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Research Paper

PROTECTIVE ROLE OF LIV.52 AGAINST RADIATION AND CADMIUM INDUCED HAEMATOLOGICAL CHANGES IN THE SWISS ALBINO MICE

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The present study was aimed to evaluate protective efficacy of a herbal drug Liv.52 against radiation and cadmium induced haematological changes in the Swiss albino mice. The animals treated with gamma radiation and/or cadmium chloride with and without Liv.52 were sacrificed by cervical dislocation at post treatment intervals of 1, 2, 4, 7, 14 and 28 days. After sacrificing them the blood was collected by cardiac puncture in heparinized tubes for various haematological studies viz. estimation of the value of RBC, WBC, haemoglobin, PCV, MCV, MCH, MCHC, SGOT and SGPT. The Values of RBC, WBC, Hb, PCV and MCV were found to decrease in all the groups as compared to normal group. The values of MCH, MCHC, SGOT, SGPT and monocytes & granulocytes percentage found to increase up to day-14 in non drug treated groups and day-7 in drug treated groups. Thereafter a decrease in the value was observed without reaching to the normal. When the animals were treated with radiation and cadmium chloride simultaneously, synergistic effects were observed. In all drug treated groups recovery started earlier than that in non drug treated groups. The Liv.52 treated animals exhibited less severe damage and early recovery as compared to non drug treated groups. Thus, it appears that Liv.52 is potent enough to check haematological changes in the Swiss albino mice.

Keywords: Radiation, Cadmium, Liv52, Blood, Mice

INTRODUCTION

The setting up of large number of nuclear reactors all over the world for power generation has resulted in additional sources of radiation exposure in the form of radioactive effluents and nuclear wastes.

Discovery of radioactive rays, over a hundred years ago, has proved a landmark in the history of medical research. Extensive studies on radio-exposure have yielded plenty of information on its biological effects on living systems at the molecular, cellular, neuro-endocrine and

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Haematological levels (Bauer, 1940; Daga *et al.*, 1995; and Ellinger, 1957). Earlier it was not understood, nor appreciated how a very small deposition of energy by atomic radiation could alter the life of an organism. But very soon, it became apparent that, unlike chemical agents, the cellular penetration of which is prevented by natural cellular barriers, the radiation can penetrate the cells and deposit its energy within them in random fashion, leading to radiation damage (Heda and Bhatia, 1986; Heineke, 1904; and Jacob and Jagetia, 1992; and Krise *et al.*, 1961). Although the injury to several different sites in the cell may be responsible for the lethal effects, but changes in blood cell counts are still considered (although imperfect indices) the most sensitive biological evidences for excessive acute exposure to both external and internal irradiation (Jacob and Jagetia, 1992; Krise *et al.*, 1961; and Kumar *et al.*, 1984). After whole-body exposure manifestation of injury to mammalian tissues is well reflected in peripheral blood (Kumar *et al.*, 1984; and Shaheen and Hassan, 1991). It is generally agreed that Haematopoietic organs i.e., Spleen, thymus and bone marrow are markedly sensitive to ionizing radiation (Ellinger, 1957). The clinical symptoms, which are largely due to damage in the radiosensitive haematopoietic organs, (Heineke, 1904), a very small dose of radiations to a blood-forming organ causes an arrest of the haematopoiesis with changes in the peripheral blood. Cadmium is reported as one of the most toxic heavy metals in the environment and its rapid uptakes and accumulation in food chain contribute to its being a potential environmental hazards (Thomson, 1962) environmental contamination of air, water, soil and food by cadmium is serious threat to all living systems, the modification of radiation and

cadmium chloride response is obtained by mean of chemical substances. That can significantly decrease the magnitude of response when present in biological system (Arvidson, 1980; Axelsson and Piscator, 1966; Bache *et al.*, 1986; Chaptwala *et al.*, 1980; Cross *et al.*, 1970; and Friberg, 1974). This type of modification is classified as chemical protection and the substances responsible for it are called chemical protectors. A large number of compounds have been investigated for protective action by different workers but these protectors are highly toxic at their effective dose levels (Prakash *et al.*, 1988a; Agarwal, 2010; Aslam and Aslam, 1979; Dave *et al.*, 1973; Pandey *et al.*, 1994; Purohit *et al.*, 2002; Purohit *et al.*, 2008; and Purohit, 2007).

