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PROTECTIVE EFFICACY OF *Moringa oleifera A*GAINST RADIATION AND MERCURIC CHLORIDE INDUCED HAEMATOLOGICAL CHANGES IN MICE

MURALIDHAR PANCHARIYA[1], POOJA RANI KHATRI[1], R. K. PUROHIT[1*], MANISHA AGARWAL[1], ARUNA CHAKRAWARTI[1] AND DEEPAK MITTAL[2]

[1]Department of Zoology, Radiation Biology Laboratory, Govt. Dungar College, Bikaner (Raj), India.
[2]Department of Zoology, SSSUTMS, Sehore (M.P.), India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author MP designed the study, performed the statistical analysis. Author PRK wrote the protocol. Author RKP managed the analysis of the study. Author MA managed the literature searches. Author AC looked after the ethical issues and author DM critically analyzed the whole paper. All authors read and approved the final manuscript.

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ABSTRACT

The present study was planned to investigate the impact of the *Moringa oleifera* against combined administration of mercuric chloride and radiation on haematogical changes in mice. The mice were exposed to mercuric chloride (0.5 ppm) and gamma radiation (2.0Gy) simultaneously and individually. The experimental groups were given *Moringa oleifera* seven days prior to radiation or mercuric chloride treatment. The changes included increase or decreae in the various heamatological parameters viz. RBC, WBC, Hb, MCH, MCHC etc. In the combined treatment groups the changes were more severe showing synergistic effect. An early and fast recovery in *Moringa* pre-treated groups may be due to the protection provided by the drug.

Keywords: Radiation; mercuric chloride; *Moringa oleifera*; mice.

1. INTRODUCTION

The threat of nuclear exposure is heightened and it is imperative to identify potential countermeasures for acute radiation syndrome. Currently no countermeasures have been approved for prophylactic administration. Effective countermeasures should function to increase survival in the short term as well as to increase the overall prognosis of an exposed individual long term. The detrimental effects of ionizing radiation involve a highly orchestrated series of events that are amplified by endogenous signaling and culminating in oxidative damage to DNA, lipids, proteins, and many metabolites. Despite the global impact of IR, the molecular mechanisms underlying tissue damage reveal that many

**Corresponding author: Email: dr_rajendra_purohit@yahoo.co.in;*

biomolecules are chemo selectively modified by radiations.

Heavy metals are metallic elements which have a high atomic weight and a density much greater than water. There are more than twenty heavy metals, but four are of particular concern to human health; Lead (Pb), Cadmium (Cd), Mercury (Hg) [1,2] and inorganic Arsenic (As)According to the U.S Agency for Toxic Substances and Disease Registry, these four heavy metals are four of the top six hazards present in toxic waste sides They are highly toxic and can cause damaging effects even at very low concentrations.

Mercury poisoning (also known as hydrargyria or mercurialism) is a type of metal poisoning and a medical condition caused by exposure to mercury or its compounds. Mercury (chemical symbol Hg) is a heavy metal occurring in several forms. All of these, except elemental liquid mercury (for which intravenous injection of a certain volume) produce toxicity or death with less than a gram. The damage done by elemental mercury is caused by blocking blood vessels.

In the present investigation, toxicological research efforts would do well to first establish the safety of consumption of the plant products and to the various extents; as this will be vital to the establishment of the use of the plant's products as standard nutritional supplements and natural or bio-medicinal products. To this end, we employed histological methods to evaluate the effects of *Moringa* leaf extracts on vital body tissue organs. The rationale is that histological methods of observations would provide a more reliable and consistent picture of the effects produced by the interactions of the photochemical with the body cells and tissue better than in vitro tests and analysis of the highly dynamic biochemical activities as contained in extracted tissue fluids. Also, the use of Histological methods of assessment of *Moringa* leaf extract effects on blood tissues is important because literatures are comparatively scarce on such methods of investigation of the plant's extracts' effects.

Moringa leaf extract at their estimated sub-lethal dosages were relatively safe for tested body organs. The aim of this particular investigation was to observe the effects of *Moringa oleifera* leaf extract on the haematological parameters of mice.

