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Chemopreventive Effect of Liv.52 Against Radiation and Cadmium Induced Hepatic Biochemical Alterations in Mice

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Abstract

In the present investigation, protective effects of herbal drug Liv. 52 against radiation and cadmium induced biochemical changes in the liver of mice have been taken into consideration. For the purpose, the Swiss albino mice were exposed to 2.5 Gy and 5.0 Gy of gamma radiation with and without Liv.52 treatment. The value of total proteins, glycogen, cholesterol, acid and alkaline phosphatase activities, DNA and RNA were observed in the form of increase or decrease. After combined treatment the changes were more severe due to synergistic effect. An early and fast recovery observed in the Liv.52 treated groups may be due to the protection provided by the drug.

Keywords: Gamma Radiation, Cadmium chloride, Swiss albino mice, Liv.52, Liver.

Introduction

Radiation causes deleterious effects in all forms of life due to increasing utilization and production of modern technology, a simultaneous exposure of organisms to heavy metals is also unavoidable. These heavy metals become toxic when present in large quantities, with increasing the industrial revolution and industrial waste, the emission of cadmium has increased into the environment. Thus concomitant exposure to cadmium chloride and ionizing radiation might produce deleterious effect upon biological system. The total environmental burden of toxicants may have greater effect as against their individual impact as expected by their nature. So interaction between radiation and other toxicants represents a field of great potential importance. In the recent years, immense interest has been developed in the field of chemoprotection against radiation and heavy metals induced changes. Liv. 52 is an Ayurvedic 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[1-5].

Each ml. of Liv.52 drops or 2.5 ml of Liv.52 syrup contains:

Extracts:

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

Processed in *Eclipta alba* (Bringharaj), *Phyllanthus amarus* (Jar-amla), *Boerhaavia diffusa* (Sant), *Tinospora cordifolia* (Giloe), *Berberis aristata* (Daruhaldi), *Raphanus sativus* (Muli), *Phyllanthus emblica* (Amla), *Plumbago zeylanica* (Chitrak), *Embelia ribes* (Baberang), *Terminalia chebula* (Hirda), *Fumaria officinalis* (Pit-papara).

Therefore our study aims to investigate the protective effect of Liv.52 (a herbal drug) against combined exposure of radiation and cadmium on liver of Swiss albino mice.

Review of Literature

The use of chemicals to protect against the harmful effects of radiation was attempted after World War II with the realization of the need to safeguard humans against the military use of atomic weapons. It has been investigated that The effect of amino-acid cysteine in rats exposed to lethal doses of X-rays has been investigated and it was found that pretreatment of rats protected them against the radiation-induced lethality. Thereafter, several chemical compounds and their analogues have been screened for their radioprotective ability however, their high toxicity at optimum protective doses precluded their clinical use. The other major drawback of these compounds was that they were unable to provide post-irradiation protection. With the recognition that normal tissue protection during radiotherapy is as important as the destruction of cancer cells, the focus of protection research became more therapy oriented. Recent terror attacks throughout the world has strengthened the idea that it its necessary to devise appropriate measures against the nuclear terror attacks by using pharmacological agents that can protect against the ill effects of radiation[6-8].

Materials and Methods

Animal care and handling

The adult healthy male Swiss albino mice (6-8 weeks old) were procured from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hissar. The Govt. Dungar College, Bikaner is registered under CPCSEA, Chennai (registration no. 1066/ac/07/ CPCSEA) and has its own Institutional Animal Ethics Committee (IAEC). In view of the fact, the present experiments were conducted under the supervision of IAEC of the College. The animals were housed in polypropylene cages and maintained on balanced mice feed and tap water *ad libitum*. They were acclimatized to laboratory conditions before use. Occasionally tetracycline water was provided as a precaution against infection. The temperature of the room was maintained between 22-27ºC.

Source of irradiation

Cobalt-60 gamma radiotherapy source (Theratron) of AECL make, obtained from Canada was used to expose the animals. This facility was provided by the Radiotherapy Department of Prince Bijay Singh Memorial Hospital, Bikaner (Rajasthan). The animals were irradiated at the dose rate ranging from 0.97 Gy/min to 1.97 Gy/min. The dose was calculated at the mid point by multiplying dose rate and tissue air ratio. The tissues of Swiss albino mice were assumed to be equivalent to human soft tissues.

