WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 846-863. Research Article ISSN 2277– 7105

AMELIORATIVE EFFECT OF *EMBLICA OFFICINALIS* IN RADIATION AND CADMIUM INDUCED ALTERATION IN MICE LIVER

Bhati SL, Ranga D, Meena DC, Agarwal M, Chakrawarti A, Purohit RK*

Radiation Biology Laboratory, Department of Zoology, Govt. Dungar College, Bikaner (Rajasthan), India-334001.

Article Received on
26 September 2014,

Revised on 21 Oct 2014,
Accepted on 14 Nov 2014

***Correspondence for Author**
Dr. Rajendra Purohit
Radiation Biology Laboratory, Department of Zoology, Govt. Dungar College, Bikaner (Rajasthan), India.

ABSTRACT

Background: All organisms living on earth are being perpetually exposed to some amount of radiation organizing from a variety of sources. Radiation causes deleterious effects in all forms of life due to increasing utilization and production of modern technology, a simultaneous exposure of organisms to heavy metals is also unavoidable. So there is a need of hour to search for an ideal herbal protector which reduce damaging effects induced by the both the toxicants. **Objective:** The present study was planned to evaluate the protective role of *Emblica* against combined effect of radiation and cadmium in the liver of mice. **Methodology:** For the present experiment, healthy male Swiss albino mice (6-8 weeks) were divided in seven groups according to treatment i.e. Cadmium chloride solution as drinking water (Group – II) or exposed to low dose 3.5 and 7.0 Gy of gamma radiation (Group – III) or combined treatment of radiation and cadmium chloride (Group - IV). The animals of experimental groups were administered *Emblica* extract orally seven days prior to radiation or cadmium chloride treatment (group V, VI and VII). The animals from all group were sacrificed by cervical dislocation at each post treatment intervals of 1, 2 ,4, 7, 14 and 28 days. **Results:** The value of total proteins, glycogen, acid and alkaline phosphatase activity increased up to day-14 in non drug treated groups and day-7 in the *Emblica* treated groups, thereafter value declined up to day-28 without reaching to the normal. The value of cholesterol declined up to day-14 in non drug treated groups and day-7 in the drug treated groups, thereafter value elevated up to day-28. In the above experiment the liver of *Emblica* treated animals exhibited less severe changes as compared to non drug treated animals at all

the corresponding intervals, The combined action of Cadmium chloride and radiation showed synergistic effect. **Conclusion:** It is concluded that *Emblica* is potent enough to check radiation and cadmium induced changes in the liver of mice.

KEYWORDS: Gamma Radiation, Cadmium chloride, Swiss albino mice, *Emblica*, Liver.

INTRODUCTION

Ionizing radiation inflicts deleterious effects to living cells through the generation of reactive oxygen species that damage vital cellular targets such as DNA and membrane. Protecting living systems from the onslaughts of ionizing radiation is of paramount importance in radiation biology. This has particular relevance in nuclear warfare, nuclear accidents and nuclear terrorism. Radiation protection is also important in the radiotherapy of cancer where normal tissues need to be protected while cancer are exposed to high doses of radiation.[1,2] The synthetic compounds having thiol group are found to be good free-radical scavengers and are effective against radiation induced damage.[3] However, these compounds produce serious side effects and were toxic at the doses required for radioprotection.[4] To reduce the toxic effects of synthetic compounds, there is a need to explore the compounds; which could be less toxic and highly effective at non-toxic dose. Heavy metals, such as lead (Pd), mercury (Hg), and cadmium (Cd) may displace or substitute for essential trace metals and interfere with proper functioning of enzymes and associated cofactors.[5] It has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential element like cadmium. Thus there is ample opportunity for exposure to cadmium both in and outside the work place.[6] Although cadmium has no redox activity, it could indirectly induce the production of hydroxyl radicals.[7] Superoxide anion, nitric oxide and hydrogen peroxide.[8] Increased lipid per-oxidation, as a result of cadmium exposure was observed in various *in vitro* and *in vivo* studies.[9,10-12] Several classes of antioxidant dietary compounds have been suggested to present health benefits and there is evidence that consumption of these products leads to a reduction of the expression of various oxidative stress biomarkers.[13-15] A positive correlation has also been established between dietary supplementation with certain vegetables and plants product to the reduction of toxic effect of various toxicants, environmental agents including heavy metals and radiation.[16,17] In our quest for an easy, safe and affordable means to combat this problem, a plant *Emblica officinalis* Linn. (Amla) (family Euphorbiaceae) fruit extract, shows a promising result in mice. It is rich in quercetin, phyllambic compound, gallic acid, pectin and others.[18] The

effective compounds are flavonoids, tannins, and vitamin C found in maximum concentration and antioxidant in action.[19] Amla extract possesses anticancer, antisclerotic, lipid lowering, hepatoprotective, anti-HIV activities.[20,21] and inhibits thioacetamide-induced oxidative stress and hyper proliferation in rat liver. [22] Liver of mammals has been reported as highly radiosensitive organ. [23] Hepatic injury can be life threatened complications when the entire or most of liver is exposed to ionizing radiation. Most instances of radiation hepatitis occur in human within 90 days after irradiation. [24, 25] The combined treatment of radiation and cadmium may cause additive effect in comparison to their individual effect. The *Emblica* may protect from the toxic effect caused by both the agents. In view of the fact, present study was planned to investigate the possible prophylactic role of *Emblica* against radiation and cadmium induced changes in the liver of Swiss albino mice.

MATERIALS AND METHODS

Animal care and handling

The adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered under CPCSEA, Chennai (registration no. 1066/ac/07/ CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). Looking into the fact, the present experiments were conducted under the supervision of IAEC of the College. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. They were acclimatized to laboratory conditions before use. Occasionally tetracycline water was provided as a precaution against infection. The temperature of the room was maintained between 22-27ºC.

