Rajgira leaf extract prevents intestinal mucosa against radiation induced oxidative stress: a quantitative study

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Abstract

The radioprotective potential of *Rajgira* leaf extract (RLE) was investigated in Swiss albino mice. The mice were divided into three (I-III) groups. Each group had an experimental set in which RLE was given at the dose of 800 mg/kg body weight orally for 15 consecutive days and a control set that received distilled water in similar manner (volume equal to that used to administered RLE in experimental set). On the last day of extract administration, the animals of I, II and III groups were exposed to 6, 8 and 10 Gy of gamma radiation respectively. Animals of experimental and control sets were autopsied at different time intervals for quantitation of mucosa cells in jejunum. A significant decline was observed in the number of mitotic figures, crypt epithelial cells (per crypt section) and villus height and an increase in frequency of dead cells / crypt section from day 1, which continued and reached at peaks at day 3 in animals of all control sets (except mitotic figures that were absent in 10 Gy exposed animals at day 3). Later on, mitotic figures, crypt epithelial cells and villus height increased and frequency of dead cells decreased continuously from day 7 and almost normal levels were observed at last autopsy interval in surviving animals. Though, the pattern of change in number of mitotic figures, crypt epithelial cells, villus height and dead cells was similar in RLE pretreated irradiated animals of all sets (I-III) to that of irradiated alone sets but the numbers of mitotic figures, crypt cells, villus height were significantly higher and number of dead cells were lesser. Thus, RLE protected the intestinal mucosa from radiation induced damage as expresed primarily by the higher number of mitotic figures, crypt epithelial cells and higher villus height and decreased frequency of dead cells. The protective and antioxidative properties of *Rajgira* leaf extract appear to be related to its provitamin A (β - carotine), vitamin C and riboflavin contents.

Keywords: gamma irradiation, Swiss albino mice, *Rajgira* and intestinal mucosa

Introduction

Intestinal mucosa is constantly renewed to replace old cells and is so dependent upon crypts stem cells. Due to this characteristic, gastrointestinal tract is highly sensitive to ionizing radiation. Irradiation of living cells with ionizing radiation leads to a rapid burst of reactive oxygen species ($O_2^{\cdot -}$, $HO^{\cdot}_2$, $OH^{\cdot}$ and H_2O_2) through radiolysis of water.

The elevated reactive oxygen species (ROS) can damage proteins, membrane lipids, nucleic acids and various other biological materials and may eventually give rise to cell death. Abdominal and pelvic irradiation during diagnostic procedure, radiotherapy of cancer and / or accidental exposure results in loss of proliferative capacity of crypt cells and hence substantial loss of intestinal mucosa occurs. Administration of synthetic radioprotectors during such exposures can protect the small intestine and other associated organs and increase patient's tolerance to radiation.

Secondly, it has also been considered that radiotherapy for cancer patients could be improved by the use of radioprotectors to protect normal tissue. But synthetic protectors, so for tested are found to be toxic at their effective dose levels, which limit their clinical application. Therefore, several studies have been carried out with several herbal preparations such as liv. 52 (Saini *et al.*, 1984) *Rasayanas* (Kumar *et al.*, 1986*), Mentat* (Jagitia and Baliga, 2002), *Abana* (Baliga *et al.*, 2004) and plant extracts such as *Ocimum sanctum* (Uma Devi and Ganasoundari, 1995); *Mentha piperita* (Samarath *et al.*, 2002) and *Rajgira* (Maharwal *et al.*, 2003) to modify radiation response without being toxic at their effective dose levels.

These herbal preparations and plant extracts contain ample quantity of vitamins (A, C, E), carotinoids, flavonoids, enzymes and minerals (Se, Zn, Cu, Mn) and most of the animals including man depend on diet for these antioxidant nutrients. Herbal perpetrations and extracts are gaining importance as less or nontoxic nutritional antioxidants and radioprotectors and several of them are being tested for biochemical and pharmacological properties.

Amaranthus paniculatus is a herb, belonging to family *Amaranthaceae* and commonly called as *Rajgira*. It is one of the world's most nutritious grain with edible stem and leaves. The leaves are rich in vitamins, especially provitamin A (β-carotene), vitamin C and riboflavin (Gopalan *et al.*, 2000). *Rajgira* contains an unusually high percentage of an amino acid (lysine), which is present at low levels in wheat, corn and rice (Kulkarni and Singhal 1992). Recently Maharwal *et al.* (2003) studied the *Rajgira* and reported it to be a potent antioxidant and inhibitor of radiation induced lipid peroxidation. In the present attempt, radiation induced alterations in numbers of mitotic figures, crypt epithelial cells and dead cells/ crypt section and villus height were studied in mouse intestinal mucosa for assessing the radioprotective potential of *Rajgira* leaf extract.

