FRUIT EXTRACT OF EMBLICA OFFICINALIS (AMLA) PROTECTS RADIATION INDUCED BIOCHEMICAL LESIONS IN THE BRAIN OF SWISS ALBINO MICE

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Abstract

The alterations in enzymatic and biochemical activities were investigated in the brain of Swiss albino mice, with (experimental) or without (control) fruit extract before exposure to 5 Gy gamma radiation. The value of serum acid phosphatase activity were significantly higher in the irradiated group throughout the experiments compared to normal. However, this activity in Emblica officinalis pretreated irradiated animals showed a significant decline over untreated irradiated animals at all the autopsy intervals and attained the normal value by day 20th of treatment conversely, a marked decrease in serum alkaline phosphatase activity was noted in both the irradiated groups, but in the Emblica officinalis pretreated group an elevation in protein and cholesterol levels was observed. Furthermore, Emblica officinalis inhibited lipid peroxidation and increased glutathione (GSH) level in brain.

Key Words : Emblica officinalis, Irradiation, Radioprotection, Lipid- peroxidation, Glutathione, Phosphatase activity.

Introduction

In recent years, the study related to the interaction of living systems with ionizing radiation has focused on physicochemical or biochemical events associated with the cell membrane. Although, radiation is beneficial for therapeutic purposes but high doses or continuous exposure can cause disastrous consequences by damaging cell membrane and causing different syndromes. Ionizing radiation causes formation of free radicals which are potentially dangerous to the cell and its constituents. The reaction of hydroxyl and peroxyl free radicals on bimolecules is important in the field of physiology and pathology.

The nervous system of adult animals is considered to be extremely radio resistant, in terms of morphologic changes. However, a variety of physiologic response have been reported following relatively low doses of radiation. Radio sensitivity of the nervous system varies greatly with the kind of radiation, the species of animal, the stages of genetic development, specific sites with in the nervous system and the criteria used for assessing the effects (1-3). A number of synthetic compounds like deoxyspergualin, MPG, WR-2721 and herbal products as Liv. 52, rasayana, mentha oil as well as some vitamins like A, C and E have been tested in mammals, and found to offer some protection against the toxicity associated with exposure to ionizing radiation, but their use in clinical field is limited due to their inherent toxicity generated by them at protective dose level (4-11). An ideal radio protective compound should have minimum or no toxicity with potential protection at optimum dose level therefore search is on the way to find out some ideal radio protector which can be used in clinical field. The herbs have been commonly used to treat various disorders in man since the advent of human history. Humans are dependent on herbs not only for medicinal use but also consume herbs and fruits for their sustenance. Therefore, use of products from natural sources could be a better choice than the synthetic compounds to reduce the effects of radiation.

Emblica officinalis, belonging to family Euphorbiacae, is extensively found all over India. The fruits of this plant are rich in vitamin C and have been used in Ayurveda as a potent rasayana (12). Clinical studies suggest that the fruits of this plant have anabolic activity and exhibit significant adaptogenic, immunopotentiating and memory facilitating effects (13). The comman usage, wide acceptability in human beings, and diverse medicinal and antioxidative properties attributed to Emblica officinalis fruits stimulated us to obtain insight into the radio protective effect of amla (E. officinalis) on brain of mice exposed to gamma radiation. No systematic work has been initiated so far to reduce brain lesions by using such agents against ionizing radiations. Therefore, the present study is an attempt to find out the efficacy of Emblica officinalis as in modulating the radiation induced biochemical alterations in the brain of Swiss albino mice.

Materials and Methods

Animals care and handling- Adult male Swiss albino mice (6-8 weeks old) weighing 25±2 g from an inbred colony were used for the present study. The animals were maintained on the standard mice feed (procured from Hindustan Lever 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 (Genava, Switzerland) and the Indian National Science Academy (New Delhi, India). The Departmental Ethical Committee has aproved the present study.

Preparation of the extract- Emblica officinalis Linn. was identified in herbarium (No. RUBL 19885), by a competent botanist of Botany Department, UOR, Jaipur. Fresh fruits of the E. officinalis plant were collected locally during February through April of the year. These were cleaned, cut into small pieces, 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 powder form. The

extract was re dissolved 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 Emblica officinalis extract (EOE) was done on the basis of our previously conducted animal survival study (14). Various doses of EOE (50, 100, 200, 400, 800 mg/kg b.wt.) were tested against gamma irradiation (9.0 Gy) for radiation sickness and mortality. Optimum dose (100 mg/kg b.wt.) thus obtained was used for further detailed experimentation.

