

Antioxidative Effect of *Aloe Vera* Leaf Extract against Radiation Induced Oxidative Stress

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Summary

Antioxidative potential of *Aloe* was studied in mice exposed to different doses of gamma radiation. In this study, adult male Swiss albino mice were divided in six groups (I, II, III, IV, V and VI). Group I and II contained sham irradiated (normal) and *Aloe* alone treated animals respectively. Each group from III-VI contained an experimental set in which *Aloe* extract was administered orally at the dose of 1000 mg/kg body weight for 15 consecutive days and a control set, which received double distilled water (volume equal to that used for administration of *Aloe* in experimental sets) in the similar manner for similar period. On the last day of *Aloe* and double distilled water (DDW) administration, animals of group III, IV, V and VI were exposed to 0.5, 1, 3 and 5 Gy gamma radiations respectively. Level of lipid peroxidation (LPO) and contents of reduced glutathione (GSH) were measured in mice liver at day $\frac{1}{4}$, $\frac{1}{2}$, 1, 5, 10 and 20 post-irradiation. Treatment with *Aloe* alone lowered the LPO level and increased the contents of GSH non-significantly. Irradiation of animals resulted in a dose dependent increase in LPO and GSH contents in liver of mice at $\frac{1}{4}$ day post irradiation. Thereafter, LPO decreased and GSH increased at later autopsy intervals but these parameters did not return to normal level even at last day of this study (day 20) in any of the irradiated alone sets. Whereas, treatment with *Aloe* prior to irradiation reduced generation of LPO and increased the contents of GSH significantly at all autopsy intervals. Conclusively Vitamins A (β -carotene), C, E, glutathione peroxidase, several isozymes of super oxide dismutase and minerals such as zinc and selenium, present in *Aloe* seem to be responsible for its antioxidative property.

Key words: Mice, Radiation, Lipid Peroxidation, Reduced glutathione, *Aloe barbadensis* (Mill.), Liliaceae

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Introduction

Reactive oxygen species (ROS) such as superoxide radical primarily arises as by product of normal metabolic activities, especially during oxidative phosphorylation in mitochondria and β -oxidation of fatty acids in peroxisomes. Exposure to ionizing radiation also triggers the generation of reactive oxygen species through radiolysis of water molecules and disturbs the balance between ROS concentration and antioxidants level in a biological system that results in peroxidation of membrane lipids¹, oxidation of proteins² and DNA³. Peroxidation of membrane lipids changes the structure, fluidity and permeability of membranes and may eventually give rise to cell death. ROS mediated damage can be prevented or minimized to some extent by raising the level of antioxidants as they detoxify or scavenge the free radicals.

To achieve this goal, various synthetic compounds have been tested since 1950 to early 1980 and were found as good radioprotectors but their high toxicity limited their clinical applications. During last 25 years several herbal preparations Liv.52, rasayanas, triphala, mentat, chyavanprash, abana⁴⁻⁹ and plant extracts Rajgira, Mentha, Ginseng, Ocimum¹⁰⁻¹³ etc have been studied extensively in animal models and were reported effective radioprotectors because these plant products are rich in several antioxidants and are always better than synthetic drugs, especially due to their non-toxic nature

Aloe barbadensis (Mill.) belongs to family Liliaceae and commonly known as *Aloe vera*. *Aloe* leaf contains two basic components, pulp (gel) and latex. *Aloe* gel is a clear mucilaginous substance produced by parenchymal cells located in central region of the leaf. AG is composed mainly of water (99%) and mono and polysaccharides (25% of dry weight of the gel). The most common monosaccharide in AG is mannose -6-phosphate and most common polysaccharides are called gluco-mannans¹⁴. The prominent gluco-mannan is named as acemannan. AG significantly stimulates collagen synthesis in dermal wound in rats¹⁵. Mannose-6 phosphate was found to be responsible in wound healing in man¹⁶. Thereafter, various biological properties of *Aloe* have been reported by several workers. Topically applied *Aloe* gel can help in healing of radiation burns¹⁷. Latex contains anthraquinone, glycosides that are potent stimulant laxatives. *Aloe* gel is rich in vitamins [A (β -carotene), C and E]¹⁸, glutathione peroxidase¹⁹, several isoenzymes of superoxide dismutase²⁰ and minerals like zinc¹⁴ and selenium.