Materials And Methods

Animals:

Adult male Swiss albino mice of 6-8 weeks old, weighing 25 ± 3g, fed on standard mice feed and water *ad libitum*, were used for this study.

Irradiation:

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, restrained in well-ventilated Perspex boxes were whole - body exposed to different doses of gamma radiation at the distance (SSD) of 77.5 cm from the source to deliver the dose-rate of 1.33 Gy / min.

***Rajgira* Leaf Extract (RLE) :**

The extract of fresh, shade dried and powdered leaves of *Rajgira (Amaranthus paniculatus:* RUBL-19869) was prepared by refluxing with double distilled water (DDW) for 36 h (12 h x 3) at 40ºC. The prepared extract was vacuum evaporated for making it in powder form. The extract obtained in powder form was dissolved in DDW just before oral administration.

Experimental Design:

Animals selected from an inbred colony were divided into 3 (I, II and III) groups. Each group contained an experimental set in which 800 mg/kg b.wt. of *Rajgira* leaf extract(RLE) was administered orally for 15 consecutive days and a control set, which received distilled water in the similar manner (volume equal to that used for extract administration in experimental animals). On the last day of RLE administration, the animals of I, II and III groups were exposed to 6, 8 and 10 Gy of Co^{60} gamma radiations respectively. A minimum of four animals from each set were killed by cervical dislocation on 1, 3, 7, 14 and 30 days post-irradiation and jejunum was fixed in Bouin's fluid. Five-micrometer thick sections were prepared, stained with hematoxylin and eosin and number of mitotic figures, dead cells and crypt epithelial cells/ crypt section were counted and villus height was measured to evaluate the radioprotective potential of *Rajgira* leaf extract.

Statistical Analysis:

The Student's t-test was used to make a statistical comparison between the groups. The results obtained were expressed as mean ±SE. Significance level was set at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

Results

The change in numbers of mitotic figures, dead cells and crypt epithelial cells/ crypt section and villus height in control sets relative to sham irradiated group and experimental sets have been shown in figs.1-4 respectively. All these cell parameters have not been studied in 8 and 10 Gy irradiated alone animals after day 7 as they did not survive beyond day 7 (i.e. upto next autopsy interval).

A decrease in mitotic figures / crypt section was observed from day 1 after exposure to 6, 8 and 10 Gy of gamma radiation and found to be very few (0.8 ± 0.33, 0.4 ± 0.21 and zero) on day 3. Later on a gradual increase was observed in mitotic figures at subsequent autopsy intervals in 6 Gy exposed animals, whereas, in 8 and 10 Gy exposed animals the mitotic figures just appeared on day 7 (Fig. 1). Study of mitotic figures in 8 and 10 Gy irradiated alone animals was not possible beyond day 7 because the animals of these two control sets did not survive till next autopsy interval (fig. 1).

The number of dead cells / crypt section increased markedly and were found to be 3.4 ± 0.06, 4.6 ± 0.45 and 3.4 ± 0.45 on day 1,which further increased and recorded as highest (4.0 ± 0.63, 5.6 ± 0.45 and 6.2 ± 0.52) on day 3 after exposure to 6, 8 and 10 Gy respectively. However, at day 7 a decrease was noticed in dead cells, which continued upto last autopsy interval only in 6 Gy exposed surviving animals. Whereas, 8 and 10 Gy irradiated alone animals did not survive till next studied interval and hence dead cells were not counted (fig. 2).

The number of crypt epithelial cells / crypt section also showed a declined from day 1 and found to be minimum (34.6 , 1.16, 29.0 ± 1.05 and 25.6 ± 1.34) on day 3 after irradiation with 6, 8 and 10 Gy respectively. This decline in crypt epithelial cells was followed by an increase on day 7, which continued till last studied interval only in 6 Gy irradiated alone surviving animals (fig. 3). There was also a marked decrease in villus height from day 1 and attained a minimal height i.e. 265.95 ± 8.83, 250.71 ± 14.40 and 215.13 ± 10.87 μm on day 3 after exposure to 6, 8 and 10 Gy respectively. Thereafter, a continuous increase has been observed in villus height in 6 Gy irradiated animals till the last autopsy interval whereas, in 8 and 10 Gy exposed animals an initiation of increase in villus height was seen on day 7 (fig. 4).

