

THE PROTECTIVE EFFECT OF *ALOE VERA* AGAINST RADIATION INDUCED DAMAGE TO INTESTINE OF SWISS ALBINO MICE

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ABSTRACT

The protection against radiation induced intestinal damage in Swiss Albino Mice by *Aloe vera* have been studied. Animals were given *Aloe vera* leaf extract orally 1000 mg/kg body weight/day for 15 consecutive days before radiation exposure (4.5 GY gamma-radiation). Mice were autopsied at 1 and 30 days after irradiation to evaluate the radiomodulatory effect in terms of protein, glycogen, cholesterol, LPO and GSH. Radiation treatment showed decrease in GSH content and an increase of LPO, protein, glycogen and cholesterol observed 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, protein, glycogen and cholesterol but values remained below normal. The result of present study suggests that *Aloe vera* has a radio protective effect due to their antioxidant and radical scavenging activity.

KEYWORDS: Radiation, Radioprotection, *Aloe vera*, Antioxidant activity.

INTRODUCTION

Radiation such as gamma and X-rays as well as various radioisotopes are being used considerably in medical science for both diagnostic and therapeutic purposes such as therapy of cancer. Radiation exposure in any of the conditions is extremely hazardous for living systems. It destroys the cells in the targeted tissue by damaging their DNA. So the amounts of ionizing radiation that can be given to treat malignant tumors are limited. On exposure the radiation energy is observed by a biological system that cause radiolysis of water and produces oxygen radicals ($O_2^{\cdot-}$, OH^{\cdot} , H^{\cdot} , H_2O^{\cdot}) and H_3O^+ (Dragaric and Dragaric 1971; Pradhan *et al.* 1973; Dragaric and Schols, 1983) which damage biomolecules such as lipids, protein and DNA. Peroxidation of membrane lipid cause changes in structure, fluidity and

permeability of biological membranes, which alter functions of cells and lastly lead to death of the cells.

In the past 30 years, plant extracts and herbal preparations have been reported to have radio protective action *in vivo* and *in vitro* studies (Shinoda 1995; Kumar *et al.* 1996; Uma Devi and Ganasoundari, 1999; Kamat *et al.* 2000). *Aloe vera* is a succulent plant species used medicinally for several thousands of years, it belongs to Aloaceae family. It has rejuvenating, healing or smoothing properties. *Aloe vera* has two parts- latex and gel. Latex contain anthraquinone glycosides (Aloin, Aloe emodin) that are potent laxatives, laxative effect from *Aloe* is stronger than any other herb.

Aloe vera has a specific anti-neuroelectrodermal tumor activity due to presence of hydroxyanthraquinone in *Aloe vera* leaves (Kostalova *et al.* 2004). It has antioxidants properties because it contains vitamin A (carotene), C and vitamin E (Atherton, 1998), glutathione peroxidase as well as several isoenzymes of superoxide dismutase (Klein and Penneys 1988; Sabeh, *et al.* 1993), it also contain vit.B complex, choline, folic acid, thiamine and niacin, it also contains minerals such as Ca, Mg, Co, Al, Na, Cl, Fe, K, and S (Shelton, 1991). Three anti-inflammatory fatty acids, cholesterol, campesterol and B-sitosterol and lupeol of *Aloe* are very effective in treatment of cuts, abrasions and rheumatic fever (Atherton, 1998). *Aloe vera* has multiple constituents possessing potential biological activities (Femenia *et al.* 1999) and is a pharmacy itself. The gel contains emollient polysaccharide, glucamannan and acemannan. Glucamannan is a good moisturizer, which accounts for its use in many cosmetics (Henry, 1979). Acemannan, the water-soluble long chain mannose polymer accelerates wound healing (Kostalova *et al.* 2004). *Aloe* gel extract has a protective effect against hepatotoxicity produced by diabetes. The increase of GSH (glutathione) and the decrease of LPO (Lipid peroxidation) in intestine with the treatment of *Aloe* gel extract are consistent with the beneficial effects of *Aloe*.

Intestine plays a key role in removing excess calcium, magnesium and iron internally and also important in food digestion.

