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Aloe vera modulates X-ray induced bone mineral loss and other deleterious effects on various tissues of mice

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The present study was designed to examine the effects of *Aloe vera* on whole body X-ray exposure induced injury to heart, lung, and bone of male balb/c mice. Animals were divided into four groups: control, *Aloe vera* (50 mg/kg body weight on alternate days for 30 days), X-ray (2Gy) and *Aloe vera*+ X-ray. X-ray irradiation led to enhanced lipid peroxidation level associated with decline in reduced glutathione concentration in pulmonary tissue of mice. Moreover, lipid peroxidation level and reduced glutathione content in cardiac tissue remained unaltered after radiation exposure. In addition, X-ray exposure caused poor and delayed uptake of ^{99m}Tc-mebrofenin as observed in hepatobiliary clearance study. Dual energy X-ray absorptiometry scan revealed a significant decrease in bone mineral density after X-ray irradiation. *Aloe vera* administration to radiation exposed animals restored pulmonary reduced glutathione content and lipid peroxidation level along with significantly improved bone mineral density and hepatobiliary clearance profile as compared to irradiated counterparts. The current observations suggest that *Aloe vera* plays vital role in modulating deleterious effects caused by X-ray exposure in various organs of mice, which may attributed its free radical scavenging ability and strengthening of antioxidant defense system.

Keywords: ^{99m}Tc-mebrofenin, *Aloe vera*, DXA imaging, X-ray.

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Introduction

Ionizing radiation have varied applications in industry, diagnostics, therapeutics, and power generation[1]. Besides clinical exposure, several people come in contact with lethal or sub-lethal doses of ionizing radiation resulting from accidents or nuclear disasters. Ionizing radiation induces biological effects via series of molecular events, set off by excessive production of reactive oxygen species (ROS). This results in damage to macromolecules like nucleic acids, proteins and lipids, leading to associated diseases such as malignancies, bone microfractures and impaired bone mineralization[2,3].

Modulation of ROS produced from the interaction of ionizing radiation with biological system by certain compounds/drugs has been shown to ameliorate associated pathological damages[4]. Although synthetic radioprotectors like amifostine and cysteine were discovered at the beginning of the nuclear era, but due to their uncontrolled side effects in clinical trials, radioprotectors with minimal unwanted effects yet remain elusive. Therefore, an effective radio-modifier that provides protection against radiation exposure in normal cells and tissues is the need of the hour. Several medicinal plants have shown potential as radioprotectors because of their primary medical benefits, low toxicity, easy availability and cost effectiveness. Several studies are reported which suggest minimal detrimental effects of ionizing radiation upon administration of certain plant products[5].

Aloe barbadensis Miller (syn. *Aloe vera*) belonging to the family of Liliaceae has been extensively used for several medicinal purposes, due to the presence of various constituents like minerals, enzymes, amino acids, vitamins, carbohydrates, anthraquinones etc. It has been documented to possess antioxidant, anti-inflammatory, and nutraceutical properties[6]. The protective role of *A. vera* gel extract in reducing the degree of pulmonary tissue injury induced by cigarette smoking in murine model was reported from the authors' laboratory earlier[7]. Various studies including the authors have explicated the

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radioprotective effects of *A. vera* gel extract against X-ray irradiation induced haematological, hepatic, splenic, renal and testicular toxicity[2,8-10].

A previously reported study by the authors explained the preventive effect of *Aloe vera* against X-ray irradiation induced damage in high and moderately radiosensitive tissues[2,8,10]. However, the current study was carried out in important but less radiosensitive organs like bone, heart and lungs of mice, to understand the effect of X-ray induced damage and its possible amelioration by *A. vera* extract.

Materials and Methods

Aloe vera gel extract preparation

Aqueous *A. vera* gel extract [Herbarium PAN no. 21953] was prepared according to the method described previously[6,9]. Qualitative and quantitative phytochemical analysis, various tracer elements, and free radical scavenging activity of *A. vera* gel extract has been reported by us previously[2,6].