Source of irradiation

The animals were exposed by the Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate ranging from 0.85 Gy/min to 1.65 Gy/min. The dose was calculated at the midpoint by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Cadmium

Cadmium in the form of inorganic cadmium chloride, obtained from S. D. Fine Chemicals Ltd. Boisar, Mumbai (India) was used for present study. The dose 20 ppm was selected for

the present experiment. For this aqueous solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20 ppm and then administered orally in drinking water. [26]

Emblica extract

Fresh fruits of the *Emblica officinalis* were cleaned, cut into small pieces, air dried, powdered and extracted with double distilled water (DDW) by refluxing for 36 hrs. (12 hrs. x3). 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 per cent yield of the extract was obtained. The drug was given orally at the dose rate of 1000mg/kg body wt/ animal / day from seven days prior to cadmium chloride treatment or irradiation.

Experimental design

The animals for the experiments were divided into the following groups:

Group – I (Sham-irradiated animals)

Group– II (Cadmium chloride treated animals)

Group- III (Irradiated animals)

Sub-group III a: 3.5 Gy

Sub-group III b: 7.0 Gy

Group- IV (Animals treated with radiation and Cadmium chloride)

Sub-group IV a: 3.5 Gy + Cadmium chloride

Sub-group IV b: 7.0 Gy + Cadmium chloride

Group – V (Cadmium chloride and Drug treated animals)

Group- VI (Radiation and drug treated animals)

Sub-group VI a: 3.5 Gy + *Emblica*

Sub-group VI b: 7.0 Gy + *Emblica*

Group-VII (Radiation, Cadmium chloride and drug treated animals)

Sub-group VII a: 3.5 Gy + Cadmium chloride + *Emblica*

Sub-group VII b: 7.0 Gy + Cadmium chloride + *Emblica*

Autopsy of animals

A minimum of three animals from each group was sacrificed after 1, 2, 4, 7, 14 and 28 days of treatment. The animals were sacrificed by cervical dislocation. After sacrificing the animals the liver was taken out, it was blotted, weighed and kept at -20^{o}C for biochemical studies.

The following biochemical parameters were taken into consideration:

1. Total proteins. [27]
2. Glycogen. [28]
3. Cholesterol. [29]
4. Acid and Alkaline phosphatase. [30]

Statistical analysis

After obtaining the mean values of control and experimental groups, the standard error (S.E.) of mean was calculated. Student's 't' test was used. The Significance levels are expressed in 'P' value as <0.05-Significant, <0.01-More Significant, <0.001- Highly Significant and N.S. -Non Significant. Linear regression analysis was done to obtain LD50/30 values and to determine dose reduction factor (DRF).

RESULT

***Emblica* tolerance study**

Mice for this study were divided into seven groups of ten animals each and were given 100, 200, 400, 800, 1000, 1500 and 2000 mg/kg body weight/day *Emblica* in double distilled water (DDW) for five consecutive days. These mice were observed regularly till 30 days for any signs of sickness, morbidity, behavioural, toxicity and mortality. During the entire experiment (30 days), no sickness and mortality were observed. This indicates that even high dose of *Emblica* (2000 mg/kg body weight) is well tolerated in Swiss albino mice. Maximum tolerance dose of *Emblica* was determined accordingly.

Selection of optimum dose of *Emblica*

The optimum dose of *Emblica* extract was selected against sub lethal gamma radiations (3.5 and 7.0 Gy) in Swiss albino mice on the basis of survival experiment where number of death and surviving animals were recorded up to 30 days post- irradiation. Mice treated with *Emblica* at the dose of 100, 200, 400, 800, 1000, 1500 or 2000 mg/kg body weight/ day orally for five consecutive days prior to irradiation exhibited 20, 25, 40, 60, 90, 40 and 30 per cent survival respectively throughout the period of study. On the other hand all animals died within 20 days of radiation exposure in control (without *Emblica* treated irradiated). On the basis of present study, 1000mg/kg body weight/ day *Emblica* was found as the optimum dose, and it was used for further detailed investigation against various doses (3.5 and 7.0 Gy of gamma radiation).

LD50/30 and Dose reduction factor

The effectiveness of any radioprotective chemical/agent can be evaluated by determination of DRF. The dose reduction factor of *Emblica* based on $LD_{50/30}$ survival test was calculated by following formula after exposing a large number of Swiss albino mice to different doses of gamma rays with or without cadmium chloride treatment in the presence (Experimental) or absence (Control) of *Emblica*.

$$DRF = \frac{LD_{50/30} \text{ for Experimental animals}}{LD_{50/30} \text{ for Control animals}}$$

In order to establish the survival dose response of Swiss albino mice to radiation in the presence or absence of *Emblica*, two groups of animals were used. The mice of one group were given orally double distilled water, volume equal to *Emblica* and were exposed to different doses (3.5 and 7.0 Gy) of gamma radiation, while the animals of other group were given orally *Emblica* (1000mg/kg body weight/ day) for seven consecutive days once daily. On 7^{th} day after half an hour of the last administration of *Emblica* mice of second group were exposed to similar doses of gamma radiations (as per group first).

Exposure of mice of gamma radiation showed 0, 33 and 100 per cent mortality within 30 days of irradiation at 3.5 and 7.0 Gy respectively, while in *Emblica* pre-treated irradiated animals, mortality was significantly decreased and it was found 0,0 and 30 per cent respectively. Data were used for the calculation of the dose reduction factor of *Emblica* extract against gamma radiation and the same was computed as 1.96.

Total Proteins

The value of total proteins increased in groups III and IV on day-1 which continued up to day 14 thereafter it declined on day- 28, whereas, in groups VI and VII the value increased up to day-7 thereafter it declined on day-14 which continued up to day- 28. Similarly in group II the value decreased up to day-14 thereafter it increased on day-28, whereas in the group V the value decreased up to day-7 thereafter it increased on day-14 and continued so up to (figure 1). This increase or decrease was comparatively lesser in the *Emblica* treated animals showing protection by *Emblica*. The combined action of Cadmium chloride and radiation showed synergistic effect.

