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Antitumor Activity of *Tinospora cordifolia* Bark Extract Against Dalton's Lymphoma Ascites In Swiss Albino Mice

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ABSTRACT:
Different parts of *Tinospora cordifolia* plant products are employed in the Indian traditional medicine for the treatment of several disorders. Our aim was to investigate the cytotoxicity, Antitumor activity and Antioxidant status of *Tinospora cordifolia* against Daltons Lymphoma Ascites in Swiss Albino Mice. Oral administration of Ethanolic and Water extract of *Tinospora cordifolia* at a dose of 800 mg/kg body weight each showed an increase in Life span. The ethanolic extract of *Tinospora cordifolia* showed more effect than water extract and was much comparable to that of 5-flurouracil (standard drug). Our results indicate that Ethanolic extract of *Tinospora cordifolia* has a prominent Antioxidant and Antitumor activity in experimental DLA bearing mice and can there for be used as an alternative remedy for the treatment of cancer and its complication, due to its lesser or no side effect.

Key words: Tinospora cordifolia**,** Dalton's Lymphoma Ascites, Antitumor activity, ethanolic extract, water extract, Swiss Albino mice

INTRODUCTION

Cancer is one of the most dreadful diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. In the United States, as the leading cause of death, it accounts for 25% of all the deaths in humans presently [1]. It is considered as an adversary of modernization and advanced pattern of socio-cultural life dominated by western medians. Multidisciplinary scientific investigations are mating best efforts to combat this disease, but the sure-sort; perfect cure is yet to be brought into world medicine.

Chemotherapy is a major treatment modality for cancer. However, most of the chemotherapeutic agents exhibit severe normal toxicity, resulting in undesirable side effects [2]**.** More over, many of the active molecules sold for the treatment of cancers, are highly expensive, mutagenic, carcinogenic and teratogenic.

Recently a greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management. Medicines derived from plants have pivotal role in health care of ancient and modern cultures. Ayurveda, a traditional Indian medicine of plant drugs has been successful from very early times in using these natured drugs and preventing or suppressing various tumors using various lines of treatment.

Many herbs have been evaluated in clinical studies and are currently being investigated phytochemically to understand their tumouricidal actions against various cancers. Thus, burdened by dry-induced toxic side effects have has turned to seek help from the complementary and alternative medicine hoping for a better cure. [3]

Tinospora cordifolia is a large glabrous, deciduous climbing shrub belonging to the family Menispermanceae. It is distributed throughout tropical Indian subcontinent and China, ascending to an altitude of 300m. The plant is commonly known as Guduchi, Giloy or Amritha.

Guduchi is widely used in veterinary folk medicine and ayurvedic sytem of medicine for its general Tonic, Antiperiodic, Antisparmodic, Antiinflammatory, Antiarthritic, Antiallergic and Antidiabetic properties [4]. The whole plant is used in ayurvedic "Rasayanas" to improve the immune system and the body resistance against infections and root is known for its antistress anti-leprotic and antimalarial activities. 'Guduchi' has been reported to be active against throat cancer in man and it has been reported to be non-toxic in acute toxicity studies *in vivo*, with almost no side effects [5].

The present study is to investigate the antitumor activity of ethanolic extract of stem of *Tinospora cordifolia* (Guduchi) in Swiss Albino Mice and to study its antioxidant activity *in vivo*.

MATERIALS AND METHODS

Animals

Studies were carried out using Swiss Albino mice weighing 25±2 grams. The mice were grouped and housed in polyacrylic cages (38×23×10cm) with not more than 12 animals per cage and maintained under standard laboratory conditions (temperature 26±20°C). The mice were acclimatized to laboratory condition for 14 days before commencement of the experiment. All the procedures described were reviewed and approved by Institutional Animal Ethic's Committee (IAEC).

Tumor cells

Dalton's Lymphoma Ascites (DLA) cells were obtained from the Amala Cancer Research Centre, Thrissur, Kerala, India. The DLA cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 1×10^6 cells/mouse. DLA cells 12 days old were used for the screening of plant extract.

Preparation of Plant Extract

Both ethanolic and water extract of *Tinospora cordifolia* were prepared using Soxhlet apparatus method [6]. A known volume of ethanolic and water extract is suspended in saline and was orally administrated to the animals by gastric incubation using a force feeding needle during the experimental period.

