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# Evaluation of radioprotective efficacy and possible mechanism of action of Aloe gel

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## ABSTRACT

The present study was undertaken to determine the optimum effective dose, dose reduction factor (DRF) and possible mechanism of action of Aloe gel. Three different doses of gel (250, 500 and 750 mg/kg body weight) were tested against 8 Gy induced damage in Swiss albino mice. A dose of 750 mg/kg body weight of Aloe was found the most effective while, 250 mg/kg body weight was the least effective in providing protection, as observed in the form of higher concentrations of blood GSH and vitamin C and lower level of serum LPO than irradiated animals at 1 h post irradiation and higher percent of survivors up to day 30 post irradiation.

Treatment of mice with Aloe before irradiation with different doses of gamma radiation (6–12 Gy) delayed the onset and reduced the severity of signs of radiation sickness. The LD<sub>50/30</sub> was calculated as 6.77 and 10 Gy for untreated irradiated and Aloe treated irradiated animals, respectively and its dose reduction factor was also calculated as 1.47. Aloe gel scavenged the free radicals, DPPH•, ABTS•• and NO in a concentration dependent manner in vitro and therefore, scavenging of free radicals seems to be its important mechanism of action.

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## 1. Introduction

Applications of radiation are continuously increasing in various fields. Rapid technological advancement in the field of medicine (radiotherapy and radiodiagnosis), agriculture, industries, research laboratories, etc. has increased the human exposure to ionizing radiations enormously. Accidental and occupational exposures are also increasing day by day and enhancing the risk for human population and will continue to enhance as the whole world is in the race of nuclear weapons' testing. To protect humans against the deleterious effects of ionizing radiations several synthetic chemical compounds such as cysteine (Patt et al., 1949), cysteamine (Bacq et al., 1951), cystamine (Bacq, 1953), WR-2721 (Stromberg et al., 1968), and MPG (Sugahara et al., 1970) have been used but none

of them gained much importance in clinical field due to their high toxicity.

Therefore, intervention of plant extracts such as *Piper betel* (Bhattacharya et al., 2007), *Amaranthus peniculatus* (Saini and Maharwal, 2007), *Boerhaavia diffusa* (Manu et al., 2007), and *Emblca officinalis* (Jindal et al., 2009), herbal preparations like Liv. 52 (Saini et al., 1985), Rasayanas (Kumar et al., 1999) and Abana (Baliga et al., 2004), natural agents like vitamins E (Borek et al., 1986), C (Konopacka et al., 1998), and A (Shrivastava, 2002) and enzymes such as super oxide dismutase (Patkau et al., 1975) could be the most prudent strategy to develop non-toxic and the most effective drugs to protect the human beings against harmful effects of ionizing radiation.

*Aloe vera* is an herb and belongs to family Liliaceae. Aloe leaf can be divided into two major parts, the outer green rind including the vascular bundles and inner is Aloe

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gel. It is a thin clear jelly like substance obtained from the parenchymal tissue of inner portion of leaf. It contains more than 75 active organic and inorganic ingredients. Glucmannan, acemannan, mannose-6-phosphate, glycoproteins, glucose, gamma-linolenic acid, saponins, sterols, cholesterol, pro-vitamin A ( $\beta$ -carotene), vitamins C, E (Atherton, 1998), glutathione peroxidase (Klein and Penneys, 1998), isoenzymes of superoxide dismutase (Sabeh et al., 1993), phenolic antioxidant, etc. are the organic ingredients and magnesium, calcium, zinc, selenium, etc. are the inorganic ingredients. Therefore, it is the most commercialized *Aloe* species and processing of the leaf pulp that has become a large worldwide industry. In food industry, it is being used for the production of gel-containing health drinks and beverages. It has been used for the production of creams, lotions, soaps, shampoos, facial cleansers and other products in the cosmetic and toiletry industry. In the pharmaceutical industry, it is being used for manufacturing of topical products such as ointments and gel preparations as well as in the production of tablets and capsules (He et al., 2005). It is taken as liquid that helps in acid indigestion and treatment of ulcers (Thomas et al., 1998). Treatment with *Aloe* gel accelerates wound contraction and increases the breaking strength of resulting scar tissue (Hegggers et al., 1996). *Aloe vera* has anti-microbial (Robson et al., 1982), anti-tumor (Imanishi and Suzuki, 1984; Saini et al., 2010), anti-rheumatoid (Davis et al., 1986) and anti-diabetic properties (Bunyapraphtsara et al., 1996). It has been shown to be effective for treating radiation induced burns (Chithra et al., 1998). It also provides protection to mouse skin against X-irradiation by scavenging hydroxyl radicals and preventing alteration in enzyme activity (Sato, 1991).

Therefore, this study was undertaken to determine optimum effective dose, dose reduction factor (DRF), and possible mechanism of action of *Aloe* gel by estimating its scavenging activity of DPPH $\cdot$ , ABTS $\cdot\cdot$  and NO *in vitro*.

