P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-3* ISSUE-12* (Part-2) March- 2019
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Modification of Serum Phosphatases Activity against Whole-Body Irradiation in Mice by *Embilica Officinalis* Fruit Extract

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Abstract

The protective effect of aquous extract of *Embilica officinalis* on serum phosphatases was studied in Swiss albino mice exposed to 5.0 Gy of ɣ-irradiation.The values of acid phosphatase activities were significantly higher as compared to normal in untreated irradiated group throughout the experiment; whereas, irradiated animals pretreated with EO showed significant decline in acid phosphatase activity as compared to untreated irradiated animals at all the autopsy intervals between 12 hrs. to 30 days, and normal level was regained at day 5. A marked decrease in serum alkaline phosphatase activity was recorded in both the groups; however, in EO pretreated irradiated group values of alkaline phosphatase activity remained significantly higher than untreted irradiated animals at all the intervals and attained normal value from day 3 onwards.

Keywords: Gamma Radiation; Acid Phosphatase; Alkaline Phosphatase; Swiss Albino Mice; *Emblica Officinalis*.

Introduction

Radiation induced hematological alterations have been studied extensively (Block, 1976; Kumar and Uma Devi, 1983; Misurova *et al.,* 1992; Malhotra, 1995; Verma, 2000). Hematopoietic tissues are among the tissues most sensitive to ionizing radiation in living beings. After whole-body exposure, manifestations of injury to mammalian tissues are well reflected in peripheral blood (Rugh and Somogyi, 1968; Shaheen and Hassan, 1991; Farooqui and Kesavan, 1992; Daga *et al.,* 1995; Samarth *et al.,* 2001). The major effects of whole-body irradiation are more obvious in the number than in morphology of circulating cells (Block, 1976; Daga *et al.,* 1995).

Extensive research has been and are being carried out on finding a suitable chemical radioprotective agent which can be administered safely before radiation exposure. Though, several synthatic compounds such as AET (Arient *et al.,* 1970), 2-mercaptopropionylglycine (Sugahara *et al.,* 1970; Ayene *et al.,* 1988), hydroxylamine 5-HTP (Srivastava, 1971), WR-2721 (Yushas *et al.,* 1980), lipoic acid (Ramakrishnan *et al.,* 1992), deoxyspergualin (Nemato *et al.,* 1995), dipyridamole and adenine monophosphate (Pospisil *et al.,* 1995), thiols and ascorbates (Svoboda and Harms-Ringhal, 1999) and vitamin E (Goyal *et al.,* 1999) have shown very promising results when tested in the laboratories, but these have not been successfully applied in the field of clinical radiotherapy, owing to their inherent toxicity at the optimum dose level.

Recently increased interest has developed on search for pontential drugs of plant origin that are capable of modifying immune response (Praveen Kumar *et al.,* 1999) and radiations responses with minimum side effects. Some plant extracts like ginseng (Pande *et al.,* 1988), garlic (Gupta, 1988), ocimum (Uma Devi *et al.,* 1999) as well as herbal preparations such as rasayanas (Ramakrishnan *et al.,* 1992; Praveen Kumar *et al.,* 1999), Liv.52 (Daga *et al.,* 1995), triphala (Jagetia *et al.,* 2002), abana (Jagetia *et al.,* 2003) and septilin (Jagetia *et al.,* 2004) etc. reduced radiation induced damage in different body systems. Plant products appear to have an advantage over synthetic compounds in terms of low/no toxicity at their effective dose level.