Invitro Cytotoxic Assay

Short term cytotoxicity was studied using DLA cells. The cells were aspirated from the peritoneal cavity on day 15^{th} and washed in ice cold PBS for 3 times. The viable cells were counted under microscope and adjusted at 1×10^6 cells/ml, and made different dilutions [7]. Different concentrations of the *Tinospora cordifolia* stem extract were incubated with tumor cells suspended in Phosphate buffered saline and cytotoxicity was determined after 5 hours using trypan blue exclusions method. [8]. The drug at toxic concentration damages the cell that makes pores on the membrane through which the trypan blue enters, which are stained blue and which are therefore be distinguished from viable cells.

% of toxicity was calculated by following equation:

$$\frac{\text{No. of dead cells}}{\text{Total no. of cells}} \times 100$$

Determination of Antitumor Activities

DLA cells were aspirated from peritoneal cavity of the tumor bearing mice. 1×10^6 cells were injected intraperitonially into Swiss albino mice. The mice were divided into 5 groups of 6 animals with DLA cells except the normal group. Group I served as normal control and Group II served as tumor control. Group III that served as positive control was treated with the suspension of 5-fluorouracil at 20mg/kg body weight. Group IV was treated with 800mg/kg of the ethanolic extract of *Tinospora cordifolia.* Group V was treated with 800 mg/kg of the water extract of *Tinospora cordifolia.*

All these treatment were given 24 hours after the tumor inoculation, once daily for 14 days. After the last doze and 24 hours of fasting, 2 mice from each group were sacrificed. The blood was collected directly from heart under slight anesthesia (Chloroform) condition and the hematological parameters such as Red blood cells (RBC), White blood cells (WBC), Differential count and Hemoglobin content were determined [9]. After collecting the blood samples, the mice were killed by cervical dislocation. The liver was excised, rinsed in ice cold normal saline solution followed by cold 0.15M Tris Hcl (pH 7.4), blotted, dried and weighed. The rest of the animals were kept to check average life span and change in body weight for 6 weeks.

Tumor Growth Response

The antitumor effect of ethanolic and water extract of *Tinospora cordifolia* was assessed by change in body weight, tumor volume, Mean Survival Time [MST] and percentage increased life span [%ILS]. MST of each group containing 6 mice was monitored by recording the mortality rate per day for 6 weeks

MST = (Day of first death – Day of last death)/ 2

Hematological studies

Hemoglobin content, Red blood cells (RBC) and White blood cells (WBC) counts were measured. Differential leukocyte count of WBC was carried out from Leishman stained blood smears.

Estimation of Hemoglobin

Hemoglobin content was estimated using Sahli's acid haematin method. In this method, Hb calibrated tube is filled up to the mark 20 with 0.1 Hcl and Hb pipette exactly up to 20 cu mm of controlled sucking. Empty the pipette into acid and mix the acid haematin well and allow it to stand for 10min [10]. At last dilute the solution of acid haematin by adding distilled water and addition of water continued till the color of solution matches perfectly with standards and the reading is taken in grams percent.

Total Red Blood Cell Count (T.R.B.C)

The oxalated blood was filled in Red cell pipette up to 0.5 mark and draw the diluting fluid up to mark 101, and mix it properly. Load the Neubauer's Chamber with the sample and allowed 2 minutes for setting of the cells and then count the red blood cells lying in 80 very small squares which comprising of 5 medium sized squares each of which is bounded by a triple line[11].

White Blood Cell Count (W.B.C)

Oxalated blood was taken in a dry pipette upto the mark 0.5 and diluting fluid upto mark 11. Load the Neubauer Chamber with the sample and allowed 2 minutes for the settling of cells and white blood cells are counted on the 4 large corner squares [12].

Differential Leukocyte Count

A small drop of blood is placed on the central line of the slide and is spreaded uniformly to form a thin film, and the smear was stained by Leishman stain. The Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils were counted with a high power microscope.

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RESULTS AND DISCUSSION

Cancer is a disease of misguided cells that have high potential of excess proliferation without apparent relation to the physiological demand of the process [14]. It is the second largest cause of death in the world [15]. Of all the available anticancer drugs during 1940-2002, 40% were natural products or natural product derived with another 8% being natural product mimics [16].

The present investigation was carried out to evaluate the antitumor activity and antioxidant status of ethanol and water extract of *Tinospora cordifolia* stem in DLA tumor bearing mice.

Cytotoxicity studies

In vitro cytotoxicity of the *Tinospora cordifolia* stem was determined against DLA cells, it can be seen from the data presented in the table 1 that the ethanolic extract of *Tinospora cordifolia* stem showed highest percentage of toxicity at a concentration of 2 mg/ml. The water extract showed highest percentage of toxicity at a concentration of 2 mg/ml

TABLE: 1 Effect of *Tinospora cordifolia* stem extract on *in vitro* cytotoxicity of DLA cells.

