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Evaluation of Radioprotective Effects of *Emblica Officinalis* Fruit Extract in Swiss Albino Mice

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Abstract

Recently, medicinal plant are being used for the development of herbal and natural radio-protector. *Emblica officinalis*, an Indian medicinal plant, has been reported to be clinically effective in treatment of various diseases. This information encouraged us to conduct experiments to find its possible radio modulator potential against radiation induced hematological alterations. For this purple, healthy *Swiss albino* mice were selected from on inbred colony and divided into four groups. Animals in Group-I were administered with double distilled water (DDW), to serve as vehicle control, Mice in groups-II were administered orally *Emblica officinalis* fruit extract (EOFE), Group-III animals were given DDW for 7 consecutive days than exposed to 2.5 Gy gamma radiation half an hour on 7th day after the last administration of EOFE. Group – IV mice were treated with EOE (as in Group-II), and were exposed to gamma radiation (as in Group-III). These animal were sacrificed on 12hrs., 24hrs., 3days, 5days, 10days, 20days and 30days post-treatment intervals and, their blood was collected for estimation of total leucocytes and differential leucocytes (lymphocytes, monocytes, neutrophils & non neutrophols count. Animals treated with EOFE alone (Group-II) did not show any significant change in leucocytes count in comparison to normal (Group-I). A marked decline as compared to normal in total lucocytes, monocytes, lymphocytes, neutrophils and non-nutrophilic granulocytes count was observed in irradiated Group (Group-III) at early intervals i.e. 12hrs. to day 3rd, afterwards counts started to regain but normal level could not be restored even till day 30th. A significant increase in all these types were noticed in EOFE pretreated irradiated animals during the entire period of study by returning almost normal value on period of last autopsy interval. The results of present investigation demonstrate that EOFE pretreatment protects the peripheral leucocytes against irradiation. Further investigations are in progress to study the exact mechanism of action and clinical applicability of *Emblica officinalis* extract as a radio-protector.

Keywords: *Radioprotection, Emblica Officinalis, Swiss albino mice, Peripheral leucocytes.*

Introduction

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 biomolecules is important in the field of physiology and pathology. Exposure to ionizing radiation results in a complex set of responses whose onset, nature and severity depends on both total radiation dose and radiation quality. In general, direct damage to tissues by ionizing radiation yields reactive oxygen species and these harmful effects can be reduced by administration of the radioprotectors in the body. A good chemical protector should be able to protect against the deleterious effect of ionizing radiation during therapeutic procedures as well as during nuclear accidents, space flight and cheap, background irradiation etc. An ideal radioprotector should be not have toxic implications in a wide dose range, orally administered, rapidly absorbed, possesses a reasonably good dose reduction factor and can act through multiple mechanisms. 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 (Goyal,P.K.et al.,1999, Samarth,.M.et al., 2001, Munis, V. and Goyal, P.K. 2004, Kumar, A. 2005, Satyavati,G.V. et al.,1976, Moron, M.S. 1979, Ghosal, et al .,1996, Singh stal., 2005, Walker, R.I., 1998, Jocobson,et al.,1999).

Radioprotector herbal plant are usually no toxic effect and easily can orally administered. Herbal plant are could act through multiple mechanisms due to the presence of many content. The advantage of natural plant products is that they are used in several traditional system of medicines. The radioprotectors have needs scientific evaluation. Herbal radioprotectors are beneficial more successful than synthetic chemical content. Radioprotector has antimicrobial, Immuno-modulatory, antioxidant, anti-inflammatory, freeradical scavenging and antis tress properties.

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 *(Valentine, W.N. and Pearse, M. l., 1952)*

Clinical studies suggest that the fruits of this plant have anabolic activity and exhibit significant adaptogenic, immunopotentiating and memory facilitating effects ((Allen, J.G.,1948).

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 radioprotective effect of amla (*E. officinalis*) on blood of mice exposed to gamma radiation. No systematic work has been initiated so far to reduce blood cells 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 modulation the radiation induced heamatological alterations in the blood of 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 effect.

Materials & Methods

Animals Care and Handling

Swiss albino mice adult male (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 *adlibitum.* 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 *Emblica 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. The extract thus obtained was vacuum evaporated so as to make it in dry powder form . The extract was redissolved in DDW just before oral administration. 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 (Singh *et al.,*2005). Various doses of EOE 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.