In the present investigation antioxidative potential of *Aloe* was evaluated by measuring level of LPO and GSH contents in irradiated alone and *Aloe* treated irradiated mice liver as one of the metabolic organs and performs several important functions in the body.

Materials and Methods

Animals

Adult male Swiss albino mice of 6-8 weeks old, weighting 25 ± 2 g were selected from an inbred colony for this study and maintained on standard mice feed (procured from Ashirwad Industry, Chandigarh, India) and water *ad libitum*.

Irradiation

A cobalt teletherapy unit (ATC - C9) at the Radiotherapy Department, SMS Medical College and Hospital, Jaipur was used for irradiation. The unanaesthetised animals were kept in well-ventilated Perspex boxes at a distance (SSD) of 77.5 cm from the source to deliver the dose at the rate of 0.98 Gy / min.

Aloe vera extract

Fresh leaves of *Aloe barbadensis* (Mill.) were collected locally and identified by the Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen (RUBL - 19886) is also deposited at the Herbarium of the same department. Fresh leaves of *Aloe vera* were dried in shade, powdered and extracted with double distilled water (DDW) by refluxing for 36 hours (12 hours \times 3) at 80 °C. The prepared extract was vacuum evaporated so as to make it in powder form. This extract was redissolved in DDW just before oral administration.

Experimental Design

Animals selected from an inbred colony were divided into six groups (I, II, III, IV, V and VI) for this study. Group I and II contained sham irradiated (normal) and *Aloe* alone treated animals respectively. Each group from III - VI contained an experimental set of mice in which *Aloe* extract was administered orally at the dose of 1000 mg/ kg. body weight for 15 consecutive days and a control set that received double distilled water (volume equal to that used for administration of *Aloe* extract in experimental sets) in similar manner for similar period.

On the last day of administration of *Aloe* and double distilled water animals of groups III, IV, V and VI were exposed to 0.5, 1, 3 and 5 Gy gamma radiation respectively. A minimum of 4 animals from each set were sacrificed at day $\frac{1}{4}$, $\frac{1}{2}$, 1, 5, 10 and 20 post irradiation and livers were taken to estimate level of lipid peroxidation (LPO) and contents of reduced glutathione (GSH).

Liver homogenates (10%) were prepared from sham irradiated, *Aloe* alone treated, irradiated alone and *Aloe* treated irradiated animals and levels of lipid peroxidation and reduced glutathione were measured.

Lipid Peroxidation (LPO) assay: Lipid peroxidation level in liver was measured by the method of Ohkhawa *et al.*²¹ The absorbance was read at 532 nm using UV-VIS systronic spectrophotometer.