Modification of radiation response : The animals selected from an inbred colony were divided into the following four groups. Animals of Group-I were orally given double distilled water (volume equal to EOE) to serve as normal (vehicle treated), and animals of Group-II (EOE treated) were fed orally with EOE (100 mg/kg/b.wt./day/animal), once in a day for 7 consecutive days. Group-III (untreated irradiated) animals received DDW (equivalent to EOE extract), EOE (as in Group-II) While the Group-IV was (EOE treated irradiated). After the last administration on day 7th, animals of group III and IV were exposed to 5 Gy gamma radiation to serve as control and experimental respectively. These animals were whole-body exposed to ^{60}Co gamma radiation source (Theratron Atomic Energy Agency, Canada) in a specially designed well ventilated acrylic box . A batch of 12 animals was irradiated each time to 5.0 Gy at a dose rate of 0.88 Gy/min. at a source-to-animal distance (from midpoint) of 77.5 cm.

The animals from the above groups were autopsied at 12 hrs, 24 hrs, 3, 5, 10, 20 and 30 days of post-irradiation. Brain was removed from sacrificed animals by dissecting the skull and homogenate of its cerebellum part was prepared in saline. Protein and cholesterol contents were measured by Lowery et al and Leiberman methods respectively, while activity of acid phosphatase (ACP) and alkaline phosphatase (ALP) was assayed in cerebellum part by using commercially available kits(15,16).

Glutathione & Lipid peroxidation estimation

Reduced Gluthathione (GSH) assay : GSH content in blood was measured spectrophotometrically using Ellman's reagent (DTNB) as a colouring agent as per the method described by Beutler et al. (17). The hepatic level of reduced glutathione (GSH) was determined by the method as described by Moron et al. (18). The absorbance was read at 412 nm using a Systronics UV-VIS spectrophotometer.

Lipid peroxidation (LPx) assay : The lipid peroxidation level in liver and blood serum was measured using thiobarbituric acid reactive substance (TBARS) by the method of Ohkhawa et al. (19). The absorbance was read at 532 nm using a Systronics UV-VIS spectrophotometer.

Analysis of Data : The results obtained from the study were expressed as mean±SE. The Student,s t-test was used to make a statistical comparison between the groups.

Results

Radiation sickness

Animal exposed to 5 Gy gamma radiations represented signs and symptoms of radiation sickness. These mice were found as lethargic and week. Food and water consumption was reduced, although general activities of such animals were apparently normal during all 20 days post-irradiation. Animals pretreated with Emblica officinalis extract (EOE) and later exposed to 5 Gy gamma radiation did not show any sign and symptoms of radiation sickness. Moreover, a significant weight gain in these animals was observed as compared to control with normal food or water consumption.

Biochemical & enzymatic activity

Protein : The level of protein decreased significantly and progressively from day 3rd to 20th. After depression of nearly 16.19 percent of normal at 12 hrs., while at the last autopsy interval, protein level remained only 67.31 percent of normal. Treatment of Emblica officinalis prior to irradiation maintained a higher percentage of protein level in comparison to control. The values were found significantly higher over control from the beginning until the end of experimentation. The level of significance were $p<0.5$ (at 12 hrs.), $p<0.05$ (at 24 hrs.) and $p<0.001$ (day 3 onwards). Normal value of protein in this group restored on day 20th by exhibiting the maximum protection (Fig. 1).

Cholesterol: The values of cholesterol were found to be

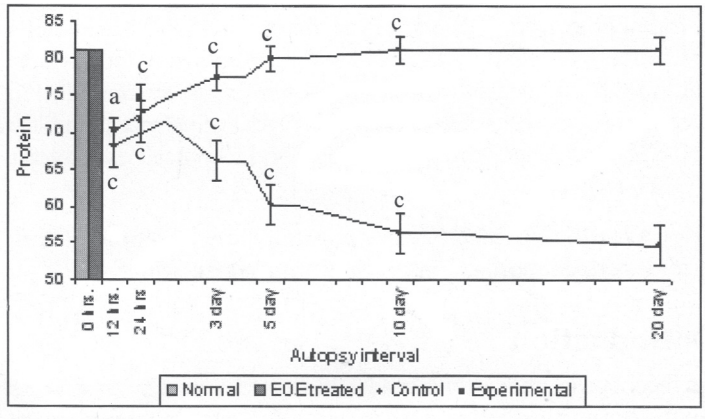

Fig. : 1 Protein level (mg/gm) in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

decreased at all the autopsy intervals but with low magnitude. Normal level could not be measured even on the last day of experimentation. The significant level observed throughout the experimentation was $p<0.5$. No significant protection in cholesterol level was found at 12 hrs. in experimental group, but afterwards, values were found significantly higher over control and restored to normal at day 20 (Fig. 2).

