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MODULATION OF RADIATION INDUCED OXIDATIVE STRESS IN SWISS ALBINO MICE BRAIN BY PRUNUS DOMESTICA

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ABSTRACT

Prunus domestica fruits extract (PDE) was evaluated for in vivo radioprotective activity against whole body gamma irradiation in Swiss albino mice. The irradiation of mice resulted in significant elevation of lipid peroxidation in terms of thiobarbituric acid reactive substance (TBARS) and depletion in glutathione (GSH), protein, superoxide dismutase (SOD) and catalase at all the intervals studied, viz 1-30 days in comparison to the control group (no treatment). Radiation also induced deficit in spatial memory as assessed by Morris Water Maze (MWM). Pre/post treatment with PDE significantly ameliorated the lipid peroxidation and activities of endogenous antioxidant enzymes in brain and improved spatial learning.

KEY WORDS:Superoxide dismutase, Morris Water Maze, Radiation, Prunus domestica

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INTRODUCTION

Several plant constituents have been proven to possess considerable free radical scavenging or antioxidant activity. The use of plant and natural products may be beneficial in protecting against the radiation induced damage, as they are less toxic or practically non toxic compared to the synthetic compounds at their optimum protective dose level. Therefore, the interest has been increased in development of most potential drug of plant origin for modification of radiation effect. Recent studies have indicated that some commonly used medicinal plants may be good sources of potent but non toxic radio protectors. The majority of studies in our laboratory report that nutritional intervention to increased intake of phytoantioxidants reduces the threat of free radicals produced by irradiation [1,2,3]. In the present investigation *Prunus domestica,* (family Rosaceae) native to northern Asia, Europe, North America and Africa's northwest coast, contains many phenolics, predominant components of which are caffeoylquinic acid (CQA) isomers [4,5,6] has been selected for the present study. The fruit also contain anthocyanins (type cyanidin-3-glucoside and cyanidine-3-rutinoside), vitamins A, C and E, flavanols (catechin) [7]. Vitamin C, especially abundant in this fruit behaves as a reactive oxygen species (ROS), scavenger, metal chelator and enzyme modulator. According to Mahmood *et al*. (2009) hentricontane, ethyl hexadecanoate and linoleic acid were identified in n-haxene extracts of *Prunus domestica.* Bioassay screening of oil showed moderate antibacterial activity against salmonella group (Gram +ve and -ve) by agar well diffusion method, moderate antifungal activity against *Microsporum canis* by agar tube dilution method and good antioxidant activity by DPPH Radical Scavenging Method [8]. Chlorogenic acid is one of the most abundant polyphenols in plum having health promoting advantages. It protects granulocytes from oxidative stress; moreover this antioxidant activity is coupled with anxiolytic-like effects[9]. In the present study, therefore radiation effects and its modulation by *Prunus domestica* were assessed through biochemical estimations of LPO, GSH, protein, SOD, and catalase and spatial learning and memory in whole brain.

MATERIALS AND METHODS

Mice

The animal care and handling was done according to the guide lines set by INSA (Indian National Science Academy, New Delhi, India). The Departmental Animal Ethical Committee (DAEC) approved this study. Six weeks adult male *Swiss albino* mice, weighing 25 ± 2 g, from an inbred colony were used for the present study. Four mice were housed in a polypropylene cage containing sterile paddy husk (procured locally) as bedding throughout the experiment. They were provided standard mouse feed (procured from Hindustan Levers Ltd, India) and water *ad libitum*.

Extract Preparation

Fresh fruits of *Prunus domestica* collected locally from Shimla were washed, shade dried and powdered after removal of seeds. Methanolic extract was then prepared by refluxing for 48 hours (4 × 12) at 50°C. The extract thus obtained was vacuum-evaporated so as to achieve powdered form. The extract was redissolved in doubled-distilled water (DDW) just before the oral administration.

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. Unanaesthetized mice were restrained in well-ventilated perspex boxes and the whole body exposed to gamma radiation at a source-to-skin distance (SSD) of 77.5 cm from the source to deliver the dose rate of 1.07 Gy/min.

Experiments

Experimental design for radio protective study

Mice were randomly divided into following groups (ten per groups) for biochemical and (six per group) learning studies.