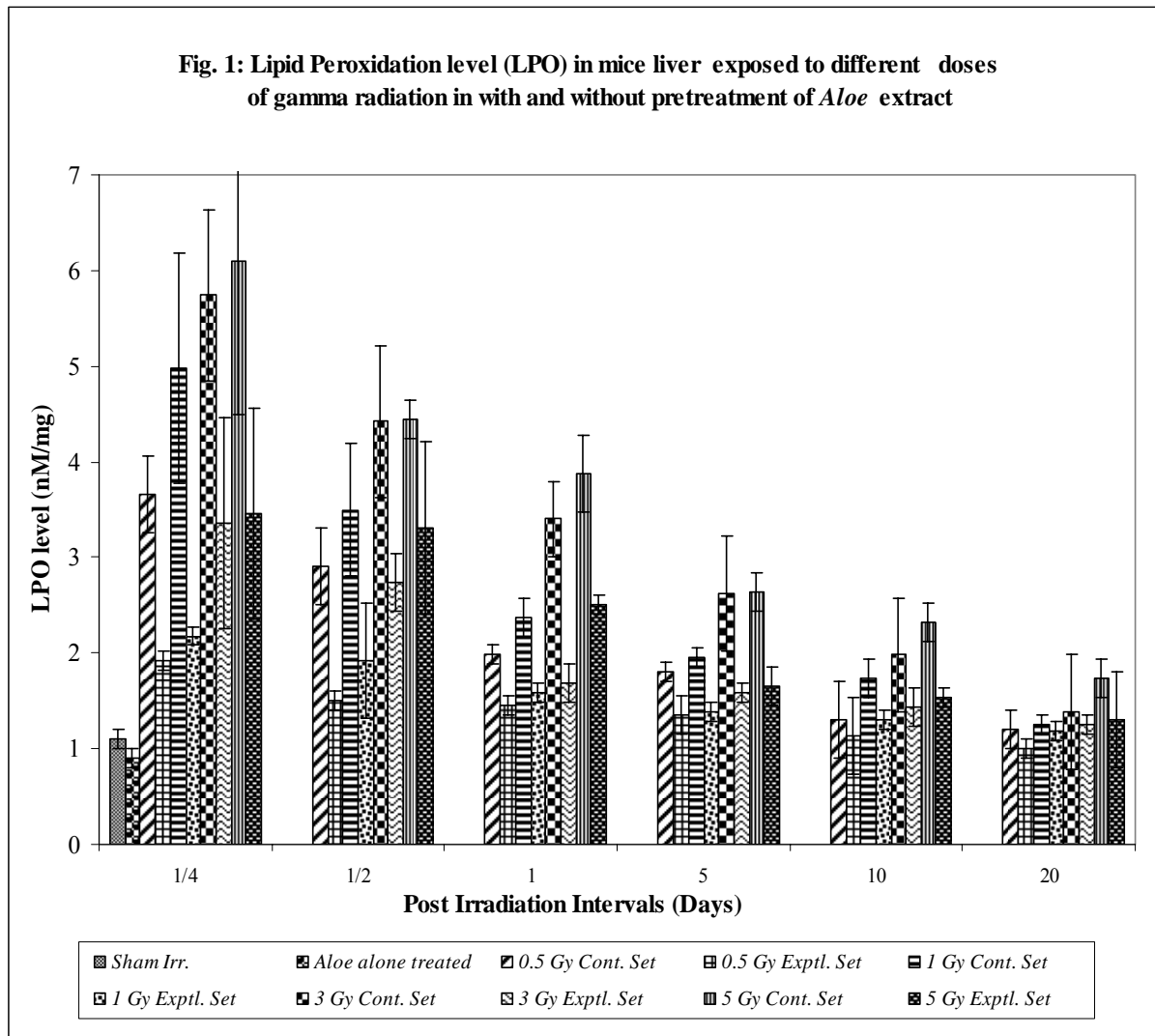
Reduced Glutathione (GSH) assay: GSH contents in liver were measured by the method of Moron *et. al.*²² . The absorbance was read at 412 nm using UV-VIS systronic spectrophotometer.