Results

Group-I (Normal/Sham-irradiated)

During the entire experimental period, the Sham-irradiated animals exhibited a consistent weight gain till day 30 by obtaining 30.85±0.55 per cent higher weight than the initial. No sign of sickness, mortality and morph metric changes were observed in this group. Erythrocytes number, total leucocyte count and differential leucocytes counts (lymphocytes, monocytes, eosinophils, basophils and neutrophils) did not show any noticeable change from 12 hrs. to 30 days after Sham-irradiation (Tables- 2, 3, 4, 5, 6 & 7).

Group -II (EOE Treatment)

Oral administration of EOE (100 mg/kg b.wt./day) for seven consecutive days to Swiss albino mice showed no significant change in body weight of these animals as compared to normal (Group-I). These animals exhibited a regular weight gain till day 30 by reaching 30.40±0.37 higher weight than the initial. No signs of sickness, morbidity and mortality were observed throughout the study (Table-1). EOE treatment did not result any significant alterations in hematological constituents of peripheral blood of these mice as total counts of RBC, WBC remained in normal range during entire period of study. Moreover, lymphocytes, monocytes, eosinophils, basophils and neutrophils percentage also did not exhibit any significant variation after EOE treatment (Table- 2, 3, 4 , 5, 6 & 7).

Group-III Control (Radiation Treatment)

Animals exposed to 2.5 Gy gamma radiation exhibited signs and symptoms of radiation sickness. These mice were lethargic and weak. Food and water consumption was also reduced, although general activities of such animals were apparently normal during all 30 days post-irradiation. After exposure, all animals survived until 30^{th} day of irradiation. A biphasic decrease in body weight, first at day 7

(21.20±.0.34) and second at day 20 (23.20±0.70), was evident. Only 24.50±0.55 weight gain from initial was recorded at day 30 against 26.57±0.66 in normal (Group-I) animals (Table-1). Animal exposed to 2.5 Gy gamma radiation represented signs and symptoms or 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 2.5 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.

Group-IV: Experimental (EOE+2.5 Gy Radiation Treatment)

Animals pretreated with EOE and exposed 2.5 Gy gamma radiation. A continuous gain in body weight was recorded soon after irradiation (12 hrs.) and it maintained upto the end of experimentation. Food or water uptake and general activities also found to be normal . All animals of this group survived till 30 days post irradiation (Table-1).

Total leucocyte count (WBC)

There was a significant decline than normal in total leucocytes count till day 3, however, no significant decrease in number of such cells was observed on day 20 and 30 post-irradiation. A maximum decline was noticed day 3 ($4.12\pm0.17\times10^3 mm^3$; $p<0.001$). A drop in total leucocyte count as compared to normal was recorded in this group till day 5 post-treatment a like control, but the values were higher than the irradiated group. After day 5, a recovery pattern was observed and a normal value ($6.18\pm0.16\times10^3/mm^3$) was restored on last autopsy interval (i.e. day 30). WBC counts were significantly higher than control from 12 hrs. to day 10th of irradiaton (Table- 2).

Differential leucocyte count (DLC)

Lymphocytes

A highly significant ($p<0.001$) increase in lymphocytes number was observed till day 3 (50.2±1.13%; $p<0.001$), however, such significant difference with normal count became narrow at later period of study (day 5 to 10). Contrarily, an appreciable decline was observed on day 30, but the lymphocyte percentage remained below normal . A similar trend was noticed in lymphocytes as in irradiated group. In experimental group, the values were higher than irradiated group and a significant protection was evident on day 12 hrs. (56.4±1.68 %; $p<0.001$), 24 hrs. (51.8±0.17 %; $p<0.005$) and 3 day (56.4±1.50 %; $p<0.005$). The lymphocytes cells number did not reach to normal even at the last autopsy interval (Table-3).

Monocytes

The normal of monocytes deceased to 50 per cent at 12 hrs. after radiation exposure and it remained significantly different from normal. Monocytes number increased till day 20 but decreased further at day 30 without returning to normal. No significant difference was observed with respect to control treatment the period of study (Table- 4).

