PROTECTIVE EFFECT OF *EMBLICA OFFICINALIS* FRUIT EXTRACT AGAINST GAMMA IRRADIATION IN MICE

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Summary

Treatment of mice with *Emblica officinalis* extract (EOE) before exposure to different doses of gamma radiation reduced the severity of symptoms of radiation sickness and mortality. EOE pretreatment protected mice against the gastrointestinal as well as bone marrow deaths, as evidenced by the greater number of survivors. The dose reduction factor (DRF) was found to be 1.9 for EOE +irradiation group. The values of serum acid phosphatase activity were significantly higher in the irradiated group throughout the experiment as compared to normal. However, this activity in *E. officinalis* pretreated irradiated animals showed a significant decline over untreated irradiated animals at all the autopsy intervals, and attained the normal value on day 5^{th}. Conversely, a marked decrease in serum alkaline phosphatase activity was noted in both the irradiated groups, but in the *E.officinalis* pretreated irradiated group, these values were found to be significantly higher than the irradiated control at early intervals but became normal at day 5 post-irradiation and onwards. Irradiation resulted an elevation in lipid peroxidation (LPx) and a decline in glutathione (GSH) level in liver as well as blood. On the other hand, treatment of animals with *Emblica officinalis* extract before irradiation caused a significant decrease in LPx and a marked elevation in GSH.

Keywords: Emblica officinalis; irradiation; radioprotection; lipid peroxidation; glutathione; phosphatases activity

Introduction

Radiotherapy is an important modality for cancer cure, and it is estimated that about half of cancer patients derive benefit from it. However, the prime importance of radiotherapy is in the treatment of loco regional growths that cannot be excised by surgery, such as those of advanced lung , head and neck cancers. Unfortunately, high doses of radiation lead to severe oesophagitis in lung cancer and acute mucositis and pharyngitis in head and neck cancer that force physicians to discontinue or lessen the treatment cycles. In such situations, an agent that can render a therapeutic differential between the cancer cell cytotoxicity and normal tissue toxicity may be of great help. Therapeutic differential may be achieved with chemical radiation protectors or sensitizers. The development of radiation protectors is important not only to enhance the effectiveness of cancer treatment but also for the study of underlying mechanisms of radiation cytotoxicity.

Several synthetic compounds such as cysteine[1], WR-2721[2], lipoic acid[3], deoxyspergualin[4], 2-mercaptopropionylglycine[5,6], dipyridamole adenine monophosphate[7], Deltiazem[8] have been tested for their protective action against radiation. Owing to their inherent toxicity, these have not been found successful in the field of clinical radiotherapy. In addition to synthetic drugs, some plant extracts and plant products such as garlic[9], ginseng[10], ocimum[11], triphala,[12] abana,[13] septilin[14] and *Syzygium Cumini* (Jamun)[15] have been found to have a radioprotective effect.

Emblica officinalis Linn. (Amla), belonging to the family Euphorbiacae, is to be one of the strongest rejuvenatives, particularly for the blood, bones, liver and heart. It is an exceptionally rich source of vitamin C. Extract of this plant has been found to have a protective effect upon radiation induced chromosomal damage[16] and also hypocholesterolemic, hypolipidemic, cardio protective and antiatherosclerolic in both humans and experimental animals[17-20]. Its extract significantly reduced the cytotoxic effects of sodium arsenite

when administered orally in experimental animals[21]. Some of the plants like *Glycyrrhiza* glablra, *Emblica officinalis, Rubia cordifolia* and *Aegle marmelos* have been found to possess antioxidant properties[22,23] . The comman usage, wide acceptability in human beings, and diverse medicinal and antioxidative properties attributed to *Emblica officinalis* stimulated us to examine the radioprotective potential in mice.

Materials and methods

Animals- Adult male Swiss albino mice (6-8 weeks old) weighing 23±2 gm from an inbred colony were used for the present study. The animals were maintained on the standard mice feed (procured from Hindustan Lever's Ltd., India) and water *ad libitum.* Four animals were housed in polypropylene cage containing paddy husk (procured locally) as bedding throughout the experiment. Animal care and handling were performed according to guidelines issued by the World Health Organization (Geneva, Switzerland) and the Indian National Science Academy (New Delhi, India). The animal Ethical Committee of this department has approved the study.

