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DEFENSIVE IMPACT OF MORINGA OLEIFERA BESIDE THE RADIATION AND MERCURY INDUCED HAEMATOLOGICAL TRANSFORMATION IN MICE

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Abstract: *Radiation induced mutilation and lethality to the ordinary tissues can be moderately condensed by the usage of radio-protectors that worse down the destructive effects of radiation. In current years, widespread investigation work has been carried out on chemical fortification contrary to radiation and heavy metals induced harmfulness. A number of artificial chemicals have been tested for their radio-protective accomplishment in animals but their practical applications is initiated to be limited in several fields owing to their great toxicity at their optimum dosage levels. Moringa oleifera (Family: Moringaceae) is a valuable tropical and sub-trobical plant. Moringa are a good source of protein, amino acids, vitamins (A, B1, B2, B3, C, and E), minerals (calcium, iron, phosphorus, magnesium), phytochemical compound (alkaloids, glycosides, sterols, flavonoids, saponin, tannins and various phenolics. In the present study protective influence of Moringa oleifera against radiation mercuric chloride induced changes in mice have been taken into consideration. The present work, is planned to explore the impact of Moringa Oleifera against radiation and mercury induced hematological transformation in Mice. We use Swiss Albino mice for experimental study which is exposed to 2.0 Gy of gamma rays with or without mercuric chloride ($HgCl_2$) treatment. Moringa leaves is given for the treatment to mice for seven days prior to radiation or $HgCl_2$. The escalation or diminution in the value of serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) value may be due to the fortification provided by the drug.*

Keywords: *Moringa Oleifera, Mercuric Chloride, SGPT, SGOT, Induced Radiation, Haematological.*

1. Introduction

Outside of nuclear power and nuclear weaponry, there remains a wide array of ways in which radioactive material and the radiation it gives off remain useful in the daily lives of people all over the world. Radiation is used in medicine, academics, and industry, as well as for generating electricity. In addition, radiation has useful applications in such areas as agriculture, archaeology, space exploration, law enforcement, geology including mining, and many others. People are exposed to natural sources of ionizing radiation, such as in soil, water, and vegetation, as well as in human-made sources, such as x-rays and medical devices. Ionizing radiation has many beneficial

applications, including uses in medicine, industry, agriculture and research. All high-energy radiation, whether particulate or electromagnetic are capable of inducing excitation or ionization of atoms or molecules of both living and non-living matter. However, the diagnostic and therapeutic use of radiations is of prime importance and hence their effects specially on haematopoietic and other systems has attracted the interest to minimize them by using some agents.

The discovery of chemical protection stimulated the interest among radiobiologists to find out its practical applications. But unfortunately the initial interest in the radioprotective compound was soon followed by disappointment because of their higher toxicity. Various chemicals, like Cysteamine [2], MPG, WR-2721 [1] have been tested for the protection of mice against harmful effects of radiation. These compounds are highly toxic at their effective dose levels except MPG and hence attempts were made to find out a non-toxic agent, which can minimize the deleterious effects of radiations. Secondly their availability and cost restricted the uses of radio-protectors so far studied.

Blood is an essential component of the circulatory system and the body at large. It is a fluid connective tissue that performs many functions in the body including regulating and/or transporting medium, maintains its constituents within defined physiological normal range and keeps the body alive. It includes erythrocytes, leukocytes and thrombocytes suspended in plasma. But due to the incidence of poverty and malnutrition and other environmental factors in Africa and other developing countries, some of these disease conditions, especially anaemia, affecting mostly ladies and kids.

An explosion of knowledge has occurred regarding molecular and cellular pathways that trigger and mediate hematologic responses to ionizing radiation. In addition to damaging DNA, ionizing radiation alters gene expression and transcription, and interferes with intracellular and intercellular signaling pathways. The clinical expression of these disturbances may be the development of leukemia, the most significant hematologic complication of radiation exposure among survivors of the atomic bomb detonations over Japan. Those at greatest risk for leukemia are individuals exposed during childhood [3].

The association of leukemia with chronic, low-dose-rate exposure from nuclear power plant accidents and/or nuclear device testing has been more difficult to establish, due in part to lack of precision and sensitivity of methods to assess doses that approach background radiation dose. Nevertheless, multiple myeloma may be associated with chronic exposure, particularly in those exposed at older ages. Life forms developed and evolved on earth in a radiation field that was much stronger than that existing today. Nevertheless, experimental exposure to ionizing radiation has been long known to be associated with changes in hematopoietic tissue and sometimes death [4].

Mainly heavy metals have a high atomic number and atomic weight. However heavy metals are considered as the oldest known toxic material harmful to human. Heavy metal toxicity remains a very general subject due to the variety of symptoms caused by heavy metal poisoning. Mercury is the most commonly found metals correlated with harmful effects to human due to its accumulation in human body caused through any dietary products like as lead affects the kidney disorders (high blood pressure levels).

2. Moringa Oleifera

Moringa oleifera (Family: Moringaceae) is a valuable tropical and sub-trobical plant. It is widely distributed in many countries of Africa, Arabia, India, South Asia, Latin America and Himalayas. The plant is referred to number of names such as miracle tree, ben oil tree, horse radish tree and drumstick tree. *Moringa* are a good source of protein, amino acids, vitamins (A, B1, B2, B3, C, E), minerals (calcium, iron, phosphorus, magnesium), phytochemical compound (alkaloids, glycosides, sterols, flavonoids, saponin, tannins and various phenolics. All the parts of this plant have medicinal and therapeutic uses. The seeds of *Moringa* contain 38-40 per cent oil (ben oil) can be used for cooking, in soaps and perfumes. Seeds are extensively used for liver, renal, hematological, cardiovascular diseases, treating inflammation and used as antidiabetic. *Moringa* seeds having efficacy in purification by flocculation of contaminants in drinking water [5].

Panchariya *et al.* (2021) also evaluated the protective influence of *Moringa oleifera* against radiation and mercury induced changes in the blood of mice. They observed synergistic effect after after combined exposure. They further mentioned that *Moringa* pre-treated mice showed less severe changes which may be due to the protective effect of the drug.[6]

No systematic work has appeared so far on the use of *Moringa* for its protective role against heavy metal like mercury intoxication or the combined use of such metal and radiation. Hence, the present study was planned to evaluate the possible prophylactic role of *Moringa* against the changes caused by radiation and mercury in the blood of mice.

3. Material and Method

The mice were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. It is pertinent to mention that the Govt. Dungar College, Bikaner has its own Institutional Animal Ethics Committee (IAEC) and is registered under CPCSEA, New Delhi.. The animals were maintained on balanced mice feed and tap water *ad libitum*. The room temperature was kept between 22-27ºC. The animals were irradiated at Acharya Tulsi Cancer Hospital and Research Centre at the dose rate ranging from 0.75 Gy/min to 1.75 Gy/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. The mercury salt in the form of mercuric chloride of analytical grade was used for the present study. It was purchased from Ranbaxy Laboratories Ltd., India. It was administered orally in drinking water at the dose of 0.5ppm.

The animals for the experiments were divided into the following groups –

GROUP1: (SHAM-IRRADIATED ANIMALS-NORMAL)

The sham-irradiated mice of this group were treated as normal.

GROUP II: (MERCURIC CHLORIDE TREATED ANIMALS)

The mice of this group were administered mercuric chloride solution at the dose of 0.5 ppm *ad libitum* in drinking water. The solution was given till the day- 28.

GROUP III: (ONLY IRRADIATED ANIMALS)

Sub- group III a: 2.0 Gy

Sub- group III b: 4.0 Gy

GROUP IV: (ANIMALS TREATED WITH RADIATION AND MERCURIC CHLORIDE)

Sub- group IV a: 2.0 Gy + Mercuric chloride

Sub- group IV b: 4.0 Gy + Mercuric chloride

GROUP V:(ANIMALS TREATED WITH MERCURIC CHLORIDE AND *MORINGA OLEIFERA*)

The animals of this group were orally fed mercuric chloride solution at the dose rate of 0.5 ppm and were also administered *Moringa oleifera* orally for seven days at a dose of 150 mg/kg body weight prior to Mercuric chloride treatment and continued up to the last autopsy interval.