Table : Biochemical alterations in brain of mice after exposure to 5 Gy gamma rays with (Experimental) or without (Control) *Emblica officinalis fruit extract*

Biochemical Parameter	Group	Post-treatment autopsy intervals					
		12 hrs.	24hrs.	3 days	5 days	10 days	20 days
Alkaline phosphatase 6.71±0.07 KAU* 6.70±0.04 KAU**	C E	5.02±0.21^{c} 5.60±0.04^{a}	5.33±0.04^{c} 5.79±0.23^{a}	5.62±0.11^{c} 5.87±0.20^{a}	5.50±0.33^{c} 5.83±0.14^{b}	5.57±0.12^{c} 6.40±0.34^{c}	5.94±0.16^{b} 6.59±0.25^{a}
Acid phosphatase 2.71±0.09 KAU* 2.72±0.06 KAU**	C E	4.63±0.30^{c} 3.06±0.21^{c}	3.85±0.22^{c} 2.99±0.17^{c}	3.89±0.24^{b} 2.95±0.11^{a}	3.75±0.10^{a} 2.83±0.20^{a}	3.12±0.11^{a} 2.74±0.16^{a}	2.91±0.02^{a} 2.70±0.07^{a}
Cholesterol 14.52±0.80 mg/gm* 14.55±0.36 mg/gm**	C E	13.56±0.49 13.65±0.57	13.39±0.14^{a} 13.75±0.45	13.06±0.60^{a} 13.88±0.05^{a}	12.87±0.11^{a} 14.15±0.80^{a}	12.58±0.15^{a} 14.06±0.46^{b}	12.44±0.16^{b} 14.49±0.66^{b}
Protein 81.14±1.07 mg/gm* 81.10±0.86mg/gm**	C E	68.09±1.08^{c} 70.18±0.19^{a}	71.49±0.82^{c} 74.76±0.22^{c}	66.16±0.13^{c} 77.44±0.22^{c}	60.16±0.86^{c} 80.02±1.12^{c}	56.41±0.14^{c} 81.18±0.23^{c}	54.62±0.71^{c} 81.10±0.54^{c}

* Normal (DDW treated)

** Emblica officinalis extract (EOE) treated

C (Control) : DDW+5 Gy gamma rays

E (Experimental) : EOE+5 Gy gamma rays

Significance level : a=P<0.5; b=p<0.05; c=p<0.001

Control v/s normal

Experimental v/s Control

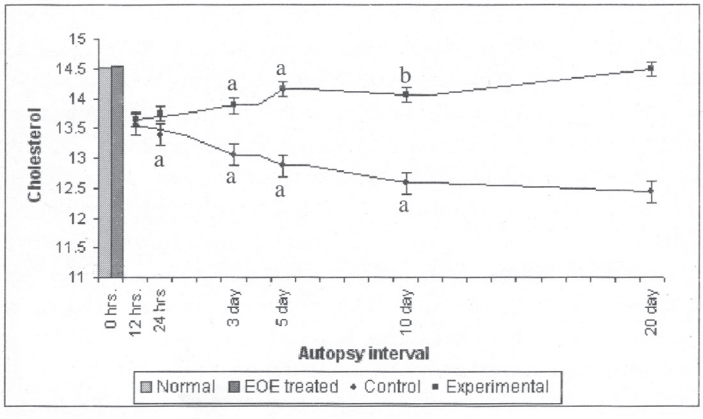

Fig. : 2 Cholesterol level (mg/gm) in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

Significance level between Normal V/s Control, Control V/s Experimental (a p<0.05, b p<0.01, c p<0.001)

Acid phosphatase (ACP): After irradiation with 5 Gy, acid phosphatase level was found to be elevated (70.84 % above normal) at first autopsy interval (12 hrs.). A second peak of elevation was observed on day 3 which was lesser than the first one (43.54%). Later, it decreased till the end of experiment but the values did not restore to normal. Activity of such enzyme was significantly (p<0.05) lower than control in experimental group.