Preparation of the Extract- *Emblica officinalis* was identified in herbarium (Identification No.RUBL-19885) by a competent botanist of Botany Department, UOR, Jaipur. Fresh fruits of the *E. officinalis Linn.* were collected locally, cleaned, air dried, powered and extracted with double distilled water (DDW) by refluxing for 36 hrs. (12 hrs. × 3). The extract thus obtained was vacuum evaporated so as to make it in power form . The extract was redissolved in DDW just before oral administration. An approximate 38% yield of the extract was obtained. Henceforth, the extract of *E. officinalis* fruit will be called EOE.

Selection of optimum dose- Dose selection of EOE extract was done on the basis of our previously conducted animal survival study[24]. Various doses of

EOE (50, 100, 200, 400, 800 mg/kg body wt.) were tested against gamma irradiation (9 Gy) for radiation sickness and mortality. Optimum dose (100 mg/kg body wt.) thus obtained was used for further detailed experimentation.

Radioprotective activity - A separate experiment was carried out to ascertain the radioprotective activity of EOE, for which animals were divided into the following groups:

(i) DDW + irradiation group

The animals of this group were administered 0.1 ml/g of sterile DDW orally for 7 consecutive days, once in a day.

(ii) EOE+ irradiation group

The animals of this group were injected orally with 100 mg/kg of EO, once in a day, consecutively for 7 days.

Half an hour after the last administration of DDW or EOE on the seventh day, animals of above groups were whole-body exposed to 2.5, 5, 7.5 and 10 Gy of ^{60}Co gamma radiation (Theraton, Atomic Energy Agency, Ontario, Canada) in a specially designed well ventilated Perspex box. A batch of 12 animals was irradiated each time at a dose-rate of 0.87 Gy/ min. at a source to animal distance (midpoint) of 77.5 cm. Immediately after irradiation, the animals were stored into individual polypropylene cages. The animals of both groups were monitored daily for the development of symptoms of radiation sickness and mortality. A total of 15 animals were used for each dose of radiation in each concurrent group. The dose reduction factor (DRF) was calculated my the method of Miller and Teinter[25] (1944).

$$\text{DRF} = \frac{\text{LD}_{50/30} \text{ of the EOE+irradiation}}{\text{LD}_{50/30} \text{ of the DDW+irradiation}}$$

Modification of radiation response: The animals selected for this experiment from an inbred colony were divided into three groups. *Group-I* (Emblica extract treated unirradiated): These were fed orally EOE (100 mg/kg/body weight/day/animal) for 7thconsecutive days. *Group-II* (Untreated irradiated): These animals were given DDW (0.1mg/kg.) orally for 7 consecutive days, once daily. This group served as a control. *Group-III* (Emblica extract treated irradiated): Mice belonging to this group also received EOE as in Group I and served as experimental. Half an hr. after injection of DDW or EOE on the last 7th day, animals of *Group-II* and *III* were exposed to 7.5 Gy gamma radiation. The animals from the above groups were autopsied at 12 hrs. 24 hrs. 3, 5, 10, 20 and 30 days post-irradiation. Blood from these animals was collected by cardiac puncture and serum was separated. The serum activity of acid phosphatase (ACP) alkaline phosphatase (ALP) was assayed using commercially available kits (Span Diagnostics Ltd., Surat).

Biochemical Determinations: Biological determinations were carried out in survivors of both EOE + irradiation and DDW + irradiation groups at 1 hr. post-exposure. These animals were killed by cervical dislocation, and their blood was collected from orbital sinus by heparinised needle. Also, their livers were profuse transcardially with ice-cold saline.

***Reduced glutathione (GSH) assay* :** The hepatic level of reduced glutathione (GSH) was determined by the method as deserved by Moron *et al.*,[26]. GSH content is blood was measured Spectrophotometrically using Ell man's reagent (DTNB) as a coloring reagent as per the method described by Beutler

et al.,[27] . The absorbance was read at 412 nm using a UV-VIS Systronics Spectrophotometer.

