PREVENTION OF RADIATION-INDUCED HEPATIC DAMAGE IN *SWISS ALBINO* MICE BY *ALOE VERA* LEAF EXTRACT

by

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The radioprotective effect of the *Aloe vera* leaf extract was studied in *Swiss albino* mice against radiation-induced changes in the liver. The mice were treated with 1000 mg/kg of body weight orally, once a day for 15 consecutive days, before exposure to a single dose of gamma radiation (6 Gy), half an hour after the last administration. The irradiation of mice caused a significant elevation in lipid peroxidation followed by a decrease in glutathione, acid phosphatase and alkaline phosphatase. The treatment of mice before irradiation elevated the glutathione, acid phosphatase and alkaline phosphatase, and was accompanied by a decline in lipid peroxidation. Recovery and regeneration from radiation damage were faster in pretreated animals than the animals in the irradiation-only group. The data clearly indicate that the *Aloe vera* leaf extract significantly reduced the deleterious effects of radiation on the liver and it could be a useful agent in reducing the side effects of therapeutic radiation.

Key words: Aloe vera, liver, gamma radiation, Swiss albino mouse

INTRODUCTION

Gamma radiation is the most commonly used source of ionizing radiation for the treatment of neoplastic disorders in clinical conditions. The clinical success of radiotherapy depends on its ability to selectively kill tumor cells while sparing the surrounding normal tissue. The response of mammalian cells to ionizing radiations at the cellular and molecular levels is a complex and irreversible process dependent on both the radiation dose and the tissue-weighting factor [1].

As is well established, radiation or pro-oxidants interact with cells and tissues through secondary ionization such as peroxidation. It is also known that peroxidation can be inhibited by antioxidants. Recently, the interest for the development of potential drugs of plant origin which could quench the reactive energy of free radicals and eliminate oxygen, one of the major participants in lipid peroxidation, and at the same time modify radiation responses (radio protectors/sensitizers) with minimum side effects is on the increase.

Due to lack of an effective protective agent, newer compounds are currently under investigation as possibly adjuvant in the radiation treatment of cancer, while herbal medicines have only recently begun to receive some attention as possible modifiers of the radiation response [2].

Studies carried out in the past 15 years have shown that herbal preparations such as Liv. 52 [3], Brahmarasayana [4], Pododphyllum [5], *Ocimum sanctum* [6], Triphala [7], *Emblica officinalis* [8], *Rosemarinus officinalis* [9] reduced radiation-induced damage in various mammal body systems.

One such well-known and widely used plant is the *Aloe vera barbadensis* belonging to the family of Liliacae and consisting of more than 250 species [10, 11]. It is commonly called "Guar-patha" or, ghee-Guar. It is rich in vitamins A, E, C, zinc, selenium, and polysaccharides. It is reported to have antioxidant, anti-tumor and anti-inflammatory properties [12, 13]. Our investigation assesses the radio-protective efficacy of the *Aloe vera* leaf extract in the hepatic constituents of *Swiss albino* mice.

MATERIALS AND METHODS

Test organism

Animal care and handling were performed according to the guidelines set by the WHO (World Health Organization), Geneva (Switzerland) and the

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INSA (Indian National Science Academy), New Delhi (India). Male Swiss albino mice (*Mus musculus*), 6-8 weeks old and weighing 20-24 g, from an inbred colony, were used for the present study. These animals were maintained under controlled conditions of temperature and light (light-dark: 10 h-14 h). They were provided with standard mouse feed (procured from Ashirwad Industries Chandigarh, India) and water *ad libitum*. Once a fortnight, as a preventive measure against infections, tetracycline water was given to them. The Departmental Animal Ethical Committee has approved the study presented here.

Irradiation

The Cobalt teletherapy unit (ATC-C9) at the Cancer Treatment Centre, Radiotherapy Department, SMS Medical College & Hospital, Jaipur, was used for irradiation. Unanesthetized animals were restrained in well-ventilated Perspex boxes and exposed to gamma radiation at the distance (SSD) of 77.5 cm from the source so as to deliver the dose-rate of 0.99 Gy per minute.