Group I (Control): Mice of this group received double distilled water.

Group II (Only PDE): Mice of this group were supplemented PDE orally once every day for fifteen consecutive days at optimum dose dissolved in double distilled water.

Group III (Irradiated): Mice in this group received double distilled water, which equalled to the dose of extract for fifteen days and then exposed to whole body γ-irradiation at the dose of 5 Gy.

Group IV (PDE+ Irradiation): Mice were supplemented orally with PDE at optimum dose for fifteen consecutive days and then exposed to 5 Gy whole body irradiation.

Group V (Irradiation +PDE): Mice in this group were exposed to 5 Gy whole body γ-irradiation and then supplemented orally with PDE at the optimum dose (400mg/k bwt.)

Spatial Learning and Memory Method by Wenk, (1998)[10]

The apparatus used was a circular water tank (100 cm diameter) filled to a depth of 30 cm with water (25°C). Four points equally distributed along the perimeter of the tank served as starting locations. The tank was divided arbitrarily into four equal quadrants and a small white coloured platform (5 cm width) was submerged in the center of one of the quadrants 1cm below the level of water and therefore invisible to animals. The water was made opaque by the addition of chalk powder. Four different starting positions, equally spaced around the perimeter of pool, were used in a fixed order. The maximum duration of the trial was 60 seconds and mice not finding the platform within these 60 seconds were placed on it. At the end of each trial the mice was allowed to remain on the platform for 20 seconds and then returned to their home cage and left there to rest for 15 minutes, before beginning of the next trial. After initially receiving trial for two days the performance of the mice were video typed for detailed analysis for next ten days (five trials/mice/day). On the technical side, the performance of the animals was quantitatively measured as escape latency, *i.e.* the time elapsed between the immersion of the animal in the tank and it's reaching the platform. This parameter was chosen on the basis of the measured uniform swimming speed of all animals over all trails. In water maze experiments escape time (*i.e.,* the time between releases in the water to landing on the platform) was measured by a stopwatch. The average escape time of 10 days of each mice were used in the data analysis.

Biochemical Assay

Protein

Estimation of protein was based on the method proposed by Bradford (1998)[11]. 10% homogenate was prepared (1 gm of tissue in 9 ml of NaCl) and 0.1ml of the sample was taken for the assay. Three repeats of the assay from each animal were carried out. The absorbance was read at 595 nm.

Lipid Peroxidation (LPO)

LPO was measured by the method of Buege and Aust (1978)[12]. Briefly, tissue homogenate was mixed with TCA-TBA-HCl and was heated for 15 min in a boiling water bath. After centrifugation the absorbance was recorded at 535 nm using a UV-Vis double beam spectrophotometer. The LPO has been expressed as MDA in n mole/ gm tissue.

Reduced Glutathione (GSH)

Spectrophotometric quantification of reduced glutathione (GSH) has been carried out using 5, 5_dithiobis- (2-nitrobenzoic acid) (DTNB) reagent according to the method proposed by Moron *et al*. (1979)[13].

Super Oxide Dismutase

Super oxide dismutase was assayed by the method of Marklund and Marklund (1974)[14] which involves inhibition of pyrogallol auto-oxidation at pH 8.0.

Catalase

It was estimated in the brain homogenate in a UV-VIS spectrophotometer as described by Aebi (1984)[15]. The difference in absorbance at 240 nm per unit time is a measure of catalase activity.

Statistical Analysis

The results obtained in the present study were expressed as mean± SEM. The statistically differences between various group was analysed by the student's t- test and the significance was observed at the $p<0.001$, $p<0.01$ and $p<0.05$ level. The following groups were compared by Student's t test:(a) control versus PDE treated, (b) control versus irradiated, (c) irradiated versus PDE treated + irradiated, (d) irradiated versus irradiated + PDE treated.

RESULTS

Learning Studies

Spatial learning ability investigated after exposure to Co_{60} rays (5Gy) by using water maze showed that initially upto 48 hours after irradiation the mice remained in the quadrant instead of reaching the platform. Nevertheless these mice were able to acquire the spatial information regarding the portion of the escape platform and effectively locate it in subsequent ten days, improving day by day as evident by decrease in time taken to reach the platform. The irradiated mice took longer time to reach the platform compared to the control which was statistically significant ($p<0.05$). Whereas mice in PDE supplemented prior/post irradiation actively tried to acquire the spatial memory as evident by their movement in the pool all over, not just staying in the quadrant area. They showed decrease in escape latency as evident by the lesser time taken to reach the target compared to only irradiated mice (Fig-1).