Exposure to ionizing radiation is known to have lethal effects in blood cells. It is predicted that an individual may spend days, weeks or even months in a radiation field without becoming alarmed. The study aimed to discuss the evaluation of low dose ionizing radiation (IR) effect on some blood components in animal model. Hematological parameters were determined for 110 animal rats (divided into 8 groups) pre- and post-irradiation. An attempt to explain the blood changes resulting from both irradiation and time is given. There was a significant reduction in WBC counts one day after irradiation at all dose levels compared to the control group and started to be affected at the dose of 0.3 Gy. The significance was increasing with increasing the dose [3].

Blood is a body fluid in humans and other animals but delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from that same cells. It is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55 per cent of blood fluid, is mostly water, and contains proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood.

The blood cells are mainly red blood cells (also called RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes) and platelets (also called thrombocytes). The most abundant cells in vertebrates' blood are red blood cells. These contain hemoglobin, an iron containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is mostly transported extra cellular as bicarbonate ion transported in plasma. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood. Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled. The immature cells and cells in active division are especially sensitive to irradiation and mercury, hence it responds rapidly to injury. The damage of blood is of particular primarily concern in cytotoxic therapies involving radiation exposure or chemical agents and in accidental release (e.g. Chernobyl).Various drugs have been used as chemoprotective like WR-2721, MPG etc. against radiation and heavy metals induced changes in intestine which are toxic at their effective dose levels.Therefore, present study was hypothesized to study the protective role of *Moringa oleifera* against radiation and mercury induced hematological changes in *Mus musculus*.

2. REVIEW OF LITERATURE

The blood cell (White and Red cell) counts of Wistar rats fed with ethanolic extract of *Moringa oleifera* leaves were evaluated. For the study, Wistar rats (18) were divided into three groups of 6 each: Group 1 served as control (not given extract), Group 2 was fed with 200mg per kg (of body weight) of the leaf extract for 15 days, and Group 3 was fed with 300 mg per kg (of body weight) of the leaf extract for the same duration. The results showed a significant increase ($P<0.05$) in both red and white blood cell counts of rats in groups 2 and 3 as compared to the ones in the control group (group 1) in both low and high doses. They concluded that 200 mg per kg and 300 mg per kg of *Moringa oleifera* leave extract contain active ingredients required for the formation and maturation of blood cells (red and white blood cells), hence the increase in the blood cells counts [4].

The combined effects of chemicals and radiation have mostly been studied on unborn babies because of their high sensitivity to these toxicants. The general aspects of the interaction between radiation and chemicals during prenatal development were summarized in the UNSCEAR Report [5] and later reviewed by other workers [6] on the interaction between ionizing radiation and lead, actomyosin D, phenols, sodium nitrate and caffeine etc.

Moringa oleifera is a tree that is exceptionally rich in medicinally relevant and immune modulating nutrients and phytochemicals. The experiment was planned to determine whether tea prepared from dried *Moringa oleifera* leaves influenced acute stress induced changes to the levels of circulating lymphocyte and neutrophils. Balb/c mice were offered either water or *Moringa* tea *ad libitum* for four days and then randomly selected for either restraint for 1h or 12 h to induce stress or left alone (unstressed). Post stress the levels of circulating neutrophils and lymphocytes in the blood were determined by differential cell staining. While, mice that consumed water had significant ($p<0.05$) increases in circulating blood neutrophils after 1h of restraint, the level of neutrophils in the mice dosed with tea remained unchanged. Post 12h of restraint stress significant ($p<0.05$) increases in neutrophil levels were observed in mice that consumed tea but not in mice that consumed water. In the case of lymphocytes, consumption of *Moringa* tea had no effect. Compared to unstressed mice, 1 h or 12 h of restraint stress resulted insignificant ($p<0.05$) decreases in circulating lymphocytes of similar magnitude in both the water and *Moringa* tea groups. Flow cytometric analysis showed no significant differences in CD3+ T cells or CD19+ B cells levels. These data suggested that consumption of the *Moringa* tea can influence stress induced variations to circulating neutrophils but not lymphocytes [7].