Therefore, this study was undertaken to evaluate the value of nutritional supplementation of *Aloe vera* against the radiation induced damage on the intestine of Swiss albino mice.

MATERIALS AND METHOD

Animals

Male Swiss albino mice 6-8 weeks old 26 ± 2 g were used, they were given standard mice feed and water. The maintenance and handling of the animals were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. All the experimental work was approved by the institutional animal ethics committee.

Source of radiation

Animal were treated with cobalt-60 source of radiation in radiotherapy Dept. SMS hospital, Jaipur. On exposure to radiation, animals were kept in a ventilated box and the radiation dose given to these animals were 4.5 Gy.

Aloe vera cold extract

The *Aloe vera* leaf is collected locally. The specimen was placed at Herbarium, Dept. of Botany, University of Rajasthan, and Jaipur. The voucher number is RUBL-19886. *Aloe vera* extract is prepared by peeling, drying in powder form and these residue of *Aloe vera* extracted with ethyl alcohol double the volume of leaf extract and keeping at a room temperature for evaporation & this process is repeated for three times and finally kept in incubator at 37°C for complete evaporation at 24 hours, then the concentrated extract are ready for oral administration.

Experimental design

Animal were divided into three groups, where the animals of group I were administrated with double distilled water only (i.e. normal). And the groups II was orally given *Aloe vera* drug (i.e. drug alone). The group III is further divided into 2 sets i.e. control and experimental, the control sets of animal were treated with radiation and experimental group of animal were treated with both drugs and radiation. The radiation dose given to animals was 4.5 Gy and the animals were autopsied at the interval of 1 and 30 days.

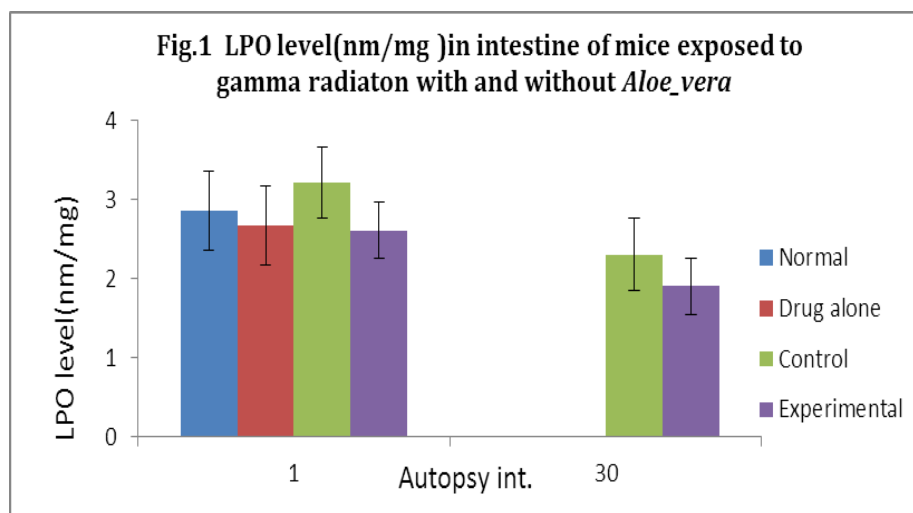
Biochemical studies

(1) **LPO (lipid peroxidation):** the LPO level in liver and intestine was measured in term of Thiobarbituric Acid Reactive Substances [TBARS] by the method of Ohkawa *et.al* 1979. These absorbance was read at 532nm by using spectrophotometer (UV-VIS).

- (2) **GSH (glutathione):** The hepatic GSH level was determined by the method described by Moron et.al. The tissue sample of liver and intestine was measured by using Ellman's reagent (DTNB) as a coloring reagent, this method described by Moron *et al* (1979). The absorbance was read at 412nm by using spectrophotometer.
- (3) **Protein:** The total proteins were measured by the method of Lowery *et.al.* (1951) and absorbance was read at 432nm by using UV-VIS spectrophotometer.
- (4) **Glycogen:** Glycogen was measured by the method Montgomery (1957) and absorbance was read at 624nm by using spectrophotometer (UV-VIS).
- (5) **Cholesterol:** The cholesterol was measured by Burchard (1959) and absorbance was read at 550nm by using UV-VIS spectrophotometer.

