mechanisms, which shift the balance between prooxidant and antioxidants in cell towards former resulting in severe oxidative stress and ultimately cell death [1]. Malondialdehyde (MDA) formation as an end product of lipid peroxidation is one of the important indices of oxidative damage [2]. One of the mechanism of protection, involving free-radical scavenging, is based on the assumption that free radicals resulting from the radiolysis of water are the main cause of radiation damage to cells [3] .Thus, scavenging free radicals and inhibiting lipid peroxidation are likely key target activities for developing successful radioprotection strategies [4]. Several molecular drugs of synthetic and

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natural origin are being tried in several experimental models to mitigate the radiation injury [5]. Among different molecular radioprotectors WR-2721 and related compounds have been found to be most promising but the side effects associated with them have restrained their use [6]. In view of this, search for newer and more effective agents is inevitable.

India has a rich heritage of medicinal plants, many of which have been explored for the various bioactivities since ages, but their radioprotective potential has hardly been explored. In this context, Cherry (*Prunus avium*) commonly known as sweet cherry, wild bird cherry, found in Jammu and Kashmir, Himachal Pradesh, Uttaranchal and hills of Tamil Nadu [7] rich in antioxidants was selected to evaluate its radioprotective efficacy. According to Wang *et al*. [8], anthocyanin content in sweet cherry is 350-450 mg/100gm of fruit. According to USDA Nutrient Database for Standard Reference [9] 100 grams of edible portion of fruits of *Prunus avium* has selenium 0.6 µg, ascorbic acid 7 mg, thiamin 0.05 mg, riboflavin 0.06 mg, niacin 0.4 mg, pantothenic acid 0.127 mg, vitamin B_6 0.036 mg, folate 4.2 µg, vitamin A 214 IU, vitamin A 21 µg RE and vitamin E 0.130 mg ATE.

According to Pandey *et al.* [10] tender stem of P*runus avium* has ethnomedicinal value in heart diseases. The fruit stalks are astringent, diuretic and act as tonic. A decoction is used in the treatment of cystitis, oedema, bronchial complaints, looseness of the bowels and anemia [11]. Fruits of P*runus avium* have digestive and antispasmodic uses. Recent study reported some evidence that cherry consumption might lower levels of urate in the blood [12]. But the radioprotective potential of this fruit has hardly been explored.

Brain is considered sensitive to oxidative damage because brain is enriched in the more easily oxidizable polyunsaturated fatty acids as it has highest rate of aerobic glycolysis of all tissues. Human brain consumes 20% of body oxygen and glucose and for this reason it is sensitive to hypoxia [13]. On the other hand, brain is not enriched in antioxidant defense system as it contains relatively low levels of superoxide dismutase, catalase, glutathione peroxidase [14]. External supplementation through antioxidants is recommended to protect cells from the deleterious effects of such oxidative stress conditions. Ample researches indicate that age-related neuronal-behavioral decrements are the result of oxidative stress that may be ameliorated by antioxidants [15].

So, we studied mitigation of deleterious effects of ionizing radiation in Swiss albino mice by *Prunus avium* extract (PAE) with special reference to brain along with free radical scavenging activity of PAE by employing nanosecond pulse radiolysis, stopped-flow spectrometer and other assays to prove the radioprotective activity of anthocyanin and vitamins rich *Prunus avium* extract (PAE).

MATERIALS AND METHODS

Animal care and handling

Swiss albino mice, 6–8 weeks old weighing 23±2 gm, from an inbred colony were used for the present study. These animals were maintained under controlled conditions of temperature and light (light: dark, 10 hrs: 14 hrs.). Four animals were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mice feed (procured from Hindustan Levers Ltd., India) and water *ad libitum*. The departmental animal ethical committee approved this study.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH*) was purchased from Aldrich Chemicals, USA. Xanthine, Xanthine oxidase, Bovine Serum Albumin (BSA), 2,2′-dinitrophenyl hydrazine (DNPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), Tetramethoxy Propane (TMP), glutathione (GSH), 5, 5 dithio-bis 2-Nitrobenzoic acid (DTNB), Potassium superoxide were obtained from Sigma Chemicals, USA. All the other reagents used were of the highest available purity. Nitrous oxide (N_2O) and oxygen (O_2) gases

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obtained from Indian Oxygen Ltd., Mumbai were of IOLAR grade purity. Nanopure water was obtained from a Millipore Elix/A-10 water purification system. Freshly prepared solutions were used for each experiment.

Extract preparation (Drug)

Fresh fruits of *Prunus avium* were washed, shade dried and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 48 hours (4×12) at 40°C. The extract thus obtained was vacuum evaporated so as to get in powdered form. The extract was re-dissolved in doubled-distilled water (DDW) just before the oral administration. For the various concentrations, a known amount of PAE was suspended in DDW and 50 µl of PAE suspension was given to each mouse by oral gavage.