GROUP VI :(ANIMALS TREATED WITH RADIATION AND *MORINGA OLEIFERA*)

Sub- group VI a: 2.0 Gy+ *Moringa oleifera*

Sub- group VI b: 4.0 Gy+ *Moringa oleifera*

GROUP VII: (ANIMALS TREATED WITH RADIATION, MERCURIC CHLORIDE AND ALOE VERA)

Sub -group VII a: 2.0 Gy + Mercuric chloride + *Moringa oleifera*

Sub –group VII b: 4.0Gy + Mercuric chloride + *Moringa oleifera*

4. Results

The present study evaluated the effect of crude extract of M. oleifera on radiation and mercury-induced toxicity and the results of the present investigation revealed that treatment with the Moringa leaves significantly protect animals from the toxic effects of both radiation and mercury. From the ancient time, Moringa leaves are used as vegetable in Indian subcontinent. Due to the deleterious effects of mercury on human body, there is an increasing interest in the development of preventive therapy for reducing mercury toxicity in human. Moringa leaf is a safe natural antioxidant containing vegetable and is found as a potential source of four natural antioxidants such as total phenolic antioxidant, vitamin A, C, and E.

The value of total proteins, glycogen, cholesterol, DNA and RNA decreased up to day-14, thereafter it increased on day-28, whereas the value of acid phosphatase activity and alkaline phosphatase activity increased up to day-14, thereafter it decreased on day-28 in the groups.

II, III and IV respectively. In the Moringa oleifera treated groups V, VI and VII the value of total proteins, glycogen, cholesterol, DNA and RNA decreased up to day-7, then it increased on day-14 and continued so up to day-28. Similarly, the value of acid phosphatase activity and alkaline phosphatase activity increased up to day-7 then decreased on day-14, which continued up to day-28.

5. Discussion

Currently the major application of blood and blood component irradiation is for the prevention of graft-versus-host disease in immune deficient patients by the abrogation of T-lymphocytes. However, a potential application of this technology would be for the sterilization and inactivation of pathogenic microbes in contaminated blood products. Transfusion associated transmission of infectious diseases as a result of contaminated blood products is now well documented and routine screening of blood donations is now common practice. Nonetheless, it is recognized that the possibility of transmission of infections due to limitations

of the screening methodology exists. The relevant literature on the effect of ionizing radiation (essentially gamma and X-rays) on whole blood, blood cells and other blood components in order that a rational decision can be made on the feasibility of their irradiation whether for sterilization (or decontamination), or alternatively, for inactivation of a particular blood component such as, for example, lymphocytes in preventing graft versus host disease [7].

The radiation doses for inactivation of T-lymphocytes, for instance, may be in the order of 10 to 50 Gy, doses used for sterilization purposes are generally a thousand-fold higher, i.e. in the 10 to 30 kGy range. Reviews have been published on the effects of sterilizing doses of ionizing radiation on polymers, pharmaceuticals, cosmetic raw materials, and biological materials [8].

The exposure of animals to ionizing radiations causes a series of physiological changes which are known as the acute radiation syndrome, which is dependent on the exposure dose and which 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 those in the normal or control animals. Therefore, the effect of the whole body irradiation is mainly felt 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 [9].

Changes in blood cell count are still considered (although imperfect indices) the most reliable biological evidences for acute exposure to both external and internal irradiation. This is understandable because of high sensitivity of blood forming tissues to ionic radiation and manifestations of injury to the blood even in the absence of demonstrable histopathological changes in blood forming or lymphatic tissues [10].

Radioprotectors prevent these alterations and protect cells and tissues from the deleterious effects of radiations. Radioprotectors are of great importance due to their possible and potential application during planned radiation exposures such as radiotherapy, diagnostic scannings, clean up operations in nuclear accidents, space expeditions etc. and unplanned radiations exposures such as accidents in nuclear industry, nuclear terrorism, natural background radiation etc. Many of the available synthetic radioprotectors are toxic to mammalian system at doses required to be effective as radioprotector. The blood is most sensitive vital cellular component to the lead and ionizing radiation. The present work pertains to studies on protective effect of *Moringa* against radiation and mercury induced haematological changes in the Swiss albino mice.

The toxicity of mercury sources can be expected to depend on its nature, i.e., salts vs. organo-mercury compounds vs. elemental mercury. One mechanism of mercury toxicity involves its irreversible inhibition of selenoenzymes, such as thioredoxin reductase (IC50=9 nM). Although it has many functions, thioredoxin reductase restores vitamins C and E, as well as a number of other important antioxidant molecules, back into their reduced forms, enabling them to counteract oxidative damage. Since the rate of oxygen consumption is particularly high in brain tissues, production of reactive oxygen species (ROS) is accentuated in these vital cells, making them particularly vulnerable to oxidative damage and especially dependent upon the antioxidant protection provided by selenoenzymes. High mercury exposures deplete the amount of cellular selenium available for the biosynthesis of thioredoxin reductase and other selenoenzymes that prevent and reverse oxidative damage, which, if the depletion is severe and long lasting, results in brain cell dysfunctions that can ultimately cause death. Mercury in its various forms is particularly harmful to fetuses as an environmental toxin in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects. Mercury exposures in excess of dietary selenium intakes in young children can have severe neurological consequences, preventing nerve sheaths from forming properly. Mercury inhibits the formation of myelin [11].

RED BLOOD CORPUSCLES (RBC)

In the present investigation the RBC count showed a decreasing trend up to day 14 in non drug treated groups and day-7 in Moringa treated groups and the count increased thereafter till day- 28. In [12] reported that decline in the number of RBC may be due to defective haemopoiesis and intravascular blood cells damage.

In [13] reported on the kinetics of the diffusion of salts into and out of X-irradiated erythrocytes. The X-irradiation of erythrocytes was reported by this group to cause osmotic disturbances. They showed that at 330 Gy, the effect of X-rays was not due to haemolytic disturbances in the red blood cells but rather to a "disturbance within the cells". This manifested itself in slow swelling of the cells. Another early study was that of Solomon, who investigated the permeability of red cells to water and ions. An *in vitro* study on the effect of X-rays on movement of sodium in human

erythrocytes, showed a loss of sodium/potassium ion balance with entry of sodium ions into the erythrocytes, and exit of potassium ions, following radiation doses in the range of 8.9 to 89 Gy. This phenomenon was due in part to discontinuation of membrane integrity. Radiation-induced changes in the properties of cell membranes, resulting in loss of ability to regulate electrolyte balance and changes in permeability have been reported for red blood cells by Goggle. Irradiation of erythrocytes with a 20 Gy dose caused a significant increase in the external potassium ion concentration and internal sodium ion concentration. The change was attributed to increased permeability of the red blood cell membrane lipid bilayer to sodium and potassium ions. These changes could be reversed by incubating the cells at 37°C. Whole body X-irradiation of rabbits with a dose of 6 Gy produced a rise in the concentrations of calcium and magnesium ions in whole blood and in erythrocytes, due to disturbances in permeability of the membrane to metal ions and other cellular components. They investigated that the fall in RBC count after irradiation might be du to changes in plasma volume, leakage of cells through capillaries secondary to thrombopenia and by severe haemorrhage in som instances. They have suggested three possible explanations for the reduction in the number of the different groups of cells following irradiation.

- There may be a cessation of mitosis lasting for a variable period.
- The cell may be show no immediate variable abnormalities but die during the next of subsequent mitosis or
- The cell may undergo degeneration and die by a mechanism which is related to the mechanism of cell division.

