



**PRUNUS DOMESTICA PROTECTS AGAINST HEPATIC AND INTESTINAL DAMAGE
INDUCED BY RADIATION IN SWISS ALBINO MICE**



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Abstract

The present study focused on the possible protective effect of *Prunus domestica* extract (PDE) against radiation induced damage in liver and small intestine of *Swiss albino* mice. Male mice were distributed into five groups of 10 mice each. *Prunus domestica* fruits extract (PDE) was evaluated for *in vivo* radio protective activity against whole body gamma irradiation in *Swiss albino* mice. Radiation induced histological lesions and antioxidant parameters were observed. PDE was found to have strong radical scavenging activity in 2, 2- diphenyl-1-picrylhydrazyl (DPPH*). PDE supplementation augmented the antioxidant defence indices, such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) lowered by radiation exposure. Histopathological observations revealed damage in irradiated animals which were found to be reduced by supplementation of PDE prior/post irradiation. These findings suggest the possible radio protective role of antioxidants present in PDE in ameliorating hepatic and intestinal injury via free radical scavenging mechanism.

INTRODUCTION

Radiation is known to produce various reactive oxygen species (ROS) in biological systems such as superoxide, hydrogen peroxide and hydroxyl radicals and various types of tissue damage due to free radical reactions¹. The nature and degree of such unwanted side effects depends upon the dose of ionizing radiation and sensitivity of the organs that are irradiated. ROS are generated by every physiological response and are scavenged by antioxidants and superoxide dismutase. However, in the case of excessive generation of ROS caused by inflammation, some kinds of stress and reperfusion following ischemia or that in the case of dysfunction of the system for scavenging ROS, all of the ROS cannot be quenched. ROS influence lipids, proteins and nucleic acids, and they contribute to many diseases including cancer and cardiovascular diseases². To protect our body from injury caused by ROS, it is therefore important to supplement our diet with antioxidant compounds rich fruits and vegetables. Administration of antioxidants may help with removing ROS and thus improving clinical outcome. The high sensitivity of the gastrointestinal (GI) tract

to ionizing radiation and other cytotoxic insults render it a possible target in accidentally exposed persons and a dose limiting tissue in some radio and chemotherapy practices, Damage to the GI system seems to be of primary concern for local X-irradiation of the abdominal and pelvic tumors as well as for whole body X-irradiation preceding bone marrow transplantation³. In this context, *Prunus domestica* (Plums) (family Rosaceae) commonly known as Alu bukhara, which has been used as a traditional medicinal food in humans to enhance immunity against infectious agents, has been used for exploring its antiradiation effect. This fruit contains immunostimulatory components that potentially may be useful in human and veterinary medicine. *Prunus domestica* (Plums) are fruits rich in phenolic compounds, characterized by relatively high antioxidant activity, higher than e.g. oranges, apples or strawberries^{4,5}. The fruit contain anthocyanins (type cyanidin-3-glucoside and cyanidine-3-rutinoside) and flavanols (catechin)⁶. According to nutrient database, 100 grams of edible portion of fruits of *Prunus domestica* has Protein 0.7g, fat 0.5g, carbohydrate 11.1g, minerals and

fibre 0.4g, calcium 10mg, phosphorus 12mg, iron 0.6mg, magnesium 147mg, sodium 0.8g, potassium 247g, copper 0.13g, sulphur 33mg, carotene (Vit A) 166 µg, thiamine (Vit. B1) 0.04mg, riboflavin (Vit. B) 0.1mg, niacin 0.3mg, vitamin C 5mg and oxalic acid 1mg⁷. Hentricontane, ethyl hexadecanoate and linoleic acid were identified in n-hexane extract 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 method⁸. *Prunus domestica* also have radioprotective activity against whole body gamma radiation in *Swiss albino* mice. Pre and post treatment with PDE significantly ameliorated the endogenous protein in brain and improved spatial learning⁹. In the present study, therefore radiation effects and its modulation by *Prunus domestica* were assessed through biochemical estimations of LPO, GSH, SOD, and catalase in liver, and intestine. After selecting optimum dose Reduction Factor (DRF) was also calculated as it clearly gives

the drug quantitative capacity to enhance the tolerance of tissue to radiation.

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.

Experimental Design for Radioprotective Study

Mice were randomly divided into following groups (ten per group) for biochemical and histopathological 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/kg bwt).

Mice were sacrificed by cervical dislocation on day 7 and various biochemical parameters were analyzed viz. GSH, SOD and catalase. Histopathological observation was carried out after fixing the tissues in bouins and cutting the sections at 5 μ after paraffin embedding. Sections were stained with haematoxylin and eosin.

