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PROTECTIVE EFFECTS OF *ALOE VERA* AGAINST RADIATION INDUCED BIOCHEMICAL DISORDERS IN LIVER OF SWISS ALBINO MICE

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Keywords:

Radiation, *Aloe vera*, Protein, Cholesterol, Glycogen, Free radicals

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ABSTRACT: In this study, radioprotection against radiation-induced hepatic damage in Swiss albino mice has been observed by *Aloe vera*. When individuals are exposed, the radiation energy is absorbed by the biological systems, which causes radiolysis of tissue water and generates free radicals. Animals were given *Aloe vera* leaf extract orally 1000 mg/kg body weight/day for 15 consecutive days before radiation exposure 0.5, 3 and 5.5 Gy gamma radiation. Mice were autopsied at day ¼, 1, 3, 5, 10 and 20 after irradiation to evaluate the radio modulator effect in terms of protein, cholesterol, and glycogen. In control set animals radiation treatment showed an increase of protein, glycogen, and cholesterol, however, the animals of experimental group, *i.e.*, *Aloe vera* and radiation combined group showed a significant decrease in protein, glycogen and cholesterol but values remained below normal. It can be concluded that *Aloe vera* modulate the radiation-induced biochemical alterations in Swiss albino mice.

INTRODUCTION: Depending on the energy of the radiated particles radiation is categorized as either ionizing or non-ionizing. Radiation pollution is the result of global changes in the climate because of human activities which result in the many severe environmental hazards, and human exposure is one of the most common incidents in the same. It has been proved that production of reactive oxygen species (ROS) such as hydroxyl radicals, hydrogen peroxide, superoxide anion radicals, etc initiated by ionizing radiations [1]. The *Aloe vera* plant, *Aloe barbadanis* Miller, family Liliaceae (Lily of the desert) is the most investigated and used of more than 300 species of *Aloe*.

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The usefulness of *Aloe vera* for the prophylaxis of radiation-induced dermatitis has demonstrated by clinical trial [2]. *Aloe vera* contains 75 potentially active constituents that include vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids [3]. Many investigators have shown that *Aloe vera* extract, induces hepatoprotective effects [4]; protects against heavy metals induce oxidative stress [5]; as well as enhances anti-inflammatory properties [6]. On the other hand, *aloe vera* induces immune-stimulation actions [7]. Given these considerations, the present study was carried out to evaluate the curative effect of *aloe vera* extract against radiation-induced some biochemical parameters in albino mice.

MATERIAL AND METHODS:

Animals: For the present study, male Swiss albino mice of 6-7 weeks old, weighing 24-26 g were selected from an inbred colony. The selected animals were maintained under controlled conditions of temperature and light during the

experimental period. The animals were provided standard mice feed (Procured from Ashirwad Industries, Chandigarh, India) and water *ad libitum*. Tetracycline was also given along with drinking water to them once a fortnight as a preventive measure against infection. All surgical and experimental procedure was performed by the recommendations found in the Guide for the Care and approved by the institutional Animal House and Use Committee of the University of Rajasthan, Jaipur.

Source of Irradiation: Animals were irradiated at Cancer Treatment Center, S.M.S. Medical College and Hospital, Jaipur by using Cobalt teletherapy unit (ATC-C9). Animals were kept properly in a well-ventilated wooden box and distance between the animals in a wooden box, and the radiation source was 77.5 cm for exposure at the dose rate of 1.33 Gy/min. The dose rate was calibrated time to time throughout the experimentation according to the decay table of Co60.

Preparation of *Aloe vera* Leaf Extract: The *Aloe vera* leaf was collected locally. The specimen was placed at Herbarium, Department of Botany, University of Rajasthan, and Jaipur. The voucher number is RUBL-19886. Extract of fresh, shade dried and powdered leaves of *Aloe* were prepared in ethyl alcohol. Powder of *Aloe* leaves was mixed with double the volume of alcohol. The mixture was stirred and left for 24 h and filtered after that through cheese-cloth. The leftover residue after filtration was again mixed with the same volume of ethyl alcohol as used earlier and the procedure was repeated two more times.

Finally, all three filtrates were mixed, and alcohol was allowed to evaporate naturally from it at the room temperature (30 ± 30 ºC) to obtain a concentrated *Aloe* extract, which was put in the oven at 40ºC for complete evaporation of alcohol. The powdered extract was redissolved in DDW just before oral administration.

Experimental Design: For this study, selected adult male Swiss albino mice were divided into five groups (I, II, III, IV, and V).