Eosinophils

Eosinophil type of WBC decreased in their number upto 24 hrs. but later counts elevated significantly till day 5. The normal number could not be seen till the end of experiment. The eosinophils percentage decreased significantly at 12 hrs. (2.2±0.21%; $p<0.001$) than normal but the values were higher than control. The number of such cells increased at later intervals towards the normal counts (Table- 5).

Basophils

A non significant difference as compared to normal was noticed in the values of basiophils during the entire period of study. Like acdiophils, basophilic count also did not show significant variation from control. However, a increase in such cells was observed first at 24 hrs. and also at day 5 (0.8±0.21%); whereas at remaining intervals, normal counts were observed except at day 5 (0.8±0.35%) (Table-6).

Neutrophils

A highly significant ($p<0.001$) increase beyound normal in neutriphilic number was observed following irradiation. Maximum rise (48.4±1.37) was noted at 24 hrs. interval. Day 5 onwards, a recovery towards normal count was evident but normal value could not be restored until day 30th post-irradiation. The neutrophils exhibited an elevated level at 24 hrs (42.6±0.96 %) in this group and thereafter such number decreased until day 20 (36.0±2.13 %). The counts remained above normal even on day 30 (37.0±1.63 %) post-treatment (Table- 7).

Table - 1 : Variations (mean ± S.E.) in leucocytes count ($x10^3/mm^3$) in peripheral blood of mice after exposure to different doses of gamma rays with (experimental) or without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
		12 hrs.	24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	4.23 ± 0.16^c	4.41 ± 0.22^c	4.12 ± 0.17^c	4.89 ± 0.27^b	5.14 ± 0.14^b	5.26 ± 0.12^c	5.63 ± 0.77^b
	Experimental	5.59 ± 70.20^c	5.49 ± 0.14^b	5.52 ± 0.20^a	5.61 ± 0.16^b	5.63 ± 0.12^b	5.73±0.22	6.18 ± 0.16^c

Normal Value = $6.210\pm0.12 \times 10^3/mm^3$

Statistical comparison :

C (Control) = DDW+Irradiation

Control v/s Normal; Experimental v/s control

E (Experimental) = EOE + Irradiation

Significance levels -

N.S. = No survival
[a]p < 0.05, [b]p < 0.005 , [c]p < 0.001

Table - 2 : Variations (mean±S.E.) in total lymphocyte (%) in mice after exposure to different doses of gamma rays with (experimental) of without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
			24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	48.2±1.77[c]	46.8±0.21[c]	50.2±1.13[c]	55.8±1.96[c]	58.4±2.23[c]	56.4±2.26[c]	59.2±1.37[b]
	Experimental	56.4±1.68[c]	51.8±0.17[b]	56.4±1.50[b]	58.6±0.45	60.6±2.23	59.4±3.16	60.4±2.42

Normal value = 67.8±1.10%
Statistical comparison : C (Control) = DDW+Irradiation
Control v/s Normal; Experimental v/s control
E (Experimental) = EOE + Irradiation
Significance levels N.S. = No survival
[a]p < 0.05, [b]p < 0.005 , [c]p < 0.001

Table - 3 : Variations (mean±S.E.) in monocyte (%) in mice after exposure to different doses of gamma rays with (experimental) of without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
		12 hrs.	24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	1.6±0.72[c]	2.4±0.72 [c]	2.2±0.33 [c]	1.4±0.21 [c]	1.8±0.21 [c]	1.6±0.53 [c]	1.8±0.43 [c]
	Experimental	2.0±0.81	2.8±0.81	2.0±0.43	1.8±0.21	1.4±0.21	2.4±0.66	1.4±0.45

Normal value = 3.2±0.25%
Statistical comparison:
C (Control) = DDW+Irradiation
Control v/s Normal , Experimental v/s Control
E (Experimental) = EOE + Irradiation
Significance levels -
N.S. = No Survival
[a]p < 0.05, [b]p < 0.005, [c]p < 0.001