The effects of dietary *Moringa oleifera* leaf (MOL) on reproductive performances in mice have also been investigated by other workers [8]. The reproductive performance of mice for six consecutive gestations was studied. Mice fed with 4% MOL diet showed improved litter size, litter birth weight, and litter survivals until weaning age compared to control mice fed with normal diet ($p < 0.05$). Mice fed with MOL diet did not change weight and organ coefficients. Serum malondialdehyde (MDA) concentrations in both male and female mice were significantly decreased by dietary MOL ($p < 0.05$), but glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) were unchanged. For male, dietary MOL significantly reduced sperm abnormality rate ($p < 0.05$) and Bcl2-associated X protein (Bax) expression in testis ($p < 0.05$), but did not affect serum testosterone and the expression levels of androgen receptor (AR), phosphoglycerate kinase 2 (Pgk2), protamine2 (Prm2), and B cell leukemia/lymphoma 2 (Bcl2) in testis. For female, dietary MOL did not change serum estradiol and the expressions of estrogen receptor beta (ERβ), Bcl2, Bax, and vascular endothelial growth factor receptor (VEGFR) in ovary. In summary, MOL increased litter size and antioxidant ability, reduced the rate of sperm abnormality and the expression of Bax. Therefore, MOL may serve as a functional feed addictive for improving animal reproductive performance.

3. MATERIALS AND METHODS

Six to eight weeks old male Swiss albino mice were procured form an inbred colony maintained in animal house of C.C.S University, Hissar. The animals were kept in the polypropylene cages in the departmental animal house. The standard mice feed and water were provided *ad libitum*. The temperature of the animal house was maintained between 20-25ºC.

The animals used in the experiment will be irradiated at the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan) by Theratron, a Cobalt-60 beam therapy unit which was a source, procured from Atomic Energy Agency Ltd., Canada.

All the mice were exposed to Co^{60} γ-radiation simultaneously in a well ventilated wooden box of size 30 cm x 30 cm x 5cm having a glass lid. The box was placed at a distance of 75cms from the radiation source. 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.

3.1 *Moringa oleifera*

The dried powder of *Moringa oleifera* was procured from the Umalaxmi organics private limited, Jodhpur (Raj.) and aqueous extract of the same was obtained in the department. Extracts obtained from leaves of *Moringa oleifera* are a rich source of many bioactive compounds: flavonoids, phenolic acids or carotenoids. It also contains such components as, vitamins (A, C, niacin, pantothenic acid), alkaloids, tannins or saponins.

The plant extract of *Moringa* was fed orally at the dose of 150 mg/kg body weight. The *Moringa* extract was given daily from seven days prior to individual or combined treatment of mercuric chloride and radiation and continued up to the last autopsy interval.

3.2 Mercury

In the present study, mercury in the form of mercuric chloride, manufactured by Ranbaxy Laboratories Ltd**.,** will be used. Mercuric chloride was given in the drinking water at the dose of 0.5 ppm.

