TINOSPORA CORDIFOLIA (MIERS) EXTRACT PROVIDES PROTECTION AGAINST RADIATION INDUCED ALTERATIONS IN INTESTINAL MUCOSA OF *SWISS ALBINO* MOUSE.

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Summary

Intestinal protection in mice against radiation injury by *Tinospora cardifolia* (TC)(5mg/kg/body weight/day) was studied from day ¼ to days 30 after whole body gamma radiation (6 Gy and 8 Gy). Villus length, goblet cells/villus Section/crypt Section, total cell population/villus or crypt Section, mitotic cells/crypt Section of jejunum are good parameters for the assessment of radiation damage. There was significant decrease in the villus length, total cell population and mitotic cells, whereas goblet cell count significantly increased after irradiation. *Tinospora cordifolia* pretreatment resulted in a significant increase in villus length, total cell population and mitotic cells, whereas goblet cells were lesser than those which were irradiated without TC at each autopsy day. The results suggest that *Tinospora cordifolia* pretreatment provides protection against radiation induced alterations in intestinal mucosa of *swiss albino* mouse.

Key words: *Tinospora cordifolia*, Radioprotection, Gamma radiation, Intestinal villi, Goblet cells, Total cell population, Mitotic cells.

Introduction

Study of plants as modifiers of radiation effects is done extensively during last two decades and several plants have been tested for the purpose. Plants being less toxic than the chemicals can be of great use in this field as all the chemical radioprotectors are toxic at the desired dose levels. There are several medicinal plants which are used in traditional and folk medicine as potent healers. TC is mentioned as “cure for all problems or “Tridosh Shamak” in ayurvedic system of medicine. It is called Guduchi or Giloe in Hindi. TC is used in a variety of combinations. During last few years scientists have worked out to test and verify various medicinal properties of this plant scientifically. TC has proved its immunomodulatory, antihepatotoxic, antistress, antileprotic and antimalarial, antioxidant, free radical scavenging, antiulcer, anticancer, antidiabetic, antispasmodic, and antiinflammatory activity. It is also found useful in chronic diarrhoea, removal of urinary stones, back aches and leg cramps in dysmenorrhoea. It is called Heart leaf moonseed in English.

Exposure to ionizing radiation has arisen as a new industrial and medicinal hazard and also a tool of experiments. The use of ionizing radiations in science and industry is now rapidly increasing. Radiations are a valuable tool in medicine. It is used for the diagnosis of disease or improper body function and for therapy of existing malignant or non-malignant conditions. Radionuclides are also being used in medical diagnosis and treatment. Persons recieving high doses of radiation suffers from their harmful effects which appear in all the organs.

Intestine is highly vulnerable to the radiation damage and severe damage to the gut may result in gastrointestinal syndrome. The cell renewal kinetics of villi of this segment are important. The renewal system lies in the crypt and villus where epithelial cell formation, migration and subsequent loss of aged cells occurs. This compartment suffers from marked damage. Destruction as well as mitotic inhibition occurs within the highly radiosensitive crypt,the proliferating cell compartment within an hour after exposer to high doses.

In the present study *Tinospora Cordifolia* was tested for its protective potential against Co^{60} gamma rays in the intestine of *swiss albino* mouse.

Materials and methods

Animals:

Adult male swiss albino mice (6-8 weeks old, weighing 25±2g) maintained in the animal house from an inbred colony were used. These were given standard mice feed and water ad-libitum. The temperature of animal house is maintained at 37±5°C and animals were kept in natural day light and dark night cycles.

Irradiation:

Irradiation was performed at Cobalt teletherapy cancer treatment centre, Radiotherapy Department, SMS Medical College and Hospital, Jaipur. 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 to deliver total dose of 6Gy and 8Gy to two different groups in a single exposure.

Tinospora Cordifolia Extract (TC):

Extract of TC (50% alcohol + 50% distilled water) obtained from Amsar Pharmaceutical Private Limited, Indore was used. The extract was dissolved in double distilled water and given at the dose rate of 5mg/kg body weight one hr. before irradiation. The dose of TC was selected on the basis of experiment conducted with different doses of plant extract for various treatment periods.

Experimental Design

Healthy animals were selected and divided into four groups. First of all the animals were divided into two groups, which were exposed to Co^{60} gamma rays. Both the groups were divided into two subgroups. The first subgroup of each group was irradiated only and the second subgroup of each group received TC extract one hour before irradiation. The third group received plant extract only at the same dose rate and the fourth group was sham irradiated only. The six animals were sacrificed at each of the ¼, 1, 2, 4, 7, 10, 14 and 28 days after irradiation and six animals were sacrificed at each interval. Intestine was removed and fixed in the Bouins fluid for histological examination. Transverse sections of 5µ thickness were cut and stained with Harris Hematoxylin and Eosin. Villus length, Total cell population, goblet cells and mitotic cells/crypt section were counted from the selected sections.

Statistical Analysis

The data were collected and on an average 60 Sections per animal were scored. The data were subjected to the student's 't' test for comparison between the groups. The values are expressed as mean ± SE and compared at $P<0.05$, <0.005 and 0.001 levels.