## 2. Materials and methods

### 2.1. Animals

Animal care and handling were done according to the guidelines set by the World Health Organization (WHO), Geneva, Switzerland and the INSA (Indian National Science Academy, New Delhi). Healthy male Swiss albino mice of 6–8 weeks old, weighing  $25 \pm 2$  g were selected from an inbred colony and maintained on standard mice feed (procured from Ashirwad Industry, Chandigarh, India) and water *ad libitum*.

### 2.2. Irradiation

The cobalt teletherapy unit (ATC-C9) at the cancer treatment centre, Radiotherapy Department, SMS Medical College and Hospital, Jaipur was used for irradiation. Unanaesthetised mice restrained in well-ventilated Perspex boxes were whole-body exposed to different doses of gamma radiation at the distance (SSD) of 77.5 cm from the source to deliver the dose at the rate of 1.62 Gy/min.

### 2.3. Test substance

*Aloe barbadensis* (Mill.) commonly referred as *Aloe vera* was collected locally, identified and placed in the herbarium, Department of Botany, University of Rajasthan, Jaipur, India. A voucher number RUBL – 19886 was also allotted by the same department. The green outer thick cuticle or rind of fresh *Aloe vera* leaves was removed, pulp (gel) was separated and dried in shade on a glass plate at room temperature ( $30 \pm 3$  °C). The dried pulp was placed in the oven at 40 °C for complete evaporation of water and then powdered. The pulp powder was dissolved in double distilled water (DDW) at the time of oral administration to experimental animals.

### 2.4. Experimental design

#### 2.4.1. Determination of optimum effective radioprotective dose

Survival of mice (%) up to day 30 post-irradiation and alteration in levels of LPO, GSH and vitamin C at 1 h post irradiation were estimated to determine the optimum effective radioprotective dose. For this purpose, 76 mice were divided into I, II, III and IV groups of 19 each and *Aloe* gel was given orally at the dose of 0, 250, 500 and 750 mg/kg body weight/day for 15 consecutive days, respectively. On the last day of the treatment with *Aloe*, animals of all groups were exposed to 8 Gy gamma radiation. Four animals from each group were sacrificed at 1 h post irradiation by cervical dislocation and the blood was collected through syringe from the heart for estimation of LPO, GSH and vitamin C. Remaining 15 animals of each group were observed daily up to day 30 for recording their survival.

2.4.1.1. Lipid peroxidation (LPO) assay. Serum lipid peroxidation level was measured in terms of thiobarbituric acid reactive substances [TBARS] by the method of Ohkhawa et al. (1979). For conducting this assay, 0.2 ml serum was mixed with 0.2 ml sodium dodecyl sulphate (SDS) and 20% trichloroacetic acid (TCA). To this solution, 1.5 ml of 0.6% aqueous thiobarbituric acid (TBA) was added and heated at 100 °C for 60 min. The mixture was cooled and n-butanol and pyridine (15:1, w/v) were added. The absorbance was read at 532 nm using a UV-VIS Systronic Spectrophotometer [Model No. 108, Naroba, India].

2.4.1.2. Reduced glutathione (GSH) assay. Blood GSH level was measured spectrophotometrically using ellmans reagent (DTNB) as a coloring reagent as per the method described by Beutler et al. (1963). Concisely, 0.2 ml blood was mixed with 1.8 ml double distilled water and glacial meta phosphoric acid (a precipitating solution) was added, centrifuged and supernatant was collected. Lastly, the supernatant was mixed with 0.3 M disodium hydrogen sulphate and DTNB reagent and was allowed to stand for 2 min at room temperature. The absorbance was read at 412 nm.

2.4.1.3. Estimation of vitamin C. Contents of vitamin C were measured in blood by the method of Roe and Kuether (1943). For this purpose, 1 ml blood was mixed with 2.5 ml acetate buffer and centrifuged. To the supernatant, 4 ml of 4% TCA

was added and kept in dark for over night. It was centrifuged on next day and 1 ml of 2,4-dinitrophenyl hydrazine (DNPH) was added in 4 ml supernatant, mixed well and incubated at 37 °C for 45 min and lastly, 7 ml of 65% H<sub>2</sub>SO<sub>4</sub> was added to it. The yellow color developed was read spectrophotometrically at 540 nm.

This optimum effective dose (750 mg/kg body weight) was used to study radiation sickness and dose reduction factor (DRF).

#### 2.4.2. Study of radiation induced sickness and determination of dose reduction factor (DRF)

To study the radiation induced sickness and to determine the DRF, 120 mice were divided into four groups (I, II, III and IV) of 30 each. Each group had an experimental set and a control set. The animals of each experimental set were administered maximum effective dose of Aloe (750 mg/kg weight/day) orally for 15 consecutive days and animals of each control set received DDW (volume equal to that used for administration of Aloe in experimental set) in similar manner for 15 consecutive days. On the last day of Aloe and DDW administration, the animals of I, II, III and IV groups were exposed to 6, 8, 10 and 12 Gy gamma radiation, respectively, and 30 days post irradiation survival was recorded.