From blood factors studied, the number of RBC, the average MCV and percentage of platelets in the treated groups were significantly increased under the influence of the electromagnetic waves. Whereas Hb level, percentage of lymphocytes and WBCs decreased. Increase in the number of RBCs might caused by the stimulating effect of waves on cell division of stem cells of bone marrow. Increase in cell volume may also be due to immature cells increase or reticulocytes increasing. A review on effects of electromagnetic waves on human peripheral blood lymphocytes reveals that these waves do not have role in affecting micronucleus frequency and cell cycle [14].

At a higher dose of 9.0 Gy, the number of RBC fell more sharply up to day -7, but recovered thereafter. It was suggested that the radiation-induced depletion of hematopoietic stem cells may be an important factor contributing to the decline in the erythrocytic population. The acute decline in the total RBC count may be caused by the leakage on account of hemorrhage caused by radiation induced lesions in blood vessels.

RBCs are extensively used in studies of aging processes 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.

The decline in haematological constituents may be caused by the direct damage by radiation. The radiation-induced depletion of haematopoietic stem cells may be an important factor contributing to the decline in the erythrocytic population. These results are in accordance with the present findings.

In [15] performed studies 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.

Exposure to ionizing radiation directly damages hematopoietic stem cells and alters the capacity of bone marrow stromal elements to support and/or maintain haematopoiesis *in vivo* and *in vitro*. Exposure to ionizing radiation induces dose- dependent declines in circulating hematopoietic cells not only through reducing bone marrow cell production, but also by redistribution and apoptosis of mature form elements of the blood cells**.**

After exposure of 2.0 Gy the erythrocyte count exhibited a fall that can be attributed to inhibition of new cells entering into blood, loss through haemmorhage and / or radiation-induced injury. A similar depression was observed in haemoglobin level without returning to normal till last day of experiment. These results suggested that the haemoglobin concentration follows a pattern similar to that of RBC in general. Similar results were observed in present study.

In contradistinction to the slow uptake of other divalent ion such as Mn^{++} by the red cell, the uptake of mercury is very fast, with equilibrium rapidly achieved. This is not indicative merely of surface-binding by stroma is shown by the relatively low capacity of stroma for the metal. In saline solutions mercuric ion exists almost entirely as an anionic complex with chloride. Such an anionic complex might be

expected to penetrate red cell membranes with much greater rapidity than a divalent cation. In attempting to relate the binding of mercury by red cells to the effects of mercury on red cell permeability to K + and glucose, it is apparent that there are functionally at least two types of binding. Decreasing permeability to glucose and increasing permeability to potassium ions are produced by concentrations of mercury which have reacted with approximately twenty five per cent of the total number of cellular sulfhydryl groups and with approximately all the stromal sulfhydryl groups.

The binding to the additional 75% of the total sulfhydryl groups in the cell, including reaction with reduced glutathione, results not only in no further increase in K + permeability, but actually results, instead, in a decrease. Because the reaction of mercury with reduced glutathione does not occur until total cellular sites are occupied by metal, it is suggested that reduced glutathione plays no role in the increasing alterations in permeability produced by mercury .

WHITE BLOOD CORPUSCLES (WBC)

Leucocytes show early response to radiationin comparison to the erythrocytes. In the present study, irradiation with different doses of gamma rays (2.0 Gy and 4.0 Gy) caused a reduction in the level of WBC reaching the minimum on day -14 in without-drug treated groups and day -7 in *Moringa* treated groups and then started increasing up to last autopsy interval. The present findings are in conformation with [16] who also reported a decline in total leucocyte count from 0.5 day till day 5 in mice exposed to 3.60 Gy gamma rays, by exhibiting 66% decline as compared to normal and even on the last autopsy interval i.e. day 28, it was 15% lesser than the normal. This depletion might be due to the direct cell killing as well as destruction of stem cells in haemopoietic organs thus causing a decline in lymphocyte as well as total leucocyte count.

In [17] stated that the rapid decline in the lymphocytes number is due the direct destruction of such cells in peripheral blood.

In combined treatment (Group IV), the number of WBC declined more drastically than mercuric chloride or radiation alone, indicating their synergistic effect. In the *Moringa* treated groups (V, VI & VII) decline in WBC level was less severe and an early recovery was also noted showing protective effect of *Moringa*. These results coincided with the findings of [18].

The administration of mercuric chloride alters the structure and number of WBCs. The nuclear arrangement was also distorted. In mercury treated groups the shape and structure of the monocyte was also altered with reniform (kidney shaped) nucleus. At higher dose level this intensity of indentation was increased so that the normal range of nucleo-cytoplasmic ratio is disturbed and appeared as reactive monocytes. These findings are also in support with the evidence of reactive monocytes enclosing the cytoplasm become more intensely basophilic and vacuolated.

In a study performed on young dogs, development of anemia, leukocytosis, monocytopenia, polychromato-philia, glycosuria, increased serum urobilinogen and hematuria have been reported. These results are is close association.

HAEMOGLOBIN (HB)

The haemoglobin content of blood decreased in all the experimental groups in present study. This decrease was dose dependent and continued up to day –14 in the without drug treated groups and day-7 in the *Moringa* treated groups. Thereafter, the value increased in all the experimental groups. The decrease was more prominent in combined treatment groups. In the *Moringa* administered experimental animals decrease was less severe which was supposed to be the protection provided by the *Moringa*.

In the present study, decrease in haemoglobin level of blood was chiefly due to the damage caused to the red blood cells by radiation and also probably due to injury to the "precursor cells" of erythroid elements of the bone marrow. In [19] observed that the total number of erythrocytes precursors reduced to about 15% of normal value within one day after exposure of rats to 600 R. Rats irradiated with a single dose of 300 R showed a decrease in iron incorporation into newly formed erythrocytes, which attained its lowest point approximately 48 hours post-irradiation. According to [20], primary cause for the initial reduction in labeled iron incorporation was the mitotic inhibition and destruction of erythropoietic tissue. In [21] found a rapid fall of mitotic index in erythroblast after irradition, which was due to the occurrence of blockage in late prophase.

In studying six weeks old weanlings and adult rats after total body dose of 800 R of x-rays, observed a rise in red blood cells number and haemoglobin concentration in weanlings by the 3rd day after irradiation. According to Cassarette, this rise in values indicated haemo concentration, and it was seen that most of the weanling rats did not survive the 4th day because of maximal intestinal damage and almost extreme depletion of bone marrow and lymphatic tissue. Whereas, in adults, red blood cell and haemoglobin values

dropped, 24 hours irradiation, and the decline progressed gradually from 2nd day to 10th day after irradiation.

They also noticed a remarkable depletion in hemoglobin concentration in the mice when exposed to 3.6 Gy gamma radiations. 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 depletion in the synthesis of haemoglobin after radiation exposure. These findings coincided with the result of [22].

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 and a significant decrease ($P<0.001$) in neutrophil value from 23.70 in the control mice to 13.00 and 14.30 in the mice exposed to UVC for on 30 and 45 d, respectively. But, a significant decrease and increase ($P<0,001$) were recorded in the haemotocrit (Ht) value from 52.50 in the control mice to 47.80, 48.50 and 55.00 in the mice exposed to UVC for 30, 45 and 60 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 .

The exposure to UVC for 60 days may lead to suppression in the activity of some haematopoietic tissues which led to a reduction in erythropoiesis and impeded the formation of RBCs. The defense reaction against toxicity through the stimulation of erythropoiesis was caused by the perturbation in these blood indices. The significant decrease ($P<0.001$) in the haemoglobin concentration may be caused due to either an increase in the rate of haemoglobin destroyed or to a decrease in the rate of haemoglobin synthesis. Haematocrit values were previously used as a tool in checking anaemic condition .