Results

Villus length

Control animals (irradiation alone) suffered from maximum damage in their intestinal mucosa. Both the 6Gy and 8Gy treated groups had extensive damage in the villi after irradiation. There was a great reduction in the size of the villi on 5th day (66.32 ± 2.48, P<0.001) in 6Gy and on 3rd day (70.00 ± 3.24, P<0.001) in 8Gy treated group.

In TC pretreated and then irradiated animals (Experimental group), the damage to the villi was less severe than the control group. The normal structure of the villi was restored by the 14th day post-irradiation (97.23 ± 2.32, P<0.05) in this group (Table 1, Fig. 1).

Total cell population in crypt

Total cell population in the intestinal crypt of the 6Gy treated animals decreased sharply till 5th day (38.16 ± 0.21, P<0.001) and on day 7th day it was (39.50 ± 0.22, P<0.001). The animals which were exposed to 8Gy the total cell population decreased upto day 3rd (34.33 ± 0.42, P<0.001) and reached to the normal within 28 days (39.50 ± 0.22). It was observed that in the TC pretreated animals total cell population was always higher in comparison to their respective controls (Table 2, Fig. 2).

Mitotic cells

Mitotic cell counts were minimum on 3rd day in both the control and experimental groups. After that they continuously increased and reached near normal on 10th day in both the experimental groups but not in the control groups. (Table 3, Fig. 3)

Goblet cells per villus section

In 6Gy treated group, the goblet cells in the villi significantly decreased at one day (P<0.05) and reached the minimum number on 5th day (51.87%) in the control group and then on the 10th day, near normal number of goblet cells in the villi was observed in the experimental group (P<0.001) but not in the control group.

In 8Gy treated group, the goblet cells had the same pattern of damage with more severity and were found minimum on the 7th day (27.4%) but animals could not survive uptill day 14th in the control group. In plant extract treated group i.e. experimental group goblet cells were maximum on 10th day and their number was not yet normal (Table 4, Fig.4)

Goblet cells per crypt section

After exposure to 6Gy gamma radiation, the goblet cell counts in the crypts decreased significantly (P<0.001) and touched the minimum level on the 5th day (Table 5). Cell counts increased at the subsequent intervals and reached near normal on 14th day in experimental animals, wich was significantly higher than the control (P<0.001).

In the group exposed to 8Gy goblet cell count was maximum on the 5th day (Table 5). Lesser recovery was observed at later intervals in comparison to the sublethal dose (6Gy). In experimental group goblet cell counts were more than the control group and minimum number was observed on day 5th. It started increasing on 7th day and continued to increase till 10th day. After that it decreased again. In only plant extract treated group goblet cells were maximum on 10th day and their number was found to be normal (Fig.5)

Table 1: Variations in the length of intestinal villi(in µm) of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	¼	1	3	5	7	10	14	28
6Gy	79.18±3.23 P<0.01*	78.82±3.22 P<0.01*	73.50±3.28 P<0.001*	66.32±2.48 P<0.001*	74.26±4.30 P<0.01*	77.03±2.89 NS	79.95±2.24 P<0.01*	91.80±2.02 P<0.01*
6Gy + Plant Extract	90.23±2.42 P<0.05**	89.92±2.41 P<0.05**	83.23±2.85 P<0.05**	78.93±3.40 P<0.05**	89.61±2.50 P<0.05**	91.10±2.50 P<0.01**	93.01±4.14 P<0.05**	95.10±2.40 P<0.01**
8Gy	86.21±3.10 NS	81.40±5.13 NS	70.00±3.24 P<0.001*	73.10±3.00 P<0.001*	76.24±2.31 P<0.001*	85.00±2.43 P<0.05*	Animals not survived	Animals not survived
8Gy + Plant Extract	98.73±3.23 P<0.05**	94.20±2.10 P<0.05**	85.30±3.05 P<0.05**	89.23±2.30 P<0.05**	90.02±3.21 P<0.01**	93.42±2.84 P<0.05**	97.23±2.32 -	99.02±4.14 -
Plant Extract Only	88.60±1.23	89.51±1.27	90.24±1.70	91.73±4.62	95.72±3.50	98.06±1.50	99.23±1.27	99.79±2.80

The length of intestinal villi in the healthy normal mouse without any treatment is = 92.20±2.02.
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

Table 2: Variations in the total cell population in intestinal crypt of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	1/4	1	3	5	7	10	14	28
6Gy	41.58±0.04 P<0.05*	39.00±1.13 P<0.05*	38.26±1.22 P<0.05*	38.16±0.21 P<0.001*	39.50±0.22 P<0.001*	40.00±0.36 P<0.001*	40.66±0.33 P<0.001*	41.33±0.42 NS
6Gy + Plant Extract	43.54±0.62 P<0.001**	43.29±0.65 P<0.01**	41.26±0.05 P<0.05**	39.33±0.42 P<0.05**	41.16±0.30 P<0.01**	41.80±0.20 P<0.01**	41.83±0.40 P<0.05**	42.00±0.25 P<0.05**
8Gy	36.00±0.44 P<0.001*	35.74±0.08 P<0.001*	34.33±0.42 P<0.001*	35.33±0.21 P<0.001*	35.83±0.30 P<0.001*	37.33±0.33 P<0.001*	Animals not survived	Animals not survived
8Gy + Plant Extract	38.16±0.30 P<0.01**	37.27±0.68 P<0.05**	35.65±0.05 P<0.05**	36.66±0.51 P<0.001**	37.16±0.47 P<0.05**	38.50±0.22 P<0.01**	38.83±0.16 -	39.50±0.22 -
Plant Extract Only	41.66±0.42	41.50±0.34	40.50±0.22	40.83±0.30	41.16±0.16	41.33±0.21	41.83±0.30	41.90±0.67

