ALOE VERA PROTECTS RADIATION INDUCED DNA DAMAGE IN MOUSE

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Summary

Present study is an attempt to investigate the radioprotective potential of ethanolic extract of Aloe vera against low dose gamma radiation induced injury in jejunal DNA of Swiss albino mice. For this study, the animals were divided into five (I-V) groups. Group I had normal animals (sham irradiated), while animals of group II were treated with Aloe for 15 consecutive days to observe Aloe toxicity, if any. Each group from III-V had two sets of animals. The animals of first set were given Aloe extract orally for 15 consecutive days at the dose of 1 g/kg b. wt. and served as experimental set. The second set of animal was given distilled water in similar manner (volume equal to that used for Aloe administration in first set) for similar period and called control set. On the last day of extract administration the animals of III, IV and V groups were exposed to 0.5, 2.5 and 5 Gy gamma radiation respectively and autopsied at day 1/4, 1, 3, 5, 10 and 20 to remove jejunum for DNA estimation.

The jejunal DNA was found to be minimum in all control and experimental sets of animals at day 1 but it was significantly higher (P<0.05) in all experimental sets as compared to that of their respective control sets. Later DNA contents increased continuously in all sets of control and experimental animals upto the last autopsy interval of this study but in irradiated alone animals decrease in DNA was more pronounced and recovery was slow and therefore, its levels remained for below to normal especially in 5 Gy irradiated animals at day 20. While increase in DNA contents was more and faster in Aloe treated 0.5, 2.5 and 5 Gy irradiated animals and it reached at normal level by day 5 and 20 in Aloe treated 0.5, 2.5 Gy irradiated animals respectively but in Aloe treated 5 Gy irradiated animals DNA remained 8.61 per cent lesser as compared to normal level even at day 20. These results provided an evidence that Aloe vera extract protected to DNA against radiation induced damage because of its high antioxidative properties.

Keywords: Aloe vera, radiation, DNA, Intestine

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**Introduction**

Planned radiation exposure during radiotherapy and radiodiagnosis as well as unplanned exposures (occupational, accidental and natural background exposure) are increasing the risk for man and will continue to increase as the whole world is in the race of nuclear weapons testing. Exposure of a biological system to ionizing radiation results in a rapid burst of reactive oxygen species (ROS) such as $O_2^\cdot$, $H^\cdot$, $HO_2^\cdot$, $OH^\cdot$ and $H_2O_2$ (Harman, 1993; Gracy et al., 1999), which are highly damaging for cellular macromolecules like DNA, proteins, lipids and to some extent for carbohydrates. Therefore, to develop effective and nontoxic radioprotective and radiotherapeutic drugs, many sulphhydryl compounds such as cysteine (Patt et al., 1949), cysteamine (Bacq et al., 1951), cystamine (Bacq, 1953), AET (Maisin and Dhortey 1963), MPG (Sugahara et al., 1970) and WR-2721 (Withers and Elkind, 1970) were synthesized and tested but none of them was found to be suitable for clinical applications due to their high toxicity. Hence, efforts have been made to develop effective, nontoxic, inexpensive and easily available radioprotective drugs of plants origin for human welfare and several plants extracts such as *Piper betel* (Batacharya et al., 2007) and *Amaranthus paniculatus* (Saini and Maharwal, 2007), herbal preparations like Liv. 52 (Saini et al., 1985), Rasayanas (Kumar et al., 1999) and Abana (Baliga et al., 2004) and natural antioxidants like vitamin A (Srivastava, 2002) C (Knopacka et al., 1998) and E (Nair et al., 2000) have been tested and are also being tested.

*Aloe barbadensis* (Mill.) is commonly called *Aloe vera*. It has been widely used in the treatment of various pathologies such as arthritis (Hanley et al., 1982), wound healing (Roboson et al., 1982), radiation burns (Chithra et al., 1998) and dermatitis (Fisher et al., 2000). The fresh *Aloe* gel (juice) and formulated products have long been also used for medical and cosmetic purposes and general health (Chithra et al., 1998; Reynolds and Dweck, 1999). Recently, it has also been reported to elicit significant protection against radiation and arsenic induced oxidative stress in mice (Maharwal, 2002) and rat (Gupta, 2005) respectively.