The dose reduction factor (DRF) was calculated by using the following formula:

$$\text{DRF} = \frac{\text{LD}_{50/30} \text{ of experimental animals}}{\text{LD}_{50/30} \text{ of control animals}}$$

#### 2.4.3. Study of mechanism of action of Aloe gel by its free radical scavenging activity

**2.4.3.1. DPPH• scavenging activity.** Ability of Aloe gel to scavenge the stable free radical, DPPH• is measured by the method of Mensor et al. (2001). To the 1 ml methanolic solution of DPPH• (0.25 mM), 1 ml of ethanolic solution of Aloe gel at different concentrations (100–900 µg/ml) was added. To prepare control, 1 ml of methanol was added to the 1 ml methanolic solution of DPPH• (0.25 mM). After 20 min, absorbance was recorded at 517 nm in a UV-VIS double beam spectrophotometer. The inhibition (%) of free radicals was calculated by using the following formula:

$$\text{Inhibition(\%)} = \frac{\text{AC} - \text{AA}}{\text{AC}} \times 100$$

where, AC is the absorbance of control and AA is the absorbance of test.

**2.4.3.2. ABTS•• scavenging activity.** ABTS•• scavenging activity of Aloe was measured by the method of Re et al. (1999). First, ABTS•• free radicals were generated through the oxidation of ABTS with potassium persulphate. For this purpose, ABTS was dissolved in deionized water to 7 mM concentration and potassium persulphate was added to a concentration of 2.45 mM. The reaction mixture was kept in dark at room temperature for 12–16 h before final use. Lastly, the ABTS•• solution was diluted with absolute ethanol till the absorbance was read 0.700 ± 0.020 at 734 nm. Aloe gel (20–160 µg/ml) in ethanol was added to 3 ml of ABTS•• solution and the absorbance was read after 6 min.

**2.4.3.3. Nitric oxide scavenging activity.** The interaction of Aloe gel with nitric oxide (NO) was assessed by the nitrate ion detection method. Sodium nitropruside (5 mM) in phosphate buffer spontaneously generates NO in an aqueous solution (Sreejayan and Rao, 1997). NO interacts with oxygen and produces nitrate ions, which can be estimated by the use of Greiss reagent (1% sulphanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% naphthylethylene diamine dihydrochloride) (Green et al., 1982). Sodium nitropruside (5 mM) in phosphate buffer was mixed with different concentrations of Aloe (10–50 µg ml<sup>-1</sup>) and incubated at 25 °C for 150 min. Prepared samples were allowed to react with Greiss reagent. The absorbance of chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was read at 546 nm. The same reaction mixture without the Aloe gel but with equal amount of distilled water served as control.

### 2.5. Statistical analysis

The results obtained in the present experiment were expressed as both mean ± SE and in percentage. Statistical analysis was carried out using one way analysis of variance followed by Tukey's post test to compare the means of the different treatment groups. The level of statistical significance was set at  $p < 0.05$ .

## 3. Results

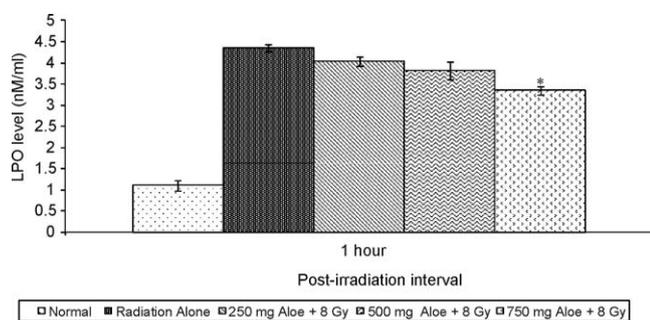
### 3.1. Determination of optimum effective radioprotective dose

#### 3.1.1. Lipid peroxidation (LPO)

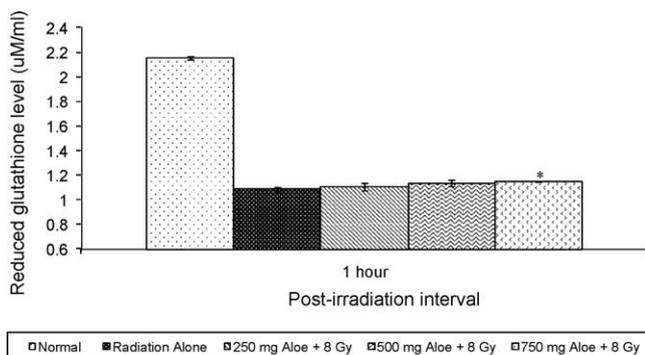
Serum lipid peroxidation level (LPO) was estimated as 1.098 ± 0.131 nM/ml (100%), in sham irradiated animals which increased and reached at the level of 4.356 ± 0.08 nM/ml (396%) in 8 Gy irradiated animals at 1 h post irradiation. However, treatment with 250, 500 and 750 mg/kg body weight of Aloe prior to irradiation with 8 Gy reduced the LPO level from 4.356 ± 0.08 nM/ml (396%) to 4.027 ± 0.11 (366%), 3.825 ± 0.21 (348%) and 3.345 ± 0.09 nM/ml, (304%), respectively, in serum at 1 h post irradiation. The decrease in serum LPO was insignificant in mice treated with Aloe at the dose of 250 and 500 mg/kg body weight prior to irradiation with 8 Gy in comparison to irradiation with 8 Gy alone, whereas treatment of mice with Aloe at the dose of 750 mg/kg body weight before irradiation with 8 Gy decreased the level of LPO significantly ( $p < 0.05$ ) in comparison to 8 Gy irradiated alone (Fig. 1).

