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PROTECTIVE ROLE OF LIV.52 AGAINST RADIATION AND CADMIUM INDUCED WHITE BLOOD CORPUSCLES (WBC) CHANGES IN THE SWISS ALBINO MICE

[1]*Sharma Ramakant and Rao Rakesh[2]

[1]Ramadevi Girls P.G. College, Harnathpura (Nua) Jhunjhunu, India.

[2]University of Kota, Kota Rajasthan, India.

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***Correspondence for Author**
Sharma Ramakant
Ramadevi Girls P.G. College, Harnathpura (Nua) Jhunjhunu, India.

ABSTRACT

The present study was aimed to evaluate protective efficacy of a herbal drug Liv.52 against radiation and cadmium induced biochemical 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. The values of WBC decrease in all the group as compared to normal group,but the decrease in these value were lesser in LIV 52treated group(V to VII) as compared to non drug treated group(II to IV). 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 biochemical changes in the Swiss albino mice.

KEYWORDS: Radiation, Cadmium, Liv52, WBC, Mice.

INTRODUCTION

In the present investigation, the combined treatment of cadmium chloride and radiation showed an "additive synergistic" effect and its intensity increases with the dose of radiation. Leucocytes show early response to radiation as compared to the erythrocytes. In the present investigation, irradiation with different doses of gamma rays (3.0 Gy and 6.0 Gy) caused a reduction in the level of white blood cells reaching the minimum on day 14 in non-drug treated groups and day 7 in Liv.52 treated groups and then started increasing upto last autopsy interwal, i.e. day-28. The present findings are in conformation with Daga *et al.* (1997) who also reported a decline in total loucocyte 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 may 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.Norris *et al.* (1966) reported that after 50 days in higher exposures (260-280 Rads), the mean white blood cell counts dropped of a fairly stable level, 5500 cells/mm3 of whole blood as compared to pre-irradiation mean count 10800 cells/mm3 and after exposure to 231- 240 and 241-260 Rads, the total leucocyte counts, were 84% of the pre-irradiation value on day 60.

Norris *et al*. (1966) also suggested that various white blood cells except lymphocytes approached the normal level in about 50 days.Reduction in the level of leucocytes after irradiation is also supported by the earlier findings of Jacobson *et al.* (1948); Krise *et al*.(1961); Kumar *et al*. (1982); Prakash *et al*. (1990); Daga *et al*. (1995) and Goyal *et al*. (1999).Mitra *et al*. (1953) observed decrease in white blood cell counts to different extent, after 0.3 µCi/g, 0.5 µCi/g, 1.0 µCi/g, 2.0 µCi/g and 4.5. µCi/g of P-32 exposure. After 1.0 µCi/g of p-32, a gradual decline in white blood cell count was observed, reaching minimum level at about 12 days and then showed recovery thereafter.Maffei and Mango (1964) found that the leucocyte value, after an initial decrease, returned to normal by 16^{th} day of the administration of 2.0 µCi/g body weight of P-32. With higher doses 5.0 µCi/g, 10.0 µCi/g and 20.0 µCi/g, there was no sign of recovery in the leucocyte value.In cadmium chloride treated animals (Group II), white blood cells exhibited a gradual decline, which continued upto day 14 in the non-drug treated groups and day 7 in the Liv.52 treated groups increasing thereafter. The present findings are similar to those observed by Prakash *et al*. (1988c). They also reported cadmium-induced decrease in white blood cell count in the blood of rat.Besides this, Kumari and Banerjee (1986) observed a significant reduction in small lymphocytes and increase in large lymphocytes along with a significant increase in total leucocyte count in $CdCl_2$ treated fish, Anabas testudineis (Bloch). Another interesting feature was the significant increase in basophil throughout the experiment. These facts indicate hypersensitive nature of leucocytes for the toxicant and a long-term toxic effect on haemogram due to sub-lethal concentrations of the treated heavy metal salts.An increase in white blood cell count after chronic exposure to cadmium was also reported by Sastry and Gupta (1994). After subcutaneous injections of cadmium chloride to male Wistar rats at doses of 0.5, 1 and 2 mg Cd/kg, 3 days/week for 4 weeks Yamano *et al.* (1998) also

observed an increase in the leucocytes count accountable by an increased percentage of neutrophils in peripheral white blood cells.