Table - 4 : Variations (mean±S.E.) in eosinophil (%) in mice after exposure to different doses of gamma rays with (experimental) of without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
		12 hrs.	24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	1.8±0.66[c]	1.6±0.21 [c]	1.8±0.17 [c]	2.4±0.45 [c]	1.4±0.21	2.0±0.52 [c]	2.0±0.40 [c]
	Experimental	2.2±0.2 [c]	2.2±0.48	1.4±0.4	2.6±0.21	2.4±0.45	1.8±0.43	2.8±0.33

Normal value = 2.8±0.28%
Statisical Comparison
C (Control) = DDW+Irradiation
Control v/s Normal; Experimental v/s Control
E (Experimental) = EOE + Irradiation
Significance levels -
N.S. = No Survival
[a]p < 0.05, [b]p < 0.005 , [c]p < 0.001

Table - 5 : Variations (mean±S.E.) in basophil (%) in mice after exposure to different doses of gamma rays with (experimental) of without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
		12 hrs.	24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	0.4±0.21	0.8±0.43	0.4±0.43	0.4±0.21	0.2±0.17	0.8±0.43	0.6±0.21
	Experimental	0.4±0.21	0.6±0.21	0.2±0.17	0.8±0.35	0.4±0.21	04±0.21	0.4±0.21

Normal value = 0.8±0.53%
Statistical Comparison :
C (Control) = DDW+Irradiation
Control v/s Normal; Experimental v/s Control
E (Experimental) = EOE + Irradiation
Significance levels -
N.S. = No Survival
[a]p < 0.05, [b]p < 0.005 , [c]p < 0.001

Table – 6 : Variations (mean±S.E.) in neuterophil (%) in mice after exposure to different doses of gamma rays with (experimental) of without (control) *Emblica officinalis* extract (EOE)

Radiation Dose (Gy)	Group	Post – treatment Autopsy Intervals						
		12 hrs.	24 hrs.	3 days	5 days	10 days	20 days	30 days
2.5	Control	47.6±0.33[c]	48.4±1.37[c]	45.0±0.89[c]	40.0±1.13[c]	37.2±1.30[c]	39.2±1.48[c]	36.4±1.23[b]
	Experimental	39.4±0.53[c]	42.6±0.96	40.0±1.75	36.2±0.17	36.2±0.17	36.0±2.13	37.0±1.63

Normal Value = 25.4±1.48%
Statistical comparison :
C (Control) = DDW+Irradiation
Control v/s Normal; Experimental v/s Control
E (Experimental) = EOE + Irradiation
Significance levels -
N.S. = No Survival
[a]p < 0.05, [b]p < 0.005 , [c]p < 0.001

Table- 7 :Body weight response of mice with or without *Emblica officinalis* treatment and/or exposed to different doses of gamma radiation.

Post-irradiation treatment	Normal	EOE treated	Body weight in grams	
			2.5 Gy	
			Control	Experimental
1	22.00±0.40	22.50±0.66	**22.33±0.40**	**22.90±0.56**
2	22.33±0.56	22.33±0.56	**22.00±0.42**	**22.00±0.50**
3	22.66±0.60	22.00±040	**22.33±0.42**	**22.33±0.60**
4	22.66±0.60	22.60±0.56	**22.56±0.37**	**22.33±0.60**
5	22.90±0.53	23.00±0.40	**21.00±0.34**	**23.20±0.43**
6	21.33±0.60	23.66±0.30	**21.40±0.40**	**23.50±0.43**
7	21.33±0.60	23.66±0.56	**21.20±0.34**	**23.50±0.43**
8	21.20±0.43	23.66±0.73	**21.20±0.34**	**24.16±0.76**
9	21.50±0.43	24.66±0.66	**21.50±0.34**	**24.50±0.43**
10	21.50±0.43	25.00±0.40	**22.20±0.56**	**24.50±0.43**
11	21.60±0.55	25.00±0.40	**22.00±0.66**	**24.50±0.43**
12	22.00±0.40	25.14±0.37	**23.42±0.56**	**25.00±0.50**
13	23.33±0.56	25.66±0.56	**24.57±0.76**	**25.00±0.50**
14	23.00±0.70	25.66±0.60	**24.28±0.76**	**25.00v0.43**
15	23.00±0.70	26.00±0.81	**24.14±0.43**	**24.50±0.70**
16	24.50±0.35	26.80±0.43	**24.85±0.43**	**25.00±0.82**
17	23.66±0.60	27.20±0.43	**26.71±0.50**	**25.50±0.50**
18	24.50±0.35	27.50±0.82	**25.60±0.50**	**26.00±0.43**
19	25.50±0.70	27.60±0.43	**25.80±0.43**	**26.28±1.11**
20	24.00±1.11	27.60±0.43	**23.20±0.70**	**27.50±0.82**
21	25.50±0.35	27.66±0.73	**24.60±0.70**	**27.50±0.55**
22	26.00±1.41	27.80±0.44	**25.80±1.41**	**27.00±0.52**
23	27.60±0.70	27.80±0.59	**25.60±1.41**	**26.66±0.55**
24	27.60±0.70	28.00±0.40	**26.00±0.70**	**26.50±0.48**
25	27.60±0.70	28.00±1.00	**26.00±0.70**	**27.00±0.66**
26	26.50±0.82	28.40±0.66	**26.00±0.70**	**27.00±0.52**
27	26.50±0.82	28.40±0.79	**25.00±0.48**	**2700±0.52**
28	26.50±0.82	29.33±0.96	**24.00±0.48**	**2751±0.66**
29	26.57±0.66	29.40±0.35	**24.00±0.55**	**28.50±0.52**
30	26.57±0.66	29.40±0.35	**24.50±0.55**	**28.50±0.52**