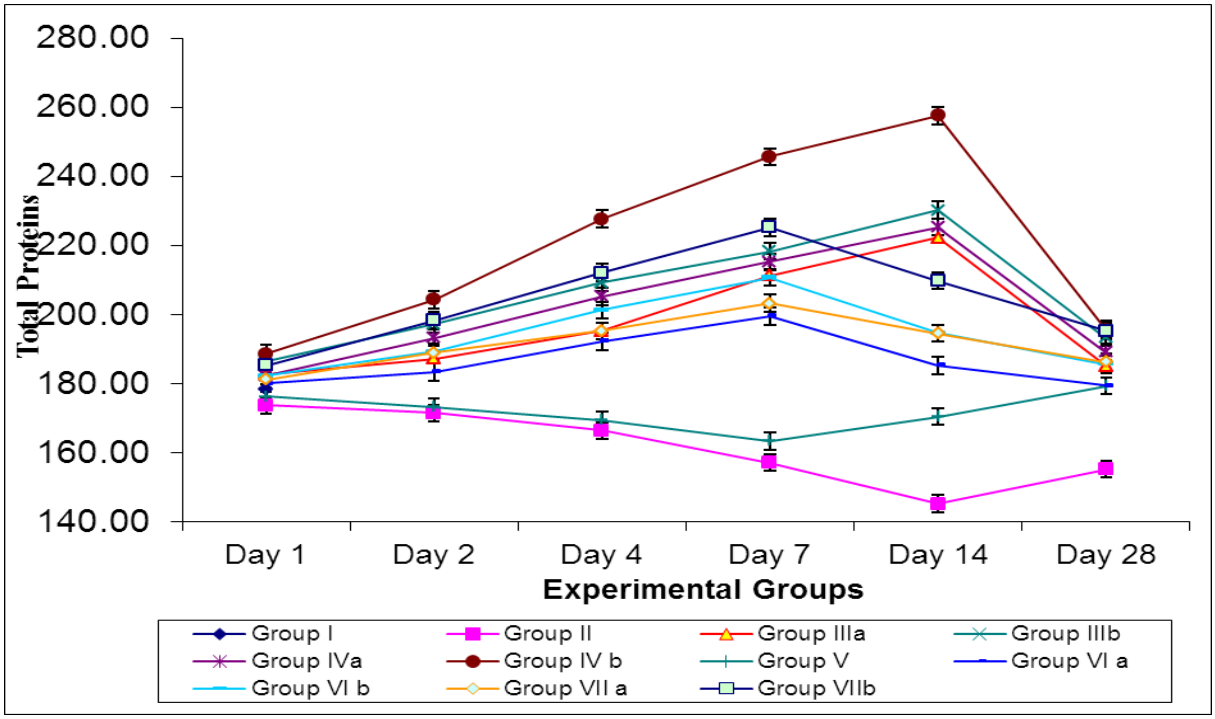

Figure 1: Variations in the Total proteins content in Liver of mice in various groups (mg/gm of tissue weight).

Glycogen

Similar trend of increase was also noticed in the value of glycogen as observed in total proteins content in the present study (figure 2).

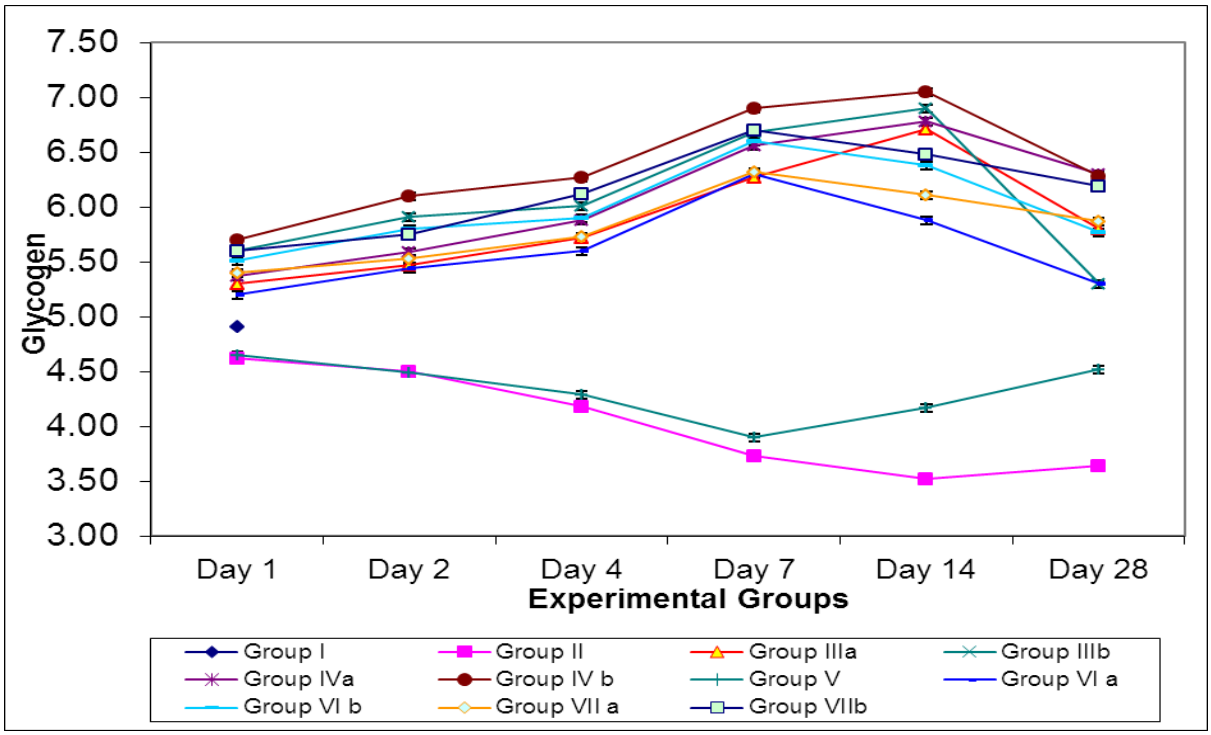

Figure 2: Variations in the Glycogen content in Liver of mice in various groups (mg/gm of tissue weight).