Antioxidant Efficacy of *Prunus domestica*

Preparation of DPPH

2, 2'-diphenyl-1-picrylhydrazyl (DPPH; C18H12N5O6; Hi media) 0.8 mg was dissolved in 10ml methanol to obtain a concentration of 0.08 mg/ml for antioxidative (qualitative and quantitative) assay. Qualitative and quantitative assay was done by Takao *et al.* (1994)¹⁰.

Data were processed using Excel and concentration that cause 50% reduction in absorbance (IC_{50}) was calculated. The same procedure was also followed for the standards quercetin and ascorbic acid.

Antioxidative Assay

Reduced Glutathione (GSH) assay

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)¹¹.

Super Oxide Dismutase

Super oxide dismutase was assayed by method of Marklund and Marklund (1974)¹² which involves inhibition of pyrogallol auto-oxidation at PH 8.0. A single unit of enzyme is defined as the quantity of superoxide dismutase required to produce 50% inhibition of auto-oxidation. The absorbance was read at 420 nm with a UV-VIS systronics spectrophotometer.

Catalase

It was estimated in the brain homogenate in a UV-VIS spectrophotometer as described by Aebi (1984)¹³. The reaction mixture (1 ml, vol.) contained 0.02 ml of suitably diluted cytosol in phosphate buffer (50 Mm.

Ph 7.0) and 0.1 ml of 30 Mm H_2O_2 in phosphate buffer. The specific activity of catalase has been expressed as μ moles of H_2O_2 consumed /min/mg protein. 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 \pm SEM. The statistically differences between various groups were analyzed 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

Antioxidant efficacy of *Prunus domestica*

Radical scavenging (antioxidant) activity of *Prunus domestica* methanolic extract IC_{50} is 6μ g/ml whereas the ascorbic acid has 4μ g/ml, which shows that the methanolic extract of *Prunus domestica* has antioxidant activity comparable to the ascorbic acid (Figure 1).

Antioxidant assay

GSH level in both intestine and liver was found to be significantly higher ($p < 0.05$) in Group II compared to control. Catalase and SOD estimation on 7th day after treatment revealed that supplementation of PDE pre/post irradiation increased the catalase and SOD levels in liver/intestine which were depleted by irradiation.

Histopathological Studies

Histologically no difference was noted between Group I and II in intestine and liver. In Group III (irradiated), mice liver showed radio lesions in the form of dilated sinusoidal spaces, cellular oedema, hydropic degeneration, hyperemia, lymphocytic infiltration, degranulated and vacuolated cytoplasm, swollen Kupffer, giant, multinucleated, enucleated and necrotic cells along with many pyknotic nuclei figure. In Group IV & V (PDE+ irradiation; Irradiation+ PDE), hepatic architecture was seen with mild cytoplasmic vacuolation and some pyknotic and shrunken nuclei and binucleated cells figure. Intestine of irradiated mice showed epithelial cells with frequently loose contact with each other and having many lateral

and basal projections. PDE pre/post treatment reduced the radiation induced damage. (Figure 2).

Photomicrograph (A) showing normal hepatocytes and K=Kupffer's cells with usual arrangements of hepatic cords and S=sinusoids of group I (control). Photomicrograph (B) T.S. of liver of mice at day 7 in (group II) after 5 Gy gamma radiation illustrating distorted hepatic arrangement, wider sinusoidal spaces, cytoplasmic vacuolation, pyknotic nuclei, enucleation and crenated nuclei. Photomicrograph (C) T.S. of intestine of control mice (40x) W= Intestinal epithelium V= Microvilli .Photomicrograph (D) T.S. of intestine of PDE supplemented mice. Photomicrograph (E) T.S. of intestine of irradiated mice. These are showing disturbed intestinal epithelium wall.

DISCUSSIONS

Oral administration of a 400 mg/kg b wt dose of PDE for 15 consecutive days, prior to radiation exposure (10 Gy), was found to be effective in terms of survivability compared to other higher and lower doses of PDE. Delayed mortality in group supplemented with PDE prior to irradiation

may be due to the effectiveness of PDE in arresting gastrointestinal (GI) death, as indicated by the increased number of survival days in all the treatment groups, compared to the irradiated mice. This reduction in GI death may also be due to the protection of intestinal epithelium, which would have allowed proper absorption of the nutrition. Several studies have demonstrated that the antioxidant properties of plant compounds could be correlated with oxidative stress defence. In present investigation screening attempts have been made to search for anthocyanin and other antioxidants rich fruit extract having potential as antioxidant agent. *Grewia asiatica* rich in anthocyanin was also reported to scavenge DPPH, NO (nitric oxide) radicals *in vitro* in a concentration dependent manner¹⁴. Methanolic extract of *Prunus domestica* showed antioxidant activity comparable to ascorbic acid. Thus, this fruit can be safely used as potent antioxidant agent and can be used for various herbal drinks to cure.