	Percentage of toxicity	
Concentration mg/ml	Ethanol extract (%)	Water extract (%)
0.2	5	3
0.5	18	15
1	32	26
1.5	40	39
2	59	52

DLA INDUCED ASCITIC TUMOR STUDIES

Effect on Hematological Parameters

As shown in the table 2 hematological parameters of tumor bearing mice on day 15th was found to be significantly altered compared to the normal group.

The haemoglobin content and RBC count in the DLA control group was significantly decreased as compared to normal group. Treatment with both Ethanolic and water extracts of *Tinospora cordifolia* stem at a doze of 800 mg/kg body. Wt significantly increased the Hb content and RBC count to more or less normal level.

The total W.B.C count was found to be increased significantly in the DLA control group when compared to normal group. Administration of *Tinospora cordifolia* stem extracts at doze of 800 mg/kg body wt. in DLA bearing mice significantly reduced WBC count as compared with DLA control group.

In a differential count of WBC, the presence of Neutrophil increased while the lymphocyte count decreased in the DLA control group. Eoisinophil and Monocyte count in the control group was found be decreased.

Treatment with the plant extract increased the lymphocyte, monocyte and eosinophil count more or less equal to the normal group, while the neutrophil count was decreased towards normal level. However the standard drug 5-FU treatment at 20 mg/kg body wt. produced better result than the extract treatment in all these parameters.

TABLE: 2 Effects of *Tinospora cordifolia* stem extract on hematological parameters of DLA bearing mice

		Treatment (Dose, mg/kg body weight)			
Parameters	Normal	Control 1×10^6 cells / ml	DLA + Standard drug 5-FU (20mg/ kg)	DLA + Ethanol extract (800mg/ kg)	DLA + Water Extract (800mg/ kg)
Total WBC ($\times 10^3$/mm^3)	8.82	13.6	7.9	10.6	9.8
RBC ($\times 10^6$/ mm^3)	12.43	7.37	9.2	9.25	10.6
Hb (g/dl)	15.2	9.3	12.3	12.3	13.4
WBC differential count (%)					
Lymphocyte	66.1	35	66.7	54	64
Monocyte	1.9	1.13	1.61	1.2	1.2
Neutrophil	26	60	35.83	40	30
Eosinophil	6.2	4	5.53	4.9	5

Effect on Mean Survival Time

The reliable criteria for judging the value of any anticancer drug is the prolongation of life span of the animals (Gupta *et al*., 2004). From the fig. 1, in the DLA control group the mean survival time was 16.5±0.53 days while it increased to 22.5±0.86 days of water extract and 25 ±0.96 days for ethanol extract respectively. Whereas the standard drug 5-FU (20 mg/kg body wt.) treated group had a mean survival time of 37.5±0.65 days.

The effect of ethanolic and water extract of *Tinospora cordifolia* stem on tumor growth was monitored and mortality rate was recorded daily for a period of 6 weeks. From the Fig. 2 the percentage increase in life span was evaluated to be 36.3and 51.5 for water and ethanol extracts respectively, while it was 127.2 % for standard drug 5-Fluoro Uracil.

Treatment of ethanolic and water extracts at a doze of 800 mg/kg body wt. significantly reduced the tumor volume as compared to the DLA control group (Fig. 3). In control animals the tumor volume induced by DLA cells was found to be 4 times increased from day 0 to 15th. However the tumor volume was found to be

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only 2.5 and 2.4 times increased in animals treated with ethanol and water extract of *Tinospora cordifolia* stem respectively at 800 mg/kg body wt. About 50% of reduction in tumor volume was observed for both the extracts on 15th day of experiment

In DLA tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. The present increase in body weight and decrease in tumor volume (Fig.4) were found to be significantly less than the tumor control animals indicating the antitumor nature of the extract. These results could indicate either a direct cytotoxic effect of ethanolic and water extract of *Tinospora cordifolia* stem on tumor cells as evidence by the *in vitro* studies.

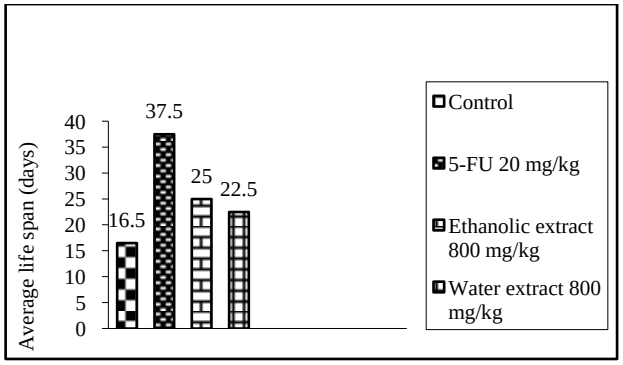

Fig. 1 Effect of *Tinospora cordifolia* stem extract on Mean Survival Time of DLA bearing Swiss albino mice

