

RADIOPROTECTION OF *ALOE VERA* AGAINST BIOCHEMICAL ALTERATIONS IN SWISS ALBINO MICE

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ABSTRACT

Objective: 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.

Methods: 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 LPO and GSH.

Results: Radiation treatment showed a decrease in GSH content and an increase of LPO in control set animals, however, the animals of experimental group i.e. *Aloe vera* and radiation combined group showed a significant increase in GSH content and decrease in LPO but values remained below normal.

Conclusion: The result of present study suggests that *Aloe vera* has a radioprotective effect due to their antioxidant and radical scavenging activity.

Keywords: Radiation, *Aloe vera*, Antioxidants, LPO, GSH

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INTRODUCTION

In the present time, gamma and x-rays, as well as various radioisotopes, are being used considerably in medical sciences both for diagnostic and therapeutic purposes. Radiation exposure in any of the conditions is extremely hazardous for living systems. The hazardous effects of radiation on biological systems may be the result of direct action of radiation in which a molecule directly absorbs energy itself and undergo changes or an indirect action of radiation in which a molecule does not absorb energy directly from radiation but receives it by transfer from other molecule. When individuals are exposed, the radiation energy is absorbed by the biological systems, which causes radiolysis of tissue water and generates free radicals. The major free radicals resulting from aqueous radiolysis are O_2 , OH, H, HO_2 , H_3O^+ [1]. Such free radicals combine with each other and dissolved oxygen to give a variety of potent oxidizing agents such as hydrogen peroxide, molecular oxygen and perhydroxy radicals.

Anyhow, whatever is the mode of exposure, protection should be provided against the harmful effect of ionizing radiation and the integrity of vital tissues must be maintained. For this purpose, different herbal plants are used in favor of radioprotection. *Vernonia cinerea* exerts its radioprotection by suppressing the formation of ROS generated as a result of irradiation, stimulating the antioxidant defense system of the body, protection of a hepatic and gastrointestinal system of the body [2].

The *Aloe vera* plant, *Aloe barbadensis* Miller, family Liliaceae Lily of the desert is the most investigated and used of more than 300 species of aloe. It is one of the most-known herbs and has been widely used for centuries. *Aloe* is commonly known for its tropical use to treat wounds and burns. A clinical trial has demonstrated the usefulness of *Aloe vera* for the prophylaxis of radiation-induced dermatitis [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]. *Aloe vera* is a medicinal plant and due to its extensive medicinal, nutraceutical and other uses it's enjoying a great demand in the market across the globe [7].

MATERIALS AND METHODS

Animals

For the present study, male Swiss albino mice of 6-7 w 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 in accordance with 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 wooden box and 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

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 thereafter 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 $30\pm 3^\circ C$ to obtain a concentrated *Aloe* extract, which was put in the oven at $40^\circ C$ for complete evaporation of alcohol. The powdered extract was re-dissolved 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 was control. Animals of the 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 hour of last administration of extract and DDW, animals of group III, IV and V were exposed to three different sub lethal doses i.e. 0.5, 3 and 5.5 Gy gamma radiation respectively.

Parameters studied

Animals were sacrificed by cervical dislocation at day ¼, 1, 3, 5, 10 and 20 post-irradiation and liver were taken for study. Liver was perfused with chilled saline 84.61% NaCl and removed immediately after scarification of animals for biochemical study. Small pieces taken from all lobes were crushed in masticator. Homogenates were prepared in Tris KCl, DDW, acetate buffer, DDW, 30 % KOH and glacial acetic acid for estimation of LPO [8], GSH [9].

RESULTS

It is a proven fact that both direct and indirect radiation interactions damage the biomolecules structurally and functionally in a living system. However, most of the damage is caused by the indirect action of ionizing radiation i.e. by reactive oxygen species ROS, generated through radiolysis of water molecules. ROS cause peroxidation of membrane lipids, oxidation of DNA, proteins and several other important macromolecules in a living system.