In last two decades, the rapid development of radiation biology led to new insight into the molecular mechanisms of ionizing radiation response to tissue including GI

tract, ample evidence shows that ionizing radiation may activate or alter multiple intracellular signaling pathway in different tissues or may affect cells via membrane generated alarm signals, activate state or excitability of different receptors¹⁵. Glutathione is an important non-enzymatic antioxidant which plays a critical role in cellular defense system against toxic chemicals of exogenous and endogenous origin. Depletion of cellular GSH increases cell vulnerability to oxidative stress¹⁶. In the present study the decrease in the activities of antioxidant enzymes GSH may be due to the damaging effect of free radicals produced following radiation exposure or alternatively could be a direct effect of formaldehyde formed from oxidation of free radicals, on these enzymes. GSH acts as a free radical scavenger and regenerator of alpha-tocopherol and plays a significant role in sustaining protein sulfhydryl groups¹⁷. Decreased hepatic GSH contents result in increased susceptibility to hepatic injury via induction of lipid peroxidation and TNF- α ¹⁸. GSH is the main antioxidant found in liver cells and plays a protective role in the metabolism of a large number of toxic

agents, including oxidative stress. Enhanced radiation toxicity has been associated with decreased hepatic/intestine GSH, which may reflect the depletion of GSH by the overproduction of ROS and subsequent oxidative stress caused by radiation. Our results showed that pre/post PDE supplementation significantly inhibited the radiation induced depletion of hepatic GSH. However, PDE treatment alone did not affect GSH levels. These findings further suggest that the protective effect of PDE against radiation to increased in cellular GSH content. The decrease in GSH activity correlates with the increase in lipid peroxidation. This may account for the increased levels of oxidized lipids in the serum lipoproteins of irradiated mice following consumption of a diet rich in oxidized lipids¹⁹ since the intestinal/hepatic GSH detoxifies dietary lipids before they enter the circulation²⁰. SOD and CAT, are the first line of defence against oxidative injury. The inhibition of antioxidant system may cause the accumulation of H₂O₂ or products of its decomposition²¹. SOD catalyzes the conversion of superoxide anion into H₂O₂. The primary role of CAT is to scavenge H₂O₂ that has been generated

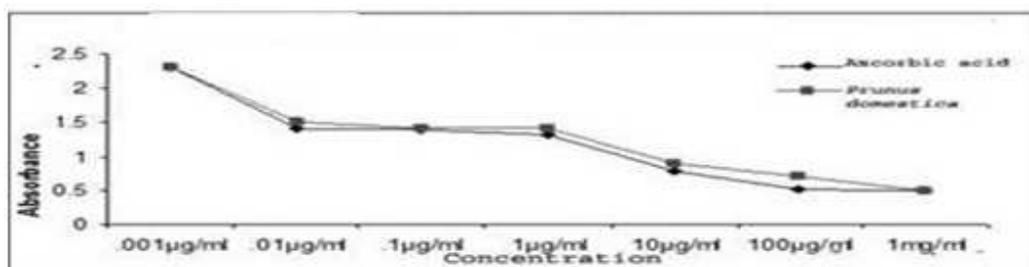
by free radical or by SOD. Importantly, administration of PDE restored the activities of enzymatic antioxidants (SOD and CAT) in liver/intestine of radiated mice. The antioxidant property of PDE has been linked to the presence of polyphenols, especially flavonoid. From these findings, it can be inferred that Pre/post treated PDE positively modulates the antioxidant status and regenerates the tissue to near control group in mice. In the present study the levels of enzymatic antioxidants (CAT, SOD, GPX) were lowered by irradiation in liver and intestine whereas the PDE treated groups exhibited an increased SOD activity to eliminate the radicals. This increased intracellular Ca⁺² can induce the irreversible conversion of xanthenes dehydrogenase (XDH) to XO, which in turn catalyses the oxidation of xanthenes to provide a source of O₂⁻. These mechanisms could be the main reasons for and reduction in activity of SOD leading to an overload of oxygen radicals and repression of antioxidant enzyme with radiation exposure²².

Oxidative stress is an important factor in cancer, cardiovascular diseases, and neuronal degeneration such as that in Alzheimer's disease². For prevention of

these ROS-induced diseases, further approaches focusing on the health aspects of functional foods are needed. The results of this study suggest that appropriate pharmacological activity results showed by the appropriate accumulation into the tissue. So it is important to deliver the functional food components to the target tissues. Further studies are needed to elucidate the relationship between pharmacokinetics and pharmacodynamics of *Prunus domestica*. Such knowledge would probably lead to the more effective and more efficient use of PDE.