The total cell population in crypts of the healthy normal mouse without any treatment are = 41.76±0.05
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

Table 3: Variations in the number of mitotic figures in intestinal crypts of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	1/4	1	3	5	7	10	14	28
6Gy	2.13±0.16 P<0.01*	2.01±0.03 P<0.001*	1.92±0.02 P<0.001*	2.00±0.03 P<0.001*	2.10±0.07 P<0.001*	2.35 ±0.06 P<0.05*	2.50±0.15 NS	2.61±0.20 NS
6Gy + Plant Extract	2.80±0.09 P<0.01**	2.60±0.09 P<0.001**	2.00±0.03 P<0.05**	2.37±0.13 P<0.05**	2.62±0.18 P<0.05**	2.80±0.12 P<0.01**	2.90±0.12 P<0.05**	3.18±0.11 P<0.05**
8Gy	1.92±0.02 P<0.05*	1.70±0.05 P<0.001*	1.40±0.36 P<0.05*	1.47±0.19 P<0.01*	1.50±0.062 P<0.001*	1.78±0.06 P<0.001*	Animals not survived	Animals not survived
8Gy + Plant Extract	2.52±0.18 P<0.01**	2.21±0.10 P<0.001**	2.11±0.30 P<0.05**	2.27±0.13 P<0.01**	2.60±0.01 P<0.001**	2.80±0.09 P<0.001**	2.85±0.12 -	2.95±0.13 -
Plant Extract Only	2.60±0.16	2.45±0.12	2.30±0.10	2.42±0.16	2.46±0.06	2.68±0.14	2.89±0.13	2.97±0.14

Mitotic figures in the intestinal crypts of healthy normal mouse without any treatment are = 2.83±0.15.
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

Table 4: Variations in the number of goblet cells in intestinal crypts of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	1/4	1	3	5	7	10	14	28
6Gy	3.39±0.04 P<0.001*	3.30±0.02 P<0.001*	3.28±0.02 P<0.001*	3.25±0.02 P<0.001*	3.26±0.06 P<0.001*	3.27±0.05 P<0.001*	3.23±0.02 P<0.001*	3.20±0.04 P<0.001*
6Gy + Plant Extract	4.57±0.06 P<0.001**	4.50±0.02 P<0.001**	4.48±0.04 P<0.001**	4.38±0.03 P<0.001**	4.40±0.03 P<0.001**	4.35±0.02 P<0.001**	4.31±0.03 P<0.001**	4.26±0.05 P<0.001**
8Gy	3.18±0.03 P<0.001*	2.96±0.02 P<0.001*	2.89±0.06 P<0.001*	2.73±0.02 P<0.001*	2.80±0.06 P<0.001*	2.82±0.06 P<0.001*	Animals not survived	Animals not survived
8Gy + Plant Extract	3.41±0.04 P<0.001**	3.36±0.06 P<0.001**	3.33±0.03 P<0.001**	3.30±0.02 P<0.001**	3.35±0.03 P<0.001**	3.38±0.07 P<0.001**	3.31±0.01 -	3.25±0.05 -
Plant Extract Only	4.28±0.03	4.26±0.02	4.23±0.03	4.20±0.05	4.24±0.01	4.29±0.06	4.27±0.02	4.26±0.03

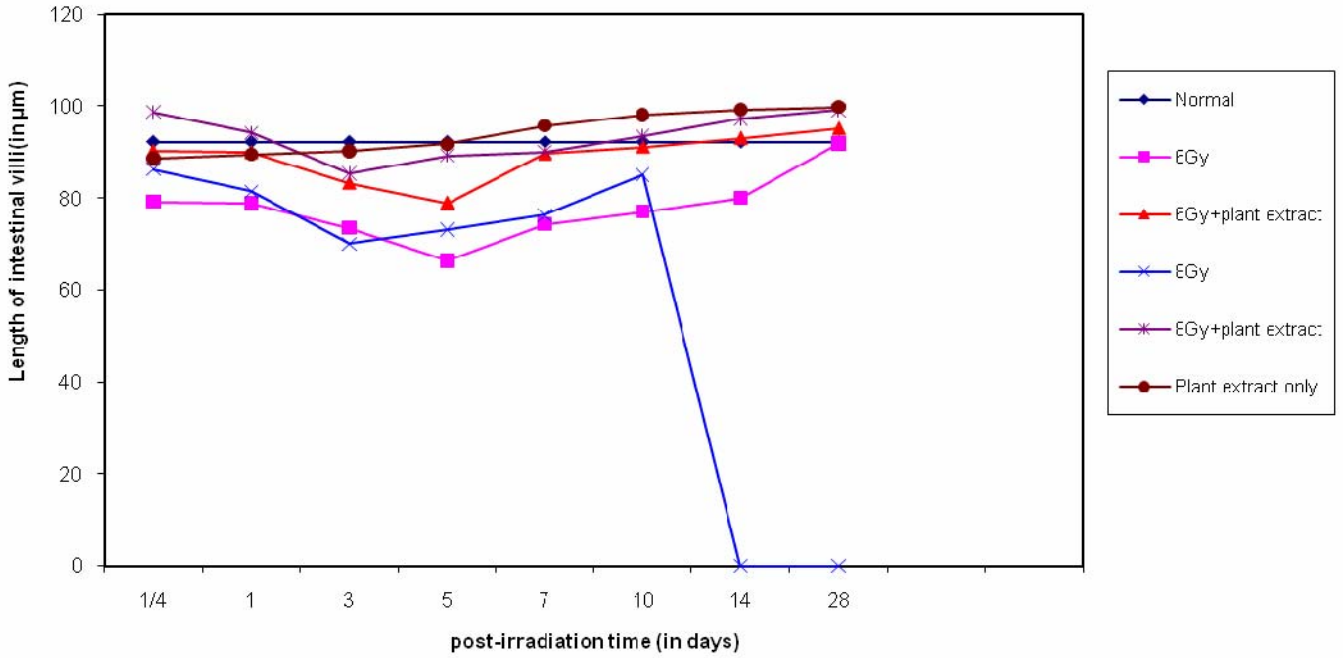
Goblet cells in the intestinal crypt of the healthy normal mouse without any treatment are = 4.30±0.08
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

