J. Phytol. Res. 23(2) : 253-258, 2010

RADIOPROTECTION OF MOUSE PERIPHERAL BLOOD BY A SMALL DOSE OF *TINOSPORA CORDIFOLIA, IN VIVO* STUDIES

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Irradiation to a lethal dose of gamma radiations causes severe changes in the peripheral blood. RBCs and various types of WBCs are affected to a great extent. Their number drops significantly in the irradiated animals causing anemia and loss of normal immune function. *Tinospora cordifolia* (Miers), a medicinal plant when administered orally at the rate of 5 mg/kg body weight one hour before irradiation prevented changes in the peripheral blood cells significantly. The animals were irradiated with and without *T. cordifolia* aqueous extract with Co^{60} gamma rays and were sacrificed at 6 h, 1, 3,5,7 and 10 days post irradiation. Blood was collected by heart puncture and analysed for cellular parameters. It was observed that there was statistically significant difference between the groups irradiated with and without *T. cordifolia*. Results obtained are discussed in detail.

Keywords : Gamma radiations; RBCs; *Tinospora cordifolia;* WBCs.

Introduction

Medicinal plants have been used to cure human illness since time immemorial. Some of these are believed to promote positive health and maintain organic resistance against infection by re-establishing body equilibrium and conditioning the body tissues. The folk use of plants in medicine is as old as the existence of the mankind. *Tinospora cordifolia* is one of them. It is a large glabrous deciduous climber belonging to family Menispermaceae. It has cordate leaves and yellow flowers. Its fresh juice is of medicinal value. All the parts of the plant are used to cure several ailments. It is native to India and distributed throughout the tropics of Asia, Africa and Australia. Besides folk medicine and traditional medicinal systems, it is now being tested by modern techniques for various purposes. Its pharmacology is now well known. *Tinospora cordifolia* (TC) is also known to reduce blood glucose level in diabetic patients[1]. Rege *et al.*[2] found it useful in reduction of Carbon tetrachloride induced hepatic damage. Pulverised fruit of *T. cordifolia* is used as a tonic and also for the treatment of jaundice and rheumatism. The stem is bitter, stomachachic, diuretic, stimulates bile secretion, causes constipation, allays thirst, burning sensation, vomiting, enriches the blood and cures jaundice. It is used as antidote of snake bile alognwith other medicines. It has immunomodulating activities also. Recently its antioxidant, free radical scavenging and anti lipid peroxidation activity is also reported[3]. It is also proved to be a tonic and vitalizer to the body.

In the present study effect of oral administration of a small dose (5 mg/kg body weight) of *T. cordifolia* was observed on peripheral RBCs and WBCs of *Swiss albino mouse* whole body irradiated with 8 Gy of Co^{60} gamma radiation.

Material and Methods

Animals - Swiss albino mouse (Mus musculus) were bred in the laboratory and kept under controlled conditions of temperature and light. They were fed pellated standard mice feed and water *ad libitum*. They were kept in polypropylene cages (15"x10"x6") on saw dust as bedding material. Healthy adult males of 6-8 weeks of age and 25±2g body weight were selected for the experiments.

Irradiation - Animals were irradiated with 8 Gy at Radiobiology and Radiotherapy Department, SMS Medical College and Hospital, Jaipur with Co^{60} teletherapy source.

Tinospora cordifolia extract (TE) - Aqueous extract of *Tinospora cordifolia* (Miers) dried extract was prepared, dried and powdered.

Dose selection was done on the basis of survival experiment. $LD_{50/30}$ was calculated and optimum dose of the plant extract was selected[4]. For this purpose one group of mice was irradiated to a lethal dose of 8 Gy Co^{60} gamma irradiation. The second group was given *T. cordifolia* extract dissolved in distilled water at different dose rates before irradiation to 8 Gy Co^{60} gamma rays. Then survival

of mice was recorded for 30 days. It was found that 5 mg/ kg body wt. of *T. cordifolia* extract provides sufficient protection to this group.

Experimental design : Selected adult male mice were divided into following four groups

Group I : Animals of this group were sham irradiated.

Group II: Animals of this group were given *T. cordifolia* extract one hour before irradiation (8 Gy) to Co^{60} gamma rays at the dose rate of 5 mg / kg body weight orally.

Group III : Animals of this group were irradiated with 8 Gy of Co^{60} gamma rays and given equal amount of double distilled waster as given with the *T. cordifolia* extract.