In combined treatment (Group IV), the number of white blood cells declined more drastically than cadmium chloride or radiation alone, indicating their synergistic effect. In the Liv.52 treated groups (V, VI & VII) decline in WBC level was less severe and an early recovery was also noted showing protective effect of Liv.52. These results coincided with the findings of Purohit *et al.* (2007) and Agarwal (2010). In the present investigation, The haemoglobin content of blood decreased in all the experimental groups. This decrease was dose dependent and continued upto day –14 in the non-drug treated groups and day-7 in the Liv.52 treated groups. Thereafter, the value increased in all the experimental groups. The decrease was more prominent in combined treated groups. In the Liv.52adminstered experimental animals decrease was less severe which may be due to the protection provided by the drug. Daga *et al.*(1995) also reported a decline in haemoglobin percentage till day 5 in 1.25 Gy gamma irradiated animals and till day 14 in 3.60 Gy irradiated animals. Kumar *et al.* (1983) also observed a gradual and continuous decrease in the haemoglobin value and the minimum value was recorded on day 14. Decreased haemoglobin content was also reported by Heda and Bhatia (1986); Shaheen and F Hassan (1991) 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 Tsuya *et al*. (1961) observed that the total number of erythrocytes precursors reduced to about 15% of normal value within one day after exposure of rates to 600 R. Rates 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. (Baum, 1961). According to Baxter *et al*.(1955), primary cause for the initial reduction in labelled iron incorporation was the mitotic inhibition and destruction of erythropoietic tissue. Odurtchenko *et el*.(1964) found a rapid fall of mitotic index in erythroblast after irradition, which was due to the occurrence of blockage in late prophase.Cassarette (1953), studying 6 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 haemoconcentration, 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. After dietary exposure to cadmium, decreased haemoglobin concentration was among the early signs of cadmium toxicity (Decker *et al*., 1958). Cadmium interferes with the formation of haemoglobin, almost certainly in the bone marrow, after the stage of protoporphyrin. The combination of iron with haeme is inhibited (Scott, 1973). In rat, it has been demonstrated that intoxication by cadmium can hinder the resorption of iron resulting in an iron deficiency anaemia (Huebers *et al*., 1987).Cadmium-induced permanent decrease in the value of haemoglobin was observed by Kumari and Banerjee (1986). The present observation also showed a significant decrease in haemoglobin content continuously upto day 14 in the cadmium chloride treated group II and day 7 in the Liv.52 pre treatment groups V but increasing thereafter. Decrease in haemoglobin content due to cadmium toxicity has been reported by many workers (Gill and Pant, 1986; Prakash *et al*., 1988 c, Mukherjee and Sinha, 1993; Patil and Dhane, 1996; Mackova *et al*., 1996).Higher cadmium doses (25-100 ppm) orally administered to rats, resulted in either a decrease or unchanged haemoglobin value (Prigge, 1978). In quail, as little as 5 ppm cadmium orally administered was sufficient to reduce duodenal Iron concentration and to produce anaemia (Jacobs *et al*., 1972). Moreover, cadmium-induced Fe deficiency may result mainly from losses of non-haeme Fe (ferritin) without any effect on blood haemoglobin (Stonard and Webb, 1976). Thus, an anaemic response of rats due to low dietary $CdCl_2$ doses cannot be observed inspite of a significantly disturbed Fe metabolism.The animals of group IV exhibited more decrease in haemoglobin percentage of blood as compared to individual effects of radiation or cadmium chloride. This may be due to combined action of radiation and cadmium. Similarly in the Liv.52 preadministered animals the decrease in haemoglobin content was less severe and an early recovery was also showing protective effect of Liv.52.

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. 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

Group1: (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+$CdCl_2$

Sub-group IV b: 6.0 Gy+$CdCl_2$

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 + $CdCl_2$ + Liv.52

Sub -group VII b: 6.0 Gy + $CdCl_2$ + 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 biochemical studies.

WHITE BLOOD CORPUSCLES (WBC)

The number of WBC in blood was estimated by visual method using improved double ruling neubauer haemocytometer as given by Dacie and Lewis (1974).

