PREVENTION OF RADIATION INDUCED HISTOPATHOLOGICAL CHANGES IN ALBINO MICE BY ALOE VERA

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ABSTRACT
The radio protection by Aloe leaf extract (1000mg/kg b.wt) was studied in liver of Swiss albino mice before radiation exposure (3 Gy gamma-radiation). Mice were autopsied at day 3 post irradiation and liver was taken for histopathological studies. In this study mice, showing distorted hepatic architecture, degranulated and vacouolated cytoplasm in both control and experimental set. But experimental set mice showed mildly crenated and shrunken nuclei as compare to control. The result of present study suggests that Aloe vera has a radioprotective effect due to their antioxidant and radical scavenging activity.

KEYWORDS: Radiation, Aloe vera, Histopathology, Radioprotection, Albino mice

INTRODUCTION
In medical science radiation such as gamma as well as various radioisotopes is being used considerably for both diagnostic as well as foe therapeutic purposes such as therapy of cancer. Radiation destroys the biological molecules due to this it is very dangerous for living systems. When individuals are exposed, the radiation energy is absorbed by the biological systems, which causes radiolysis of tissue water and generates free radicals. The major free radicals such as $O_2^\cdot$, OH$^\cdot$, $H^\cdot$, $HO_2^\cdot$, $H_2O^\cdot$ combine with each other and dissolved oxygen to give a variety of potent oxidizing agents such as hydrogen peroxide, molecular oxygen and perhydroxy radicals (Dragaric I.G. and Dragaric Z.D., 1971; Pradhan D.S., Nair C.K.K., Sreenivassan A., 1973; Dragaric I.G. and Scholes T.S., 1983).

The damage of tissue varies with dose of radiation exposure. It also depends on what kind of radiation is given and on some other factors such as age, sex, species and nature of tissue.
Chemical agents emerge to be a consequence of the anatomical position of liver and it plays an important role in the metabolism (Plaa, 1988). Recent studies have recognized that hepatotoxicity may be inflicting by thousands of synthetic chemicals, environmental pollutants such as radiation and naturally occurring toxicants.

Latent liver damage, evoked in adult animals by preceding irradiation, manifests itself during the course of liver regeneration after partial hepatectomy by various biochemical and cytological changes, mainly by the inhibition of DNA-synthesis and mitotic activity and by an increase in the occurrence of chromosomal aberration.

Several studies have shown histological and biological changes in liver after irradiation. Dettmer et al. (1968) have noted that the condition of hepatocytes at the time of irradiation is a determining factor for the subsequent damage. Grad and Stevens (1950) and Mehta et al. (1975) have reported cytoplasmic degranulation, pyknosis and loss of architecture as a result of irradiation. Low and moderate fractionated doses of X-rays lead to significant increase in liver mass.

Therefore, interest has generated among scientists to develop the potential drugs of plant origin for modification of radiation effects and hence the search for more effective, less expensive, less toxic and easily available radioprotectors is still going on. To fulfill this need various plant extracts and preparations are being investigated to evaluate their radioprotective effects, because it is believed that a plant product has no or minimum side effects.

*Aloe barbedensis* (Mill.) commonly known as *Aloe vera* and belongs to family Liliaceae. From several thousands of years *Aloe* has been used medicinally and polysaccharides which are present in *Aloe* are always considered effective radioprotectors on radiation induced skin damage (Wang et al. 2004). In treatment of acute radiation dermatitis *Aloe* may be useful (Wickline, 2004). *Aloe vera* is widely used by patients with inflammatory bowel disease and it has been claimed to have anti-inflammatory effects Langmead et al. 2004).

Liver is an important metabolic organ. It plays a key role in detoxification of pollutants, toxic foods and drugs. Therefore, this study was undertaken to evaluate the value of nutritional supplementation of *Aloe vera* against the radiation induced damage on the liver of Swiss albino mice.
MATERIALS AND METHODS

Animals
Swiss albino mice of 6-7 weeks old, weighing 24-26 gm were selected for this experimental study. Animals were housed in polyvinyl chloride cages (290 × 320 × 390 mm) and maintained under standard laboratory conditions. The animals had free access to food (mice feed) and water. Tetracycline was also given along with drinking water to them once fortnight as a preventive measure against infection. The maintenance and handling of the animals were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, animal ethics committee Ministry of Environment and Forests, Government of India.

Source of Irradiation
Animals were irradiated at Cancer Treatment Center, S.M.S. Medical College and Hospital, Jaipur by using Cobalt teletherapy unit (ATC-C9). Animals were kept properly in a well-ventilated wooden box and distance between the animals in wooden box and radiation source was 77.5 cm for exposure at the dose rate of 1.33 Gy / min. The dose rate was calibrated time to time throughout the experimentation according to the decay table of Co$^{60}$.

*Aloe vera*
*Aloe barbadensis* (Mill.) belongs to family Liliaceae and commonly known as *Aloe vera*. Plant was collected from surrounding area, identified by the botanist, Department of Botany, University of Rajasthan, Jaipur, allotted identification/voucher number RUBL-19886 and same plant was placed in the departmental herbarium.

Experimental Design
For this study, selected adult male Swiss albino mice were divided into three groups (I, II and III).

**Group I:** Animals of this group were given double distilled water (DDW) orally (volume equal to that used for *Aloe* administration in experimental mice) for 15 consecutive days and called sham irradiated (normal) group.

**Group II:** Animals of this group were administered *Aloe* extract orally at the dose of 1000 mg /kg body weight (once in a day) for 15 consecutive days to study its toxic effects on liver.

