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Modulation of radiation and cadmium induced biochemical changes in mouse kidney by *Emblica officinalis* Linn

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Background: Protective effect of *Emblica* against radiation and cadmium induced biochemical changes in mouse kidney has been studied. ***Materials and Methods:*** Adult male mice were divided into seven groups: I (sham-irradiated), II (cadmium chloride), III (irradiated with 2 Gy gamma rays), IV (radiation and cadmium chloride), V (Cadmium chloride and *Emblica*), VI (radiation and *Emblica*), VII (radiation, cadmium chloride and *Emblica*). The animals were autopsied after 1-28 days of treatment. The kidney was taken out and different biochemical parameters such as total proteins, glycogen, cholesterol, acid phosphatase activity, alkaline phosphatase activity, DNA and RNA were estimated. ***Results:*** The value of glycogen, RNA, acid phosphatase and alkaline phosphatase activity increased up to day-14 in non drug treated groups and day-7 in the *Emblica* treated groups and thereafter decreased up to the last autopsy interval. The value of cholesterol and DNA decreased up to day-14 in non drug treated groups and day 7 in the drug treated groups then increased in all the groups. In groups III, IV, VI and VII the value of total proteins increased during early intervals and decreased thereafter, but the animals of groups II and V, which were given only cadmium chloride with or without *Emblica*, showed an opposite trend. The biochemical parameters showed highly significant values ($p<0.001$) as compared to normal ones. ***Conclusion:*** Results indicated that combined treatment of radiation and cadmium chloride exerts synergistic effect. The drug treated animals showed less severe biochemical changes and an early and fast recovery, which may be due to protection provided by *Emblica*. **Iran. J. Radiat. Res., 2010; 8 (1): 3-10**

Keywords: *Radiation, cadmium, Emblica, kidney, mice.*

INTRODUCTION

Radiation and radioisotope based tools and technologies are increasingly contributing to progress in the quality of human health, agriculture and development in industries and have provided powerful methods in research. Therefore, extensive radiobiological research is needed in generating new basic knowledge and understanding, in setting standards for safety, in establishing protocols for regulation and in providing guidance for effective protection of public health.

Apart from ionizing radiation human beings are continuously exposed to a wide range of metallic pollutants from the environment. Many chemicals are released into the environment by mining, smelting, discharging industrial agricultural and domestic waste, burning fossil fuel and using pesticides. Cadmium is reported as one of the most toxic elements in the environment and its rapid uptake and accumulation by food chain crops contribute to its being a potential environmental hazard [1].

The wide variety of tissues constituting the kidney together with its importance and accessibility has made it a favourite site of study among the radiobiologists. Amongst numerous problems pertaining to the biological effects of ionizing radiation, which have been carefully investigated in recent years in many countries, radiation injury to the kidney occupies a special place, for it is adapted to filtering wastes from the blood. Proper knowledge of the response of kidney to ionizing radiation appears as a problem of great clinical and biological importance. Perhaps in no field of radiation biology are opinions as greatly divergent as in considering the kidney to be radio-resistant.

Kidney is resistant anatomically but probably most radiosensitive physiologically

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from standpoint of serious or fatal damage. Kidney is a moderately sensitive organ. The characteristic radiation response of kidney is acute radiation nephritis which appears 6 months to one year after completion of radiation therapy. The major complaints are swelling of legs, shortness of breath, head-ache and vomiting.

The inhibition of kidney uptake of radiolabelled somatostatin analogue: amino acid or gelufusine has been studied. Gelofusine significantly inhibited kidney uptake of octreotide to a level comparable to the level of inhibition by currently applied amino acid solutions. It was reported that amino acid infusion for kidney protection may have several side effect such as vomiting and potentially fatal hyperkalamia [2].

Modification of radiation and cadmium chloride induced response is obtained by means of chemical substances that can significantly decrease the magnitude of response when present in biological system. This type of modification is called as chemical protection and the substances responsible for it are termed chemical protectors.

A large number of compounds have been investigated for protective action by different workers, but these protectors are highly toxic at their effective dose level. *Emblica* is found to be a good herbal radio protector and at the same time non-toxic, inexpensive and easily available.