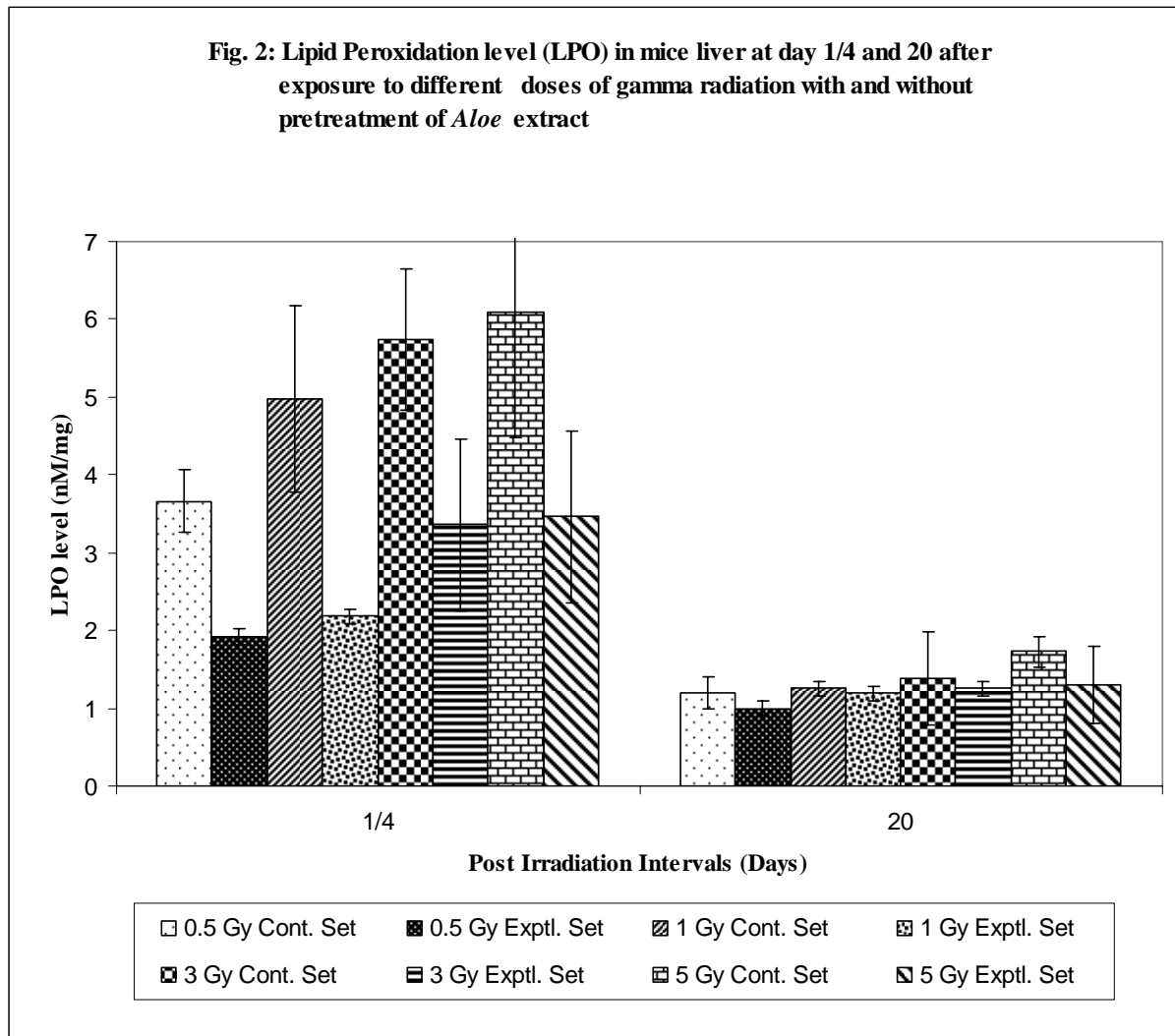
Statistic Analysis

The data were subjected to the Student's 't' test for comparison between different groups. The values were expressed as mean \pm standard error. Significance level was set at $p < 0.05$.