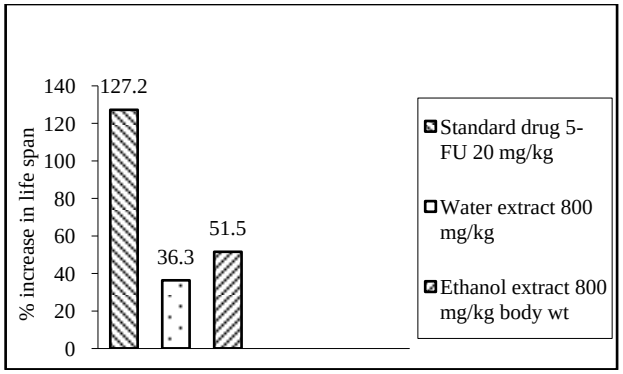

Fig.2 Effect of *Tinospora cordifilia* stem extract on % increase in life span of DLA bearing Swiss albino mice

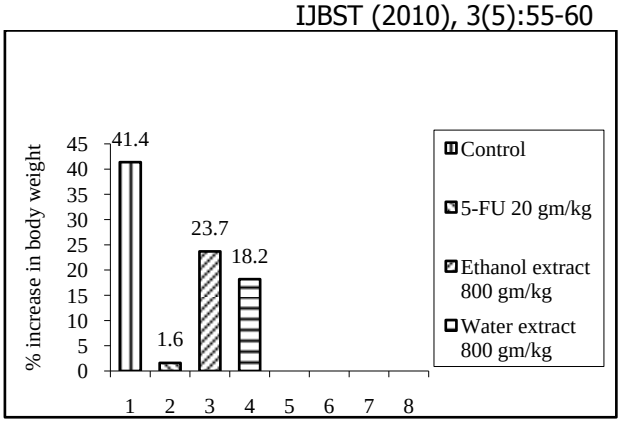

Fig.3 Effect of *Tinospora cordifolia* stem extract on % increase in body weight of DLA tumor bearing Swiss albino mice

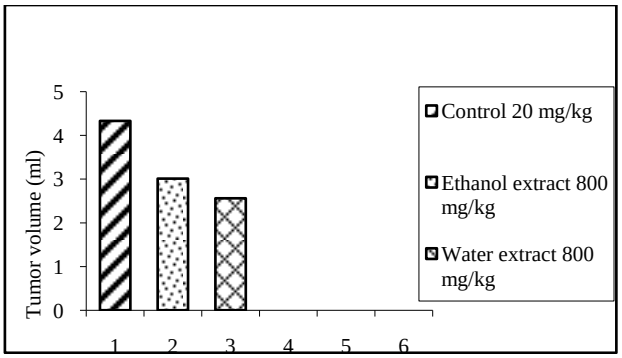

Fig.4 Effect of *Tinospora cordifolia* stem extract on tumour volume of DLA tumor bearing Swiss albino mice

CONCLUSIONS

The present study demonstrates that the ethanol extract of *Tinospora cordifolia* stem increased the life span of DLA tumor bearing mice by a significant decrease in the total WBC and neutrophil count and an increase in the RBC, Haemoglobin and Lymphocyte towards the normal values. All these parameters suggest that the ethanolic extract of *Tinospora cordifolia* stem exhibits potential antitumor activity.

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KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India.
Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)
Institutional Animal Ethics committee (IAEC).

CERTIFICATE

Title of the Project	: Antitumor activity of *Tinospora cordifolia* bark extract against dalton's lymphoma ascites in swiss albino mice
Proposal Number	: KMCRET/B.Pharm/01/2007
Date received after modification (if any)	: -NA -
Date received after second modification	: - NA -
Approval date	: 08-08-2007
Animals	: Wister strain rats and Mice Balb C or Albino
No. of animals sanctioned:	: 300 mice and 300 rats
Expiry date (Termination of the Project)	: August 2008
Name of IAEC chairperson	: Dr. Abhay Dharamsi

Signature of IAEC Chairperson 08/08/07

Date:

(Mr. E.P. Kumar)
CPCSEA Inspector & Nominee

(Dr.P. Chinnaswamy)
Biological Scientist

(Dr.Thavamani D. Palaniswami)
Scientist

(Mr.A. Mohan)
Society Awareness Member

(Dr. O.T. Buvaneswaran)
IAEC Member

(Dr. B. Showkath Ali)
Veterinarian

(Mr. A. Shyam Sundar)
Scientist in-charge of
Animal house and Convener