Lipid peroxidation (LPx) ***assay* :** The lipid per oxidation level in liver and serum was measured using Thiobarbituric acid Reactive Substances (TBARS) by the method of Ohkhawa *et al.*,[28]. The absorbance was read at 412 nm.

Statistical analysis : The data were subjected to Student's test for comparison between the groups. The values are expressed as mean ±SE. Significance level was computed at $p<0.05$, $p<0.005$ and $p<0.001$.

Results

Radioprotective effect : The radioprotective action of EOE was evaluated using an optimal dose of 100 mg/kg orally administered for 7 consecutive days before exposure to 2.5, 5, 7.5 and 10 Gy of γ-radiation. The irradiation of animal using different doses resulted in the development of symptoms of radiation sickness within 2 to 4 days after exposure, depending on the irradiation dose for DDW + irradiation group. Exposure of animals to higher irradiation doses resulted in an early onset of symptoms of radiation sickness and mortality. The symptoms included reduction in the food and water intake, irritability, epilation, weight-loss, emaciation, lethargy, diarrhea, and ruffling of hairs. Facial edema was also observed in a few animals between 1 and 2 weeks after exposure to 7.5 and 10 Gy.

Pretreatment of mice with EOE provided protection against radiation sickness and mitigated suffering. The delay in the onset of death was 5 to 7 days for the EOE + irradiation group when compared with the DDW + irradiation group. Pretreatment of mice with EOE reduced the 10 day mortality significantly for 7.5 and 10 Gy irradiation. Administration of EOE resulted in 52% survivors with 10 Gy dose, whereas no animals survived beyond day 12 post-irradiation

in the control group. Pretreatment of mice with EOE reduced the 30-day mortality by 1.4 and 2.3 fold for 7.5 and 10 Gy irradiation, respectively.

Table:-I Variation in serum phosphatase activity of mice treated with *Emblica officinalis*/or 7.5 Gy Gamma radiation.

Autopsy Interval	Group	Serum acid phosphatase (KAU)	Serumalkalinephosphatase (KAU)
12 hrs.	GR-I	2.9295±0.06	7.3321±0.13
	GR-II(Con.)	6.6920±0.28[c]	4.5913±0.33[c]
	GR-III(Exp.)	3.9876±0.29[c]	6.4012±0.02[c]
24 hrs.	GR-I	2.6253±0.03	7.5489±0.89
	GR-II(Con.)	6.2186±0.12[c]	4.2314±0.22[c]
	GR-III(Exp.)	3.6453±0.07[c]	5.9378±0.14[c]
3 days	GR-I	2.3436±0.06	7.2675±0.32
	GR-II(Con.)	7.6248±0.11[c]	3.5943±0.07[c]
	GR-III(Exp.)	4.1242±0.22[c]	6.5483±0.09[c]
5 days	GR-I	2.5198±0.04	6.5199±0.10
	GR-II(Con.)	5.3451±0.36[c]	4.2351± 0.22
	GR-III(Exp.)	2.5821±0.36[c]	7.6081±0.06
10 days	GR-I	2.5180±0.15	6.9139±0.17
	GR-II(Con.)	6.3214±0.06[c]	3.5623±0.18
	GR-III(Exp.)	3.6019±0.08[c]	8.0098±0.07
20 days	GR-I	2.9195±0.08	8.8651±0.34
	GR-II(Con.)	6.9716±0.22[c]	3.7231±0.19
	GR-III(Exp.)	3.1129±0.11[c]	8.1102±0.17
30 days	GR-I	2.3737±0.06	6.9565±0.26
	GR-II(Con.)	NS	NS
	GR-III(Exp.)	2.9514±0.24	7.4513± 0.21
	Normal	2.7882± 0.25	7.6436±0.17

GR-I = Emblica treated unirradiated;

GR-II = (Control), untrerated irradiated;

GR-III = (Experimental.), Emblica treated irradiation;

Normal = DDW treated

NS = not survived

Each value represents mean±SEM.

