

DELETERIOUS EFFECTS OF IONIZING RADIATION & LEAD ON BLOOD OF SWISS ALBINO MICE. THIS PROTECTED BY EMBLICA OFFICINALIS. LINN.

¹K.K. NAYAK, ²A. CHAKRAWARTI, ³R.K. PUROHIT

¹NAVJEEVAN SCIENCE COLLEGE

¹LABORATORY OF ZOOLOGY

¹SIKAR (INDIA) PIN 332001

^{2,3}RADIATION BIOLOGY LABORATORY,

^{2,3}DEPARTMENT OF ZOOLOGY,

^{2,3}GOVT. DUNGAR COLLEGE, BIKANER (INDIA) PIN 334001

Abstract- The extensive use of ionizing radiation recent in various branches of natural economy, medicine, Physiology and science- Technology has made radiation injury on urgent problem attracting the attention not only of specialists in a variety of clinical disciplines but also of a vast army of theoretical scientists. The heavy metals such as Lead enter in human body via food chain and cosmetics. Various public health measures have been undertaken to control, prevent and treat metal toxicity. Emblica is a natural herbs which have been proved to be patent enough to check the radiation and heavy metals induced histopathological histochemical, biochemical and Hematological levels in swiss albino mice. the recent study will shed light upon the modulatory influence of Emblica against the deleterious effects induced by simultaneous exposure of radiation and heavy metal in the blood of Swiss albino mice. Here, six to eight week old male swiss albino mice were selected and divided in to seven group: Group I (Sham. irradiated), Group II (Treated with Lead acetate 20 ppm), Group III (Irradiated with 3.0 Gy and 6.0 Gy gamma rays) Group IV (Irradiated and Lead acetate treated), Group V (Lead acetate and Emblica treated), Group VI (radiation and Emblica treated) Group VII (radiation and Lead acetate and Emblica) were sacrificed at each post treatment intervals of 1,2,4,7,14 and 28 days. The blood, was taken out quantitatively analyzed for different biochemical parameters. Value of blood cells and Hb were found to decrease in all groups as compared to normal group and Biochemical value of M.CV, PCV, SGOT/AST and SGPT/ALT were also found. to decrease and combined treatment of radiation and Lead acetate synergistic effects were observed but Emblica treated animals exhibited less severe damage as compared to nondrug treated animals at all. the corresponding Intervals. Emblica reduced the severity of damage and made the recovery of biochemical Parameters. Therefore Emblica has reported as a good radioprotection in radiotherapy during clinical application of human beings.

Keywords: Mice, Blood, Radiation, Lead, Emblica

INTRODUCTION

Since the discovery of the deleterious effects of ionizing radiation, studies have been focused on developing chemical radio protectors that have to ability to decrease the ill effects of radiation on normal tissues. Several phytochemicals and plant extracts with innumerable pharmacological property in recent past have been reported to at as good radio protectors due to the ability of scavenging the free radical and modulating antioxidant defense system of the body up/ down regulation of the antioxidant the gene expression. Emblica officinalis Gaertn. or Phyllanthus emblica Linn, commonly known as Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda. Plant extracts of *Emblica Officinalis* has been found to have protective effects against the radiation induced disorders in mammals.

In light of the above, the present study was aimed to evaluate the Protection provided by *Emblica officinalis* Linn. against radiation and lead induced Haematological changes in the Swiss albino mice Modification of radiation and lead acetate 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 classified as chemical protection and the substances responsible for it are called 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 levels except MPG

METHODOLOGY

Animal

For the purpose, six to eight weeks old male Swiss albino mice were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered (1066/ac/07/CPCSEA) under CPCSEA, Chennai. The College has its own Institutional Animal Ethics Committee. All the experiments in present investigation conducted under the supervision and guidelines of IAEC. The animals were kept in the polypropylene cages in the departmental animal house. The standard mice feed and water was provided *ad libitum*. The temperature of animal house was maintained between 20-25⁰c.

Amla (*Emblica officinalis*)

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.x 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. The drug was given orally in the form of *Emblica* extract at a dose of 1000mg/kg body weight/animal/day from seven days prior to Lead acetate treatment or irradiation till last autopsy day there for the present study was undertaken to evaluate the role of *Emblica* in modifying the radiation and lead induced hematological changes in the Swiss albino mice.

Lead

Lead salt in the form of Lead acetate was procured from Ranbaxy Laboratory Limited, India. Lead acetate was given in the drinking water at the dose of 20 ppm.

Source and procedure of irradiation

Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada was used to expose the animals. 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 0.69 Gy/min to 1.22Gy/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.