Cholesterol

The value of cholesterol decreased On day-1 and continued so up to day-14 in groups II, III and IV respectively. Thereafter it increased on day-28 without reaching to the normal level. In the *Emblica* treated groups V, VI and VII the value decreased up to day-7. Thereafter it increased on day-14, which continued up to day-28(figure 3). The decrease was found dose dependent. In *Emblica* treated groups a less severe decrease and early recovery in the cholesterol level was observed.

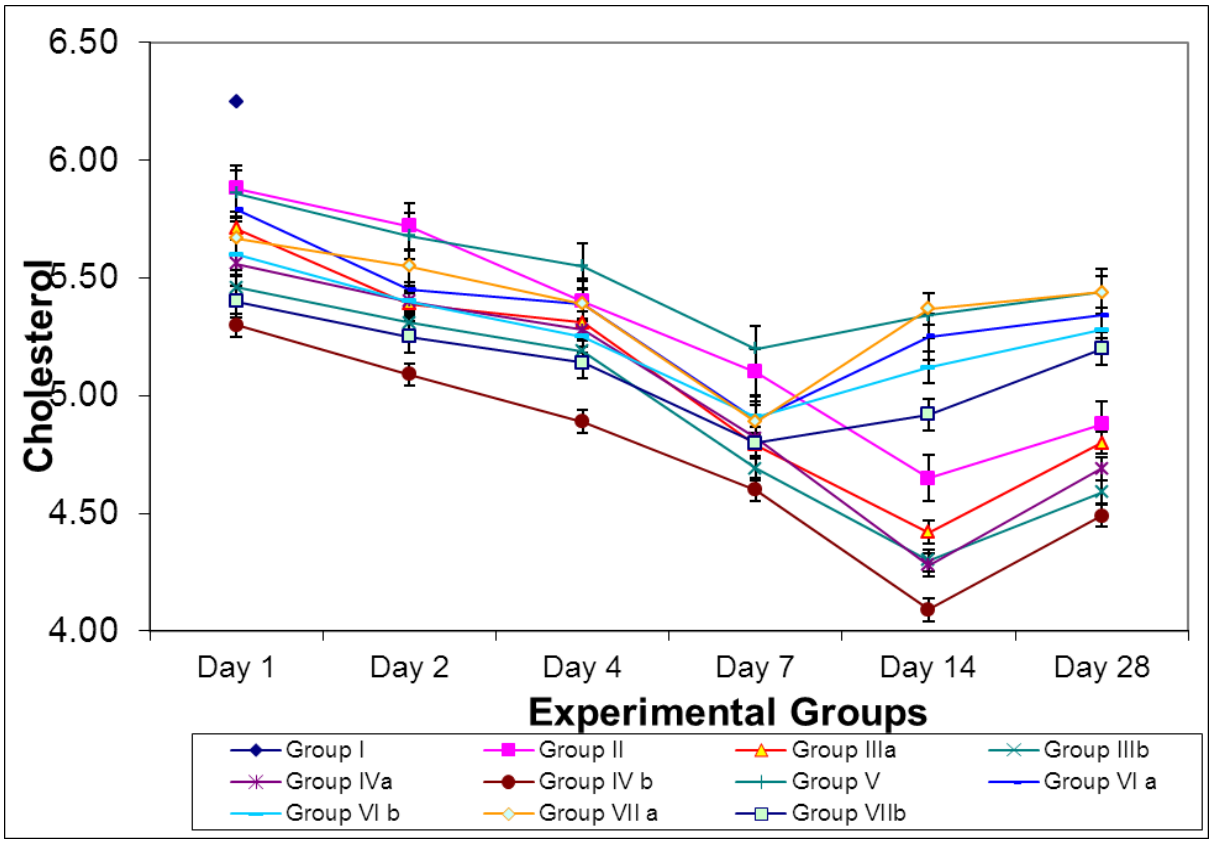

Figure 3: Variations in the Cholesterol content in Liver of mice in various groups (mg/gm of tissue weight).

Acid and Alkaline Phosphatase Activity

The acid and alkaline phosphtase activity increased on day-1 in the groups II, III and IV which continued up to day-14. On day-28 the value decreased. On the other hand in the *Emblica* treated groups V, VI and VII the value increased up to day-7 thereafter it decreased on day-14 and Continued to decrease up to day-28 without reaching to the normal. A less severe increase was observed in the drug treated groups (figure 4 and 5).