*Aloe vera* is rich in vitamin-A ($\beta$-carotene), E and C (Atherton, 1998) and zinc (Shelton, 1991), selenium and enzymes such as glutathione peroxidase (Sabeh et al., 1993) and isoenzymes of superoxide dismutase (Klien and Penneys, 1998). Therefore, this study was carried out to assess the radioprotective potential of *Aloe vera* against radiation induced DNA damage.
Materials And Methods

Source of animals:

Male Swiss albino mice of 6-8 weeks old weighing 25±2g were selected from an inbred colony. The selected animals were maintained at suitable temperature and light during the whole experimental period. The animals were provided standard mice feed (procured from Ashirwad Industries, Chandigarh, India) and water ad libitum. Tetracycline water was also given once a fortnight as a precaution against infection.

Source of radiation

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 were restrained in well-ventilated perspex boxes and whole-body exposed to different doses of gamma radiation at a distance (SSD) of 77.5 cm from the source to deliver the dose rate of 1.42 Gy/min.

Aloe vera leaf extract

Extract of Aloe leaf was prepared in ethyl alcohol. For this purpose, fresh leaf powder of Aloe was mixed with double the volume of alcohol. The mixture was stirred and left for 24 hours, after which it was filtered through a cheese cloth. The left over residue after filtration was again mixed with same volume of ethyl alcohol as used earlier and the procedure was repeated two more times. Finally all three filtrates were mixed and the alcohol was allowed to evaporate naturally from it at room temp. (30±3 °C) to obtain a concentrated Aloe extract, which was put in the oven at 40°C for complete evaporation of alcohol. The powdered extract was redissolved in DDW for oral administration to experimental animals.

Experimental design:

The animals were divided into five groups as per the mode of treatment.

Group I: This group included sham irradiated (normal) animals.

Group II: Animals of this group were given ethanolic Aloe vera leaf extract at the dose of 1 g/kg body weight to observe its toxic effects, if any.

Group III, IV, V: Each group from III-V was divided into two sets, one was experimental and another was control. In experimental set Aloe vera extract was administered orally at the dose of 1g/kg body weight for 15 consecutive days and control set received distilled water (volume equal...
to that used for administration of Aloe in experimental set) in similar manner for similar period. On the last day of Aloe vera administration, animals of III, IV and V groups were exposed to 0.5, 2.5 and 5 Gy gamma radiation respectively and autopsied at 1/4, 1 3, 5, 10 and 20 days to remove jejunum for DNA estimation.

**DNA Estimation**

The DNA was estimated by the method of Burton (1963).

**Statistical analysis**

The results were expressed as mean±SE. Student’s-test was performed for making statistical comparison between results obtained from (1) Aloe treated and sham irradiated (normal) animals (2) irradiated alone (control) and normal animals and (3) Aloe treated irradiated (experimental) and control animals. Significant level was set at <0.05.

**Results**

Aloe alone treated animals (group II) for consecutive days did not show signs of Aloe toxicity at the given dose level (1g/ kg/ b.wt./ day) during the whole study period whereas, but this treatment caused a slight increase in DNA contents as compared to sham irradiated (normal) animals (fig.1). The intestinal DNA declined from 6 hours and attained a minimum level (81.94, 72.57 and 45.56%) at day 1 in animals irradiated with 0.5, 2.5 and 5 Gy respectively. Thereafter a recovery was seen from day 3 and DNA contents were found to be 91.32, 82.83 and 63.98 per cent at day 10 and 91.49, 90.78 and 81.38 per cent at day 20 after exposure to 0.5, 2.5 and 5 Gy respectively. Thus, increase in DNA was found to be dose dependent and remained lower than normal value (100%) even at last autopsy interval of this study. DNA contents also declined from 6 hrs and found to be 91.46, 90.50, 70.32 per cent at day 1 in those animals treated with Aloe before exposure to 0.5, 2.5 and 5 Gy gamma radiation respectively. This decrease in DNA was lesser than that of irradiated alone animals. Secondly, in Aloe pretreated 0.5 and 2.5 Gy irradiated animals DNA damage was less and recovery was fast as evidenced by attending normal level at day 5 and 20 respectively but in Aloe treated 5 Gy irradiated animals DNA value did not approach normalcy and remained 91.39 per cent of normal even at last day of this study (Fig. 1-2).
Fig. 1: DNA contents in jejunum of Swiss albino mice exposed to 0.5, 2.5 and 5 Gy with and without pretreatment of *Aloe*