In conclusion, supplementation of PDE exerts a significant protective effect against radiation induced hepatic / intestinal damage in mice. The protective effect of the extract may be attributed to the active components such as anthocyanins, polyphenols, and carotenoids which elicit antioxidant and detoxifying effects. Therefore, a dietary intake of *Prunus domestica* fruit may supply necessary components that offer protection against radiation induced toxicity. Further studies are warranted to isolate the active component in this fruit that is responsible for the observed effect.

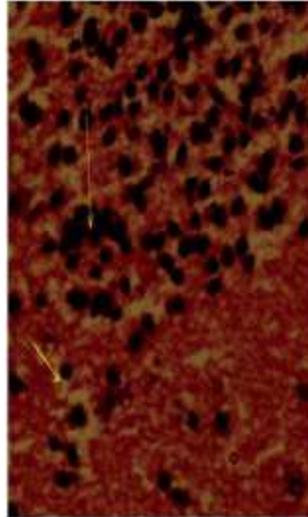
CONCLUSION



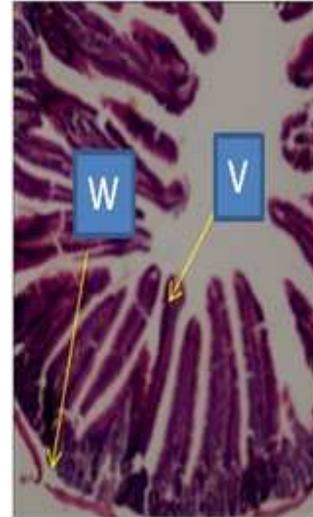
Graph -1 Showing antioxidant potentials of *Prunus domestica*



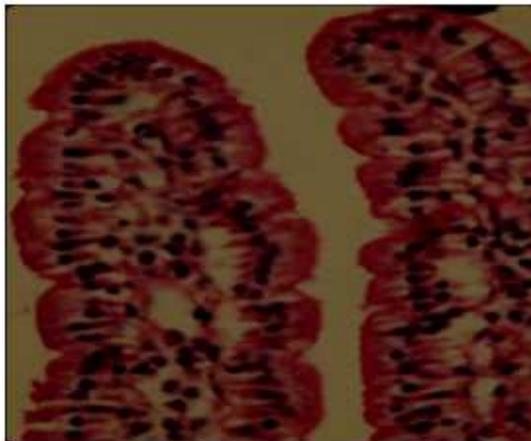
(A)



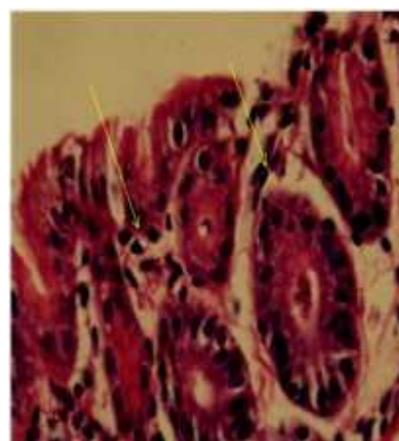
(B)



(C)



(D)



(E)

Table 1
Variation in the activity of different biochemical parameters in liver and intestine of various experimental groups

Tissue	Experimental group	GSH (nM/100mg)	SOD (μ /mg tissue)	Catalase (nmol/ml)
Liver	Control	27.92 \pm .253	3.11 \pm .298	49.09 \pm .842
	(PDE)	28.63 \pm .285 <i>p</i> <0.05	4.36 \pm .278 <i>p</i> <0.01	52.18 \pm .623 <i>p</i> <0.01
	Irradiation	16.87 \pm .163 <i>p</i> <0.001	3.02 \pm .275 <i>p</i> <0.001	39.58 \pm .366 <i>p</i> <0.001
	PDE+IR	19.14 \pm .098 <i>p</i> <0.001	2.39 \pm .246 <i>p</i> <0.001	40.94 \pm .423 <i>p</i> <0.05
	IR+PDE	24.03 \pm .450 <i>p</i> <0.001	2.60 \pm .243 <i>p</i> <0.001	47.36 \pm .485 <i>p</i> <0.001
Intestine	Control	10.39 \pm .224	3.54 \pm .429	29.27 \pm .759
	(PDE)	8.43 \pm .311 <i>p</i> <0.001	4.07 \pm .248 N	30.29 \pm .281 N
	Irradiation	19.18 \pm .310 <i>p</i> <0.001	1.67 \pm .136 <i>p</i> <0.001	20.66 \pm .165 <i>p</i> <0.001
	IR+PDE	11.66 \pm .207 <i>p</i> <0.001	2.92 \pm .219 <i>p</i> <0.001	21.11 \pm .222 <i>p</i> <0.001
	PDE+IR	13.94 \pm .266 <i>p</i> <0.001	2.41 \pm .144 <i>p</i> <0.001	26.05 \pm .531 <i>p</i> <0.001

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