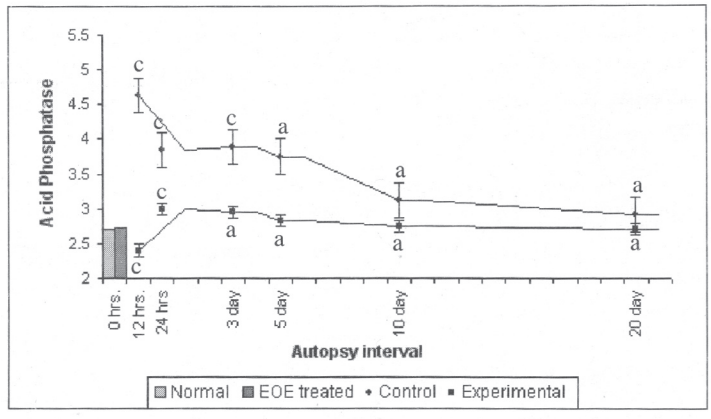

Fig. : 3 Acid phosphatase (KAU) level in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

The value was found to be elevated beyond normal at 12 hrs., but afterwards it showed a continuous decline till the last autopsy interval, however the normal value was restored on day 14. At 12 hrs., values were found to be nearly 60 percent higher than the control by exhibiting maximum protection (Fig. 3).

Alkaline phosphatase (ALP): The maximum decrease (22.51% below normal) in alkaline phosphatase activity was observed at 12 hrs. post-irradiation time. Later, a significant elevation in such enzyme activity was noted till the end of experimentation (18.04%) but it remained higher over control.

A significant increase in alkaline phosphatase activity over control (without EOE irradiated) was measured throughout the period of study in mice who received EOE before 5 Gy gamma irradiation. Increasing pattern persisted till day 3rd (12.52%) then it decreased slightly on day 10th (13.12%). At the last day of observation, the activity of alkaline phosphatase was measured nearly normal (Fig. 4).

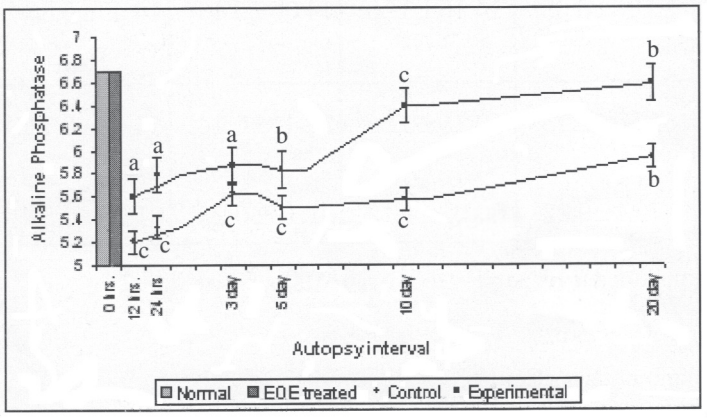

Fig. : 4 Alkaline phosphatase (KAU) level in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

Significance level between Normal V/s Control, Control V/s Experimental (a $p<0.05$, b $p<0.01$, c $p<0.001$)

Glutathione & lipid peroxidation estimation

No significant alterations in the hepatic and blood GSH contents were observed in the normal and EOE treated animals. However, a statistically significant decrease in GSH was evident in the control animals. Experimental animals exhibited a significant increase in the GSH content (Blood and liver) with respect to controls, but the values remained below normal (Fig. 5).

Lipid peroxidation remained unaltered in DDW or EOE treated animals (Group I & II). Exposure of animals to 5 Gy gamma rays resulted in a significant increase in LPx. EOE pre-treatment significantly reduced LPx induction in this group although the level remained above normal (Fig. 6).