Significance level [a]p<0.05; [c]p<0.001

Normal v/s Control; control v/s Experimental

Modification of radiation response : In the control group, a significant elevation in serum acid phosphatase with respect to Group - I was noticed. A considerable increase was evident at 12 hrs. (6.6920+0.28) reaching highest at day 3 (7.6248+0.11). The acid phosphatase level decreased subsequently on day 5 but elevated further and remained higher than normal.

In the experimental group (EOE + irradiation), a significant increase over control (4.1242+0.22) in serum acid phosphatase activity was noted on day 3, however the level came down to normal on day 5 but increased further by returning towards normal at the end of experimentation (Table. 1; Fig-1).

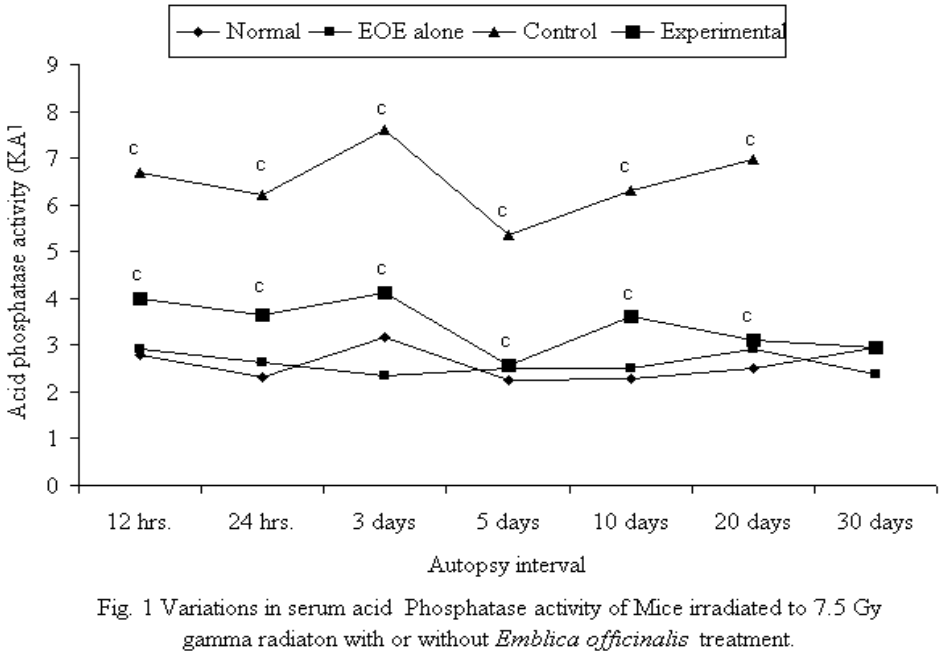

Fig. 1 Variations in serum acid Phosphatase activity of Mice irradiated to 7.5 Gy gamma radiaton with or without *Emblica officinalis* treatment.

The value represents mean ± S.E.. The statistical significance was obtained between normal V/s control and Control V/s Experimental (bp < 0.005; cp <0.001)

A remarkable decrease in serum alkaline phosphatase activity was recorded at all the autopsy intervals. However, maximum decline was noted on day 3 and 10 after post-irradiation in untreated irradiated animals. No animal survived till day 30 in this group. In experimental animals (EOE pretreated irradiated), the activity of this enzyme exhibited a significant rise above control and attained the normal value at day 5 (7.6081 ± 0.06), but elevated further on days 10 and 20, and decreased finally on day 30 (7.4513 ± 0.21) (Table.1; Fig-2).