A. Total phenolic content

The total phenolic content present in PAE was measured by using a modified colorimetric Folin-Ciocalteu method [16]. Briefly, 0.5 mL of water and 0.125 mL of the methanolic extract of PAE (concentration range 15–30 µg/mL) were added to a test tube. The Folin-Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. To that 1.25 mL of the sodium carbonate solution (7%) and 3 mL of water was added and allowed to stand for 90 min. The absorbance of the solution was measured at 760 nm. The measurements were compared with the standard calibration curve plotted for the gallic acid solution (2-10 µg/mL) and the total phenolic content in PAE was expressed as % gallic acid equivalents.

B. In-vitro free radical scavenging studies radical scavenging assays

a. DPPH radical scavenging

DPPH· (2,2′-diphenyl-1-picrylhydrazyl) is a stable free radical [17]. The odd electron present in the DPPH gives a strong absorption maximum at 517 nm. For steady state DPPH experiments, 0.6 ml of 200 mM DPPH in methanol was mixed with PAE (200-700 mg/ml) in methanol in 3 ml reaction mixture, and kept in dark for 20 min. The absorbance at 517 nm was monitored both in the presence and absence of PAE. Blank experiment was also carried out to determine the absorbance of DPPH before interacting with the extract.

b. Superoxide radical scavenging assay

The superoxide assay was studied spectrophotometrically using xanthine/ xanthine oxidase system [18]. Briefly, 3 ml solution consists of 38 mM tris- HCl buffer pH 7.4, 16 mM xanthine, 10 mM cytochrome C (Fe^{3+}) and about 0.02 units/ml of xanthine oxidase enzyme. The decrease in the absorbance of Fe^{2+} was monitored at 550 nm in the absence and presence of 2-25 mg/ml of the PAE.

c. Hydroxyl radical scavenging assay

Using nanosecond pulse radiolysis technique, employing 500ns pulses of 7 MeV electron, with an absorbed dose of 8 Gy, the reactions of ·OH radicals with PAE were carried out in microsecond timescale and the transients detected by absorption spectrometry as described by Sharma *et al.* [17]. Rate constant for the reaction of any antioxidant with a free radical indicates its reactivity towards the free radical. However, in the case of plant extracts like PAE, which is a mixture of components with unknown concentrations, estimation of rate constant is difficult. However, it is possible to estimate their relative reactivity in comparison with a standard whose rate constant with ·OH radical is accurately known. Here we employed potassium thiocyanate (KSCN) as the reference solute. In this method, ·OH generated by radiolysing water is made to react with 2 mM KSCN at pH 7, in the absence and presence of various concentrations of PAE. In the absence of PAE, ·OH reacts completely with SCN^- to produce $(SCN)_2^{\cdot-}$. The absorbance was calculated by monitoring the formation of $(SCN)_2^{\cdot-}$ at 500 nm. However in the presence of PAE, decrease in the absorbance of $(SCN)_2^{\cdot-}$ was observed. From the extent of decrease, the rate of scavenging of hydroxyl radical by PAE was calculated with respect to SCN^-. The reactions involved in this competition kinetic method are:

$$\cdot OH + 2SCN^- \rightarrow (SCN)_2^{\cdot-} k_1 = 1.1 \times 10^{10}\ M^{-1} s^{-1}, \quad (1)$$

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$^{\cdot}OH + PAE \rightarrow$ Products $k_2 = ?$ (2)

The absorbance due to $(SCN)_2^{\cdot-}$ at 500 nm in the absence (A_0) and presence of varying concentrations of PAE or PA (A), is related to the rate constants by the following equation

$$\frac{A_0}{A} - 1 = \frac{K_2[PAE]}{K_1[SCN^-]} \quad (3)$$

Slope of the linear plot for $[(A_0/A)-1]$ vs [PAE]/[SCN^-], both the concentrations expressed in mg/ml, is used to estimate the comparative ability of the PAE to react with the $^{\cdot}OH$ radical with respect to KSCN.

