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Evaluation of Tissue Protective activity of *Tinospora cordifolia* stems in irradiated Swiss albino mice with ^{60}Co radiation

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Abstract

In the present study, wholesome tissue protective efficiency of the ethanolic extract of *Tinospora cordifolia* stems in mice in terms of whole body survival, genotoxicity, cell proliferation and hematological profile have been investigated. The animals were divided into four groups having six animals in each. Group I, (Control), received vehicle, Group II received orally ethanolic extract of *Tinospora cordifolia* (500mg/kg), Group III was irradiated with ^{60}Co radiation (external gamma radiation of 4 GY for 24h), and animals of Group IV were irradiated with ^{60}Co radiation (external gamma radiation of 4 GY for 24 h) and given orally ethanolic extract of *Tinospora cordifolia* (500mg/kg), for seven days. On the 8th day all the animals were sacrificed, the blood was collected and hematological cell profile was analyzed. Intestine was removed and its histopathology was done and their mitotic index and crypt of villi was counted. The drug shows significant tissue protective activity ($P<0.05$) and shows normalization in hematological cell profile in irradiated animals.

Keywords: Tissue protective, *Tinospora cordifolia*, ^{60}Co radiation

1. Introduction

Tinospora cardifolia Miers (Menispermaceae) is an Indian medicinal plant a perennial climber, is well used for the treatment of various ailments for its adaptogenic and immunomodulatory activity in fighting infections [1, 2]. Stem is used as a general tonic, anti-inflammatory, antitumor, antiarthritic, antimalarial, antidiabetic, antiallergic and as an aphrodisiac. The roots are used for its antistress, antileprotic and antimalarial activity. Alkaloids belonging to isoquinoline group viz. berberine, palmatine, tembetarine, magnoflorine, cordifolisides A-E and two phenylpropane derivatives and some sterols have been isolated from stem [3, 4, 5].

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Many synthetic and natural agents have been investigated in the recent past for their efficacy to protect against radiation damage. Synthetic molecules induce inherent toxicity in doses sufficient to produce radioprotective action and this warrants further search for safer and effective radioprotective molecules. Some isolated plant products and crude extracts may have radioprotection through various mechanisms such as free radical scavenging, calcium channel blocking, inhibition of lipid peroxidation, enhancement of DNA repair and stimulation of stem cell proliferation are considered important. The present study is proposed to investigate the tissue protective efficiency in experimental animals, in term of whole body survival, genotoxicity, cell proliferation and hematological parameters.

2. Materials and Methods

2.1 Plant material

The stems of *Tinospora cordifolia* were collected from the local area of Bhopal and authenticated by the taxonomic division, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic and Resources, New Delhi. A voucher specimen (NHCP/NBPGR/2005/90/4071) was retained in our laboratory for further reference.

2.2 Plant extract

The plant material was dried under shade and then reduced to moderately course powder in a mechanical grinder. The powdered material was extracted successively with petroleum ether (60-80°C) and ethanol using soxhlet apparatus.

Table 1. Effect of the ethanolic extract of *Tinospora cordifolia* stems in irradiated mice on different Hematological Parameters. The values are expressed as Mean ± SE.

Sl. No	Groups Hematological Parameters	Group I (Control)	Group II (Irradiated with ^{60}Co radiation)	Group III (Treated with EETC)	Group IV (Irradiated with ^{60}Co radiation + Treated with EETC)
1.	RBC (millions/mm3)	5.09 ± 0.111	4.198 ± 0.097	5.072 ± 0.106	5.27 ± 0.162
2.	Hb (g/dl)	16.2 ± 0.406	12.8 ± 0.406	16.1 ± 0.331	16.5 ± 0.180*
3.	TLC (Cells/mm3)	15540 ± 317.18	11960 ± 578.46	15400 ± 291.55*	15106 ± 108.79
4.	PCV (%)	51.0 ± 2.549	39.4 ± 3.544	52.2 ± 1.200*	51.2 ± 2.709
5.	MCV (mm3)	100.01 ± 3.168	67.82 ± 8.419	103.15 ± 3.655	92.27 ± 4.812
6.	Platelet Count (Cells/mm3)	94000 ± 7483.54	67000 ± 6945.71	100000 ± 8366.85	108000 ± 7348.69*
7.	Differential LeucocytesCount				
	Lymphocytes (%)	67.8 ± 0.860	548.0 ± 0.547	63.4 ± 2.271*	62.6 ± 3.010
	Neutrophils (%)	28.0 ± 0.707	21.8 ± 1.462	33.6 ± 2.064	33.8 ± 2.799*
	Eosinophils (%)	2.2 ± 0.200	2.8 ± 1.019*	2.1 ± 0.663	2.6 ± 0.244
	Monocytes (%)	2.0 ± 0.707	1.8 ± 0.400	2.3 ± 0.800	2.0 ± 0.316
	Basophils (%)	00	00	00	00

*$p<0.05$ Significant compared to control. n = 6, EETC: Ethanolic extract of *Tinospora cordifolia*

