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EFFECT OF GAMMA RADIATION AND LEAD ON TOTAL ERYTHROCYTE COUNT (TEC) AND HAEMOGLOBIN OF MICE AND ITS MODIFICATION BY *EMBLICA OFFICINALIS* LINN.

Venkteshwar Songara, Seema Singariya, Manisha Agarwal, Aruna Chakrawarti, R. K. Purohit*

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

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***Correspondence for Author**
Dr. R. K. Purohit
Radiation Biology Laboratory, Department of Zoology, Govt. Dungar College, Bikaner, Rajasthan, India- 334001.

ABSTRACT

Haematopoetic system is the one of the most radiosensitive part in the mammals. Exposure to ionizing radiation increases the production of the reactive oxygen species (ROS) leading the irradiated cells into a state of oxidative stress. Furthermore, Lead exposure along with ionizing radiation can potentially become toxic to the tissues due to the heightened oxidative stress. In the present study adult male Swiss albino mice were procured and divided into seven groups. Group (II to IV) serving as control, received sub lethal dose (3.0 Gy or 6.0Gy) and /or lead acetate (20ppm) in drinking water *ad libitum*. The experimental groups(V to VII) were given aqueous solution of *Emblica* (1000 mg/ Kg b.wt./ animal/ day) orally seven days prior to radiation and/or lead acetate treatment. Sham- irradiated animals of Group I served as normal. Animals of all the groups were autopsied at each post treatment interval of 1,2,4,7,14 and 28 days. Blood was collected for total erythrocyte count (TEC) and haemoglobin estimation. The TEC and haemoglobin showed a dose- dependent decreasing trend in control groups up to day- 14 and thereafter it increased up to day-28. The Combined treatment showed synergistic effect. In experimental groups less severe radiolesions and an early onset of recovery was observed. Therefore it may be deduced that *Emblica* is a good herbal radioprotecter and may be useful for the clinical applications in human beings during radiotherapy.

KEYWORDS: Mice, Radiation, Lead acetate, *Emblica*, Radioprotector.

INTRODUCTION

The widespread use of radiation in the diagnosis, industry and the energy sector and its inadvertent exposure during air and space travel, nuclear accidents and nuclear terror attacks requires a safeguard against human exposures. Hence, a pharmacological intervention could be the most prudent strategy which can be used to protect humans against the harmful effects of ionizing radiations. Radiation increasingly being used for medical and occupational purposes and it is an established weapon in the diagnosis and the therapy of cancer. Radiation therapy injures or destroys the cells in the areas which are being treated ("target tissues") by damaging their genetic materials.

Lead enters the human body in many ways. It can be inhaled in dust from lead paints, or waste gases from leaded gasoline. It is found in trace amounts in various foods, notably fish, which are heavily subjected to industrial pollution. Plants can absorb lead from soils and tetra ethyl lead traffic-induced air pollution (90 per cent of total lead emissions into the atmosphere). Lead can contaminate water and consequently enter the aquatic food chains.[1]

Toxic effects caused by lead exposure are usually detected in the kidney, the nervous, hematopoietic and gastrointestinal systems, male and female reproductive organs as well as other soft tissues, with a long term deposition of lead usually accumulating into the bones.[2] A significant decreased RBC counts, hemoglobin levels and hematocrit values were reported in male and female mice given dietary lead[3]. Phagocytic cells, such as macrophages, may be used as a biomarker of immunotoxicity in wildlife studies. Several studies have shown that maternal exposure to lead is highly responsible for miscarriages and birth defects in the fetuses and also has adverse effects on the cognitive development of children.[4-6]

In recent years, radiaoprotective agents with novel modes of action have been under investigation; in particular, the compounds that can affect the hematopoietic stem cell regeneration have attracted significant interest. The traditional Indian system of medicine, Ayurveda, gives a detailed account of several diseases and their treatments. The majority of drugs and/or drug formulations which are used in Ayurveda are principally derived from herbs and plants. Plant extracts such as Garlic, Ginsing, *Emblica officinalis*, *Aloe vera*, *Podophyllum*, *Ocimum*, *Spirulina*, *Mentha piperita* and various herbal drug preparations have been found to have protective effects against the radiation induced disorders in mammals.[7-15] *Emblica officinalis* enjoys a hallowed position in Ayurveda an Indian indigenous system of medicine. According to believe in ancient Indian mythology, it is the first tree to be created in

the universe. It belongs to family Euphorbiaceae. It is also named as *Amla, Phyllanthus emblica* or Indian Gooseberry. The species is native to India and also grows in tropical and subtropical region. The fruits of *Emblica officinalis* are widely used in the Ayurveda and are believed to increase defense against diseases. It has beneficial role in cancer, diabetes, liver treatment, heart trouble, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastro protective. Additionally, it is useful in memory enhancing, ophthalmic disorder and lowering cholesterol level.

