



ISSN No: 0975-7384
CODEN(USA): JCPRCS

J. Chem. Pharm. Res., 2011, 3(6):877-881

Evaluation of Anticarcinogenic and Antimutagenic Effect of *Tinospora cordifolia* in Experimental Animals

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ABSTRACT

Tinospora cordifolia is used to treat various diseases in the traditional medicinal system in India. Its chemopreventive potential for cancer was the subject of present study. In this study the anticarcinogenic and antimutagenic activity of *T. cordifolia* extract was used, in C57 Bl mice and Swiss albino mice respectively. In antimutagenic study, a single application of *T. cordifolia* extract at a dose of 200, 400 and 600 mg/kg dry weight, 24 hrs prior the i.p. administration of cyclophosphamide (at the 50 mg/kg), significantly prevented the micronucleus formation in bone marrow of mice, in a dose dependent manner. In melanoma assay, C57 Bl mice when received 50% methanolic extract of *T. cordifolia* at a dose 750 mg/kg body weight for 30 days showed increase in life span and tumor size was significantly reduced as compared to control.

Key words: *Tinospora cordifolia*, Antimutagenic assay, Melanoma Assay.

INTRODUCTION

Tinospora cordifolia is an Indian medicinal system and has been used in ayurvedic preparation for the treatment of various ailments throughout the countries. Ancient Hindu Physicians prescribed it for gonorrhoea. European medical men in India became interested in tonic and diuretic properties of *T. cordifolia*. Today the drug and tincture prepared from it received official recognition in the Indian Pharmacopoeia [1,2]. Evidence hints that *T. cordifolia* may have anticancer [3,4], immune stimulating [5], antidiabetic [6,7], cholesterol lowering [8] and Liver protective [9] actions. *T. cordifolia* has also shown some promising speed in healing the diabetic foot ulcers [10]. Since there is a paucity of information on the antimutagenicity and

anticarcinogenicity of compounds widely used in Ayurvedic medicine, we have therefore undertaken to study the anticarcinogenicity of plant extracts using melanoma tumor model and antimutagenicity using micronucleus test in bone marrow cells of mice. Here we present our finding with a methanol extract of *T. cordifolia*.

EXPERIMENTAL SECTION

Collection of plant: The plant was collected from Raffik Ahmed Kidwai College of Agriculture Campus, Sehore (M.P.) in August 2010.

Preparation of Extract: Fresh stems of *Tinospora cordifolia* extract was dried under the shade and then powdered with grinder. The powder was extract with 50% methanol using separating funnel. After 24 hrs. The upper layer of solvent was collected in a beaker and a procedure was repeated 3 times in the interval of 24 hrs. Continuously till the color of solvent was disappear. Now the extract was dried at 60°C in water bath. The semi solid paste formed was transferred to Petri plate and kept in hot air oven till it gets in powder form. The total weight of powder was measure and stored in air tight container for further use.

Solubility testing: The drug is tested for solubility in different solvent like acetone, alcohol (methyl alcohol), dimethyl sulfoxide etc. but its solubility in double distilled water was accepted for further experimental work.

After that 50% methanolic extract was prepared using separating funnel, the project was approved by institutional animal ethic committee (IAEC) of Jawahar Lal Nehru Cancer Hospital and Research Centre, Bhopal (Project No. 5, Ref. No. 670/2251). The experiment was carried out according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines and Institutional animal ethic committee approved all the procedure. Cyclophosphamide was purchased from sigma chemicals Co., U.S.A. and other chemicals were reagents grade and purchased locally.

Micronucleus Assay Protocol: It was done as per the method prescribed by Schmid (1975) [11]. Male Swiss albino mice of 15-20 g body weight were obtained from the animal colony of our research centre and were housed in plastic cage. The animals were provided standard pallet diet and water ad libitum.

Animals were sacrificed by cervical dislocation and bone marrow cells were harvested from femur bones of freshly killed mice. Bone marrow was aspirated by flushing with HBBS (Hanks Balance Salt Solution) with the help of syringe and was collected in centrifuge tube. Now, the tubes were centrifuged at 1000 rpm for 10 min. and procedure is repeated three times one after one by discarding the supernatant. The slides are prepared by smear method. The small drops of viscous suspension were drop on the end of the slide and spread by pulling the material behind the polish cover glass held at the angle of 45°. The preparation was dried for 5-10 minutes. In the dried slides the cells are fixed with methanol which is kept in coupling jar for 10 minutes. After the fixing of cells the slides are kept into Maygrunwald reagent for 5 minutes and then in Giemsa stain for 10 minutes.

Methanol	:	Maygrunwald	:	Giemsa
10	:	5	:	10

Polychromatin erythrocytes (PCE) were scored for the micronucleus under the microscope and the ratio of PCEs and Normochromatin erythrocytes (NCEs) is also determined by counting 1000 cells.

The animals were divided into 6 different groups and each group comprises of 6 animals:

Group 1: Cyclophosphamide (50 mg/kg).

Group 2: It is subdivided into 3 groups:

(a) *Tinospora cordifolia* (200 mg/kg)+ Cyclophosphamide (50 mg/kg).

(b) *Tinospora cordifolia* (200 mg/kg)+ Cyclophosphamide (50 mg/kg).