MATERIALS AND METHODS

Animals

Six to eight weeks old healthy male Swiss albino mice were procured from CCS Agricultural University, Hissar and maintained at 20-25^0 Celsius .The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum.*

Source of radiation

A cobalt-60 gamma radiotherapy source Theratron of AECL makes Canada was used to irradiate the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan), India. The animals were irradiated at the dose rate of 0.96 Gy/ min.

Cadmium chloride treatment

The aqueous solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, giving a concentration of 20ppm and given as drinking water.

Emblica

Emblica officinalis Linn. Juice was procured from Vritika herbotech, Jaipur (India). The drug was fed orally at the dose rate of 0.01 ml/animal/day. The drug was given from seven days prior to cadmium chloride treatment or irradiation and continued up to the last autopsy interval.

Plan of experimentation

The animals were divided into the following groups:

Group-I Sham-irradiated animals
Group-II Only cadmium chloride treated animals
Group-III 2.0 Gy of gamma irradiated animals
Group-IV Cadmium chloride + 2.0 Gy of gamma radiation
Group-V Cadmium chloride + *Emblica*
Group-VI 2.0 Gy of gamma radiation + *Emblica*
Group-VII Cadmium Chloride + 2.0 Gy gamma radiation+ *Emblica*

Autopsy of animals

Five animals from each group were autopsied after 1, 2, 4, 7, 14 and 28 days of treatment. The animals were sacrificed by cervical dislocation. Immediately after autopsy, the kidney was taken out and it was blotted and weighed on electrical monopan balance. The kidney was kept at -20° Celsius for various biochemical

estimations viz., total proteins, glycogen, cholesterol, acid and alkaline phosphatase activities, DNA and RNA [3-7].

RESULTS AND DISCUSSION

In groups III and IV the animals of which were irradiated with or without cadmium chloride, the value of total proteins content of the mouse kidney showed an increasing trend up to day-14, but only up to day-7 in *Emblica* treated groups VI and VII, thereafter the content decreased up to day-28. On the contrary, the animals of groups V and II, which were given only cadmium chloride with and without *Emblica* the value of total proteins declined (figure 1). These observations indicated that the amount of total proteins is adversely affected by the cadmium. The changes were less severe in the *Emblica* treated groups showing protection provided by drug. A significant increase in the number of ribosome may occur due to their increased mobilisation from ER and this leads to the increased protein synthesis [8].

An increase in the glycogen content was noted on days 1 and 2 which continued up to day-14 in non drug treated groups and day-7 in the *Emblica* treated groups. Thereafter the value declined up to the last autopsy interval i.e. day-28. After combined treatment synergistic changes were seen. An earlier and faster recovery was noted in *Emblica* treated groups showing protection provided by the drug (figure 2). An increase in the value of liver glycogen in rats with 450 R exposures for the first 6-days was observed which might be due to gluconeogenesis [9, 10].

Decrease in the value of cholesterol was observed from day-1 up to day-14 in non drug treated groups II, III and IV and day-7 in *Emblica* treated groups in which almost normal value was observed on day-28. An earlier and faster recovery showed protection by *Emblica* (figure 3). Decrease in the cholesterol level after irradiation has also been reported by many workers [11-14].

An increase in the value of acid phosphatase activity was observed up to day -14 in the non drug treated groups II, III and IV and day-7 in the *Emblica* administered groups then the value decreased on day-28