MATERIALS AND METHODS

Procurement of animals and their maintenance

In the present study, adult healthy male Swiss albino mice (6-8 weeks old) were purchased from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar (India). The animals were housed in cages under good ventilation and illumination condition. They were fed with standard mice feed and water was given *ad libitum*. The temperature of the room was maintained between 22-27ºC. The Govt. Dungar College, Bikaner is registered under CPSCEA, Chennai (Registration no. 1066/ac/07/CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). All the experiments conducted in the present investigation were performed strictly under the supervision of IAEC of the college.

Lead

Lead salt in the form of Lead acetate was procured from Ranbaxy Laborataries Ltd. Lead acetate was given in the drinking water at the dose of 20 ppm.[16]

Emblica [EOE]

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 powdered form. The extract was redissolved in DDW just before oral administration. An approximate thirty eight per cent yield of the extract is obtained.[17] The EOE was given from seven days prior to Lead acetate treatment or irradiation in all the experimental groups.

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 of 0.69 Gy/min during first year and 1.22Gy/min during the subsequent year.

Experimental Design

Group	Chemical	Radiation Dose	Herbal Drug (EOE)	Treatment to mice
I (Normal)	-------	Sham Irradiated (control)	----------	Normal Group
II (Chemical)	Lead acetate	----------	----------	In this experimental group animals were received lead acetate orally at a dose of 20ppm
III a (Radiation)	----------	Sub lethal dose (3.0 Gy)	----------	Animals were whole body gamma irradiated with sub lethal dose
III b (Radiation)	----------	Sub lethal dose (6.0 Gy)	----------	Animals were whole body gamma irradiated with sub lethal dose
IV a (Chemical + Radiation)	Lead acetate	Sub lethal dose (3.0 Gy)	----------	Animals were received lead acetate orally at a dose of 20ppm and also treated with the gamma radiation.
IV b (Chemical + Radiation)	Lead acetae	Sub lethal dose (6.0 Gy)	----------	Animals were received lead acetate orally at a dose of 20 ppm and also treated with the gamma radiation.
V (Chemical + EOE)	Lead acetate	----------	*Emblica* (EOE)	Animals of this group were treated with lead acetate orally at the dose of 20 ppm and also received *Emblica* at a dose of 1000mg/ kg body weight/animal / day from seven days prior to lead acetate treatment and continued up to the last autopsy interval.
VI a (Radiation+ EOE)	----------	Sublethal dose(3.0 Gy)	*Emblica* (EOE)	Animals were treated with whole body gamma radiation to sub lethal dose and also received *Emblica* at a dose of 1000 mg /kg bodyweight/ animal /day from seven days prior to radiation and continued up to the last autopsy interval.
VI b (Radiation+ EOE)	----------	Sub lethal dose (6.0 Gy)	*Emblica* (EOE)	Animals were treated with whole body gamma radiation to sub lethal dose and also received *Emblica* at a dose

				1000 mg / kg body weight/ animal /day from seven days prior to radiation and continued up to the last autopsy interval.
VII a (Radiation+Chemical+ EOE)	Lead acetate	Sub lethal dose (3.0 Gy)	*Emblica* (EOE)	In this experimental group animals were treated with 3.0 Gy of gamma radiation and lead acetate at the dose of 20 ppm. They also received *Emblica* at a dose of 1000mg/ kg body weight / animal/day from seven days prior to treatment and continued up to the last autopsy interval.
VII b (Radiation+Chemical+ EOE)	Lead acetate	Sub lethal dose (6.0 Gy)	*Emblica* (EOE)	In this experimental group animals were treated with 6.0Gy of gamma radiation and lead acetate at the dose of 20ppm. They also received *Emblica* at a dose of 1000mg/kg body weight /animal/day from seven days prior to treatment and continued up to the last autopsy interval.

Autopsy of the animals

Three animals from each group (II to VII) were autopsied by cervical dislocation at each post-treatment interval of 1, 2, 4,7,14 and 28 days. Three sham-irradiated animals (group-I) were also autopsied. Prior to autopsy the animals were weighed. Immediately after the autopsy the blood was collected by cardiac puncture in heparinized tubes for TEC and haemoglobin estimation.