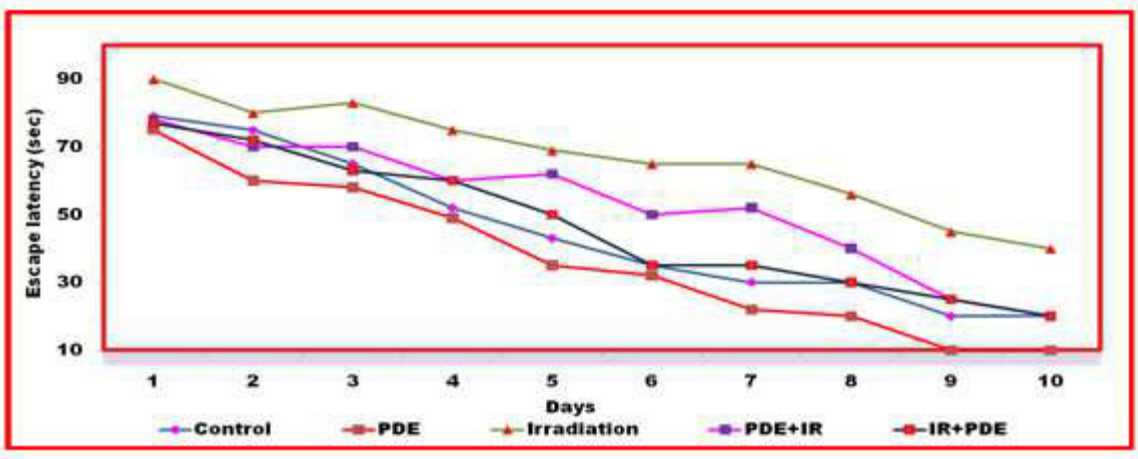

Figure 1
Average escape latency (i.e. time to reach the platform after release into the water) during the 10 days training sessions

Protein

In the whole brain the total protein content increased by 1.43% by oral supplementation of the drug for 15 consecutive days. In Group III (irradiated) in whole brain initially protein content decreased after radiation upto 7 day and maximum protein loss noted was by 13.8% and Group V the protein content was significantly higher at all autopsy intervals in comparison to Group III (Irradiated) studied in the whole brain (Fig.2).

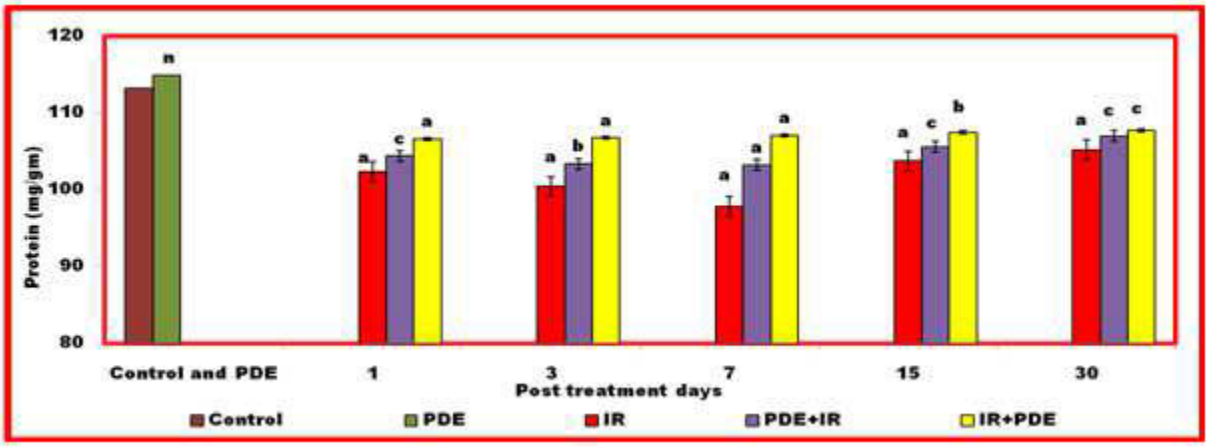

Figure 2

Protection of protein level of mice brain after 5 Gy gamma irradiation by PDE pre and post-administration. t-test applied between: Control vs. PDE treated; Control vs. Irradiated; PDE treated +Irradiated vs. Irradiated; Irradiated+ PDE treated vs. Irradiated. a=p<0.001; b= p<0.01; c= p<0.05; n= Non significant