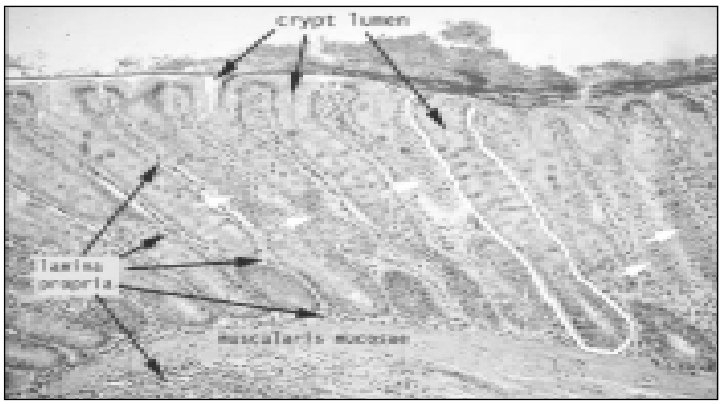

Fig 1. Histopathological observations of the T.S. of intestine of control groups and showing normal histological appearance in crypt of villi and mitotic index

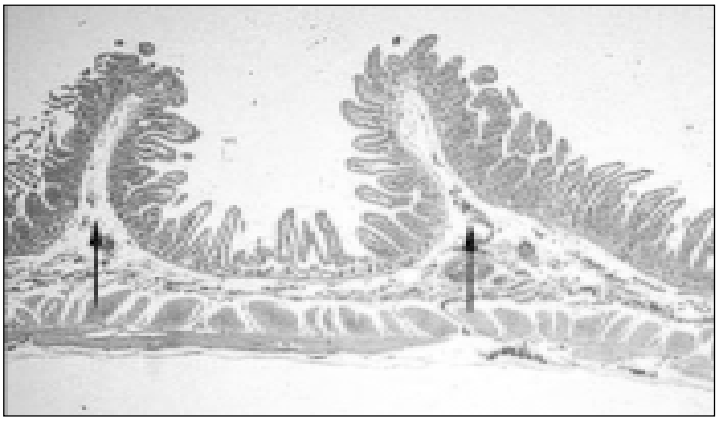

Fig 2. Histopathological observations of the T.S. of intestine of irradiated groups and disturbances in crypt of villi and mitotic index

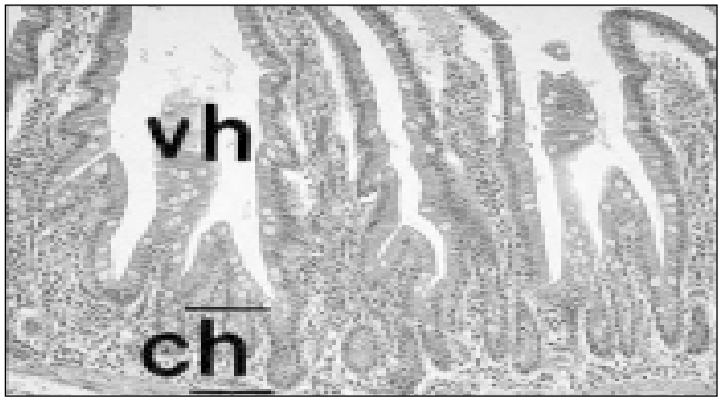

Fig 3. Histopathological observations of the T.S. of intestine of treated groups and showing recovery in crypt of villi and mitotic index. vh - villi hyphen, ch - crypt of villi hyphen.

The ethanol extract was dried under vacuum (yield 9.1% w/w). Preliminary qualitative analysis of the extract showed the presence of alkaloid, tannin, glycoside, saponins, reducing sugar and triterpenes. The ethanolic extract of *Tinospora cordifolia* (EETC) was used for the tissue protective studies.

2.3 Animals

The Institutional Ethical Committee approved the use of animals (Ethical clearance number: 711/02/a/CPCSEA). Swiss albino mice of both sexes (20±2g) were housed in polypropylene cages (38X23X10 cm) with six animals per cage under standard laboratory condition (25±2°C, 12/12 h dark and light cycle). They were allowed free access to standard pellet diet (Hindustan Lever, Kolkata, India) and water ad libidum.

2.4 Assessment of tissue protective activity

For tissue protective activity animals were divided into four groups. Group I served as control and received vehicle (2% gum acacia 1ml/kg), Group II received ethanolic extract of *Tinospora cordifolia* (500mg/kg), Group III irradiated with ^{60}Co radiation (external gamma radiation of 4 GY for 24 h), and animals of Group IV were irradiated with ^{60}Co radiation (external gamma radiation of 4 GY for 24 h) + ethanolic extract of *Tinospora cordifolia* (500 mg/kg). All the doses were given by gastric intubations for seven days. On the 8th day all the animals were sacrificed under light ether anesthesia, blood was collected, and hematological cell profile such as WBC, RBC, DLC, TLC, Hb% were recorded [6]. Intestine was removed and its histopathology was done and their mitotic index and crypt of villi were counted [7].

3. Results and Discussion

Cancer is a basically a disease of cell characterized by loss of normal cellular growth, maturation, multiplication and thus their homeostasis is disturbed. Application of radiotherapy inevitably leads to fall in normal hematological cell profile and consequent damage to normal body tissues. The animals of Group III irradiated with ^{60}Co radiation registered a fall in hematological cell profile, compared with control, whereas the group IV which is irradiated and given EETC shows a significant normalization in hematological cell profile (Table 1). Further the histopathological investigation of intestine of the animals of group III irradiated shows decrease in no of villis and mitotic index (Figure: 2) where as the group which received its ethanolic extract after the radiations shows the recovery in histopathological observations (Figure: 3). Thus the EETC shows a significant tissue protective activity in irradiated animals.

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