Discussion

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 was demonstrated by increased body weight and survival rate. A

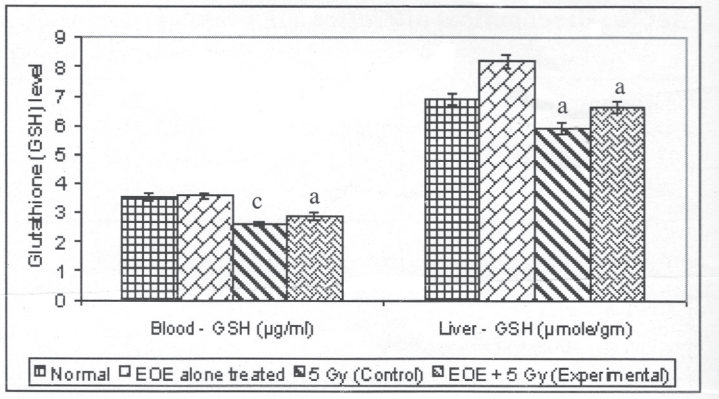

Fig. : 5 Glutathione (GSH) level in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

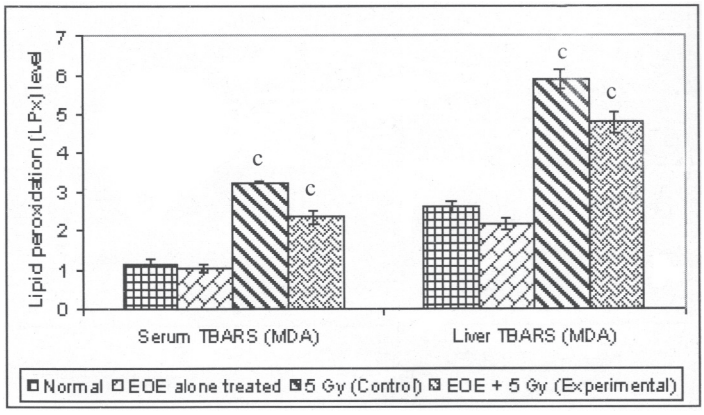

Fig. : 6 Lipid peroxidation (LPx) level in Swiss albino mice after 5 Gy gamma radiation in the presence (Experimental) or absence (Control) of EOE

Significance level between Normal V/s Control, Control V/s Experimental (ap<0.05, cp<0.001)

significant radioprotection was achieved when EOE was given orally 100 mg/kg body 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 irradated animals showed recovery in body weight until 30 day post-irradiation. Only 12.5% mortility was observed in such group; whereas cent per cent mortality was evident in animals irradiated without EOE (Group-I). It is due to the damage to the gastrointestinal tract (20), and protection of the intestinal mucosa against radiation damage might be one of the reasons for the greater survival time in EOE pretreated animals because it may facilitate digestion and absorption in the post-irradiation period. This drug is considered as one of the foremost 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.

Maximum depletion from normal in alkaline phosphatase enzyme activity was observed after 12 hrs. of irradiation. Alkaline phosphatase plays an important role in maintenance of cell membrane permeability and acts on monophosphoesters. The

damage to cell membrane caused by radiation may be one of the reasons for decrease in activity of alkaline phosphatase. Such decline in level may be attributed to the several lysosomal enzymes. Post-irradiation reduction in ALP may be due to damage of brush border cells and increased permeability of villi cells (21-23). Such changes in ALP level in intestine can make changes in brain ALP level through blood. Lynn and Skinner observed a non-exponential loss of activity in alkaline phosphatase at centers of secondary importance for the enzymatic activity and there is a notable destruction of component amino acid residue during radiolysis (24). Although the activation of lysosomal enzymes in tissues with interphase death is well documented, however information on lysosomal enzymes in liver, kidneys and brain are merged and often contradictory (25). It is known that lysosomes from different cell types or even from the some tissue vary greatly in their susceptibility to damage by radiation (26).

Imbalance between cellular production of free radicals and the ability of cell to defend against them is referred to as oxidative stress (OS). OS has been implicated as a potential contributor to pathogenesis of acute CNS injury (27-29). The possible role of acid phophatase in active protein synthesis has been suggested (30-31). Novikoff further stated involvement of acid phosphatase in pinocytosis and related process (30). An elevated golgi activity and peroxidation of membrane as well as oozing out of this enzyme are attributed to an increased acid phosphatase level (32-34). Singh and Singh found that acid phosphatase activity appears to be relatively more intense in purkinje cells of cerebellum(35). Increase in acid phosphatase level in present study is similar to the observation of De et al. (36). They may be one of the reasons for the rupture of lysosomal membrane in the gamma irradiated mice brain.