Plant material

Aloe leaves were collected locally over a one year period. The *Aloe vera* plant was identified by the curator at the Herbarium of Botany, University of Rajasthan, Jaipur, India (RUBL Number: 19886).

AV extract

To prepare the aqueous extract, fresh shade-dried leaves of *Aloe vera* were powdered and refluxed with double distilled water (DDW) for 36 hours (3 12 hours) at 40 °C and vacuum evaporated so as to produce the powder form. The extract was then dissolved in DDW, immediately before oral administration.

EXPERIMENTAL DESIGN

Determination of AVE tolerance

For this experiment, mice were divided into seven groups of 10 animals each and orally given 100, 200, 400, 800, 1000, 1500, and 2000 mg/animal/day of the *Aloe vera* leaf extract (AVE) in DDW, for fifteen consecutive days. The animals were then continuously observed for any signs of sickness, morbidity, body weight change, mortality or behavioural toxicity up to 30 days after the last treatment.

Selection of the optimal AVE dose against irridation

The mice were divided into seven groups of ten animals each and administered with 100, 200, 400, 800, 1000, 1500, and 2000 mg/animal/day of AVE for fifteen consecutive days, once a day. Thirty minutes after the last administration, they were exposed, whole body, to 8 Gy gamma radiation and for the next 30 days monitored for any signs of radiation sickness, weight change, mortality or behavioural changes.

Modification of radiation response

Mice were randomly divided into four groups.

Group I (normal/Sham-irradiated): Five mice were given a volume of DDW equal to AVE through oral gavage, once a day for 15 consecutive days.

Group II (drug alone): Five mice were treated with 1000 mg/kg of body weight per day of AVE dissolved in DDW through oral gavage for 15 consecutive days.

Group III (irradiated control): Thirty five mice were given distilled water for 15 days and then exposed to a 6 Gy dose of gamma radiation.

Group IV (experimental): Extract of *Aloe vera* was given orally to thirty five mice, at the dose of 1000 mg/kg of body weight per day, for 15 days. After 30 minutes of the last administration, these animals were exposed to 6 Gy dose of gamma radiation.

Following various treatments, five mice from groups III and IV were necropsied by cervical dislocation on 12 and 24 hours, as well as 3, 5, 10, 20, and 30 days. A homogenate of the liver was prepared and the activity of acid and alkaline phosphatase measured by using commercially available kits (procured from Span Diagnostics Ltd, India). A spectrophotometer (Systronix UV-VIS-108) was used to measure optical densities.

Glutathione (GSH) assay

The GSH level in liver and blood was measured using the method described by Moron *et al*. [14] and Beutler *et al*. [15], respectively. Using a Systronix UV-VIS-108 Spectrophotometer, the absorbance was read at 412 nm.

Lipid peroxidation (LPx) assay

LPx levels in liver and blood were estimated by the method of Ohkawa *et al*. [16] as thiobarbituric acid (TBA) reactive substances. The absorbance was read at 532 nm with a Systronix UV-VIS-108 Spectrophotometer.

Statistical analysis

The statistical significance of the difference between normal and DDW + irradiated control, as well as irradiated control and experimental, was evaluated by using the Student's t-test.

RESULTS

Radioprotective effect of AVE: Animals of the 6 Gy irradiated group resulted in radiation sickness within 10 days after exposure. The symptoms induced reduction in food and water intake, diarrhea, lethargy, emaciation, epilation, and ruffling of hairs. Daily administration of the AVE for 15 consecutive days did not cause any radiation-induced mortality. AVE administration delayed the appearance of radiation sickness symptoms such as diarrhea, irritability, lethargy, and the reduction in food and water intake.

Acid phosphatase (ACP): A significant increase in acid phosphatase activity over normal was measured up to day 5th after 6 Gy irradiation. On day 5th, the value was found to be 76.26 % higher as compared to normal. After this, the level of the enzyme declined, but remained higher than normal even on the last day of experimentation (fig. 1).