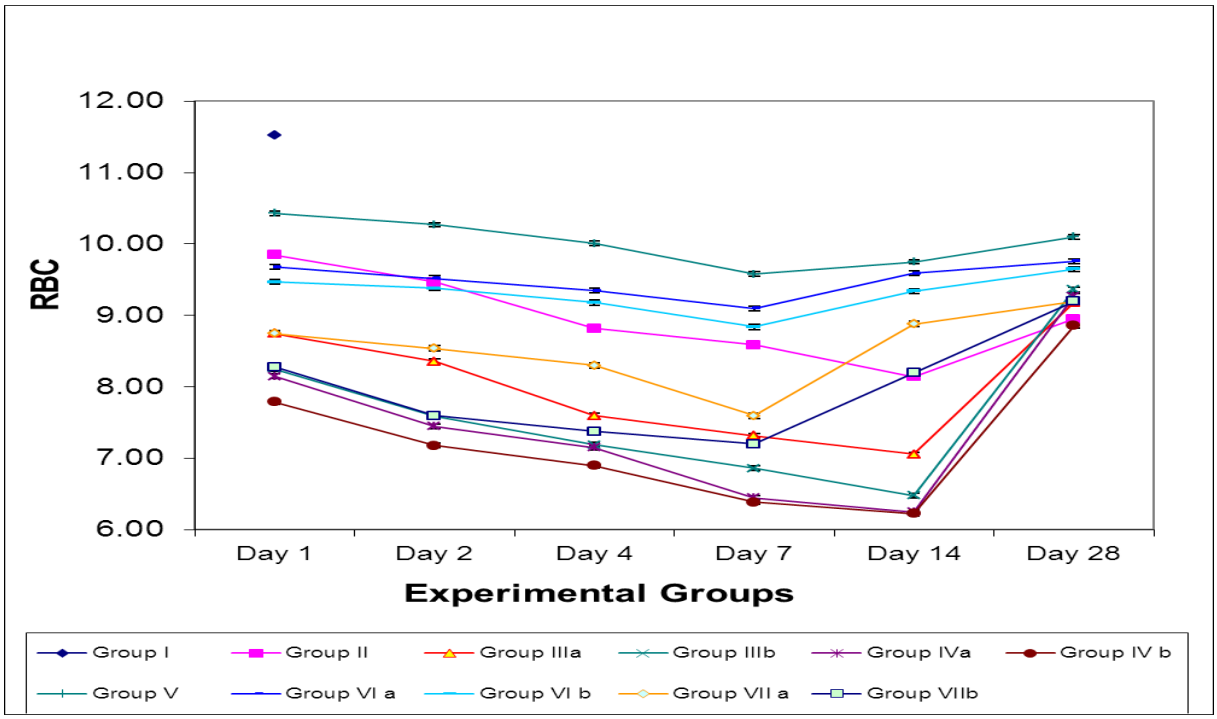
RESULTS

The value of TEC showed a decreasing trend in the control groups (II to IV). The value declined significantly on day-14. On day-28, the value increased significantly, but it was below than the normal value. In the EOE treated groups V, VI and VII, the value declined from day-1 to day-7. On day -14, the value increased and continued so up to day -28.