Lipid peroxidation LPO

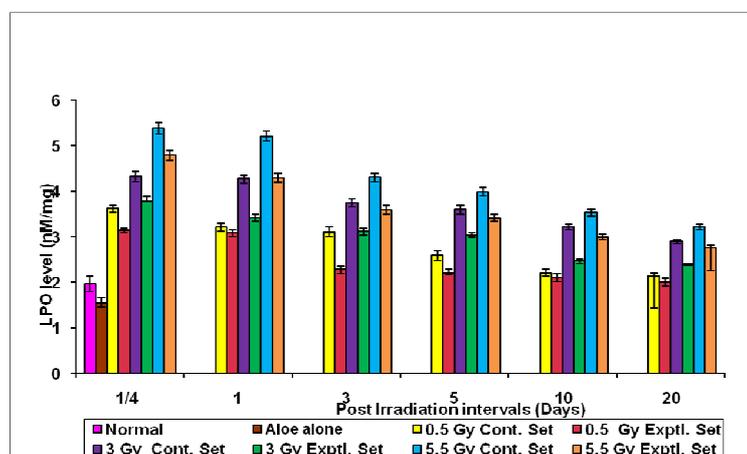
Level of LPO in sham-irradiated normal mice group I was considered as 100 percent, which decreased to 79.29 percent in mice treated with *Aloe* alone group II table 1, fig. 1.

LPO level increased in liver and found to be 184.55, 220.19 and 274.47 percent at 6 h after irradiation with 0.5, 3 and 5.5 Gy respectively which was followed by a continuous decrease at all subsequent autopsy intervals table 1, fig. 1. Decrease was found to be 163.68, 158.38, 131.57, 112.72 and 108.85 percent at day 1, 3, 5, 10 and 20 respectively in 0.5 Gy table 2, fig. 1. In both 3 and 5.5 Gy irradiated animals LPO level was followed by a significant $p < 0.05$ decrease from day 1 to day 20 but did not reach the normal level and remained 147.32 and 164.34 percent at day 20 respectively table 1, fig. 1. In *Aloe* pretreated 0.5, 3 and 5.5 Gy irradiated animals, LPO level was found 159.60, 191.20 and 240 percent at 6 h but this level was significantly lesser $p < 0.05$ than irradiated alone animals. Thereafter, lipid peroxidation decreased gradually at all subsequent autopsy intervals and noticed as 157.10, 115.93, 112.77, 107.27 and 102.27 percent at day 1, 3, 5, 10 and 20 respectively table 1, fig. 1. Later, LPO level decreased continuously in all autopsy intervals i.e. day 1 to 20 in *Aloe* pretreated 0.5, 3 and 5.5 Gy irradiated animals and found to be 116.64, 145.89 percent at day 20.

Thus, a dose-dependent increase and time-dependent decrease were observed in the LPO levels in all controls as well as experimental sets.

Table and fig. 1: Lipid peroxidation LPO level nM/mg in liver of swiss albino mice exposed to different doses of gamma radiation with and without pretreatment of *Aloe*

Autopsy intervals days	Group III		Group IV		Group V	
	Cont. set III DDW+0.5 Gy	Exptl. set III <i>Aloe</i> +0.5 Gy	Cont. set IV DDW+3 Gy	Exptl. set IV <i>Aloe</i> +3 Gy	Cont. set V DDW+5.5 Gy	Exptl. set V <i>Aloe</i> +5.5 Gy
¼ 6 h.	3.621±0.08*	3.132±0.05*	4.321±0.11*	3.782±0.10*	5.386±0.13*	4.786±0.11*
1	184.55	159.60	220.19	191.20	274.47	243.89
3	163.68	157.10	217.59	174.02	265.60	218.56
5	158.38	115.93	191.04	158.63	219.33	182.74
10	131.57	112.77	183.30	155.02	203.12	173.41
20	112.72	107.27	164.04	125.41	179.99	152.31
	2.212±0.09 ^{NS}	2.105±0.09 ^{NS}	3.219±0.09*	2.461±0.04*	3.532±0.10*	2.989±0.06*
	112.72	107.27	164.04	125.41	179.99	152.31
	2.136±0.11 ^{NS}	2.007±0.10 ^{NS}	2.891±0.10*	2.289±0.02*	3.225±0.09*	2.863±0.07*
	108.85	102.27	147.32	116.64	164.34	145.89



Reduced glutathione GSH

GSH contents in sham-irradiated normal mice group I was taken as 100 percent, which increased to 106.88 percent in mice treated with *Aloe* alone group II table 2, fig. 2.