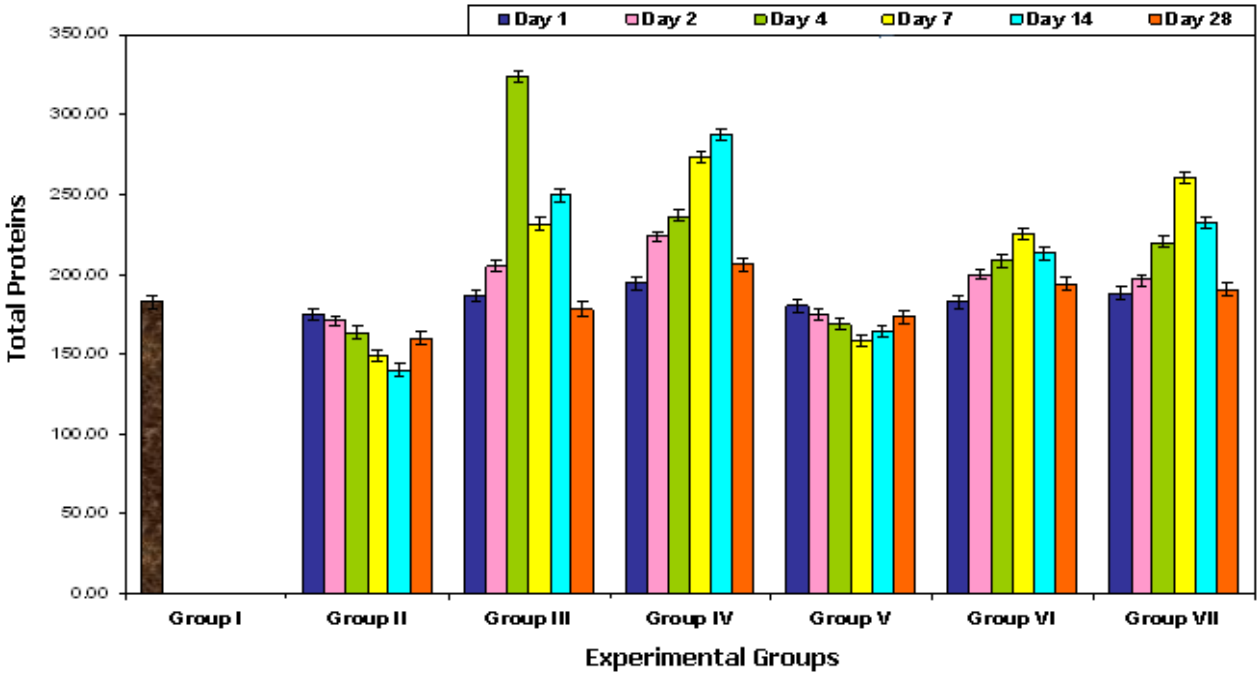

Figure 1. Variations in the total proteins content in kidney of mice in various groups (mg/gm of tissue weight).

significantly (P<0.001). A less prominent increase was noted in the *Emblica* treated animals as compared to non drug treated animals showing protection by *Emblica* (figure 4). The increase was found severe after combined treatment of radiation and cadmium chloride showing synergistic effect. The increased acid phosphatase activity seems to be characteristics of tissue damage by radiation [15]. Lysosomal hydrolases are thought to contribute to the degradation of damaged cells, hence facilitate their replacement with normal tissue. The cellular damage might cause rupture of lysosomes and hence acid phosphatase activity increases due to heavy metal toxicity and irradiation.

In the present study an increase in the value of alkaline phosphatase activity was observed till day-14 in the non drug treated