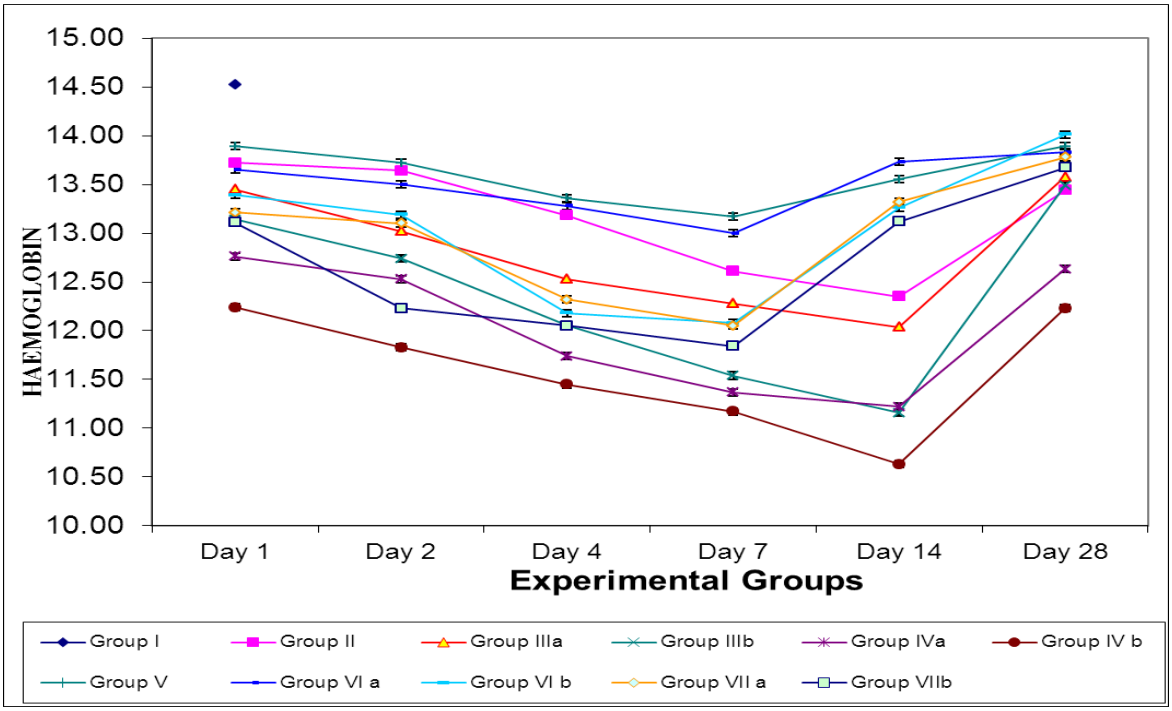
The haemoglobin content of blood decreased in all the experimental groups. This decrease was dose dependent and continued up to day –14 in the control groups and day-7 in the *Emblica* treated groups. Thereafter, the value increased in all the experimental groups. The decrease was more prominent in combined treatment groups. In the *Emblica* administered

experimental animals decrease was less severe which may be due to the protection provided by the drug.

Variation in the values of RBC (million/Cu.mm) of mice in various experimental groups (Mean ± S.E.)



Variations in the Haemoglobin content (gm/100ml.of blood) of mice in various experimental groups (Mean ± S.E.)



PHOTOMICROGRAPHS OF BLOOD SMEAR

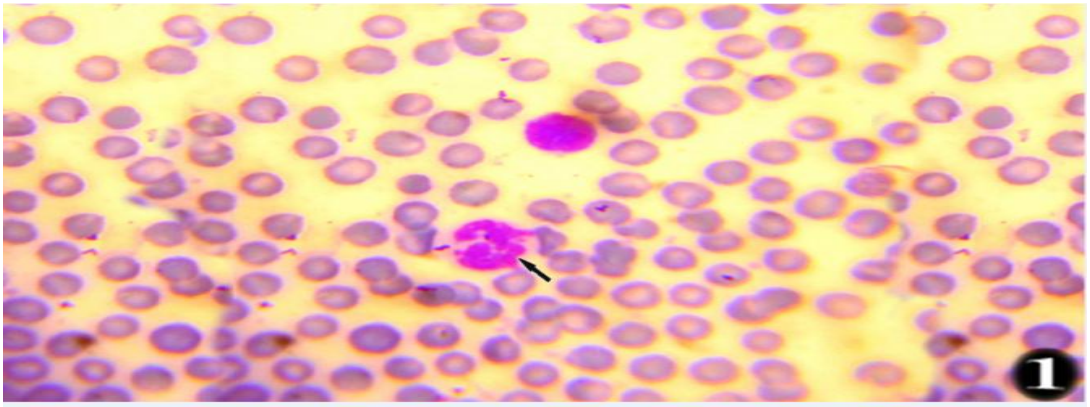


Fig 1: Sham-irradiated group showing normal RBCs, complete lymphocyte and neutrophil.