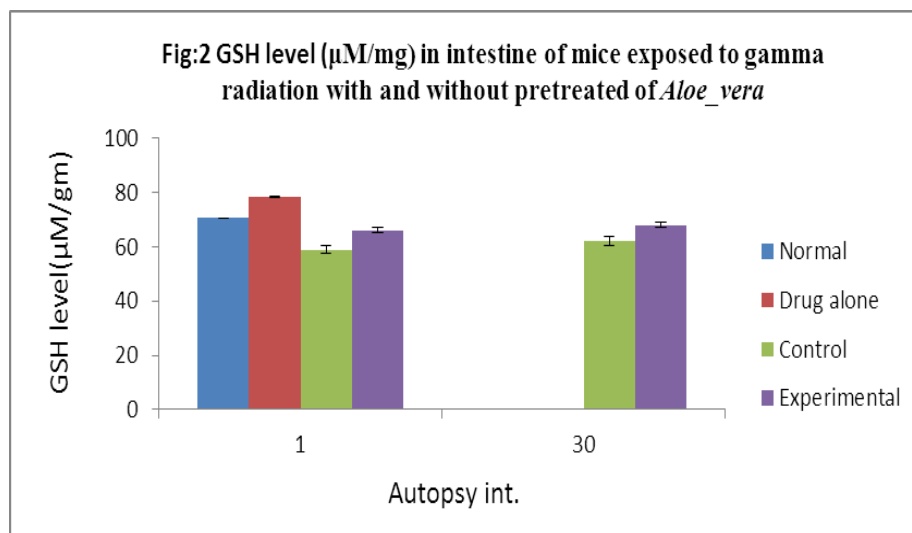
RESULT

Lipid peroxidation (LPO): In the present study LPO increases in control set of intestine due to increase on malanoaldehyde (MDA) formation in peroxidation of membrane lipid after radiation dose and in experimental set *Aloe vera* decreases the MDA formation & provided the protection to cell membranes against free radical induce oxidative damage .So the experimental set is decrease as compare to control set. (Fig. 1)

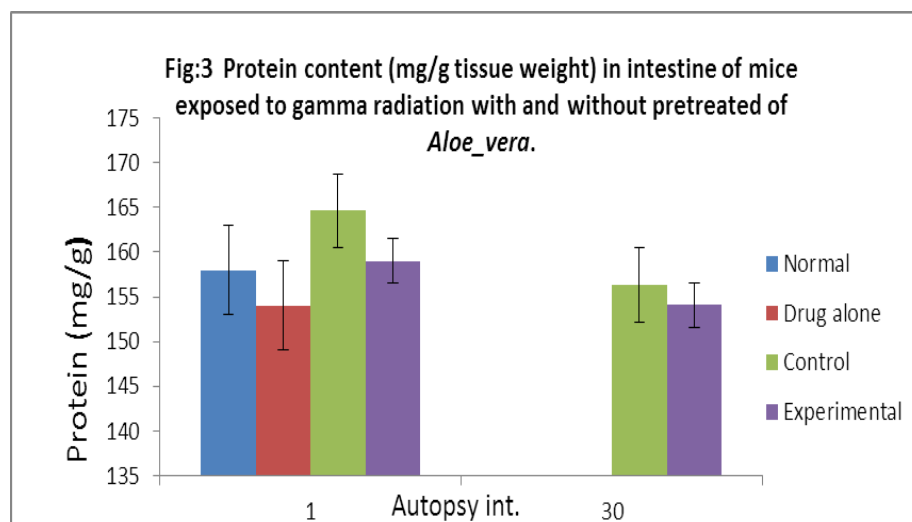


Glutathione (GSH)