Such decrease in haemoglobin and haematocrit may be due to haemolysis as a consequence of toxicity, severe anemic state or stress. Some researchers suggested that in toxicity experiment the decrease in haemoglobin and haemetocrit level could be related to the conditions of confinement or stress induced by the lack of food. In [6] also reported decrease in the haemoglobin content in mice after exposure of 2.0 Gy of gamma radiation in the presence or absence of mercuric chloride treatment. *Moringa* treated experimental mice showed an early and fast recovery which was supposed to be the protection provided by the *Moringa*.

PACKED CELL VOLUME (PCV)

The packed cell volume decreased in all the experimental groups. The PCV declined up to day-14 in the without drug administered groups and day-7 in the *Moringa* treated groups. Thereafter, the value increased up to day-28 in all the experimental groups. The decrease was found to be dose-dependent. They also observed a dose-dependent decrease in haematocrit value in C57BL/6 mice after whole-body proton-irradiation at varying doses and at low and high-dose rates. Similar findings were also reported by Daga *et al*. (1995) who noticed decreased haematocrit value up to day 7 in 2.50 Gy gamma-ray exposed group and till day 14 in 3.60 Gy gamma ray exposed group.

In this, they reported decline in the PCV which can be attributed to total cell depletion in peripheral blood aided by disturbances in steady state mechanisms in blood forming organs as well as an increase in plasma volume after irradiation. These findings are in close association with [6]. In the present investigation, mercuric chloride treated animals exhibited a similar decline in PCV values, which continued up to day-14 increasing thereafter during the subsequent intervals. In the *Moringa* administered mice (Group V) the value decreased up to day-7. Decrease in the value of PCV due to mercury toxicity has also been reported by [6].

The immediate toxic effect of heavy metals on the blood of Swiss albino mice was the significant decrease in total erythrocyte count, haemoglobin, small lymphocytes and coagulation time. This decrease in total erythrocyte count will give decreased value of PCV, as, these are positively co-related .

They reported that alterations in the erythrocytes count, haemoglobin level and haematocrit percentage show a parallel pattern. These were markedly reduced in the 3.0 Gy irradiated animals and a normal value could not be achieved till the last autopsy interval (i.e., day-30). They further reported that prior administration of *Rosmarinus officinalis* leaves extract enhanced the recovery in these parameters, and a normal value was registered by day-30 post-interval. Leucocytes were recorded minimum on day 3 post-irradiation with a progressive increase till the last autopsy interval, but a normal value could not be attained.

The failure of erythropoiesis, destruction of mature cells, and internal bleeding may lead to the decrease in the PCV. The reduction in the haematocrit value can also be attributed to the total cell depletion in peripheral blood aided by disturbances in steady-state mechanisms in blood forming organs, as well as an increase in plasma volume after irradiation. They investigated that radiation exposure reduces the number and functional activity of circulating lymphocytes and changes the distribution and ratio of their sub populations.

The animals of group IV also exhibited the similar way of decline in the value of PCV, but here the decrease was

greater as compared to individual effects of radiation or mercuric chloride. This may be due to combined effect of radiation and mercury. In the *Moringa* pre-treatment groups this decline was less prominent showing protective effect of *Moringa*. These results are in conformation with [6].

MEAN CELL VOLUME (MCV)

The present study showed an increasing trend in MCV of all the experimental groups after the exposure to 2.0 and 4.0 Gy of gamma rays. This increase was dose-dependent and became more pronounced with higher radiation doses. The value increased up to day-14 in the without drug administered groups and day-7 in the *Moringa* pre-treatment groups, declining thereafter during the subsequent intervals.

It is observed that the increase in the MCV as the dose increased, and the decrease in the dispersion of hemolysis reflects the presence of unusually flattened cells (i.e. the well-defined discoid shape vanished). They further reported that the change in the shape of red blood after exposure to 6.0 Gy gamma radiation altered cell permeability, and developed echinocytes and spherocytes with the progressive appearance of the regularly spaced spicules on cell surface. The decrease in the complete solubilization may be due to the radiation induced damage in the RBC membrane, which facilitate the membrane interaction and decrease the detergent concentration needed to solubilize the membrane completely.

The macrocytic anemia happened in irradiated groups because of DNA synthesis inhibition result in the production of red blood cells. They observed that when DNA synthesis was interrupted, then the cell cycle could not be grown from the growth phase (G2) to mitosis phase (M). This led to continued cell growth without division and seen as a macrocytic anemia.

Ionizing radiation was suspected of causing interference with DNA synthesis in this study. Defects in DNA synthesis of RBCs were most often caused by hypovitaminosis, particularly deficiencies of vitamin B12 and folic acid and they investigated that in the anemia macrocytic, usually immature red blood cells were released by the bone marrow into the circulation to meet an increasing need.

The mice of combined treatment of group fourth also showed the similar trend of elevation in the values of mean cell volume but here the increase was more pronounced as compared to the individual effects of radiation and mercuric chloride indicating their "additive-synergistic" action. In the *Moringa* pre-treated experimental animals the increase was less severe showing protection provided by the drug.

MEAN CELL HAEMOGLOBIN (MCH)

In the present study the changes in the levels of mean cell haemoglobin (MCH) of mice after irradiation with different doses of gamma rays have been observed.MCH showed an initial elevation in the values which lasted up to day-14 in control groups and day-7 in the *Moringa* treated experimental groups and then continued to decline up to the last autopsy interval i.e. day 28.

The MCV relates to the average size of the red blood cell. The amount of haemoglobin in a single red blood cell is indicated by the MCH. The level of both MCV and MCH decreased due to decrease in size of RBC, destruction of number of RBC or impaired biosynthesis of heme in bone marrow due to radiation effect. In the present investigation, *Moringa* pretreatment showed a gradual recovery of haematological constituents in the peripheral blood so it also maintains the level of MCV and MCH of irradiated Swiss albino mice.

The combined treatment group also exhibited increase in the values of mean cell haemoglobin but in this group the increase was much higher as compared to individual effect of radiation and mercuric chloride. This may be due to combined action of radiation and mercury. In the *Moringa* treated animals the increase was less severe and an early recovery was also found showing protective effect of *Moringa*.

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

In the present study, the mean corpuscular haemoglobin concentration (MCHC) declined in all the experimental groups. The value declined up to day-14 in the non-drug treated groups and day-7 in the *Moringa* treated groups.

MCHC values indicate the concentration of haemoglobin in 100 ml of erythrocytes. Haemoglobin's role is maintaining hemoglobin oxygen transport function of the lungs to body tissues. Iron is a substance needed to heme formation that develop the hemoglobin. Interference in the absorption of elemental iron results in a lack of iron in the blood circulation, so that is reducing the amount of hemoglobin.

In the present study, treatment of radiation and mercuric chloride simultaneously has shown an "additive synergistic" type of nature and its severity increased with the dose of radiation. In the *Moringa* administered groups,

a less severe decline and an early recovery noted showing protection provided by the drug. These findings are in conformation with [6].

DIFFERENTIAL LEUCOCYTE COUNT (DLC)

In [23] reported that the lymphocytes are among the most radiosensitive cells in the living organisms which are involved in the immunological response and are of immense interest to researchers and clinicians.

In the present findings, the leucocytes in general showed an initial decline after irradiation, in all the experimental groups. The changes in lymphocyte number also showed a similar behaviour, decreasing up to day-14 in the non-drug treated groups and day-7 in Moringa treated groups. After which it increased moderately.

An increased incidence of chromosomal aberrations and micronuclei were found in peripheral blood lymphocytes from individuals who were occupationally exposed to radiofrequency radiation but in other investigations , this was not found. Some studies indicate that electromagnetic waves does not have genotoxic effect on human lymphocyte while increases micronucleus frequency in human fibroblasts cultured. Some researchers believe that electromagnetic waves can affect the homeostasis of iron in biological systems, thus leading to increased cytoplasmic and nuclear free iron that may through a chemical reaction produce hydroxyl radicals increase and can lead to DNA damage.