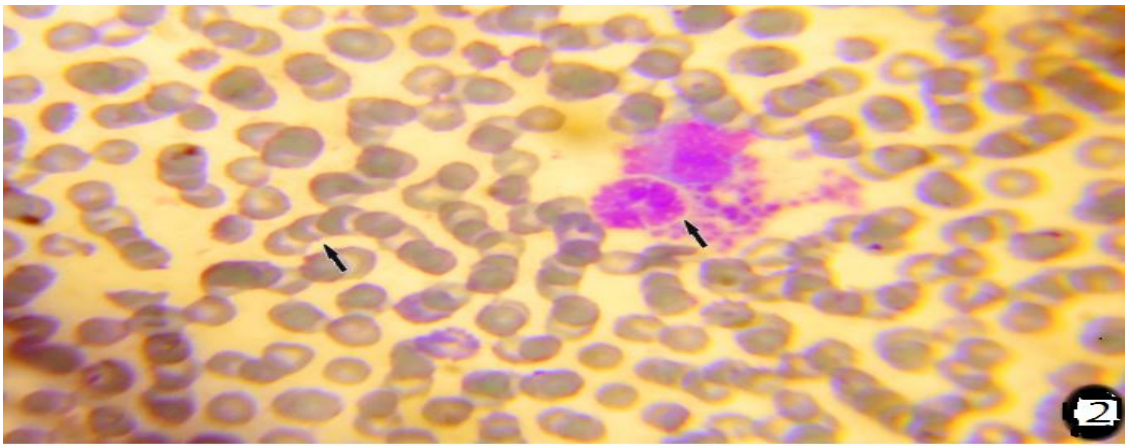


Fig: 2: After 7- day (lead acetate treatment) exhibiting clusters in RBCs and Lysing Neutrophils.

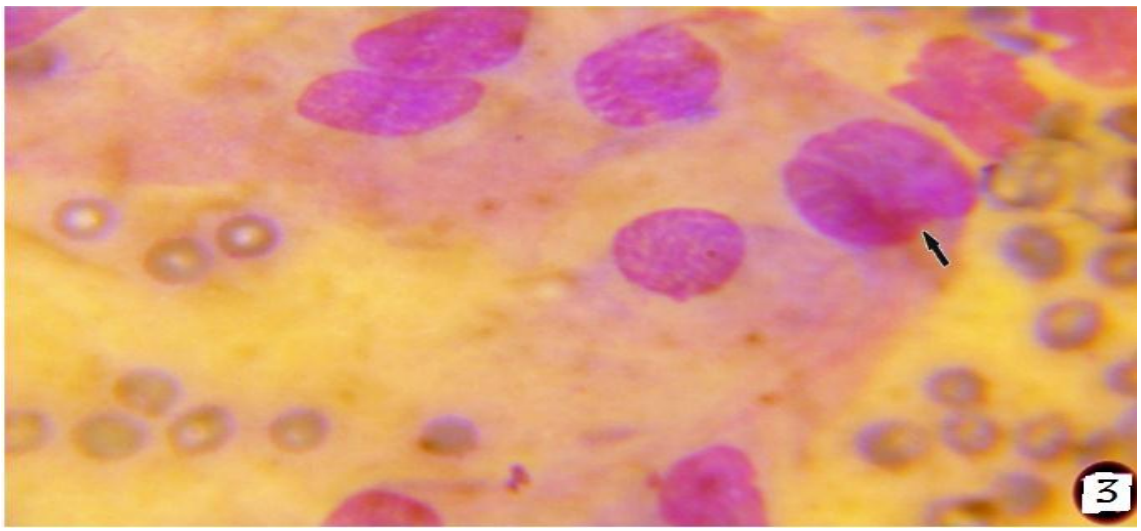


Fig 3: After 7-day (3.0 Gy + lead acetate) exhibiting complete dissolution of RBC, monocyte, reactive lymphocyte and segmented neutrophil.