Liv.52 is a herbal drug containing constituents from plants that are described in Ayurvedic literature. The herbal preparation is available in the form of drops, syrup and tablets and is prescribed in hospitals in India for the treatment of various types of liver dysfunctions. Secondly, it is claimed to be completely non-toxic even at higher dose levels. It is also being used as detoxicating agent (Aslam and Aslam, 1979).

Each ml. of Liv.52 drops or 2.5 ml of Liv.52 syrup contains: Exts : *Capparis spinosa*, (Kabra), 17mg, *Cichorium intybus*, (Kasni), 17mg *Solanum nigrum* (Makoi) 8 mg, *Cassia occidentalis* (Kasondi) 4 mg, *Terminalia arjuna* (Arjun) 8 mg, *Achillea millefolium*, (Gandana) 4 mg, *Tamarix gallica* (Jhau) 4 mg. The Liv.52 has a similar action in producing nitrogen balance as the anabolic steroids. This drug has multiple actions like hepatic stimulant, choleric, stomachic, anabolic, etiotrophic and encourages, normal growth in children. It also enhances the level of

glutathione in the body of animal (Dave *et al.*, 1973).

MATERIALS AND METHODS

Experimental Animals

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

SOURCE OF IRRADIATION

A cobalt-60 gamma radiotherapy source (Theratron) of AECL make obtained from Canada was used for irradiating the animals in the present investigation. 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 0.97 Gy/minute. The dose was calculated at mid point by multiplying dose rate and tissue air-ratio. The tissue of Swiss albino mice was assumed to be equivalent to human soft tissue.

CADMIUM CHLORIDE TREATMENT

Cadmium salt in the form of Cadmium chloride (SDS Chemicals, India) was prepared by dissolving 20 mg of cadmium chloride in 1000 ml of the glass distilled water, thus giving a concentration of 20ppm and then administered orally in drinking water (Friberg, 1974; Gupta *et al.*, 1989; Hilmy *et al.*, 1985; Kazantzis, *et al.*, 1963; Muller *et al.*, 1982; Murata *et al.*, 1970; and Prakash *et al.*, 1988a).

LIV.52

Liv.52 drops were procured from Himalaya drug company, Mumbai, India. The drug was fed orally at the dose rate of 0.05 ml/animal/day seven days prior to irradiation and cadmium chloride treatment till the last autopsy day of experiment (Friberg, 1974; Gupta *et al.*, 1989; Hilmy *et al.*, 1985; Kazantzis *et al.*, 1963; Muller *et al.*, 1982; Murata *et al.*, 1970; and Prakash *et al.*, 1988a).

EXPERIMENTAL DESIGN

The animals for the experiments were divided into the following groups

Group 1: (Sham-irradiated animals-normal)

Group II: (Cadmium chloride treated animals)

Group III: (Only irradiated animals)

Sub- group III a: 3.0 Gy

Sub- group III b: 6.0 Gy

Group IV: (Animals treated with radiation and cadmium chloride)

Sub-group IV a: 3.0 Gy+CdCl₂

Sub-group IV b: 6.0 Gy+CdCl₂

Group V: (Animals treated with cadmium chloride and Liv.52)

Group VI : (Animals treated with radiation and Liv.52)

Sub- group VI a: 3.0 Gy+Liv.52

Sub- group VI b: 6.0 Gy+ Liv.52

Group VII: (Animals treated with radiation, cadmium chloride and Liv.52)

Sub -group VII a: 3.0 Gy + CdCl₂ + Liv.52

Sub -group VII b: 6.0 Gy + CdCl₂ + Liv.52

AUTOPSY

Five animals from each group were autopsied by cervical dislocation at each post-treatment interval of 1, 2, 4, 7, 14 and 28 days. The weight of animals

was recorded before the autopsy. Five normal mice were also autopsied. Immediately after the autopsy the blood was collected by cardiac puncture in heparinized tubes for various haematological studies.