The decrease in cholesterol level in present study might be due to the stress response caused by irradiation to stimulate synthesis of steroid hormones via hypothalamic-pituitary system. The decreased concentration of cholesterol might be due to increased ACTH secretion by pituitary leading to decreased cholesterol concentration (37). Neurons appear to produce enough cholesterol to survive and to grow, but require external cholesterol to form a sufficient number of synaptic contacts. Since cells in the brain cannot access the cholesterol supply in the blood, they need to synthesize cholesterol by themselves. Glial cells have been found to produce surplus cholesterol and deliver it to nervous tissue via lipoproteins (38-39). The decrease in cholesterol level in present study may be because of radiation induced free radicals that can damage the glial cells leading to depletion in cholesterol level.

A significant reduction in protein level after exposure to radiation has been observed in the present study. Depletion started from first autopsy interval (12 hrs.) and kept on decreasing till the end of experiment (30 days) which is supported with the finding of others (39-40). Goutan et al. reported radiation-induced apoptosis in the external granular cell layers which results in transient decrease in the expression of synaptic proteins in developing rat cerebellum(41). Contrary to this some authors stated that protein synthesis is known to be unaffected by a low dose of radiation, but its rate of synthesis is reduced within a short period in tissue subjected to high doses (42-45). It is well known that free radicals generated during radiolysis of water play the most significant role in indirect biological damage induced by ionizing radiation (46). The GSH/GST detoxification system is an important part of cellular defence against a large array of injurious agents. GSH offers protection against oxygen derived free radicals and cellular lethality following exposure to ionizing radiation (47). Under normal conditions, the inherent defense system including glutathione and antioxidant enzymes, protects against the oxidative damage.

GSH is versatile protector and executes its radio protective function through free radical scavenging restoration of the damage molecule by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the damage state (48). The present study demonstrates a significant reduction in liver and blood GSH 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. Oral administration of Emblica officinalis extract did not significantly influence the endogenous GSH level either in liver or blood, but its presence while 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 in the present study 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 (49). In the present study, although 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 biomembrane can be caused by antioxidants (50, 51). It has been shown that more a-tocopherol is needed in the membranes to protect polyunsaturated fatty acids (PUFA) against radiation induced lipid peroxidation when low dose-rates are applied (52). Several mechanisms, including a potent antioxidant activity, immune response and enhanced recovery of bone marrow have been suggested for radioprotection by vitamin E (53). In the present study, it was observed that Emblica officinalis pretreated 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 (54). The in vitro antioxidant activity of tannoids was demonstrated as well (13). Some of the plants like glycyrrhiza glabra, rubia cordifolia, phylanthus emblica have also been reported to possess antioxidant and free radical scavenging activities (55-57). Treatment of mice with EOE before, during and after the topical application of DMBA carcinogen on skin in mice exhibited chemo

preventive activity in this laboratory (58). 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 (13) and in vivo (54,59). A combination of antioxidant activities via modulation of DNA repair processes may be responsible for the radioprotective affects of Emblica officinalis (Linn.) fruit extract.

