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MODIFICATION OF RADIATION INDUCED CHANGES IN THE INSTESTINAL VILLI OF THE *SWISS ALBINO MOUSE* BY *CENTELLA ASIATICA* PRE TREATMENT

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Radiosensitivity of the instestine leads to the death of the animal 10 to 15 days after radiation exposure to the lethal dose. Lower doses also create intestinal injury leading to the loss of fluids and electrolytes. Digestive disturbances are also caused. Being a proliferative organ intestine is highly sensitive to radiation exposure. In order to prevent radiolesions in irradiated animals *Centella asiatica* (CA) was given to the animals one hour before radiation exposure. The animals were irradiated in two sets and each set was divided into two groups. Animals of the first set where irradiated with 6Gy and the animals of the second set were irradiated with 8 Gy of Co^{60} gamma rays with and without CA pretreatment. It was observed that length of the interstinal villi decreases after radiation exposure. In all the groups it reaches to the normal at day 14. It was observed that CA pretreatment prevents the decrease in the villus length to a significant extent and leads to faster recovery.

Keywords : *Centella asiatica;* Gamma radiation; Intestine; Vill.

Introduction

Generally, the effect of radiations is hazardous, even if given in a small dose. The damage cause by radiation is influenced by the relative radiosensitivity of the tissue.

According to the law of Bergonie and Tribondeau[1] actively proliferating tissues are the most sensitive to radiation. It is well known that irradiation of the whole animal produces damage to the intestine. The death resulting from large does of irradiation is probably due to the intestinal damage and has been called "acute intestinal death"[2].

Small intestine is the most radiosensitive organ of the gastrointestinal tract[3,4]. The high radio sensitivty shown by it is due to the presence of vegetative intermitotic cells in its epithelial lining and because cell population on the villi is dynamic and under normal conditons. It is in a steady state[5].

Histologically, radiation damages the crypt cells and their nuclei first and then affects other parts of mucosa[6]. Early destructive changes in intestinal epithelium may be visible at 1 Gy. The net effect of radiation on normal proliferating tissue *in vivo* depends both on the degree of cell killing and the efficacy and temporal course of the subsequent compensatory response.

There are a number of synthetic and herbal products which are tested to modulate radiation induced damage[7-11]. Some of them are radiosensitive and others are antiradiation protective agents[12], *Centella asiatica* (CA) is one of them.

Centella asiatica (Linn.) Urban; syn. *Hydrocotyle asiatica* (L) is a herbal medicinal plant which belong to the family Apiaceae (Umbellifereae).

It has shown antileprotic, antitumor, antiulcer tonic, wound healing, antibacterial, nerve tonic, antiprotozoal, antifertility and antispasmodic properties[13-15]. The plant is digestible, laxative, cooling, alexiteric, antipyretic, improves aptitude, voice, memory[16] cures leucoderma, anaemia, urinary discharge, diseases of blood, bronchitis, inflammation, fever, biliousness, enlargement of spleen, thirst, asthma, small pox and is used in insanity and somatic and Psychic anxiety[17]. It is reported to increase blood protein, Nitrogen, mean level of RBC, blood sugar, serum Cholesterol and total proteins.

Materials and Method

Adult male *Swiss albino mice* 6-8 weeks of age were selected from an inbred colony maintained in the laboratory. These were given standard mice feed and water *ad-libiitum*.

The cobalt teletherapy unit (ATC-CZ) at Cancer Treatment Centre, Radiotherapy Department, SMS Medical

College and Hospital, Jaipur was used for irradiation. Unanaesthesised mice restrained in well ventilated per spex boxes and the whole body of these animals was exposed to gamma radiation at the dose rate of 1.59 Gy/min. at a distance (SSD) of 77.5 cm from the source.

Aqueous and Alcoholic extract (50 : 50) obtained from Amsar Pharmaceutical Private Limied, Indore was used. The extract was dissolved in glass distilled water and given at the dose rate of 100 mg/kg body weight one hr before irradiation. The dose of *Centella asiatica* was selected on the basis of experiment conducted with various doses of the drug and various treatment periods[18].

Healthy animals were selected and divided into four groups. First of all animals were divided into two groups, which were exposed to Co^{60} gamma rays. The first group received 6 Gy and the second received 8 Gy of Co^{60} gamma rays. Both the groups were divided into two subgroups. The first subgroup of each group ws irradiated only and the second subgroup of each group received CA extract one hour before irradiaton. The third group received plant extract only at the same dose rate and the fourth group was sham- irradiated only. The animals were autopsied at 1/4 1,2, 4, 7, 10, 14, and 28 dyas after irradiation. Intestine was removed and fixed in the Bouins fluid for histological examination. T.S. of the intestine were cut at 5 μ in thickness and stained with Harris Hematoxylin and Eosin. Villus length was measured with the help of occular micrometer from the selected sections. The data were collected and on an average 60 sections per animals were scored. The data were subjected to the students 't' test.