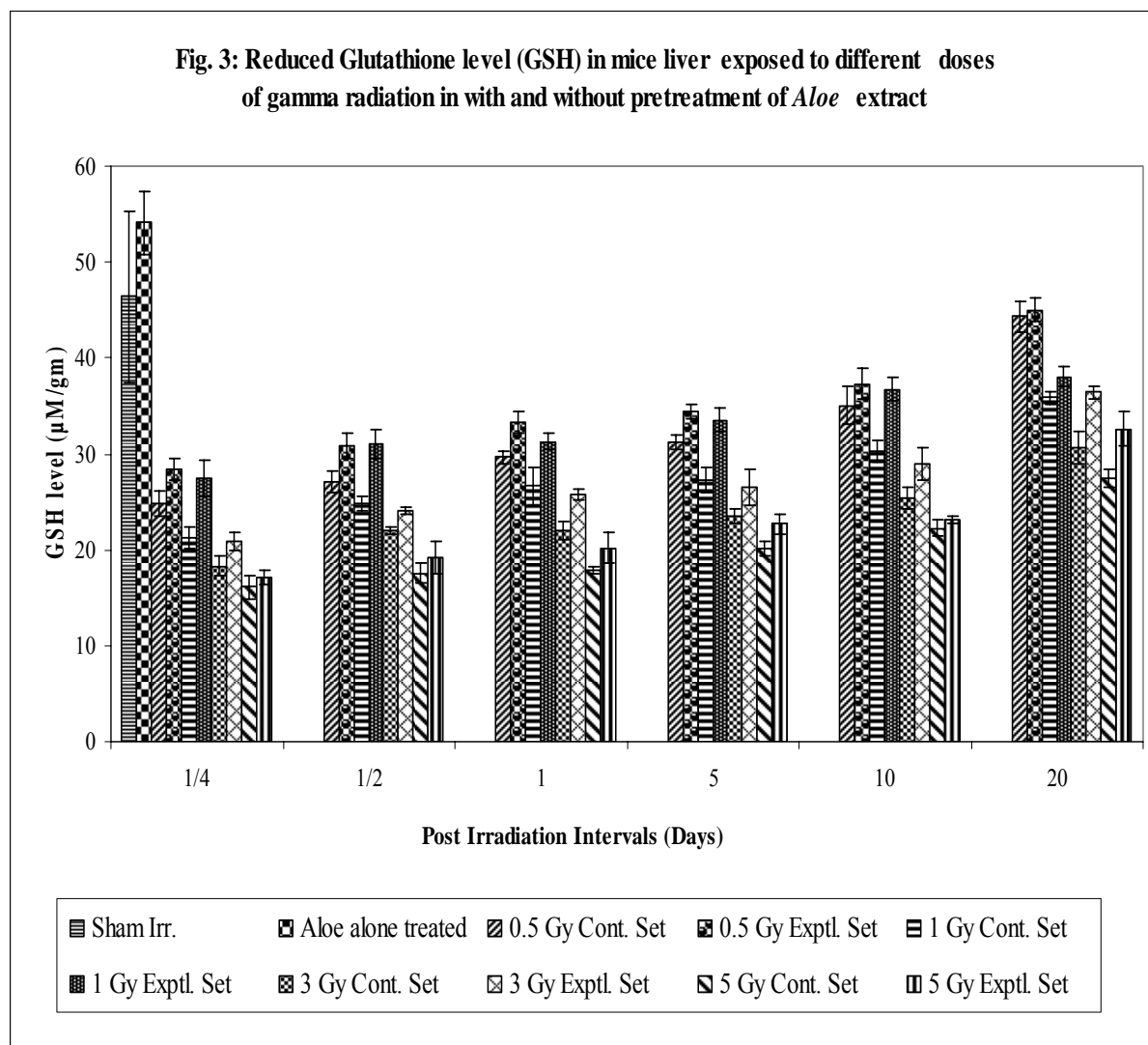
Results

Although, treatment with *Aloe* to mice for 15 consecutive days decreased the LPO level and increased the contents of GSH but difference between levels of LPO and GSH in sham irradiated (group I) and *Aloe* alone treated animals (group II) was insignificant. Exposure of mice to 0.5, 1, 3 and 5 Gy resulted in dose dependent increase in lipid peroxidation and decrease in GSH contents at day ¼. Therefore, LPO decreased gradually at later autopsy intervals but did not return to normal level even at day 20 in any of the control sets (Fig.1). Figure 2 indicates that level of LPO was the highest at day ¼ and lowest at day 20 post-irradiation in all control sets. The pattern of change in LPO level was similar in *Aloe* treated and irradiated alone mice liver but it was significantly lower in *Aloe* treated irradiated mice liver (experimental sets) in comparison to irradiated alone mice (control sets).