C. In-vitro radioprotective activity

a. Lipid peroxidation

Lipid peroxidation (LPO) studies were carried out in phosphatidyl liposomal models induced by the radiation from a ^{60}Co g-source. N_2O/O_2-purged liposomal solution was exposed to g-radiation in the absence and presence of different concentrations (25-200 mg/ml) of the PAE at physiological pH 7.4 (phosphate buffer). The detailed methodology used for the lipid peroxidation is given in our earlier references [17]. The extent of lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS) using 15% w/v trichloroacetic acid, 0.375% w/v TBA, 0.25 N hydrochloric acid, 0.05% w/v BHT as TBA reagent measuring the absorbance at 532 nm ($e_{532} = 1.56\times10^5\ M^{-1}\ cm^{-1}$).

b. Protein carbonyl estimation

Protein oxidation was carried out in Bovine Serum Albumin (BSA). The formation of carbonyl groups due to exposure in g-irradiation in the absence and the presence of (10-50 mg/ml) of PAE were assayed by a standard DNPH-coupled Spectrophotometric method [17-18]. Samples were saturated with N_2O and exposed to g-radiation to an absorbed dose of 60 Gy from ^{60}Co g-source with a dose rate of 30 Gy min^{-1} as measured by standard Fricke Dosimetry. Percentage protection calculations for lipid peroxidation and protein carbonylation calculated by using equation 4.

$$\%\ protection = 100 - \left\{\frac{\Delta OD_{Irradiation}(Test-compound)}{\Delta OD_{Irradiation}(Blank)} \times 100\right\} \quad (4)$$

Here ΔA (Test compound) and ΔA (Blank) indicate the absorbance either at 532 or 370 nm in the presence and absence of PAE respectively. For the estimation the concentration (in mg/ml) required to inhibit lipid peroxidation and protein carbonylation, by 50 % was plotted as a function of the concentration of PAE or and from the plot, the concentration required to reduce the activity by 50% was identified.

D. In-vivo radioprotective studies

a. Source of irradiation

The cobalt teletherapy unit (ATC-C9) at Cancer Treatment Center, Radiotherapy Department, SMS Medical College and Hospital, Jaipur, Rajasthan, India was used for irradiation. Unanaesthestized animals were restrained in well-ventilated Perspex boxes and whole body exposed to 5 Gy gamma radiation.

b. Drug dose selection

Single dose of PAE at the rate of 450-mg/kg b.wt was given orally to mice one hour before the radiation exposure as used by Sisodia *et al.* [19] earlier.

c. Experimental design

Mice selected from an inbred colony were divided into 5 groups

Group 1. Control (vehicle treated): Mice of this group received only DDW water for 15 days.

Group 2. PAE treated: Mice of this group received PAE (450 mg/kg of b.wt./day) for 15 days.

Group 3. Irradiated: Mice received DDW (volume equal to PAE solution) one hour before the whole body exposure to 5 Gy of gamma-radiation.

Group 4. PAE treated + Irradiated: Animals in this group were orally administered PAE (450 mg/kg of b.wt./day) once daily for 15 consecutive days before they were whole body exposed to single dose of 5 Gy gamma-radiation.

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Group 5. Irradiated + PAE treated: The mice were whole body exposed to single dose of 5 Gy gamma-radiation thereafter oral administration of PAE (450 mg/kg of b.wt./ day) was made once daily for 15 consecutive days.

Removal of brain tissue

Mice were sacrificed by cervical dislocation. An incision was made at the sides of the jaws to separate the upper and the lower palates. The upper palate was cut in the middle and after having cleared of the surrounding tissue; brain was excised and separated from the spinal cord at the decussating of the pyramids. The intact whole brain was then removed carefully and homogenate was prepared and used for quantitative estimations.

Mice from each group were necropsied at various intervals, i.e. 1, 3, 7, 15, 30 and 45 days post irradiation. Whole brain was used to estimate various alterations in biochemical parameters viz. glutathione, lipid peroxidation and protein content.

d. Lipid peroxidation (LPO) assay

LPO was measured by the method of Buege and Aust [20]. A standard curve was prepared by using TMP. After comparison with standard curve the LPO level were expressed in n mole TBARS/ g tissue.

e. Reduced glutathione (GSH) assay

The reduced glutathione (GSH) content of tissue samples was determined in testis by the method of Moron *et al.* [21]. The results were expressed as n mol of GSH/100mg of tissue.

f. Protein assay

Estimation of protein was based on the method proposed by Bradford [22].

Statistical analysis

The results obtained in the present study were expressed as mean ± SEM. The statistical difference between two groups were analyzed by the Student's *t-test* and difference between various group were analyzed by one way ANOVA with Graph Pad Prism-5 software at different autopsy intervals and the significance was observed at the $p < 0.01$, $p < 0.001$ and $p < 0.000$ level.

RESULTS AND DISCUSSION

Radiation protection is an important aspect in radiotherapy of cancer where normal cells have to be protected while cancers are exposed to radiation [23]. The interaction of ionizing radiation with biological system results in the generation of many highly reactive oxygen species (ROS) mainly due to the hydrolysis of water. These ROS attack cellular macromolecules like DNA, RNA, proteins, membranes, etc., and cause its dysfunction and damage. ROS increased the membrane lipid peroxidation, which in turn can alter the integrity of membrane structure leading to inactivation of membrane-bound enzymes, loss of permeability of the membranes and decrease in membrane fluidity. Various disease conditions are also associated with free radical induced oxidative stress [24]. As our previous study showed that 5 Gy gamma radiation shows significant change in brain protein and LPO level which also correlates with change in brain histology alteration and behavioral imbalance [25], Similarly we found significant deleterious change in protein, LPO and GSH level in group 3 i.e. irradiated only and at this exposure (5 Gy) mice no mortality has been noticed as we have shown already that at 10 Gy mice hardly survive beyond 10-12 days.