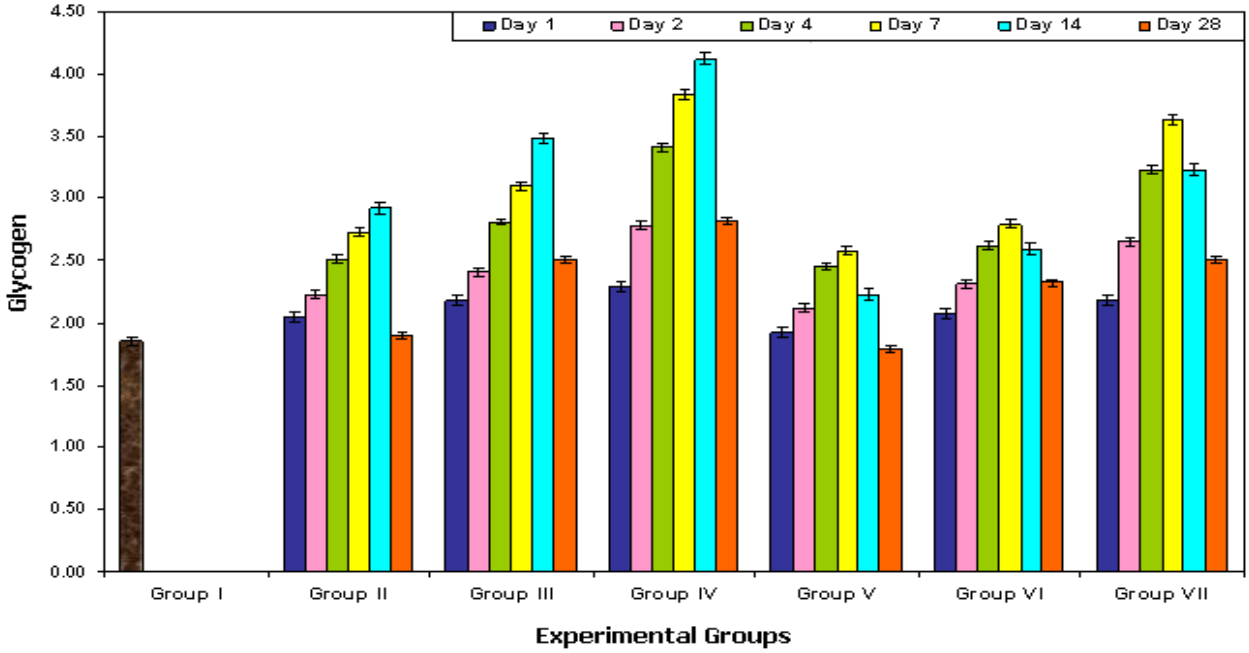

Figure 2. Variations in the glycogen content in kidney of mice in various groups (mg/gm of tissue weight).

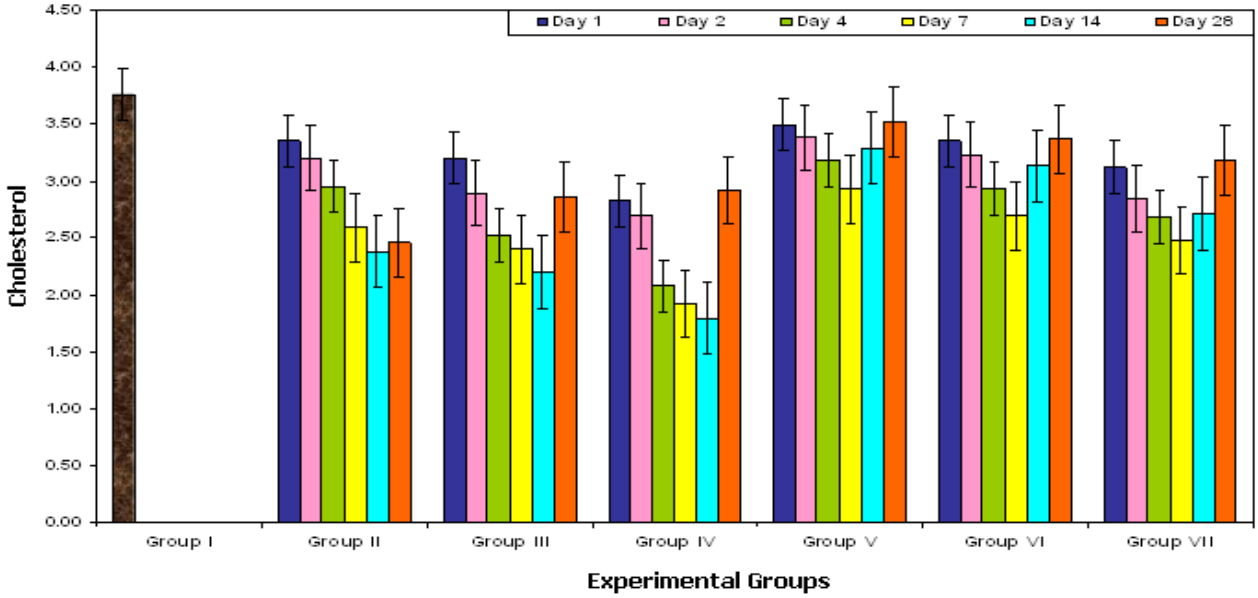

Figure 3. Variations in the total cholesterol content in kidney of mice in various groups (mg /gm of tissue wt.).

groups and day-7 in *Emblica* treated groups and thereafter it decreased up to day-28 in all the groups. After combined treatment changes were more severe showing synergistic effect, whereas a less prominent increase was observed in *Emblica* treated groups (figure 5). The efficacy of *Emblica* in modifying acute cytotoxicity of cadmium in male rats has also been evaluated. The results suggested cytoprotective potential of *Emblica* fruit in acute cadmium toxicity which could be due to its multiple roles in biological system [16].