Group I: Animals of this group were given double distilled water (DDW) orally (volume equal to that used for *Aloe* administration in experimental mice) for 15 consecutive days and called sham-irradiated (normal) group.

Group II: Animals of this group were administered *Aloe* extract orally at the dose of 1000 mg/kg body weight (once in a day) for 15 consecutive days to study its toxic effects on the liver if any.

Group III, IV, and V: Each group from III –V was divided into two sets, one was experimental, and another control. Animals of experimental set were administered *Aloe* extract orally at the dose of 1000 mg /kg body weight (once in a day) for 15 consecutive days, whereas animals of control set were given double distilled water (DDW) orally (volume equal to that used for *Aloe* administration in experimental sets) for 15 consecutive days.

Just after 1 h of last administration of extract and DDW, animals of group III, IV and V have exposed to sublethal doses, i.e. 0.5, 3 and 5.5 Gy gamma radiations.

Parameter Studied:

- ✓ Total Proteins were measured by the method of [8].
- ✓ Glycogen was measured by the method of [9].
- ✓ Cholesterol was measured by the method of [10].

RESULT:

Total Proteins: Contents of total proteins in normal mice (group I) were considered as 100 percent, which increased to 103.02 percent in mice treated with *aloe* alone (group II) **Fig. 1**. Total proteins contents increased gradually from 6 h in 0.5, 3 and 5.5 Gy irradiated alone animals and reached at the highest level (111.21%, 130.59%, 158.31% respectively) on 5 days post-irradiation. After that level tended to decrease at day 20 **Fig. 1**. In *Aloe treated* irradiated (0.5, 3, 5.5 Gy) animals total proteins contents also increased from 6 hours to day 5 but this increase was significantly lesser ($p<0.05$) than that of irradiated alone animals. After it, total proteins level decreased and was found 103.29, 113.75, and 145.20 percent at 20 days respectively in 0.5, 3 and 5.5 Gy **Fig. 1**. After radiation, the protein increases in control set because the permeability of plasma membrane increases and amino acid transport is also extended.

Treatment with *Aloe vera* before radiation exposure (Experimental set) protected the plasma membrane against free radicals. This induces alterations in its permeability, which inhibited amino acid transport and ultimately decreased protein synthesis [11].

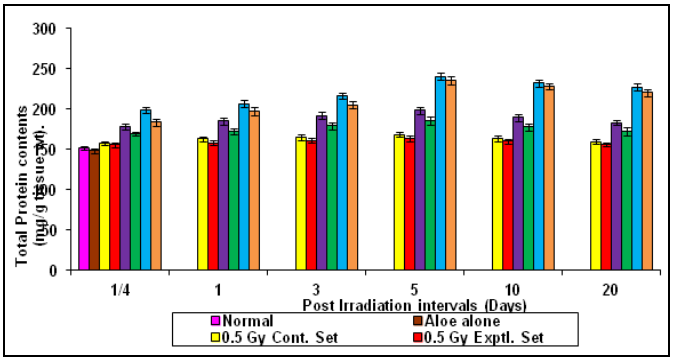

FIG. 1: TOTAL PROTEIN CONTENTS (mg/g TISSUE wt.) IN LIVER OF SWISS ALBINO MICE EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION WITH AND WITHOUT PRETREATMENT OF *ALOE*

Glycogen Contents: Glycogen contents in mice of group I was considered as 100 percent, which decreased to 96.06 percent in mice treated with group II **Fig. 2**. The contents of glycogen increased in mice liver and found 102.05, 109.75 and 116.68 percent at 6 h after irradiation with 0.5, 3 and 5.5 Gy respectively, which continued to increase and reached at maximum level (119.43%, 122.73%, and 137.07%) on day 5 in all three groups. After that, a gradual decreased was noticed at day 20 **Fig. 2**. An increase in hepatic glycogen in control set is due to increased rate of protein catabolism & the release of substances from radiosensitive cells after radiation dose [12].

