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RESEARCH ARTICLE

RADIOPROTECTIVE POTENTIAL OF *ALOE VERA* AGAINST RADIATION AND CADMIUM MEDIATED ALTERATIONS IN DIFFERENTIAL LEUCOCYTE COUNT OF SWISS ALBINO MICE

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Abstract

Haematopoietic system is among the most radiosensitive part in the body and it has a highest cell turn over. *Aloe vera* has been claimed to contain several important therapeutic properties including anticancer effects, antioxidant effects, mutagenesis and immunemodulation. Various studies showed the prevention of radiation induced suppression of immunity by *Aloe vera* components. Having these unique properties, *Aloe vera* could be used in clinical field as a protector against radiation and heavy metal toxicity in human beings.

For the purpose, six to eight weeks old male Swiss albino mice were divided into seven groups. Control groups (II to IV) were administered cadmium chloride at the dose of 20ppm ad libitum and irradiated at the dose rate of 3.5 Gy and 7.0 Gy. Experimental animals (V to VII) were given *Aloe vera* with heavy metal and radiation alone as well as in combination. Five 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. The differential leucocyte count was estimated .

The value of lymphocytes declined up to day-14 in control groups and day-7 in the *Aloe vera* treated groups. The values of monocytes and granulocytes percentage increased up to day-14 in the control animals and day-7 in the drug treated animals. Thereafter, a decrease in the value was noted and continued up to day-28.

INTRODUCTION

Ionizing radiation in interaction with living cells causes a variety of changes depending on absorbed dose, duration of exposure, interval after exposure and susceptibility of tissues. The exposure of mammals to ionizing radiation, such as gamma-radiation, can cause the development of a complex, dose-dependent series of potentially fatal physiological and morphological changes, such as nausea, vomiting, loss of appetite, decreased leucocyte count and weakened immunofunction. Oxidative stress contributes to normal tissue damage during tumor therapy with irradiation. (Sankaranarayanan, 2006 ; Song *et al.*, 2006).

The interaction of ionizing radiation with mammalian cells induces several types of molecular damage to cellular macromolecules and especially to DNA, where it causes single-strand breaks, double-strand breaks, bases damage, cross-linking between DNA and protein and a combination of all of these lesion types. Only DNA double-strand breaks may leads to chromosome aberrations (Belli *et al.*, 2002).

The hematopoietic system is exquisitely sensitive where the acute exposure to radiation causes a sequence of changes that lead to disturbance in the red blood cells function including intravascular hemolysis (Kotb *et al.*, 1990) and decrease in the erythrocytes (John and Gray, 1992).

In more recent times, toxicologists concerned with metal poisoning attempted to elucidate the metabolism and range of effects induced by metals. They can disturb important biochemical processes, constituting an important threat for the health of plant and animals. Plants and animals absorb these elements from soils, sediments and water by contact with their external surfaces, through ingestion and also from inhalation of airborne particles and vaporized metals (Madaan and Mudgal, 2009 ; Mudgal *et al.*, 2010).

The exact mechanisms responsible for the immune toxicity of As, Cd and Pb have not yet been elucidated. It has been reported that lead and cadmium cause the destruction of the cell membrane of human lymphocytes and monocytes (Steffensen *et al.*, 1994). In addition, cadmium and arsenic are able to induce apoptosis of hamster ovary cells and rat testicular tissue (Wang *et al.*, 1996 ; Yan *et al.*, 1997).

Recently interest has increased in the development of potential drugs of plant origin for the modification of radiation effects. Plant extract such as Garlic, Ginseng, *Aloe vera*, Podophyllum, Ocimum sanctum, Amaranthus, Emblica, Spinacea and Mentha have been found to have an advantage over the synthetic compounds in terms of low and no toxicity at the effective dose with minimum side effects (Gupta, 1988; Pande *et al.*, 1998a,b; Goel *et al.*, 1999; Umadevi *et al.*, 2000; Bhatia and Jain, 2003a,b ; Samarth and Kumar, 2003 ;Agarwal *et al.*, 2013).