Lipid Peroxidation (LPO)

Radiation treatment significantly *(p*<0.001) increased the LPO levels in the whole brain of *Swiss albino* mice. Conversely, the LPO levels were significantly depleted in Group IV (IR+PDE) and Group V (PDE+IR) compared with Group III (radiation alone) at all the autopsy intervals. PDE alone was able to lower the LPO contents by 25.41% in the whole brain compared to control. LPO levels were found to be significantly lower in Group V (IR+PDE) compared to Group IV indicating the greater efficacy of PDE (Fig.3).

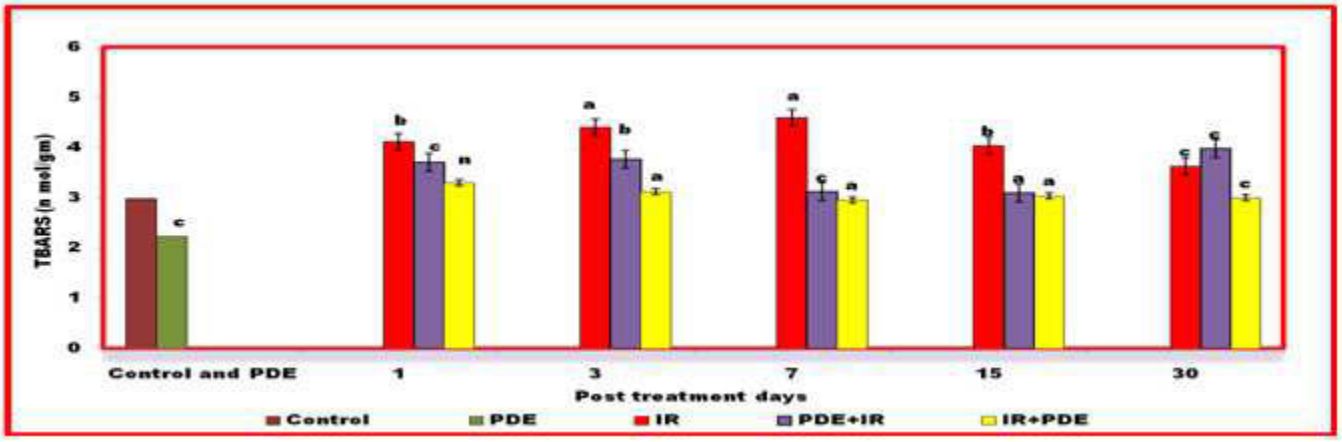

Figure 3

Protection against lipid peroxidation in mice brain after 5 Gy gamma irradiation by pre and post administration of PDE. t-test applied between: Control vs. PDE treated; Control vs. Irradiated; PDE treated +Irradiated vs. Irradiated; Irradiated+ PDE treated vs. Irradiated. a=p<0.001; b= p<0.01; c= p<0.05; n= Non significant

Reduced Glutathione (GSH)

Supplementation of only PDE in Group II raised the baseline GSH values significantly by 7.77% (*p*<0.001) in whole brain in comparison to the control. In Group III irradiation induced continuous

depletion in glutathione concentration up to 7 days in whole brain, it increased. Maximum decrease noted at day 7 post treatment was 43.2% in whole brain (Fig-4).