#### 3.1.2. Reduced glutathione (GSH)

GSH concentration was found to be 2.156 ± 0.014 µM/ml (100%) in sham irradiated animals, which decreased in 8 Gy irradiated animals and estimated as 1.086 ± 0.020 µM/ml (50.37%) at 1 h post irradiation, whereas GSH concentration was found to be 1.109 ± 0.029 (51.43%), 1.135 ± 0.025 (52.64%) and 1.148 ± 0.005 µM/ml (53.24%) at 1 h post-irradiation in mice treated with 250, 500 and 750 mg/kg body weight of Aloe, respectively, before irradiation with 8 Gy gamma radiation. The increase in GSH concentration was insignificant in mice treated with Aloe at the dose of 250 and 500 mg/kg body weight



**Fig. 1 – Lipid peroxidation level (LPO) in Swiss albino mice treated with different doses of Aloe prior to irradiation with 8 Gy gamma radiation.**



\* = Significant ( $p=0.05$ )

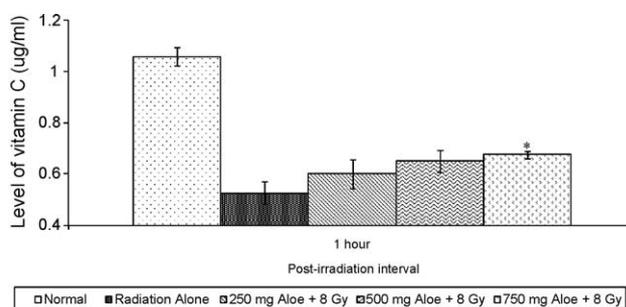
**Fig. 2 – Reduced glutathione (GSH) level in Swiss albino mice treated with different doses of Aloe prior to irradiation with 8 Gy gamma radiation. \*Significant ( $p < 0.05$ )**

prior to irradiation with 8 Gy in comparison to irradiation with 8 Gy alone, whereas treatment of mice with Aloe at the dose of 750 mg/kg body weight before irradiation with 8 Gy increased the concentration of GSH significantly ( $p < 0.05$ ) in comparison to 8 Gy irradiated alone (Fig. 2).

### 3.1.3. Vitamin C

Contents of vitamin C decreased in blood of 8 Gy exposed mice from  $1.058 \pm 0.036$  (found in sham irradiated animals) to  $0.525 \pm 0.043$   $\mu\text{g/ml}$  (49.62%) at 1 h post irradiation, whereas vitamin C contents were estimated as  $0.600 \pm 0.057$   $\mu\text{g/ml}$  (56.71%),  $0.650 \pm 0.043$   $\mu\text{g/ml}$  (61.43%) and  $0.675 \pm 0.014$   $\mu\text{g/ml}$  (63.79%) at 1 h post irradiation in mice treated with 250, 500 and 750 mg/kg body weight of Aloe, respectively, before irradiation with 8 Gy gamma radiation. The increase in vitamin C concentration was insignificant in mice treated with Aloe at the dose of 250 and 500 mg/kg body weight prior to irradiation with 8 Gy in comparison to irradiation with 8 Gy alone, whereas treatment of mice with Aloe at the dose of 750 mg/kg body weight before irradiation with 8 Gy increased the concentration of vitamin C significantly ( $p < 0.05$ ) in comparison to 8 Gy irradiated alone (Fig. 3).

Administration of 0, 250, 500 and 750 mg/kg body weight/day of Aloe for 15 consecutive days prior to irradiation with 8 Gy gamma radiation resulted in 20, 53.33, 86.66 and 93.33% survival of mice within a period of 30 days. The



\* = Significant ( $p=0.05$ )

**Fig. 3 – Level of vitamin C in Swiss albino mice treated with different doses of Aloe prior to irradiation with 8 Gy gamma radiation. \*Significant ( $p < 0.05$ ).**

results indicate that the treatment of mice with Aloe prior to irradiation delayed the onset of radiation induced mortality in a dose dependent manner. The longest delay was observed for 750 mg/kg body weight, where the first mortality occurred at day 15 post irradiation and shortest delay was observed for 250 mg/kg body weight, where the first mortality occurred at day 4 post irradiation (Fig. 4).