Antioxidants can protect against the damage induced by free radicals acting at various levels [26]. Herbal drugs containing free radical scavengers like phenolics, tannins and flavonoids are known for their therapeutic activity [27]. In the present study, preliminary phytochemical testing showed the presence of phenolics in PAE by Folin-Ciocalteu method. Subsequent quantification with respect to gallic acid revealed that the total phenolic content in PAE is equivalent to 8.38 mg/ml of gallic acid.

The reaction of DPPH radical with PAE was studied by steady state. Figure 1 shows

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the change in absorbance of DPPH radical in methanol at 517 nm in the absence and presence of different concentration of PAE. From this figure the IC_{50} value of PAE estimated was 413 mg/ml (table 1).

Xanthine oxidase is one of the main enzymatic sources of reactive oxygen species *in-vivo*. In this study PAE has been found to possess superoxide radicals scavenging property. The superoxide radicals generated from xanthine/xanthine oxidase assay were examined for the scavenging by PAE in comparison with cytochrome C and the percentage inhibition of superoxide radical formation was estimated. Figure 2 shows the percentage inhibition of superoxide radicals at different concentrations of PAE. From this study, the IC_{50} value of PAE for the inhibition of superoxide radicals was calculated to be 7.63 mg/ml (table 1).

Here the activity for the inhibition of superoxide radical can be either due to direct scavenging of the superoxide radical or due to the inhibition of the activity of xanthine oxidase.

Among the several free radicals, hydroxyl radical (·OH) is the most potent oxidant produced during radiation exposure as well as Fenton reactions [17]. It is a strong oxidizing radical with standard reduction potential of 1.9 V vs NHE at pH 7. Using nanosecond pulse radiolysis technique, the reaction of ·OH radical with PAE was carried out in microsecond time scale and the transient spectrum obtained in these reactions is shown in figure 3. The spectra obtained from the reaction of ·OH radical with PAE showed a broad spectrum in the region of 350 – 600 nm. The spectrum has no characteristic absorption bands, indicating presence of many unidentified species in PAE extract. The reactivity of the PAE towards ·OH radical was estimated by competition kinetics methods using KSCN as a reference solute. Inset of figure 3 shows the linear plot, for the variation of the absorbance due to $SCN_2^{\cdot-}$ as a function of the ratio of concentrations of PAE with SCN^- according to the equation 3, from the slope of this linear plot, calculated relative rate of scavenging ·OH radical by PAE was found to be 0.57 times slower that of SCN^-.

Table 1. IC_{50} (i.e. 50% inhibition concentration) values of *methanolic extract of Prunus avium fruits* (PAE) obtained by various *in-vitro* free radical scavenging and radioprotective studies.

Free radical scavenging assays	IC_{50} values (mg/ml)
DPPH scavenging assays	413 mg/ml
Superoxide radical scavenging assays	7.63 mg/ml
Lipid Peroxidation	136.18 mg/ml
Protein Carbonyl assays	16.94 mg/ml

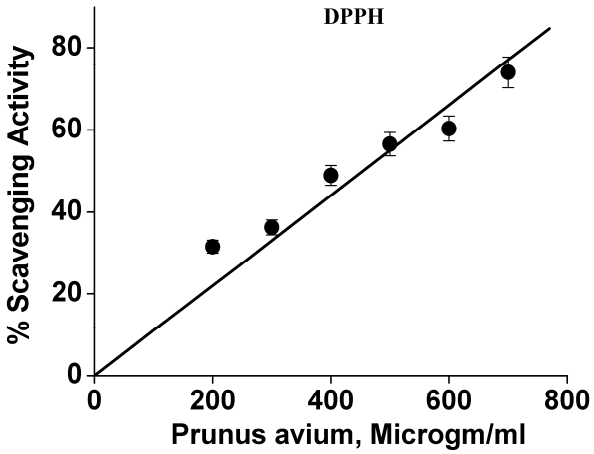


Figure 1. Linear plot shows the scavenging ability of DPPH radical by different concentrations (200µg/ml to 700 µg/ml) of PAE (methanolic extract of *Prunus avium* fruits). The DPPH radical scavenging activity was measured by following the decrease in absorbance at 517nm at DPPH concentration of 100 µM.