Decrease in the DNA content was noted up to day-14 in non *Emblica* treatment groups and day-7 in *Emblica* treated groups

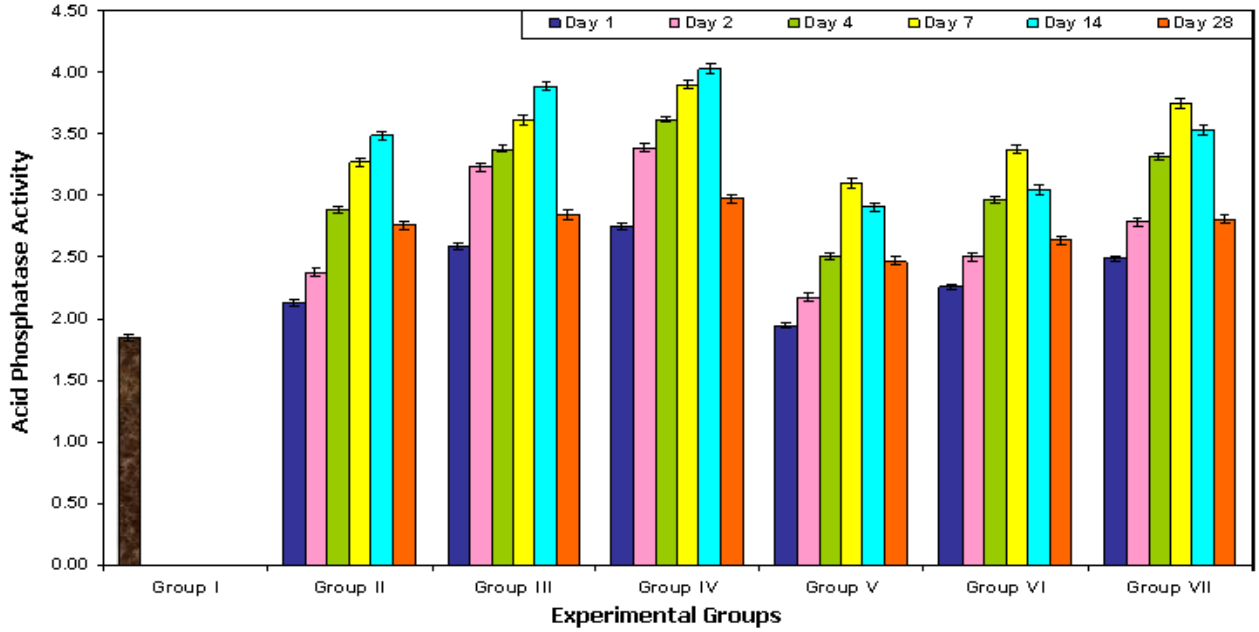

Figure 4. Variations in the Acid Phosphatase Activity in kidney of mice in various groups (mg pi/gm/hr.).