Plan of experimentation

The animals were divided into the following

Group – I: Sham-irradiated animals (normal).

Group - II: Lead acetate treated animals

Group - III: Only irradiated animals

Sub-group III a 3.0 Gy Sub-

Group III b : 6.0 Gy

Group - IV: (Radiation + lead acetate)

Sub-group IV a : 3.0 Gy + lead acetate

Sub-group IV b : 6.0 Gy + lead acetate

Group - V : Lead acetate + *Emblica*

Group - VI: (Radiation + *Emblica*)

Sub-group VI a : 3.0 Gy + *Emblica*

Sub-group VI b : 6.0 Gy + *Emblica*

Group - VII: (Radiation + lead acetate + *Emblica*)

Sub-group VIIa : 3.0 Gy + lead acetate + *Emblica*

Sub-group VIIb: 6.0Gy + lead acetate + *Emblica*

Autopsy of animals

A minimum of three animals from each group were autopsied after 1,2,4,7,14 and 28 days of treatment. Three sham-irradiated mice were also be autopsied. The animal were sacrificed by cervical dislocation. Prior to autopsy the animals were weighed.

1. Haematological Studies:

1. Red blood corpuscles (RBC)
2. White blood corpuscles (WBC)
3. Haemoglobin (Hb)
4. Packed cell volume (PCV)
5. Mean cell volume (MCV)
6. Mean corpuscular haemoglobin (MCH)
7. Mean corpuscular haemoglobin concentration (MCHC)
8. Differential Leucocyte Counting

2. Biochemical Studies :

1. Serum glutamic oxaloacetic transaminase (SGOT) /
2. Aspartate amino transferase (AST)
3. Serum glutamic pyruvic transferase (SGPT) /
4. Alanine amino transferase (ALT)

Result and Discussion

In this experiment Haematological change observed which is dose dependent in the blood of Swiss albino mice. The severe changes seen in the blood of model animal after treated with heavy metal and 3 Gy, and 6 Gy gamma radiation the symptom appeared within 3 to 05 days after exposure in model animal such as low intake of food and water, weight loss, weakness, irritability and ruffling of hair. These changes were more marked on day 7 but on day 14 the signs of recovery were observed and day 28, comparatively better Hematological value was observed.

Hematological Parameters

The values of RBC, WBC, Hb and PCV were found to decrease in all the groups as compared to normal group, but the decrease in these values was lesser in *Emblica* treated groups (V to VII) as compared to non-drug treated groups (II to IV).

The values of MCV were also found to decrease but the difference from normal value was significant at previous intervals and it was non-significant on later intervals. The values of MCH increased in all the groups as compared with normal group after days 1, 2, 4, 7 and 14. Thereafter a fall in the values was noted on day-28 without reaching to the normal. The increase in the value of MCH was lesser in *Emblica* treated groups (V to VII) as compared to non drug treated groups (II to IV). Besides this values of MCHC increased in all the groups at various intervals but the values were lower in the *Emblica* treated groups (V to VII) as compared to non-drug treated groups (II to IV). The difference from the normal was non-significant in all the groups. The value of lymphocytes declined up to day-14 in non drug treated groups and day-7 in the *Emblica* treated groups. Similarly the values of monocytes and granulocytes percentage increased up to day-14 in the non drug treated animals and day-7 in the drug treated animals thereafter, a decrease in the value was noted up to day-28 without reaching to the normal. The values of SGOT/AST and SGPT/ALT elevated up to day-14 in the non drug treated groups and day-7 in the *Emblica* treated groups, thereafter a fall in the value was seen up to day -28. After exposure to a higher dose (6.0 Gy) similar changes were noticed but they were more pronounced and there was late manifestation of recovery. In the combined treatment of radiation and lead acetate synergistic effects were observed. The *Emblica* treated animals exhibited less severe damage as compared to non-drug treated animals at all the corresponding intervals. An early and fast recovery was also noticed in *Emblica* pretreated animals.

Lead Effect:

Lead toxicity is a particularly insidious hazard with the potential of causing irreversible health effects. It is known to interfere with number of body functions and it is primarily affecting the Hematopoietic disorder in animal.