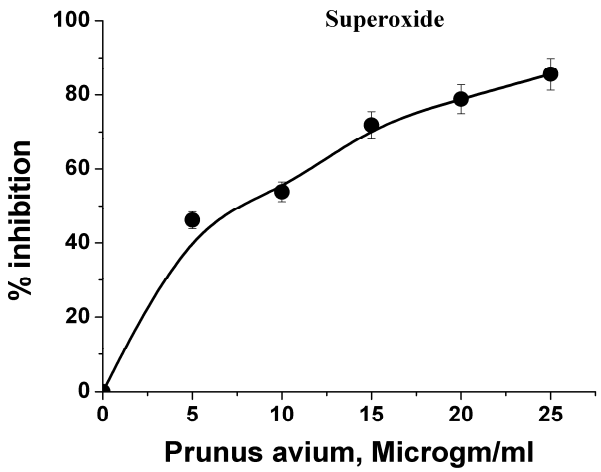


Figure 2. Inhibition of the superoxide radical scavenging activity with increasing concentrations (2 µg/ml to 25 µg/ml) of PAE (methanolic extract of *Prunus avium* fruits).

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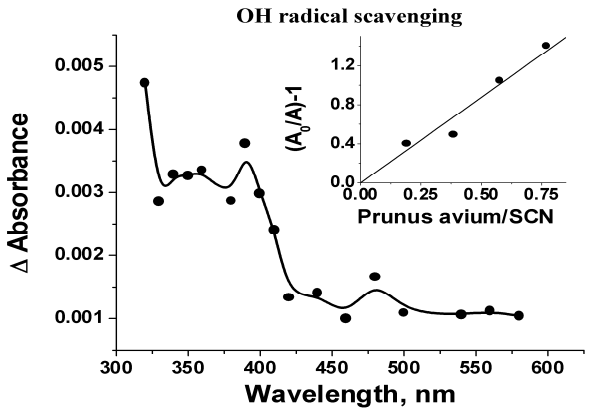


Figure 3. Difference in absorption spectrum of the transient obtained on pulse radiolysis of N_2O saturated aqueous solutions containing 100µg/ml of PAE (methanolic extract of *Prunus avium* fruits) at pH 7. Inset shows linear plot for competition kinetics between SCN^{2-} and PAE towards hydroxyl radicals. Lines show fitting according to equation 3.

Oxidative stress leads to lipid peroxidation, protein and carbohydrate oxidation and metabolic disorders [28]. The peroxyl radical formed through lipid peroxidation attacks protein membrane and enzymes and reinitiates lipid peroxidation. The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions such as lipid peroxidation. Inhibition of iron-induced lipid peroxidation in egg phosphatidylcholine liposomes as assessed by the formation of TBARS by PAE was shown concentration dependent (figure 4) and IC_{50} value of PAE was found to be 136.18 µg/ml (table 1). This result indicates that PAE has the ability to inhibit the lipid peroxidation as shown by decreasing amount of TBARS content.

Lipid peroxidation product (figure 5) as reflected by TBARS equivalent in *in-vivo* studies was significantly ($p<0.001$) higher after 5 Gy gamma radiation exposure in Group 3 mice brain when compared to the control mice (Group 1) brain at all autopsy intervals. Such increase in lipid peroxidation product was not stable and continuously decreased till the last autopsy interval except at day 7 where slight increase was noticed. TBARS content was significantly lower ($p<0.001$) in Group 4 and 5 as compared to Group 3 mice at all autopsy intervals and values almost reached the control levels by the last autopsy interval i.e. day 45 (figure 5). Statistically significant reduction ($p<0.001$) in the level of TBARS equivalent was seen in only PAE treated group (Group 2). When all the four groups i.e. group I, III, IV and V were compared to each other on each autopsy interval by one-way ANOVA, highly significant differences were observed on day 1 ($F_{3,44}$=792.11, p=0), 3 ($F_{3,44}$=265.55, p=0), 7 ($F_{3,44}$=2002.69, p=0), 15 ($F_{3,44}$=62.35, p=0), 30 ($F_{3,44}$=46.45, p=0) and 45 ($F_{3,44}$=162.17, p=0) *p.i.* between all the groups.

The preservation of cellular membrane integrity depends on protection or repair mechanisms capable of neutralizing oxidative reactions [17]. Present study reveals that prior/ post supplementation of PAE against radiation exposure shows beneficial effect against the formation of free lipid radicals and thus prevents the formation of endoperoxidation.