3.3 Experimental Design

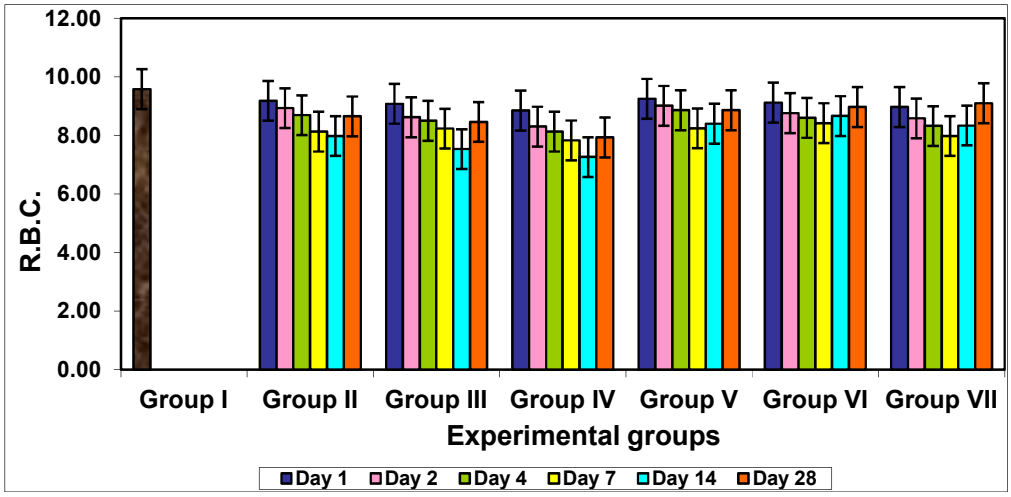
For the study the mice were divided into seven groups. The animals of group first were Sham-irradiated and served as normal. The group second were treated with mercuric chloride at the dose of 0.5 ppm. The animals of group third were exposed to 2.0 Gy of gamma radiation from Cobalt-60 source. The group fourth included combined treatment of radiation and mercuric chloride. The animals of group fifth were orally fed with mercuric chloride solution at the dose of 0.5 ppm and were also administered *Moringa* orally for seven days at a dose of 150 mg/kg body weight prior to mercuric chloride treatment and was continued up to the last autopsy interval. The group sixth included 2.0 Gy radiation exposed and *Moringa* treated mice. The group seventh animals were treated with treated with radiation, mercuric chloride and *Moringa.* The experiments lasted up to one month.

The parameters included were

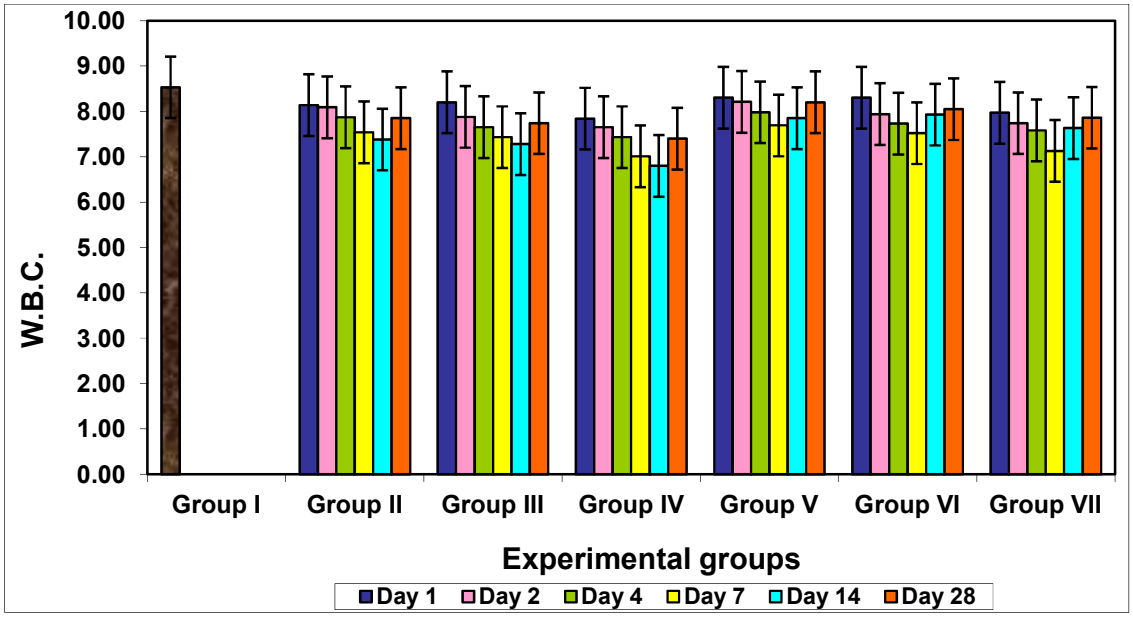
1. Red blood corpuscles (RBC)
2. White Blood corpuscles(WBC)
3. Haemoglobin (Hb)
4. Packed cell volume(PCV)
5. Mean cell volume(MCV)
6. Mean corpuscular Haemoglobin(MCH)
7. Mean corpuscular Haemoglobin concentration (MCHC)
8. Differential leucocyte count (DLC)