Group IV : Animals of this group received *T. cordifolia* extract only at the dose rate of 5 mg / kg body weight orally.

The animals of all the groups were sacrificed by cervical dislocation at 1/4, 1, 3,5,7 and 10 days post treatment. At least 6 animals of each group were sacrificed at each interval.

The blood was collected by heart puncture and blood cells were counted by automatic blood analyzer. The data were subjected to students 't' test. Significance level of the difference is expressed in the form of P value.

Results and Discussion

Results are presented in Table 1 and 2. Whole body irradiation to 8 Gy of Co^{60} gamma rays causes drastic reduction in the weight of spleen and thymus of *Swiss albino mouse* till 5th day post irradiation. This decrease was also observed in *Tinospora cordifolia* extract (TE) preteated and then irradiated animals but it was lesser as compared to those which were irradiated without *T. cordifolia* extract. The animals which were irradiated without *T. cordifolia* extract could not survive after 10th day post irradiation while those, which were irradiated with *T. cordifolia* extract pretreatment survived during the whole experimental period. Total leucocyte counts (TLC) decreased significantly after irradiation and remained below normal till the end of the experimental period. In *T. cordifolia* extract pretreated animals decrease in TLC was significantly lesser and it reached to near normal 5 days after irradiation. Differential leucocyte counts (DLC) show that all the types of WBCs except neutrophils decrease in number after irradiation. It seems to be due to direct destruction of these cells in the peripheral blood. Lymphocytes are the most radiosensitive cell type amongst blood cells.

The neutrophils increased 6 hrs after irradiation and this increase continued which may be due to abortive rise phenomenon. Early maturation of granulocyte precursors in bone marrow and their release in circulation may be another factor.

Neutrophils are phagocytic cells, when they are activated during phagocytosis, they generate O_2^- and H_2O_2 through NADPH oxidase. Neutrophils accumulate in the inflamed tissue and oxidative damage due to generation of ROS (reactive oxygen species) occurs to the tissue. It is one of the reasons of the increased neutrophils after irradiation while lesser number of neutrophils in *T. cordifolia* extract pretreated animals can be attributed to lesser inflammation and damage in those animals. Total number of leucocytes decreased in irradiated animals, while this decrease was lesser in the *T. cordifolia* extract pretreated animals at all the post irradiation intervals. Changes in DLC are a reflection of damage to the haematopoietic organs and precursors of WBCs. Haematopoetic tissue is highly sensitive to gamma radiations and gets damaged even at 0.5 Gy of irradiation. Besides this, the utilization, production and destruction of the blood cells following whole body irradiation are affected indirectly also. Radiation induced hypoxia, damaged vasculature and metabolic aberrations may lead to low blood cell counts. Irradiation disturbs steady state of cell renewal system. All the blood cells have a definite life span and after completion of it they die off naturally. Irradiation may cause immediate death or reduction in their life span. Haematopoietic tissues suffer great damage at 8 Gy and it takes enough time to recover, if animal does'nt die.

Death of RBCs and haemorrhage cause reduction in RBC counts and haemoglobin content in peripheral blood. As soon as haematopoietic system starts new cell formation, cell circulation in the blood also reaches to normal in due course of time. Irradiation also induces genomic instability in the bone marrow which magnifies with number of cell divisions after radiation exposure.

According to Petcu[6] irradiation induces apoptosis in lymphocytes. *T. cordifolia* is well known for its immunopotentiating capabilities. Blood cells are major part of the immune system of the body. Antioxidant activity is also reported to play a vital role in radioprotection of blood cells[7]. *T. cordifolia* contains several constituents which cope up with radiation induced damage through several mechanisms. *T. cordifolia* root contains alkaloids, glycosides, sterols, lactones and fatty acids. The major constituents are Berberine, Tinosporin, Giloinin, Giloin and Giloisterol. *T. cordifolia* is reported to enter in almost all the tissues of the body. It is a vitalizer and generate tonic used since a long time.

An arabinogalactan polysaccharide isolated from *T. cordifolia* has antioxidant activity and provided

Table 1. Variation in peripheral blood of Swiss albino mice with or without *T. cordifolia* extract (TE) pretreatment and exposed to 8 Gy of gamma irradiation.