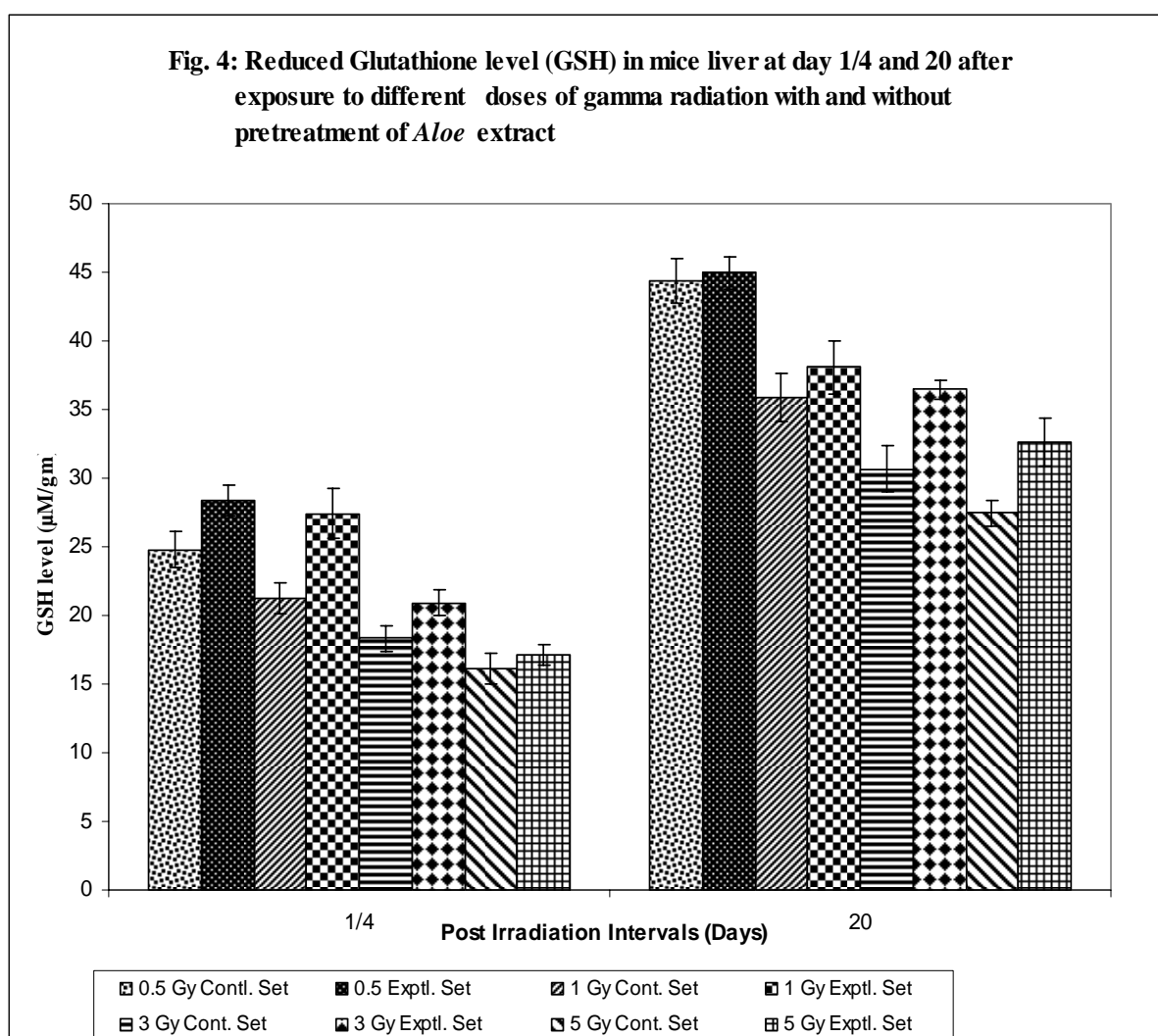


Conversely, contents of GSH decreased by 0.53, 0.45, 0.39 and 0.34 folds at day ¼ post irradiation in mice exposed to 0.5, 1, 3 and 5 Gy respectively. This decrease in GSH contents was followed by a gradual increase up to last day of this study without returning to normal level (Fig.3). Unlike LPO, reduced glutathione contents were the lowest at day ¼ and the highest at day 20 post-irradiation in all control and experimental sets but contents of GSH were significantly higher in experimental set than control sets at all autopsy intervals (Fig.4). Secondly, increase in GSH was the highest in *Aloe* treated low dose exposed animals (0.5 Gy) and was the lowest in *Aloe* treated high dose exposed animals (5 Gy) (Fig.3).