Table 5: Variations in the number of goblet cells in intestinal crypts of Co^{60} Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment.

Treatment	Post-irradiation time(in days)							
	1/4	1	3	5	7	10	14	28
6Gy	6.21±0.55 P<0.05*	6.10±0.46 P<0.01*	5.42±0.82 P<0.05*	4.15±0.24 P<0.001*	6.01±0.27 P<0.001*	5.93±0.30 P<0.001*	5.62±0.22 P<0.001*	5.47±0.40 P<0.001*
6Gy + Plant Extract	8.40±0.48 P<0.05**	7.88±0.60 P<0.05**	7.70±0.42 P<0.05**	6.22±0.30 P<0.001**	7.50±0.11 P<0.001**	7.54±0.23 P<0.01**	7.30±0.14 P<0.001**	6.90±0.33 P<0.05**
8Gy	4.36±0.26 P<0.001*	3.64±0.17 P<0.001*	3.12±0.16 P<0.001*	2.80±0.13 P<0.001*	2.20±0.10 P<0.001*	2.90±0.20 P<0.001*	Animals not survived	Animals not survived
8Gy + Plant Extract	5.42±0.32 P<0.05**	4.20±0.18 P<0.05**	4.01±0.15 P<0.01**	3.81±0.18 P<0.01**	3.50±0.20 P<0.001**	3.81±0.10 P<0.001**	3.10±0.17 -	2.90±0.13 -
Plant Extract Only	7.62±0.43	7.31±0.12	6.95±0.30	6.84±0.36	7.21±0.13	8.27±0.23	8.00±0.76	7.82±0.83

Goblet cells in the intestinal villi of the healthy normal mouse without any treatment are = 8.00±0.14.
P value = Control Vs Normal* Control Vs Experimental** NS= Not Significant
Experimental = The plant extract was given 1 hr before irradiation at the dose rate of 5mg/kg body weight.
Control = Irradiated only

Fig 1: Variations in the length of intestinal villi (in µm) of C060 Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment