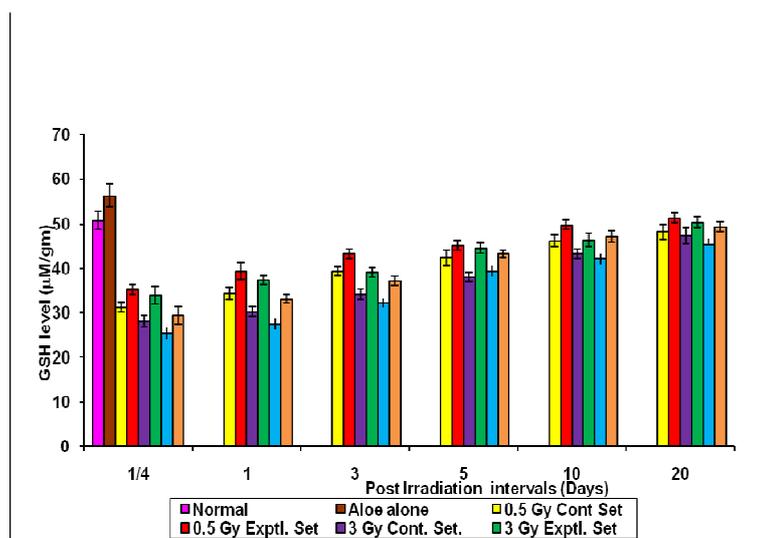
When mice were exposed to 0.5, 3 and 5.5 Gy gamma radiations without *Aloe* pretreatment, GSH contents decreased and found to be 59.27, 53.38 and 48.52 percent respectively at 6 h postirradiation. Thereafter, a significant $p < 0.05$ increase was marked in GSH levels at all subsequent autopsy intervals and reached as 91.32, 89.82 and 85.87 percent at day 20 respectively in all doses table 2, fig. 2.

In *Aloe* pretreated 0.5, 3 and 5.5 irradiated animals GSH contents also decreased and found to be 66.86, 64.45 and 55.85 percent at first autopsy interval. However, a decrease in GSH was significantly lesser $p < 0.05$ than that of the without *Aloe* pretreated irradiated animals. Thereafter, GSH increased gradually and reached at a peak level 97.28, 95.43 and 93.52 % at day 20 of this study which was significantly higher $p < 0.05$ than that of their respective control set table 2, fig. 2.

These results indicate that GSH did not reach to a normal level even at day 20 but in *Aloe* pretreated animals it was more near to normal table 2, fig. 2.

Table and fig. 2: Reduced Glutathione peroxidase GSH level $\mu\text{M/gm}$ in the liver of Swiss albino mice exposed to different doses of gamma radiation with and without pretreatment of *Aloe*

Autopsy intervals days	Group III Cont. set III DDW+0.5 Gy	Exptl. set IV <i>Aloe</i> +0.5 Gy	Group IV Cont. set IV DDW+3 Gy	Exptl. set IV <i>Aloe</i> +3 Gy	Group V Cont. set V DDW+5.5 Gy	Exptl. set V <i>Aloe</i> +5.5 Gy
¼ 6 h.	31.23±1.06*	35.23±1.13 ^{NS}	28.13±1.32*	33.96±1.97*	25.36±1.22*	29.43±1.97 ^{NS}
	59.27	66.86	53.38	64.45	48.52	55.85
1	34.39±1.32*	39.35±1.86*	30.23±1.05*	37.38±1.09*	27.53±1.34*	33.15±1.12 ^{NS}
	65.26	74.68	57.37	70.94	52.24	62.91
3	39.38±1.02*	43.21±1.18*	34.18±1.19*	39.13±1.02*	32.13±1.06*	37.12±1.03 ^{NS}
	75.59	82.00	64.86	74.26	61.03	70.44
5	42.28±1.78*	45.13±1.04*	38.04±1.12*	44.53±1.15*	39.32±1.18*	43.18±1.04 ^{NS}
	80.24	85.65	72.19	78.26	74.62	81.95
10	46.13±1.30*	49.79±1.10 ^{NS}	43.17±1.08*	46.32±1.38*	42.16±1.08*	47.13±1.18 ^{NS}
	87.54	94.49	81.93	87.91	80.01	89.44
20	48.12±1.72 ^{NS}	51.26±1.08 ^{NS}	47.33±1.79*	50.28±1.26 ^{NS}	45.25±1.32*	49.28±1.21 ^{NS}
	91.32	97.28	89.82	95.43	85.87	93.52