Exposure of mice with 5 Gy gamma radiation declined the glutathione (GSH) content upto day 7 thereafter recovery was noticed (figure 6). In groups (4 and 5) supplemented with PAE prior/ post irradiation magnitude of recovery from oxidative damage was higher in comparison to group 3 at all the autopsy intervals but significant statistical difference was present at later intervals only, whereas in Group 5 (irradiated + PAE treated), after non-significant

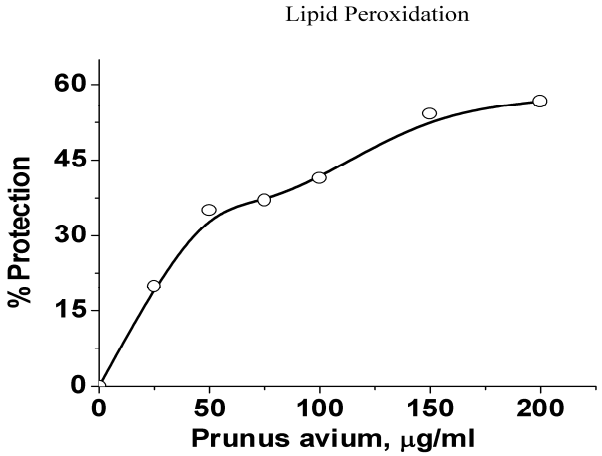


Figure 4. Inhibition of γ radiation (240 Gy) induced lipid peroxidation in phosphatidyl choline liposome (1mg/ml) in presence of different concentrations of PAE (methanolic extract of *Prunus avium* fruits) (25µg/ml to 200µg/ml).

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difference noticed at day 1, the values of GSH were found significantly higher ($p<0.001$) than Group 3 mice till the last autopsy interval where the values reached near control level (figure 6). Statistically non-significant change in GSH content was noted in group 2 in comparison to group 1. When all the four groups i.e. group I, III, IV and V were compared to each other on each autopsy interval by one-way ANOVA, highly significant differences were observed on day 1 ($F_{3,44}=7.388$, $p=0.0004$), 3 ($F_{3,44}=8.347$, $p=0.0002$), 7 ($F_{3,44}=10.199$, $p=0.0000$), 15 ($F_{3,44}=6.45$, $p=0.001$), 30 ($F_{3,44}=4.567$, $p=0.0072$)

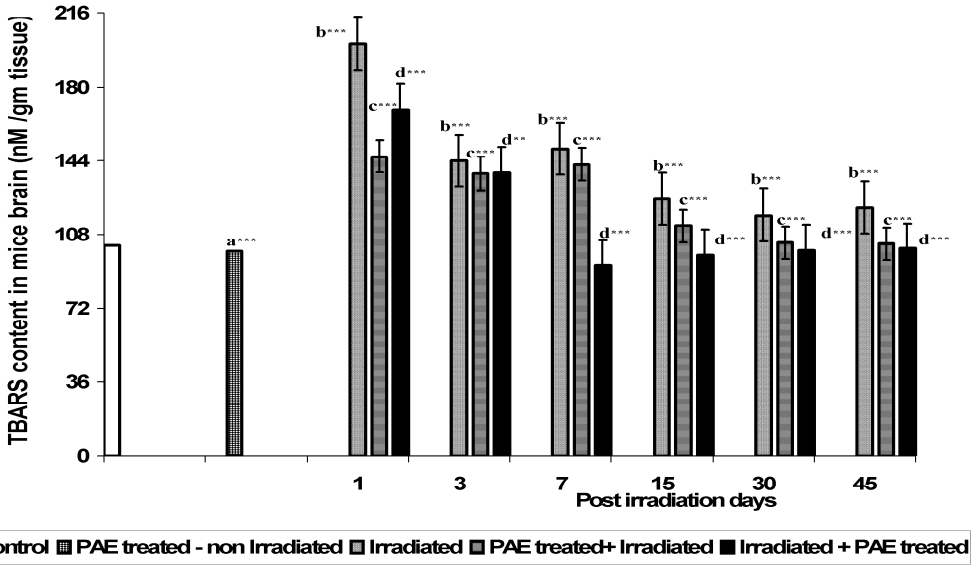


Figure 5. Graph showing protection of lipid peroxidation in mice brain after 5 Gy gamma irradiation by prior and post administration of PAE (methanolic extract of *Prunus avium* fruits). The lipid peroxidation was measured in terms of nM TBARS/g tissue by LPO assay. Following groups were compared by student's t-test- a: Control v/s PAE treated b: Control v/s Irradiated, c: Irradiated vs PAE treated + Irradiated, d: Irradiated vs Irradiated + PAE treated. Symbols for p values of t-test $<0.05,*<0.01, **<0.005, ***<0.001, n= non significant.