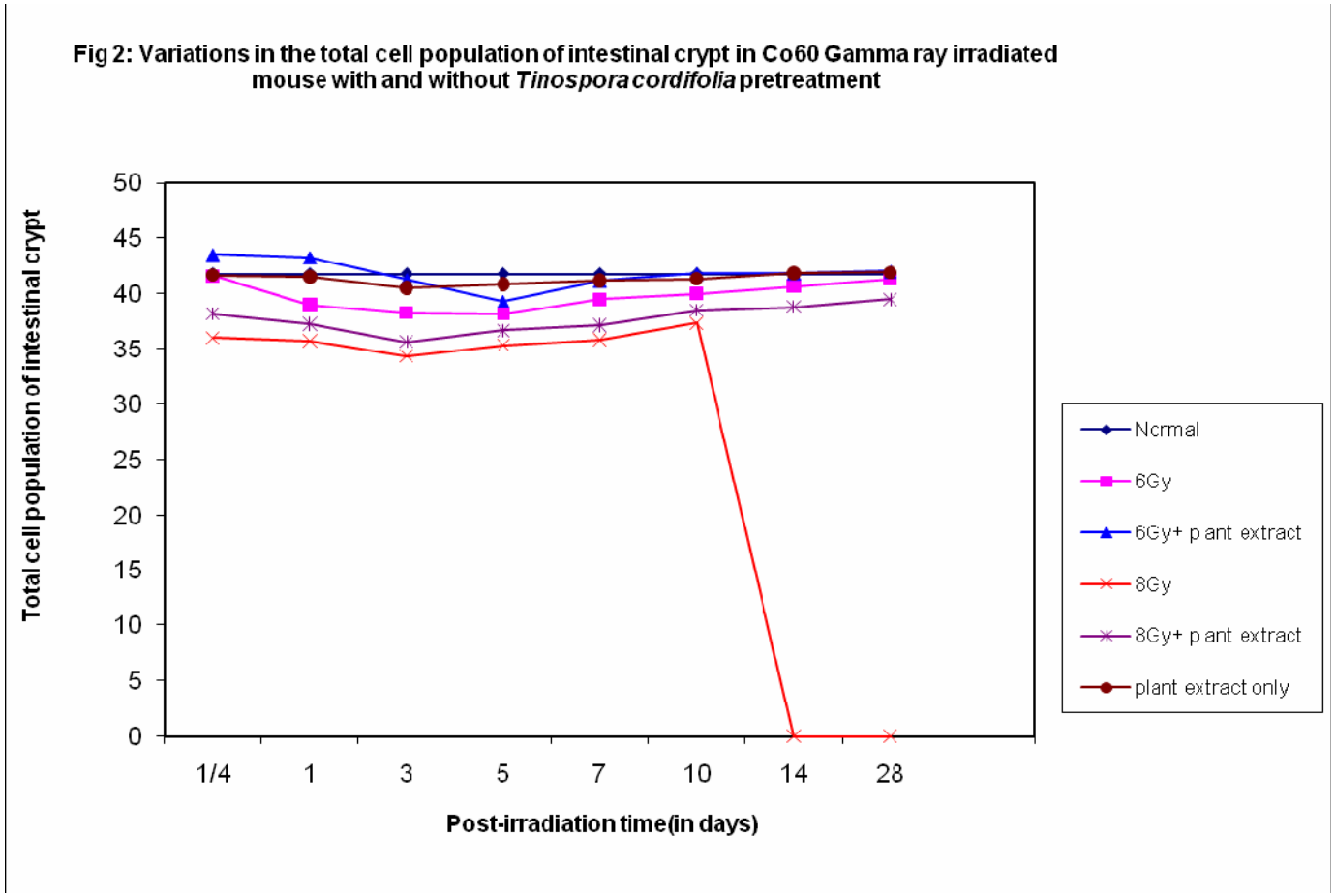
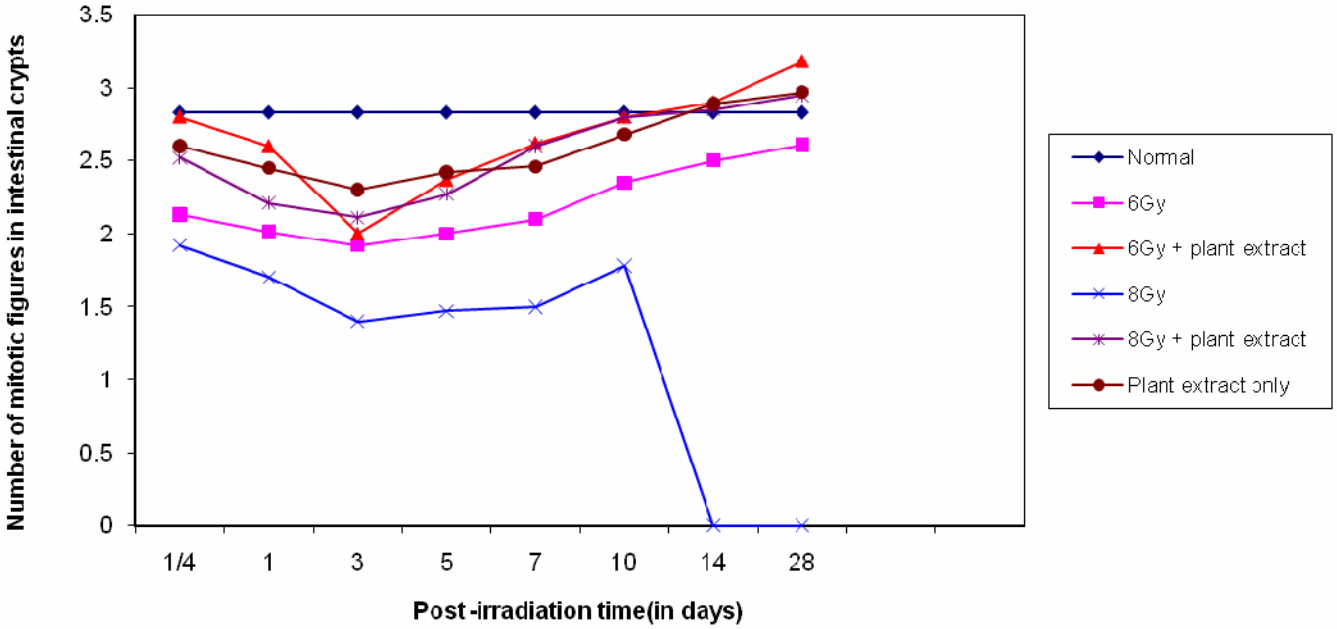


Fig 2: Variations in the total cell population of intestinal crypt in Co60 Gamma ray irradiated mouse with and without Tinospora cordifolia pretreatment
Total cell population of intestinal crypt
50
45
40
35
30
25
20
15
10
5
0
1/4
1
3
5
7
10
14
28
Post-irradiation time(in days)
Ncrmal
6Gy
6Gy+ p ant extract
8Gy
8Gy+ p ant extract
plant extract only

Fig 3: Variations in the number of mitotic figures in intestinal crypts of Co60 Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment