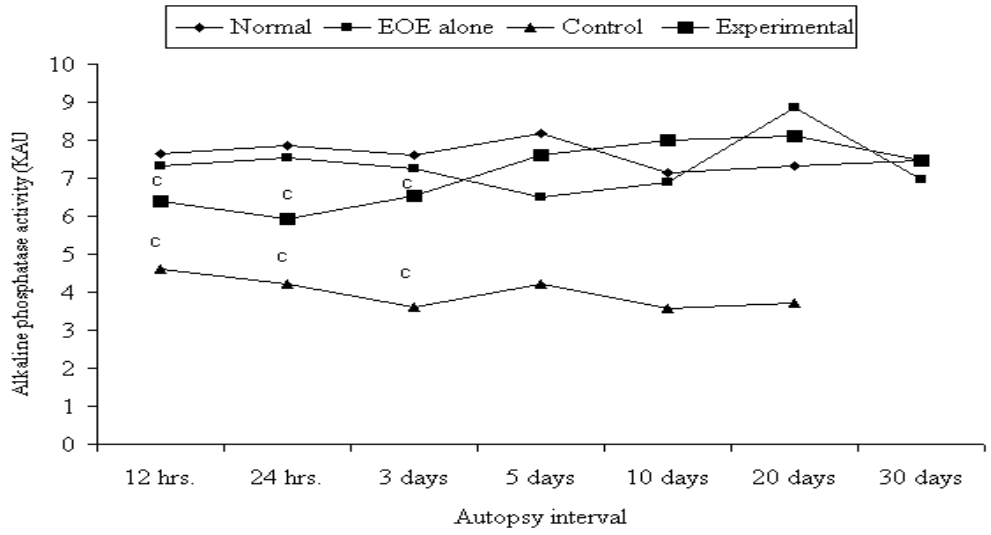

Fig. 2 Variations in serum alkaline phosphatase activity of Mice irradiated to 7.5 Gy gamma radiaton with or without *Emblica officinalis* treatment.

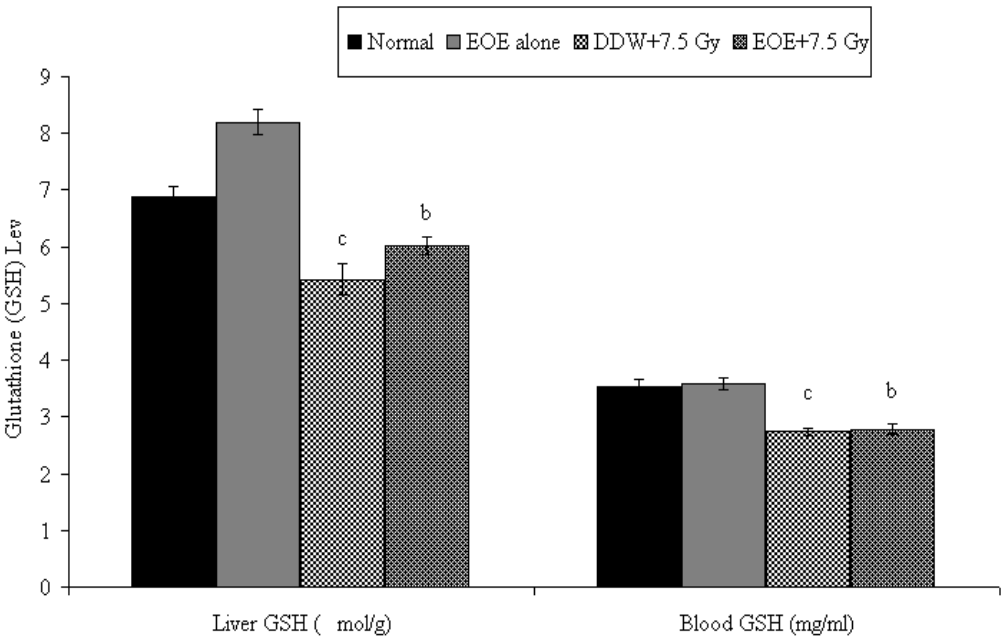

Fig. 3 Glutathione (GSH) level in mice after 7.5 Gy gamma irradiation in the presence (experimental) or absence (control) of Emblica Officinalis (EOE) fruit extract.

The value represents mean ± S.E.. The statistical significance was obtained between normal V/s control and Control V/s Experimental ($bp < 0.005$; $cp < 0.001$)

Biochemical determinations: Administration of EOE before Sham-irradiation did not alter GSH content significantly. Exposure of mice to gamma radiation resulted in a significant decline in GSH in blood and liver in DDW+ irradiation group. EOE pretreatment restored normal GSH Level in blood as well as liver in the animals of Group-IV (Fig-3).