DISCUSSION

Lipid peroxidation LPO is a free radical chain reaction and mainly involves three distinct steps i.e. initiation, propagation and termination [10]. It results in a loss of biochemical and structural architecture of cellular organelles and therefore, it is a highly destructive process. Lipid peroxidation is initiated by hydroxyl radical $\text{OH}\cdot$. It has sufficient energy and can abstract a hydrogen atom from methylene carbon of polyunsaturated fatty acid PUFA and initiates lipid peroxidation. Lipid radiolytic products such as Alkozyll $\text{LO}\cdot$ and peroxy $\text{LOO}\cdot$ radicals also initiate lipid peroxidation process by attacking on fresh lipid molecules [11]. The alkozyll and peroxy can also attack on proteins and enzymes besides reinitiating lipid peroxidation. Peroxidation of membrane lipids is one of the main causes of radiation-induced membrane damage [12]. Peroxidation of membrane lipids has a devastating effect on the functional state of the membrane because it alters membrane fluidity and permeability

typically decreasing it and thereby allowing ions such as Ca^{2+} to leak into the cell. Present Findings of Patil *et al.*, 2013 demonstrate the potential of rutin and quercetin in mitigating radiation-induced oxidative stress, which may be attributed to the inhibition of radiation-induced decline in the endogenous antioxidant levels and scavenging of radiation-induced free radical. Berberine might have enhanced radiation-induced DNA damage either by increasing the radiation-induced ROS, lipid peroxidation, lactate dehydrogenase release or by suppressing topoisomerase II [14].

Results of the present study showed that lipid peroxidation LPO level was maximum in the liver of all irradiated alone mice controls sets at 6 h postirradiation. Thereafter, LPO level decreased continuously at all successive autopsy intervals and returned almost to normal at day 20 in 0.5 Gy exposed mice, while in 3 and 5.5 Gy exposed mice it was still 1.47 and 1.64 folds higher than normal level table 2, fig. 1. Increase in LPO level with an increase of exposure

dose of radiation suggested increased peroxidation of membrane lipids in the liver. Radiation exposure resulted in a considerable rise in LPO level in blood, which increased up to day 7 in both experimental and control groups being significantly higher $p < 0.001$ in control group than the respective experimental group [15]. Afterwards, these values started to decrease in both the groups and tend to be normalized. Although the values were significantly lower $p < 0.01$ in experimental animals than irradiated controls, but the normal level could not be recovered even by the end of experimentation and found 109.56% higher than the normal. *Garcinia indica* as the modulation of the radiation-induced decrease of reduced glutathione and the radiation-induced increase in lipid peroxidation assessed in the liver and the blood [16].

Earlier workers have also reported the elevated level of LPO in mice liver after whole-body exposure to different doses of ionizing radiation [17, 18, 19, 20, 2, 21 and 13]. Thus, level of LPO gives an index of free radical activity. In the present study, *Aloe* treatment to Swiss albino mice before exposure to 3 and 5.5 Gy gamma radiations lowered the LPO level significantly between 6 h to day 10 post-irradiation. On the other hand, treatment with *Aloe* before exposure to the lowest dose 0.5 Gy reduced the LPO level significantly at 6 h postirradiation, while between days 1-20 reduction was non-significant that might be because of lesser TBARS formation in 0.5 Gy exposed mice liver as compared to 3 and 5.5 Gy exposed mice liver. These results indicated that treatment with *Aloe* provided protection to cell membranes against free radical-induced oxidative damage.