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References

1. Sharma M. Investigation on β-carotene vs radiation effect on mice cerebellum. PhD thesis, University of Rajasthan, Jaipur, 2001.
2. Bencizk J, Tenhumen M, Snellman M, et al. Late radiation effects in the dog brain : Correlation of MRI and histological changes. Radiother Oncol 2002; 63 : 107-20.
3. Taki S, Higasi K, Oguehi M, et al. Changes in regional cerebral blood flow in irradiated region and normal brain after stereotactic radiosurgery. Ann Nucl Med 2002; 16 : 273-77.
4. Yuhas JM, Spellman JM, Cullo F. The role of WR-2721 in radiotherapy and/or Chemotherapy. Cancer Clin Trials 1980; 3 : 221.
5. Mathur VB, Uma Devi. Effect of MPG on the radiation induced changes in the intestinal activity of alkaline phosphatase in mice. Nat Acad Sci Lett 1981; 4 : 183-85.
6. Daga MK, Prabash K, Malhotra K, Mishra TK. A Study of lipid peroxide and alpha-tocopherol in acute myocardial infraction. JAPI 1995; 147: 676-79.
7. Nemato K, Horiuchi K, Miyamoto T. Deoxyspergualin is a new radioprotector in mice. Radiat Res 1995; 141: 223-26.
8. Goyal PK, Singh N, Gajawat S, et al. Embryotoxic effect of gamma radiation in mice and their modulation by vitamin. In : Schneeweiss FHA, Sharan RN, Eds. In Recent Apects of cellular and Applied Radiobilogy. Germany Forscungszentrurn Julich Gmbh, 1999; 104-107.
9. Samarth RM, Goyal PK, Kumar A. Modulation of serum phosphatases activity in Swiss albino mice against gamma irradiation by Mentha piperita. Phytotherapy Res 2001; 15 : 1-4.
10. Nunia V, Goyal PK. Prevention of gamma radiation induced aneamia in mice by Diltiazem. J Radiation Res 2004; 45 : 11-17.
11. Kumar A, Ram J, Samarth RM, Kumar M. Modulatory influence of adhatoda vasica nees leaf extract against gamma irradiation in Swiss albino mice. 2005; 12 : 285-93.
12. Satyavati GV, Raina MK, Sharma M. Medicinal Plant of India, New Delhi: Indian Council of Medical Research, 1976, 1 : 377.
13. Ghosal S, Tripathi VK, Chauhan S. Active constituents of Emblica officinalis, the chemistry and antioxidative effects of two new hydrolysable tannis, emblicanin A and B. Indian J Chem 1996; 35: 941.
14. Singh I, Sharma A, Nunia V, Goyal PK. Radioprotection of Swiss albino mice by Emblica officinalis. Phyto Res 2005; 19 : 444-46.
15. Lowery GH, Rousbarough MJ, Farr AL, Randall RF. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193 : 365-71.
16. Leiberman B, Microanalysis. In : Kings EJ, Woltan I Eds., Medical Biochemistry, London : Churchill Pub, 1959; 42-57.
17. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-88.
18. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, GR and GST activities in rat lung and liver Biochim Biophys Acta 1979; 582 : 67-68.
19. Ohkhawa H, Ohishi N, Yogi K. Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction. Analyt Biochem 1979; 95: 351-57.
20. Walker RI. Acute radiation injuries. Pharmac Ther 1998; 39: 9-12.
21. Highman B, Hanks AR. Serum intestinal alkaline phosphatase in rats after 800R whole body regional X-irradiation. Soc Exp Biol Med 1970; 133: 1201.
22. Baijal K. Biochemical and histopathological effects of external irradiation of small intestine of mammals. PhD thesis, University of Rajasthan, Jaipur, 1978.
23. Akoev IG, Pashovkina MS, Dolgacheva LP, et al. Enzymatic activity of some tissues and blood serum from animals and humans exposed to microwaves and hypothesis on the possible role of free radical process in nonlinear effects and modification of emotional behaviour of animals. Radiat Biol Radioecol 2002; 42 : 322-30.
24. Lynn KR, Skinner WJ, Radiolysis of an alkaline phosphatase. Radiat Res. 1979; 57: 358.
25. Gajawat S. Role of vitamins in altering the hepatic toxicity against combined administration of lead radiation in mice: a histopathological and biochemical approach. PhD. thesis, University of Rajasthan, Jaipur, 2000.
26. Beck F, Lloyd JB, Squier C. A Laboratory hand book, Amersterdam: North Holland Publishing Co. 1975.
27. Poeggeler B, Reiter RJ, Tan D, Chan LD, Mancherster LC. Hydroxyl radical mediated damage and aging : A hypothesis. J Pineal Res 1993; 14: 168-81.
28. Ravikumar A, Arun P, Devi Deepa KV, et al. Isoprnoid pathway and free radical generation and damage in neuropsychiatric disorders, 2000; 38: 438-46.
29. Sherkigy Rosenbaum Z, Malamed E, Offen D. Antioxidant therapy in acute central nervous system injury; Current State Pharmacol Rev 2002; 54: 271-84.
30. Novikaff AB. GERL its form and functions in neurons of rat spinal ganglion. Biol Bull 1963; 127: 358-59.
31. Kokko A. Histochemical and cytometric observations esterases in the spinal ganglion of rat. Acta Physiol Scand 1965; 66: 1-76.
32. Wills ED, Wilkinson AE. Release of enzymes from lysosomes by irradiation and the relation of lipid peroxide formation to enzyme release. Biochem J 1966; 99: 657.
33. Rene AA, Dorden JH, Parker JL. Radiation induced ultrastructural and biochemical changes in lysosomes. Lab Invest 1971; 25: 230.
34. Shah VC, Gadhia PK. Effects of sublethal dose of R- irradiation on lysosomal enzymes in tissue of pigeon. Radiat Res 1979; 20: 322.
35. Singh SP, Singh R. Acid phosphatase and monoamine oxidase activity in the cerebellar cortex of brain dove , Streptopelia Senegalensis and bat, Rhinopoma microphyllum. Ad Bios 2002; 21 : 35-40.
36. De AK, Chipalkatti S, Aiyar AS. Effect of chronic irradiation on age - related biochemical changes in mice. Radiat Res 1983; 95: 637-45.
37. Wexlar BC, Pencharz R, Thomas SF. Adrenal ascorbic acid and histological changes in male and female rats after half body X-rays irradiation. Amer J Physiol 1955; 183: 71-74.
38. Nagler K. March D, Pfriegar FW. Glia derived singals induced synapse formation in neurons of the rat central nervous system. J Physiology 2001; 533: 665-67.
39. Yadav R. Possible role of Calcium antagonists in modifying brain lesions induced by heavy metal and ionizing radiation in mice. PhD thesis, University of Rajasthan, Jaipur, 2003.
40. Ekstram R, Liu DSH, Richardson A. Brain protein synthesis during the life span of male fisher rats. Gerontology. 1980; 26: 121-28.
41. Goutan E, Marti E, Ferrer I. Expresson of synaptic proteins in the developing rat cerebellum following ionizing radiation. Teratology, 1999; 59 222-26.