(c) *Tinospora cordifolia* (200 mg/kg)+ Cyclophosphamide (50 mg/kg).

Group 3: *Tinospora cordifolia* alone (200 mg/kg).

Group 4: (untreated group): No treatment is given.

Melanoma assay protocol: It was done as per the method described by Agrawal (2009) [12]. Melanoma cell lines were obtained from National Cell Science Centre, Pune, and maintained in our laboratory. The C57Bl hybrid mice of both sexes of the mean weight of 25±2 gm were obtained from the animal colony of our institute. They were housed in good laboratory condition and given standard mouse pellet diet and water ad Libitum. All the animals were kept at controlled light and temperature conditions. Hairs of dorsal sides in a particular area were removed by hair remover cream of each mice and were kept in laminar air flow cabinet under pathogen free conditions. The implanted tumor from donor (having melanoma tumor) was removed from the mice. Prepared cell suspensions having 5×10^5 cells/ animals were injected subcutaneously. After implantation of melanoma cell lines, animals were kept under observation and experiment was started after 10 days when the tumours were seen. The treatment of *Tinospora cordifolia* extract (750 mg/kg) was given orally and cyclophosphamide (50 mg/kg) was given intraperitoneally. The tumor volume and survival time of each animal were recorded. The implanted tumour bearing mice randomly divided into 4 groups with 4 mice in each group.

Group 1: *Tinospora cordifolia* (750 mg/kg).

Group 2: *Tinospora cordifolia* (750 mg/kg) orally + Cyclophosphamide (50 mg/kg) I.P.

Group 3: Cyclophosphamide (50 mg/kg) I.P.

Group 4: Untreated.

RESULTS

Antimutagenic activity: In Antimutagenic studies, the single application of *Tinospora cordifolia* at the dose of 200, 400, 600 mg/kg dry wt, 24 hrs prior the i.p. administration of cyclophosphamide (at the dose of 50 mg/kg) have significantly prevented the micronucleus formation in dose dependent manner.

Table 1: Effect of *Tinospora cordifolia* Extract on micronucleus formation in mouse bone marrow cells

Group	MNPCE±SE	PCE/NCE Ratio
Cyclophosphamide (50 mg/kg)	1.5±0.45	0.88±0.08
T.C Extract+Cyclophosphamide (50mg/kg)		
(a) 200 mg/kg	0.83±0.30	0.68±0.04
(b)400 mg/kg	0.5±0.22	0.63±0.05
(c)600 mg/kg	0.33±0.21	0.66±0.08
<i>Tinospora cordifolia</i> alone (200 mg/kg)	0.33±0.20	0.69±0.17
Untreated	0.00	0.57±0.10

Anticancerous activity: In Anticarcinogenic studies, C57BL mice which received *Tinospora cordifolia* extract at the dose of 750 mg/kg for 30 days showed increase in life span of animals and tumor size was significantly reduced in *Tinospora cordifolia* treated mice as compared to control.

Table 2: Anticancerous activity of *Tinospora cordifolia* extract on Hybrid mice

S. no	Group	Tumor volume (mm ³)	Survival days	Increases life span (%)	% Inhibition rate
1.	T.C	426.94	22	18.91	47.96
2.	T.C+ Cyclophosphamide	125.98	29	61.11	56.32
3.	Cyclophosphamide	160.99	23	24.32	44.20
4.	Untreated	288.55	18		

DISCUSSION

The present study demonstrated that the, *Tinospora cordifolia* prevents the micronucleus formation in dose dependent manner while in melanoma tumor model, *T. cordifolia* have a preventive effect on tumor volume. It also showed that the mean survival time and increased in life span have increased in test group where animals were treated with *T. cordifolia* extracts as compared to control group.

T. cordifolia plant extracts made with water, ethanol/methanol, or methylene chloride extracts have been evaluated for antineoplastic effects in various animal experiments. Tumor mass reduction and increased survival time have been observed with administration of the extract in several experiments in mice with induced carcinomas. [13,14] At low doses, an ethanol extract of *T. cordifolia* increased bone marrow cell counts, while higher doses resulted in decreased counts in mice with induced lymphoma. [15]

Intraperitoneal injection of the alcoholic extract of *T. cordifolia* has been shown to Dalton's lymphoma (DL) bearing mice e stimulated macrophage functions like phagocytosis, antigen-presenting ability and secretion of Interleukin-1 (IL-1), tumour necrosis factor (TNF) and Reference Nutrient Intake (RNI) as well as slowed tumor growth and increased lifespan of the tumor-bearing host.[3] *T. cordifolia* was has been shown effective in several other tumour models including Ehrlich ascites carcinoma (EAC) in mice[16]. It induces proliferation and myeloid differentiation of bone marrow precursor cells in a tumor-bearing host [17], activates tumor-associated macrophages-derived dendritic cells [4] is effective against various cancers

[18], killing the cancer cells very effectively in vitro [19,20] inhibits skin carcinogenesis in mice [21] and inhibits experimental metastasis [22]. *T. cordifolia* may offer an alternative treatment strategy for cancer in combination with gamma radiation [23,24].

In conclusion, the present result suggest Anticarcinogenic and Antimutagenic activity of *T. cordifolia* extract.

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