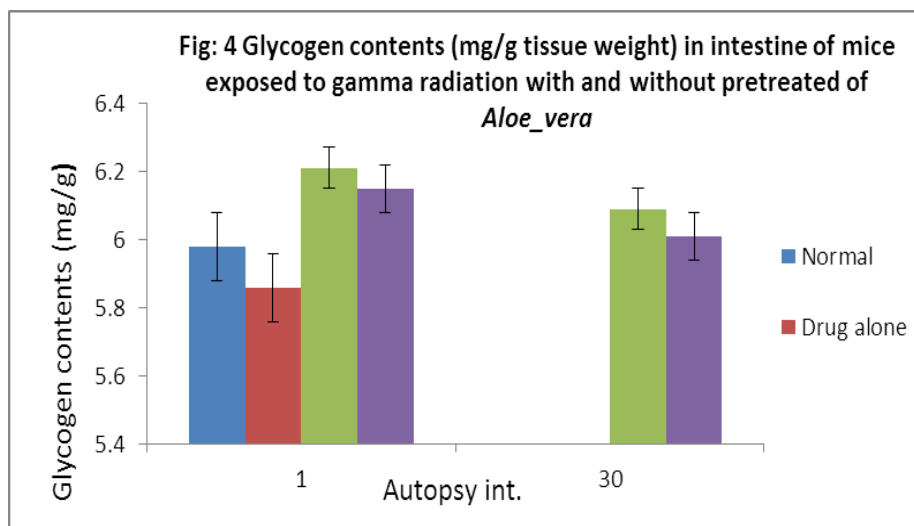
The GSH decreases in control set of intestine due to radiation dose but increases in experimental set from the control set due to *Aloe vera* scavenging properties. (Fig.2)



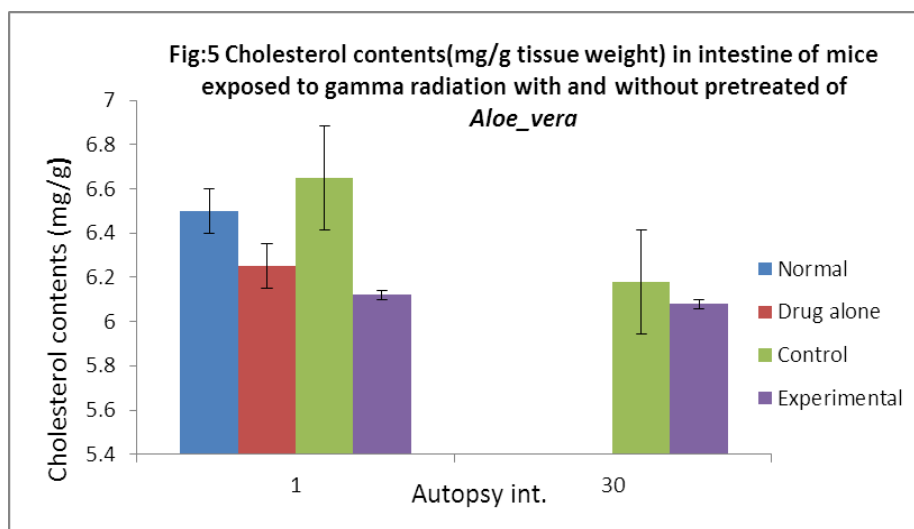
Protein: The protein increases in control set of intestine after radiation dose because due to radiation the permeability of plasma membrane increases and amino acid transport is also extend, endoplasmic reticulum & ribosome mobilization is also increased. (Fig.3)



Glycogen: The glycogen increases in control set of intestine due to increased rate of protein catabolism & the release of substances from radiosensitive cells after radiation dose (Dixit *et al.* 1976). And in experimental set *Aloe vera* pretreated protect the tissue proteins by disulphide formation, which in turn limit the breakdown products to enter into intestine & prevent excessive glycogen synthesis. So that it is lesser as compare to control set. (Fig.4)



Cholesterol: Cholesterol increases in control set due to radiation dose because it enhances the activity of HMG CO-A reductase, which is a rate limiting enzyme of cholesterol synthesis (Pugalendhi *et al.* 1992). Conversely in experimental set *Aloe vera* probably, inhibited HMG CO-A reductase activity and hence decreases the cholesterol concentration. (Fig.5)



DISCUSSION

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 indirect action of ionizing radiation i.e. by ROS generated through radiolysis of water molecules. ROS cause peroxidation of membrane lipid, oxidation of DNA, protein & several other important macromolecules in living system.