Thus, ROS damage structural and functional integrity of the cell membranes, break DNA strands and denature cellular proteins [22].

Preservation of cellular membrane integrity depends on protective or repairing mechanisms of antioxidants. Antioxidants like vitamins A β -carotene, C and E [23], glutathione peroxidase [24], several isozymes of superoxide dismutase [25], and minerals such as selenium [24] present in *Aloe* seem to be responsible for inhibiting lipid peroxidation level in liver. These results suggest that the basic cause of lipid peroxidation is not only the free radicals but also the low level of antioxidants in a biological system, which removes them.

Glutathione GSH, a tripeptide of glutamic acid, cysteine and glycine is the most abundant intracellular thiol compound present in virtually all mammalian tissues and GSH is essential for the protection of the cells against reactive oxygen species and free radicals produced even in normal metabolism [26, 27]. By its multifunctional properties, GSH plays an important role in drug metabolism [28], radiation and cancer [29], immunology, aging and exercise [30]. In the present study, GSH level depleted and reached at a minimum level in mice liver at 6 h after exposure to different doses of gamma radiation. However, GSH level showed a continuous increase at all subsequent autopsy intervals thereafter and was found to be 91.32, 89.82 and 85.87 percent at day 20 in 0.5, 3 and 5.5 Gy irradiated alone mice respectively table 2, fig. 2. The maximum decrease in GSH level was in those animals exposed to the highest dose of radiation 5.5 Gy as compared to those irradiated with the lowest dose 0.5 Gy. Depletion in GSH level in Swiss albino mice after exposure to different doses of gamma radiation have also been reported by several workers [17, 31, 32, 33].

GSH depletion does not have direct consequences in the form of acute toxicity but the cells become more susceptible to chemical or oxidative stress. Severe depletion affects numerous cell functions. The GSH status affects the synthesis of two major cellular polymers, i.e. proteins and DNA. Depletion of GSH may decrease protein biosynthesis. Numerous enzymes like glutathione synthetase, glutathione peroxidase, glutathione S-transferases, leukotriene C4 synthetase 3-iodinase, glutaredoxin and glyoxylase are GSH dependent. The activities of these enzymes may be regulated by thiosulphide exchanger and thus depend on the GSH status. Therefore, the conclusion can be drawn that cells not only become more susceptible to any further challenge, but their basic functions are also perturbed by the extensive GSH depletion [34].

In the present study, although the level of GSH was always higher in *Aloe* treated 0.5, 3 and 5.5 Gy irradiated mice liver as compared to 0.5, 3 and 5.5 irradiated alone mice but it was significantly higher between day 1-5 in *Aloe* treated 0.5 Gy irradiated mice, while in 3 Gy

exposed animals, level of GSH was significantly higher between 6 h to day 10 post-irradiation in comparison to *Aloe* untreated irradiated animals. Similarly, level of GSH was also higher at all autopsy intervals in animals treated with *Aloe* before exposure to the highest dose of gamma radiation 5.5 Gy but it was statistically non-significant. Earlier workers have also reported that treatment with plant extracts like *Rajgira* [17], *Aloe* [18], *Aegle marmelos* [35], *Hemidesmus indicus* [36], *Adhatoda vasica* Nees [2], *Spirulina fusiformis* [32] *Rosmarinus officinalis* [33], herbal preparations such as *Aloe vera* [21], *Tinospora* [15], *Garcinia indica* [16] to animals before exposure to different doses of ionizing radiation elevated the level of GSH.

Aromatic plants and their products have gained momentum globally during recent times, with wide applications in the herbal drug industry. Natural resources such as wastelands and forests could serve as a reservoir for the same [37].

CONCLUSION

Results of the present study conclude that treatment of mice with *Aloe* extract for 15 consecutive days did not exhibit toxic effects in the liver at biochemical levels and *Aloe vera* modulate the radiation-induced biochemical alterations such as LPO and GSH in Swiss albino mice.

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AUTHOR CONTRIBUTION

All the work have been carried out by me

CONFLICT OF INTERESTS

Declared none

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