Whole body X-ray Irradiation

Whole body X-ray exposure was carried out using X-ray machine (Philips, Model no. 9890 000 86 101) at the Department of Radiodiagnosis and Imaging, PGIMER, Chandigarh (India). The animals were subjected to whole body X-ray irradiation at cumulative dose of 2Gy (0.258Gy twice a day for four days in the last week of the study). During exposure, animals were restrained in well ventilated perspex box with dose area product (DAP) of 776.57dGycm^2 at 90KV, 500mAs and source to surface distance was 49 cm. The dose of X-ray was standardized in the Department of Radiodiagnosis and Imaging which was further verified in the Department of Radiotherapy, PGIMER, (Chandigarh) as described in our previous reports[2,8,9].

Animal treatment and experimental design

Healthy male balb/c mice ranging 25-30 g were selected from inbred colony at the Central Animal House, Panjab University, Chandigarh. These animals were maintained under controlled conditions of temperature and light (i.e. 21±1 ^{o}C, 50-60% humidity and 12 h day/night cycle) during the experimental period. These animals were provided with standard pellet diet as obtained by Ashirwad Industries Ltd., Ropar, Panjab (India) and water *ad libitium*. All the experiments were performed in accordance with the recommendations found in the Indian National Science Academy guidelines. The maintenance and handling of animals were approved by (approval no. PU/45/99/CPSEA/IAEC/2017/77) Institutional Ethics Committee of Panjab University, Chandigarh. The animals were kept in polypropylene cages containing sterile rice husk throughout the experiment. For the present study, male balb/c mice were randomly assorted into four groups (n=10 each). Group I served as control animals and received no special treatment. Group II mice were orally administered with 50 mg/kg body weight of *A. vera* gel extract on alternate days for 30 days. Group III mice were irradiated with whole body X-ray exposure (twice a day) for four consecutive days in the last week of the experimental protocol. Group IV mice were administered with *A. vera* gel extract (as in Group II) followed by whole body X-ray exposure (as in Group III). Dosage of the *A. vera* gel extract 50 mg/kg body weight was standardized in the laboratory as mentioned in previously reported studies[2,6].

After 24 hours of the above mentioned different treatment groups, heart and lung tissues were excised out for estimation of biochemical alterations. Hepatobiliary clearance profile by using ^{99m}Tc-mebrofenin was done to analyse the functional status of heart and liver of mice. Dual Energy X-ray Absorptiometry (DXA) scan was also performed for estimation of bone mineral density in mice.

Reduced glutathione (GSH)

GSH concentration was estimated in heart and lung tissues by the method as described previously[11]. GSH content was determined by using 5,5′-dithiobis-2 nitrobenzoic acid (DTNB), which forms a yellow-coloured complex where absorbance was read at 412 nm. Reduced GSH was expressed as nanomoles of GSH content/mg protein.

Lipid peroxidation (LPO)

LPO level was determined in the heart and lung tissues by estimating the formation of thiobarbituric acid-reactive substances (TBA-RS), according to a previously described method[12]. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532 nm. The extinction coefficient of 1.56 x10^5 per molar per centimetre as used to calculate MDA-TBA chromophore and expressed as nanomoles of MDA-TBA chromophore formed/mg protein.

Physiological status of heart and liver

^{99m}Tc-mebrofenin hepatobiliary clearance profile

^{99m}Tc-mebrofenin hepatobiliary clearance was performed according to the method described previously[13]. ^{99m}Tc-sodium pertechnetate (185-200 MBq) labelled with mebrofenin was prepared in the Department of Nuclear Medicine, PGIMER (Chandigarh). About 100-200 μCi of ^{99m}Tc-mebrofenin/ animal was injected intravenously at different time intervals. The scintillation counter was calibrated at 10% window centred at 140 KeV, using ^{99m}Tc as a source. Pre syringe counts and post syringe counts were taken to calculate the radioactivity administered to the animal. These animals were positioned anteriorly by placing liver and mediastinum in the field of view. The perfusion phase, uptake phase and clearance phase were monitored at different time intervals (i.e. 0, 10, 20, 30, and 60 min) for estimating the functional status of the heart and liver tissues.

DXA scan (Dual Energy X-ray Absorptiometry)

Quantification of Bone mineral density (BMD) was estimated by using DXA scanner in the Department of Radiodiagnosis and Imaging, PGIMER, Chandigarh (Fig. 1). For DXA scan, anaesthetized mice were put on the centre of the table with respect to the centre lines at the head and foot of the pad. Using the arm motion controls on the control panel, the laser indicator was set in vertical line to approximately 2 inches below the iliac crest. The laser indicator horizontal line was coinciding with the midline of the subject and whole body scan was then performed. BMD was expressed as g/cm^2.