The *Aloe vera* plant has been known and used for centuries for its health, beauty, medicinal and skin care properties. The name *Aloe vera* derives from the Arabic word "Alloeh" meaning "shining bitter substance," while "vera" in Latin means "true." Two thousand years ago, the Greek scientists regarded *Aloe vera* as the universal panacea. The Egyptians called Aloe "the plant of immortality" (Rai *et al.*, 2011 and Himesh *et al.*, 2011).

The present study was focused on the protection and modulation provided by the *Aloe vera* against radiation and cadmium induced changes in differential leucocyte count.

Materials and Methods

Procurement of animals and their maintenance

For the study, adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar (India). The animals were housed in polypropylene 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.

Cadmium chloride treatment

Cadmium chloride was procured from S.D. Fine Chemicals Private Limited, Boisar (Mumbai). Cadmium, in the form of cadmium chloride was administered orally in drinking water at the dose of 20ppm (Agarwal *et al.*, 2011). 20 ppm solution of cadmium chloride was prepared by dissolving 20 mg of cadmium chloride in 1000 ml. DDW.

Aloe vera extract (AVE)

Fresh leaves of the *Aloe vera* were cleaned, cut into small pieces, air dried, powdered and extracted with double distilled water 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 **(**Gehlot and Goyal, 2007). The drug was given from seven days prior to Cadmium chloride treatment or irradiation.

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. 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.

Experimental Design

In the present study, the animals were grouped as under

Group I : (Sham-irradiated animal-normal)
Group II : (Cadmium Chloride treated animals)
Group III : (Only irradiated animals)
Sub-group IIIa : 3.5 Gy
Sub-group IIIb : 7.0 Gy
Group IV : (Animals treated with radiation and Cadmium Chloride)
Sub-group IVa : 3.5 Gy + $CdCl_2$
Sub-group IVb : 7.0 Gy + $CdCl_2$
Group V : (Animals treated with Cadmium Chloride and *Aloe vera*)
Group VI : (Animals treated with radiation and *Aloe vera*)
Sub-group VIa : 3.5Gy + *Aloe vera*
Sub-group VIb : 7.0Gy + *Aloe vera*
GroupVII :(Animals treated with radiation, Cadmium Chloride and Aloe vera)
Sub-group VIIa : 3.5Gy + $CdCl_2$ + *Aloe vera*
Sub-group VIIb : 7.0Gy + $CdCl_2$ + *Aloe vera*

Autopsy of animals

Five 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. Five 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 differential leucocyte count (DLC).

Hematological Analysis

Certain Haematological parameters were taken into consideration:-
Differential Leucocyte Count:
(i) Lymphocyte percentage
(ii) Monocyte percentage
(iii) Granulocyte percentage

Results

The leucocytes in general showed an initial decline after irradiation in all the experimental groups. The variation in lymphocyte number also showed a similar behaviour, decreasing up to day-14 in the non-drug treated groups and day-7 in *Aloe vera* treated groups, thereafter it increased moderately.