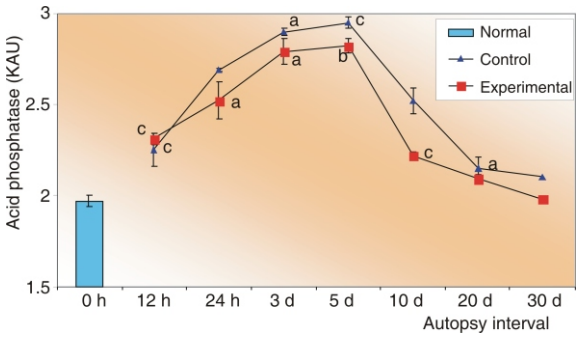

Figure 1. Acid phosphatase level in liver after 6 Gy of gamma-irradiation with (experimental) or without (control) AVE
Control = DDW + irradiation; experimental = AVE + irradiation; statistical comparison: control vs. normal; experimental vs. control; significance levels: (a) p ≤ 0.05, (b) p ≤ 0.01, (c) = p ≤ 0.001

Alkaline phosphatase (ALP): Alkaline phosphatase values elevated sharply from the very first up to day 5th, when the value was twice higher than the normal. After the 5th day, a declining trend was observed, but the normal value could not be restored even on the 30th day (fig. 2).

Glutathione (GSH): No significant variations in hepatic and blood GSH contents were observed in the normal (Group I) and AVE treated animals (Group II). However, a statistically significant decrease in GSH content in both liver and blood was evident in irradiated control animals (Group III). On the contrary, a significant increase in GSH values was measured in AVE pretreated experimental animals, as compared to the irradiated control group (fig. 3).

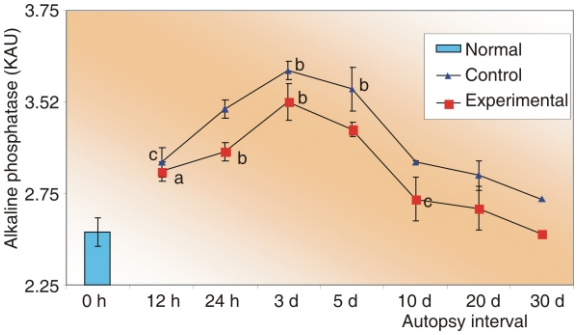

Figure 2. Alkaline phosphatase level in liver after 6 Gy of gamma-irradiation with (experimental) or without (control) AVE
Control = DDW + irradiation; experimental = AVE + irradiation; statistical comparison: control vs. normal; experimental vs. control; significance levels: (a) p ≤ 0.05, (b) p ≤ 0.01, (c) p ≤ 0.001

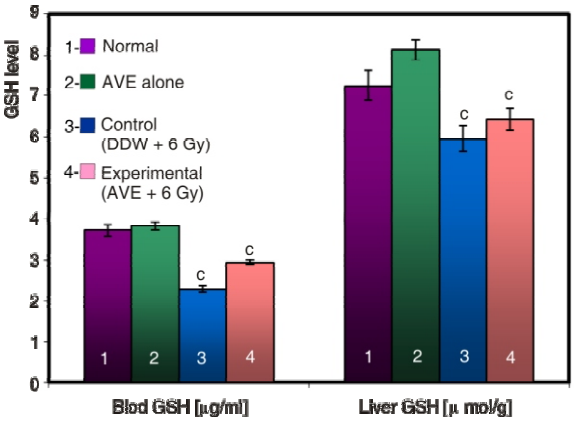

Figure 3. Glutathione level in blood and liver after exposure to 6 Gy of gamma radiation with (experimental) or without (control) AVE
Control = DDW + irradiation; experimental = AVE + irradiation; statistical comparison: control vs. normal; experimental vs. control; significance levels: (a) p ≤ 0.05, (b) p ≤ 0.01, (c) p ≤ 0.001

Lipid peroxidation (LPx): When compared with the DDW treatment, the administration of AVE did not alter the LPx level. A significantly increased level of LPx was measured in both serum and liver of mice after gamma irradiation (Group III). On the other hand, AVE pretreatment significantly reduced LPx induction in the experimental animals (Group IV) (fig. 4).