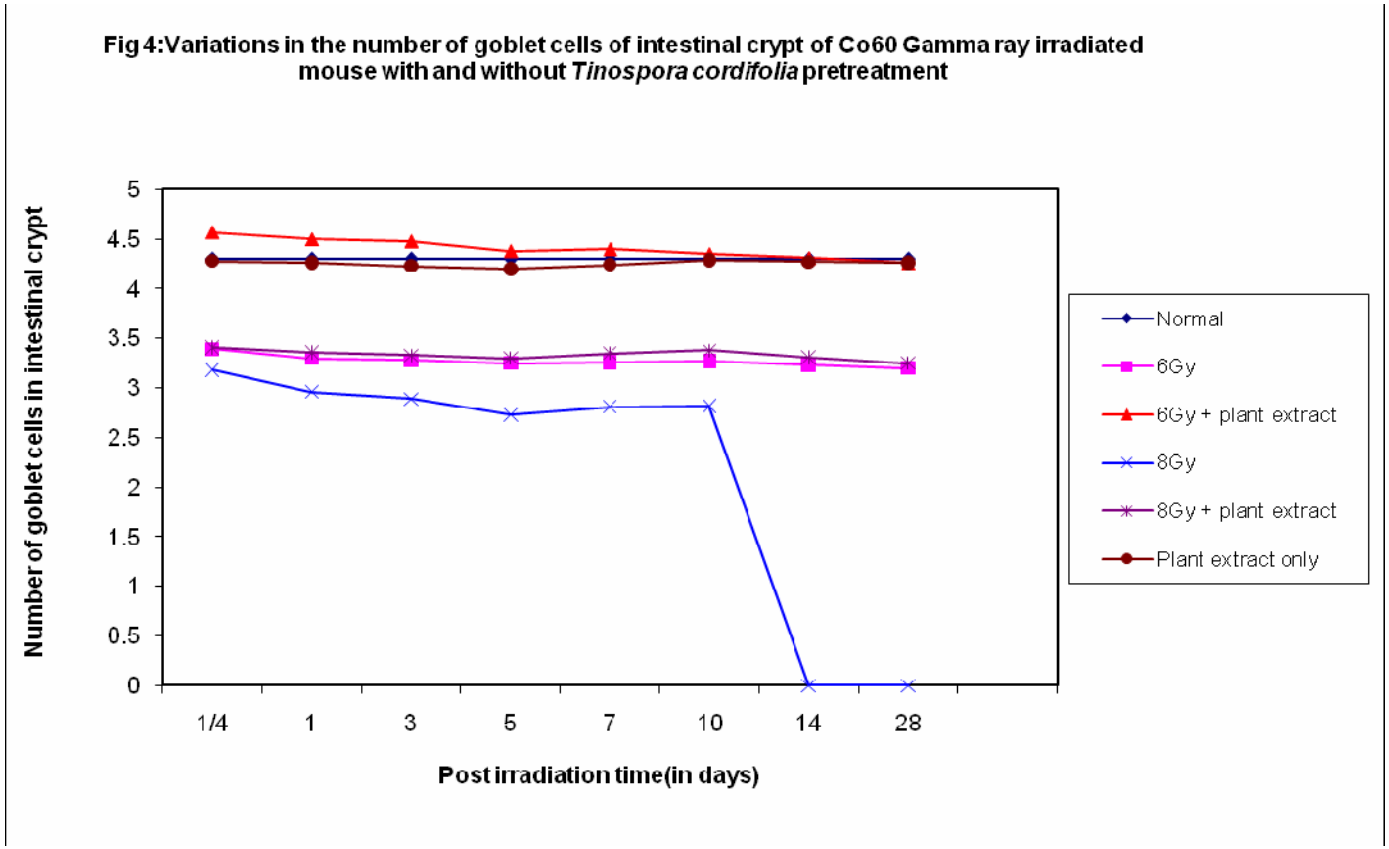
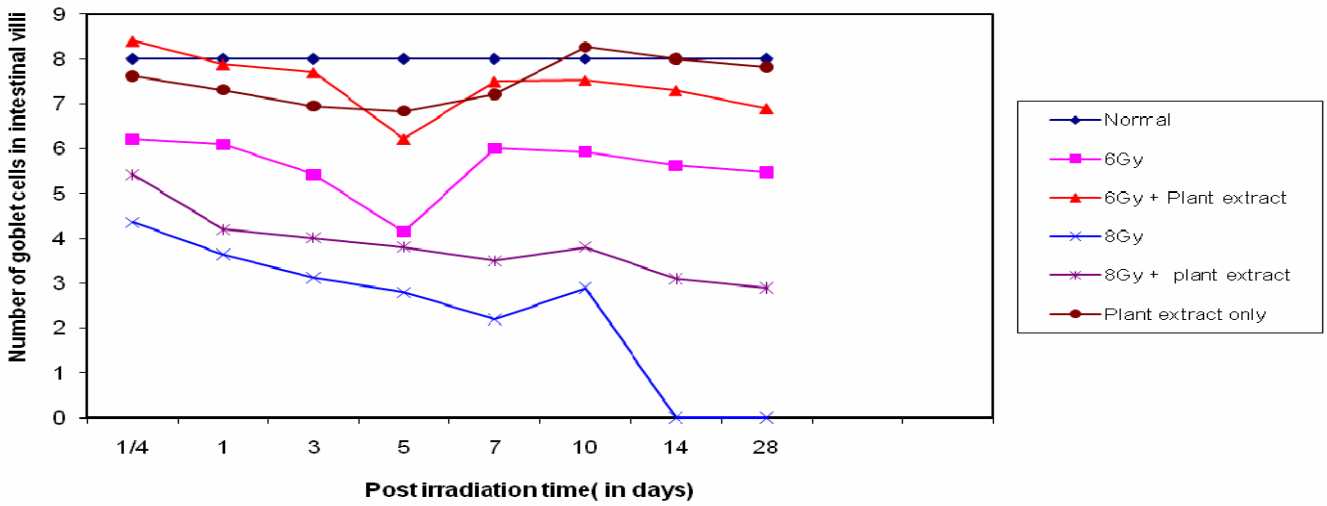