Control = Irradiation alone; Experimental = EOE+Irradiation
Normal = No treatment
EOE alone = 100mg/kg b.wt./day for 7 days
N.S = No survival

Discussion

The result of the present study are as follows, The pretreatment of EOE protects of the mice. The radioprotective effect of EOE is demonstrated by increased weight & survival rate. When EOE was administered orally 100 mg/kg b.wt for 7 consective days prior to irradiation, A significant radioprotection was achieved. A significant loss of

body weight was evident in control animals. EOE treated animals showed significant recovery in about 30 days post-irradiation with EOE only 12% mortality rate was observed. EOE is considered as a foremost rejuvenating drug which promotes increased protection of RBC cells. Thus the hematological constituents such as (RBC & WBC) can be significantly higher in animals irradiated whithout EOE.

In the present study there were no adverse effects, in terms of sickness or mortality, in the animals administered with different doses of *Emblica officinalis* fruit extract. Also, there was no significant change in body weight, urination and defecation pattern. After the preliminary experiment conducted with different dose of *Emblica officinalis* extract (EOE) against irradiation, it was observed that 100 mg/kg b. wt./daily for 7conscutive days of EOE was the most effective dose for radioprotection. Therefore, a detailed investigation was performed at the optimum dose of EOE (100 mg/kg b. wt.)

The animals of DDW + irradiation group (Control) exhibited sings of radiation sickness within 2-4 days after exposure to different doses of gamma radiation depending on the irradiation dose. Exposure of mice to radiation doses (2.5 Gy) resulted in an early appearance of the no signs of sickness. They mainly included reduction in the food and water intake, irritability, epilation, weight loss, emaciation, lethargy, diarrhoea and ruffling of hairs.

For this purpose, mice were treated with EOE half an hr. prior to dose of gamma radiation. The protective effect of EOE against radiolesions injury was assessed by the endogenous spleen colony assay (CFU-S) and survival assay (DRF=1.96). There was a significant increase in number of radiation induced spleen colonies and weight of the spleen in EOE pretreated irradiated animals.

The radioprotective effect of EOE on the peripheral blood of mice was also studied between 12 hrs to 30 days post-irradiation. A significant increase in the hematological parameters such as total WBC count, Leucocytes showed a typical response to irradiation. Maximum decline in WBC counts was observed at day 3 (2.5 Gy). A decrease in the WBC described first by Jacobson *et al.* (1949) and later it has been observed by others in cats (Valentine and Pearse, 1952), dogs (Allen 1948), pigs (Cronkite *et al.,* 1969), rats (Suter, 1947; Cohin, 1952) and in mice (Samarth *et al.* 2001); (Singh and Goyal, 2005; Yadav, 2005).