![Graph showing DNA contents in jejunum of Swiss albino mice](image1)

Fig. 2: DNA contents in jejunum of Swiss albino mice exposed to 0.5, 2.5 and 5 Gy with and without pretreatment of *Aloe*

![Graph showing DNA contents in jejunum of Swiss albino mice](image2)
Discussion

DNA is first vital target molecule for radiation injury in biological systems. Therefore, efforts have been made in the present study to see whether Aloe treatment prior to irradiation provides protection to DNA or not. DNA contents decreased and found to be minimum at day 1 in the animals, exposed to different doses (0.5, 2.5 and 5 Gy) of gamma radiation, indicated the damage and/or inhibition of its synthesis. On day 3, DNA showed slight increase, that can be correlated with the first sign of its synthesis. Later a continuous increase was seen in DNA at all subsequent intervals but remained lesser than normal level even upto last autopsy interval (Fig.1-2). Both decrease as well as increase in DNA contents was found to be dose dependent. These results are in good agreement with those of Quastler (1959), Sherman and Quastler (1960) who also reported a post-irradiation reduction in labelled thymidine incorporation in the intestinal DNA. Similarly Wrigglesworth and Pover (1967) stated that a decrease in DNA after irradiation in the intestine represented a decrease in cell number or nuclear contents of the cells. Lesher and Bauman (1968) and Lesher and Lesher (1974) suggested that a reduction in the rate of DNA synthesis was due to a reduction in the movement of cells from G₁ to S-phase, which was blocked by the high doses of radiation. They also stated that reduction increased in magnitude as the dose was increased. According to Prasad (1974), radiation induced depression in DNA synthesis is due to decrease in the DNA polymerase activity and alteration in the pool size of DNA precursors either by a change in membrane permeability or by an alteration of other biochemical factors necessary for the synthesis of nucleosides and nucleotides. Kwok and Chapman (1977) explained that diminution in DNA synthesis was due to impairment of carrier transport system of precursors incorporated into DNA.

Although, ROS (O₂・, H⁺, HO₂・, OH’ and H₂O₂) are being produced continuously during normal respiration as well as through other biological pathways in biological systems but exposure to ionizing radiations raises the generation of ROS in these biological systems and therefore are causative factor for such molecular damage. ROS can alter DNA at molecular level. The attack of ROS results in degradation of bases and sugars, breakage of hydrogen and sugar phosphate bonds and crosslinkage, all of which are deleterious to structural and functional integrity of a DNA molecule.
Superoxide radical ($O_2^{•−}$) results in an increase in chromosome breaks, rearrangement and sister chromatid exchanges (SCEs) (Emerit et al., 1982). OH• is known to induce conformational changes in DNA including strand breaks, base modifications, damage to tumor suppresser gene and enhanced expression of proto-oncogene (Helliwell and Aruoma, 1991; Cerrutti et al. 1994). Hydroxyl radical (OH•) interacts with DNA and causes many types of oxidized nucleosides. 8-hydroxy-2’-deoxyguanosine (8-OHdG) is one of the most common occurring products of these DNA modifications.

H$_2$O$_2$ is known to cause DNA breaks in intake cells and purified DNA (Tsuda, 1981). 1-mMol H$_2$O$_2$ produced significant toxicity and caused DNA damage in the form of SSBs and DSBs (Thibodeau and Paquette, 1999), chromosomal aberrations (Sofni and Ishidate, 1984) and SCEs (MacRac and Stich, 1979). A cell depends on its DNA for coding information to synthesize various types of proteins that includes enzymes, certain hormones, transport proteins and structural proteins, which support life. When the genetic information containing the "blueprint" for these substances is disturbed, cell homeostasis is disrupted resulting in a wide range of alterations that can lead mutations in genetic coding and lastly to cancer.