All *Rajgira* leaf extract pretreated irradiated animals of all sets also showed a reduction in mitotic figures / crypt section from day 1 and found to be maximum on day 3. But this reduction in mitotic figures was significantly lesser than that of all irradiated alone animals.

Fig. 1: Number of mitotic figures / crypt section in Swiss albino mice after exposure to 6, 8 and 10 Gy of gamma rays with (experimental) and without (control) treatment of RLE

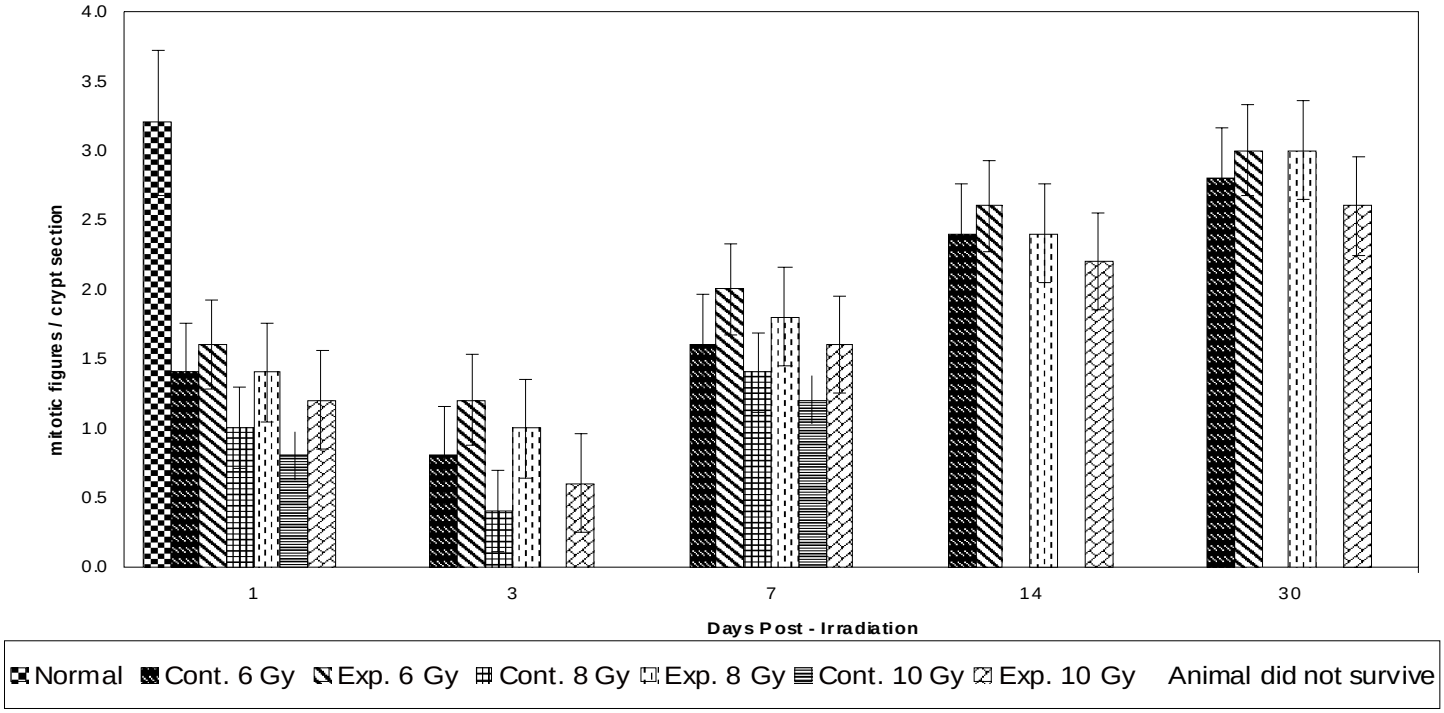


Fig. 2 Number of dead cell/crypt section in Swiss albino mice after exposure to 6,8 and 10 Gy of gamma rays with (experimental) and without (control) tgreatment of RLE