**Group III:** Group III was divided into two sets, one was experimental and another was control. Animals of experimental set were administered *Aloe* extract orally at the dose of
1000 mg/kg body weight (once in a day) for 15 consecutive days, whereas animals of control set were given double distilled water (DDW) orally (volume equal to that used for Aloe administration in experimental sets) for 15 consecutive days.

Just after 1 hour of last administration of extract and DDW, animals of group III, was exposed to sublethal dose 3 Gy gamma radiation.

**Histopathology**

A minimum of 5 animals from group II and each set of control and experiment of group III were sacrificed by cervical dislocation on day 3 of post irradiation and liver was taken for histopathological observations.

**OBSERVATIONS**

In present investigation animals of group I showed normal hepatic architecture of liver. There were no changes in the cytoplasm and nuclei. A normal hepatic structure of liver is seen in animals of group II which were supplemented Aloe alone for 15 consecutive days.

Several histopathological changes were found in the liver of Swiss albino after exposure to 3 Gy of gamma radiation individually as well as with pretreatment of Aloe vera. The changes observed on day-3 after exposure was cytoplasmic degranulation, vacuolation, hyperaemia, pycnotic and crenated nuclei. Many cells were lacking their nuclei. In the combined treatment of radiation and Aloe vera similar changes were observed but they were more pronounced showing synergistic effects. The liver of Aloe vera pretreated animals exhibited less severe damage as compared to non-drug treated animals. An earlier and faster recovery was also noticed in Aloe vera pretreated animals. (Figs.2).

Radiolesions like distorted hepatic architecture, few enucleated hepatocytes, degranulated and vacuolated cytoplasm, mildly crenated and shrunken nuclei were observed in liver of Aloe treated 3 Gy irradiated mice (experimental set) along with some normal hepatocytes at day 3 post irradiation. However, severity of these histopathological changes was certainly lesser than control set IV. (Fig.3).
Fig 1: Drug alone.

Fig 2: Control Set.

Fig 3: Experimental Set.
DISCUSSION

From the beginning, effects of radiation on liver were not clear and therefore due to its radiosensitivity it remained as a controversial organ for a long time. Even the eminent workers failed to observe any visible pathological change in hepatic tissue following exposure to moderate or large doses of radiations (Hall and Whipple, 1919). Liver is relatively resistant organ against radiation (Koletsky and Gustafson (1952); Kelly and Hirsch (1955); and Gupta (1980).

Earlier workers such as Gupta (1972) and Bhatia et al. (1978) reported that mammalian liver is a sensitive organ to internal irradiation at different post-natal ages (1 to 6 weeks).

In 3 Gy irradiated mice there were no radiation sickness and 30 days mortality was observed. However, several workers such as Saharan (1977), Saini (1977), Maharwal (2002), Jagetia and Baliga (2003) have reported various severe signs of radiation sickness and 30 days mortality because of whole body irradiation of mice with high doses of gamma radiation. Results of their study indicated that exposure of mice to 3 Gy did not because severe damage in both bone marrow and gastrointestinal tract and therefore, signs of radiation sickness did not appear.

Severity of hepatolesions increased and maximum damage was seen at day 3 in the form of distorted hepatic architecture, karyorrhexis, chromatolysis, crenation, shrinkage and fragmentation of nuclei and degranulation and vacuolization of cytoplasm. Similarly, Bhartiya (1970), also reported maximum histopathological changes such as hyperaemia, oedema, lymphocytic infiltration, pycnosis, cytoplasmic degranulation and vaculation at day 2 post irradiation in liver of gerbils exposed to 3 Gy gamma radiation and but resumption of almost normal hepatic picture was reported after one week.

To provide protection against radiation induced deleterious effects cysteine was used for the first by Patt et al. in 1949. Subsequently, several chemicals have been synthesized and tested to evaluate their protective effects on different organ systems including liver, which is an important metabolic organ and performs several functions.

Reports of Friedburg (1956) and Doul et al. (1961) also showed that herbicide, 3 amino1, 2-triazole provides slight protection to mouse liver against radiation induced damage. Similarly, treatment with cystamine (Chatterjee and Bose, 1962), cysteamine (Eldjarn, 1964) and
serotonin (Vittorio et al. 1963) provided protection to liver against radiation induced damage, which is in agreement with present findings.

It has been observed that chemical radioprotectors, which generally provide maximum protection, have to be given in high doses, which are toxic to animals. Therefore, various natural products, herbal preparations and plant extracts have been tested by several workers during last 30 years and reported that such protectors have various advantages over synthetic protectors as they are nontoxic or less toxic, easily available, less expensive and easy to take being a part of regular diet. Studies on such protectors are relatively a new area of research and can be very promising for human beings.

Jain (2002) reported that oral administration of Amaranthus and Spinacia extracts singly and/or in combination to Swiss albino mice reduced the 5.5 Gy induced damage in liver and enhanced the recovery process. Similar findings have also been reported by Maharwal et al. (2005) in liver of Rajgira extract treated 6, 8 and 10 Gy irradiated Swiss albino mice.

Thus, results of this study indicate that radioprotective effect of Aloe manifested in various forms in liver. Treatment with Aloe prior to irradiation reduced the magnitude (severity) of radiation induced various histopathological changes (distortion in hepatic architecture, crenation, shrinkage and loss of liver cells nuclei, degranulation and vacuolization of cytoplasm, lymphatic infiltration and dilation of sinusoids) and therefore, increase in kupffer cell population was inhibited in mice liver. In this study, Aloe extract was tested for providing protection to mice liver against radiation induced injury.

CONCLUSION
Results of this study suggest that pretreatment of mice with Aloe reduced the severity of radiation induced various histopathogical changes in liver.

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