HAEMATOLOGICAL PARAMETERS

The various haematological parameters estimated were as follows:

- (i) Red Blood Corpuscles (RBC)
- (ii) White Blood Corpuscles (WBC)
- (iii) Haemoglobin (Hb)
- (iv) Packed Cell Volume (PCV)
- (v) Mean Cell Volume (MCV)
- (vi) Mean Corpuscular Haemoglobin (MCH)
- (vii) Mean Corpuscular Haemoglobin Concentration (MCHC)
- (viii) Differential Leucocytes Count (DLC)

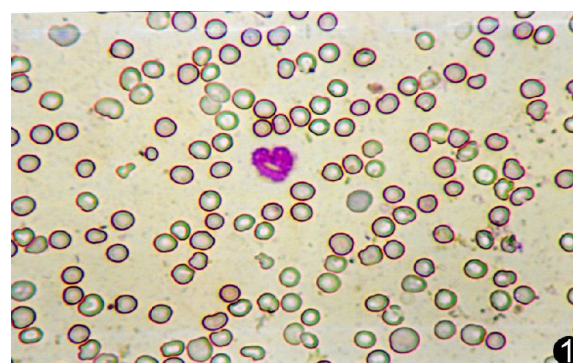
RESULTS AND DISCUSSION

All these parameters were exhibited modulations, in the form of increase or decrease following treatment of cadmium chloride and radiation exposure independently as well as in combination with or without Liv.52. 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 were lesser in Liv.52 Treated groups (V to VII) as compared to non-drug Treated groups (II to IV). Mitra *et al.* (1953) and Norris *et al.* (1966) the values of MCV were also found to decrease but the difference from normal value was non significant at previous intervals and it was non significant on later

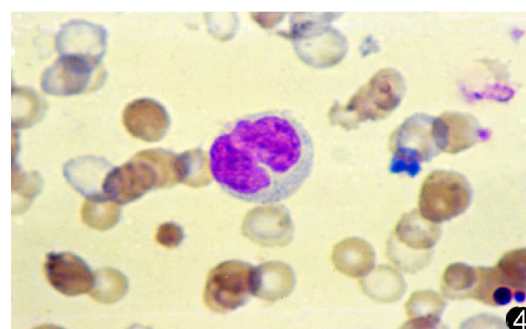
intervals. The values of MCH increased in all the groups as compared with normal groups.

The increase in the value of MCH was lesser in Liv.52 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 Liv.52 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 (Mitra *et al.*, 1953; Norris *et al.*, 1966; Prasad, 1980; Sastry and Gupta, 1993; and Verma, 1991).

Normal RBCs and Small Neutrophil



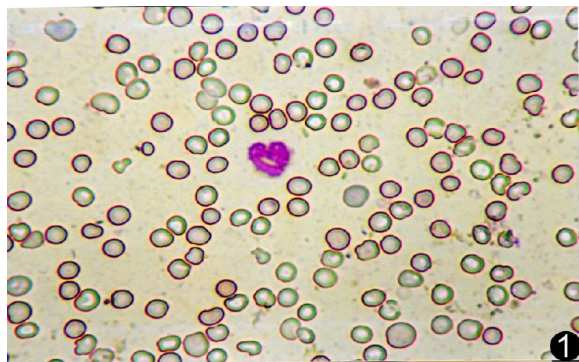
After 1 Day of Cadmium Chloride Treatment Showing Crenation in RBCs and a Complete Monocyte



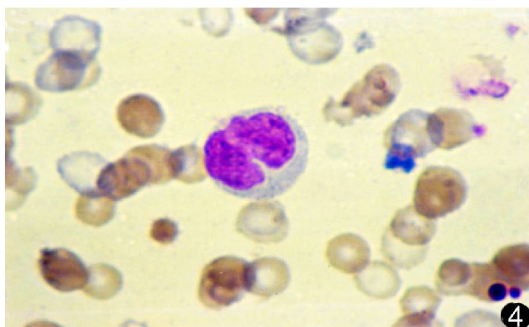
The value of lymphocytes declined up to day-14 in non drug treated groups and day-7 in the Liv.52 treated groups. Similarly the values of monocytes and granulocytes percentage increased up to day-14 in the non drug treated animals and day-7 in the drug treated animals thereafter; a decrease in the value was noted up to day-28 without reaching to the normal.