Haematological Parameters	Groups	Post Irradiation time (in days) 1/4	1	3	5	7	10
RBCs $\times 10^6$ mm^3	Control	4.20 ± 0.07***	3.64 ± 0.10***	3.30 ± 0.06***	3.95 ± 0.08***	8.10 ± .14***	5.00 ± .14***
	Experimental	7.56 ± 0.19	7.29 ± 0.06	6.40 ± 0.14	7.32 ± 0.12	9.10 ± 0.14	9.20 ± 0.06
Hb (gm/dl)	Control	8.75 ± 0.05***	5.56 ± 0.07***	5.50 ± 0.09***	6.25 ± 0.07***	12.90± 0.08***	7.50 ± 0.08***
	Experimental	12.50 ± 0.14	12.42 ± 0.12	11.42 ± 0.17	12.84 ± 0.19	14.17 ± 0.08	14.24 ± .08
HCt (%)	Control	21.60 ± 0.10***	21.70 ± 0.05***	20.20 ± 0.71***	22.24 ± .010***	27.23 ± 1.05*	20.10 ± 0.44***
	Experimental	28.20 ± 1.29	27.30 ± 0.27	26.42 ± 0.39	29.30 ± 0.49	33.50 ± 1.53	26.15 ± 0.70
MCH (Pg)	Control	21.90 ± 0.42***	29.60 ± 0.62***	29.64 ± 0.70***	26.25 ± 0.45***	15.50 ± 0.33	25.53 ± 1.00***
	Experimental	16.50 ± 0.45	17.0 ± 0.15	19.0 ± 0.17	18.10 ± .46	15.0 ± 0.41	15.50 ± 0.15
MCV (μ^3)	Control	55.80 ± 1.52***	68.50 ± 3.30***	67.61 ± 2.55*	60.02 ± 0.95	40.25 ± 0.60*	45.40 ± 1.78***
	Experimental	40.50 ± 0.70	40.90 ± 0.37	48.50 ± 1.19	44.69 ± 0.98	39.15 ± 1.27	42.30 ± 0.56
MCHC (%)	Control	40.50 ± 0.36*	46.55 ± 0.47***	46.65 ± 1.59*	45.35 ± 0.40	47.30 ± 1.32*	61.21 ± 1.40***
	Experimental	43.50 ± 1.23	44.0 ± 0.15	42.20 ± 0.70	42.10 ± 1.46	41.05 ± 1.40	38.40 ± 0.30

Values in untreated healthy mouse

RBC = 9.35 ± 0.06 × 106/mm3 MCH = 15.70 ± 0.26 (pg)
Hb = 14.89 ± 0.90 gm/dl MCV = 48.30 ± 0.70 3
Hct = 41.90 ± 0.20% MCHC = 33.20 ± 0.41%

Significance level
* = P < 0.05
** = P < 0.01
*** = P < 0.001

Abbreviations :
RBC = Red Blood Corpuscle Hb = Hemoglobin
Hct = Hematocrit MCH = Mean corpuscular hemoglobin
MCV = Mean corpuscular volume MCHC = Mean corpuscular hemoglobin concentration

Table 2. Variation in total leucocyte counts and differential leucocyte counts in peripheral blood of *Swiss albino mice* with or without *T. cordifolia* extract (TE) pretreatment and exposed to 8 Gy of gamma irradiation.

Haematological Parameters	Post Irradiation time (in days)						
	Groups	1/4	1	3	5	7	10
WBC (10^6/mm^3	Control	4.20 ± 0.07**	4.45 ± 0.17*	4.65 ± 0.14***	4.85 ± 0.19	4.20 ± 0.17***	4.45 ± 0.11***
	Experimental	5.90 ± 0.30	5.23 ± 0.15	5.85 ± 0.20	6.12 ± 0.18	6.12 ± 0.15	6.10 ± 0.15
Lymphocytes (%)	Control	49.5±1.50	55.3 ± 1.65	50.5 ± 1.24	47.9 ± 1.80	59.8 ± 2.25	51.2 ± 2.50*
	Experimental	56.5±1.30	54.3 ± 2.30	51.5 ± 1.50	48.4 ± 2.70	60.0 ± 1.20	60.5 ± 1.80
Neutrophils (%)	Control	43.2±0.50***	41.5 ± 1.40	44.9 ± 0.82	47.0 ± 1.50	40.0 ± 1.75	45.10 ± 1.80**
	Experimental	39.5±0.60	41.3 ± 1.65	42.85 ± 1.20	46.0 ± 1.60	38.20± 1.50	36.1 ± 1.35
Monocytes (%)	Control	3.8±0.60**	2.0 ± 0.50	2.0 ± 0.50	2.6 ± 0.90	1.6 ± 0.70	2.0 ± 0.50
	Experimental	1.2±0.40	1.1 ± 0.51	1.3 ± 0.40	1.7 ± 0.30	1.3 ± 0.40	1.1 ± 0.50
Eosionophils (%)	Control	4.2±0.60	2.0 ± 1.70	3.8 ± 1.20	1.7 ± 0.26	2.0 ± 0.70	2.7 ± 0.85
	Experimental	2.1±0.20	1.7 ± 0.22	2.8 ± 0.30	1.6 ± 0.50	1.20 ± 0.30	1.9 ± 0.26
Basophils (%)	Control	0.5±0.30	0.9 ± 0.17	0.7 ± 0.40	0.5 ± 0.30	0.4 ± 0.27	1.0 ± 0.20
	Experimental	0.7±0.31	0.9 ± 0.20	0.9 ± 0.40	0.5 ± 0.20	0.3 ± 0.40	0.42 ± 0.25