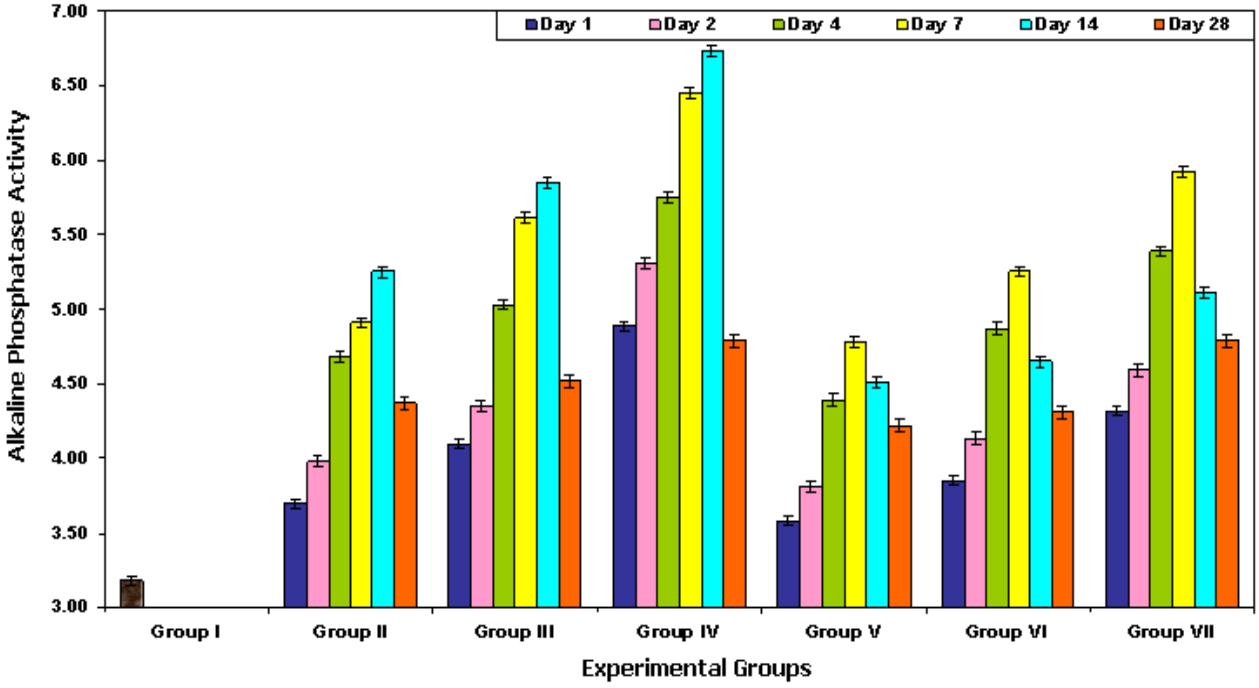

Figure 5. Variations in the Alkaline Phosphatase Activity in kidney of mice in various groups (mg pi/gm/hr.).

and then an increase was observed on day-28 without reaching the normal value (figure 6). After combined treatment synergistic changes were observed. Depletion in the DNA content of a tissue *in vivo* due to reduction in or absence of the essential factors controlling DNA synthesis was also reported [17]. These factors are the substrate (Four Deoxyribonucleoside tri phosphates), enzymes (Polymerase), and template activity of deoxyribo nucleoproteins activators (Mg^{++} and other divalent ions).

The concentration of RNA increased on day-1 and continued so significantly ($P<0.001$) up to day -14 in non drug treatment group and day-7 in *Emblica* treatment groups thereafter, it declined on day-28 (figure 7). This increase in the cellular RNA may be due to:

1. Ability of DNA to transcribe RNA is not affected quantitatively [18-20] but the length of the chain of RNA molecules reduces [21].
2. Increase in the nuclear RNA polymerase activity may contribute to the post-

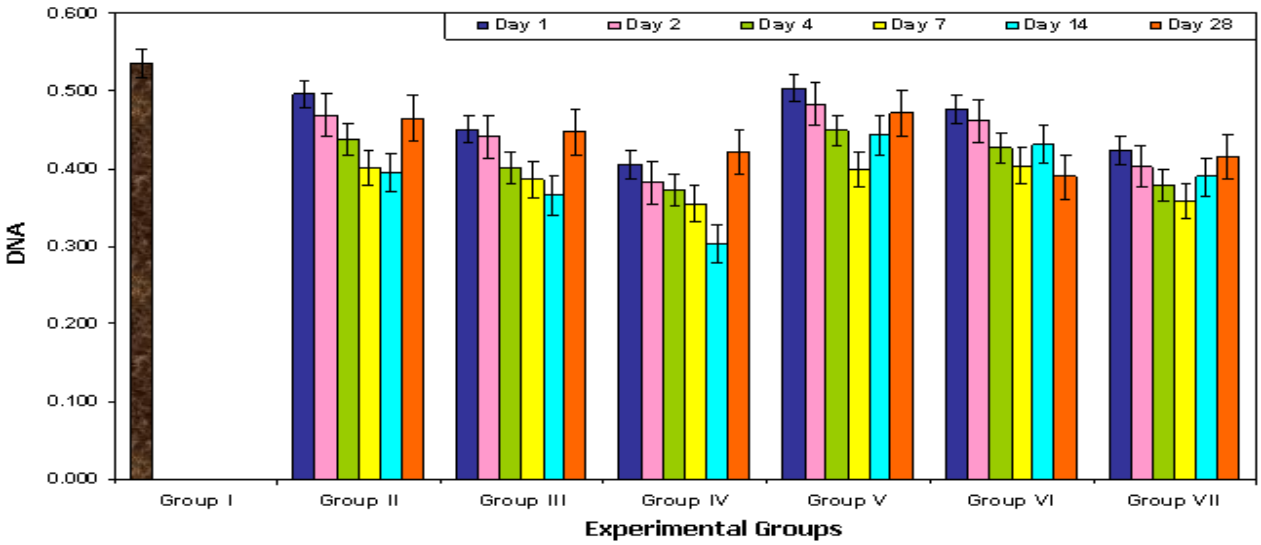

Figure 6. Variations in the DNA content in kidney of mice in various groups (mg/gm of tissue weight).

