

## **Alterations in Oxidative Stress in Testes of Swiss Albino Mice by *Aloe vera* Leaf Extract after Gamma Irradiation**

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### **Summary**

The *Aloe vera* has been used as traditional medicine for past several years for the treatment of various ailments. Modulatory effects of *Aloe vera* leaf extract against radiation –induced changes in terms of histological alterations in testes, reduced Glutathione(GSH), Lipid per oxidation(LPO), Acid and Alkaline phosphatase levels in Swiss albino mice was studied at various post-irradiation intervals between 6 hrs. and 21 days. There was lesser damage to testes tissue architecture and various cell populations including spermatogonia, spermatids and leydig's cells in irradiated animals. Correspondingly, a significant decrease in the LPO and an increase in the GSH level were observed in testes, liver and blood of *Aloe vera* pre-treated irradiated mice. Similarly, a significant decrease in level of acid phosphatase and increase in level of alkaline phosphatase were observed. The study suggests that *Aloe vera* plant extract has significant radioprotective effects on testes that warrants further mechanistic studies aimed at identifying the role of major ingredients in the extract.

Keywords: - Gamma radiation, testes, *Aloe vera*, LPO, GSH, acid and alkaline Phosphatase activity

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## Introduction

Applications of ionizing radiation in difficult areas are constantly increasing including the use for medicinal and industrial purposes. Ionizing radiation induces testicular damage in mammals which may cause sterility and transmit to next generation. Testes are main reproductive organ in male and it is responsible for sperm production. It is one of the sensitive organs because of cell renewal system. Ionizing radiation was found to produce marked effects on testes in terms of lethality and impaired spermatogenesis. Testes are also known to be reduced in their size and weight after radiation exposure<sup>1-4</sup>.

In recent years, extensive research work has been carried out a chemical protection against radiation. Chemicals like Cysteine, Cysteamine, 2-MPG, WR-2721, AET and Superoxide Dismutase role have been tested for their radioprotective role but application of these compounds is limited in radiotherapy owing to their high toxicity at optimum dose level<sup>5, 6</sup>. A world wide hunt is on to find suitable detoxifying agent against environmental toxicants.

Recently, some plant extracts have been screened out against radiation like Podophyllum<sup>7</sup>, herbal preparations like Liv.52<sup>8</sup>, ocimum<sup>9</sup>, mentha<sup>10</sup> are found to be quite promising.

One such popularly known used plant is *Aloe vera barbadensis*<sup>11</sup> belonging to family Liliaceae and consists of about more than 250 species<sup>12</sup>. It is commonly called “Guar – patha” or Ghee-Guar. It is rich in vitamin A, E, C and Zinc and Selenium. It is reported to have antioxidant, anti-tumor and anti-inflammatory activities<sup>13, 14</sup>.

The present investigation has been made to assess the radioprotective efficacy of *Aloe vera* leaf extract in the testicular constituents of Swiss albino mice.

## Materials and Methods

**Animals:** Swiss albino mice (*Mus musculus*), 6-8 weeks old with body weight of 24±2 gm, were used. Mice were obtained from Hamdard University, New Delhi, India and maintained and bred in mouse house, as an inbred colony as per norms laid down by an Institutional Ethical Committee, given standard mouse food and water *ad libitum*.

**Irradiation:** Mice were irradiated by <sup>60</sup>Co source in the cobalt teletherapy unit (ATC-C9) at radiation oncology department, Sawai Man Singh Medical College and Hospital, Jaipur, India. The mice were kept in ventilated box with a distance of 77.5 cm from the source to deliver a dose rate of Gy min<sup>-1</sup>.

**Plant material:** *Aloe* leaves were collected locally during the whole year. The *Aloe vera* plant was identified by the curator at the Herbarium of Botany, University of Rajasthan, and Jaipur, India. (RUBL Number 19886)

**AV extract:** To prepare aqueous extract, fresh shade dried leaves of *Aloe vera* powdered and refluxed with double distilled water (DDW) for 36 hours at 40<sup>0</sup>c and vacuum evaporated so as to get in powder form. The powder of extract was redissolved in DDW just before oral administration.