3.4 Observations

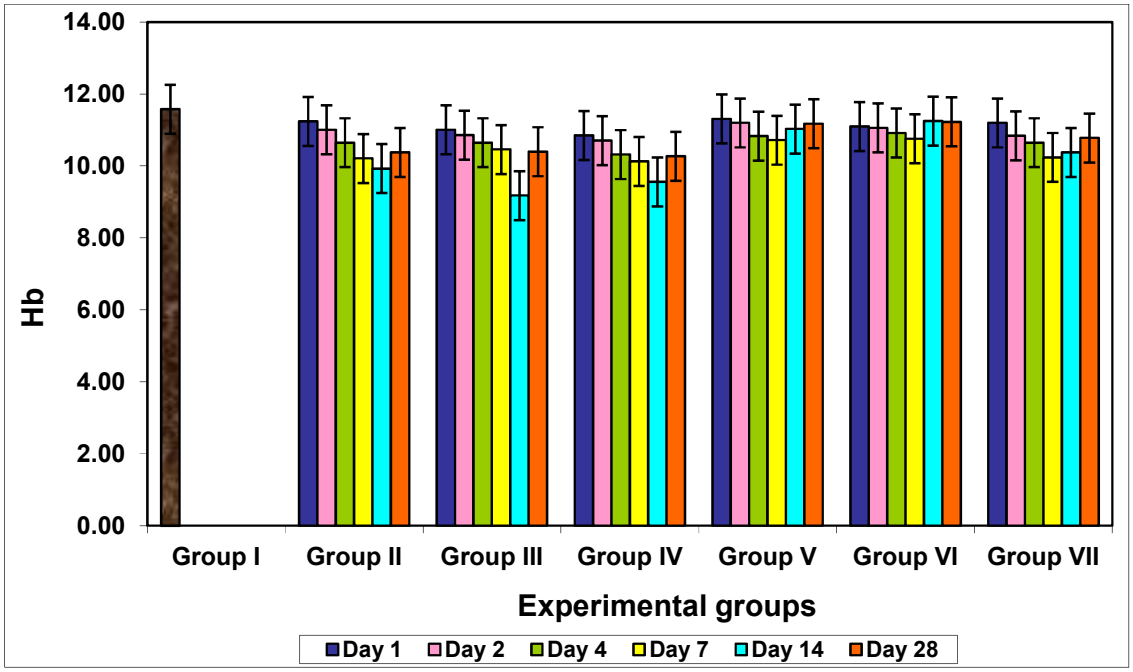
The values of RBC, WBC, Hb and PCV were found to decrease in all the groups as compared to normal group, but the decrease in these values was lesser in *Moringa oleifera* treated groups (V to VII) as compared to non-drug treated groups (II to IV). The values of MCV were also found to decrease but the difference from normal value was significant at previous intervals and it was significant on later intervals. The values of MCH increased in all the groups as compared with normal group after 1, 2, 4, 7, 14 and 28 days of post-treatment intervals.



Histogram 1. Variations in the R.B.C. (million/cu.mm) of mice in various groups



Histogram 2. Variations in the W.B.C. (thousand /cu.mm) of mice in various groups



Histogram 3. Variations in the Hb (gms/100ml. of blood) of mice in various groups