Radiation Effect:

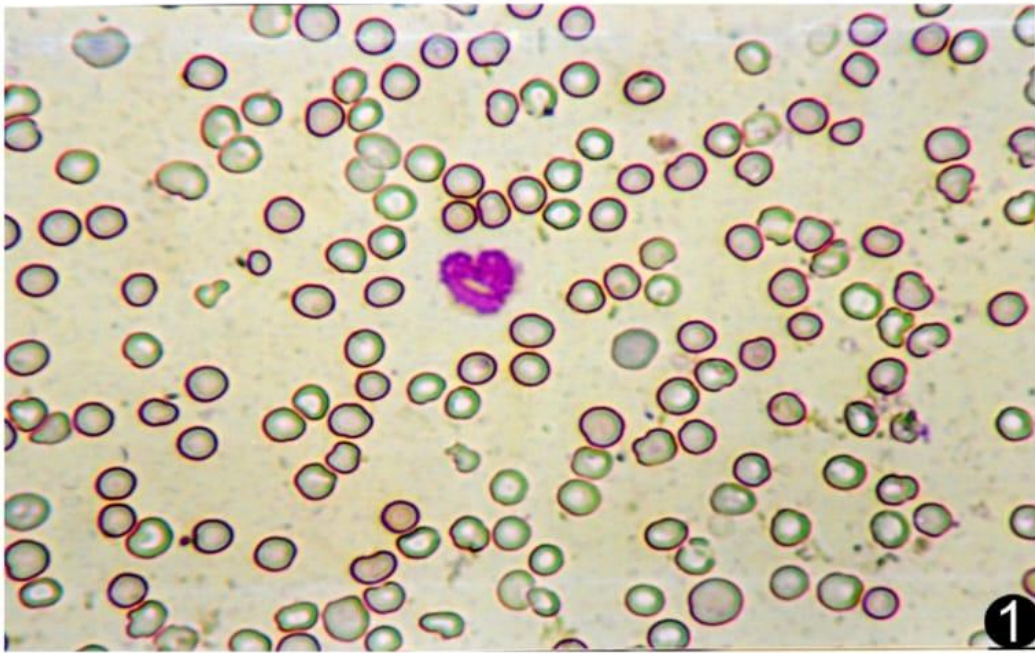
The interaction of ionizing radiation with biological system results in the generation of many highly reactive short lived reaction oxygen species (ROS) mainly due to the hydrolysis of water. The major reactive species resulting from aqueous radiolysis include H, OH, RO₂, H₂O etc. These ROS attack cellular macromolecules like, DNA, RNA, Protein membranes etc. and cause its dysfunction and damage, ROS increased the membrane lipid peroxidation which can alter the integrity of membrane structure leading to inactivation of membrane bound enzymes, loss of permeability of the membrane and decrease in fluidity and DNA is the critical target of radiation damage in living cells, which may lead to alternation in the functional state of cell and further to cell death.

Radio Protective Role of *Emblica officinalis*

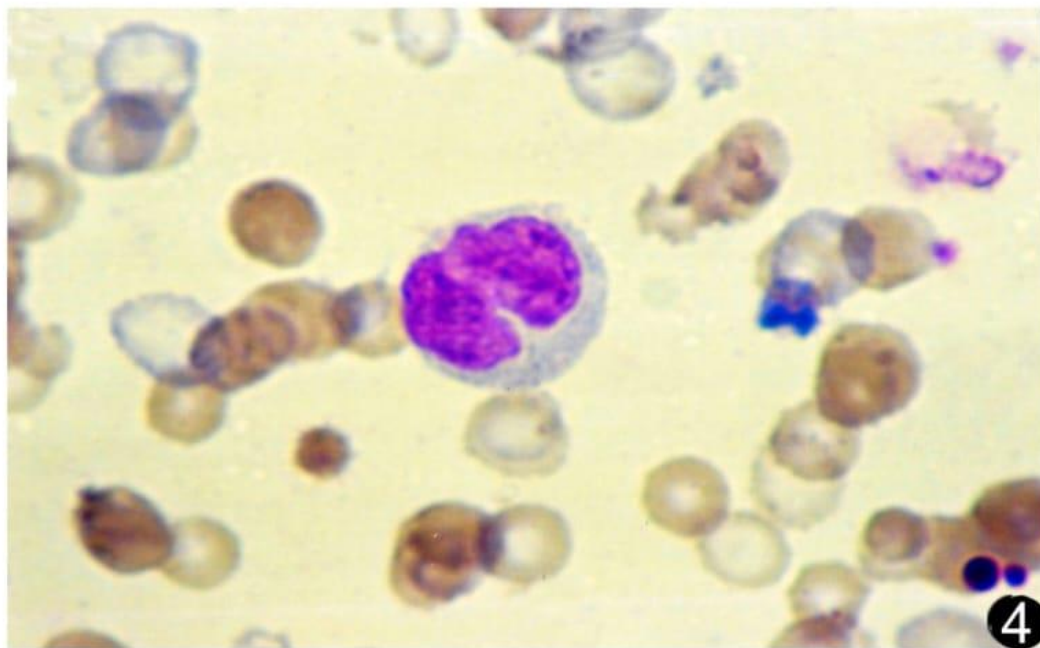
1. "DNA strand breaks and mutation after exposure of radiation and also sign peroxidative changes in lipid and Protein.
2. *Emblica* extracts work as an antioxidant which reduces the oxidative change and DNA damage.
3. *Emblica* extract was also found to inhibit mutagenesis by direct bindings to certain mutagens as well as by inhibiting carcinogen. Activation treatment of *Emblica* extract increased the 75H levels this herb showed good antioxidant activity in vitro.
4. Polyphenols are content of *Emblica* extract and these Polyphenols work as scavengers on free radicals which produced in the cell by radiation.
5. *Emblica* induced Haemopoiesis thus reducing the myelo suppression induced by radiation.
6. It stimulates antioxidant activity and potent induced of Haemopoietic system, it is an excellent natural radioprotector.