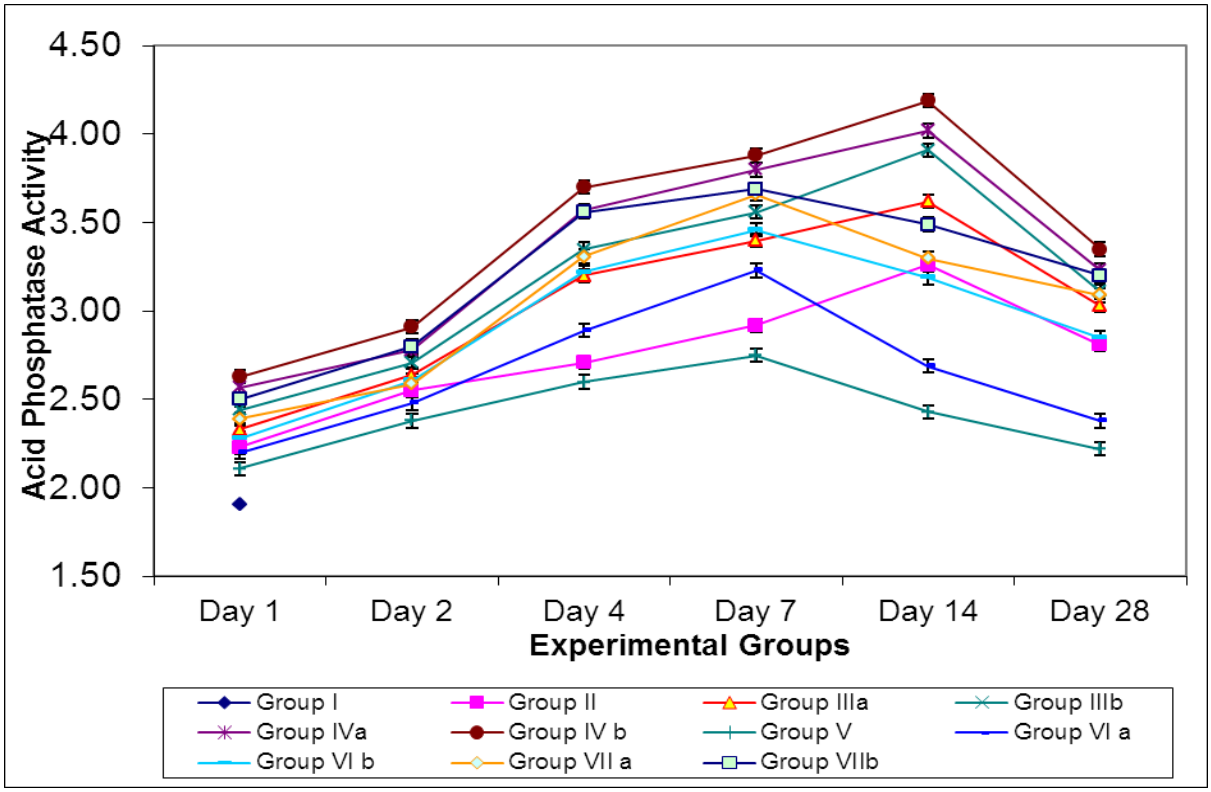

Figure 4 Variations in the Acid Phosphatase activity in Liver of mice in various groups (mg pi/gm/hr.).

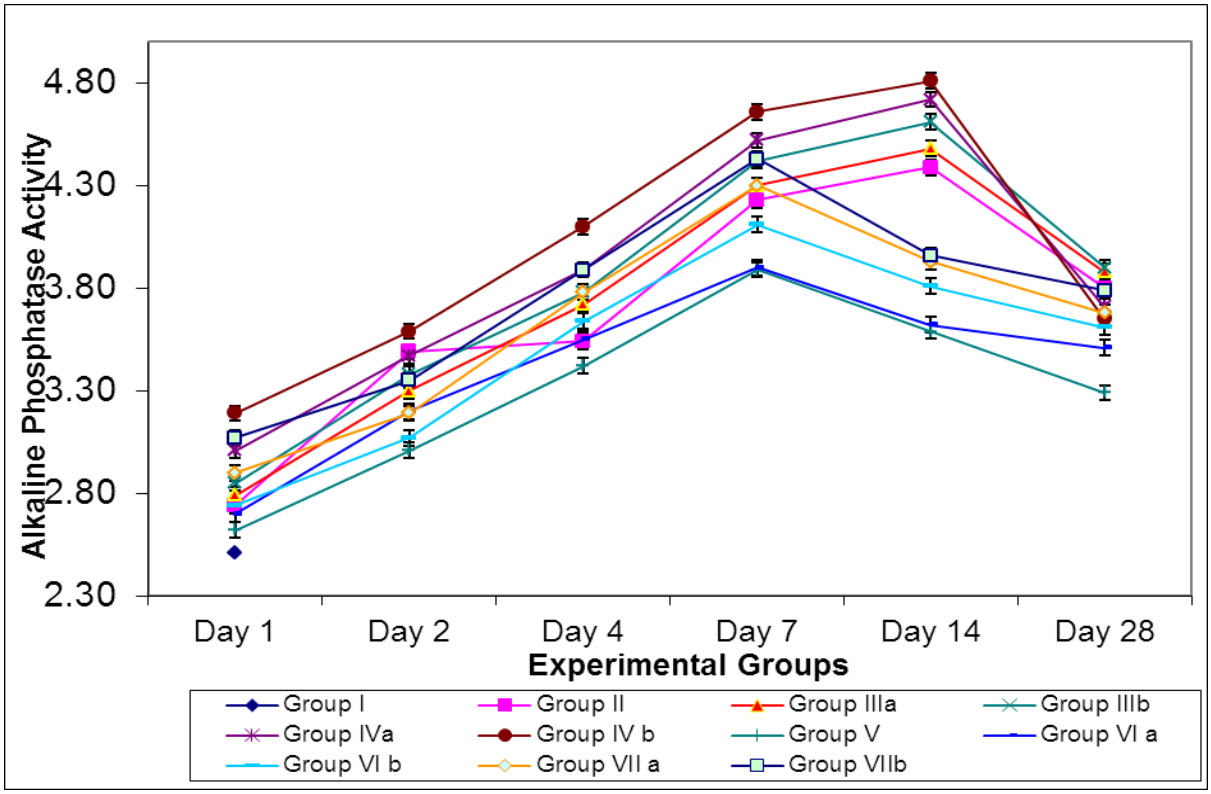

Figure 5 Variations in the Alkaline Phosphatase Activity in Liver of mice in various groups (mg pi/gm/hr.).