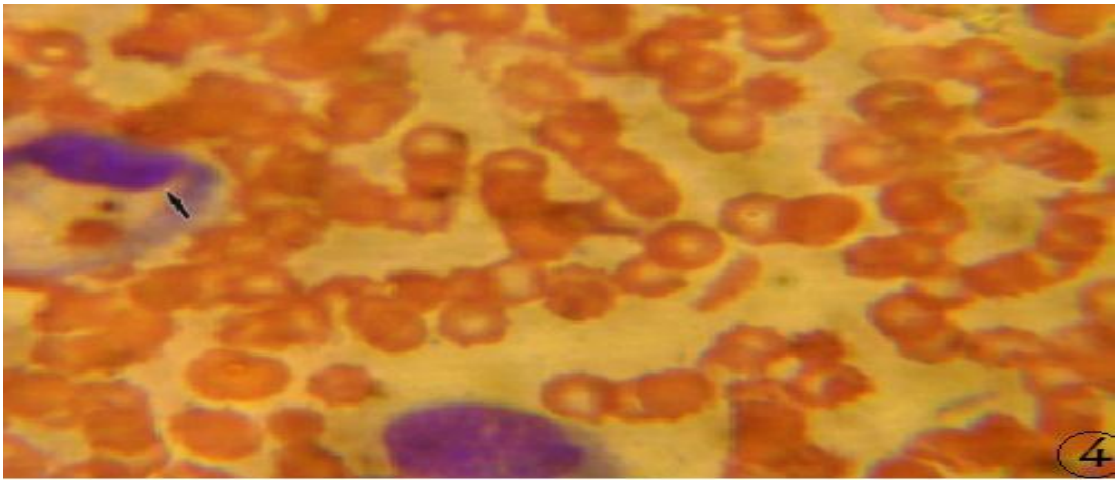


Fig: 4: After 14-day (6.0 Gy + lead acetate) exhibiting dissolving lymphocyte and Echinocyte shaped RBCs.

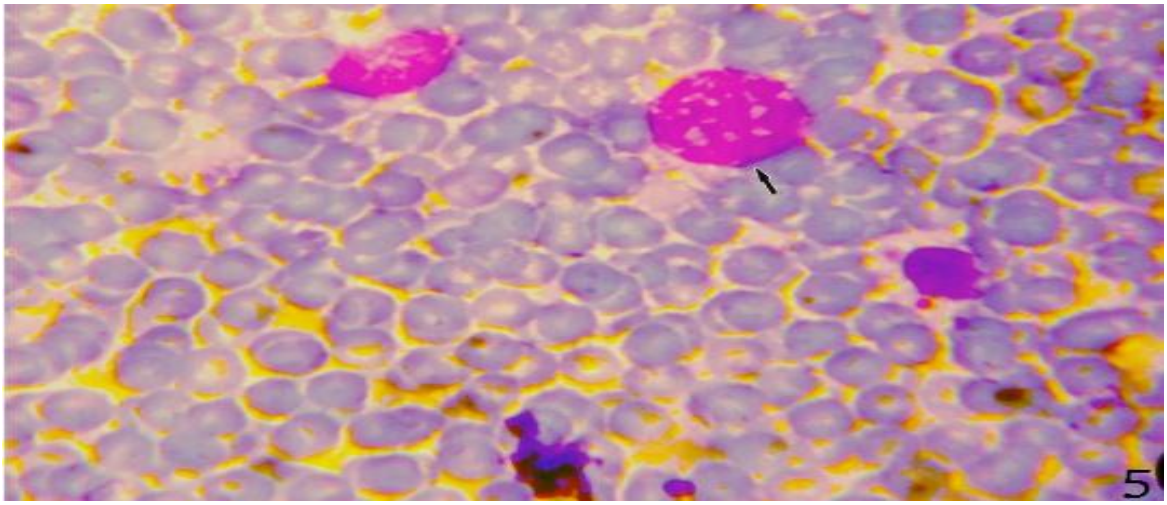


Fig: 5: After 1-day (6.0Gy + *Emblica*) showing bursting multi segmented neutrophil, normal lymphocyte and Slightly damaged RBCs.

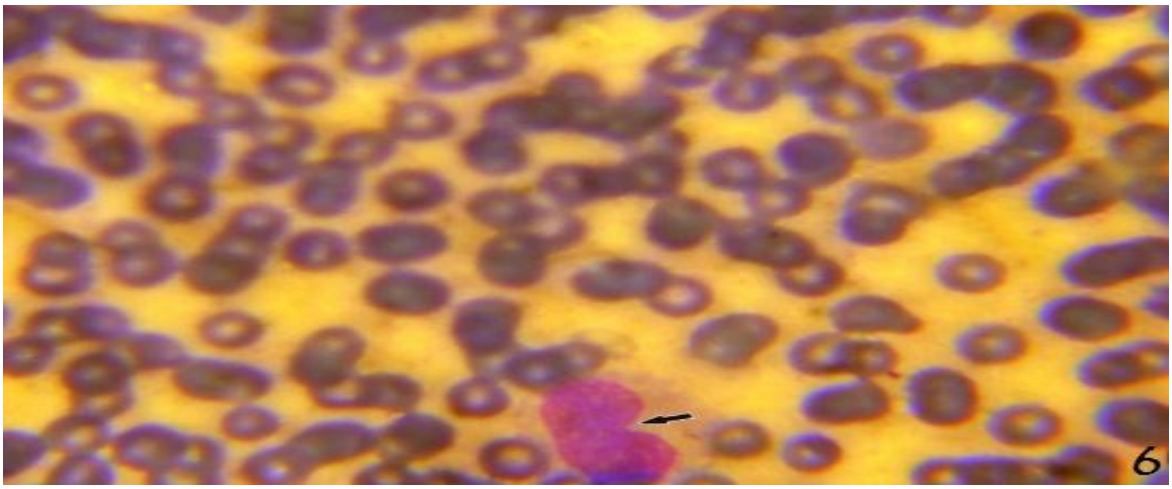


Fig: 6: After 1-day (3.0 Gy + lead acetate + *Emblica*) showing Band neutrophil and stock piling of RBCs.