Statistical analysis

Data is expressed as mean±standard deviation (SD) and was used to determine significant differences between the means of two or more independent groups followed by LSD post-hoc test by using Statistical Package for the Social Sciences (SPSS) software.

Results

GSH

GSH content remain unaltered in X-ray irradiated group when compared to control group, but exhibited significant decline when compared to AV administered group ($P \leq 0.001$; $P \leq 0.01$) in cardiac tissue. AV+X-ray group exhibited unaltered GSH concentration when compared to the control, AV and X-ray irradiated groups. However, significant increase in GSH concentration was observed in AV group when compared to control group (Table 1).

A significant decline was observed in GSH content of X-ray irradiated group in lung tissue when compared to control ($P \leq 0.001$; $P \leq 0.01$) and AV groups ($P \leq 0.001$; $P \leq 0.01$). Moreover, AV+X-ray treated group showed significant rise in GSH content of lung when compared to X-ray irradiated group ($P \leq 0.001$; $P \leq 0.01$). However, no change in the GSH concentration was found between AV and control groups of lung (Table 1).

LPO

No change in the LPO level in heart tissue was observed in any of the treatment groups. X-ray exposed group showed significant increase in LPO level in lung tissue when compared to control ($P \leq 0.001$; $P \leq 0.01$) and AV administered groups

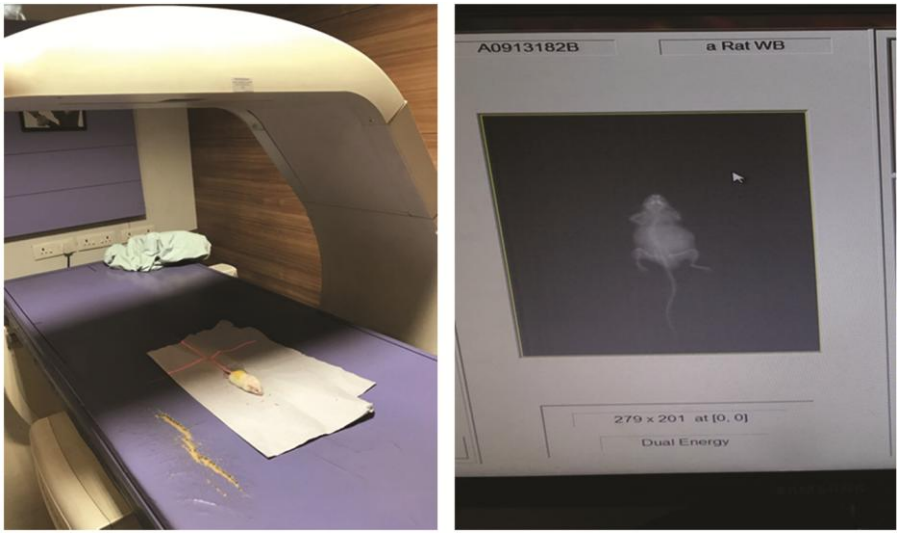

Fig. 1 — Experimental set up for DXA scan.

($P \leq 0.001$; $P \leq 0.01$). AV+X-ray treated group showed significant decrease in LPO level when compared to X-ray irradiated group ($P \leq 0.001$; $P \leq 0.01$). However, no change in the LPO level was found between AV and control groups (Table 1).

^{99m}Tc-mebrofenin hepatobiliary clearance assay

^{99m}Tc-mebrofenin was found to be perfused in heart after 20 to 30 seconds and t_{max}(time at which uptake is maximum) was observed at around 130 seconds in heart and 300 seconds in liver. ^{99m}Tc-mebrofenin perfusion and uptake was not affected in heart tissue of different treatment groups, but delayed clearance was observed in heart and liver of X-ray irradiated animals.