DISCUSSION

Once taken up enterally, Cd (Cadmium) reaches the liver where it binds to metallothioneins (MTs), glutathione (GSH) and other proteins or peptides. [31] Metallothioneins induced upon Cd exposure can act as a "double - edge sword". On one hand MTs bind to Cd, thereby detoxifying and removing it from the cellular environment. On the other hand, due to its thiol groups, MTs can scavenge reactive oxygen species (ROS) that are produced as result of Cd – induced oxidative stress. [32] However, the latter results in Cd dissociation from MTs due to the corresponding decreased metal binding stability.[33] intracellular Cd , in bound or unbound form, culminates in mitochondrial damage, and/or cell death.[31]

Ionizing radiation is known to induce various physiological, and biochemical changes in humans and animals. Several molecular mechanisms of ionizing-radiation have been proposed, including cumulative damage by ROS, dislocation in replicative cells, genome instability, mutation, or altered expression of specific enzymes and cell death.[34] The oxidative stress due to free radical-formation was greatly augmented during ionizing-radiation exposure.[35] It was likely that animal particular antioxidants generally decreased the level of oxidation in such systems by transferring hydrogen atoms to the free radical structure.[36]

Total Proteins

Total serum proteins are, diagnostically, of relative importance in assessing the state of health of an organism, their increase appearing especially in inflammatory process and tissue dysfunction after irradiation.[37] The present study revealed that irradiation resulted in continuous augmentation in total proteins in liver tissue up to day 7th that probably as a result of an increased transport of amino acid through plasma membrane as a consequence of permeability changes in irradiated cell membrane.[38] In addition, increased synthesis of m-RNA and ribonucleoprotein could also be added to the radiation induced increased level in proteins.[39]

Cadmium attacks on –SH group of proteins and it may be possible that *Emblica* providing some protection by additional –SH groups against the destructive action of Cadmium. The total protein decreased after cadmium chloride treatment on day-1 and this trend continued till the last autopsy interval due to dysfunction of the hepatocytes as induced by chemical lorded.[10] *Emblica* treated animals prior to irradiation showed a significantly lower concentration of protein in liver than control. Increased level of it was observed up to day-14

after 7.0 Gy irradiation, respectively. Thereafter, the protein level tended to recover on later autopsy intervals. It is suggested that protection of protein is due to the hydrogen atom donation by the protector. [40]

Glycogen

Higher level of hepatic glycogen after irradiation could be due to the stimulation of the pituitary-adrenal system.[41,42] since it has been shown that irradiation did not increase the hepatic glycogen in the hypophysectomized rats.[43] Fatty degeneration, necrosis, increase in connective tissues are the changes produced by heavy metals, which have been described by a number of workers.[44] Result of our experiments on Swiss albino mice treated with cadmium chloride exhibit a fall in glycogen values. The loss of glycogen from hepatocytes was statistically Significant ($p<0.001$)when compared with the values of normal group. The loss of glycogen in liver takes place before the cell necrosis and it can also drop in physiological circumstances. The present observations are in agreement with those of [45]who also reported decrease in glycogen content due to cadmium toxicity, this change attributed to the increased glycogenolysis after cadmium treatment. In the present study, when *Emblica* extract was given before Cadmium treatment, the change in glycogen content remained similar to that of control group (without *Emblica*), but the values were found to less prominent than the controls.

Cholesterol

After irradiation the reduction of cholesterol concentration in liver during early intervals might be due to the stress response caused by radiation which stimulates the synthesis of steroid hormones via hypothalamic-pituitary system. [46] In the present investigation cholesterol showed a significantly declining pattern till day-14 in the cadmium chloride treated group II and day-7 in the drug treated groups V but afterwards there was a significant elevation in cholesterol. It was suggested that the decrease in cholesterol level may be related to its enhanced utilization in corticosteroidogenesis and/or a decreased *de novo* synthesis. Involvement of thyroid hormones has also been suggested in cholesterol metabolism and an enhanced breakdown in hyperthyroidism is known to result in hypochoelesterolemia. [47] In the present study *Emblica* treated groups showed decreasing trend in value of cholesterol up to day-7 then increased on day-14 which continued up to day-28. *Emblica officinalis* is major antioxidant which affect cholesterol metabolism through its antioxidant effect. [48] In the present experiments animals treated with both radiation and Cadmium also exhibited a

decrease in the level of cholesterol in liver. While *Emblica* minimizes the level of variation of cholesterol in liver showing its protective effect.

Acid and Alkaline phosphatase activity

Radiation exposure resulted in elevation of liver phosphatase activity which may be attributable to the tissue impairment and per- oxidation of membrane lipids leading to activation of suppressed acid hydrolyases. [49,50] An increase in acid phosphatases activity after radiation exposure in the present experiment could be ascribed either to a direct effect of radiation which results in enhanced Golgi activity[51] and per- oxidation of lysosomal membranes by cadmium causing lysis of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in both enzyme activity in liver.[52-54]

From the present study, it appears that *Emblica* caused an early recovery to normalcy in both the enzyme level, which was evident as statistically lowered values in these groups in comparison to control. Active principles of *Emblica officinalis* fruits in terms of augmentation of oxidative free radical scavenging enzymes, concomitant with reduction in radiation induced lipid peroxidation. It is quite possible that the fruit extract of *Emblica* retards the formation of the toxic lipid peroxidase responsible for radiation damage.

Protective mechanism of *Emblica officinalis*

The possible mechanisms of action of *Emblica* may be as under:

- Radiation has been shown to induce DNA strand breaks and mutation and induced peroxidative changes to lipid and proteins. *Emblica* extracts has been shown to have significant antioxidant activity, which reduces the oxidative changes induced by radiation.
- *Emblica* extract was also found to inhibit mutagenesis by direct binding to certain mutagens as well as by inhibing carcinogen activation.
- It stimulates haemopoiesis thus reducing the myelosuppression induced by radiation.
- Moreover, it produces a protective layer in stomach thus reduces the mucosal damage of gastrointestinal linings during irradiation.
- Presence of a variety of polyphenols are reported in *Emblica*. These polyphenols are excellent scavangers of oxygen radicals produced in the body by radiation, thus affording protection to the body.[55]

- The protection afforded by *Emblica officinalis* may be associated with its antioxidant capacity and through its modulatory effect on hepatic activation and detoxifying enzymes.[56]
- Administration of *Emblica* extract increased the GSH levels. *Emblica* showed excellent antioxidant activity *in vitro* [57] and present study also revealed its antioxidant potential.
- It can be hypothesized that antioxidant activity, potent stimulation of haemopoietic system, nontoxicity as well as the easy availability of *Emblica* make it as an excellent choice for further development as a natural radioprotector. [58]