Fig 4:Variations in the number of goblet cells of intestinal crypt of Co60 Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment
Number of goblet cells in intestinal crypt
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1/4
1
3
5
7
10
14
28
Post irradiation time(in days)
Normal
6Gy
6Gy + plant extract
8Gy
8Gy + plant extract
Plant extract only

Fig 5: Variations in the number of goblet cells of intestinal villi of Co60 Gamma ray irradiated mouse with and without *Tinospora cordifolia* pretreatment



Discussion

Small intestine constitutes one of the most radiosensitive segment of the gastrointestinal tract, because its epithelium is one of the cell renewal system which is readily perturbed by ionizing radiation.[1)]Animals usually die due to radiation induced gastrointestinal syndrome(GI syndrome) resulting from a combination of direct cytocidal effects on intestinal crypt and endothelial cells and subsequent loss of the mucosal barrier, leading to in electrolyte imbalance, diarrhoea, weight loss, infection and mortality.[2)]Death of animals due to GI syndrome occur on 10th day after irradiation.

Normal homeostasis of intestinal epithelium is maintained by an intricate cell replacement process in which terminally differentiated epithelial cells are continuously and rapidly replaced by replication and differentiation of epithelial cells (transit cells) located within the intestinal crypt. Radiation induced gastrointestinal syndrome is due in part to the killing of clonogenic crypt cells with eventual depopulation of the intestinal villi.[3-4)]

Intestinal crypt is the proliferative unit supplying cells for the maintenance of villus integrity and as such assumes a central role in the intestinal response to radiation exposure.[5)] Crypt epithelial cells proliferate rapidly and are highly sensitive to irradiation. Loss of this regenerating population of clonogenic cells following irradiation prevents the normal reepithelization of the intestinal villi. This impairment leads to varying degree of villus blunting and fusion, with attenuation and hypertrophy of the villus epithelial cells.[6)]

The late side effects and the sequelae of severe acute intestinal radiation injury include varying degrees of intestinal inflammation, mucosal thickening, collagen deposition, and fibrosis, as well as impairment of mucosal and motor functions.[7-9)]

Ionizing radiation affects the cells in all phases of the proliferating cycle but the degree is dependent on the phase in which they were at the time of irradiation.[10)] Decline in the percentage of mitotic cells at early intervals may be attributed to a block of cells in G_2 phase of cell cycle and to prolongation of mitotic process.[11)] Dividing cells are highly radiosensitive and their direct killing by radiation exposure may be one of the major factors of reducing the percentage of dividing cells in the present study.

The intestinal stem cells reside in the crypts of Lieberkuhn at the base of the finger like villi that compare the intestinal epithelium. Epithelial cells differentiate as they migrate from the crypt and to the villus and are eventually sloughed off at the tip into intestinal lumen. The transit time is 3-4 days. In the small intestine of mouse , a wave of apoptosis is observed in what are thought to be crypt stem cells.[12)]

Epithelium which is destroyed by radiation is regenerated by restoration of normal mucosal barrier. First, the epithelial layer is re-established by migration of adjacent epithelial cells over the wound or damaged area. Stem cells within surviving crypts proliferate to give rise to progenitors of more rapidly proliferating transit cell population. The transit cell population then quickly expands to form a regenerative crypt. If some crypts are destroyed, the surviving crypt stem cells can divide to increase their numbers and subsequently, restore sufficient number of crypts by crypt fission, to maintain epithelial homeostasis.[13)] Potten *et al.*[14)] explained the reduction in the crypt cellularity on the basis that migration of cells from the crypt to the villus continues over the first 24hrs after irradiation , inspite of the fact that the cell division is inhibited or severely reduced. The increase in goblet cell number can be attributed to discharge of these cells after irradiation. Radiation exposure slows down the normal diffentiation process[15)] and reduction in the number of goblet cells was observed during early intervals.

Withers *etal*[16)]found a marked increase in the number of regenerating crypts after experimental injury. This not only occurred in area of inflammation , but also throughout the small intestine. These regenerating crypts, arise after irradiation from the survival of one or more clonogenic stem cells that were subsequently able to proliferate.

Haton *etal*[17)]administered Dexamethasone to investigate the relationship between variations in mucosal antioxidant capacity and radiation induced inflammation. Six hours after exposure, only mitochondria associated antioxidant systems were induced and the antioxidant mechanisms of the small intestinal mucosa were not markedly mobilized during the very acute tissue radiation response. During the radiation induced acute inflammatory response, the antioxidant capacity appeared to be dependent on inflammatory status to a certain extent. According to Vander *et al*[18)]a systemic inflammatory reaction was found after abdominal and total body irradiation. Radiation induced changes in the production of cytokines and chemokines and in the expression of adhesion molecules, indicate a possible abscopal effect of radiation in intestine.