Values in untreated healthy mouse

WBC	=	6.42 ± 0.08 × 10^6/mm^3	Monocytes	=	2.6 ± 0.90%	Significance level	
Lymphocytes	=	68.5 ± 2.50%	Eosinophils	=	2.4 ± 0.60%	* =	P < 0.05
Neutrophils	=	22.5±2.30%	Basophils	=	0.5±0.30%	** =	P<0.01
						*** =	P<0.001

significant protection against gamma radiation.[3] Stanely *et al.*[8] and Singh *et al.*[9] observed that *T. cordifolia* increases activity of enzymes involved in the primary defense mechanisms of the body. Leyon and Kuttan[10] found that it regulates activity of cytokines responsible for inflammation. Jagetia and Baliga[11] observed nitric oxide scavenging activity *in vitro.* Jagetia and Rao[7] reported antineoplastic activity of its extract in dichloromethane. *T. cordifolia* have also shown free radical scavenging activity against ferrous sulphate mediated lipid peroxidation *in vitro*[12]. *T. cordifolia* also inhibited chemically generated superoxide anions[13]. According to Goel *et al.*[13] intraperitoneal administration of 200 mg/kg body weight of *T. cordifolia* to mice protects against gamma radiation in terms of survival, spleen colony forming units, hematology, cell cycle progression and micronucleus induction. Alcoholic extract of *T. cordifolia* is effective in restoration of thymus homeostasis through IL-2 and interferon - gamma *T. cordifolia* also exhibits immunomodulatory activity. Antioxidant activity *in vivo* of ethanolic extract of *T. cordifolia* is reported in Alloxan diabetic rats[14].

T. cordifolia prevented radiation induced changes in cell counts significantly, which is definitely due to its protective effect. Changes in the RBCs heamoglobin and hematocrit value were significantly protected by *T. cordifolia*. It clearly means that death of RBCs is prevented.

Mean corpuscular hemoglobin (MCH) which was increased due to irradiation is kept towards normal side in *T. cordifolia* pretreated animals and it was lesser in experimental animals. Mean corpuscular volume is also high in irradiated animals, which just like MCH decreased till 10th day. MCV in experimental animals was lesser than the control as well as normal animals. It seems to be due to anti-inflammatory activity of *T. cordifolia.*

Mean corpuscular hemoglobin concentration (MCHC) was also higher in control animals in comparison to experimental animals. It appears that MCH and MCHC both are lesser in *T. cordifolia* pretreated animals but more towards normal side.

Total leucocyte count (TLC) and lymphocyte counts were less than the normal in irradiated animals of both the control and experimental groups. In the experimental group TLC and leucocyte and are higher than the control. Leucocytes are nucleated cells and their increased number at early intervals is indicative of their increased survival, which is due to *T. cordifolia* pretreatment.

Basophil counts remained unchanged in both the control and experimental groups. No significant difference between control and experimental groups was observed.

Neutrophils, monocytes and eosinophils increased significantly in the control animals. Their number was lesser in *T. cordifolia* pretreated animals.

At later intervals it appears that normal functioning of the hematopoetic cells is regained and hence cell counts reached to the normal. As *T. cordifolia* has immunomodulatory, antioxidant, free radical scavenging, anti lipid peroxidation and above all balancing properties, it helps in keeping blood counts towards normal level which is indicative of better health of the animal.

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