The values of SGOT and SGPT elevated up to day-14 in the non drug treated groups and day-7 in the Liv.52 treated groups, thereafter a fall in the value was seen up to day-28 (Kapoor, 1971; Chaptwala *et al.*, 1982; and Sastry and Sharma, 1980) from the present findings followings could be deduced.

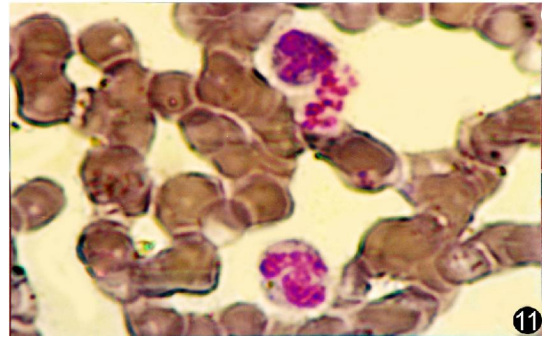
After 7-Days of Cadmium Chloride Treatment Showing Clusters and Crenation in RBCs Neutrophils Are Also Seen



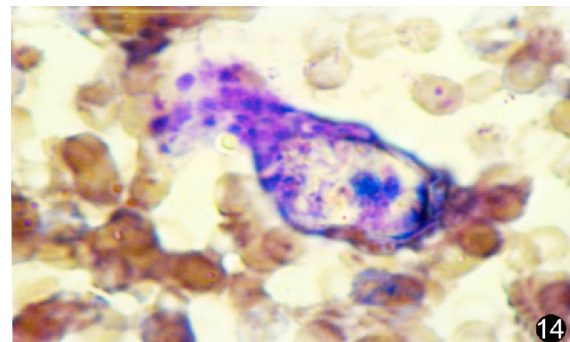
After 1 Day of Gamma Rays (3.0gy) Exposure Displaying Neutrophil and Lymphocyte Distorted RBCs are Also Seen



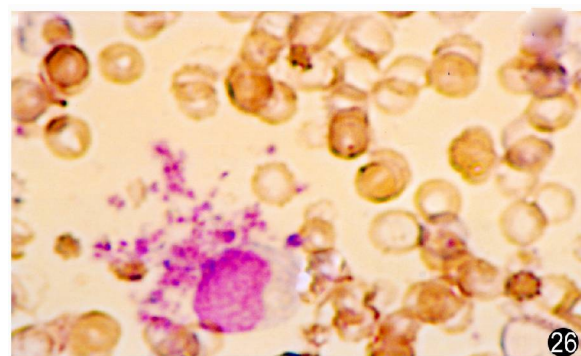
After 4 Days of Gamma Rays (3.0 Gy) Exposure Showing a Complete Neutrophil and Lysing Neutrophil, Crenated RBCs are Also Seen



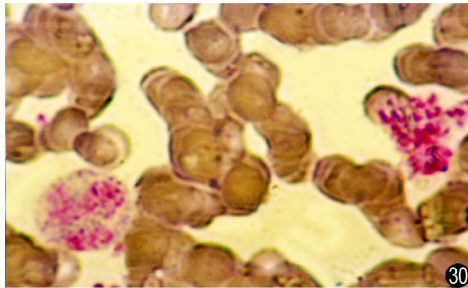
After 4-Days of Gamma Rays (6.0 Gy) Exposure Showing Bursting Lymphocyte, Crenated and Clusters of RBCs