Figure 6 Protective mechanisms of *Emblica officinalis.*

CONCLUSION

1. The combined treatment of radiation and Cadmium chloride showed synergistic changes.
2. The liver of *Emblica* treated animals showed less severe radiolesions and early and fast recovery in comparison to non-drug treated animals. Thus, it seems that *Emblica* has protected the liver at both the dose levels with and without Cadmium chloride treatment.
3. The *Emblica* might have protected the animals from radiation by more than one mechanism due to multiplicity of its properties.
4. Thus, *Emblica* is a good herbal radioprotector and can be given to cancer patients during radiotherapy to minimize the side effects of exposure.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the Head, Department of Zoology and the Principal, Govt. Dungar College, Bikaner (India) for providing necessary facilities in the department. The irradiation facility provided by the Radiotherapy Department, Acharya Tulsi Cancer Hospital and Research Centre, PBM Hospital, Bikaner (India) is also gratefully acknowledged.

REFERENCES

1. Nair CKK, Parida DK, Nomura T. Radio-protector in Radiotherapy. J Radiat Res, 2001; 42: 21-37.
2. Maisin JR. Chemical radioprotection: Past, present and future prospectus. Int. J. Radiat. Biol, 1998; 73: 443-50.
3. Jaiswal SK, Bordia A. Radio-protective effect of garlic *allium sativum* linn, in albino rats. Original Contribution, 1996; 50(7):231-3.
4. Sweeney TR. A survey of compounds from the antiradiation drug development program of the U.S. army medical research and development command. Government printing office, Washington D.C Publication, 1979; 308-18.
5. Ash C, Stone R. "A question of dose," Science, 2003; 300:925.
6. Vinoth Kumar P, A Amala Bricey, V Veerathamari Selvi, C Sudheer Kumar, N. Ramesh. Antioxidant effect of green tea extract in cadmium chloride intoxicated rats. Advances in Applied Science Research, 2010; 1: 9-13.
7. O'Brien P, Salacinski HJ. Evidence that the reactions of cadmium in the presence of metallothionein can produce hydroxyl radicals. Arch Toxicol, 1998; 72: 690-700.
8. Wang Y, Fang J, Leonard S, Rao MK. Cadmium inhibits the electron transfer chain and induces reactive oxygen species. Free Rad Bio Med, 2004; 36: 1434-43.
9. Casalino E, Sblano C, Landriscina C. Enzyme activity alteration by cadmium administration to rats: the possibility of iron involvement in lipid peroxidation. Arch Biochem Biophys, 1997; 346: 171-9.
10. Müller L. Consequences of cadmium toxicity in rat hepatocytes: Mitochondrial disfunction and lipid peroxidation. Toxicology, 1986; 40: 285-92.
11. Manca D, Ricard AC, Trottier B, Chevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology, 1991; 67: 303-23.

12. El-Maraghy SA, Gad MZ, Fahim AT, Hamdy MA. Effect of cadmium and aluminium intake on the antioxidant status and lipid peroxidation in rat tissues. J Biochem Mol Toxicol, 2001; 15: 201-14.
13. Halliwell B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radic Biol Med, 2002; 32:968–74.
14. Peng J, Jones GL, Watson K. Stress proteins as biomarkers of oxidative stress: effects of antioxidant supplements. Free Radic Biol Med, 2000; 28:1598–606.
15. Jacob RA, Aiello GM, Stephensen CB *et al.* Moderate antioxidant supplementation has no effect on biomarker of oxidative damage in healthy men with low fruit and vegetable intakes. J Nutr, 2003; 133:740–3.
16. Nandi P, Talukder G, Sharma A. Dietary supplementation with leaf extract of *Beta vulgaris* L. var. *benghalensis* Hort. in modifying cytotoxicity of lead subacetate in mouse *in vivo*. Phytother Res, 1997; 11:273–76.
17. Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidant nutrients and phytochemicals. Toxicol, 2003; 189:1-20.
18. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, Council of Scientific and Industrial Research, New Delhi, 1956.
19. Haque R, Bin-Hafez B, Ahmad I, Parvez S, Pandey S, Raisuddin S. Protective effect of *Emblica officinalis* Gaertn Incyclophosphamide-treated mice. Hum Exp Toxicol, 2001; 20:643-50.
20. Khan MT, Lampronti I, Martello D, Bianchi N, Jabbar, Choudhuri MS, Datta BK, Gambari R. Identification of pyrogallol as an antiproliferative compound present in extracts from the medicinal plant *Emblica officinalis*; effects on *in vitro* cell growth of human tumor cell lines. Int J Oncol, 2002; 21(1):187-92.
21. El-Mekkawy S. Inhibitory effects of Egyptian folk Medicines on human immuno deficiency virus (HIV) reverse transcriptase. Chem Pharm Bull, 1995; 43:641-8.
22. Sultana S, Ahmed S, Sharma S, Jahangir T. *Emblica officinalis* reverses thioacetamide-inducedoxidation stress and early promotional events of primary hepatocarcinogenesis. J Pharmacol, 2004; 56(12):1573-6.
23. Bhatia AL, Gupta ML, Singh RP. Response of mice liver to continuous β-irradiation from tritiated water. J Radiat Res, 1978; 19:197-204.
24. Lewin K, Mills RR. Human radiation hepatitis. A morphologic study with emphasis on the late changes. Arch Pathol, 1973; 96**:** 21-6.