^{99m}Tc-mebrofenin hepatobiliary clearance profile for control and AV animals in heart and liver was found to be normal as shown in (Fig. 2a-d). ^{99m}Tc-

Table 1 — Effect of X-ray and/ or *Aloe vera* on GSH content and LPO level in heart and lung tissues

	Organs	Control	*Aloe vera*	X-ray	*Aloe vera* + X-ray
GSH	Heart	10.5±2.04	$14.8\pm2.07^{a}{}_{2}$	$10.5\pm1.421^{b}{}_{2}$	11.4±1.44
	Lung	10.9±1.22	10.6±1.14	$8.38\pm0.862^{a}{}_{3}{}^{b}{}_{3}$	$9.82\pm1.02^{c}{}_{2}$
LPO	Heart	8.63±1.69	8.47±0.555	9.62±1.09	8.23±0.971
	Lung	5.42±0.757	5.69±0.735	$10.34\pm1.09^{a}{}_{3}{}^{b}{}_{3}$	$5.42\pm0.808^{c}{}_{3}$

Data is represented as mean±SD (n= 8) is analysed by one way ANOVA followed by post- hoc test. $^{a}{}_{3}P \leq 0.001$, $^{a}{}_{2}P \leq 0.01$ significant with respect to control group, $^{b}{}_{3}P \leq 0.001$, $^{b}{}_{2}P \leq 0.01$ significant with respect to *Aloe vera* group, $^{c}{}_{3}P \leq 0.001$, $^{c}{}_{2}P \leq 0.01$ significant with respect to X-ray group

Units: GSH (nanomoles per mg protein), LPO (nanomoles of TBA-MDA chromophore formed per mg protein)

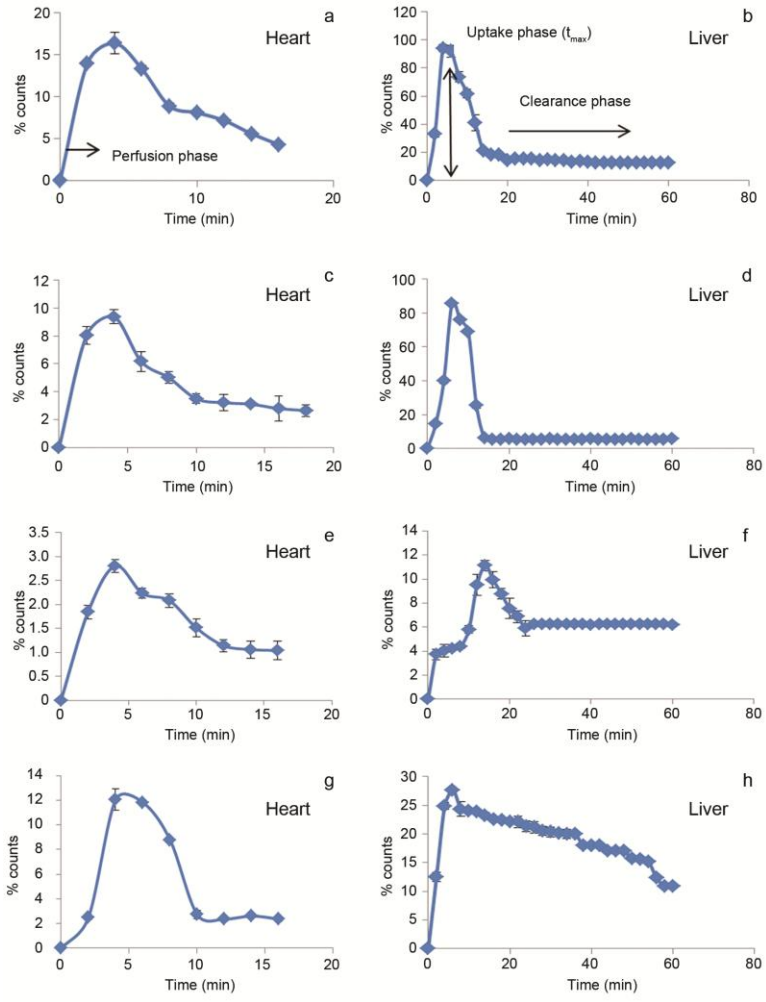

Fig. 2 — Heart and liver time activity curves derived for ^{99m}Tc-mebrofenin hepatobiliary clearance assay, a-b) Control, c-d) *Aloe vera*, e-f) X-ray, g-h) *Aloe vera* + X-ray. Data is represented as mean±SD (n= 6) and analysed by one way ANOVA followed by post- hoc test.

mebrofenin uptake and retention in liver was found to be poor and delayed in X-ray irradiated animals (Fig. 2e, f). However, liver uptake was found to be recovered in AV+X-ray group when compared to X-ray irradiated animals, indicating normal clearance of radioactive tracer as in control animals (Fig. 2g, h). ^{99m}Tc-mebrofenin clearance profile was found to be delayed in heart and liver tissues of X-ray irradiated and AV+X-ray groups in comparison to control animals.