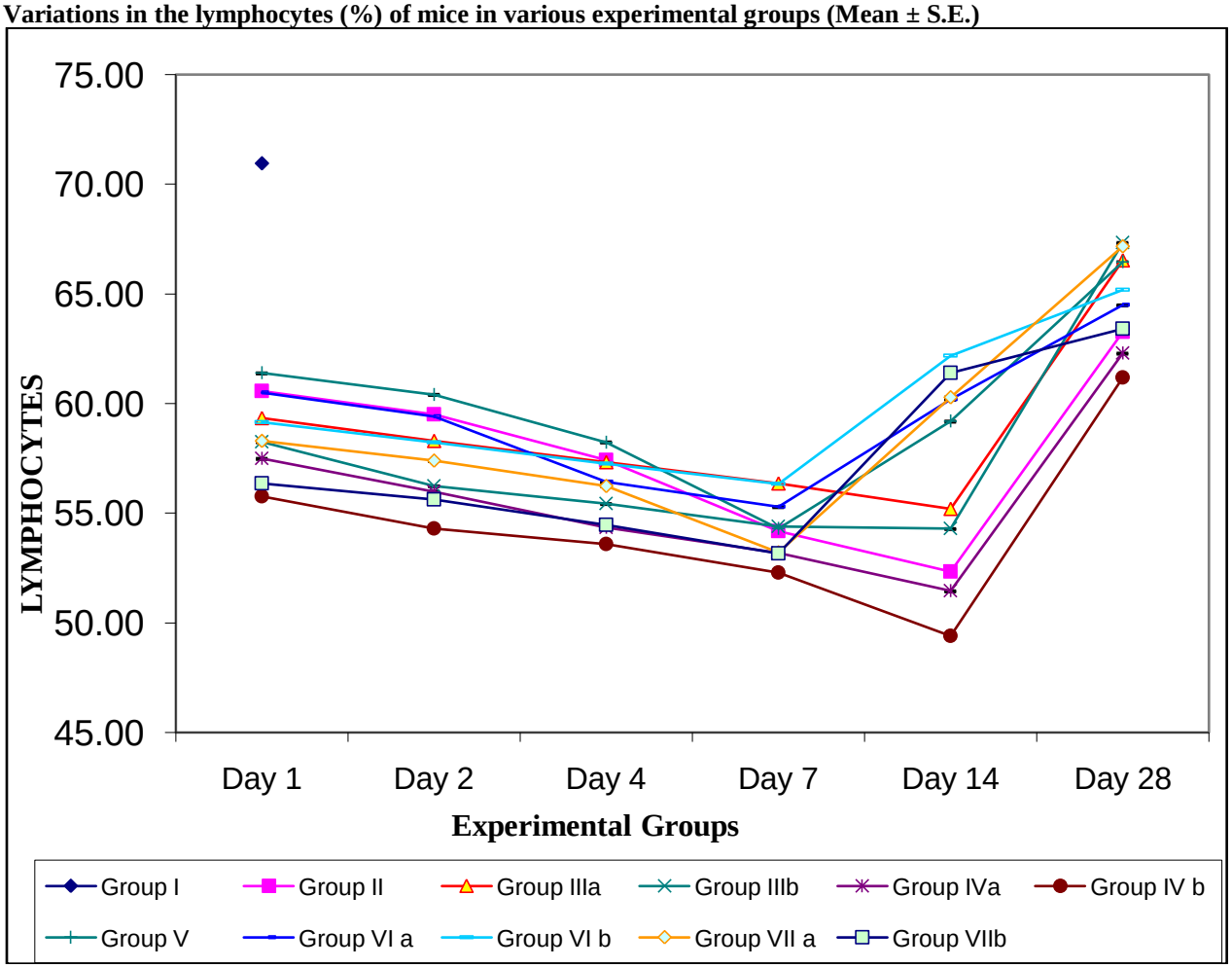
The percentage of monocytes increased up to day-14 in the non-drug treated groups and day-7 in the *Aloe vera* treated groups, thereafter the value decreased up to day -28 without reaching to the normal. The increase in the value was found to be dose-dependent. These findings are upholded by the results of Purohit *et al.* (2007) and Agarwal *et al.* (2011).

The granulocytes percentage increased up to day-14 in the non drug treated animals and day-7 in the drug treated animals. Thereafter, a decreased in the values was noted up to day-28 without reaching to the normal.