In a recent study Battle and Colleagues[19], defined some of the molecular mechanisms that regulate the inflammatory phenomenon. Their work demonstrated that cell positioning in the intestine is mediated by the interaction of ephrins and their Eph receptors, which are localized on the surface of intestinal epithelial cells.

A number of cytokines, growth factors, and other mediators associated with intestinal inflammation or injury can induce increase in crypt stem cell survival when exogenously administered before radiation. These include TNF-□, TGF-β3, TFF-3, IL-1, IL-II, Cox-1, Cox-2, FGF-2, FGF-7 and FGF-10.[20-21]

Administration of recombinant IL-II before irradiation can enhance intestinal stem cell survival. R-spondin 1 (Rspo1) acts as a mitogenic factor for intestinal stem cells. Rspo1 has protective effect and thereby increase the therapeutic ratio of chemoradiation therapy in patients undergoing abdominal irradiation for gastrointestinal malignancies.[22]

When fibroblast growth factor (FGFC) was intraperitoneally administered to BALB/c mice prior to gamma irradiation, survival of small intestinal crypts was significantly enhanced as compared to control mice and also showed greater structural stability, biological activity, resistance to trypsinization and augmented activity useful for epithelial proliferation. These results showed that FGFC could be useful in wound healing and protection against radiation induced damage.[23]

Tinospora cordifolia pretreated animals had a significant by more villus length. The number of total cells and mitotic figures was also found to be significantly higher than the control but the number of goblet cells was higher in control group as compared to *Tinospora cordifolia* pretreated irradiated animals. *Tinospora cordifolia* (Miers) is considered to be a Rasayana, Medha and anti-aging drug in ayurveda. TC has antioxidant activity, free radical scavenging and metal chelation activity .[24] TC is reported to activate macrophages (attenuation of radiation-induced decrease in adherence and spreading), Reduced apoptosis increased cell proliferation and increased IL-1B and GM-CSF levels may be responsible for impressive radioprotective efficacy.[25] According to Nair *etal*[26] that ¼ /alpha/D/glucan present in TC activates the macrophages through TLR6 signaling, NF/kappa B translocation and cytokine, production. Alcoholic extract of TC restorted in a tumor bearing mice by down regulating Thymocyte apoptosis through modulation of caspase pathway.[27] Enhanced humoral and cell mediated immunity is reported by Syringin (TC-4) and Cordial (TC-7) in rats. These substances reduce immunohaemolysis and significantly increase IgG antibodies.[28]

The aqueous stem extract of *Tinospora cordifolia* has been reported to prevent abdominal infections and sepsis, to improve kupffer cell function and poly-morphonulear cell-mediated phagocytosis in rats with chronic liver damage and in the patients of surgical jaundice.[29] According to Wazir *et al*. it is due to arabinogalactan polysaccharide present in the extract. *Tinospora cordifolia* contains β-sitosterol and a polysaccharide □-D- Glucan which have also shown radioprotective and antioxidant properties.[30-31] A polysaccharide obtained from TC is reported to activate natural killer cells, T cells and B cells at 100 micro g/ml. It is reported to enhance cell mediated immunity and

complement system.[32] Cordioside, cordiofolioside, cordiofolioside B and clerodane furanoditerpene glucosides have been reported to have immunostimulatory properties.[33]

The extract quenched radiation-mediated 2–deoxy-ribose degradation in a dose dependent fashion and inhibited the formation of Fe^{2+} bipiridyl complex and formation of the comet tail in irradiated thymocytes by chelating Fe^{2+} ions. It also inhibited ferrous sulphate mediated lipid peroxidation in liver homogenate. Their further work concluded that aqueous extract of TC increases survival of the mouse in the animals irradiated with 10Gy of gamma radiation.[34]

A small dose of TC (5mg/kg body weight per day) to swiss albino mice 1 hr prior to lethal (6Gy) dose of gamma radiation increased the survival of animal to a significant extant.[35]

TC is also reported to interfere with the activity of P- Glycoprotein in the intestine which functions as an ATP- dependent transporter pump in the apical brush border of the intestinal membrane. This altered permeability might have facilitated absorption of nutrients. [36]

It is clear from the results that TC not only prevented radiation induced damage to the intestine but also speeded up the recovery process .Radiation induced inflammation, cell death, discharge of goblet cells and shrinkage of the villi were prevented by TC which is visible at early intervals. At later intervals early and speeded recovery is seen which is due to presence of TC.

Aqueous extract of TC is effective against fructose induced insulin resistance and oxidative stress, hence TC could be used as an adjuvant therapy for the prevention and/or management of chronic disease characterized by hyperinsulinemia, hypertriglyceridemia , insulin resistance and aggravated antioxidant status.[37]
Thus, the results from the present study suggests that pretreatment of TC extract protects mouse small intestine against the radiation induced reduction in villus length, total cell population and mitotic figures/crypt section and increase in goblet cells in small intestine of mouse. It works at very low dose without causing side effects. Hence, it can be used as a radio protector.

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