Emblica afficinalis Geartn., commanly known as amla, is a member of a small genus *Emblica* (Family Euphorbiaceae) extensively

found all over India, as well as Srilanka, Malaya, China, Pakistan and Bangladesh. The fruits of the plant have been used in Ayurveda as a potent rasayana (Satyavati *et al.,* 1976; Sharma, 1978). Experimental studies conducted with extract of the fruits of *E. afficinalis* indicate that it has significant cytoprotective effect against isoprenaline -induced myocardial injury, radiation induced chromosomal damage and heavy metal induced hepatoxicity and nephrotoxicity (Bhattacharya *et al.,* 1999). As far as authers are aware no study has been appeared so far on the role of this plant extract against radiation induced haematological alterations. Therefore, based on the above properties and significance, the present study have been undertaken to investigate the radiomodifying effect of *Emblica afficinalis* fruit extract on serum phosphatases activity against a sub - lethal dose of gamma radiation in Swiss albino mice.

Aim of the Study

To developed potential drug of plant origin (*Emblica officinalis*) that is capable of modifying immune responses and radiation responses with minimum side effects.

Materials and Methods

Animals

Young-adult male Swiss albino mice (6-8 weeks old), weighing 25□2 g, from an inbred colony were used for the present study. These 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.

Irradiation

The Cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Centre, Radiotherapy Department, S.M.S. Medical College and Hospital, Jaipur was used for irradiation. Unanaesthetized mice were restrained in a well- ventilated perspex box and whole-body exposed to 5.0 Gy gamma radiation at the dose-rate of 0.88 Gy/min. from the source.

Emlica Extract Preparation

Emblica officinalis Linn. was identified (No. RUBL 1988) by a competant 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 hr ×3). The extract thus obtained was vacuum evaporated so as to make it in powder 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.

Dose Selection

The dose selection of *Emblica officinalis* (EOE) extract was done on the basis of our previously conducted animal survival study (Singh *et al.,* 2005). Various doses of EOE (50, 100, 200, 400 and 800 mg/kg b.wt) were tested against gamma irradiation (9.0 Gy). Optimum dose (100 mg/kg b.wt./day/animal) thus obtained was used further for the detailed experimentation.

Experimental Design

The animals selected from an inbred colony for the present study were divided into the following three groups. *Group-I (Emblica treated unirradiated)*: The animals of this group were fed orally *Emblica afficinalis* (100 mg/kg b.wt/day) in double distilled water (DDW) for seven consecutive days (only once in a day). *Group -II (Untreated irradiated)*: These animals were given DDW orally (volume equal of EOE) for seven consecutive days. Half an hour after last administration on 7th day, mice were exposed to 5.0 Gy gamma radiation. This group served as a control. *Group-III (Emblica trreated irradiated)* Mice belonging to this group also recieved EOE as in Group-I. After half an hour of administration, on the last day 7th, these animals were also exposed to 5.0 Gy gamma radiation (as in Group-II) to serve as experimental.

The animals from the above groups were autopsied at 12 hrs., 24 hrs., 3 days, 5 days, 10 days, 20 days and 30 days post -irradiation. Blood of these animals was collected from orbital sinus using haematocrit capillaries, and serum was separated out. The serum activity of acid phosphatase (ACP) and alkaline phosphates (ALP) was assayed by using commercially available kits.

Statistical Analysis

The results obtained from the present study were expressed as mean ± SE and the Student's t-test was used to make a statistical comparison between the groups.

Results

The animals receiving *Emblica officinalis* extract alone (Grpup-I) did not show any significant change in serum acid phosphatase as well as alkaline phosphatase activity and their values were found near normal (Table-1). In the control (Group-II), a significant increase in serum acid phosphatase with respect to Group-I was noticed. A considerable elevation in the value was evident at 12 hrs. ($p<0.001$) reaching the highest at day 3rd (6.5103±0.14 KAU). However, the level started to decrease subsequently from day 5 (5.1461±0.02) but could not restore the normal value until day 30 (2.9854±0.05).

In the Group-III (EOE pretreated irradiated), a significant decrease in serum acid phosphatase activity was observed throughout experimentation as compared with the control (Group-II) and the level depleted to normal on day 5th but increased further on successive intervals and returned towards normal at the end of experimentation (Fig.-1).