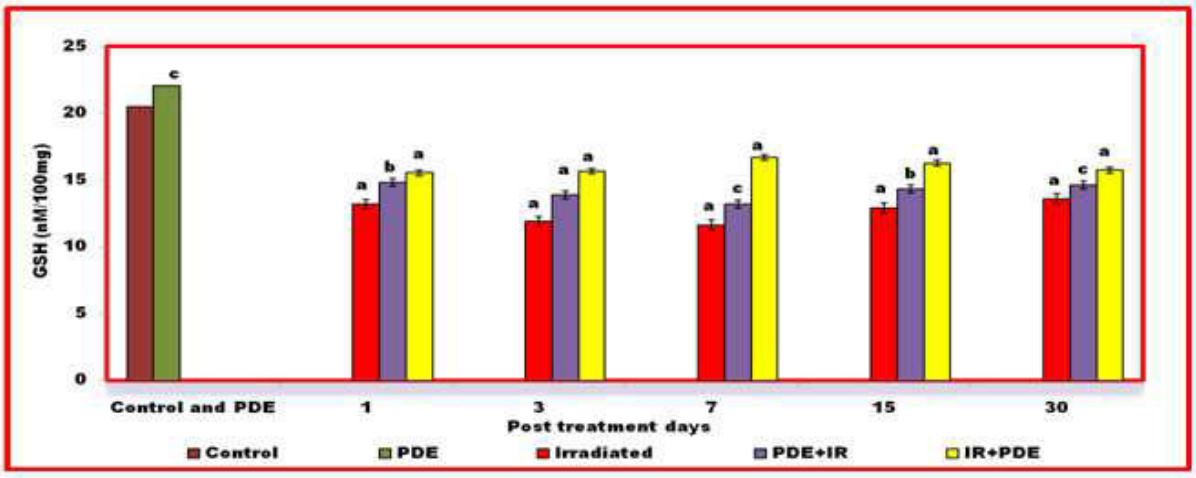

Figure 4

Protection of glutathione (GSH) level of mice whole brain after 5 Gy gamma irradiation by pre and post administrations by PDE. t-test applied between Control vs. PDE treated; Control vs. Irradiated; PDE treated +Irradiated vs. Irradiated; Irradiated+ PDE treated vs. Irradiated. a=p<0.001; b= p<0.01; c= p<0.05; n= Non significant

SOD and catalase

In Group III radiation induced significant (p<0.001) depletion of SOD at all the autopsy intervals which could be augmented by pre/post supplementation of PDE. In Group III the level of SOD on 7th day was 69.77% in whole brain. Statistically no significant difference was observed between Group I (control) and Group II (PDE treated) in catalase level. Post PDE supplementation after irradiation (Group V) provided more protection in comparison to pre supplementation (Fig.5,6).

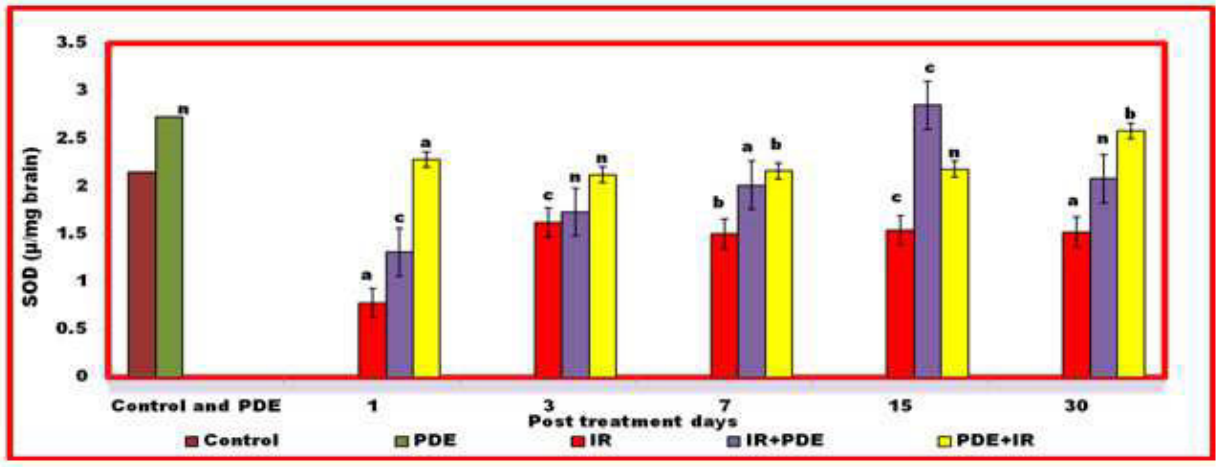

Figure 5

Variation in SOD levels (µ/mg tissue) with and without PDE supplementation in irradiated mice. t-test applied between.Control vs. PDE treated; Control vs. Irradiated; PDE treated +Irradiated vs. Irradiated; Irradiated+ PDE treated vs. Irradiated. a=p<0.001; b= p<0.01; c= p<0.05; n= Non significant