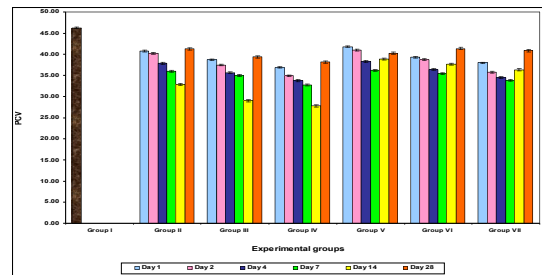
After 4-Day (3.0 Gy + Liv.52) Showing Bursting Monocyte and Crenated RBCs



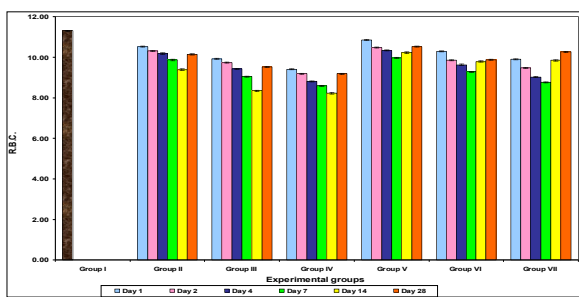
After 7-Days (6.0 Gy + Liv.52) Showing a Complete Neutrophil and Lysing Neutrophil



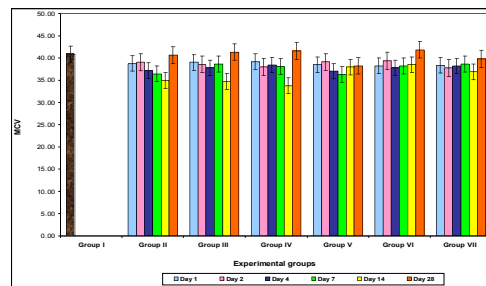
Histogram 4: Variations in the Values of PCV (%) of Mice in Various Experimental Groups (Mean ± S.E.)



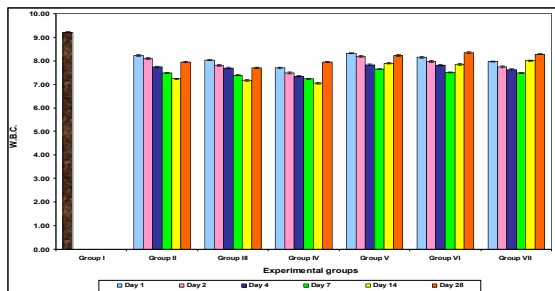
Histogram 1: Variation in the Values of RBC (thousand/Cu.mm) of Mice in Various Experimental Froups (Mean ± SE)



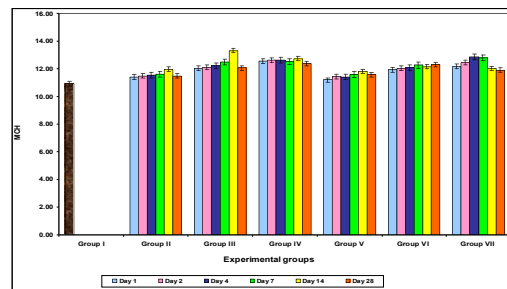
Histogram 5: Variations in the values of MCV (cubic micron) of Mice in Various Experimental Groups (Mean ± S.E.)



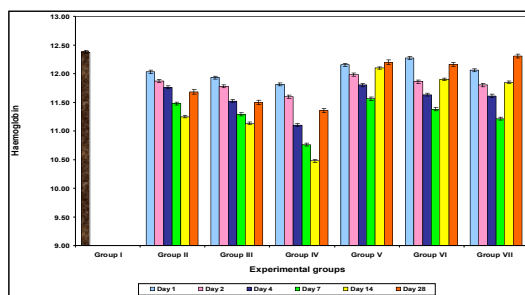
Histogram 2: Variations in the Values of WBC (Thousand/Cu.mm) of Mice in Various Experimental Groups (Mean ± SE)



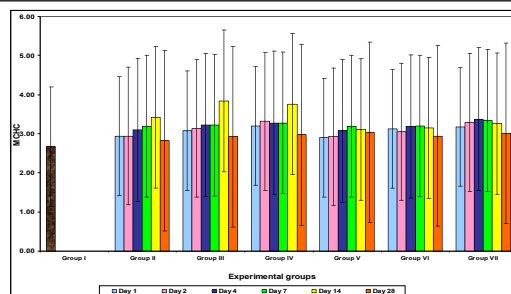
Histogram 6: Variations in the MCH (Micro micro gms) of Mice in Various Experimental Groups (Mean ± S.E.)