Cadmium

The aqueous solution of the 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 20 ppm and then administered orally in drinking water.

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.

Experimental design

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

Group – I (Sham-irradiated animals)
Group - II (Cadmium chloride treated animals)
Group – III (Irradiated animals)
Sub-group III a: 2.5 Gy
Sub-group III b: 5.0 Gy
Group - IV (Animals treated with radiation and Cadmium chloride)
Sub-group IV a: 2.5 Gy + Cadmium chloride
Sub-group IV b: 5.0 Gy + Cadmium chloride
Group - V (Cadmium chloride and Drug treated animals)
Group - VI (Radiation and drug treated animals)
Sub-group VI a: 2.5 Gy + Liv.52
Sub-group VI b: 5.0 Gy + Liv.52
Group – VII (Radiation, Cadmium chloride and drug treated animals)
Sub-group VII a: 2.5 Gy + Cadmium chloride + Liv.52
Sub-group VII b: 5.0 Gy + Cadmium chloride + Liv.52

Autopsy

A minimum of five animals from groups II to VII were sacrificed by cervical dislocation and autopsied at each post-treatment intervals of 1, 2, 4, 7, 14 and 28 days. Five sham-irradiated mice were also sacrificed in the similar manner. After sacrificing the animals, pieces of the liver were taken out and at -20^{0}C for the estimation of following biochemical parameters :

1. Total proteins[9]
2. Glycogen[10]
3. Cholesterol[11]
4. Acid and Alkaline phosphatase activities[12]
5. DNA[13]
6. RNA[14]

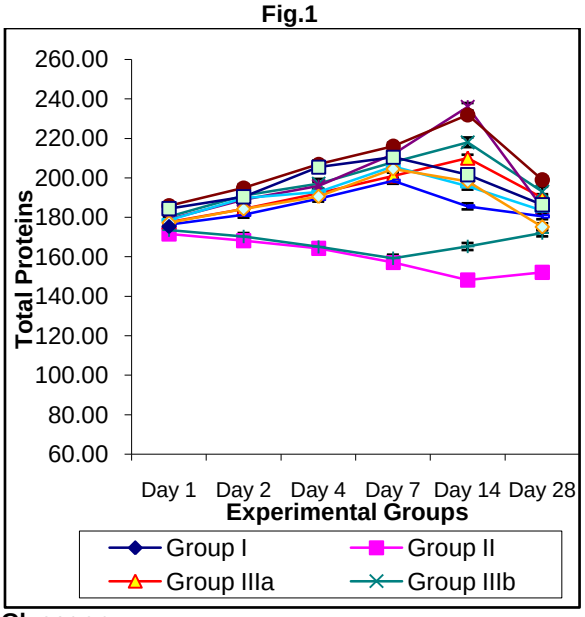
Results

Total Proteins

The total proteins content showed an increasing trend in non-drug treated groups III and IV as well as Liv.52 treated groups VI and VII respectively. In the groups II and V the value of total protein showed a decreasing trend. This increase or decrease was comparatively lesser in the Liv. 52 treated animals showing protection by the drug. The value of total proteins increased in groups III and IV on day-1 and continued so up to day 14, thereafter, it declined on day- 28, whereas, in drug treated groups VI and VII the value increased up to day-7 thereafter it declined on day 14 and continued to decline up to day- 28. Similarly in group II the value decreased up to day-14 thereafter it increased on day-28, whereas in the group V the value decreased up to day-7

hereafter it increased on day-14 and continued to increase up to day-28 (Fig.1).