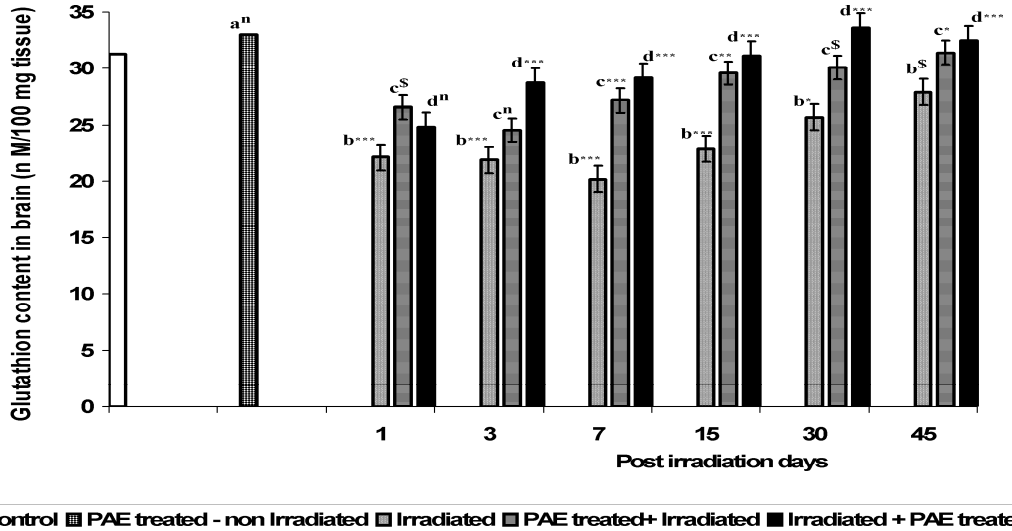


Figure 6. Graph showing protection of glutathione content in mice brain after 5 Gy gamma irradiation by prior/ post administration of PAE (methanolic extract of *Prunus avium* fruits). The glutathione level was measured in terms of nM /100mg tissue. Following groups were compared by student's *t-test*- a: Control v/s PAE treated b: Control v/s Irradiated, c: Irradiated vs PAE treated + Irradiated, d: Irradiated vs Irradiated + PAE treated. Symbols for p values of t-test $<0.05,*<0.01, **<0.005, ***<0.001, n= non significant.

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and 45 ($F_{3,44}$=3.32, p=0.028) post treatment between all the groups.

Glutathione plays important role in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation. Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and pathogenesis of many diseases, including Alzheimer's disease (AD) [29]. Most of the researchers have observed that glutathione levels decrease with ageing [30]. The present study demonstrates a significant reduction in GSH content in brain following radiation exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Our studies are in line with those of Sisodia *et al.* [31] who also showed the augmentation of lipid peroxidation and reduction in GSH content in mice cerebrum after radiation exposure. The increased GSH level suggests that protection by PAE may be mediated through the modulation of cellular antioxidant levels.

In-vitro radioprotecting ability of PAE was also evaluated in terms of inhibition of radiation induced protein carbonylation by DNPH method. figure 7 shows that the formation of γ radiation (60Gy) induced protein carbonylation in BSA decreased as concentration of PAE increases

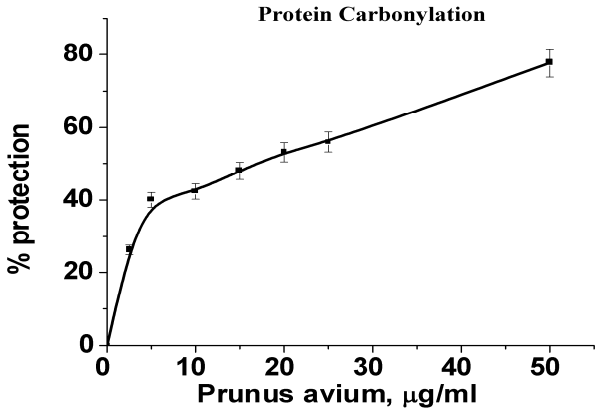


Figure 7. Inhibition of γ radiation (60Gy) induced protein carbonylation in BSA in presence of different concentrations (10- 50μg/ml) of PAE (methanolic extract of *Prunus avium* fruits).

which indicates the radioprotecting ability of PAE. The IC_{50} value for PAE to protect 50 % of BSA in comparison to radiation control was estimated as 16.94 mg/ml for PAE (table 1).

Protein estimated in *in vivo* studies also showed statistically significant decrease in mice brain after radiation exposure. Such decline in protein content was noted continuously till day 30 post irradiation at significance (P<0.001) but at day 45 p.i. this reduction was significant at level p<0.05. In Group 4 recovery was evident after day 7 *p.i.* Protein content estimated in mice brain was significantly higher (p<0.001) in both the Groups (4 and 5) supplemented with PAE (prior/ post) irradiation in comparison to Group 3 at all the autopsy intervals (figure 8). PAE administration alone for 15 days could raise the baseline values of protein concentration. The increase was statistically significant (P<0.001). When all the four groups i.e group I, III, IV and V were compared to each other on each autopsy interval by one-way ANOVA, highly significant differences were observed on day 1 ($F_{3,44}$=33.53, p=0), 3 ($F_{3,44}$=45.85, p=0), 7 ($F_{3,44}$=485.67, p=0), 15 ($F_{3,44}$=167.16, p=0), 30 ($F_{3,44}$=132.31, p=0) and 45 ($F_{3,44}$=32.42, p=0) *p.i.* between all the groups. Increased protein concentration recorded in our study, shows that PAE supplementation in irradiated mice is a beneficial effect.