Damage to bone marrow is known to be the main cause of death in animals following whole body doses of radiation between about 2.0 and 10 Gy. Radiation death in the mid-lethal dose range is due to impairment of bone marrow hematopoietic function such as leucopenia, erythropenia and thrombocytopenia which will ultimately lead to whole body infection, hemorrhage and even death.

Naruka (1986) observed two phases of decline in bone marrow lymphocytes in the mice exposed to 2.5 Gy, 5.0 Gy. and 10 Gy gamma radiation. The first decline was noticed at 12-72 hours and the second phase of decline after 17 days of irradiation. Biphasic decline in lymphocytes count of blood was also observed at 1 to 14 days with 4.0 Gy exposures. Our present study also revealed same observations.

Decrease in lymphocyte percentage in the present study may be due to direct cell killing by radiation as well as destruction of stem cells in haematopoietic organs. The lymphocyte post-irradiation death may take place before any mitosis and has been characterized as interphase death. The decrease in hematological constituents may be attributed to a direct damage by radiation dose and hematopoietic recovery after whole body irradiation is dependent on the presence of spared haematopoietic stem and progenitor cells in the bone marrow.

The irradiation of lymphocytes with different doses of gamma radiation not only increased Micronuclei binucleate cells (MNBC) with one Micronuclei (MN) but also MNBNC bearing two and multiple MN. Irradiation has been reported to increase the frequency of cells bearing one, two or multiple MN in lymphocytes and various cultured cell lines.

They observed an increase in granulocyte percentage in the bone marrow up to day 3 after 1.0 Gy and 2.0 Gy fractionated doses. Similar results were obtained, this increase in count was not an actual increase but was due to relatively more damage to other cell series (lymphocytes and pronormoblast/ normoblast).

The sensitivity of monocytes is of the same order as the granulocytes. In the present study, the percentage of monocyte increased up to day-14 in the non-drug treated groups and day-7 in the *Emblica* 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. They reported a threefold increase in neutrophil and monocyte count along with severe leucocytosis in the young rats that were exposed to lead. The present investigation revealed that administration of mercuric chloride alters the appearance and cause structural changes. The nuclear arrangement was distorted with intermingled lobes and in some cases formed a sessile nodule.

The circulating WBC, platelet and RBCs were reduced after 4.0 Gy whole-body gamma irradiation in mice. The primarily cause of mortality during the early phase of the radiation-induced hematopoietic syndrome is due to low numbers of neutrophils and platelet**.** The low number of peripheral blood cells observed after ionizing radiation due to bone marrow stem cell's damage, which are more sensitive to radiation damage than the other cells. The maintenance of the blood cells happens through the stimulation and production of bone marrow progenitor cells which are the main ways for protection of mammalins from mortality induced by gamma irradiation.

In combined treatment (radiation + mercuric chloride), variations in the percentage of lymphocytes, monocytes and granulocytes were more marked as compared to individual effects *of* radiation or mercuric chloride indicating their 'synergistic' effect. Radiation causes direct cell killing as well as destroys stem cells in haematopoietic organs thus causing a decline in lymphocyte counts.

They evaluated the radioprotective potential of Aloe vera against radiation and cadmium mediated alterations in diffrenctial lecucocytic count of Swiss albino mice after exposing the animals with 3.5/7.0 Gy of gamma rays with

or without camium chloride treatement. They reported that the value of lymphocytes declined up to day-14 in control groups and day-7 in the Aloe vera trated groups. The percentage of monocytes and granulocytes increased up to day-14 in the non-drug treated and day-7 in Aloe vera pre-treated groups. Theareafter a decrease in the value was noted and continued up to day-28. The synergistic changes were noticed after combined treatment and an early and fast recovery was also observed in *Aloe vera* pre-treatment groups.

BIOCHEMICAL STUDIES

SGOT/ALT AND SGPT/ AST

AST is a mitochondrial enzyme that is also present in heart, muscle, kidney and brain etc. In many cases of liver inflammation, the ALT and AST activities are elevated roughly in a 1:1 ratio. The excessive production of free radicals and lipid peroxides due to irradiation might have caused the leakage of cytosolic enzymes such as aminotransferases (AST and ALT), lactate dehydrogenase (LDH), creatine kinase (CK) and phosphatase. Irradiation produced a sharp increase in AST level by an average approximately 49.49 ± 15.06% in control group. Our present study also revealed above observation.
The rise of serum ALT and AST activities ($P<0.01$) occurred on the 75^{th} day of the exposure and was related to hepatic cell destruction. The important fall in serum GGT and ALP values could be related to the inhibitory effect of the radiation on enzyme activity, because it was reported that UV radiation decreased serum ALP level. These two enzymes are related to the endothelial cell membranes, which are arranged through hepatic gall channels and it can be thought that they are more easily affected by the cytoplasmic enzymes (AST and ALT). It is thought that endothelial cell death caused by radiation is connected with apoptosis and these cells are too sensitive to radiation and the basal membrane that can protect the cell from the radiation.

RADIOPROTECTIVE MECHANISM OF *MORINGA OLEIFERA*

The possible mechanisms of action of *Moringa* may be as under:

1. Radiation affects the biological systems in more than one ways. Herbals have been reported to act as a radioprotector under *in vivo* and *in vitro* conditions. The properties of bioactive compounds can facilitate therapeutic drugs to act differentially towards tumour and normal tissues, thus displaying selective metabolic effects. The alkaloids and flavonoids are among naturally occurring phytochemicals helpful in radioprotection and radiosenstization. They exhibit antimutagenic and anticarcinogenic properties, and prevent cancer as they are an ingredient of human diets.

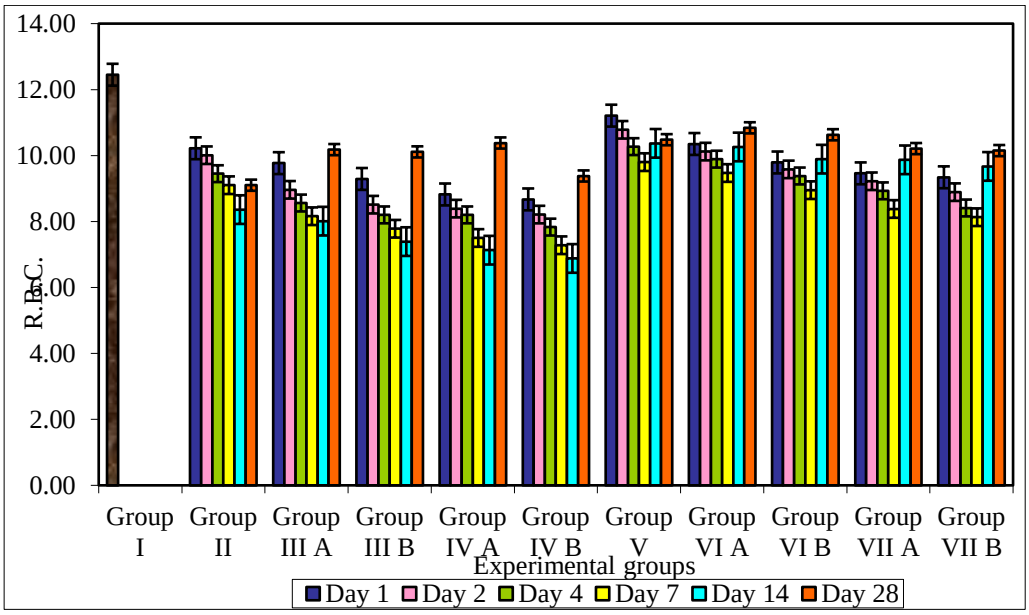
2. Phytochemical analysis showed that *Moringa oleifera* possess various phytochemicals such as ascorbic acid, phenolics (Catechin, epicatechin, ferulic acid, ellagic acid, myricetin) etc. which may play the key role in prevention of lipid peroxidation by scavenging radiation-induced free radicals.
3. The leaves of *Moringa oleifera* contain nitrile glycosides such as niazirin and niazirnin and mustard oil glycosides. These glycosides are reported to have antioxidant activities.
4. In the present study, inhibition of LPO in biomembranes has been caused by antioxidants present in *Moringa oleifera*. It was also observed that, radiation caused depletion in GSH levels in entire test period. Under normal conditions, the inherent defense system like glutathione protects against oxidative damage. 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.
5. The present study clearly demonstrated that *Moringa* leaf extract protected liver 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 we showed 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.