Fig.1

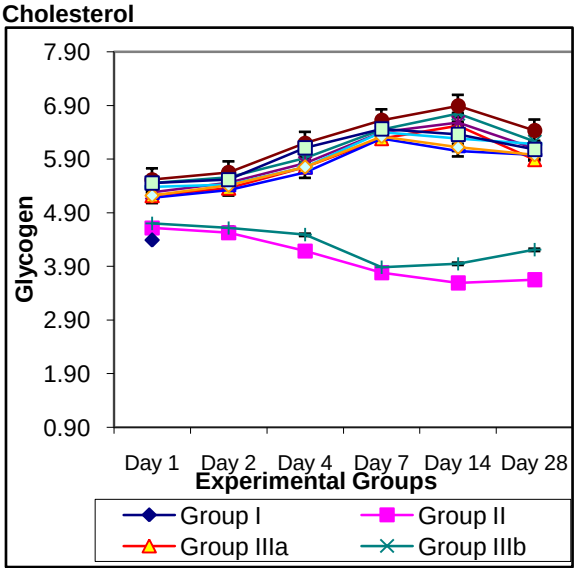


Glycogen

The glycogen content showed an increasing trend in non-drug treated groups III and IV as well as Liv.52 treated groups VI and VII respectively. In the groups II and V the value of glycogen showed a decreasing trend. The value increased in groups III and IV on day-1 and continued so up to day 14 thereafter it declined on day 28, whereas in groups VI and VII the value increased up to day 7 thereafter it declined on day 14 and continued so up to day 28. Similarly in group II the value decreased up to day 14 thereafter it increased on day 28, whereas in the group V the value decreased up to day 7 thereafter it increased on day-14 which continued up to day-28**(Fig.2)**.

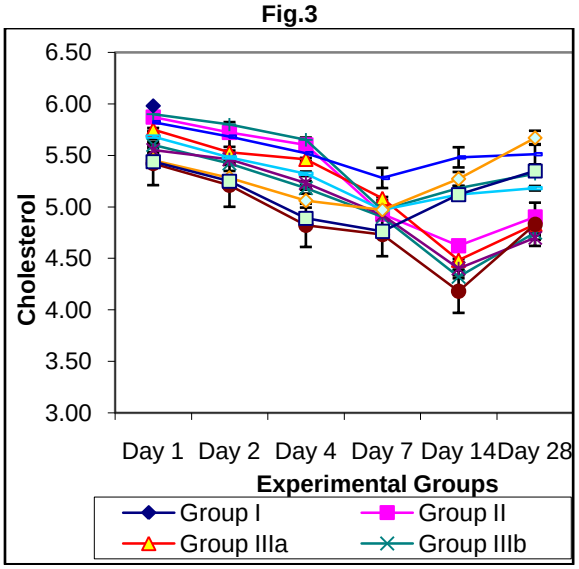
Fig.2

Cholesterol



The value of cholesterol decreased on day-1 and continued to decrease till day-14 in groups II, III and IV respectively. It increased on day-28 without reaching to the normal level. In the Liv. 52 treated groups V, VI and VII the value decreased up to day-7. Thereafter it increased on day-14 and continued to increase till day-28. The decrease was found dose dependent. In Liv. 52 treated groups a less severe decrease and early recovery in the cholesterol level was noticed (Fig.3).

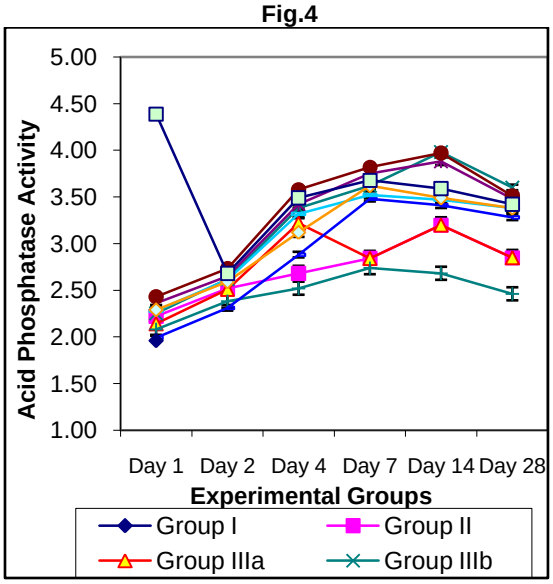
Fig.3



Acid Phosphatase Activity

The acid phosphatase activity showed an increasing trend on day-1 and continued to increase up to day-14 in the groups II, III and IV respectively. The value decreased on day-28. In the Liv. 52 treated groups the value of acid phosphatase activity increased up to day-7 and then decreased on day-14 and continued so up to day-28(Fig.4).