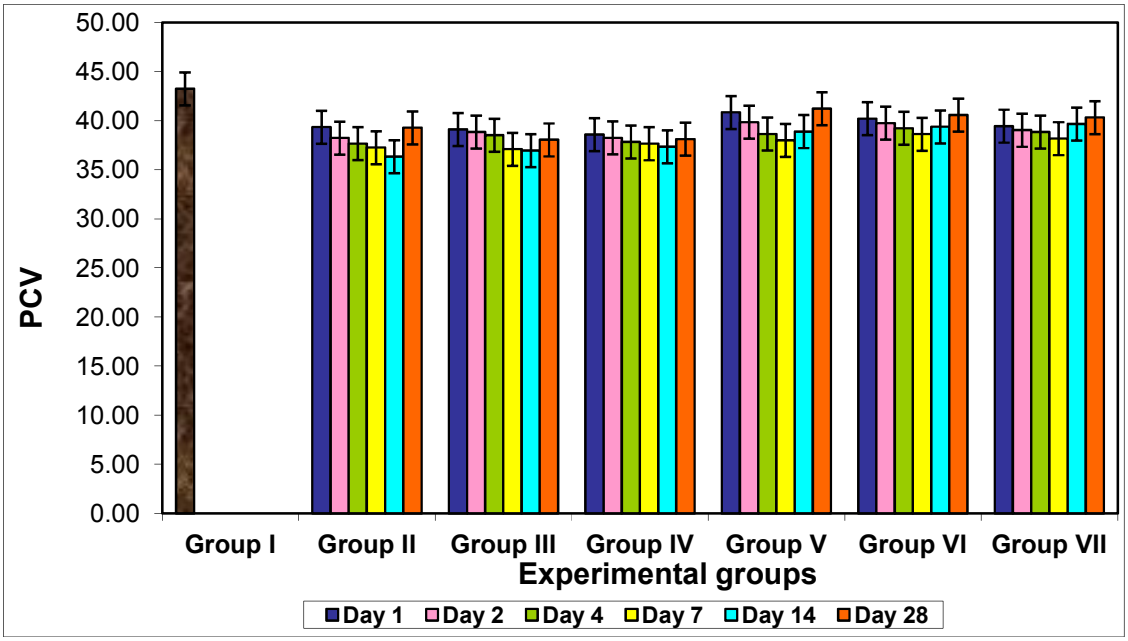
The increase in the value of MCH was lesser in *Moringa oleifera* treated groups (V to VII) as compared to non drug treated groups (II to IV). Besides this values of MCHC increased in all the groups at various intervals but the values were lower in the *Moringa oleifera* treated groups (V to VII) as 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 upto day-14 in non drug treated groups and day-7 in the *Moringa*

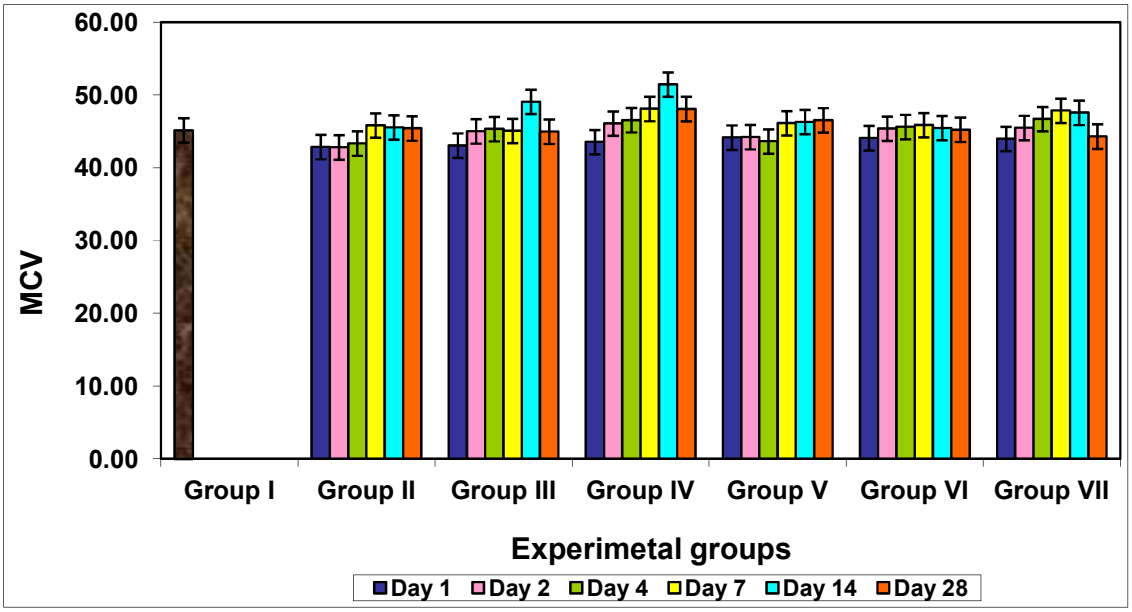
oleifera treated groups. Similarly the values of monocytes and granulocytes percentage increased up to day-14 in the non drug treated animals and day-7 in the drug treated animals thereafter, a decrease in the value was noted up to day-28 without reaching to the normal.