Treatment of mice with 750 mg/kg body weight of Aloe was found to be more effective than the treatment with 250 and 500 mg/kg body weight as observed in the form of higher contents GSH and vitamin C and lower LPO level at 1 h post irradiation and higher survival number of mice up to day 30 post irradiation. Therefore, the dose of 750 mg/kg body weight was considered as the optimum effective radioprotective dose of Aloe in this study.

### 3.1.4. Radiation induced sickness

Further, to evaluate the radioprotective efficacy of Aloe, this optimum effective dose (750 mg/kg body weight) was administered orally once daily for 15 consecutive days prior to irradiation with 6, 8, 10 and 12 Gy gamma radiation. The animals irradiated with different doses of radiation exhibited various signs of radiation sickness such as reduction in food and water intake, weight loss, diarrhoea, excessive lacrimation, letharginess, irritability, ruffling of hair followed by epilation during initial period of 2–6 days. The severity of these signs was found to be dose dependent, i.e., severity was lesser in 6 Gy exposed animals as compared to those exposed to higher doses (8, 10 and 12 Gy). Facial oedema also observed in some of the mice exposed to doses above 6 Gy between 6 and 10 days post-irradiation. The onset of signs was early and severity was more in mice exposed to the highest dose (12 Gy) than the lowest dose (6 Gy) of radiation.

The mean body weight of mice decreased from day 1 and reached at a peak on day 4 (15.24%), 11 (21.87%), 8 (21.07%) and 7 (21.96%) after exposure to 6, 8, 10 and 12 Gy, respectively. Thereafter, a gradual increase in percent mean body weight was observed but it remained 10.33 and 17.72% lesser than initial body weight in 6 and 8 Gy exposed animals, respectively, at day 30 post irradiation, whereas animals exposed to 10 and 12 Gy did not survive beyond day 8 and 7, respectively (Fig. 5).

The pattern of decrease in mean body weight was also found similar in mice treated with 750 mg/kg body weight of

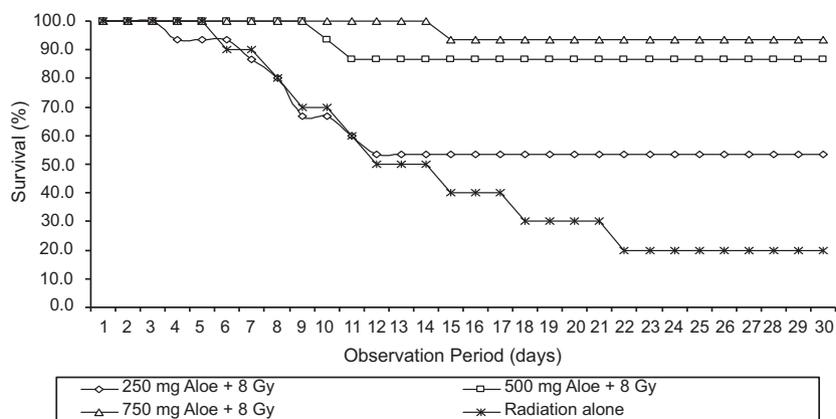


Fig. 4 – Thirty-days survival (%) of Swiss albino mice treated with different doses of Aloe prior to irradiation with 8 Gy gamma radiation.

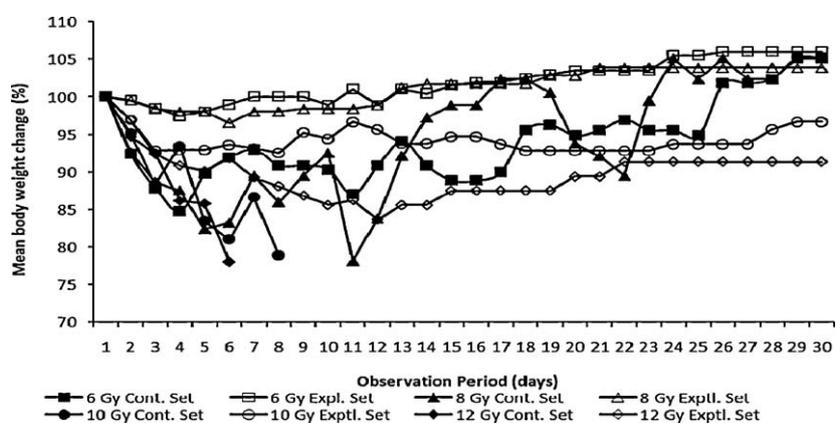


Fig. 5 – Mean body weight change (%) of Swiss albino mice irradiated with 6, 8, 10 and 12 Gy gamma radiation with and without Aloe treatment (750 mg/kg body weight).