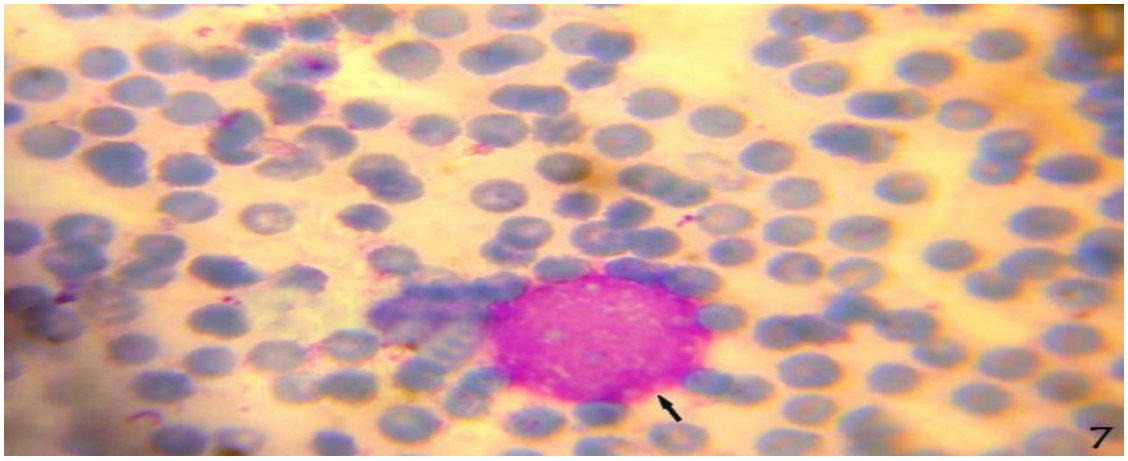


Fig: 7: After 28-day (3.0 Gy + lead acetate + Emblica) showing recovery in RBCs and a complete lymphocyte.

DISCUSSION

The problem of radiation hazard to living being has risen due to natural background radiations, increasing use of nuclear energy in industry, occupational and medical field such as radiotherapy and it produces severe side effects developed due to damage to normal tissue. Free radicals are generated by radiation energy in the cells and their reactions with DNA, RNA, and organelle cause cell dysfunction, mortality, mutagenesis or carcinogenesis.[18] Evidently, rapidly dividing cells like epithelial and haemopoietic system are prone to early and marked damage to chromosomes as well as other organelles due to higher content of oxygen and water with a higher level of free radical generation resulted as an impact of radiation energy.[19]

The exposure of animals to ionizing radiations causes a series of physiological changes which are known as the acute radiation syndrome, which depend upon the exposure dose and may even lead to death. The damage to the haematopoietic system is a major factor in the mortality, following an acute radiation exposure which might be due to the fact that the proliferating cells are highly sensitive to irradiations. In the present study, there was a significant decrease in the levels of the haematological variables in the irradiated animals as compared to normal or control animals. Therefore, the effect of the whole body irradiation is mainly caused by the highly proliferating bone marrow progenitor cells. Since the bone marrow progenitor cells are crucial for life, any damage to these cells can impair the normal physiological processes, thus causing an irreversible effect on the survival of an individual.[20] RBCs are related to the ROS action because their membrane is rich in polyunsaturated lipids and hemoglobin is a strong catalyst of free radical reactions which may initiate lipid

peroxidation. Therefore the RBC, being a unique carrier of oxygen, is highly susceptible to oxidative stress. It is known that peroxidation of lipids and membrane proteins alters membrane fluidity, ion transport and defensive enzymatic activities in the cell.[21-22]

Radiation was also shown to affect the biochemical structure of the red blood cells membrane. It increases membrane cholesterol level, causes oxidation of membrane protein, thiol groups and lipid peroxidation, and impairment of membrane permeability barrier.[23]