42. Abrams R. Effect of x-rays on nucleic acid and protein synthesis. Arch Biochem 1951; 30: 90.

43. Ivanov II, Radakov VV. Effect of whole-body gamma rays irradiation on tissue protein synthesis. Bull Exp Biol Med 1968; 66: 1340.

44. Panke TW, Michaelson. Effect of external X-irradiation on serum protein in beagle. Amer J Physiol 1971; 220: 174.

45. Bhatwedeker JM,Chinoy NJ, Shah VC. A study on the levels of nucleic acids, proteins and phosphatase in normal and X-irradiation guinea pigs. Strahlentherapie, 1970; 145: 83-87.

46. Hall EJ. Radiobiology for the radiologists, 2nd edition, Philadelphia: Harper and Row Publisher, 1978.

47. Biaglow JE, Varnes ME, Epp ER, et al. In: Anticarcinogenesis and radiation protection, New York, Plennum Press, 1987; 387.

48. Bump EA, Brown JB. Role of glutathione in the radiation response of mammalian cells in vitro and in vivo. Pharmaceut Ther 1990; 47: 117.

49. Raleigh JA, Walden Jr T, Huges C. Prostaglandin and lipid metabolism in radiation injury, New York : H N Plenum Press, 1989: 3.

50. Konings AWT, Drijver EB. Radiation effect on membranes. Vitamin E deficiency and lipid peroxidation. Radiat Res 1979; 80 : 494.

51. Konings AWT, Osterloo SK. Radiation effect on membranes. A comparison of the effect of X- irradiation and ozone exposure with respect to the relation of antioxidant concentration and the capacity for lipid peroxidation. Radiat Res 1979; 81: 200.

52. Konings AWT, Demen J, Trieling WB. Protection of liposomal lipids against radiation induced oxidative demage, Int J Radiat Biol 1979; 35: 343

53. Malick MA, Roy RM, Sternberg J. Effect of Vitamin E on post-irradiation death in mice. Experientia. 1978; 34: 1216.

54. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant actavity of active tannoid principles of Emblica officinalis (amla). Ind J Exp Biol 1999; 37: 676-80.

55. Jose K, Kuttan R. Inhibition of oxygen free radical by Emblica officinalis extract and Chanvanprash. Amala Res Bull 1995; 15: 46-52.

56. Tripathi YB, Sharma M, Manickam M. Rubiadin, A new antioxidant from Rubia cordifolia. Ind J Biochem Biophys 1997; 34: 302-306.

57. Korina LG, Afanasav IB. Antioxidant and chelating properties of flavonoids. Adv Pharmacol 1997; 38: 151-63.

58. Sancheti G, Jindal A, Kumari R, Goyal PK. Chemopreventive action of Emblica officinalis is on skin carcinogenesis in mice. Asian Pacific J Cancer Prevntion 2005; 6 : 197-201.

59. Bhattacharya A, Kumar M, Ghosal S, Bhattacharya SK. Effect of bioactive tannoid principales of Emblica officinalis on iron induced hepatic toxicity in rats. Phytomedicine 2000; 7: 173-76.