A marked decrease in serum alkaline phosphatase activity from normal was recorded at all the intervals, however, the maximum decline was noted first at 12 hrs. and later at day 5th post-irradiation. The value of such enzyme was noted to be lower than normal even at the last autopsy interval (i.e. 30th days).

In the experimental group (EOE pretreated irradiated), a significant decline towards normal in serum alkaline phosphatase activity was observed throughout experimentation as compared with the control, however, such level restored near to normal on day 3 but increased further subsequently and returned to normal only at the last autopsy interval (Fig.-2).

Discussion

The present study exhibited an increase in serum acid phosphatase activity after irradiation with gamma rays. A similar elevation in activity of acid phosphatase after radiation exposure has also been reported at sublethal doses (Shah and Gadhia, 1979; Samarth, 2001; Shekhawat, 2004). Acid phosphatase is localized in cellular lysosomes and changes in activity of lysosomal enzymes take place following whole-body irradiation. An increased Golgi complex activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and leakage of the enzymes is attributed to an increased acid phosphatase level (Wills and Wilkinson, 1966).

The release of such enzyme from lysosomes may be due to activation of pre-existing latent enzymes or due to the synthesis of new lysosomes as a consequence of radiation (Rene *et al.,* 1971). It is well known that radiation enhances the permeability of membranes for several materials and hence an increase in the serum acid phosphatase activity is seen after irradiation. In the untreated irradiated mice (Group-III), rise in acid phosphatase activity until day 3^{rd} may be attributed to the haematopoietic injury, with recovery at day 5^{th}. The acid phosphatase activity remained higher than normal. Thus, *Emblica offcinalis* extract pretreated animals showed protection against haemapoietic injury.

In the present study, serum alkaline phosphatase was found to decline after irradiation at all the intervals studied. This observation is in agreement with the findings of others (Jacob and Maini, 1994), who also reported a deterioration in serum alkaline phosphatase activity in male mice after irradiation with 5.0 Gy gamma rays. Highman and Hanks (1970) have already shown that injury to the intestinal mucosa is chiefly responsible for the decrease in circulating alkaline phosphatase following irradiation. Similarly, Lynn and Skinner (1974) observed non-exponential loses of activity in alkaline phosphatase after gamma rays exposure and suggested redical attack on phosphatase at centres of secondary importance for the enzymatic activity and there was a notable destruction of the component amino acid residue during radiolysis.

Alkaline phosphatase performs an important role in the maintenance of cell permeability and acts on monophosphoesters. The destruction to the cell membrane caused by radiation may be the reason for the lower activity of serum alkaline phosphatase. In the control mice (Group-III), the lower alkaline phosphatase level may be attributed to severe damage to the gastro-intestinal tract. The lesions affecting the villi are reflected in the form of diminished enzyme activity (Mathur and Uma Devi, 1981; Shekhawat, 2004). The post-irradiation reduction in alkaline phosphatase may be due to damage of brush border cells and increased permeability of villi cells (Baijal, 1978). A significant decrease in the alkaline phosphatase activity in heart of rats was noted, after 28 to 70 days of irradiation with a single dose of 17.5 and 20 Gy radiation (Akoeva *et al.,* 2002). Daga (1996) have also reported a declining pattern in alkaline phosphatase activity in mice after gamma irradiation.

In the present investigation, a significant decline in acid phosphatase activity and a considerable rise in the serum alkaline phosphatase activity at various periods of study in experimental animals (EOE pretreated irradiated) as compared with the respective control (EOE untreated irradiated) have been observed. The exact mechanism of *Emblica officinalis* is not clear. It has been reported that *Emblica officinalis* contains tannoid complexes as emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%) and rutin (3%) which significantly increase the concentration of the antioxidant enzymes and reduce lipid peroxidation. Furthermore, fruits of such plant are the rich source of vitamin C that is regarded as the first line natural antioxidant defence and it also regenerates the major antioxidant α-tocopherol (Vit E) in lipoproteins and cell membranes (Katiyar, 1997; Bhattacharya *et al.,* 1999). It has also been observed that *Emblica officinalis* pretreatment exhibited a significant increase in glutathione (GSH) and decrease in lipid peroxidation level in blood and liver after irradiation. The increased GSH level suggests that protection by EOE may be mediated through the modulation of cellular antioxidant levels (Singh and Goyal, 2005). *Emblica officinalis* have been found to possess antioxidant properties (Jose and Kuttan, 1995). *Phyllanthus emblica* has also been reported to possess antioxidant and free radical scavenging activities (Korina and Afanasev, 1997), which in turn is reflected in the form of declined serum acid phosphatase and increased serum acid phosphatase activities as compared with the untreated irradiated animals.