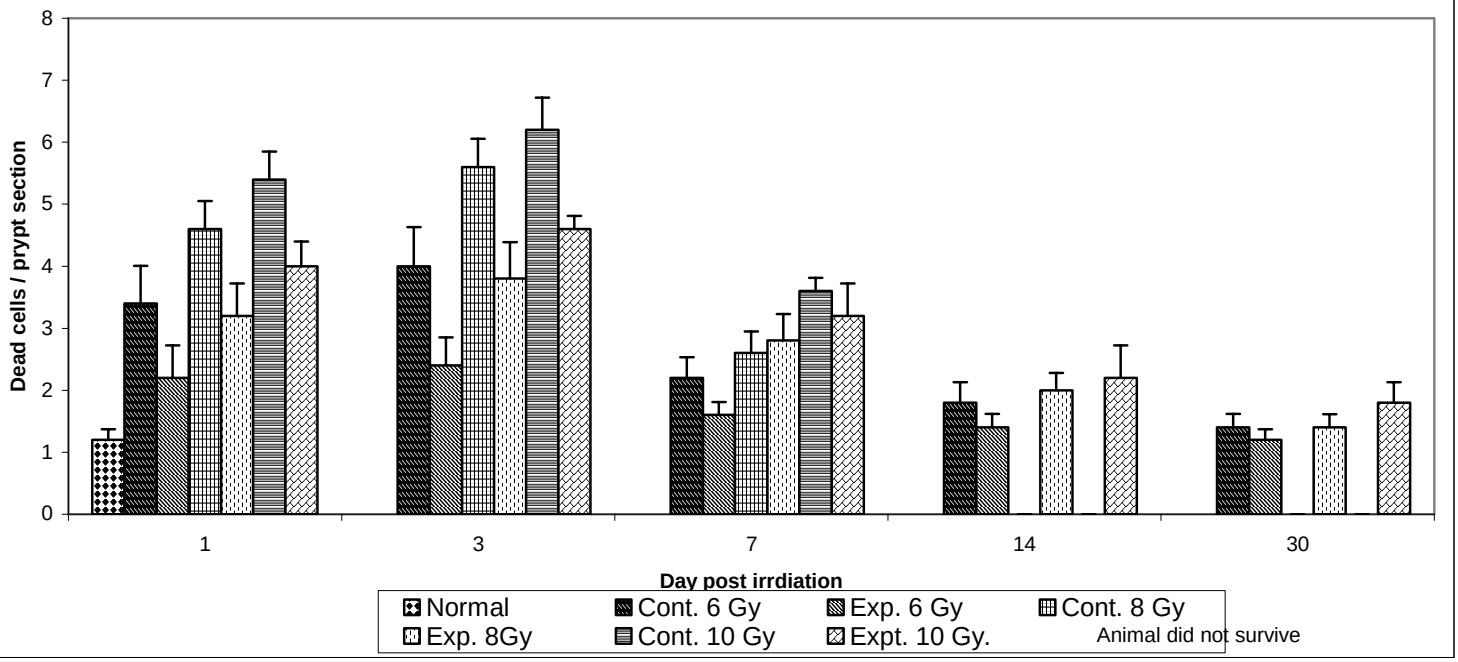
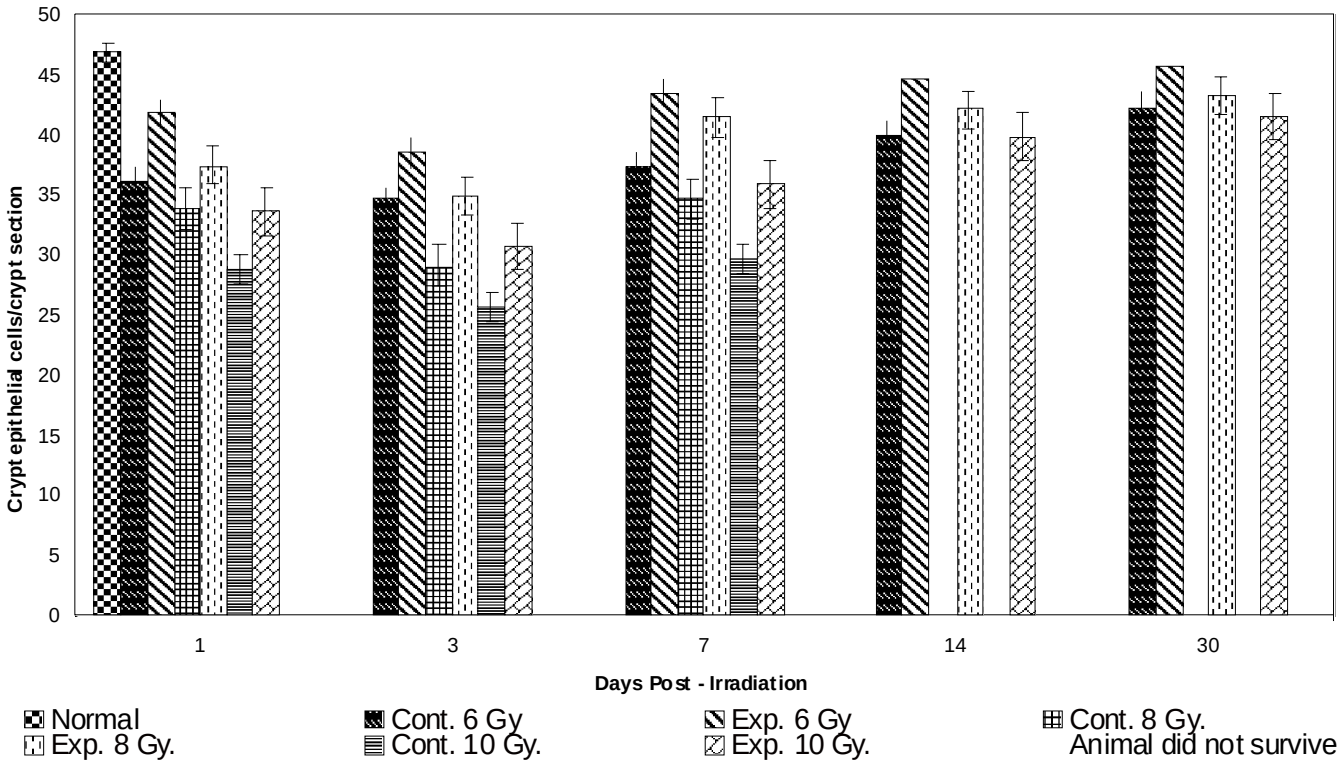