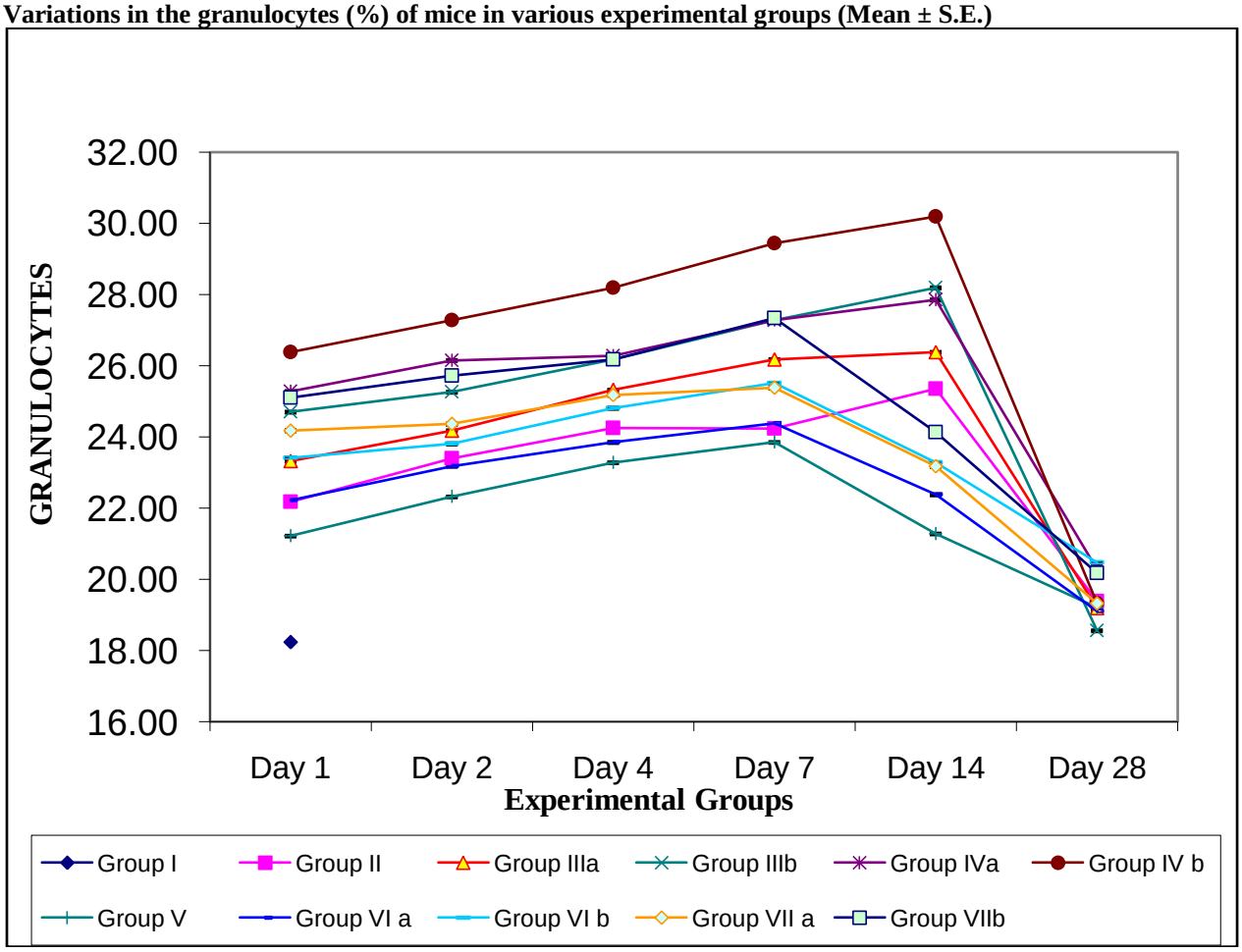
It has earlier been demonstrated that *Aloe vera* gel increases production of lymphocytes and macrophages (McDaniel *et al.*, 1997) and also stimulates lymphocyte cell division (blastogenesis) (Winters, 1991).

The increase in lymphocytes and monocytes counts could be linked mainly to the immune boosting activities of the gel constituents, since they are the major cells of the immune system (Ulbricht *et al.*, 2007).