The results of the present investigation suggest that the measurement of serum phosphatases activity could be used not only as a simple biochemical diagnostic and prognostic criterion of the radiation effects in mice, but also for the evaluation of the radiomodulatory influnce of *Emblica officinalis* extract.

Acknowledgements

Authors are thankful to Prof. D.P. Agarwal (Head) and Dr. A. K. Chougule (RSO), Department of Radiotherapy, SMS Medical College and Hospital, Jaipur, for radiation facility and dosimetry respectively.

Table-1: Variations in serum phosphatases activity of mice irradiated to 5.0 Gy gamma radiation with or without *Emblica officinalis* treatment

Autopsy Interval	Group	Acid phosphatase (KAU)	Alkaline phosphatase (KAU)
12 hrs.	GR-I	2.9295±0.06[a]	7.3321±0.13[a]
	GR-II	5.0306±0.29[c]	6.4892±0.06[c]
	GR-III	3.5211±0.08[c]	6.5672±0.13
24 hrs.	GR-I	2.6253±0.03[a]	7.5489±0.89
	GR-II	5.5808±0.13[c]	6.5242±0.39[b]
	GR-III	3.6453±0.07[c]	7.7232±0.20[b]
3 days	GR-I	2.3436±0.06	7.2675±0.32
	GR-II	6.5103±0.14[c]	6.6094±0.24[b]
	GR-III	2.9882±0.05[c]	7.7019±0.14[b]
5 days	GR-I	2.5198±0.04	6.5199±0.10
	GR-II	5.1461±0.02[c]	6.6156±0.12 [c]
	GR-III	2.7129±0.07[c]	7.8292±0.33[b]
10 days	GR-I	2.5180±0.15	6.9139±0.17
	GR-II	4.0256±0.08[c]	7.4849±0.28
	GR-III	2.8952±0.10[c]	8.1256±0.30
20 days	GR-I	2.9195±0.08	8.8651±0.34
	GR-II	3.0254±0.09	7.1127±0.09[b]
	GR-III	2.8125±0.07	8.2068±0.20[c]
30 days	GR-I	2.3737±0.06	6.9565±0.26
	GR-II	2.9854±0.05	7.0992±0.26
	GR-III	2.7451±0.26	7.5124±0.11
	Normal	2.7882± 0.25	7.6436±0.17

Normal : No treatment
GR-I : Emblica treated unirradiated;
GR -II : (Control) untrerated irradiated;
GR-III : (Experimental) Emblica treated irradiated;

Statistical compairson
Normal v/s Control; Control v/s Experimental
Each valuerepresents mean ±SEM
Significance level :[a]p<0.05; [b]p<0.005; [c]p<0.001