LPx remained unaltered in the animals who received EOE alone for 7 consecutive days. Exposure of mice to radiation increased LPx for both control and experimental groups, however, EOE pretreatment significantly reduced LPx level after irradiation (Fig-4).

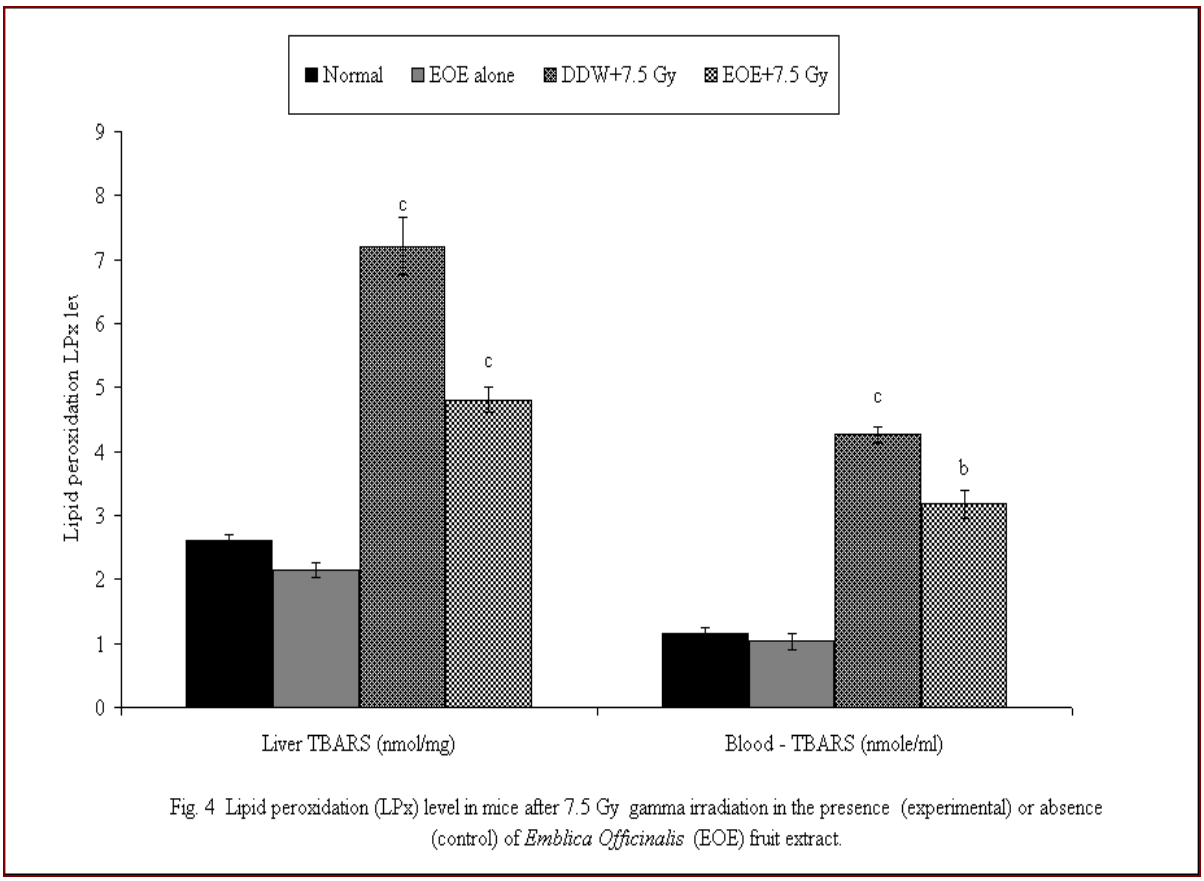

Fig. 4 Lipid peroxidation (LPx) level in mice after 7.5 Gy gamma irradiation in the presence (experimental) or absence (control) of *Emblica Officinalis* (EOE) fruit extract.

The value represents mean ± S.E.. The statistical significance was obtained between normal V/s control and Control V/s Experimental (bp < 0.005; cp <0.001)

Discussion

Ingredients present in our diet may be very useful if they are found to protect against the deleterious effects of ionizing radiation, as they will be widely acceptable, and would not add any extra foreign substance into the body, and can be safely manipulated without toxic manifestations. Amla fruits are consumed in India as such or in various forms and also possess several potentially useful medicinal properties[29]. In this study, we have attempted to evaluate the radioprotective effect of *Emblica officinalis* in mice.