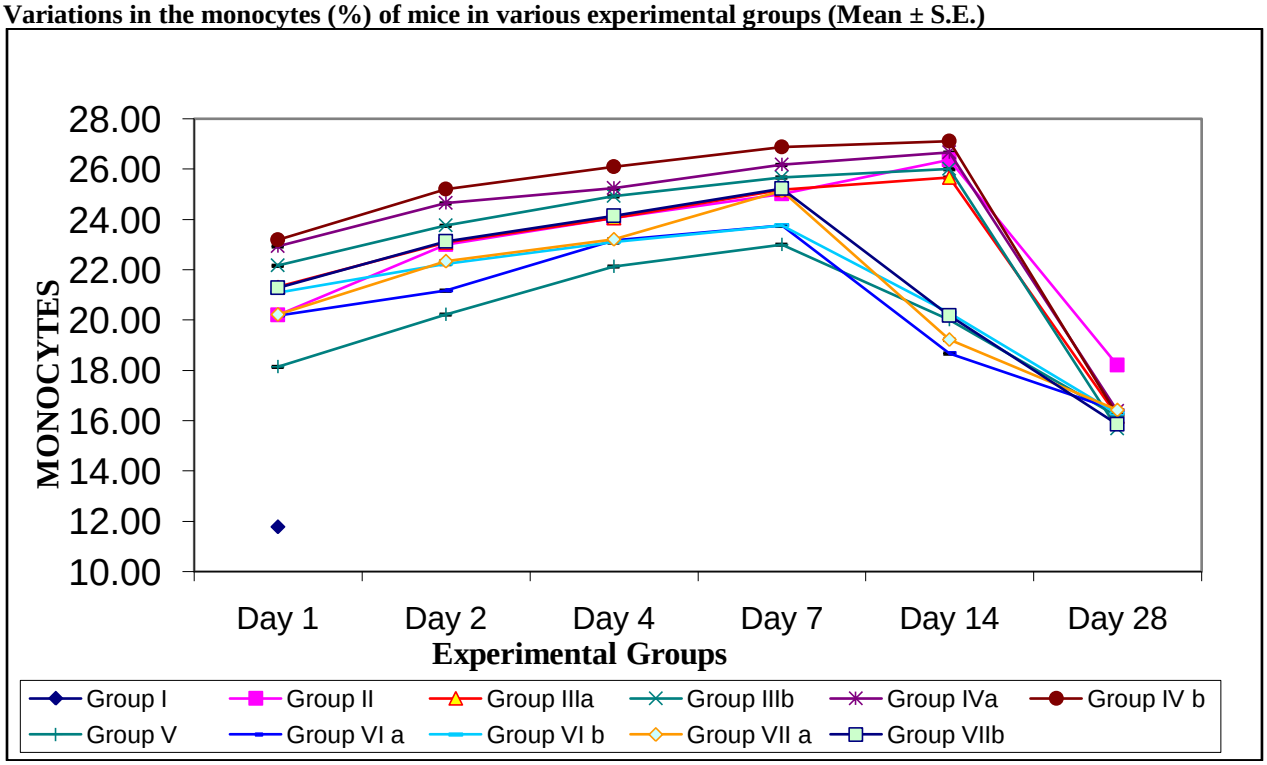
Variations in the lymphocytes (%) of mice in various experimental groups (Mean ± S.E.)