Fig. 3 : Number of crypt epithelial cells / crypt section in Swiss albino mice after exposure to 6, 8 and 10 Gy of gamma rays with (experimental) and without (control) treatment of RLE

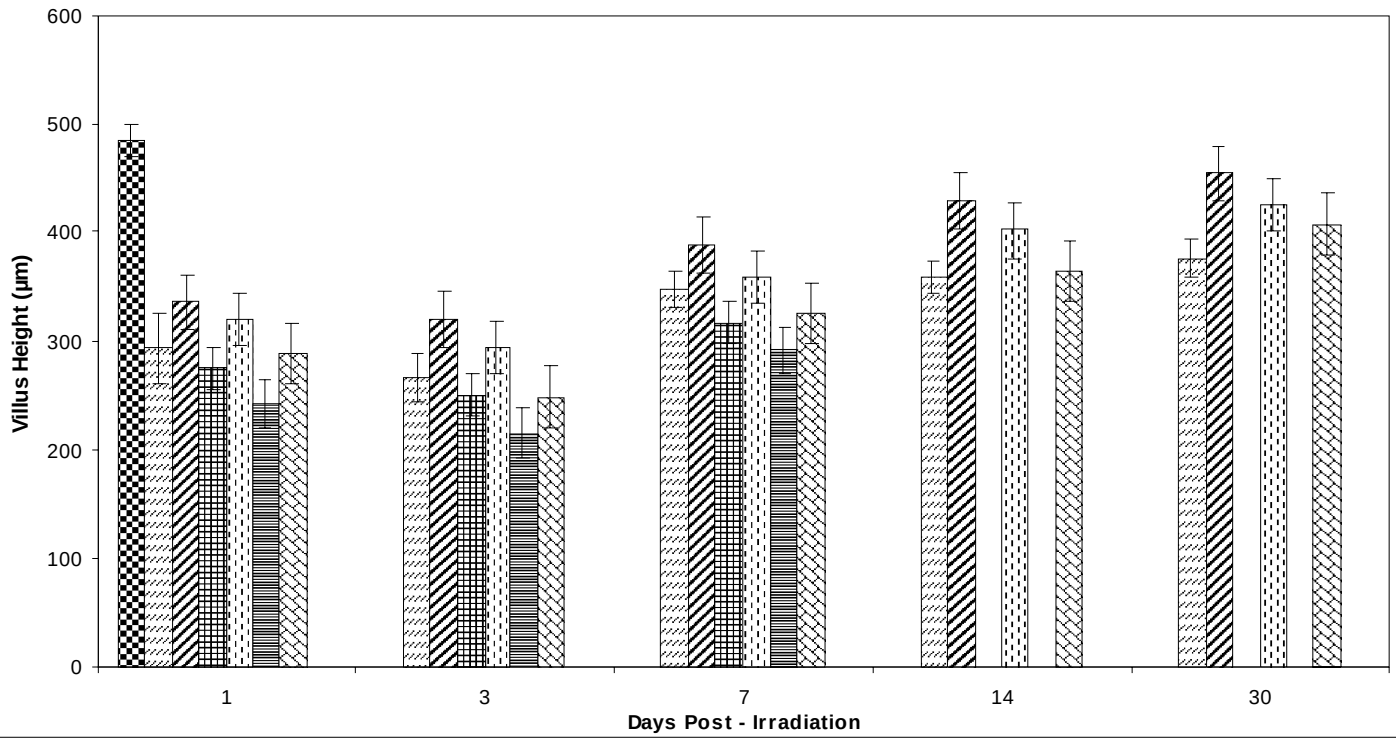


From day 7, mitotic figures increased continuously but normalcy was not restored even up to day 30 (fig.1). The pattern of change in dead cells / crypt section was also similar in both irradiated alone (control) and *Rajgira* pretreated irradiated (experimental) animals but their number was found to be significantly lesser in experimental animals in comparison to that of the control animals at all successive autopsy intervals (fig. 2).

A decrease in crypt epithelial cells / crypt section was also seen between day 1-3 in all experimental sets but to a lesser extent as compared to their respective control sets. Thereafter a continuous increase was seen in crypt epithelial cells from day 7 to the end of experiment but still they remained below the normal number. The villus height has also decreased in *Rajgira* pretreated 6, 8 and 10 Gy irradiated animals from day 1 and found to be minimum i.e. 320.16 ± 22.00, 293.06 ± 25.75 and 249.0 ± 25.53 μm respectively on day 3, but this decrease in villus height was also significantly lesser as compared to that of the respective *Rajgira* untreated

irradiated animals. Later a gradual increase in villus height has been observed till the last studied interval (day 30) but without attending the normal value (fig. 4).

Fig. 4: Villus height (µm) in Swiss albino mice after exposure to 6, 8 and 10 Gy of gamma rays with (experimental) and without (control) treatment of RLE



Discussion

Mitotic figures, dead cells and crypt epithelial cells / crypt section and villus height were studied quantitatively in mouse intestinal mucosa with and without treatment of *Rajgira* leaf extract (fig. 1-4). A decrease in mitotic figures, crypt epithelial cells and villus height and an increase in dead cells has been observed upto day 3 in all sets of both control and experimental animals.