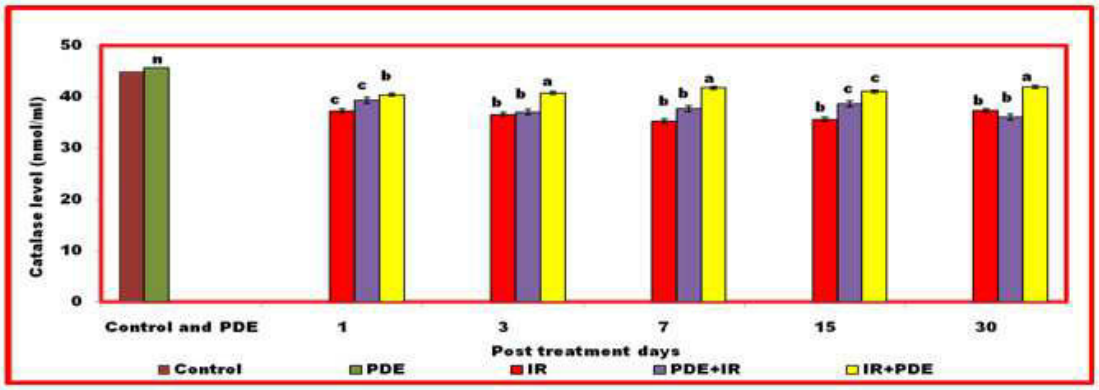

Figure 6

Variations in CAT (nmole/ml) with and without PDE supplementation in irradiated mice. t-test applied between. Control vs. PDE treated; Control vs. Irradiated; PDE treated +Irradiated vs. Irradiated; Irradiated+ PDE treated vs. Irradiated. a=p<0.001; b= p<0.01; c= p<0.05; n= Non significant

DISCUSSION

The neuro anatomical and neurochemical processes associated with water maze performance are well studied. It has been suggested that stress affects learning and memory[16]. Deficit in spatial learning and memory occurs by radiation stress. It is highly possible that these changes cause alteration in congnitive functions, related protein, such as androgen receptors and apolipoprotein A[17]. In our study we found that radiation induced deficits in spatial memory could be improved by PDE supplementation. Supplementation of *Centella asiatica* has shown to increase the neuronal connections in CA3 neurons of hippocampus and acetylcholine activity and enhanced the learning and memory in adult mice[18]. Increase in protein concentration after PDE supplementation is assigned as the compensatory beneficial effect. It suggested that decline in learning and memory is associated with a very significant increase in two parameters of oxidative stress in the brain, levels of lipid peroxidation and of protein oxidation[19]. It is reported that the behavioural suppression was associated with similar differential increases in malondialdehye (MDA) and decreases in glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) levels in males and females[20]. These results support the hypothesis that oxidative stress contributes to radiation related impairment in learning. The nervous system cells of both humans and animals are especially vulnerable to oxidative damage, and the changes in the brain's antioxidative ability are accompanied by enhanced oxidative modification of proteins. Learning ability is always the formation of new protein molecules. Decrease in the the protein contents after exposure has been reported in the present study as well as in an earlier study of our lab[21]. Decrease noticed with time in irradiated group may be probably due to lysis or inhibition of protein synthesis or may be the depression of enzyme involved in the activation of amino acid and transfer to t-RNA or by the inhibition of release of synthesized polypeptide from polysomes [22.] This could be due to excessive damage to the genetic machinery. Increased protein concentration in the present study may be due to impaired ribosomal activities, which enhance protein synthesis. Due to their high polyunsaturated lipid content, schwann cells and axons are particularly sensitive to oxygen free radical damage. The increase in LPO levels in the brain induced by radiation in the present study suggested enhanced lipid peroxidation leading to tissue damage and failure of the antioxidant defense mechanism to prevent formation of excessive free radicals. Reactive