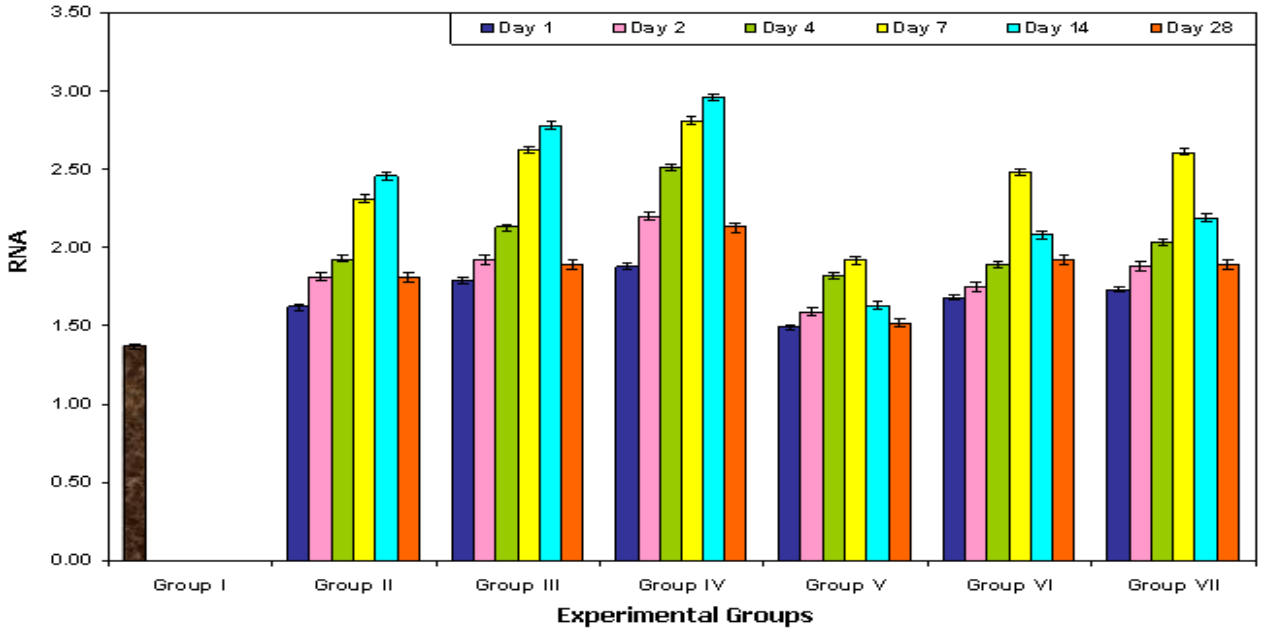

Figure 7. Variations in the RNA content in kidney of mice in various groups (mg/gm of tissue weight).

irraadiation increase in the cellular RNA [22]. 3. After irradiation with higher doses secreation of gonadotropin is increased [23]. This increased gonadotropin secreation may accelerate the RNA synthesis [24].

Biochemical estimations of various parameters in the animals indicated that in *Emblica* treated groups the values were nearer their normals than those in non-*Emblica* treated ones. In addition to it, recovery in *Emblica* treated animals started earlier, i.e., on day-14 as against day 28 in non-drug treated animals. Thus it shows that cadmium and radiation produced toxic effect on kidney and *Emblica* juice reduces these toxic effects [25-27].

Presence of variety of polyphenols is reported in *Emblica*. These polyphenols are excellent scavengers of oxygen radicals produced in the body by radiation, thus affording protection to the body. It can be hypothesized that antioxidant activity, potent stimulation of haemopoietic system, non toxicity as well as the easy availability of *Emblica* make it as an excellent choice for further development as a natural radio protector.