The radioactivity uptake and clearance was found to be 95, 60-80, 15-30, 15, and 10% at different time intervals (t_{max}, 10, 20, 30 and 60 min) in control and AV groups. However, X-ray exposed group showed 15% t_{max}, 15% uptake at 10, 2, 30 min and 10% clearance of tracer at 60 min after administration of ^{99m}Tc-mebrofenin intravenously. AV+X-ray group animals revealed 30% t_{max}, 20-25%, 20% uptake at 10, 20, 30 minutes and 5% clearance of radioactive tracer at 60 minutes time interval (Fig. 3).

Assessment of bone mineral density

Bone mineral density was found to be declined in X-ray exposed animals when compared to control ($P \leq 0.001$; $P \leq 0.01$) and AV groups ($P \leq 0.001$; $P \leq 0.01$). AV+X-ray treated group showed significant elevation in bone mineral density when compared to X-ray irradiated group ($P \leq 0.01$). However, significant increase in bone mineral density was observed in AV group when compared to the control group (Fig. 4).

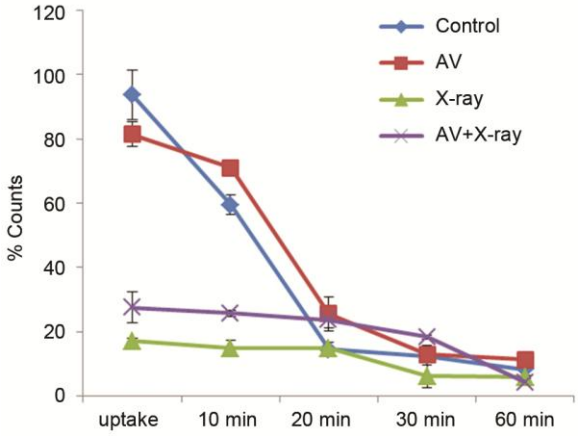

Fig. 3 — Effect of X-ray and/ or *Aloe vera* on ^{99m}Tc-mebrofenin hepatobiliaryretention. Data is represented as mean±SD (n= 6) is analysed by one way ANOVA followed by post- hoc test.

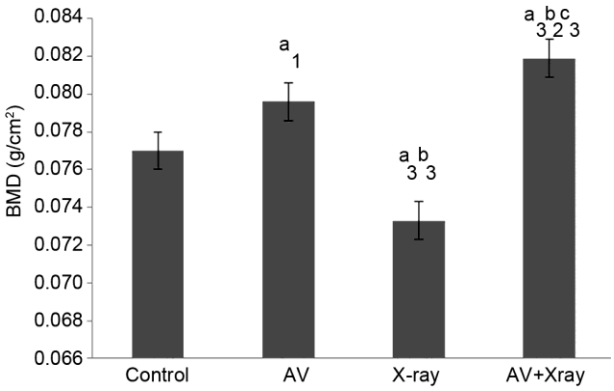

Fig. 4 — Effect of X-ray and/ or *Aloe vera* on BMD. Data is represented as mean±SD (n= 5) and analysed by one way ANOVA followed by post- hoc test. $^{a}{}_{3}P \leq 0.001$, $^{a}{}_{1}P \leq 0.05$ significant with respect to control group, $^{b}{}_{3}P \leq 0.001$, $^{b}{}_{2}P \leq 0.01$ significant with respect to *Aloe vera* group, $^{c}{}_{3}P \leq 0.001$ significant with respect to X-ray irradiated group. Unit: (g/cm^2).