**Experimental design:** Mice were randomly divided into following groups (five per Groups):

**Group I:** Normal / sham-irradiated mice were given distilled water (DDW) through oral gavage once in a day for 15 consecutive days.

**Group II:** Mice were treated with 1000 mg/kg body weight of AV dried extract dissolved in distilled water through oral gavage for 15 consecutive days.

**Group III:** Mice were given distilled water for 15 days and then exposed to 6 Gy dose of gamma radiation. This group served as positive control.

**Group IV:** Extract of *Aloe vera* was given 1000mg/kg body weight of mouse orally for 15 days and after 30 min. of last dose; they were exposed to 6 Gy dose of gamma radiation. Following various treatments, mice were autopsied by cervical dislocation on days 6hrs. 24 hrs. 3,7,14 and 21 days. Testis were surgically removed, weighed and fixed in Bouin's fluid. The tissue was embedded in paraffin block after dehydrating with increasing concentrations of 70, 90 and 100% ethanol. Five micrometer sections were cut using hand microtomy, were placed on glass slide and were stained with Harris hematoxyline and Eosin. Stained tissue sections were observed under light microscope (Olympus) to determine histopathological changes.

### **Biochemical parameters**

**Glutathione (GSH) Assay:** GSH in tissue and blood was measured using the method described by Moron *et al.* (1979)<sup>15</sup>. tissue homogenates were treated with 0.1ml of 25% trichloroacetic acid (TCA) and the resulting precipitate was pelleted by centrifugation at 3900rpm for 10 min. free endogenous sulfhydryl was assayed in a total 3 ml volume by adding 2 ml of 0.5Mm 5, 5' -dithio-bis (-2-nitro benzoic acid) (DTNB) prepared in 0.2 M phosphate buffer pH8 to 1 ml of the supernatant. The GSH reacts with DTNB and forms a yellow -colored complex with DTNB. The absorbance was read at 412nm UV-VIS systronic spectrophotometer.

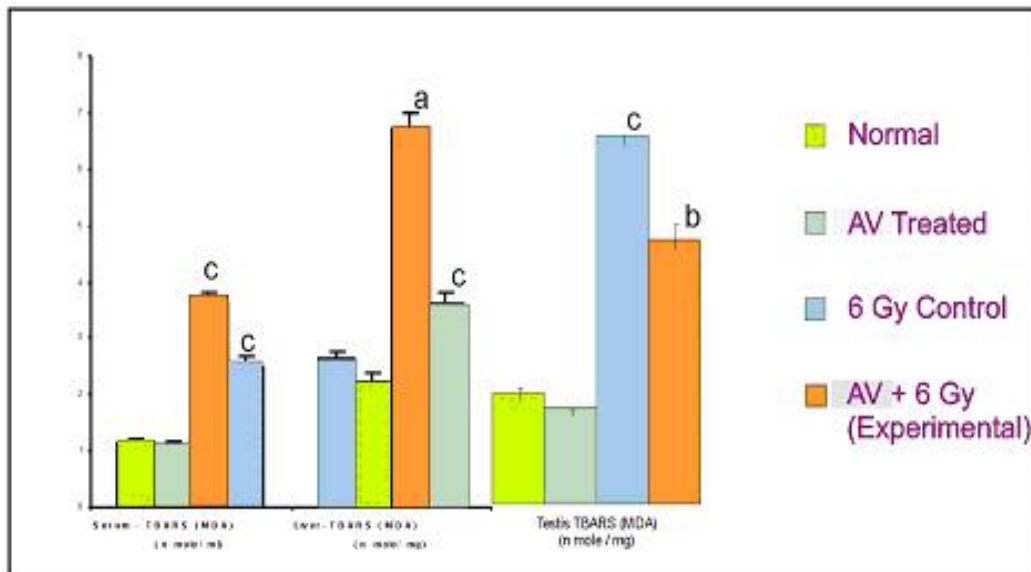
**Lipid peroxidation (LPO) Assay:** LPO levels in tissue and blood were estimated by the method of Ohkawa *et al.* (1979)<sup>16</sup> as thiobarbituric acid (TBA) reactive substances. The testes were dissected out and chilled in ice cold 0.9% NaCl. Homogenate of testes were prepared in 1.15%KCl (1 g tissue in 9 ml of 1.15% Kiln). Sodium dodecyl sulfate (8.1%; 0.2 ml) was added to 0.2 ml of sample in test tubes and pH was adjusted to 3.5 with 5 M NaOH. To this, 1.5 ml of 0.8 % aqueous solution of TBA was added. The mixture was heated at 95<sup>0</sup>c for 60min. after cooling under tap water, 1 ml of distilled water and 5 ml a mixture of n- butanol and pyridine (15:1) were added and shaken vigorously. The solution was centrifuged at 3900 rpm for 10 min. the upper organic layer