The result of the present study showed that LPO level was maximum in intestine of all irradiated alone (control set) at one day post irradiation, thereafter LPO level decreased at 30

days of autopsy interval from the normal mice (DDW alone) at 4.5 Gy radiation exposure. Increase in LPO level with increase of exposure dose of radiation suggested increased peroxidation of membrane lipid.

Hence the *Aloe vera* treatment to Swiss albino mice before exposure to 4.5 Gy gamma radiations lowered the LPO level in experimental set because *Aloe vera* provided the protection to cell membranes against free radical induced oxidative damage. Antioxidants like vitamin A, C, E, glutathione peroxidase, several isoenzymes of superoxide dismutase, minerals such as zinc & selenium present in *Aloe vera* seem to be responsible for inhibiting LPO level in intestine Uma Devi, P. and Ganasoundari, A. (1999).

Glutathione (GSH) is essential for protection of the cells against ROS and free radicals produced even in normal metabolism; it plays an important role in drug metabolism, radiation and cancer. In the present study GSH deplete and reach at minimum level at 1 day exposure to dose of gamma radiation. However it increases at 30 days autopsy interval in control set animal. GSH depletion does not have direct consequences in the form of acute toxicity but the cells became more susceptible to chemical or oxidative stress. Although the level of GSH was increased in experimental set due to *Aloe vera* treatment before exposure to gamma radiation, which elevated the level of GSH and has important role in scavenging radiation induce free radicals & prevented suppression of GSH. GSH is essential for protection of the cells against ROS (Reactive Oxygen Species) and free radicals produced even in normal metabolism (Sen 1997), it play important role in drug metabolism, radiation and cancer (Sen *et al.* 1994).

In the present study a radiation dose increases the total protein contents was observed up to day one in 4.5 Gy irradiated alone animal therefore a decreasing pattern was notice up to day 30 autopsy but it was still higher than normal in intestine . This may be due to increased transport of amino acid through the plasma membrane as a consequence of change in permeability of irradiated cell membranes. The pattern of increase in total protein contents was also similar in *Aloe Vera* treated 4.5 Gy irradiated mice in intestine at 1 day but significantly lesser in 30 day autopsy. These finding suggested that treatment with *Aloe vera* before radiation exposure provided protection to plasma membrane against free radicals induce alterations in its permeability which inhibited amino acid transport and ultimately protein synthesis. But in experimental set treatment with *Aloe vera* before radiation exposure provided protection to plasma membrane against free radicals induce alterations in its

permeability, which inhibited amino acid transport and ultimately decreases the protein synthesis (Chetty *et al.* 1977).

A dose depended increase in glycogen contents was notice in intestine with 4.5 Gy radiation at one day and the continuous decline was notice at 30 day autopsy but still remain higher than normal level in control set , this is due to the increase rate of protein catabolism & the release of substances from radiosensitive cells . In this study increase in glycogen contents also increased in intestine of *Aloe vera* treated 4.5 Gy irradiated mice at 1 day but it is significantly decreases at 30 day autopsy. *Aloe vera* protected it by disulphide formation, which in turn limit the breakdown products to enter into intestine`& prevented excessive glycogen synthesis dose (Dixit *et al.* 1976).

In this study cholesterol increased in intestine was observed at 1 day post irradiation (control set) & also in experimental set because the radiation dose enhance the activity of HMG CO- A reductase while increase was lesser in experimental set in comparison to control sets, but it decreases at 30 day autopsy because *Aloe vera* probably inhibited HMG CO- A reductase, which is a rate limiting enzyme of cholesterol synthesis (Pugalendhi *et al.* 1992). So the present study suggested that the *Aloe vera* shows antioxidants and scavenging activity because it protected from radiation induced damage to intestine of Swiss albino mice.

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CONCLUSION

The present study concluded that the administration of *Aloe vera* to mice probably helped in maintaining the balance up to some extend between free radicals and antioxidant level and therefore provided protection to mice intestine.

Finally, it can be stated that oxidative stress can be minimized in human beings by the regular use of *Aloe vera*.

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