Fig.4

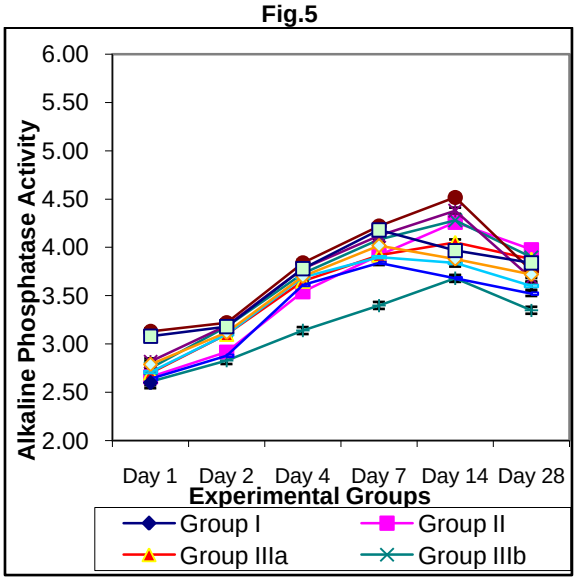


Alkaline Phosphatase Activity

The alkaline phosphtase activity increased on day-1 in the groups II, III and IV which continued so up to day-14. On day-28 the value decreased. On the other hand, in the Liv. 52 treated groups V, VI and VII the value increased up to day-7 thereafter it

increased on day-14 and continued to increase up to day-28. A less severe increase was observed in the drug treated groups (Fig.5).

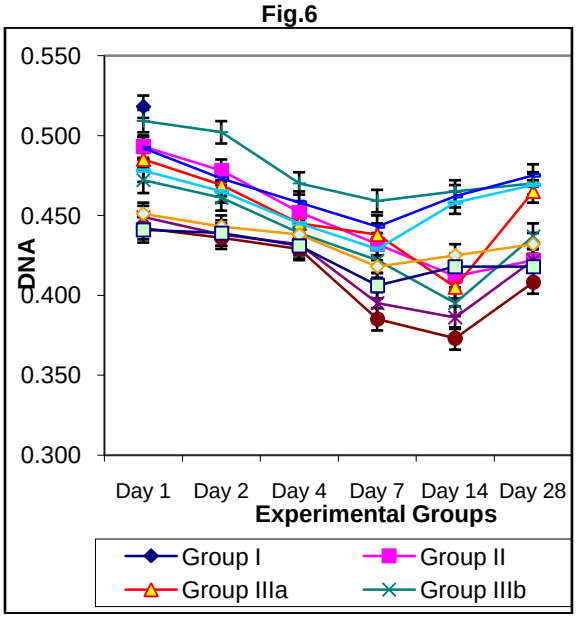
Fig.5



DNA

The DNA content decreased in all the groups. The decrease was found dose dependent. The value of DNA declined up to day-14 in groups II, III and IV thereafter, it increased on day-28. In the Liv. 52 treated group V, VI and VII the DNA content decreased up to day-7 then it increased on day-14 which continued up to day-28. In Liv. 52 treated animals there was lesser decrease and early recovery observed(Fig.6).

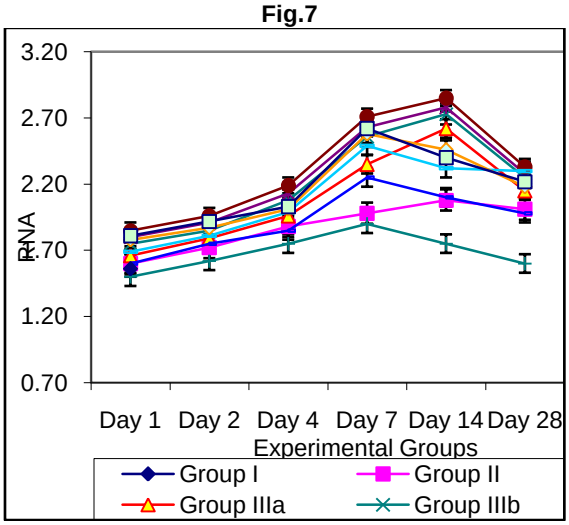
Fig.6



RNA

The concentration of RNA increased in all the groups. The RNA content increased on day-1 and continued so up to day-14 in the non drug treated groups II, III and IV respectively. The value declined on day-28 without reaching to the normal level. In the Liv.52 treated groups V, VI and VII the RNA content increased up to day-7, thereafter it declined on day-14 which continued up day-28. The increase was found dose dependent(Fig.7).