Aloe prior exposure to different doses of gamma radiation. But maximum decrease was only 2.6, 3.5, 7.4 and 16.3% at day 4, 6, 8 and 12 post-irradiation in 6, 8, 10 and 12 Gy exposed mice, respectively. Thereafter, mean body weight increased and found to be 6 and 3.9% higher than initial weight at day 30 post irradiation in Aloe pretreated 6 and 8 Gy irradiated animals, whereas mean body weight was 3.3 and 8.7% lesser than their initial body weight in Aloe pretreated 10 and 12 Gy irradiated animals at day 30 post irradiation (Fig. 5).

### 3.1.5. Radiation induced 30 days mortality and determination of dose reduction factor (DRF)

Mice exposed to 6 and 8 Gy exhibited 26.66 and 80% mortality, respectively, during a period of 30 days, whereas animals exposed to 10 and 12 Gy gamma radiation died at day 8 and 7 post irradiation, respectively. On the contrary, mortality was not observed in Aloe pretreated 6 Gy irradiated animals but 6.6, 46.6 and 86.6% animals died among Aloe treated 8, 10 and 12 Gy irradiated animals, respectively, up to day 30 post irradiation (Fig. 6). Thus, treatment of mice with a dose of 750 mg/kg body weight of Aloe before irradiation delayed and decreased the radiation induced mortality when compared to the mortality occurred with irradiation alone.

The LD<sub>50/30</sub> was found to be 6.77 Gy for untreated irradiated animals and 10 Gy for Aloe treated irradiated animals and dose reduction factor (DRF) was calculated as 1.47.

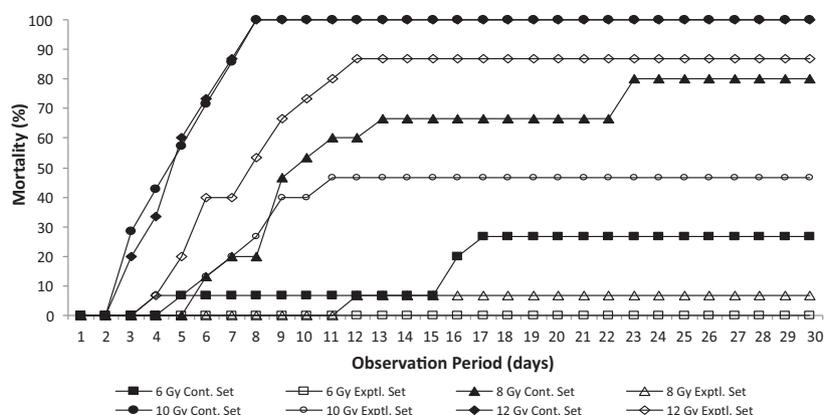
## 3.2. Mechanism of action of Aloe gel by free radical scavenging activity

### 3.2.1. DPPH• scavenging activity

Aloe gel inhibited the generation of DPPH radical in a dose dependent manner and its IC<sub>50</sub> value was found to be 572.14 ± 2.21 µg/ml (Table 1), which is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color). A lower value of IC<sub>50</sub> indicates the greater antioxidant activity of a test substance.

### 3.2.2. ABTS<sup>•+</sup> scavenging activity

ABTS<sup>•+</sup> scavenging activity of Aloe gel was estimated, which determines the decolorization of the ABTS<sup>•+</sup> through measuring the reduction of the radical cation at 734 nm. Aloe scavenged the ABTS<sup>•+</sup> in a dose dependent manner and its IC<sub>50</sub> value was found to be 105.26 ± 0.22 µg/ml (Table 2).



**Fig. 6 – Mortality (%) of Swiss albino mice irradiated with 6, 8, 10 and 12 Gy gamma radiation with and without Aloe treatment (750 mg/kg body weight).**

**Table 1 – DPPH\* scavenging activity (%) of Aloe gel and its IC<sub>50</sub> value.**

S.N.	Concentration of Aloe (μg/ml)	% inhibition	IC <sub>50</sub> (μg/ml)
1.	100	27.91 ± 0.23	572.14 ± 2.21
2	200	33.33 ± 0.12	
3	300	38.54 ± 0.48	
4	400	41.66 ± 0.23	
5	500	48.33 ± 0.24	
6	600	52.50 ± 0.36	
7	700	51.66 ± 0.12	
8	800	60.41 ± 0.36	
9	900	66.25 ± 0.36	

**Table 2 – ABTS\*\* scavenging activity (%) of Aloe gel and its IC<sub>50</sub> value.**

S.N.	Concentration of Aloe (μg/ml)	% inhibition	IC <sub>50</sub> (μg/ml)
1	20	24.64 ± 0.16	105.26 ± 0.22
2	40	34.31 ± 0.08	
3	60	36.83 ± 0.20	
4	80	42.71 ± 0.08	
5	100	46.07 ± 0.16	
6	120	52.80 ± 0.24	
7	140	62.04 ± 0.08	
8	160	65.82 ± 0.16	

**Table 3 – NO scavenging activity (%) of Aloe gel and its IC<sub>50</sub> value.**

S.N.	Concentration of Aloe (μg/ml)	% inhibition	IC <sub>50</sub> (μg/ml)
1	10	14.73 ± 1.21	46.36 ± 1.35
2	20	32.63 ± 0.60	
3	30	36.84 ± 1.21	
4	40	43.15 ± 1.82	
5	50	52.63 ± 1.21	

### 3.2.3. Nitric oxide scavenging activity

Nitric oxide scavenging activity of Aloe gel was measured at various concentrations (10–50 μg/ml). The gel resulted in inhibition of NO generation *in vitro* in a concentration dependent manner and its IC<sub>50</sub> value was calculated as 46.36 ± 1.35 μg/ml (Table 3).