was removed and absorbance was measured at 532 nm using UV-VIS systronic spectrophotometer.

**Acid and alkaline phosphatase Activity:** acid and alkaline phosphatase activities in testes were estimated using the described by Fiske and Subbarow (1925)<sup>17</sup>. The tissue homogenates were mixed with TCA and then centrifuged at 3900 rpm for 10 min. the supernatant was then treated with molybdate solution. (molybdate solution prepared by dissolving 25 g of ammonium molybdate into 200 ml glass distilled water (GDW) and combining with 300 ml of 10 N H<sub>2</sub>SO<sub>4</sub> and then was made up to 100 ml with GDW). This resulted in the formation of phosphor-molybdic acid from the phosphate present in the tissue. The phosphomolybdic acid (ANSA) to produce a blue color whose intensity was proportional to the amount of phosphate liberated. The alkaline phosphatase activity is the difference between inorganic phosphate content of the incubated and control samples expressed as Bodansky units. One Bodansky unit corresponds to the liberation of 1 mg of inorganic phosphorous from the tissue in mg g<sup>-1</sup>h<sup>-1</sup>.

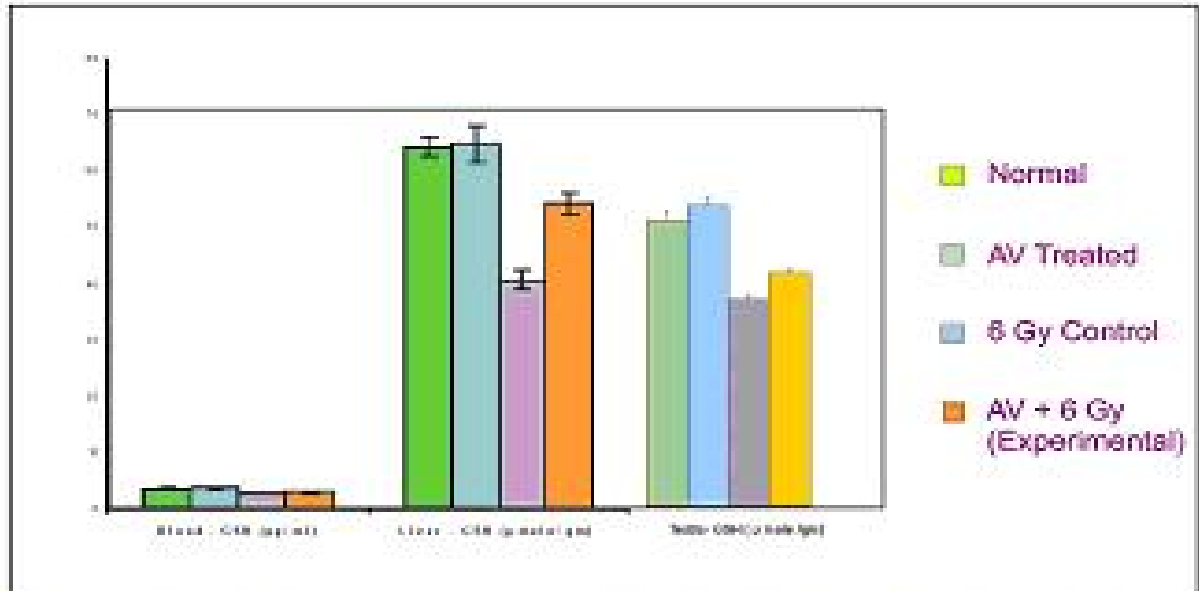
**Results**

**Lipid Peroxidation:** Administration of *Aloe* when compared to normal did not alter the lipid peroxidation. Exposure of animals to gamma radiation increase LPx in control and experimental animals. *Aloe* pre- treatment significantly reduced LPx induction in the *Aloe* + irradiation group (Fig.1).