The significance to this decrease is not known, however, it has been suggested that, it may represent a multiplication of the cells which were injured at the time of irradiation and died after a limited number of divisions. The initial and rapid fall in leucocytes counts may be due to a fast decline of lymphocytes in the peripheral blood which are the most radiosensitive as revealed by differential leucocytes count. A quick fall in total WBC count within 24 hrs. after moderate doses (150 and 200 R) of X-rays was reported by Baum *et al.* (1969). However, in the present study, an increase in WBC count was observed after day 5th of irradiation, as reported by others (Samarth, 2001; Shekhawat and Goyal, 2004).

These findings are in agreement with those of Rough and Pardo (1963) who observed a fall in total WBC count by 48 hrs. in 6-8 weeks old weanling mice. The results of the present study are also in favor with those of Kumar (1981) who found a minimum count of WBC on day 3rd and 7th in 250 and 500 R gamma irradiated mice respectively.

In the present investigation, it was observed that the lymphocyte count declined sharply after irradiation and reached to minimum at day 3 (2.5 Gy), day 5 (5.0 and 7.5 Gy) and 24 hrs. (10 Gy). It is well known that radiation exposure reduce the number and functional activity of circulating lymphocytes and changes the distribution and ratio of their sub populations (Stjernsward *et al.,* 1972; Blomgren *et al.,* 1976 and Kohorn *et al.* 1978). Patt *et al.* (1967) suggested that the rapid decline in lymphocytes number is due to direct destruction of such cells in peripheral blood. Konings (1981) suggested that lymphocytes normally do not lead to a reproductive death after irradiation but killed in interphase. Lysis of these cells after irradiation might be caused by damage to the plasma membrane. If lipid peroxidation in the plasma membrane is the predominant damaging process in lymphocytes irradiated at low dose-rates, then an inverse dose-rate dependency of radiation induced lymphocytes lysis may be expected.

The present study revealed that the depletion in lymhocytes count is dose-dependent and rapid depression was observed at early intervals mainly due to their direct killing by radiation. Huges and Walden (1988) reported lymphocytes to be quite radiosensitive and their number declined in a dose-dependent manner after exposure to 2.5 Gy. In the present study also, the lymphocytes behaved as the most radiosensitive blood cells after exposure to dose of gamma radiation (2.5 Gy). The lymphopenia seen shortly after irradiation is either due to direct cell killing of circulating lymphocytes or due to destruction of stem cells which give rise to lymphocytes having shorter life span or both. No significant changes in monocytes, eosinophils and basophils percentage were observed in the present study, after exposure to 2.5 Gy gamma radiation. However, Jacobson (1954) reported that monocytes follow a pattern similar to that of lymphocytes in peripheral blood, but regained normaly or recorded an increase between day 4 and 6 after exposure to 100 R or above. Daga (1995), Samarth (2001) and Shekhawat (2004) have also reported a significant decline in manocytes after irradiation.

In the present investigation, it was observed that neutrophilic percentage increased over normal from first autopsy interval and was found to be maximum at 24 hrs. (2.5 Gy) Thus, the neutrophilic granulocytes altered inversely as compared to lymphocytes. This abrupt increase in neutrophilic percentage may be due to an abortive rise phenomenon as described earlier by Bloom and Jacobson (1948), Valentine and pears (1952) and Nachtwery *et al.* (1967). Errera and Forssberg (1968) reported an increased neutrophilic percentage within first 24 hrs. after irradiation with

moderate to high doses of radiation which has been interpreted as stimulation effect. A hastening of maturation of granulocyte precursors in bone marrow and their release in circulation can be attributed to a rise in neutrophilic count in the present study. It was suggested that first peak of neutrophils can be attributed to a hastening of maturation in bone marrow and second peak by a mobilizing phenomenon in response to widespread tissue injury by irradiation (Daga, 1996; Nunia and Goyal, 2004., Jisha joy et al. 2015., Tyler et al. 2017 and Ravindra et al, 2017)

Conclusion

The possible mechanism of radioprotection by *Emblica officinalis* expract (EOE) may be by stmulating/ protectimg the hematological stem cells against the radiation induced free radical damage by EOE. The radio protective effect of EOE may be attribeeted to the antioxidant, and antiperoxident and radioprotection properties due to the presence of emblicanin A, emblicnin B, punigluconin, pedunculagin, lipoic acid and vitaminC.

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