## 4. Discussion

The results of the present study indicate that oral administration of Aloe gel to mice at the dose of 750 mg/kg body weight prior to irradiation with 8 Gy enhanced the concentrations of GSH and vitamin C in blood significantly ( $p < 0.05$ ) and therefore, depleted the serum LPO level at 1 h post irradiation. When Aloe gel was administered at the dose of 250 and 500 mg/kg body weight, the increase in GSH and vitamin C concentrations and depletion in LPO level were insignificantly ( $p > 0.05$ ) higher (Figs. 1–3). These findings are in good agreement with the results of Saada et al. (2003) who reported that oral administration of Aloe vera gel (leaf juice filtrate) before and after irradiation with 7 Gy significantly minimized the radiation induced increase in the amount of malondialdehyde in liver, lungs and kidney. A significant amelioration in activities of superoxide dismutase (SOD) and catalase was also noticed from day 3 to 10 in lungs, at day 7 and 10 in kidneys and at day 10 in liver. It has been also reported that Aloe treatment increased DNA, catalase and SOD activities in the skin and GSH in the liver and blood and decreased the LPO in liver and serum of mice significantly (Goyal and Gehlot, 2009).

Present results strongly support our earlier findings where administration of Aloe vera aqueous leaf extract to mice prior to irradiation with different sub lethal doses (0.5–5 Gy) of gamma radiation prevented the MDA formation and increased the contents of GSH significantly in liver at different (1/4–20 days) post irradiation intervals (Bhaya and Saini, 2008). Similarly, oral administration of ethanolic leaf extract of Aloe vera (1 g/kg body weight) for 15 consecutive days has also been reported an effective drug against 0.5, 2.5 and 5 Gy induced DNA damage (Shekhawat et al., 2007).

When Aloe gel was administered at the dose of 750 mg/kg body weight, it also resulted in a higher number of survival mice up to day 30 post irradiation as compared to rest of the two doses, i.e., 250 and 500 mg/kg body weight (Fig. 4). Therefore, this dose was considered as the optimum effective dose and has been used to study the protection provided by Aloe gel against radiation sickness and for the determination of dose reduction factor.

Though, the signs of radiation sickness were noticed in both *Aloe* untreated irradiated and *Aloe* treated irradiated animals but onset of these signs was early and severity was more in untreated irradiated animals than *Aloe* treated irradiated animals. In this study, a considerably lesser body weight loss was observed in *Aloe* pretreated irradiated animals in comparison to untreated irradiated animals, which is a good indicator of protection provided by *Aloe* gel against different doses of radiation (Fig. 5). Our findings are also in concordance with those of Pande et al. (1998) who reported that intraperitoneal administration of *Aloe vera* leaf extract at a dose of 50 mg/kg to mice before irradiation with 8 Gy improve body weight, survival time and reduce the incidence of abnormalities in cells of the testes significantly.

In the present investigation, irradiation of mice with 6 and 8 Gy resulted in 24.98 and 66.66% deaths, which occurred within initial 10 days, respectively, and rest of the animals died between 11 and 30 days post irradiation, whereas all mice irradiated with 10 and 12 Gy, died within initial 8 days post-irradiation period. On the contrary, treatment with *Aloe* gel before irradiation with 6, 8, 10 and 12 Gy reduced the mortality from 26.66 to 0, 80 to 6.6, 100 to 46.6 and 100 to 86.6%, respectively (Fig. 6). Similarly, Pande et al. (1998) also reported that intraperitoneal administration of *Aloe* extract at a dose of 50 mg/kg before irradiation with 8 Gy resulted in 90% survival of animals. Treatment with *Aloe* polysaccharides (AP) at a dose rate of 50 mg/kg body weight 30 min before irradiation with 7.5 Gy improved the 30 days survival rate of mice from 10 to 86% (Wang et al., 2004).

The present study indicate that gastrointestinal damage seems to be responsible for deaths, occurred within initial 10 days post irradiation, whereas deaths occurred between 11 and 30 days post irradiation were probably due to haemopoietic damage. Results also indicate that the pattern of mortality was similar in both *Aloe* gel treated irradiated and untreated irradiated mice but onset of mortality was certainly delayed and also decreased with *Aloe* gel treatment. Treatment with *Aloe* gel increased the radiation tolerance dose from 6.77 (LD<sub>50/30</sub> of untreated irradiated) to 10 Gy (LD<sub>50/30</sub> of *Aloe* treated irradiated) and the dose reduction factor (DRF) was calculated as 1.47.