Results and Discussion

Length of the villi in the 6 Gy treated animals (Table 1) decreased sharply after gamma ray exposure. This decrease continued up to 2nd day. Then it increased very sharply at day 4 and reached to the normal level within 14 days. In the animals which were exposed to 8 Gy it decreased up to day 4 and then reached to the normal within 28 days. It was observed that in the CA pretreated animals villus length was always higher in comparison to their respective controls. CA pretreatment prevented the sharp decrease in the 8 Gy treated animals.

Intestine is a vital organ with a very efficient cell renewal system and due to this intestinal mucosa is completely renewed within a definite time period. Crypts have the cells which divide mitotically at a constant rate and these cells reaches to the villi and they continue to migrate through out the villus length and are finally dropped off from the tip[19,20]. This process continues in the healthy animals. The time taken in this migration and the life of the epithelial cells on the villi is species specific. Gamma Radiation above 5 Gy causes intestinal injury. A variety of cells specifically cells of the crypts and villus epithelium are killed. Membrane of the cells and other components of the mucosa are also affected adversely. A large number of pycnotic nuclei and necrotic cells are seen after radiation exposure. Mitotic figures disappeared completely and villi appeared denuded. Membrane permeability is altered, hence causing difficulty in the absorption of nutrients. The major damage occurs due to the preoxidation of the lipid components of the cellular membrane. Intestinal tissue contains a very high amount of water in it. Irradiation of this water results in ionization and formation of free radicals. Besides this, all the molecules of biological importance like DNA, RNA and proteins are damaged by radiation exposure, depending upon the dose of exposure.

These animals were irradiated whole bodily thus complete physiology, of the animals was disturbed[21]. There are clear cut evidences that heamatopoietic syndrome also interferes with the gastrointestinal syndrome. The decreased height of the villi is an expression of all these states of affairs[22,23]. As soon as animals recover from the radiantion injury the length of the villi reaches to its normal level. Proliferation of

Table 1. Variations in the length of intestinal villi of Co^{60} Gamma ray irradiated *Swiss albino mouse* with and without *Centella asiatica* pretreatment.

Treatments	Post Irradiation time (in days)							
	¼	1	2	4	7	10	14	28
6 Gy	69.9 ± 3.46 < 0.05	68.84 ± 3.66 < 0.05	56.9 ± 3.84 < 0.01	56.5 ± 2.48 < 0.05	63.3 ± 4.50 < 0.05	64.06 ± 2.63 < 0.001*	66.0 ± 2.85 < 0.01	67.93 ± 2.48 < 0.01
6 Gy + Plant Extract	69.9 ± 2.84 < 0.05	68.84 ± 2.83 < 0.05	56.9 ± 2. 87 < 0.01	56.5 ± 3.84 < 0.05	63.3 ± 2.50 < 0.05	64.06 ± 2.64 < 0.01	66.0 ± 2.40 < 0.01	67.93 ± 2.45 < 0.01
8 Gy	75.5 ± 3.28 < 0.001	70.20 ± 5.15 < 0.05	59.13 ± 3.48 < 0.01	62.2 ± 3.40 < 0.05	64.3 ± 2.66 < 0.05	66.0 ± 2.85 < 0.01	Animal not survived	Animal not survived
6 Gy + Plant Extract	85.7 ± 3.59 < 0.001	83.40 ± 2.20 < 0.05	75.6 ± 3.08 < 0.01	77.77 ± 4.6 < 0.05	78.03 ± 3.65 < 0.05	79.2 ± 2.84 < 0.01	80.1 ± 2.21 -	80.8 ± 4.14 -
Plant Extract Only	77.6 ± 2.36	78.5 ± 2.28	79.2 ± 2.84	80.73 ± 5.02	85.7 ± 3.59	87.06 ± 1.50	88.48 ± 1.27	88.84 ± 0.99

The length of intestinal villi in the healthy normal *Swiss albino mouse* without any treatment is = 83.40 ± 2.20

P value = Control vs Experimental

The Plant extract was given 1 hr before irradiation at the dose rate of 100 mg/kg body weight.

the cells in the surviving crypts begins and denuded villi appear normal. Necrotic cells and pycnotic nuclei also disappear from the scene. The lesser decrease and faster recovery in the CA pretreated animals reveal the protection offered by the plant extract. It has been observed that CA pretreatment increases the survival of irradiated animals to a significant extent[24]. CA is a well known Ayurvedic medicine used in the treatment of a variety of diseases, specifically in the skin diseases and in wound healing. the presence of its extracts in the intestinal lumen and in the intestinal tissue might have prevented the radiation induced changes. It has been known to activate certain physiological reactions in the body which leads to the activation of natural body defences and secretion of several natural chemicals of the body that can cope up with the damage caused by irradiation. CA is also known for its antioxidative, free radical scavanger and antiperoxidant activities[25-27]. These properties might also have acted in the prevention of radiation induced damage.

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