25. Fajardo LF, Berthrong M. Radiation injury in surgical pathlogy (Part-I), Am. J Surg Pathol, 1978; 2:159-99.
26. Chakrawarti A, Purohit RK, Agarwal M, Joshi P, Basu A, Bhartiya KM, Bhardwaj A. Mudulation of radiation and cadmium induced biochemical changes in mouse kidney by *Emblica officinalis* Linn. Iran J Radiat Res, 2010; 8(1): 3-10.
27. Lowry OH, Rosenbrough NJ, Ferr AL, Randell RJ. Protein measurement with the folin phenol reasent. J Biol Chem, 1951; 194:265-75.
28. Montgomery R. Determination of glycogen. Arch Biochem Biophys, 1957; 67(2):378-86.
29. Oser BL. Hawk's physiological chemistry, New York; McGraw-Hill, 1965; 246.
30. Fiske CH, Subbarow Y. The colorimetric determination of phosphates. J Biol chem, 1925; 66: 375-400.
31. Thevenod F. Cadmium and cellular signaling cascades: To be or not to be? Toxicol Appl Pharmacol, 2009; 238:221–39.
32. Hart BA, Potts RJ, Watkin RD. Cadmium adaptation in the lung—A double-edged sword? Toxicology, 2001; 160: 65–70.
33. Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ. Cadmium stress: An oxidative challenge. Biometals, 2010; 23: 927-40.
34. Tawfik SS, Abbady MI, Azab KhSh, Zahran AM. Anticlastogenic and haemodynamic efficacy of flavonoid mixture challenging the oxidative stress induced by gamma rays in male mice. Egypt J Rad Sci Applic, 2006; 19: 195-210.
35. Bhatia AL, Jain MJ. *Amaranthus paniculatus* (Linn.) improves learning after radiation stress. Ethnopharmacol, 2003; 85(1):73-9.
36. Kitazuru ER, Moreira AVB, Mancini-Filho J, Delincee H, Villavicencio ALCH. Effects of irradiation on natural antioxidants of cinnamon (*cinnamomum zeylanicum* N.). *Radiat Phys Chem* 2004, 71**:**39-41.
37. Nwokocha CR, Nwokocha M, Mounmbegna P, Orhue J, Onyezuligbo O, Olu-Osifo EH, Okojie E, Asuquo E, Ejimofor T, Ikenna N, Judith M. Proteins and liver function changes in rats following cumulative total body irradiations. West Indian Med J, 2012; 61(8):773-7.
38. Mukerjee H, Goldfeder A. Protein biosynthesis in the liver of x-irradiated mice. Int J Radiat Biol, 1974; 25: 445.
39. Hidvegi EJ, Bolonic E, Holland J, Lonal P, Antoni F, Verteresz V. The effect of whole body x-irradiation of guinea pigs on liver ribosomes. Biochem J, 1968; 109(4): 495-505.

40. Grant GA. Mechanism of action of aminothiol radioprotectors. In "Radiation Damage and Sulphydryl Compounds". IAEA, Vienna, 1969; 95.
41. Kay RE, Entenman C. The conversion of glycine –1-C14 to protein C14 and 14Co2 in liver slices from x-irradiated rats. Arch Biochem Biophys, 1956; 62:419.
42. Hansen L. The increase in liver glycogen in non-fasted rats after irradiation. A comparison with pair fed animals. Int J Radiat Biol, 1967; 12:367-72.
43. North N, Nims IF. Time dose study of biochemical response of rats to x-irradiation. Fed Proc, 1949; 8:119.
44. Shastri D, Kumar M**,** Kumar A. Modulation of lead toxicity by *Spirulina fusiformis.* Phytother Res, 1999; 13(3):258-60.
45. Dhane RR, Patil GP. Blood glucose and hepatic glycogen interrelationship in *Channa punctatus* (Bloch), exposed to heavymetal pollutants. Proc. 84tj session. Ind Sci Cong, 1977; 77.
46. Gould RG, Lotz LV, Lilly EM. Effect of x-irradiation on hepatic cholesterol synthesis. Fed Proc, 1956; 15: 264.
47. Wassermann M, Wassermann D, Aronovaski I. The effects of organochlorine insecticides on serum cholesterol level in people occupationally exposed. Bull Environ Contam. Toxicology, 1970; 5:368-72.
48. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. (1999): Antioxidant activity of active tannoid principles of *Emblica officinalis* (Amla). Ind J Exptl Biol, 1999; 37:676-80.
49. Sharma A, Goyal PK. Radioprotective capacity of *Emblica officinalis* against radiation induced biochemical alterations in liver of Swiss albino mice. J cell Tissue Res, 2005; 51:341-4.
50. Rene AA, Dorden JH, Parker JL. Radiation induced ultra-structural and biochemical changes in lysosomes. Lab Invest, 1971; 25: 230-3.
51. Bhartiya HC, Khan AS. Modification of acid phosphatase activity in the liver of Swiss albino mice by 2-mercaptopropionylglycine against gamma irradiation. Radiobiologia Radiotherapia, 1983; 24(2):235-8.
52. Wilson R, Doell BH, Groger W, Hope J, Gellatey JB. The physiology of liver enlargement. In "Metallic aspects of food safty." Pub-black well scientific oxford.1970.
53. Ramadan LA, Roushdy HM, Abu Senna GM, Amin NE, El-Deshw OA. (2002): "Radio protective effect of silymarin against radiation induced hepatotoxicity". Pharmacol Res, 2002; 45(6): 447-54.

54. Shastry KV, Shukla V. Acute and chronic toxicity of some heavy metals to Heteropneustes fossils. Threat Hab, 1988; 321-34.
55. Zhang Y, Abe T, Yang C, Kouno I. Phyllanemblinins A-F, New ellagitannins from *Phyllanthus emblica.* J Nat Prod, 2001; 64: 1527-32.
56. Banu SM, Selvendiran K, Singh JP, Sakthisekaran D. Protective effect of *Emblica officinalis* ethanolic extract against 7, 12 dimethylbenz(a) anthracene (DMBA) induced genotoxicity in Swiss albino mice. Hum Exp Toxicol, 2004; 23: 527-31.
57. Jeena KJ, Kuttan R. Antioxidant activity of *Emblica officinalis.* J Clin Biochem Nutr, 1995; 19: 63-70.
58. Hari kumar KB, Sabu MC, Lima PS, Kuttan R. Modulation of haematopoetic system and antioxidant enzymes by *Emblica officinalis* Gaertn and its Protective role against γ-radiation induced damages in mice. J Radiat Res, 2004; 45:549-55.