References

[1] Jirtle, R.L. and Noell, K.T. (1982) : The radioprotection of parenchymal hepatocytes with WR-2721 (meeting abstract). 24th Annual Meeting of the American Society of Therapeutic Radiologists. Orlando, Florida.
[2] Chatterjee, P.D. and Bose, A. (1962): Determination of dry mass or rat liver cells to study the protective mechanism of

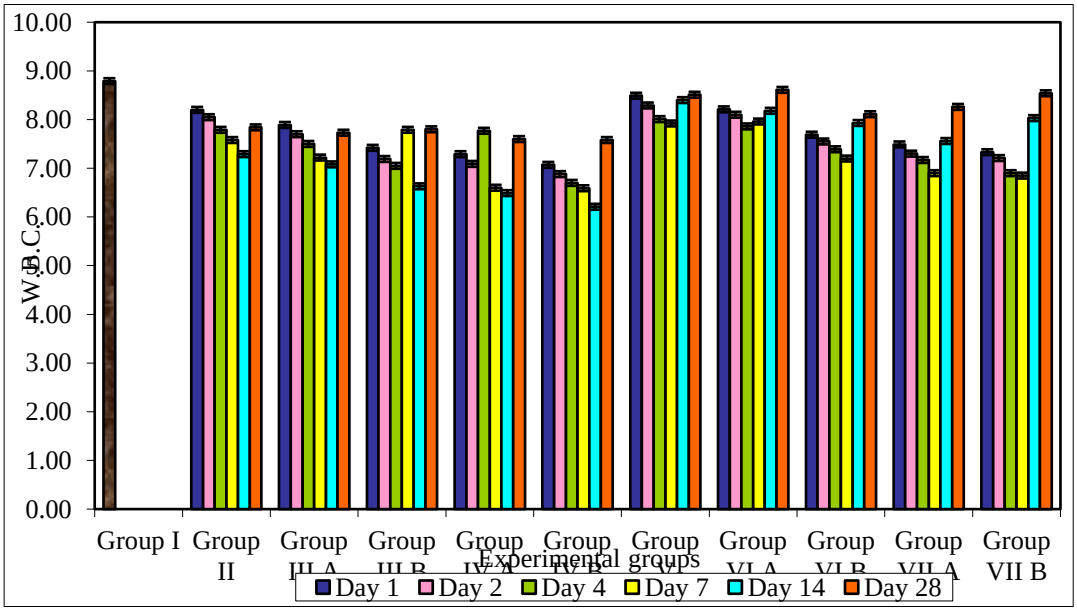
cysteamine against whole body x-irradiation. Exp. Cell Res., 27: 168-169.
[3] Dainiak, N. (2002): Hematological consequences of exposure to ionizing radiation. Exp. Hematol., 30: 513–528.
[4] Gwozdzinski, K. (1991): Ionizing radiation-induced structural modification of human red blood cells. Radiat. Environ. Biophys., 30: 45–52.
[5] Mehta, A., Flora, G., Dube, S. and Flora, S.J.S. (2004): Succimer and its analogues: antidotes for metal poisoning. In: Flora S.J.S., Romano, J.A, editors. Pharmacological perspectives of some toxic chemicals and antidotes. New Delhi: Narosa Publication., 445-66.
[6] Panchariya, M., Khatri, P.R., Purohit, R. K., Agarwal, M., Chakrawarti, A. and Mittal, D. (2021): Protective efficacy of Moringa oleifera against radiation and mercuric chloride induced haematological changes in mice. UPJOZ, 42(8): 29-37.
[7] Barr, R., Stubbe, P., Rietveld, M.T. and Kopka, H. (1986). ELF and VLF signals radiated by the ‘‘polar electrojet antenna’’: Experimental results. Journal of Geophysical Research 91. doi: 10.1029/JA091iA04p04451. issn: 0148-0227.
[8] Button, L., DeWolf, W., Newburger, P., Jacobson, M., & Kevy, S. (1981). The effects of irradiation on blood components. Transfusion, 21(4), 419–426. doi:10.1046/j.1537-2995.1981.21481275998.x
[9] Jagetia, G.C. and Aruna, R. (1997): The herbal preparation abana protects against radiation-induced micronuclei in the mouse bone marrow. Mutat. Res., 393: 157-63.
[10] Jacobson, L.O. (1954): The hematological effects of ionizing radiations. In “Radiation biology” ed. Hollaende, A. Vol I Part II, McGraw Hill New York, PP. 1029.
[11] Nicholas, V.C.R. and Laura J. R. (2010): "Dietary selenium's protective effects against methyl mercury toxicity. Toxicology., 278 (1): 112-123.
[12] Stohlman, C.J., Bredner, G., Scheidermann, H. and Gronkite, E.P. (1957): The hemolytic effects of ionizing radiations and its relationship to the haemorrhagic pluse of irradiation injury. Blood., 12: 1061.
[13] Ting, T.P. and Zircle, R.E., J. Cell Comp. Physiol. B, 1940, vol. 16, p. 197.
[14] Duncan, R., Prass, K.W. and Mahaftey, E.A. (2001): Veterinary laboratory Medicine, Clinical Pathology (6th ED). Translated by Nazifi S Mc.G Hill.
[15] Soliman, M.S. (2004): Whole body gamma radiation effects on rheological behaviour deformability of rat erythrocyte, Egypt. J. Rad. Sci. Applic., 17: 345–363.
[16] Daga, S.S., Jain, V.K. and Goyal, P. K. (1997): Lympho-toxicity induced due to combined treatment of radiation and mercury and its inhibition by Liv.52. J. med. Physics., 22: 145.
[17] Patt, H. M. (1969): Species Differences in Leucocyte Restoration after Irradi ation, in: Comparative Cellular and Species Radiosensitivity (Eds. V. P. Bond, T. Sughara), Lgaku Shoin Ltd., Tokyo., pp. 119.
[18] Purohit, R.K., Chakrawarti, A. and Bhartiya, K.M. (2007): Radiation and cadmium induced biochemical alterations in mouse kidney. Iran J Radiat Res, 5: 125-130.
[19] Tsuya, A., Bond, V.P., Fliendner, T.M. and Feinendegen, L.E. (1961): Cellularity and DNA synthesis in bone marrow after total and partial body irradiation. Radiat. Res., 14: 618.
[20] Baxter, D.E., Belcher, E.M., Harris, E.B. and Lamerton, L.F. (1955): Anemia and erythropoiesis in the irradiated rat. An experimental study with particular reference to techniques involving radioactive iron. Brit. J. Hematology., 1: 86.
[21] Odurtchenko, N., Cattier, H., Feinendenger, L.E. and Bond, M.P. (1964): Mitotic delay in more mature erythroblasts of the dog induced in vitro by sublethal dose of x-rays. Radiat. Res., 21: 413.
[22] Panchariya, M. D., Purohit, R.K. and Mittal, D. (2019): Protective efficacy of Moringa oleifera against radiation and mercury induced biochemical changes in the blood of mice. Glimpses. 9(1):409-416.
[23] Goldin, E.M. and Neff, R.D. (1975): Lymphocyte depletion in peripheral blood of Gamma irradiated rats. Int. J. Radiat. Biol., 27: 337.