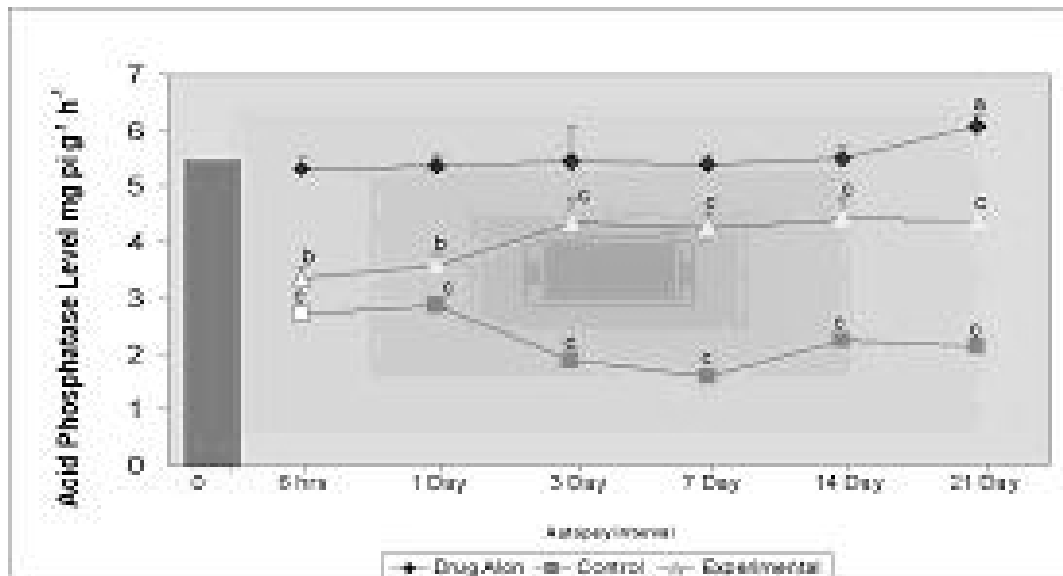
**Fig.1 :** Variations in LPO level in the testis of Swiss albino mice after exposure to 6 Gy gamma radiation with (experimental) or without (control) *Aloe vera*. The value represented mean ± S.E. The statistical significance was obtained between Normal v/s Control and Control v/s Experimental (p < a=0.05, b=0.01, c = 0.001 )

**Glutathione:** No significant difference in the testicular GSH contents was observed between normal and *Aloe* treated animals. However, a statistically significant decrease in GSH was evident in radiation treated control animals. *Aloe* treated irradiated animals showed a significant increase in GSH contents, with respect to control, but the values remained below normal (Fig. 2).



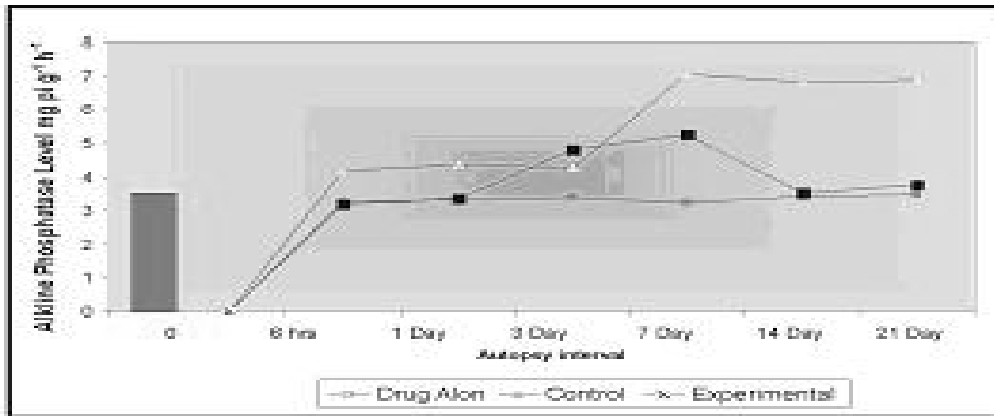
**Fig.2:** Variations in GSH level in the testis of Swiss albino mice after exposure to 6 Gy gamma radiation with (experimental) or without (control) *Aloe vera*. The value represented mean  $\pm$  S.E. The statistical significance was obtained between Normal v/s Control and Control v/s Experimental ( $P < a = 0.05, b = 0.01, c = 0.001$ )