Discussion

The deleterious effects of ionizing radiation on various tissues resulting through excessive production of ROS are well documented[14]. Also, there is a close correlation between increase in LPO level due to excessive ROS production and inhibition in antioxidant defense system[14]. Reduction in GSH concentration and rise in LPO has been suggested as one of the main causes of radiation induced membrane damage[2,9,15]. The present study revealed that whole body exposure of X-ray caused increased levels of LPO along with reduced GSH content in pulmonary tissue of mice. Enhanced production of lipid peroxides may cause increased glutathione consumption as reported previously[7,16]. Interestingly, LPO level and GSH content remained unaltered in heart of mice exposed to X-ray irradiation. This could be due to radioresistant nature of heart/ late responding organ as mentioned in the literature[17,18].

In the present investigation, GSH concentration and LPO levels significantly improved in lung tissue of AV+X-ray group when compared to X-ray irradiated group. The results are in agreement with the previously reported study in which GSH content and LPO level in pulmonary tissue were found to be improved by administration of *Panax ginseng* and *Myrtol*extract upon X-ray exposure to mice[19,20]. It seems that *Aloe vera* has ability to restore the levels of cellular thiols by virtue of its antioxidant property[21].

Iminodiacetic acid (IDA) derivative such as mebrofenin labelled with ^{99m}Tc, is routinely used for non-invasive evaluation of liverfunction[22]. ^{99m}Tc-mebrofenin has excellent hepatobiliary specificity and differed primarily in blood clearance and urinary excretion[23]. In the present study, ^{99m}Tc-mebrofenin

perfusion and uptake was not affected in heart tissue of different treatment groups. However, uptake, retention and clearance was found to be poor and delayed in liver of X-ray irradiated animals. Increased oxidative stress and altered histopathology of the liver in X-ray group, as reported previously from our laboratory, maybe the reason for obstructions in the hepatobiliary system[2] and consequent alterations observed in ^{99m}Tc mebrofenin uptake and clearance. However, liver uptake was found to be improved in AV+X-ray group when compared to X-ray irradiated animals. In our previous studies, hepatobiliary clearance of ^{99m}Tc-mebrofenin in heart and liver tissues were found to be delayed after DMBA and NDEA administration to mice[13,24]. To the best of the authors knowledge, no reports are available which indicate the status of cardiac and hepatic function by using ^{99m}Tc-mebrofenin after X-ray exposure to mice. Previously reported studies including the authors showed that X-ray exposure caused histopathological alterations in mice liver which was mitigated with *A. vera*[2,10]. The present data showed that certain constituents such as polysaccharides present in *A. vera* may improve the functional status of various organs[25].

Dual-energy X-ray absorptiometry (DXA) or (Bone density scanning) is an enhanced form of X-ray technology that is used to measure bone loss or bone mineral density. It is a sensitive and well established technique for early detection of osteoporosis and osteopenia[26]. Increased fracture risk is commonly reported in cancer patients receiving radiotherapy, particularly at sites within the field of treatment[27,28]. Healthy nearby tissue, including bone, is estimated to absorb up to one-half of the dose of 30 Gy[27]. Despite efforts to minimize dose-limiting side effects by protecting healthy tissues, the incidence of pathological fracture at sites in the direct path of therapeutic irradiation is reportedly increased relative to non-irradiated skeletal sites in cancer patients and survivors[28]. In the present study, BMD was found to be decreased in X-ray exposed animals. This might be due to changes in vasculature and damage to bone cells (osteoblast, osteoclast, and osteocytes) after irradiation that make bone more fragile which may lead to osteoporosis[29]. However, AV+X-ray exposed group showed improvement in BMD when compared to X-ray irradiated animals. Previously reported study showed similar results in which *Panax notoginseng* supressed the radiation induced osteoporosis by inducing bone formation and resorption[30]. These results suggest that *A. vera* may be capable of attenuating the bone turnover rate induced by radiation exposure.

Conclusion

The present investigations suggests that *A. vera* exerts prophylactic effect against X-ray induced biochemical perturbations in pulmonary tissue, alterations in bone mineral density and physiological status of cardiac and hepatic tissues of mice. These observations and those reported previously illustrate the possible mechanism by which *A. vera* gel extract offers protection against radiation exposure induced damage. This study may be helpful in devising protocols for managing occupational hazards to radiation workers and patients receiving radiotherapy.

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Conflict of interest

The authors declare that there is no conflict of interest.

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