Histogram 1



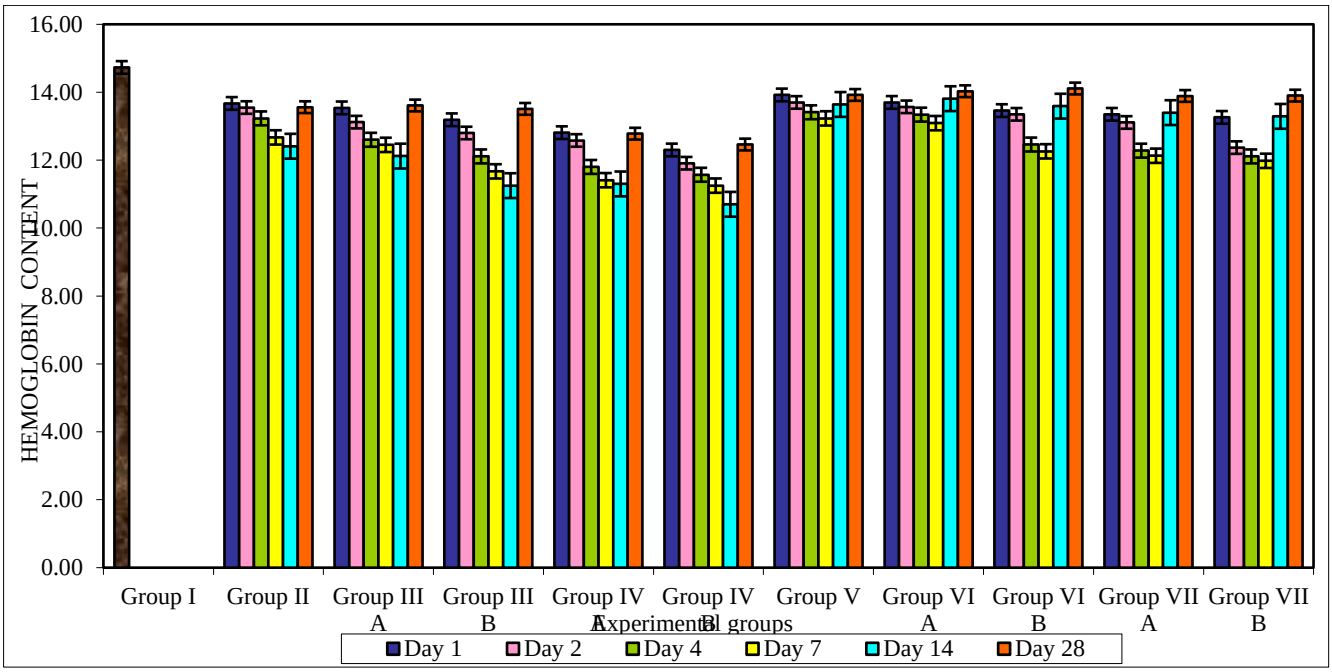
Variations in the values of R.B.C (million/cu.mm) of mice in various experimental groups (Mean ± S.E.)

Histogram 2



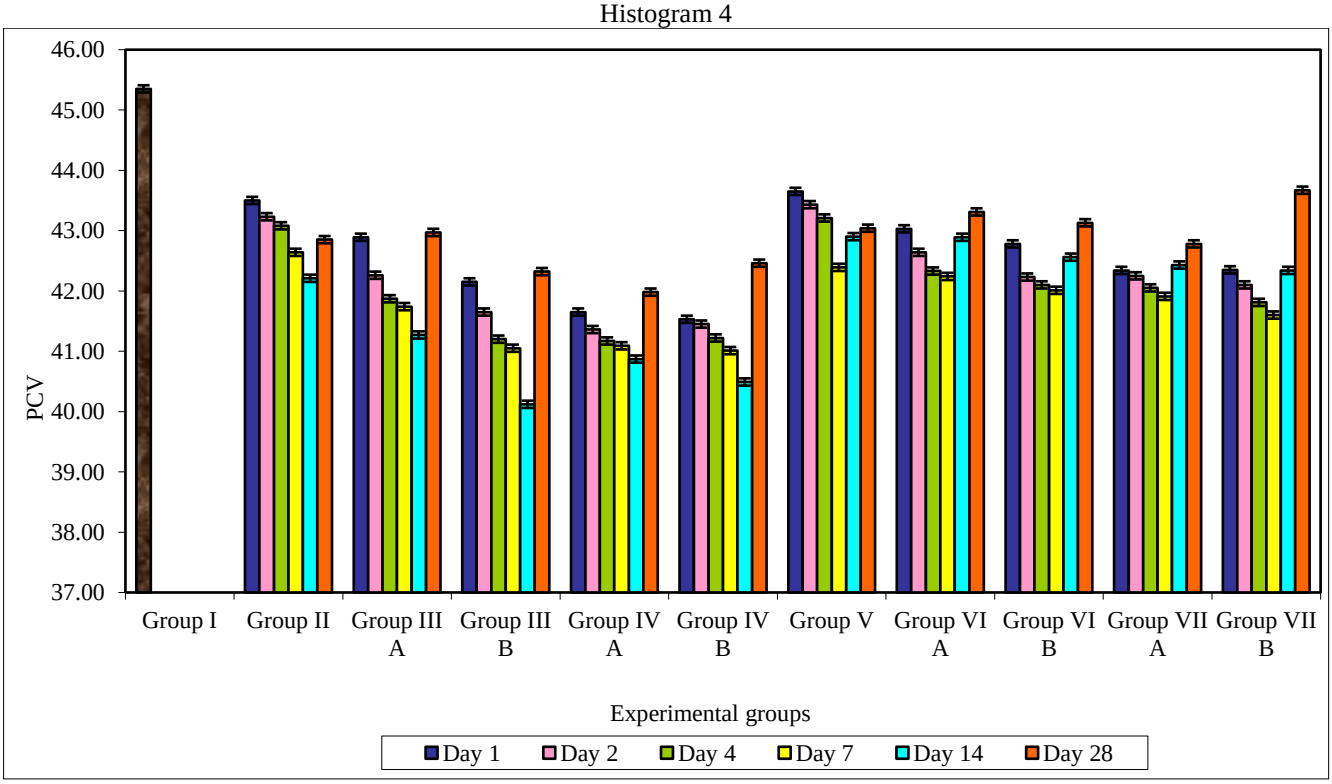
Variations in the values of W.B.C (thousand/cu.mm) of mice in various experimental groups (Mean ± S.E.)

Histogram 3



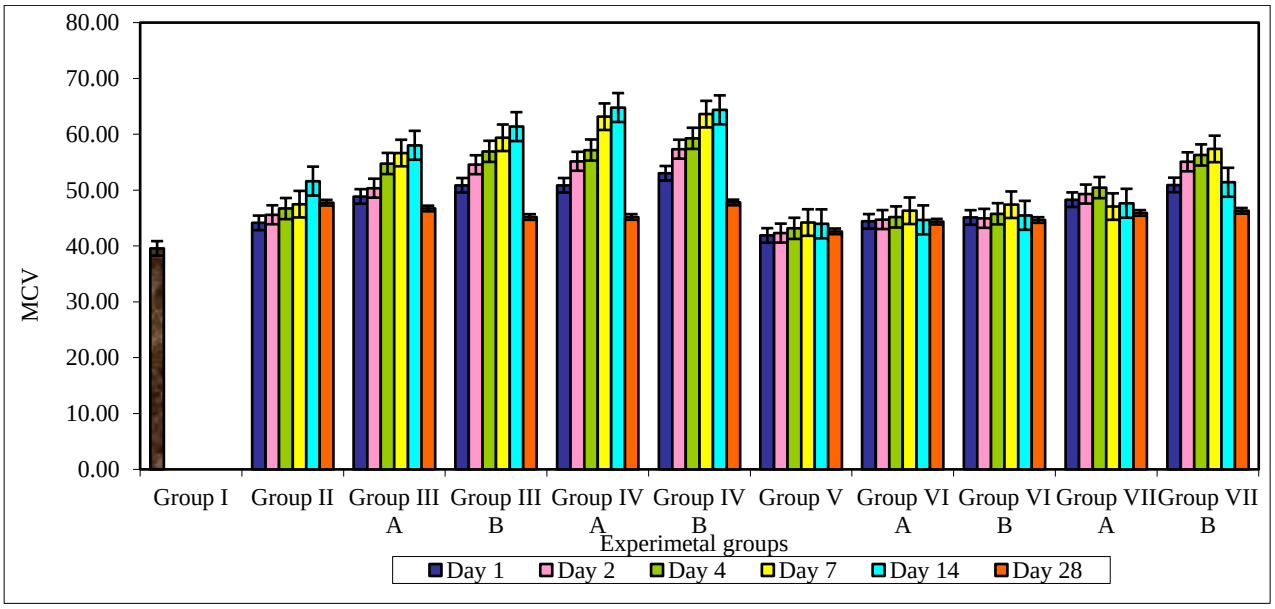
Variations in the values of Hemoglobin content (g/100ml. of blood) of mice in various experimental groups (Mean ± S.E.)