The interaction of ionizing radiation with biological system results in the generation of reactive oxygen species (ROS), i.e., free radicals, mainly due to hydrolysis of water. The major free radicals resulting from aqueous radiolysis include H<sup>+</sup>, OH<sup>•</sup>, HO<sub>2</sub><sup>•</sup>, H<sub>3</sub>O<sup>+</sup> and so on (Harman, 1993; Gracy et al., 1999). These free radicals interact with critical biomolecules such as membrane lipids, DNA and proteins and bring about cell damage that leads to cell dysfunction and death. In the present study, *Aloe* gel scavenged the free radicals, DPPH<sup>•</sup>, ABTS<sup>•+</sup> and NO in a concentration dependent manner *in vitro* (Tables 1–3). This effect of *Aloe* gel can be correlated to scavenge the radiation induced free radicals. Thus, scavenging of free radicals seems to be an important mechanism of radiation protection of *Aloe* gel.

Secondly, *Aloe* gel is rich in polysaccharides, pro-vitamin A (β-carotene), vitamin C, E (Atherton, 1998), glutathione peroxidase (Klein and Penneys, 1998) as well as several isoenzymes of superoxide dismutase (Sabeh et al., 1993) and zinc (Shelton, 1991). Treatment with 50 μg/ml *Aloe* polysaccharides (AP) has

been reported to improve the surviving fraction of three normal cell lines 293, ECV 304 and Chang liver (C. liver) from 41.5%, 46.5% and 40.9% to 49.4%, 72.1% and 89.1%, respectively, and also reduced the apoptotic rate of C. liver cells from 9.5% and 43.0% to 2.2% and 10.9% 48 h and 78 h after irradiation with 2 Gy, respectively. Further, Western blot analysis showed that pretreatment with AP blocked the upregulation of pro-apoptosis p53, Bax and Bad and the down regulation of Bcl-2 by irradiation (Wang et al., 2004).

Pro-vitamin A (β-carotene) is an established and excellent scavenger of singlet oxygen (O<sub>2</sub><sup>•</sup>). The ability of β-carotene to act as an antioxidant is due to the stabilization of organic peroxide free radicals within its conjugated alkyl structure and may play a role in trapping peroxy free radical. Since β-carotene is effective at low oxygen concentration, it complements the antioxidative properties of vitamin E, which is effective at higher oxygen concentration.

According to Palozza and Krinsky (1992) β-carotene and vitamin E (α-tocopherol) cause an additive beneficial effect in inhibiting radical initiated lipid peroxidation. Vitamin E (α-tocopherol) functions as a chain breaking antioxidant (Burton et al., 1983) and has been implicated in the activity of catalase (Masugi and Nakamura, 1976), glutathione peroxidase (Chow et al., 1973) and possibly superoxide dismutase (Serfass and Ganther, 1976). Vitamin C acts as a reducing agent and plays an important role in regeneration of α-tocopherol from the α-tocopheroxyl radical, produced during scavenging of hydroperoxyl (LOO<sup>•</sup>) and alkoxy (LO<sup>•</sup>) radicals (Packer, 1977). Vitamin C has also been shown to regenerate urate, glutathione and β-carotene radical cations (Edge and Truscott, 1997).

Glutathione peroxidase (GP<sub>x</sub>) is a seleno-enzyme. It catalyses the reaction of hydroperoxides (LOOH and H<sub>2</sub>O<sub>2</sub>) with GSH to form glutathione disulphide (GSSG) and the reduction product of hydroperoxide (Chance et al., 1979), which is ultimately converted back to GSH. Enzymes like SOD (dimer with two copper and two zinc atoms Cu–Zn SOD), glutathione peroxidase and catalase provide primary defense against ROS in mammals, whereas pro-vitamin A (β-carotene), vitamin C (ascorbate), vitamin E (α-tocopherol) and reduced glutathione (GSH) provide a secondary defense against ROS in addition to primary defense. The exact mechanism of radiation protection of *Aloe* gel is not known. It is commonly suggested that there may be some synergistic reaction taking place between various components and polysaccharides, which is responsible for the action (Pande et al., 1998; Leung, 1977; Henry, 1979).

*Aloe vera* gel exhibits anti-inflammatory and anti-cancer properties due to presence of mannose-6-phosphate and glycoprotein and polysaccharides especially acemannan, respectively. Jo et al. (2004) stated that a plant with anti-inflammatory and chemopreventive properties is known to possess radioprotective properties. *Aloe* also exhibits antimicrobial activity and can stimulate radiorecovery via its antimicrobial effect as bacteremia is one of the important causes of death.

## 5. Conclusions

Present findings indicate that *Aloe* gel is an effective radioprotective agent. It can be very useful in increasing the tolerance

dose of radiation to cancer patients and in preventing the diarrhoea which occurs during radiotherapy of pelvic and abdominal cancer as gastrointestinal tract represents one of the major dose limiting organs in radiotherapy.

### Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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