**Alkaline Phosphatase:** Alkaline Phosphatase activity showed no significant change in *Aloe* treated mice as compared to normal group. In irradiated group, the alkaline phosphatase activity in testis showed remarkable decline on all days of observation. In *Aloe* treated irradiated mice, a significant recovery in alkaline phosphatase activity was observed (Fig. 3).



**Fig.3:** Variations in alkaline phosphatase level in testis of swiss albino mice after exposure to 6 Gy gamma radiation with (experimental) or without (control) *Aloe vera*. The value represented mean  $\pm$  S.E. The statistic significance was obtained between Normal v/s Control and Control v/s Experimental ( $p < a=0.05, b=0.01, c=0.001$ )

**Acid phosphatase:** The acid phosphatase activity in testis was found at normal level in *Aloe* treated group as compared to normal. In irradiated group, an elevation in the enzyme level was observed. In *Aloe* pretreated irradiated animals, decline in acid phosphatase was observed at all the autopsy intervals in comparison to irradiated mice (Fig. 4).



**Fig. 4:** Variations in acid phosphatase level in testis of swiss albino mice after exposure to 6 Gy gamma radiation with (experimental) or without (control) *Aloe vera*. The value represented mean  $\pm$  S.E. The statistic significance was obtained between Normal v/s Control and Control v/s Experimental (  $p < a=0.05, b=0.01, c=0.001$  )

**Spermatogonium:** Spermatogonic counts in *Aloe vera* treated group were found to be significantly higher than the irradiated control animals. Similarly the *Aloe* leaf, drug initiated an early recovery from radiation induced damage to restore normal architecture of testes (Table 1).

Autopsy Interval	Control	Experimental
6 hrs	44.82 $\pm$ 2.73	55.97 $\pm$ 1.40 <sup>C</sup>
1 Day	19.45 $\pm$ 2.57	30.16 $\pm$ 2.21 <sup>C</sup>
3 Day	9.41 $\pm$ 1.32	18.5 $\pm$ 1.94 <sup>C</sup>
7 Day	7.49 $\pm$ 1.22	16.11 $\pm$ 1.49 <sup>C</sup>
14 Day	14.25 $\pm$ 1.97	23 $\pm$ 2.53 <sup>C</sup>
21 Day	31.94 $\pm$ 0.89	41.17 $\pm$ 1.61 <sup>C</sup>

**Table1:** Variations in Spermatogonium counts in testes of Swiss albino mice after exposure to 6 Gy gamma radiation with (experimental) or without (control) of *Aloe vera* leaf extract. The values represented mean  $\pm$  S.E. The statistical significance was between normal v/s control and control v/s experimental ( $p < a=0.05, b=0.01, c=0.001$ ).

### **Discussion**

In the present study, after exposure to 6 Gy gamma radiations to animals resulted in radiation sickness within 3-5 days after exposure. The symptoms included reduction in the food and water intake, weight loss, diarrhea, ruffling of hairs and irritability. The similar symptoms have been observed in mice after gamma irradiation by others also<sup>18</sup>.

Male germ cells are highly susceptible to reactive oxygen species (ROS) attack produced by any agent or xenobiotic; hence, they are equipped with an effective defense system to combat the damaging effects of ROS<sup>19,20,21</sup>.

The present study indicated alterations in the activities of some key enzymes reflecting the oxidative stress. Acid phosphatase is localized in cellular lysosomes and change in activity of lysosomal enzymes take place following whole – body irradiation. An enhanced golgi activity and peroxidation of lysosomal membranes after irradiation causing lysis of membrane and oozing out of the enzymes are attributed to an increase acid phosphatase level<sup>22</sup>. The discharge of enzymes from lysosomes may be due to activation of pre-existing latent enzymes or due to synthesis of new lysosomes as a consequence of irradiation<sup>23</sup>. It is already known that radiation enhances the permeability of membranes of several cellular organelles, and hence increase in blood, liver and testes acid phosphates activity till day 3 can be attributed to the gastro –intestinal syndrome, with started recovery at day 7. However, a further rise in ACP can be assigned to other factors like hematopoietic injury.