Radiation-induced changes in permeability and membrane elasticity were examined by the osmotic fragility test. Osmotic fragility is considered to be a function of the osmotic pressure gradient between intra and extracellular media, initial surface area to volume ratio, membrane tension of hemolysis and ionic content of the cell.[24] The observed decrease in the dispersion of hemolysis can be attributed to the presence of unusually flattened red cells in which the surface area to volume ratio is increased.[25]

Several studies have been performed on the effect of gamma radiation on blood and red blood cells. They showed that the exposure to gamma radiation produces lipid peroxidation, cross linking in membrane proteins and induces change in the membrane permeability. Other investigators reported gain of sodium and calcium and loss of potassium by the RBCs as a general effect of exposure to ionizing radiation. They stated that radiation can alter the metabolism or active transport (inhibition of ATPase activity) and also may lead to loss of membrane sulphydryl groups.[26]

In comparison to normal control mice, a significant ($p<0.001$) decline in the RBC count, WBC count and Hemoglobin concentration were recorded in lead nitrate exposed mice. The RBC count became lower with lead treatment, due to hemolysis.[27] The decrease in RBC count observed here is also in agreement with other previous reports.[3] The effects of erythrocyte membranes in particular, are analyzed because erythrocytes have a high affinity for lead, and are more susceptible to oxidative damage than other cells.[28] Osmotic susceptibilities of erythrocyte were reported to be increased in lead toxicity accompanied by decreased deformability and a shortened life span.[29] Decrease in WBCs by lead in the present study is in favor with earlier report.[30] Some researchers have observed decrease in hemoglobin concentration in lead treated animals.[31] These findings are in agreement with the results of present investigation.

Lead impairs the rate of incorporation of iron into mature and immature RBC in cases of human lead poisoning. Lead affects the hematopoietic system and reduce the hemoglobin synthesis, but this occurs only with high levels of exposure. It might be due to decrease in heme and globin synthesis or erythrocyte formation and function. Erythrocyte survival also decreases by lead due to inhibition of membrane bound Na+-K+- ATPase.[32-33]

Lead caused a significant decrease in hematocrit, RBC, WBC, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocyte and monocyte count and significant increase in neutrophil count.[34]

A noticeable depletion in hemoglobin concentration in Swiss albino mice was found when exposed to 3.6 Gy gamma radiations.[35] The decrease in the hemoglobin content may be due to the decrease in the number of red blood cells and/or the leakage of RBC or depletion in the synthesis of haemoglobin after radiation exposure.[36] These findings coincided with the result of present investigation.

The haematological analysis revealed a highly significant reduction ($P<0.001$) in haemoglobin concentration from 15.57 (g/dl) in the control mice to 13.02 (g/dl) and 13.27 (g/dl) in the mice exposed to UVC for 30 and 45 d, respectively. Decrease in Red Blood Cells (RBCs) count (from 9.19 million/mm^3 to 8.24 million/mm^3) on day 45 of the experiment was found insignificant. Similar results were described from some studies done with UVA and UVB.[37]

The phytochemicals, such as gallic acid, ellagic acids, emblicanin A and emblicanin B are also reported to possess free-radical-scavenging effects in the 2,2-diphenyl-1-picrylhydrazyl assay and efficacy was as follows: A emblicanin > B emblicanin > gallic acid > ellagic acid > ascorbic acid.[38]

The antioxidant enzymes, superoxide dismutase, GPx, and catalase, cooperate or, in a synergistic method, work to protect cells against oxidative stress. The superoxide dismutase catalyses the dismutation of superoxide radicals, a major form of ROS, into hydrogen peroxide, which is acted on by the GPx and catalase to give water. When an appropriate balance exists between these three enzymes, oxidative stress is reduced and the cells are protected from the cytotoxic and mutagenic effects of the ROS.[39]

Preclinical studies have conclusively shown that amla ameliorates the oxidative and xenobiotic-induced stress, mutagenesis, and carcinogenesis by increasing the anti- oxidant enzymes. Reports suggest that amla increases the antioxidant enzymes and prevents benzo[a]pyrene and cyclophosphamide,[40] DMBA,[41] gamma radiation,[42] hexachlorocyclohexane,[43] and ethanol (Pramyothin *et al*., 2006)-induced toxic effects.[44]

CONCLUSION

Owing to its abundance, low cost, and safety in consumption, amla remains a species with tremendous potential and countless possibilities for further investigation. The outcomes of our studies may be useful for the clinical applications of amla in humans against different cancers and may open up a new therapeutic avenue.

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