4. DISCUSSION

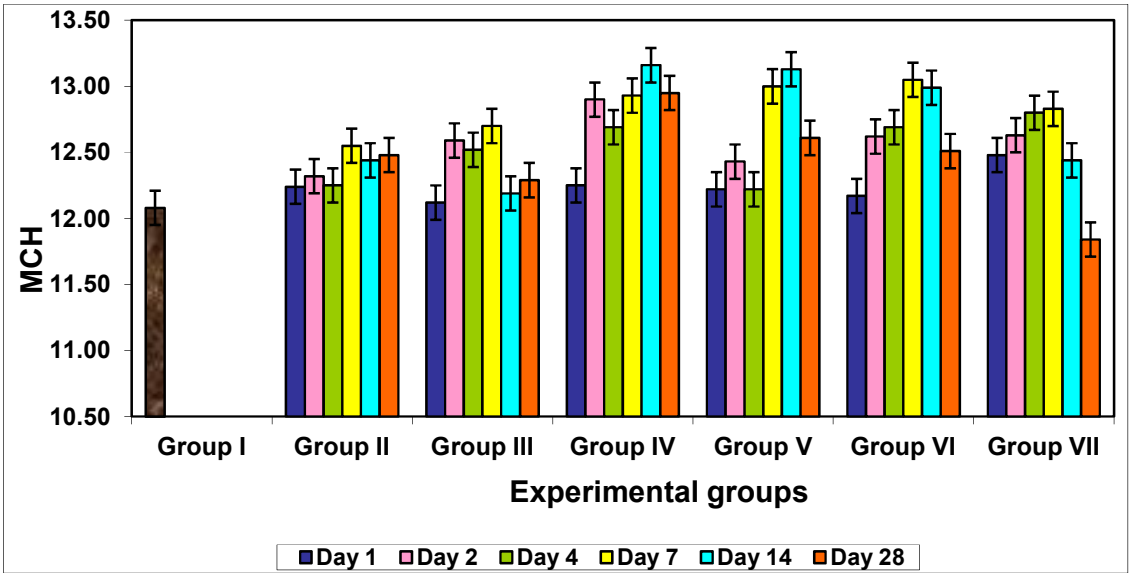
The present study is an attempt to understand the toxicity and the potential role of gamma-radiation as a therapeutic tool, the effects of different Gamma-radiation doses on haematological and dimensional