CONCLUSION

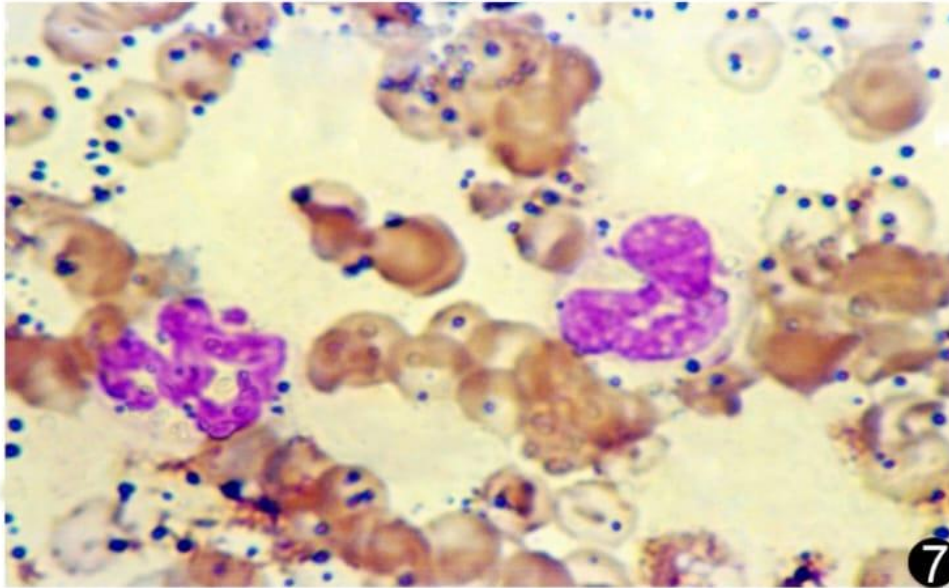
• Ionizing radiation and Lead damage blood through a series of molecular events as the haematopoietic system is among the most radiosensitive in the body and it has a highest cell turn over. Alteration in cell division, cell death, depletion of stem cell pools is followed by haematological system dysfunction. The combined treatment of radiation and Lead showed synergistic changes. The blood of *Emblica* treated animals showed less severe radio lesions and early and fast recovery in comparison to non-drug treated animals. Thus, it seems that *Emblica* has protected the blood at both the dose levels with and without lead treatment. The *Emblica* might have protected the animals from radiation by more than one mechanism due its multiple properties of scavenging hydroxyl radicals and reducing alterations in enzyme activity. Thus, *Emblica* is a good herbal radio protector and future prospectus emphasize the potential in the area of natural product based radioprotector during discovery.