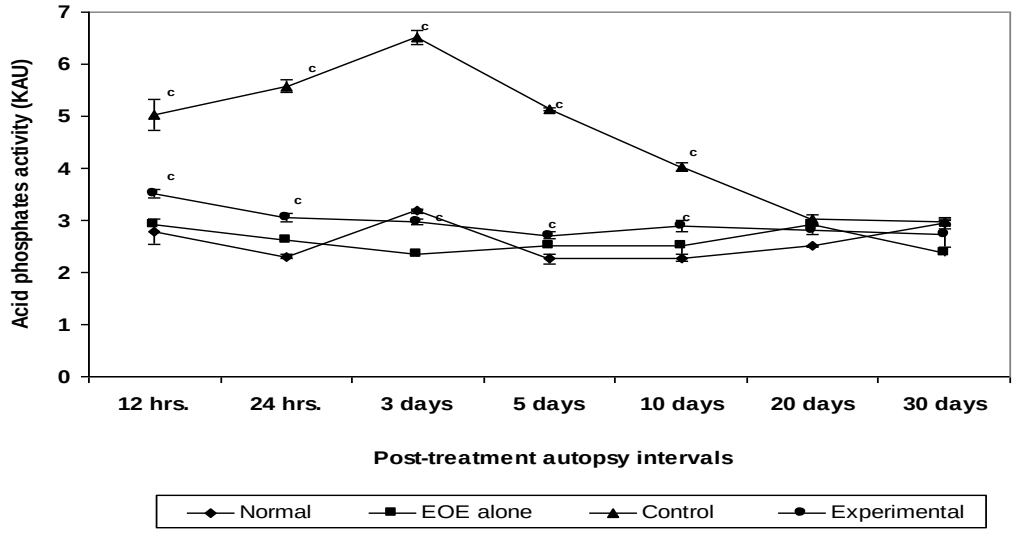

Fig. 1 - Variations in serum acid phosphatase activity of mice irradiated to 5.0 Gy gamma radiaton with (experimental) or without (control) *Emblica officinalis* treatment. The values represent mean ± S.E.. The statistic significance was obtained between Normal V/s Control and Control V/s Experimental ([c]p <0.001)

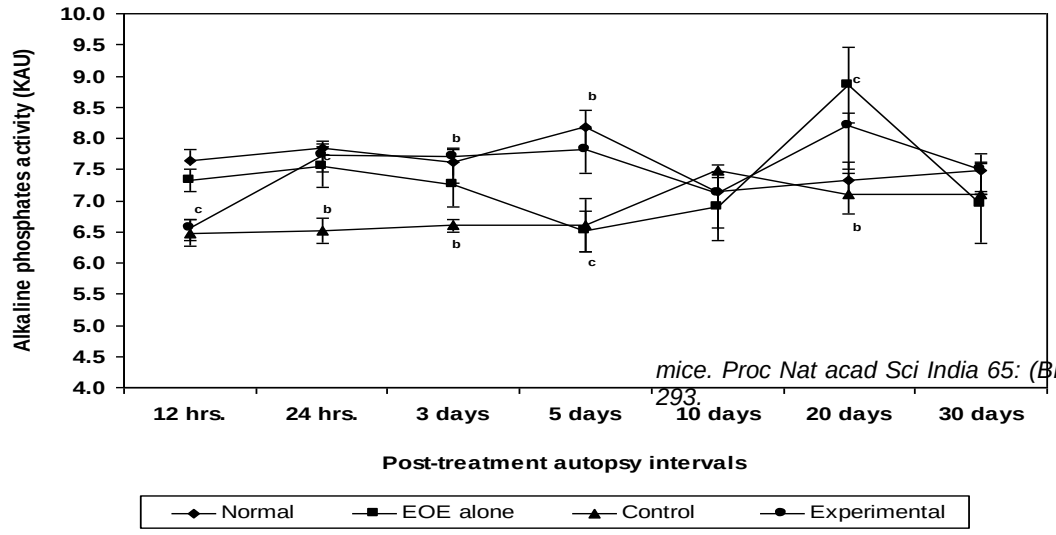

Fig. 2 - Variations in serum alkaline phosphatase activity of mice irradiated to 5.0 Gy gamma radiaton with (experimental) or without (control) *Emblica officinalis* treatment. The values represent mean ± S.E.. The statistic significance was obtained between Normal V/s Control and Control V/s Experimental ($^{b}p < 0.005$; $^{c}p < 0.001$)

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