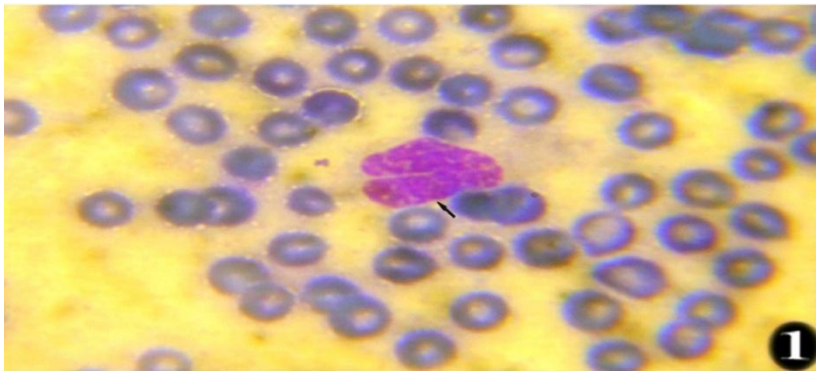
Variations in the granulocytes (%) of mice in various experimental groups (Mean ± S.E.)



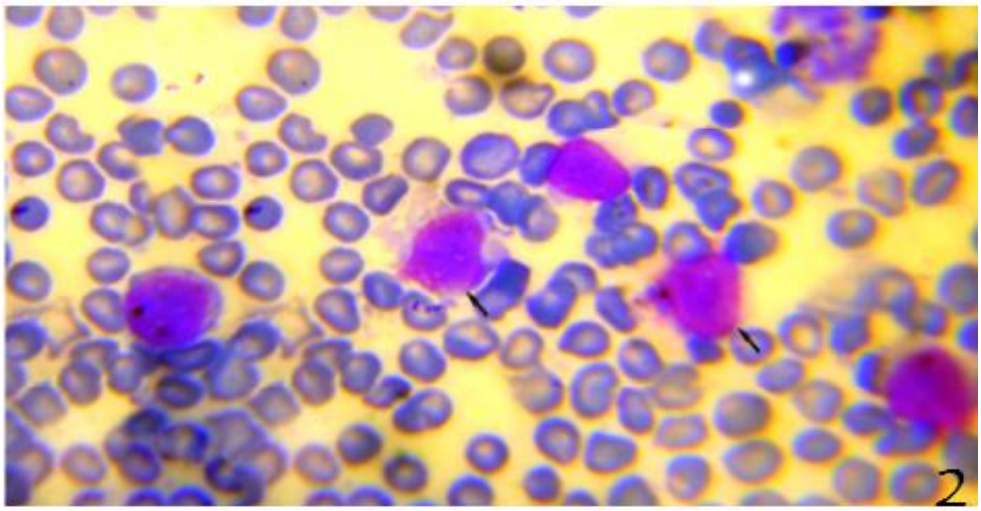
Variations in the monocytes (%) of mice in various experimental groups (Mean ± S.E.)