Principle

The W.B.C. in 1 cu. mm being in thousands makes the dilution of blood essential. This is done by using a diluting fluid which contains acetic acid for the lysis of RBC and Gentian violet which stains the nucleus of WBC's.

Reagent

Turck's fluid (WBC diluting fluid)

Gentian violet –10mg.

Glacial acetic acid –1.0ml.

Gentian violet was dissolved in glacial acetic acid and the final volume was made to 100ml by adding glass-distilled water.

Procedure

Well mixed whole blood was drawn to 0.5 mark in a white blood cell diluting pipette and diluting to "11'' mark with WBC diluting fluid. The pipette was shaken for a few minutes and the first few drops were discarded. The counting chambers of the haemocytometer were charged with blood mixture from the pipette. The cells were allowed to settle in the chamber for 1 to 2 minutes and were then counted under the microscope at the magnification of 150x. The count was made in the four large squares situated at the corner of the chambers in the

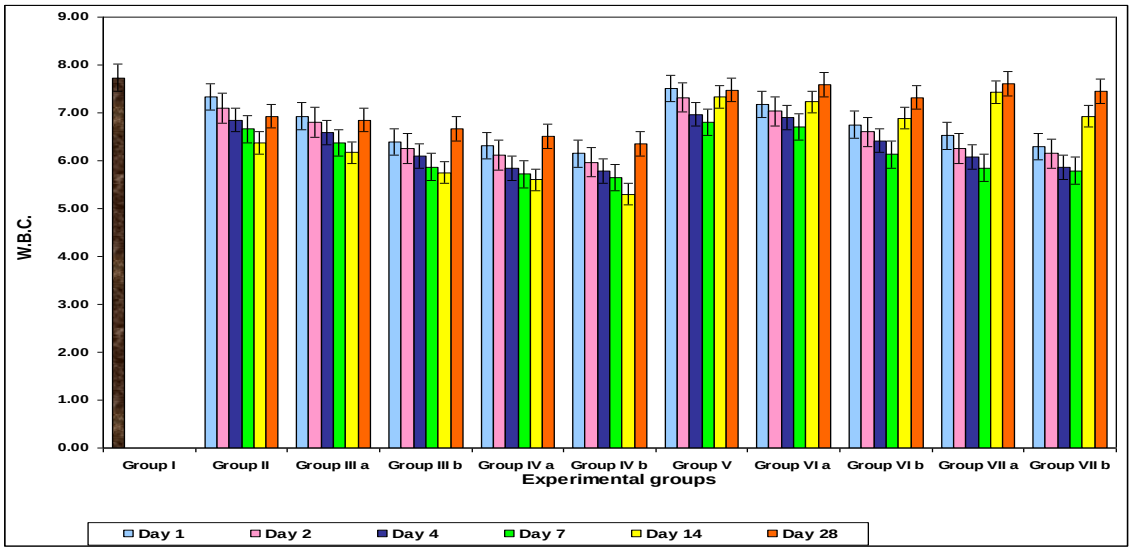
haemocytometer, each consisting of 16 smaller squares. Each large square has an area of one sq. mm.

Calculations

The average number was obtained by dividing the total count by five.

RESULTS AND DISCUSSION

The values of WBC, were found to decrease in all the groups as compared to normal group, but the decrease in these values was lesser in Liv.52 treated groups (V to VII) as compared to non-drug treated groups (II to IV).



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 physiolog ical 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 radioprotective action (Revesz *et al.*, 1963). Same mechanism of action of Liv.52 was proposed by Sarkar *et al.* (1989) who stated that it restores the intracelluar GSH level to normal in rats exposed 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 radioprotectors 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 peroxidation in mouse liver (Ganapathi and Jagetia, 1994). They further stated that radioprotective activity of Liv.52 may be due to the inhibition of lipid peroxidation by increasing the levels of α-tocopherol and glutathione.
5. Thus, it can be concluded that Liv.52 may inhibit the lipid peroxidation by (i) reducing the formation of free radicals; (ii) destroying the free radicals already formed; (iii) by supplying a competitive substrate for unsaturated lipids in the membrane, and (iv) exudating 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.

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