DISCUSSION

In the present study, after exposure to 6 Gy gamma radiation, animals exhibited radiation sickness within 3-5 days after exposure. The symptoms included reduction in food and water intake, weight loss, diarrhea, ruffling of hairs and irritability. Others have

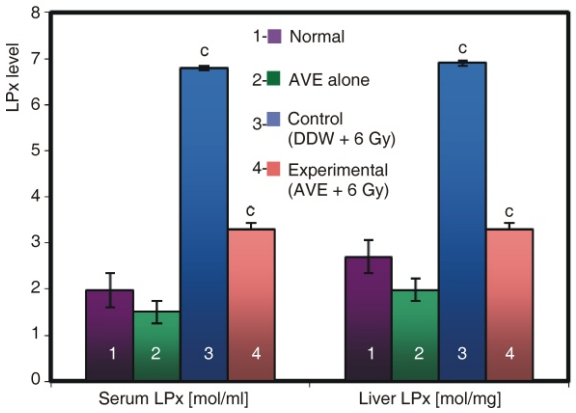

Figure 4. Variation in the lipid peroxidation (LPx) level after exposure to 6 Gy gamma radiation with (experimental) or without (control) AVE
Control = DDW + irradiation; experimental = AVE + irradiation; statistical comparison: control vs. normal; experimental vs. control; significance levels: (a) p = 0.05, (b) p ≤ 0.01, (c) p ≤0.001

also observed similar symptoms in mice after gamma irradiation [17, 18].

The liver is the primary organ of drug metabolism. It plays a key role of the detoxification agent in the body. Any damage to this organ may cause serious diseases observable in the form of histopathological and biochemical lesions.

An increase in acid phosphatase activity in the liver of mice due to irradiation was observed in the study. A similar increase in such enzymes was observed earlier, after the exposure of mice or rats to various doses of radiation [19]. Aikman *et al*. [20] suggested that the lesions are produced in the membrane lipids due to irradiation, possibly by peroxides, which lead to the activation of latent acid hydrolases which could result in the digestion of the membrane itself, with the consequent activation and release of other lysosomal enzymes; the same may be responsible for an increase in ACP levels after irradiation in the present study.

It has been observed that, after AVE administration, a radiation-induced increase in ACP was significantly lower. Hamdy *et al*. [21] observed the protective properties of acid phosphatase in cases in which solutions of lysosomal enzymes were exposed to high doses of radiation containing cysteine and 4-aminoaphthal. Nam [22], too, observed significant protective properties against the rise of serum acid phosphatase at 6 and 130 hours, if methylene blue was injected to rats prior to irradiation with 360 R. Soyal *et al*. [23] also found an increase in ACP and ALP activities in the liver of mice after exposure to gamma radiation.

A rise in ALP activity in the liver after gamma exposure, as evident in our experiment, corresponds quite closely to the early report of Khan *et al*. [24]. ACP and ALP are the enzymes linked to the biosynthesis of fibrous proteins [25] and mucopolysaccharides [26] which also act as hydrolytic enzymes with an important role in the body in the dissolution of dead cells [27]. Radiation-induced cell death may be the reason for the increased activity of ACP and ALP. Post-irradiation damage to the liver is another factor contributing to the increased levels of these enzymes. As observed in the present study, radiation-induced stress should also be taken into account when the increased activity of the said enzymes is concerned. It has also been observed that in experimental animals, compared to respective control groups, at various stages of the study, AVE provided protection by significantly decreasing the radiation-induced increase in ACP and ALP. A similar increase of said enzymes was observed in mice livers after exposure to gamma rays earlier [28]. Samarth *et al*. [29] reported that the activity of ACP increased, but that the ALP decreased in the blood of mice after gamma-irradiation.

In the present study, increased levels of the enzyme were found to be restored in the liver of experimental animals following the administration of various plant extracts believed to have protective properties against irradiation [23, 28]. This is in good agreement with the studies on the aqueous extract of *Aloe barbadensis,* found to be a significant factor in restoring the integrity of hepatocytes, indicated by an improvement in physiological parameters such as the excretory capacity of hepatocytes, as well as by the stimulation of bile flow secretion [30].