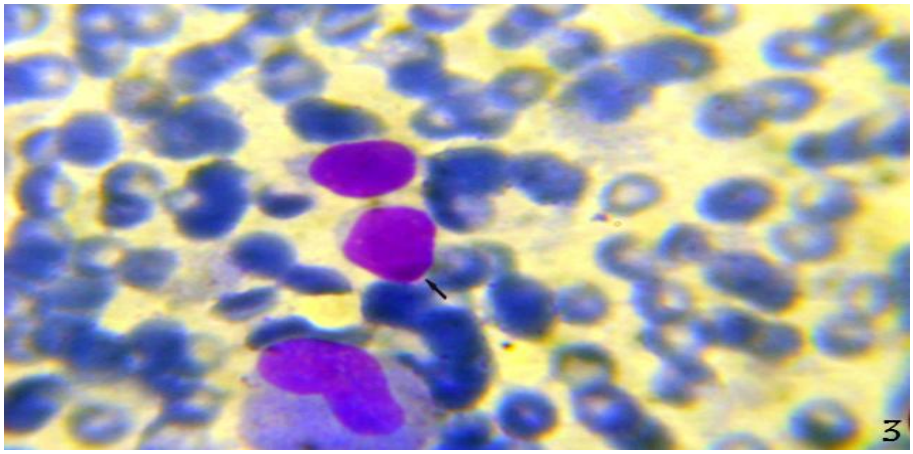
Photographs of Blood Smear



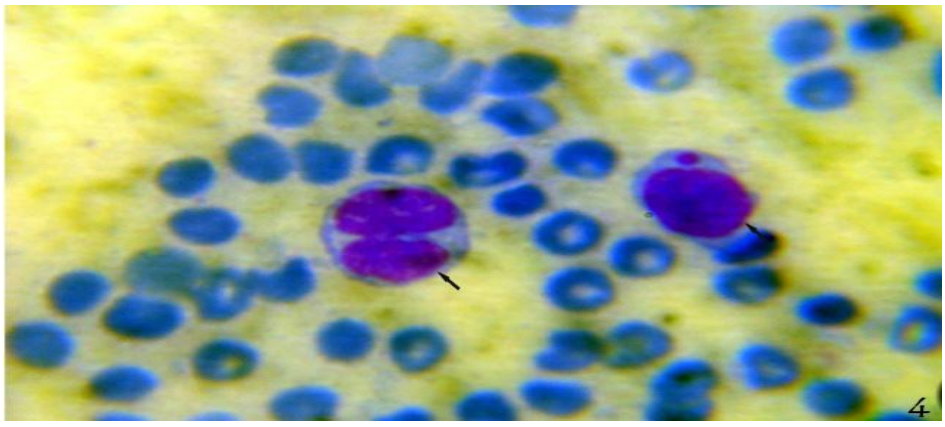
Sham-irradiated group showing normal neutrophil.



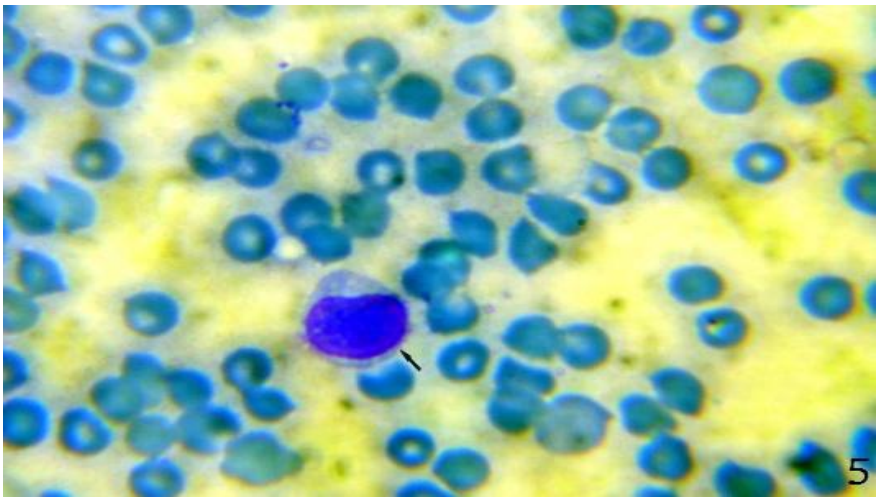
After 4- day of Cadmium chloride treatment displaying some hyperchromic RBCs, distorted and normal monocytes. A reactive lypmphocyte is also seen.



After 1- day (3.5 Gy + cadmium chloride) depicting band neutrophil and reactive lymphocyte.



After 28- day (Cadmium chloride + *Aloe vera*) displaying recovery in RBCs.
Slightly affected lymphocyte with micronucleus and normal eosinophil.



After 7- day (7.0 Gy + cadmium chloride + *Aloe vera*) displaying complete monocyte and normal RBCs.

Discussion

Radiation can have tremendous therapeutic benefits for humans. It is also associated with the risk of serious adverse effects (Borek, 2004 ; Jagetia *et al.*, 2006). It produces reactive oxygen species (ROS) that damage proteins, lipids and nucleic acid (Nair *et al.*, 2001). Haematopoietic system mainly bone marrow is known to be one of the most radiosensitive and its damage may be critical for the survival due to haematopoietic syndrome (Tukov *et al.*, 2002).