Dose reduction factor (DRF) in the present study, based on survivality experiment has been computed as 1.9[24]. The dose of fruit pulp extract found most effective against radiation was 100 mg/kg b.wt. and this dose increased the survival time and reduced mortality rate of mice significantly. Further more, body weight loss in EOE administered irradiated animals was significantly lesser in comparison to animals who were given radiation only. The results from the present study indicate that pretreatment of *Emblica officinalis* extract (EOE) protects the mice from the lethal effect of ionizing radiation . The radioprotective effect of (EOE) protects the mice from the lethal effect of ionizing radiation. The radio protective effect of EOE was demonstrated by increased body weight and survival rate. A significant radioprotection was achieved when EOE was given orally 100 mg/kg b.wt. for 7 consecutive days prior to irradiation. In the present study, a significant loss in body weight was evident in control animals (Irradiation alone). EOE pretreated irradiated animals (100 mg/kg b.wt.) showed recovery in body weight 30 post-irradiation. Only 12.5% mortality was observed in such group, whereas all animals died within 30 days in animals irradiated without EOE (Group-I). This was due to damage to the protection of the intestinal mucosa against radiation damage might be one of the reasons for the greater survival time in EOE pretreated animals because in may facilitate digestion and absorption in the post-irradiation period.

This drug is considered as one of the fore most rejuvenating drugs imparting a long healthy life and weight gain, improved hematological picture like increased production of red RBC cells. The hematological constituents (RBC, WBC and Hb etc.) were found higher in the EOE pre-treated irradiated animals than the animals irradiated without EOE.

The present study revealed an increase in serum acid phosphatase activity after irradiation. A similar increase in activity of acid phosphatase after irradiation has also been reported at sub lethal doses[30-35]. This resulted into an increased acid phosphatase activity. These findings are in close agreement with present investigation where the plasma acid phosphatase level was also found to be elevated till day 7 in 5 or 7.5 Gy irradiated mice. An increased activity of acid phosphatase after irradiation has also been reported by others [36, 37, 34, 38, 39, 35].

Acid phosphatase is localized in cellular lysosomes and change in activity of lysosomal enzymes takes place following whole-body irradiation. An enhanced golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of the enzyme are attributed to an increased acid phosphatase level[40]. The discharge of enzymes from lysosomes may be due to activation of preexisting latent enzymes or due to synthesis of new lysosomes as a consequence of irradiation[41] . It is already known that radiation enhances the permeability of membranes of several cellular organelles, and hence increase in serum acid phosphatase activity till day 3 can be attributed to the gastro-intestinal syndrome, with recovery at day 5. However a further rise in ACP can be assigned to other factor like hematopoietic injury. It has already been reported in our laboratory[24] that aqueous extract of *Emblica officinalis* shows radioprotection in Swiss albino mice against lethal dose gamma radiation.

In the present investigation, serum alkaline phosphatase activity was found to decline after irradiation at all the autopsy intervals studied. This is in agreement with the findings of Jacob and Maini[42], who have also reported a

depletion in serum ALP activity in male mice after irradiation with 5 Gy gamma rays. Injury to intestinal mucosa has been found to be chiefly responsible for the fall in circulatory alkaline phosphatase after irradiation[43]. Non exponential loses of activity in alkaline phosphatase after gamma irradiation has also been observed earlier and it was suggested that radical attacks on phosphatase at canters of secondary importance for the enzymatic activity and there is notable destruction of the component amino acid residue during radiolysis[44] .