Ionizing radiation induces lipid peroxidation (LPx) which can lead to DNA damage and cell death [31, 32]. Therefore, an agent that acts as a protective shield against such damage should provide protection against damages resulting from exposure to radiation. In comparison to the control group, the administration of AVE before irradiation significantly reduced the amount of LPx. The inhibition of LPx through the administration of AVE may also be responsible for the observed level of radioprotection. Earlier reports on the same model of animal experimental groups also proved that *Aloe vera* is a good radioprotector against mouse intestinal mucosa, if low level (0.5 Gy) exposure to gamma radiation is applied [33].

The present study demonstrates a reduction in hepatic and blood GSH following radiation exposure. This may be due to the enhanced utilization of the anti-oxidant system as an attempt to detoxify the free radicals generated by irradiation. The low-end of the depletion of GSH in AVE treated irradiated animals could be due to the high availability of GSH which increases the ability to cope with the free radicals produced by irradiation. The increased GSH level suggests that protection by *Aloe vera* may be mediated through the modulation of cellular antioxidant levels. The increase in the glutathione (GSH) level in the experimental group may be due to the scavenging of radiation-induced free radicals by antioxidants present in AVE.

It has been reported that LPx starts to increase as soon as the endogenous GSH is exhausted, and the addition of GSH promptly stops further peroxidation [34]. Different fractions of *Aloe vera*, as well as the fractionated whole gel, have anti-oxidant effects. Glutathione peroxidase activity, superoxide dismutase enzymes and a phenolic anti-oxidant were found to be present in the AVE extract which may be responsible for these anti-oxidant effects [35].

Aloe vera is rich in vitamins A, E, and C. Ascorbic acid is a good scavenger of free radicals and it protects cellular membranes, thereby preventing degenerative processes. Vitamin C undergoes a synergistic interaction with tocopheroxyl radicals in the regeneration of α-tocopherol. Vitamin E protects cell membranes from oxidative damage caused by irradiation. The free radical clearing capacity of vitamin E is due to the localization of an unpaired electron on its conjugated double bond. From results obtained, it can be inferred that AVE positively modulated antioxidant activity by quenching and detoxifying the free radicals induced by irradiation. It is worth emphasizing that AVE may have a protective role against the side effects of radiotherapy.

Finally, all of the above said leads us to the conclusion that the AVE provides a significant protection against radiation-induced biochemical alterations in the liver of mammals.

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СПРЕЧАВАЊЕ ЗРАЧЕЊЕМ ИЗАЗВАНОГ ОШТЕЋЕЊА ЈЕТРЕ *SWISS ALBINO* МИШЕВА ЕКСТРАКТОМ ЛИСТА БИЉКЕ *ALOE VERA*

Испитивани су радиопротективни учинци екстракта листа *Aloe vera* на зрачењем изазване промене на јетри *Swiss albino* мишева. Мишевима је једном дневно у току 15 узастопних дана орално давано 1000 mg/kg телесне масе екстракта, пре њиховог излагања једној дози гама зрачења (6 Gy) која је уследила пола часа после последњег апликовања екстракта. Озрачивање мишева изазвало је значајан пораст липидне пероксидазе праћене смањењем глутатиона, киселе фосфатазе и базне фосфатазе. Деловање екстрактом на мишеве пре озрачивање повисило је глутатион, киселу фосфатазу и базну фосфатазу и било је праћено смањењем липидне пероксидазе. Опоравак и регенерација од зрачењем изазваног оштећења били су бржи у животиња претходно третираних екстрактом листа *Aloe vera* од оних које су припадале групи озрачених без претходне заштите. Подаци јасно указују да екстракт значајно умањује штетне ефекте зрачења на јетру те да би могао бити користан у смањењу узгредних последица у терапији зрачењем.

Кључне речи: Aloe vera, јетра, гама зрачење, Swiss albino миш