In the present study, DNA contents were found to be higher in Aloe treated irradiated animals at each autopsy interval as compared to irradiated alone indicating prevention of DNA molecules from radiation induced damage and its enhanced synthesis (Fig). Aloe vera has high contents of vitamin-A (β-carotene), E (Atherton, 1998), C (Shaman et al., 1998), minerals like zinc (Shelton, 1991) and selenium and glutathione peroxidase (Sabeh et al., 1993) and isoenzymes of superoxide dismutase (Klien and Penneys, 1998 ), which seem to be responsible factors for providing the protection to DNA molecules against radiation induced damage. Supplementation of vitamin A (β-carotene), C, and E for 20 weeks significantly decreased endogenous oxidative DNA damage in human lymphocytes. London et al. (1992) also suggested a decrease risk of breast cancer with increase vitamin E intake from food sources.

Antonio et al. (2005) stated that carotenoids are 40 carbon molecules with conjugated double bonds, making them particularly effective for quenching free radicals. Vitamin E is thought to be important in protecting the free radical-induced oxidative DNA damage and genetic mutations. It is an important chain-breaking antioxidant, which plays an important role in prevention of various stages of carcinogenesis through its contribution to immunocompetence, membrane and
DNA repair. *In vitro* studies showed that vitamin E can prevent oxidation of DNA by inhibiting activated neutrophils. Vitamin C may also protect a cell through inhibition of DNA oxidation. DNA oxidation, as determined by 8-OHdG in cells, is increased in case of oxidative stress and is correlated with reduced plasma concentration of vitamin C and E. Antioxidant enzymes such as SOD, GPx and CAT can directly counter the oxidant attack and may protect cells against DNA damage.

Sharygin *et al.* (2005) reported that radioprotectants and antioxidants significantly increase ribonucleotide reductase activity, which increase intracellular concentrations of the four deoxyribonucleotides (dNTPs) in animal tissues exposed to ionizing radiation. In irradiated alone animals ribonucleotide reductase activity was inhibited by 40-45% during initial three days period as compared to normal (control) animals. However, treatment of a radioprotectant to irradiated animals provided a high pool of dNTPs for high performance repair of DNA damage. The radioprotectant-induced activation of dNTP synthesis during the development of compensatory and restorative responses provides an early restoration of cellular composition and functioning of organs. Activation of protein synthesis in organs by radioprotectants may increase the pool of $\text{Fe}^{3+}$ transferrin and $\text{Cu}^{2+}$ ceruloplasmin in blood and activate dNTP for DNA synthesis. Activated synthesis of dNTP, DNA and proteins in organs and increase of plasma proteins underlay the formation of resistance to DNA damaging factors. Aloe treatment before irradiation might have prevented the ribonucleotide reductase activity and therefore has increased the synthesis of dNTPs, which has resulted in enhance synthesis of DNA.

Shetty *et al.* (2005) reported that *Hemidesmus indicus* root extract offered protection to DNA *in vitro* against gamma radiation induced strand breaks, which was observed as reduction in quantity of supercoiled form (ccc) of plasmid DNA. This form of plasmid DNA reduced by 40 per cent on exposure to 500 Gy gamma radiation. Presence of H. indicus (20 ug/ml) with DNA during irradiation resulted in almost complete protection of DNA as 90 per cent of ccc form of plasmid DNA was found to be unaffected.

Similarly *Aloe* treatment to mice before irradiation with different (0.5, 2.5 and 5.0 Gy) doses of gamma radiation prevented DNA from radiation induced damage and initiated its early synthesis, which was evidenced by the higher contents of DNA contents at all autopsy intervals in *Aloe* treated irradiated animals as compared to nontreated irradiated animals. It can be concluded that
Aloe vera provided the protection to DNA molecule against radiation induced injury, which may help to human population in prevention of cancer.

Reference:


32. Lesher S Bauman J 1968 Recovery of reproductive activity and the maintenance of structural integrity in the intestinal epithelium of the mouse after single dose whole-body co 60 gamma rays exposures. In “Effects of radiation on cellular proliferation and differentiation” 507, IAEA Vienna.


36 Emerit I Keck M levy A Feingold J Michelson A 1982 Activated oxygen species at the origin of chromosome breakage and sister-chromatid exchanges Mutat Res. 103: 165-172.


42. MacRac W D Stich H F 1979 Induction of sister-chromatid exchange in Chinese hamster ovary cells by thiol and hydrazine compounds. Mutation Res. 98: 351.