Prunus avium contains anthocyanin, vitamin C etc as its constituents which are potent antioxidant. DelgadoVargas *et al.* [32] demonstrated that anthocyanins have scavenging properties against •OH and O^{-2} and are better agents against lipid peroxidation than α-tocopherol (up to seven times). Mechanisms of antioxidative action of vitamin C are direct scavenging and blocking of ROS, as well as regeneration of other antioxidative systems [33]. Protective effects of vitamin C against ionizing radiation DNA damage have also been extensively documented [34]. Earlier studies in our laboratory showed that anthocyanin, vitamin C rich *Grewia asiatica* fruit posses radioprotective efficacy in brain [35], cerebrum[31)], liver[36], blood[6] and testis[18] when supplemented prior/ post irradiation.

The results of the present investigation

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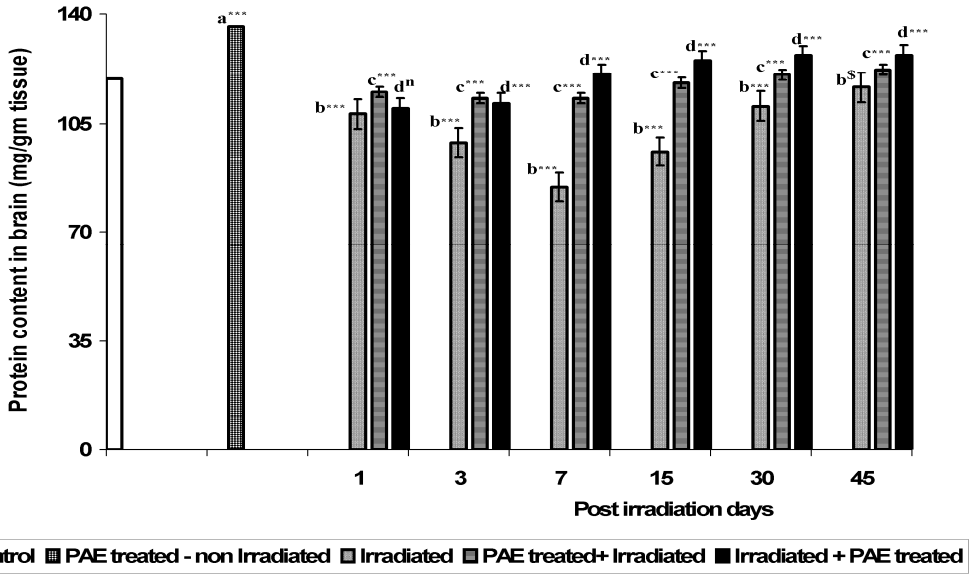


Figure 8. Graph showing protection of protein content in mice brain after 5 Gy gamma irradiation by prior/ post administration of PAE (methanolic extract of *Prunus avium* fruits). The protein level was measured in terms of mg/g tissue by Bradford method. Following groups were compared by student's t-test- a: Control v/s PAE treated b: Control v/s Irradiated, c: Irradiated vs PAE treated + Irradiated, d: Irradiated vs Irradiated + PAE treated. Symbols for p values of t-test $<0.05,*<0.01, **<0.005, ***<0.001, n= non significant.

demonstrate that PAE pre/ post treatment protects the mice brain against radiation induced damage by inhibiting the lipid peroxidation levels and ameliorating the glutathione and protein depletion. PAE also shows the free radical scavenging activity in various *in-vitro* studies, which may be responsible for the radioprotecting ability of PAE noticed.

CONCLUSIONS

Results obtained from the present study indicate that the constituents found in PAE substantially protect the brain from radiation induced oxidative stress. Free radical scavenging property of PAE, noticed in the present study, may be one of the mechanistic aspect responsible for its radioprotective efficacy.

ACKNOWLEDGEMENT

Authors humbly acknowledge Dr. K. I. Priyadarsini, Mr. Amit Kunwar and Late Mr. Umang Singh Radiation & Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai for their valuable suggestions during the course of this study. Authors are also thankful to Dr. A. Chougle, Radiotherapy Unit, SMS Medical College and Hospital, Jaipur (India) for providing irradiation facility and for help in radiation dosimetry.

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