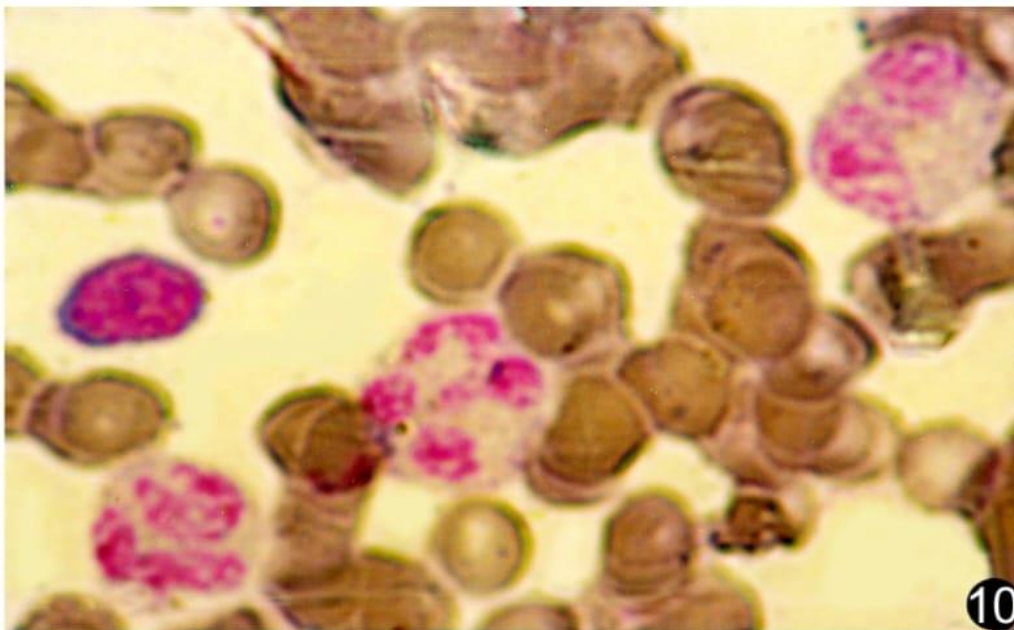
Normal RBCs and small Neutrophil



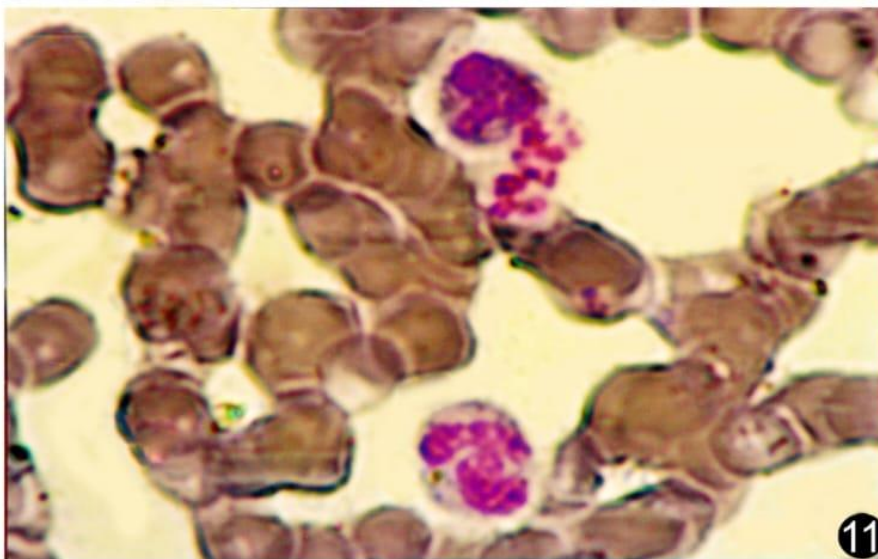
After 1 day of Lead acetate treatment showing crenation in RBCs and a complete monocyte.



After 7-days of Lead acetate treatment showing clusters and crenation in RBCs. Neutrophils are also seen.

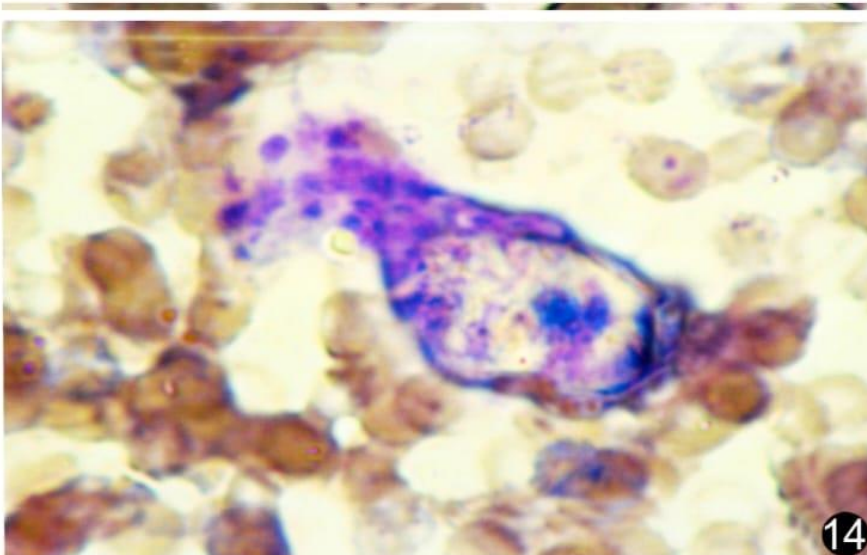


After 1 day of gamma rays (3.0Gy) exposure displaying neutrophil and lymphocyte. Distorted RBCs are also seen.