oxygen species (hydrogen peroxide, super oxide anions and hydroxyl radicals) are required for its initiation as NAPQI is expected to be incapable of initiating a radical hydrogen abstraction from lipid molecules. However, reduction of NAPQI, which could occur in the presence of flavoproteins, followed by reoxidation by oxygen could give rise to superoxide anions with a consequent formation of reactive reduced oxygen species[23]. Even protein bound NAPQI was suggested to be liable to one electron reduction. LPO is regarded to be an important initiation event in the toxicity mechanism of radiation. Supplementation of PDE pre/post irradiation significantly reduced the elevated levels of LPO.Glutathione (GSH) is the main intracellular non protein sulfhydryl compound which plays an important role in the maintenance of cellular proteins and lipids in their functional states. When GSH levels are lowered, the toxic effects of oxidative insult are exacerbated, resulting in increased membrane and cellular damage. The present study indicated that the exposure to ionizing radiation induced a progressive decrease in the GSH activity which was ameliorated by the PDE pre/ post treatment, which further validates the hypothesis that PDE may scavenge the free radicals formed during oxidative stress. Glutathione deficiency contributes to oxidative stress, which plays a key role in ageing and the pathogenesis of many diseases, including alzheimer's disease[24] . If mild oxidative stress occurs, normal tissues often respond with extra antioxidant defence. However, severe or persistent oxidative stress can cause cellular component injury with degeneration and finally, brain cell death. Besides, brain utilizes about one fifth of the total oxygen demand of the body and has the lowest level of protective antioxidant enzymes (CAT and GPx). In the present study the levels of enzymatic antioxidants (CAT, SOD, GPX) were lower in irradiated mice than in control. In the present study irradiation resulted in decrease of SOD and catalase activity whereas the PDE treated groups exhibited an increased SOD activity to eliminate the radicals. During oxidative stress in the neuronal cells there is an increase in intracellular Ca^{2+} levels in the brain[25]. This increased intracellular Ca^{2+} can induce the irreversible conversion of xanthine dehydrogenase (XDH) to XO, which in turn catalyses the oxidation of xanthine to provide a source of O_2^-. These mechanisms could be the main reasons for the increased levels of XO and reduction in activity of SOD leading to an overload of oxygen radicals and repression of an antioxidant enzyme with radiation exposure[26].

Present study showed that post treatment is more effective then pre treatment specially at later interval. A possible explanation for the post-irradiation protective effects of antioxidants is a model based on electron spin resonance studies suggesting that radiation exposure results in short-lived radicals, such as OH, responsible for cell death, and long lived radicals, which can cause mutations and transformations[27]. This concept is also supported by experiments using cells irradiated with a microbeam[28]. If PDE is to produce antioxidant effect, its antioxidant constituents must be absorbed by the body and available to the tissue exposed to oxidative stress. Anthocyanins could permeate the blood-brain barrier, in accordance with a recent in vitro study showing that brain endothelial cell lines took up cyaniding 3-rutinoside and pelargonidin 3-gluocosid[29.] cyanidin 3-glucoside, which was the predominant form (84%) in the brain, has been shown to possess a high antioxidant capacity [30,31]. Following consumption of anthocyanins rich diet, anthocyanins enter the brain and can exert protective activities against the oxidative damages responsible for numerous neurological disorders[32]. Mechanisms of antioxidative action of vitamin C are direct scavenging and blocking of reactive oxygen species (ROS), as well as regeneration of other antioxidative systems [33]. The anthocyanin and other antioxidants may up regulate mRNAs of antioxidants enzymes as well as up regulation of DNA repair genes may also protect against radiation induced oxidative stress by bringing error free repair of DNA damage and thus may counteract the oxidative stress induced by irradiation. It may also inhibit activation of protein kinase C (PKC), mitogen activated protein kinase (MAPK), cytochrome P-450, nitric oxide

and several other genes that may be responsible for inducing damage after irradiation [34].

CONCLUSION

The radio protective activity of PDE may be mediated through several mechanisms since it is a complex mixture of many chemicals. The presence of these chemicals in PDE elevates the cellular antioxidant and enables it to scavenge free radicals in the irradiated system, which could be the leading mechanism for radioprotection.

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