Fig.7



Discussion

Once taken up enterally, Cd (Cadmium) reaches the liver where it binds to metallothioneins (MTs), glutathione (GSH) and other proteins or peptides[15]. Metallothioneins induced upon Cd exposure can act as a " double - edge sword". On one hand MTs bind to Cd, thereby detoxifying and removing it from the cellular environment. On the other hand, due to its thiol groups, MTs can scavenge reactive oxygen species(ROS) that are produced as result of Cd – induced oxidative stress[16]. However, the latter results in Cd dissociation from MTs due to the corresponding decreased metal binding stability[17] intracellular Cd , in bound or unbound form, culminates in mitochondrial damage, and/or cell death.

Ionizing radiation is known to induce various physiological, and biochemical changes in humans and animals. Several molecular mechanisms of ionizing-radiation have been proposed, including cumulative damage by ROS, dislocation in replicative cells, genome instability, mutation, or altered expression of specific enzymes and cell death[18]. The oxidative stress due to free radical-formation was greatly augmented during ionizing-radiation exposure[19]. It was likely that animal particular antioxidants generally decreased the level of oxidation in such systems by transferring hydrogen atoms to the free radical structure[20].

Total Proteins

Total serum proteins are, diagnostically, of relative importance in assessing the state of health of an organism, their increase appearing especially in inflammatory process and tissue dysfunction after irradiation[21]. The present study revealed that irradiation resulted in continuous augmentation in total proteins in liver tissue up to day 7th that probably as a result of an increased transport of amino acid through

plasma membrane as a consequence of permeability changes in irradiated cell membrane[22]. In addition, increased synthesis of m- RNA and ribonucleoprotein could also be added to the radiation induced increased level in proteins[23].

Cadmium attacks on –SH group of proteins and it may be possible that Liv.52 providing some protection by additional –SH groups against the destructive action of Cadmium. The total protein decreased in cadmium chloride treatment on day-1 and this trend continued till the last autopsy interval due to dysfunction of the hepatocytes as induced by chemical lorded[24].

Liv.52 treated animals prior to irradiation showed a significantly lower concentration of protein in liver than control. Increased level of it was observed up to day-14 after 5.0 Gy irradiation, respectively. Thereafter, the protein level tended to recover on later autopsy intervals. It is suggested that protection of protein is due to the hydrogen atom donation by the protector[25].

Glycogen

Higher level of hepatic glycogen after irradiation could be due to the stimulation of the pituitary-adrenal system[26,27]. since it has been shown that irradiation did not increase the hepatic glycogen in the hypophysectomized rats[28].

Fatty degeneration, necrosis, increase in connective tissues are the changes produced by heavy metals, which have been described by a number of workers[29]. Result of our experiments on Swiss albino mice treated with cadmium chloride exhibit a fall in glycogen values. The loss of glycogen from hepatocytes was statistically significant when compared with the values of normal group. The loss of glycogen in liver takes place before the cell necrosis and it can also drop in physiological circumstances. The present observations are in agreement with those of[30] who also reported decrease in glycogen content due to cadmium toxicity, this change attributed to the increased glycogenolysis after cadmium treatment.

In the present study, when Liv.52 extract was given before Cadmium treatment, the change in glycogen content remained similar to that of control group (without Liv.52), but the values were found to less prominent than the controls. Similar results were also observed with the Liv.52[31].

Cholesterol

After irradiation the reduction of cholesterol concentration in liver during early intervals might be due to the stress response caused by radiation which stimulates the synthesis of steroid hormones via hypothalamic-pituitary system[32].