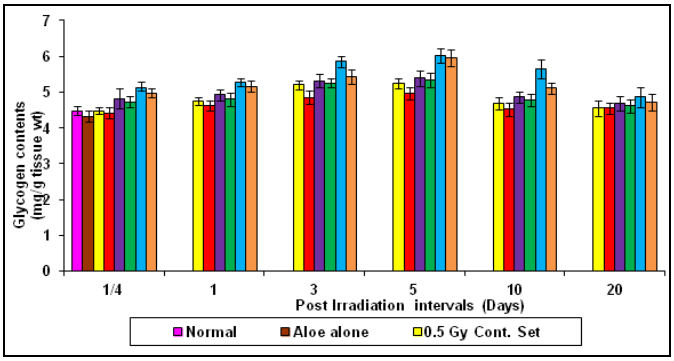

FIG. 2: GLYCOGEN CONTENTS (mg/g TISSUE wt.) IN LIVER OF SWISS ALBINO MICE EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION WITH AND WITHOUT PRETREATMENT OF *ALOE*

In *Aloe* pretreated 0.5 Gy irradiated animals, glycogen contents remained almost normal (100.68%) at 6 h postirradiation. Later, glycogen increased and found 112.93 percent on day 5, which was followed by a decrease and estimated as 102.92 and 103.60 percent at 10 and 20 days respectively **Fig. 2**. Glycogen contents also increased in the remaining two experimental sets (IV, V) from 6 h to day 5. After that, it declined progressively up to last autopsy interval (day 20) and found 104.96 and 107.47 percent respectively **Fig. 2**, but it was significantly lesser ($p<0.05$) than that of irradiated alone animals.

Cholesterol: Cholesterol contents in mice of group I was considered as 100 percent, which decreased to 96.17 percent in mice treated with *Aloe* alone **Fig. 3**. Cholesterol contents decreased at 6 hours post irradiation in 0.5, 3 and 5.5 Gy irradiated mice. After that, it increased from day 1 and attained a peak on day 3, which was followed by a further decrease from day 5 **Fig. 3**.

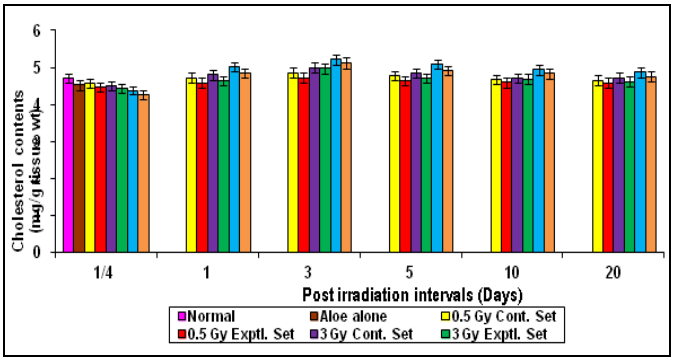

FIG. 3: CHOLESTEROL CONTENTS (mg/g TISSUE wt.) IN LIVER OF SWISS ALBINO MICE EXPOSED TO DIFFERENT DOSES OF GAMMA RADIATION WITH AND WITHOUT PRETREATMENT OF *ALOE*

In *Aloe* treated irradiated (0.5, 3 and 5.5 Gy) mice also, cholesterol decreased at first autopsy interval and then increased from day 1-3. Decrease at 6 h postirradiation was more while increase between day 1- 3 was significantly ($p<0.05$) lesser as compared to the control set III. Later, a decreasing trend was noticed at subsequent autopsy intervals, and lastly, it declined to a normal level at day 20 and was found to be 97.41, 98.05 and 107.47 percent at day 20 post-irradiation **Fig. 3**. In control set cholesterol increases due to radiation dose because it enhances the activity of HMG CO-A reductase, which is a rate-limiting enzyme of cholesterol synthesis [13]. Conversely in experimental set *Aloe vera* probably, inhibited HMG CO-A reductase activity and hence decreased the cholesterol concentration.

DISCUSSION: It has been proved that in a living system both direct and indirect radiation interactions damage the biomolecules structurally and functionally. Reactive oxygen species (ROS), generated through radiolysis of water molecules caused most of the damage. Peroxidation of membrane lipids, oxidation of DNA, damage in proteins and several other important macro-molecules caused by ROS.

Alteration in total proteins synthesis pattern after irradiation is one of the earliest sign in animals to radiation response, which in some of the tissues has been reported to be retarded following whole-body radiation exposure. On the contrary, reports also state that whole body exposure to sublethal and near-lethal doses step up the total proteins synthetic capacity of the liver. In the present study, a dose-dependent increase in total proteins contents was observed up to day 5 in 0.5, 3 and 5.5 Gy irradiated alone animals, which was maximum and significantly higher between day 3-5 post-irradiation. After that, a decreasing pattern was noticed up to day 20, but it was still higher than normal **Fig. 1**.