Radiation directly affects the cell nucleus causing atomic ionization and indirectly reacts with water molecules to form free radicals that lead to chromosome breakage and genetic damage (Bomford *et al.*, 1993).

The circulating WBCs were affected by fractionated whole body gamma irradiation dose of 6.0 Gy. It is known that lymphocytes are replication components that normally survive in the blood for 2-4 days and are highly radiosensitive (Sado *et al.*, 1998). Radiation induced depletion in lymphocytes is primarily due to apoptosis, although necrotic death occurs (Kajioka *et al.*, 2000).

Lymphocytes have been found to be one of the most radiosensitive mammalian cells (Vanbekkum, 1974). Although irradiation has been shown to alter neither lymphocyte count nor their viability, Wong *et al.* (1979) reported impairment of their function following a 30.0 Gy radiation dose, as evidenced by a decrease in response to phyto haemagglutinin and mixed leucocyte culture. In fact Pelezynski *et al.* (1991) reported that to effectively inactivate lymphocytes in red blood cell units, a minimal gamma radiation dose of 25.0 Gy is required.

The change in neutrophil count was inverse to that of lymphocytes. It increased during first 24 hours, which can be attributed to "abortive" rise in the neutrophils after irradiation (Hulse, 1961 ; Nachtwery *et al.*, 1967). A second peak of neutrophilic elevation was noted on day -14 after irradiation and Jacobson *et al.* (1949) suggested that the first peak can be possibly due to hastening of maturation in bone marrow and for the second peak a mobilization phenomenon in response to radiation-induced tissue injury can be held responsible.

Treatment with cadmium chloride showed a decrease in the rate of white blood cells (WBC) treated with 30 ppm and an increase with 60 ppm. These results are consistent with the results of Anibal *et al.* (2004) who noticed a decrease in B cells by doses of 5.0 and 10 ppm cadmium chloride and increase by 25 ppm, T cells were increased by doses of 25, 50 and 100 ppm cadmium chloride. Distribution of subsets of blood lymphocytes suggested that cadmium chloride prevents immune cell and hormonal response with low doses of the metal used and opposite effects with higher doses.

Cadmium chloride has significant effect on the abnormalities of haematological parameters especially on lymphocytes in normal and control EAC bearing mice. This compound at doses of 1.25 mg/kg, 2.5 mg/kg and 5 mg/kg have been used for the experiment. F values for cadmium chloride indicate that blood chemistry varied significantly with different doses ($P<0.001$). When the normal mice treated with cadmium chloride, the total count of RBC, WBC, Hb, neutrophil and monocytes percentage were decreased only the lymphocytes count were more as compared with untreated normal mice. Whereas in the case of EAC bearing mice the total count of RBC, percentage of Hb and lymphocytes were decreased but the total count of WBC, neutrophil, and monocytes were increased from the normal mice (Sarkar *et al.*, 2013).

Aloe vera gel was observed to decrease the number of neutrophils and eosinophils, this may be due to interference of aloe emodin and aloin (barbaloin) present in the crude gel with processes of activated polymorphonuclear white blood cells (Hart *et al.*, 1998 ; Avila *et al.*, 1997). Although, the mechanism by which this occur in not well understood.

It has earlier been demonstrated that *Aloe vera* gel increases production of lymphocytes and macrophages (McDaniel *et al.*, 1997) and also stimulates lymphocyte cell division (blastogenesis) (Winters, 1991).

The increase in lymphocytes and monocytes counts could be linked mainly to the immune boosting activities of the gel constituents, since they are the major cells of the immune system (Ulbricht *et al.*, 2007).

Conclusion

Persistent consumption of *Aloe vera* gel increases concentrations of lymphocytes, monocyte count as well as granulocyte count in rats. This action of *Aloe vera* extract is probably due to its phytochemical constituents like acemannans that stimulate interleukins and interferons which induces cells growth. It therefore implies that, in human prolonged consumption of *Aloe vera* gel could boost blood parameters and useful for immune-suppressed or cancer patients.

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