Alkaline phosphatase plays an important role in maintenance of cell permeability and acts on mono phosphatase. Damage to cell membrane caused by radiation may be the reason for declined activity of serum alkaline phosphatase. In untreated irradiated group (control), declined alkaline phosphatase level may be attributed to the severe damage to GI tract. Post-irradiated reduction in alkaline phosphatse may be due to damage of brush border cells and increased permeability of villi cells[45]. Khan and Samarth *et al.,*[46, 34, 38] too found a rise in alkaline phosphatase activity on day 3 after exposure to 5 and 10 Gy of gamma rays respectively . Similarly, Mathur and Uma Devi[47] noted a elevated concentration of alkaline phosphatase in ileum of mice after irradiation. The increase in alkaline phosphatase may be due to altered physiological conditions such as liver function mediated by serum alkaline phosphatase destruction of an inhibitor by irradiation can also be attributed to the plasma alkaline phosphatase level in the present study. It means that the higher is the dose, greater is the damage and longer is the time of recovery.

It is well known that free radicals generated during radiolysis of water play the most significant role in the indirect biological damage induced by ionizing radiation[48]. The GSH/GST detoxification system is an important part of cellular defense against a large array of injurious agents. GSH offers protection against oxygen derived free radicals and cellular lethality following

exposure to ionizing radiation[49]. Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against the oxidative damage. GSH is versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damage molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state[50]. The present study demonstrates a significant reduction in liver and blood GSH following exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Oral administration of EOE did not significantly influence the endogenous GSH level either in liver or blood, but its presence during radiation exposure protects the endogenous GSH depletion due to irradiation. The lower depletion of liver and blood GSH in the *Emblica officinalis* pre-treated irradiated animals could be due to the higher availability of GSH, which increases the ability to cope up with the free radicals produced by irradiation. The increased GSH level suggests that protection by *Emblica officinalis* may be mediated through the modulation of cellular antioxidant levels.

The basic effect of radiation on cellular membranes is believed to be the peroxidation of membrane lipids. Radiolytic products, including hydroxyl and hydroperoxyl radicals, can initiate lipid peroxidation[51]. In the present study, however, *Emblica officinalis* treatment did not significantly alter the lipid peroxidation level in unirradiated animals, but it significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants[52,53]. As chronic *Emblica officinalis* intake augmented endogenous antioxidants in rat heart, the myocardial adaptogenic property was tested by subjecting these hearts to oxidative stress, associated with *in vitro* myocardial SOD , CAT and GSH contents as observed in control heart in the present study have been previously documented in conditions of both clinical and experimental myocardial ischemicreperfusion[54-57].

Aqueous extract of *Emblica officinalis* has been previously reported as a potent inhibitor of lipid peroxide formation and scavenger of hydroxyl and super oxide radical *in vitro*[58]. *Emblica officinalis* was found to significantly increase the cortical and striatal concentrations of the antioxidant enzymes SOD, catalase and GPx, and to reduce lipid peroxidation in rat brain[29].

It has been shown that more α-tocopherol is needed in the membranes to protect polyunsaturated fatty acids (PUFA) against radiation induced lipid peroxidation when low dose rates are applied[59]. Several mechanisms, including a potent antioxidant activity, immune response and enhanced recovery of bone marrow have been suggested for radioprotection by vitamin E[60]. In the present study, it was observed that *Emblica officinalis* pre-treated irradiated animals exhibited a significant increase in GSH and decrease in LPx level.

Emblica officinalis extract has been shown to have antioxidant and antiperoxidant properties due to the presence of low molecular weight tannoids, mainly emblicanin A (37%), emblicanin B (33%), punigluconin (12%), pedunculogin (14%), and galic acid. The *in vitro* antioxidant activity of tannoids was demonstrated as well[61] concomitant with reduction in lipid peroxidation[29]. Some of the plants like *Glycyrrhiza glabra, Rubia cordifolia, Phylanthus Emblica* etc. have also been reported to possess antioxidant and free radical scavenging activities[22,23,62]. Treatment of mice with EOE before, during and application of DMBA carcinogen, exhibited chemopreventive activity[63] in this laboratory. The emblicanins are likely to the major antioxidant principles, not only because they are the major constituents of *E. officinalis* but also because of their reported antioxidant actions *in vitro*[61] and *in vivo*[29,64]. A combination of antioxidant activities via modulation of DNA repair processes increased GSH and decreased LPx may held responsible for the radioprotective effect of *Emblica officinalis (Linn.)* fruit extract in present study.

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