Histogram 3: Variations in the Haemoglobin Content (g/100ml.of Blood) of Mice in Various Experimental Groups (Mean ± SE)



Histogram 7: Variations in the Values of MCHC (%) in Blood of Mice in Various Experimental Groups (Mean ± S.E.)



RADIOPROTECTIVE MECHANISM OF LIV.52

The exact mechanism by which Liv.52 prevents the animals from radiation induced damage is not known and secondly, it may not have a single mechanism of radioprotection. It seems that Liv.52 may protect by different mechanisms because of its various physiological and biochemical properties which are as follows:

1. The depletion of intracellular glutathione (GSH) has been reported to be one of the causes of radiation induced damage while increased levels of intracellular GSH are responsible for the radio protective action (Agarwal, 2010; Aslam and Aslam, 1979; Dave *et al.*, 1973; Pandey *et al.*, 1994; Purohit *et al.*, 2002; Purohit *et al.*, 2008; and Purohit, 2007). Same mechanism of action of Liv.52 was proposed by Sarkar *et al.* (1989) who stated that it restores the intracellular GSH level to normal in rats ex-posed to 4.0 Gy of gamma radiation.
2. Saini and Saini (1985a) stated that Liv.52 may neutralize the peroxides formed from water molecules after irradiation which are toxic and cause the damage to the organs.
3. A significant enhancement in the -SH levels in animals treated with Liv.52 has also been observed by Kumari (1989). It is an established fact that only those compounds are potent radio protectors which are having -SH groups in their structures.
4. Pandey *et al.* (1994) stated that Liv.52 decreases lipid peroxidation in liver induced by CCl_4 in albino rats. It has also been reported that the drug inhibits the radiation induced lipid

per oxidation in mouse liver (Jacob and Jagetia, 1992). They further stated that radio-protective activity of Liv.52 may be due to the inhibition of lipid per oxidation by increasing the levels of alpha-tocopherol and glutathione.

5. Thus, it can be concluded that Liv.52 may inhibit the lipid per oxidation by (i) reducing the formations of free radicals; (ii) destroying the free radicals already formed; (iii) by supplying a competitive substrate for unsaturated lipids in the membrane, and (iv) exudation the repair mechanism of damaged cell membrane.

CONCLUSION

From the present findings following could be concluded:

1. The blood of Swiss albino mice suffered with radiation and cadmium induced changes at haematological levels.
2. Alterations in the histological structures followed the biochemical changes.
3. The combined treatment of radiation and cadmium chloride showed synergistic changes.
4. The blood of Liv.52 treated animals showed less severe radio lesions and an early and fast recovery in comparison to non-drug treated animals. Thus, it seems that Liv.52 has protected the blood at both the dose levels with and without cadmium chloride treatment.
5. The Liv.52 might have protected the animals from radiation by more than one mechanism due to multiplicity of its properties.
6. Thus, Liv.52 is a good herbal radio protector and can be given to cancer patients during

radiotherapy to minimize the side effects of exposure.

ACKNOWLEDGMENT

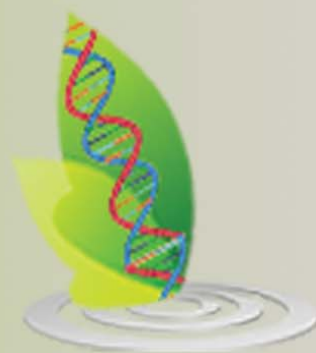
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