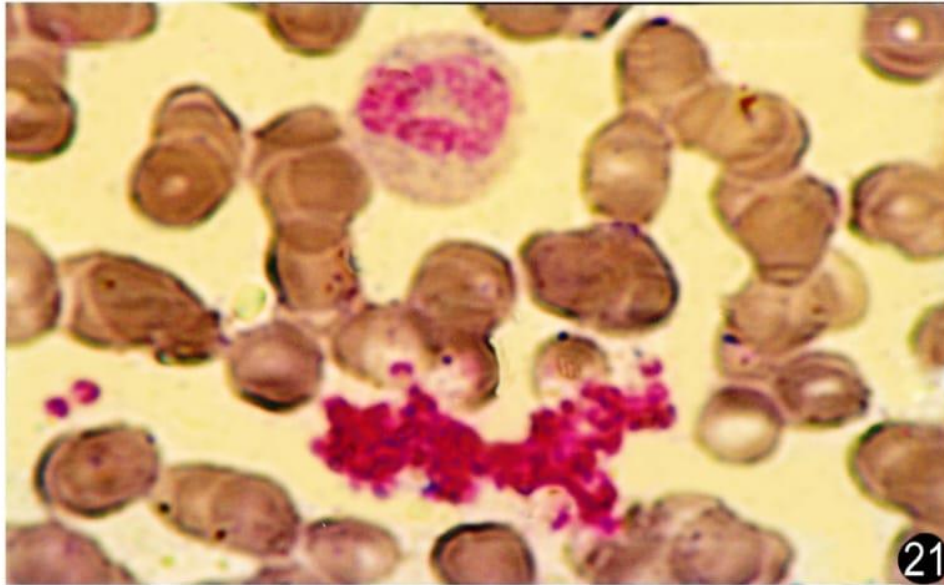
21

After 4 days of gamma rays (3.0 Gy) exposure showing a complete neutrophil and lysing neutrophil. Crenated RBCs are also seen.



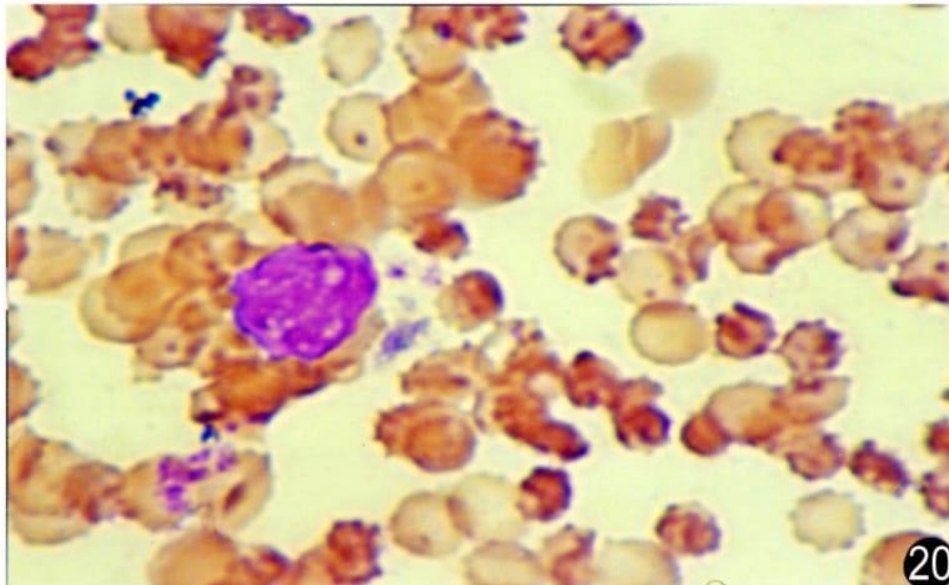
22

After 4-days of gamma rays (6.0 Gy) exposure showing bursting lymphocyte, crenated and clusters of RBCs.