But mitotic figures, crypt epithelial cells and villus height were significantly higher and dead cells were significantly lesser in all experimental sets in comparison to control sets in the present study (fig.1-4).

However, from day 7 first three cell parameters increased and dead cells decreased continuously upto day 30 in all experimental sets upto day 30, while this cell kinetic study was not possible in 8 and 10 Gy irradiation alone sets of animals due to their death. Employing a cell kinetic approach, earlier workers have also described similar alterations in a number of cell parameters of mouse intestinal epithelium after irradiation (Quastler and Sharman, 1959; Devik, 1968; Hagemann *et al.*, 1970, 1971; Sigdestad *et al.*, 1970; Tsubouchi and Matsuzawa, 1973; Uma Devi *et al.*, 1979 and Delaney *et al.*, 1994). Exposure to different doses (6,8 and 10 Gy) markedly damaged the stem cells and ceased the mitotic activity in jejunal crypts (fig. 1). Therefore, a significant and consistent decrease in mitotic figures, crypt epithelial cells / crypt section and villus height and increase in dead cell number was noticed from day 1- 3 (figs.1- 4). The changes in these cell parameters were found to be dose and time dependent. Similarly Devik (1971), Uma Devi (1979) and Samrath et al. (2002) have also reported the dose dependent reduction in mitotic activity and thereby a decrease in crypt epithelial cells and villus height. A constant flow of cells occurs from crypt to villus in intestine to maintain the steady state relationship between proliferative (crypt) and nonproliferative (villus) zone to ensure an unbroken mucosal covering of the villi.