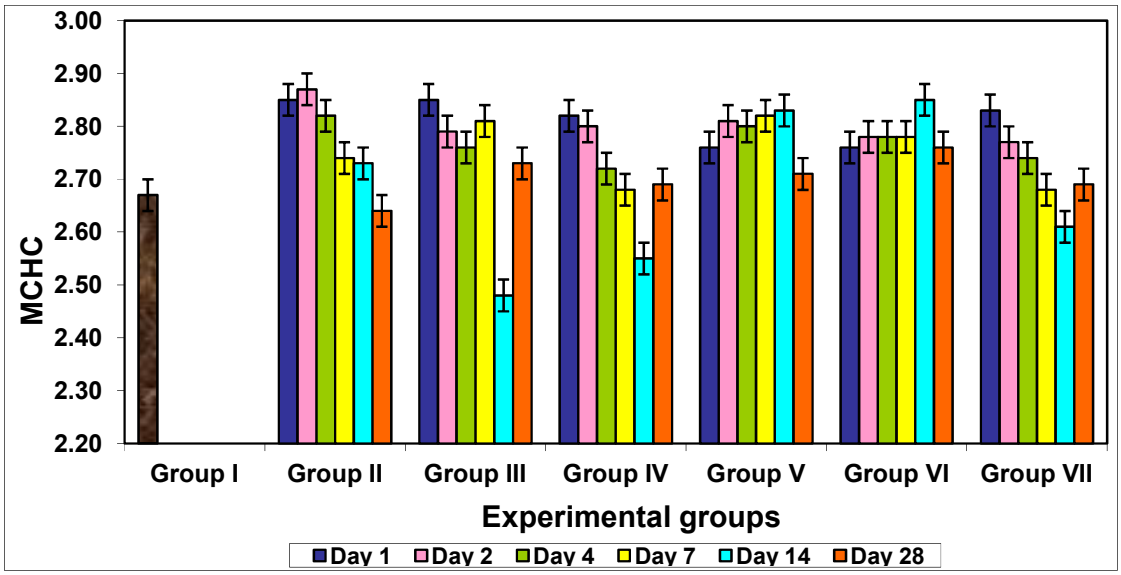
Histogram 4. Variations in the PCV (%) of mice in various groups



Histogram 5. Variations in the MCV (cubic micron) of mice in various groups



Histogram 6. Variations in the MCH (micro micro gms) of mice in various groups



Histogram 7. Variations in the MCHC (%) of mice in various groups

properties of rats blood were investigated. A significant decrease in red blood cells (RBCs) count, haemoglobin (HGB), and haematocrit (HCT) was observed compared with the control. While a significant increase in mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), red distribution width (RDW) were observed compared with the control. This study suggested that low RBCs, HGB, and HCT might produce anemia and cessation of erythrocytes production in the bone marrow. Moreover, the RBCs size increase might be attributed to changes in the morphology and deformability of RBCs. After combined treatment of radiation and mercuric chloride the changes were more severe showing synergistic or additive effect. An early and fast recovery in the *Moringa* treated group may be due to the protection provided by the herbal extract.

4.1 Protective Mechanism of *Moringa oleifera*

In the present study, inhibition of LPO in biomembranes has been caused by antioxidants present in *Moringa oleifera*. Under normal conditions, the inherent defense system like glutathione protects against oxidative damage [9]. GSH is a versatile protector and executes its radio protective function through free radical scavenging, restoration of the damaged molecules by hydrogen donation or by reduction of peroxides and maintenance of thiols in the reduced state. The decrement of GSH could be due to an enhanced utilization of the antioxidant system during detoxification of the free radicals generated by radiation. This depletion of glutathione further enhanced the lipid peroxidation [10]. The present study clearly demonstrated that *Moringa* leaf extract protected blood from radiation induced lipid peroxidation in entire study period. The result also showed that *Moringa* leaf extract quenched the hydroxyl radical which is the key mediator of lipid peroxidation. Moreover,it was shown that *Moringa* leaves possess variety of phytochemicals such as ascorbic acid, phenolics etc. It has also been revealed that *Moringa* leaf extract contains a range of important antioxidant molecules such as catechin, epicatechin, ferulic acid, ellagic acid and myricetin.

5. CONCLUSION

Moringa oleifera have both ameliorating and protecting potentials against radiation and mercury chloride induced damage in the blood of mice through regulation of antioxidant, anti-apoptotic, and inflammatory properties. *Moringa oleifera* leaf may be considered as a promising radiopreventive agent especially for the nuclear workers or for defense personals assuming possibility of nuclear exposure. The present study showed the capability of a vegetable phyto extract against the whole body radiation generated systemic stress in the mammalian model. The leaf extract efficiently prevented the lipid peroxidation and restored the GSH levels, two immediate markers of radiation stress. This popular vegetable may also be used as supplement for the patients who undergo total body irradiation during different clinical maneuvers such as bone marrow transplantation, to prevent higher levels of lipid peroxidation. However, this needs further investigation before clinical trials.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

The study was approved by animal ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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