A significant increase in protein synthesis in mice liver was observed by after exposure to 6 Gy X-rays, according to them enhanced protein biosynthesis was attributed by the higher amino acid precursor pool in X-irradiated liver [14]. This may be due to increased transport of amino acid through the plasma membrane as a consequence of a change in permeability of irradiated cell membranes. They further reported that a significant increase in the number of ribosomes might occur due to their increased mobilization from the endoplasmic reticulum, which leads to increased protein synthesis.

These findings suggest that treatment with *Aloe* before radiation exposure protected plasma membrane against free radical-induced alterations in its permeability, which inhibited amino acid transport and ultimately protein synthesis. The present findings are in conformation with those of [15, 16, 17, 18, 19] who had also reported that treatment with *Emblica Officinalis*, *Rosmarinus officinalis*, *Aloe vera*, and *Moringa oleifera* respectively to mice before irradiation with different doses of gamma radiation decreased the total protein contents in the liver.

It is a proven fact that irradiation of animals results in a decrease in food intake in rats together with the impairment of gastric digestion. It would be expected from these findings that hepatic glycogen should decrease after irradiation, but it was not so in this study. Results of this study are also in agreement with the findings of several earlier workers [20, 21] who have also reported an increase in glycogen contents in the liver of irradiated animals. Similarly, [12, 22] also reported an increase in liver glycogen contents in house rats exposed to 4 Gy and Swiss albino mice exposed to 2.5, 5 and 10 Gy respectively. Several workers [22, 15, 16] also reported that treatment with MPG, *Emblica officinalis* and *Rosmarinus officinalis* to mice before irradiation with different doses of gamma radiation decreased the glycogen contents in the liver. Protective effects of *Moringa oleifera* Lam. leaves studied against arsenic-induced toxicity in mice [17]. A dose depended on increase in glycogen contents was a notice in the liver with 4.5 Gy radiation at one day, and the continuous decline was noticeable at 30-day autopsy but remain higher than normal level in control set, this is due to the increased rate of protein catabolism & the release of substances from radiosensitive cells [23].

A dose-dependent decrease in liver cholesterol was observed at 6 hours post irradiation in mice irradiated without *Aloe* (control sets) and with *Aloe* (experimental sets). After that, it increased from day 1 and attained a peak at day 3 post-irradiation, which was followed by a second decrease from day 5 and return almost too normal level in all experimental sets, also in III and IV control sets at day 20 post-irradiation. The decrease in cholesterol was more, while an increase was lesser in experimental sets in comparison to control sets **Fig. 3**. Decreased concentration of liver cholesterol at 6 h postirradiation in mice exposed to different doses of gamma radiation may enhance the activity of HMG Co. A reductase, a rate-limiting enzyme of cholesterol synthesis, which resulted in an increase of total cholesterol at day 3 post-irradiation. Conversely, treatment with *Aloe* probably inhibited HMG Co. A reductase activity and hence lowered cholesterol concentration at this autopsy interval. Secondly, nicotinic acid (niacin), a constituent of *Aloe* also decreased the lipolysis in adipocytes and prevented the cholesterol synthesis in this investigation.

The log of the rate of cholesterol synthesis in rat liver slices was inversely proportional to the total cholesterol contents in normal and cholesterol-fed rats [24]. Results of the control sets of this study are in agreement with the findings of [25] who reported a decline in total cholesterol and a rise in hepatic cholesterol biosynthesis after whole body irradiation. They postulated that irradiation resulted in a decrease in concentration of free cholesterol and that the decreased concentration then stimulates biosynthesis. Study of [26] reported that cholesterinum 3X trituratium, a homeopathic preparation lowered the increased VLDL cholesterol up to 32 percent within 48 h, while total cholesterol and phospholipids reduced to 62 and 45 percent respectively. They suggested that inhibition of free fatty acid mobilization from adipocytes resulted in a decrease of triglycerides formation and thus lowered the level of VLDL cholesterol. The decreased activity of HMG Co. A reductase might have been compensated by the decreased metabolism in the liver maintaining liver cholesterol [13, 18, 19] also reported that *Aloe vera* and *Moringa oleifera* including antioxidant and other phytonutrients, substantially protect the living tissues from radiation damages. Thus, regulation of cholesterol homeostasis by variation in the rate of synthesis appears to be primarily a function of the liver.

CONCLUSION: Results of the present study conclude that prior treatment of mice with *Aloe* extract for15 consecutive days did not exhibit toxic effects in the liver at biochemical levels, and *Aloe vera* modulate the radiation-induced biochemical alterations such as total proteins, glycogen, cholesterol in Swiss albino mice.

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CONFLICT OF INTEREST: Nil

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