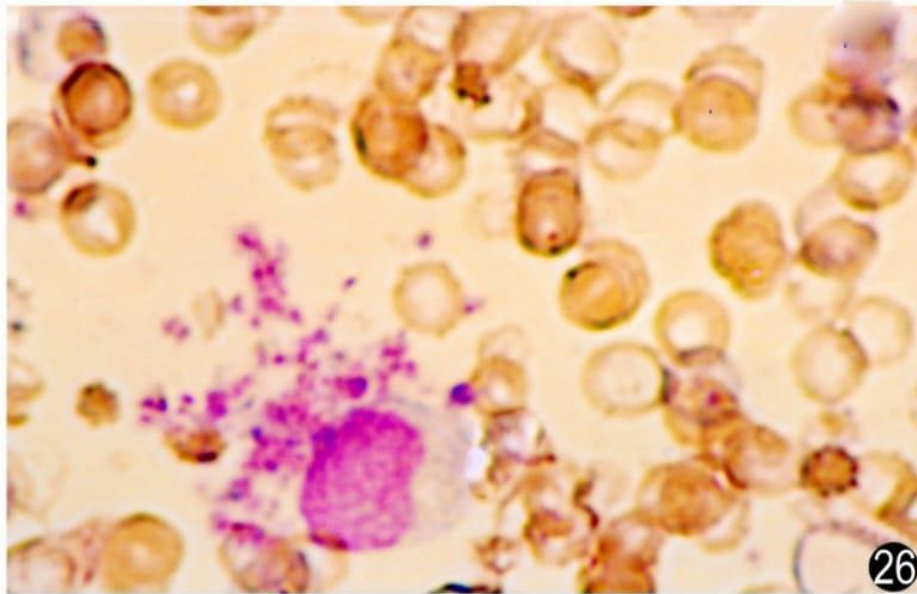
After 7-days (Lead acetate+ 6.0 Gy) showing crenated and lysed RBCs with dissolving neutrophil.

23



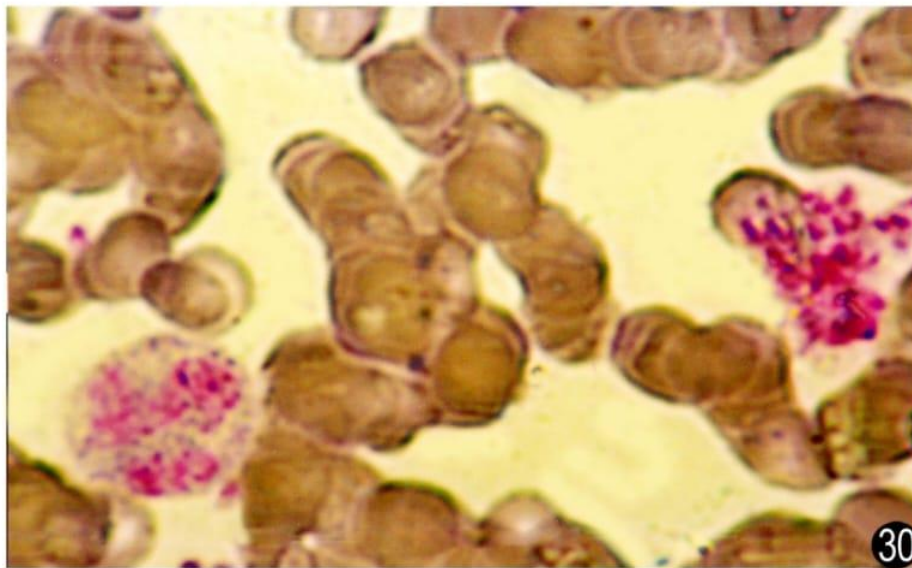
After 14-day (6.0 Gy + lead acetate) showing normal neutrophil and dissolving neutrophil. Clusters of RBCs are also seen.

24



25

After 4-day (3.0 Gy + *Emblica*) showing bursting monocyte and crenated RBCs.



26

After 7-days (6.0Gy + *Emblica*) showing a complete neutrophil and lysing neutrophil.

REFERENCES:

1. Stankovel, Riges D. Herde, BT, Begley R: Determinants of Resistance do radiation injury in blood granulocytes from normal donors and from Patients with myeloproliferativ disorders, Radioat Res. (1979) 80,49-60
2. Harrison RD, Lead. In (Ed) Hamilton Hardy's industrian toxicology 5th edition, Philadelphia, PA: Mosby, 1998, PP 70-6
3. Altman, Kid, berber, B. Okada: Th," Radiation Biochemistry Val. I, Acad. Press NV 1970, P 187
4. Bhati SL. Agarwal M. ChakrwardiA, and purohit. Chemopreventive action of *Emblica officinalis* against radiation and cadmium induced hepatic Lesion in Swiss albino mice, int.. J. of Phorma sci and Res. (2016)-1-370-376
5. mugaki MN, Heidariz, Sagheb HM: barbarestans effects of Chronic Lead acetade intoxication on blood indices of Male adult rat DARV(2003) 11: 147-51