In the present investigation cholesterol showed a significantly declining pattern till day-14 in the cadmium chloride treated group II and day-7 in the drug treated groups V but afterwards there was a significant elevation in cholesterol. It was suggested that the decrease in cholesterol level may be related to its enhanced utilization in corticosteroidogenesis and/or a decreased *de novo* synthesis. Involvement of thyroid hormones has also been suggested in cholesterol metabolism and an enhanced breakdown in hyperthyroidism is known to result in hypochoelesterolemia[33].

In the present study Liv.52 treated groups showed decreasing trend in value of cholesterol up to day-7 then increased on day-14 which continued up to day-28. Liv.52 is major antioxidant which affect cholesterol metabolism through its antioxidant effect[34]. In the present experiments animals treated with both radiation and cadmium also exhibited a decrease in the level of cholesterol in liver. While Liv.52 minimize the level of variation of cholesterol in liver showing its protective effect.

Acid and Alkaline phosphatase activity

Radiation exposure resulted in elevation of liver phosphatase activity which may be attributable to the tissue impairment and per- oxidation of membrane lipids leading to activation of suppressed acid hydrolyases[35,36]. An increase in acid phosphatases activity after radiation exposure in the present experiment could be ascribed either to a direct effect of radiation which results in enhanced Golgi activity[37] and per- oxidation of lysosomal membranes by cadmium causing lysis of cellular membranes of hepatocytes, which in turn leads to an increase in the permeability of cell membranes and facilitates the passage of cytoplasmic enzymes outside the cells leading to the increase in both enzyme activity in liver[38-40].

From the present study, it appears that Liv.52 caused an early recovery to normalcy in both the enzyme level, which was evident as statistically lowered values in these groups in comparison to control. Active principles of Liv.52 in terms of augmentation of oxidative free radical scavenging enzymes, concomitant with reduction in radiation induced lipid peroxidation. It is quite possible that the Liv.52 retards the formation of the toxic lipid peroxidase responsible for radiation damage.

DNA

The depletion in the DNA content of a tissue *in vivo* is due to reduction in or absence of the essential factors controlling the DNA synthesis[41]. These factors are the substrates (Four deoxyribonucleoside triphosphates); enzymes (Polymerase), template activity of deoxyribonucleo proteins activators (Mg^{++} and other divalent ions). The enzymic incorporation of deoxyribonucleotides into DNA by the mammalian testis has been studied. The principal type of enzyme involved seems to be a replicative DNA nucleotidyl transferase, which catalyzes the incorporation in DNA of deoxribonucleotides. There was a general agreement that interference with DNA was one of the important biological effects of irradiation[42]. Similar results were also observed with *Aloe vera*[43].

RNA

The results of the effects of ionizing radiation *in vivo* synthesis of nucleic acids in a mammalian radiosensitive tissue depends to a great extent on two important factors[44]:

1. More or less rapid cytolysis of large proportion of cells, and

2. Changes in population of cells after irradiation.

RNA metabolism may be influenced by a number of factors. Reasons for the increase in RNA due to irradiation could be due to an increase in the RNA concentration of the surviving cells after radiation insult. Causes of this increase in the cellular RNA may be:-

1. Ability of DNA to transcribe RNA is not affected quantitatively but the length of the chain of RNA molecules reduces[45].
2. Increase in the nuclear RNA polymerase activity may contribute to the post-irradiation increase in the cellular RNA[46].
3. Increased gonadotropin secretion after irradiation may accelerate the RNA synthesis after higher doses of irradiation[47].

Protective 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 radioprotective action[48]. Same mechanism of action of Liv.52 was proposed in the form that it restores the intracelluar GSH level to normal in rats exposed to 5.0 Gy of gamma radiation[49].
2. Liv.52 may neutralize the peroxides formed from water molecules after irradiation which are toxic and cause the damage to the organs[50].
3. A significant enhancement in the –SH levels in animals treated with Liv.52 has also been observed[48]. It is an established fact that only those compounds are potent radioprotectors which are having -SH groups in their structures.
4. 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[49]. 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[51].
5. Liv.52 also exerts protective effects against radiation and cadmium induced biochemical changes in the blood of mice[54].

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.

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