Histogram 4



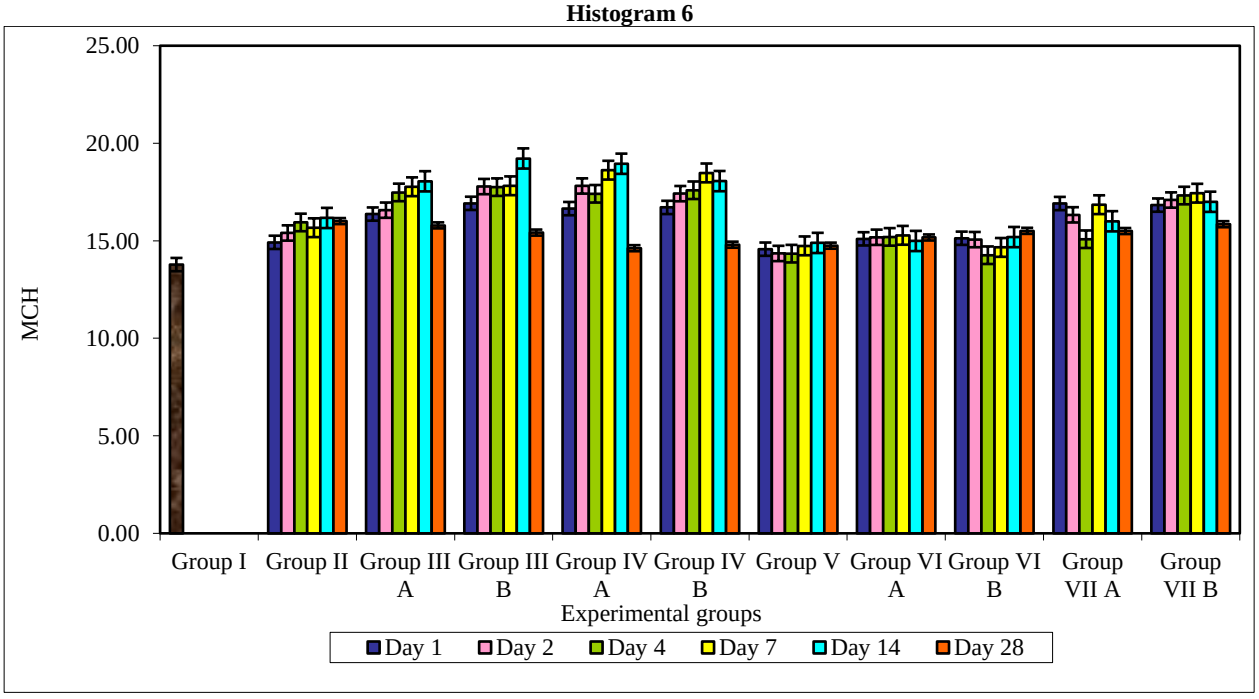
Variations in the values of PCV (%) of mice in various experimental groups (Mean ± S.E.)

Histogram 5



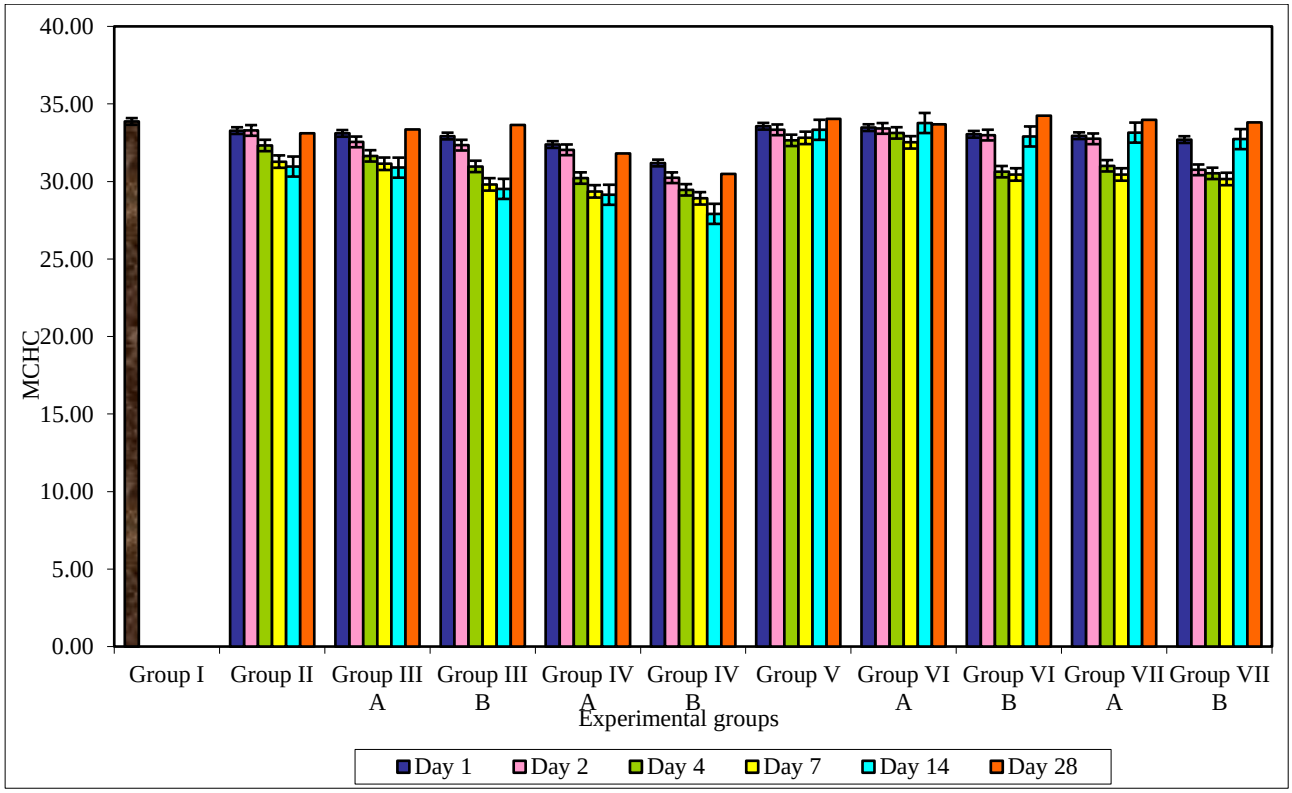
Variations in the values of MCV (cubic micron) of mice in various experimental groups (Mean ± S.E.)

Histogram 6



Variations in the values of MCH (micro gms) of mice in various experimental groups (Mean ± S.E.)

Histogram 7



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