6. Goyal, gargv 2,3,4 Triamino azobenzene induced hematological anomalies in fish *Channa punctatus* Bull. Environ. Contam. toxicol (1980) as: 136-14)
7. Suradkar sh, Ghodasara DJ Vinod, Patel, Jaiswall, prajapati, Haematobiochemical alteration Induced by Lead acetate toxicity in wistar rats, vet world (2009) 2: 429-31
8. Atomic Energy of Canda LTD- Radio chemical department Blood Irradiation bibliography A.E.CL Kanta ontario, 1984-185
9. singh I, Sharma, A, Nuniavi, goyal PK., Radio protection of swiss albino mice by *Emblica officinales*. Phytother Res. 1998 (S) 144-6
10. Shalan Mg, Mostafa M.S. Hassouna MM, El-Nabise. Amelioration of lead toxicity on rat liver with vitamin c and silymarin. supplements toxicology (2005) 206: 1-15
11. Rosenberg CE, fint NE, SalibianA: Humoral immune. alteration caused by lead, studies on an adults. Model: Acta. toxicol Argent (2007) 15 (1, 16-23
12. Abdou ZA, Attia MH, Raafat MA: Protective effect of citric acid and thiol compounds against cadmium. and Lead toxicity in experimental animals. J Biol Che Environ Sci (2007) 2:481-97
13. Morrey RK, granner DK Rodwell vw: Harper's Illustrated Biochemistry, 27th ed. New York: McGraw Hill 2006
14. Purohit RK, Chakrawarti A, Agarwal, M Jagir A, Purkharam, Pyarelal and Nayak-K.K.: Role of Protection provided by *Emblica officinales* tim. against radiation and Lead induced histological changes in the jejenum of albino mice; Int. Phair. Res. Biosci. (2013) V₂(4) 117-130
15. Pelszynskimm, Molroffg to, Lubann, Taylor Limiting dilution analysis of lymphocyte inactivation In Irradiated red Blood cells. Units Blood 78 (Suppt) 1991-2754 a
16. Kishore K, Moorthy PN, RaoKN; Radiation protection of vitamin in aqueous system, part II A Comparitive study in fluid and frozen aqueous systems. Radiat. effects 29(1976) 165-170
17. Halley TR, Van EPPS, Harvey RL, Aderson. Effect of high does of radiation on Human rictsophil chemotaxis, Phagocytosis and morphology - AMJ Pathal 75. (1974) 61-72
18. Witas H, Dudaw, katelba, witkowska B. Leykowy Changes of adenine nucleotides content and release reaction of Human blood platelets following gamma irradiation. Radiali Environ. Biophys. 14 (1977) 2317-2332
19. Huberk, Braselmann, H Bauchinger M., Intra and Inter Individual variation of background and radiation- induced micronucleus frequencies in Human lymphocytes int J Radiation Bio-61 (1992) 655-66)
20. schifter LM, etal. Extracorporeal irradiation of the blood in human effects upon erythrocyte survival. Blood 27 (1966) 831-843
21. Om Kanwar, shika ojha, Kamal N., PK. Joshi Aruna C, Purohit R Manisa A, Modulatory influence of Aloe vera Against Radiation and Cadmium induced Biochemical changes in the Brain of Swiss albinomice. Int. J. Pharms. Res. Bio. Sci. 2013, 2 (4) 117-130
22. Hilyer CD, Tiegermanko, Berkman EM Evaluation of the red cell storage Lesion after irradiation Packed, red cell unit transfusion 31 (1991) 497-499
23. Anderson , Baldwin: Quality assurance of the Irradiation process of blood components. American association of Blood Bank Bethesda MD (1992) 63-76
24. wilskih, The Radiation induced degradation of Polymers, Radict Phy. Chem. 29 (1987) 1-14
25. Jajetia GC, Radio protective potential of Plants and herbs against the effects of ionizing radiation Jclin. Biochem. Nutr (2007), 40(2)74-81
26. Gehlot & Goyal PK.: Radio protective effects of Aloe vera leaf extract on swiss alding mice against whale body. gamma irradiation. Environ. Pathology, toxi and oncology (2009), 28: 1
27. Scherishus, 6 Hommes, Malenaar: Radiation-Induced Structural changes in membrane protein of Human erythrocyte and ghosts and the relation to cellular morphology. Int radiat. Bio. 45 (1984) 159-177
28. Verma P, Sharma P, Parmar I, Agrawal A goyal PK: Amelioration of radiation-induced Haematological and Biochemical alteration in swiss albino mice by Panaxginseng Extract. · Int. Cancer Ther. (2011) 10 77-84
29. Samarth RM, Kamar A; Radioprotection of swiss albino mice by plant extract mentha piperita (Linn): Radiat. Res. 2003, 44: 101-109
30. Singh I, Dhanray, goyal p.k. *Emblica officinalis* (linn) fruit extract Provides protection against radiation. Induced Hematological and Biochemical alteration in mice.j Environ. Pathal toxicol conCal. (2006) 2514, 643-654