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REFERENCES

1. Thompson, CR and Taylor OC (1969) Effects of air pollutants on growth, leaf drop, fruit drop, and yield of citrus trees. *Environ Sci Technol,* ***3:*** *934.*
2. Rolleman EJ, de jong M, Valkema R, Kwekkboom D, kam B, Krenning EP (2006) Inhibition of kidney uptake of radiolabelled Sometostatin Analogs: Amino acids or Gelofusine. *J N u Med,* ***47:*** *1730-1731.*
3. Lowry OH , Rosenbrough MJ , Ferr AL, Rendell Rj (1951) Protein measurement with the folin phenol reagent. *J Biol Chem,* ***194:*** *265-275.*
4. Montgomery R (1957) Determination of glycogen. *Arch Biochem Biophys,* ***67:*** *378-381.*
5. Oser BL (1965) Hawk's physiological chemistry. Mc Graw Hill, New York, USA, 246.
6. Burton K (1963) Reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J,* ***62:*** *315.*
7. Fiske CH and Subbarow Y (1925) The colorimetric estimation of phosphates. *J Biol Chem,* ***66:*** *375-400.*
8. Mukerjee H and Goldfeder A (1974) Release of ribosomes from endoplasmic reticulum (ER) or X- irradiated livers. *Radiat Res,* ***58:*** *253-261.*
9. Ravdin IS, Varts HM, Goldschmidt S, Lingensmith LF (1968) *J Pharmacol Exptl Ther,* ***64:*** *111.*
10. Hansen L (1967) The increase in the liver glycogen in non fasted rats after irradiation. A comparison with pair fed animals. *Int J Radiat Biol,* ***12:*** *367-372.*
11. North N and Nims IF (1949) Time dose study of biochemical response of rats to X-irradiation. *Fed Proc,* ***8:*** *119.*
12. Gould RG and Cook RP (1958) Cholesterol metabolism in liver, In" Cholesterol" Cook RP (ed). *Acad Press, Inc N Y* ***pp:*** *145.*
13. Khan AS (1980) Radioprotective effect of 2-mercaptopropionylglycinc (MPG) on liver of swiss albino mice. Ph.D. Thesis university of Rajasthan, Jaipur. (India).
14. Purohit RK, Chakrawarti A, Bhartiya KM (2007) Radiation and cadmium induced biochemical alterations in mouse kidney. *Iran J Radiat Res,* ***5:*** *125-130.*
15. Kumari S (2007) A study on chemoprotection of liv. 52 against cadmium and radiation induced changes in the kidney of swiss albino mice. Ph.D thesis, MD S university, Ajmer. India.
16. Khandelwal S, Shukla LJ, Shanker R (2002) Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat. *Indian J Exp Biol,* ***40:*** *564-70.*
17. Altmann KI, Gerber GB, Okada S (1970) In " Radiation Biochemistry", Vol. I. Acad. Press, NY., pp187.
18. Hagen U, Ulrich M, Jung H (1969) Transcription on irradiated DNA. *Int J Radiat Biol,* ***16:****597-601.*
19. Leon SA, Kollmann G, Shapiro B (1973) Properties of DNA irradiated in the presence of protective agents bis (2-guanidiethyl) disulfide (GED). *Int J Radiat Biol Relat Stud Phys Chem Med,* ***23:****325-332.*
20. Leadon SA and Ward JF (1981) The effect of y-irradiated DNA on the activity of DNA polymerase. *Radiat Res,* ***86:*** *445-458.*
21. Mee LK, Weiss JJ, Wheelar CM (1973) Inhancement of tempelate activity of calf thymus and rat liver chromatin by γ-irradiation. *Radiat Res,* ***54:*** *539-548.*
22. Vandergoten R and Gouteir R (1966) Effect of total body X-irradiation and of chemical protector AET administration on the activity on nuclear RNA polymerase in regenerating rat liver. *Int J Radiat Biol Relat Stud Phys Chem Med,* ***11:*** *449-454.*
23. Ellis LC (1970) Radiation effects, In the testis, Vol. III, AD Johnson, WR Gomes and NL VanDemark (Eds), Academic Press, New York, p. 333.
24. Mann T (1964) Biochemistry of the semen and of the male reproductive tract. John Willey and Sons INC, London.
25. Jagetia GC (2007) Radioprotective potential of plants

and herbs against the effects of ionizing radiation. *J Clin Biochem Nutr,* ***40:*** *74-81.*

26. Singh I (2008) Modulation of radiation induced alterations by *Emblica officinalis* fruit extract in mice. *Indian J Rad Res,* ***5:*** *146.*

27. Verma R and Chakraborty D (2008) Alteration in DNA, RNA and protein contents in liver and kidney of mice treated with ochratoxin and their amelioration by *Emblica officinalis* aqueous extract. *Acta Pol Pharm,* ***65:*** *3-9.*