Discussion

It is a proven fact that radiation induced damage in a biological system is initiated by reactive oxygen species (ROS) generated through radiolysis of water. Synthetic chemical agents like thiols can interact with free radicals and inhibit lipid peroxidation but such synthetic agents are highly toxic and cannot be used in clinical field. Therefore, several studies on plant products are being carried out all over the world by different workers to develop an orally active and non-toxic drug that can mitigate the effects of radiation in biological system. For the first time Collins and Collins²³ used *Aloe vera* gel for the treatment of radiodermatitis.



An antioxidant protein, metallothioneine was induced in skin and liver within 24 hours of AG administration. AG scavenges hydroxyl radicals and prevents suppression of SOD and GPx in the skin²⁴⁻²⁵. AG prevents immune suppression in mouse skin by reducing the production and release of interleukin-10(IL-10)²⁶.

Acemannan derived from AG stimulates the synthesis and release of interleukin-1 (IL-1), which increases tumour necrosis. The cytokines in turn initiate immune attack on sarcoma cells that results in necrosis and regression of cancerous cells²⁷. AG reduces croton oil induced swelling in rats after topical application²⁸. C- glucosyl chromone is the anti-inflammatory compound isolated from AG extract²⁹.

In this study, vitamins A (β -carotene), C and E¹⁸, glutathione peroxidase¹⁹, several isozymes of superoxide dismutase²⁰ and minerals like Zinc¹⁴ and selenium present in *Aloe* probably lowered the TBARS formation significantly in mice exposed to different doses of ionizing radiation (Fig.1). Results also indicate that irradiation of mice with different doses of gamma radiation without treatment with *Aloe* resulted in dose dependent increase in TBARS formation in liver i.e. increase in LPO level was the highest in 5 Gy and the lowest in 0.5 Gy exposed mice (Fig.2). Maharwal¹⁰ and other earlier workers have also reported an increase in TBARS formation after that demonstrates the involvement of membrane lipid peroxidation. On the other hand, level of GSH was significantly higher in *Aloe* treated irradiated mice as compared to that of the control animals in this study (Fig. 3). Supplementation of *Aloe* might have increased the concentration of antioxidants [vitamins A (β -carotene), C, E, zinc and selenium], which participated in scavenging of radiation induced free radicals. Thus, relatively higher concentration of antioxidants seems to be a responsible factor for lowering the lipid peroxidation and increasing the GSH level in experimental mice because the basic cause of lipid peroxidation is not only the free radicals but also the low levels of antioxidants that scavenge them. These low molecular weight antioxidants (GSH, vitamins A, C and E) may act synergistically. Antioxidant property of β -carotene is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure. β -carotene plays an important role in trapping peroxy and alkoxy radicals in a biological system. Antioxidative role of β -carotene in plants occurs through quenching of singlet oxygen (1O_2) formed during photosynthesis. This is believed to be the same in human beings³⁰. Since β -carotene is effective at low oxygen concentration, hence it complements the antioxidative properties of vitamin E, which is effective at higher oxygen concentration. A combination of β -carotene and α -tocopherol exhibits a greater protective effect against radiation induced lipid peroxidation³¹. Vitamin E (α -tocopherol), present in cellular and sub-cellular membrane phospholipids functions as a chain breaking antioxidant³² and has been implicated in the activity of catalase³³, glutathione peroxidase³⁴ and possibly superoxide dismutase³⁵. Once tocopherol radical is formed (during chain breaking process) it can migrate to membrane surface and is reconverted in α -tocopherol by reacting with ascorbate (vitamin C) or GSH. The resulting ascorbate radical can regenerate vitamin C by reduction with GSH, which can also directly scavenge free radicals and resulting GSSG can regenerate GSH through NADPH-glutathione reductase system. Thus, GSH plays an important role in metabolism, free radical scavenging and regeneration of antioxidant like vitamin C.

Conclusion

Results of this study suggest that supplementation of *Aloe vera* can be one of the best approaches to control ROS-mediated pathogenesis and its antioxidative properties might account for possible anti-cancer activities. Secondly, *Aloe* is also a source of acemannan, which stimulates synthesis and release of inter-leukin-1 (IL-1). These cytokines initiate an immune attack on cancer cells that results in necrosis and regression of tumor.

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