In the present study when animals were exposed to radiation, cells in both crypts and villi were damaged but villi were having differerntiated cells and hence were not involved in DNA synthesis and mitosis; therefore damage was not seen immediately, where as cells in proliferative zone (crypt) were undifferentiated and therefore, damage was seen immediately in crypts in the form of pyknotic nuclei and necrotic (dead) cells (fig.2), which ultimately resulted in shortening of villi (fig.4). A major part in radiation damage to the intestinal mucosa seems to be played by interphase damage, which caused death of cells immediately after irradiation and resulted in depletion of crypt cell population between 1-3 days post irradiation. The period from 3 to 10 days was the average time of animal death with substantial manifestation of the gastrointestinal syndrome (shortening of villi, disturbances in balance of body fluid and electrolytes due to diarrhoea and damage to blood vessels).

The higher numbers of mitotic figures, crypt epithelium cells and lower number of dead cells/ crypt section and a higher villus height in experimental animals as compared to that of the control animals at all autopsy intervals are significant findings of this study.

This quantitative study of intestine mucosa of mouse indicated that *Rajgira* treatment prevented the undiffentiated crypt cell population from radiation damage, which helped in maintaining the steady state relationship between proliferative and nonproliferative zones. Survival of crypt is an important feature in *Rajgira* pretreated irradiated animals, as a crypt is a dividing zone, which supplies cells for maintennce of villus integrity.

It is well known that a crypt contains the radiosensitive stem cells, whose survival is essential to that of the animal. Ionozing radiations affect the cells in all phases of the proliferative cycle but the degree of the effect depends upon that phase in which they were present at the time of irradiation (Elkind and Whitmore,1967; Gillette et al. 1970). The tendency of a cell to develop pyknosis is high in G_2 phase, intermediate in G_1 and early S phase and low in mid and late S phase (Tsubouchi and Matsuzawa, 1974).

Exposure of a living organism to ionizing radiation generates various free radicals ($OH^{\cdot}$, $O_2^{\cdot -}$, $HO_2^{\cdot}$ and H_2O_2). Among them, hydroxyl radical ($OH^{\cdot}$) is the most noxious free radical, which rapidly initiates lipid peroxidation in a cell membrane due to high sensitivity of lipids to $OH^{\cdot}$ and changes fluidity, permeability and structure of membranes. Peroxidation of lipids, oxidation of proteins and nuclic acids alter the function of a cell, which ultimately dies. The presence of ß-carotene in *Rajgira* leaf extract plays an important role in protecting the mouse intestine against radiation induced damage. The β- carotene is very important for human beings not only as a precursor of vitamin A but also for having good antioxidant properties. The antioxidative mechanism of β- carotene has been suggested to be singlet oxygen ($^1O_2$) quenching, free radical scavenging and chain breaking during lipid peroxidation (Gerster, 1993). *Rajgira* extract is also rich in vitamin C (Gopalan *et al.,* 2000), which has been reported a potent protector of lipids and lipoprotein against oxidation damage (Frei, 1991) and enhances the host immunological functions (Kelley and Bendich, 1996). Vitamin C also act as a co-antioxidant by regenerating α-tocopherol from α-tocopherol radical. The α- tocopherol is a free radical chain breaking antioxidant and scavenges peroxyl radicals rapidly by transferring a phenolic hydrogen to a peroxyl free radical of a peroxidized polyunsaturated fatty acids and

itself get converted into α- tocopherol radical, which can cause damage if it is not recycled by other antioxidants such as vitamin C (Willson, 1983; Helliwell, 1996).

Vitamin C regenerated active tocopherol further reacts with peroxyl free radical (Packer., 1997). Vitamin C has also been shown to regenerate urate, glutathione and β-carotene *in vitro* from their respective one electron oxidation product i.e. urate radicals, glutathiyl radicals and β- carotene radicals cations (Edge and Truscott, 1997). Thus, ascorbic acid (Vitamin C) scavenges physiologically relevant ROS and reactive nitrogen species (Helliwell, 1996; Noroozi *et al*., 1998). Thus, pretreatment of *Rajgira* has provided considerable protection to inestinal mucosa by preventing crypt stem cells and causing an early recovery through early restoration of mitotic figures, and that has resulted in increase in crypt cell population and thereby an increase in villus height.

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