In present investigation, alkaline phosphatase activity was found to be declined after irradiation at all autopsy intervals. This investigation is concur with previous studies of Jacob and Maini<sup>24</sup>, who have also reported a depletion in serum ALP activity in male mice after irradiation with 5 Gy gamma rays. Non exponential loses in activity of alkaline phosphatase after gamma irradiation has also been observed earlier and it was suggested that radical attacks on phosphatase at canters of secondary importance for the enzymatic activity and there is notable destruction of the component amino acid residue during radiolysis<sup>25</sup>.

Alkaline phosphates play an important role in maintenance of cell permeability and acts on mono- phosphatase. Damage to cell membrane caused by radiation may be the reason for decline activity of alkaline phosphates. In untreated irradiated group (control), decline alkaline phosphate level may be attributed to the severe damage to GI tract. Post – irradiated reduction in alkaline phosphates may be due to damage of brush border cells and increased permeability of villi cells<sup>26</sup>. Similarly, Mathur and Uma Devi noted a elevated concentration of alkaline phosphatase in ileum of mice after irradiation<sup>27</sup>. The increase in alkaline phosphates may be due to altered physiological conditions such as liver function mediated by serum alkaline phosphates destruction of an inhibitor by irradiation can also be attributed to the plasma alkaline phosphates level in the present study. It means that the higher is the dose, greater is the damage and longer is the time of recovery.



Glutathione is widely distributed tripeptide and found mainly in the cell cytosol<sup>28</sup>, which plays a crucial role in the detoxification process<sup>29</sup>. Rat and mouse testis have been reported to contain high concentrations of GSH content was found to be depleted by gamma radiation<sup>30</sup>. There exists an inverse relationship between GSH content and lipid peroxidation levels. The present study demonstrates a significant reduction in testis, liver and blood GSH following exposure. This could be due to the enhanced utilization of the antioxidant system as an attempt to detoxify the free radicals generated by radiation. Oral administration of AVE did not significantly influence the endogenous GSH level either in testis, liver and blood, but its presence during radiation exposure protects the endogenous GSH depletion due to irradiation. The lower depletion of testis, liver and blood GSH in *Aloe vera* pre-treated irradiated animals could be due to the high availability of GSH, which increases the ability to cope up with the free radicals produced by irradiation. The increased GSH level suggests that protection by *Aloe vera* may be mediated through the modulation of cellular antioxidants levels.

The elevated levels of lipid peroxidation in the present investigation are indicative of the oxidative damage caused by gamma radiation in testicular tissue. Inhibition of lipid peroxidation in biomembranes can be caused by antioxidants<sup>31, 32</sup>. In the present study, it was observed that *Aloe vera* pre-treated irradiated animals exhibited a significant increase in GSH and decrease in LPx level

Membrane of mammalian spermatozoa is rich in high unsaturated fatty acid and is sensitive to oxygen –induced damage mediated by lipid peroxidation. The excessive generation of reactive oxygen species (ROS) by abnormal spermatozoa and by contaminating leukocytes (leukocytospermia) has been identified as one of the few defined etiologies for male infertility.

Our results confirm the contention of earlier reports that irradiated induced cell death may be a result of accumulation in the membranes including cellular, nuclear and organelle, changes in the surface properties of chromosomes leading to stickiness, breakage of double – strand s of DNA and chromosomal aberrations.

Thus, it can be documented that the extracts of *Aloe vera* significantly restore the glutathione level in the liver, blood and testes, and LPO, acid phosphates and alkaline phosphates in liver, testes and blood of mice exposed to irradiation. The phytochemicals that is responsible for the observed effects of *Aloe vera* is far from clear although the extracts contain several amino acids and vitamins like A, C & E. There is a need to test these individual responsible substances for their ability to protect radiation –induced accumulation of free radicals, membrane damage.

Our future goal is to characterize the relative role of the growth substances and vitamins in radioprotection.

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