



STUDIES ON ANTITUMOR ACTIVITY OF *TINOSPORA TOMENTOSA* MIERS AGAINST EHRlich ASCITES CARCINOMA IN SWISS ALBINO MICE

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ABSTRACT

The aim of the present study is to evaluate the antitumor effect of *Tinospora tomentosa* Miers. (Family: Menispermaceae) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The effect of methanol and aqueous extracts of *Tinospora tomentosa* Miers on tumor (ascetic fluid) growth was studied by the following parameters: percentage inhibition of ascetic cells and percentage inhibition of tumor (ascetic fluid) weight. Methanol and aqueous extracts were administered at doses of 100, 200 and 400 mg/kg body weight intraperitoneally once a day for 7 days, after 24 hours of tumor inoculation. Decrease in ascitic cell count and ascetic fluid weight were observed in extract treated animals, when compared to EAC treated animals. Methanol extract of the plant at a dose of 200 and 400 mg/kg showed significant ($p < 0.05$) results while aqueous extract at a dose of 400 mg/kg also showed significant ($p < 0.05$) antitumor activity. The results were dose dependent and suggest that both the extracts of the stem of *Tinospora tomentosa* Miers. exhibit significant antitumor effects in EAC bearing mice.

Key words : *Tinospora tomentosa* Miers., Ehrlich Ascites Carcinoma (EAC), Antitumor activity.

INTRODUCTION

Tinospora tomentosa Miers. (Family: Menispermaceae) is a large deciduous climbing shrub found in tropical thickets in Bengal and almost throughout India, ascending to an altitude of 1000 meter. It is locally known as 'padma-gulanha' and has long been used in Ayurvedic medicine. Traditionally, the different parts like stems, leaves, and roots are used as stomachic, bitter tonic, anti-periodic, mild diuretic, emetic, anti-purgative, analgesic, antipyretic, anti-inflammatory, antidiabetic, antileprotic, antigout, febrifuge, hepatic stimulant and demulcent¹⁻⁵. Although, various activities of *Tinospora* genus has been reported earlier⁶⁻¹⁴, hitherto, there is no report on the antitumor activity of *Tinospora*

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tomentosa Miers. The aim of the present study is to evaluate the antitumor activity of methanol and aqueous extracts of the stem of *Tinospora tomentosa* Miers.

EXPERIMENTAL

Materials and methods

Plant material

The plant was identified (Ref. No. CNH/I-I (53)/2004-Tech-I/885) by the taxonomists of Botanical Survey of India, Shibpur, Howrah. After authentication, the fresh stems were collected in bulk from young matured plants at the rural areas of Howrah during July-August 2004 and washed, shade dried and milled into coarse powder by a mechanical grinder. The powder was passed through sieve number 40 (B.P standard) and used for further studies.

Preparation of extracts

The powdered plant material (1000 g) was extracted with 900 mL of redistilled petroleum ether (40-60°C), followed by redistilled methanol at 40°C for 72 hour by hot continuous extraction method. The solvent was evaporated under reduced pressure at 50°C and dried in vacuum (Yield of methanol extract: 14.1% w/w on dried plant material basis). The aqueous extract was then prepared by decoction process using double distilled water, filtered, evaporated and dried under reduced pressure (yield: 22.5% w/w on dried plant material basis). The dried extracts thus obtained were dissolved in phosphate buffer (pH 7.2) solution and used directly for the assessment of antitumor activity.

Preliminary phytochemical characterization of extracts

The extracts prepared in different solvents were taken and standard methods were used to detect the nature of phytoconstituents present in them¹⁵⁻¹⁸.

Ethical clearance

Protocol used in this study for the use of mice as an animal model for cancer was approved by the University Animal Ethical Committee.

Animals

Female Swiss albino mice of about 10 weeks of age with an average body weight of 18-20 g were used for the experiment. The animals were bred and brought up in our laboratory facility with 12 hour cycles of light and dark at 24°C. They were kept on basal

metabolic diet with water ad libitum.

Tumor cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal (i.p.) transplantation of 2×10^6 cells/mouse after every 10 days. EAC cells of 9 days old were used for the screening of the extracts.

Protocol

Standard experimental protocol was followed for the evaluation of antitumor activity of the extracts¹⁹⁻²¹. Female Swiss albino mice were divided into five groups of five animals each. The methanol and aqueous extracts were dissolved in phosphate buffer solution and used directly in the assay. EAC cells were collected from donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 10×10^6 cells/mL. 0.1 mL of EAC cells per 10 g body weight of the animals was injected (i.p.) on day zero. A day of incubation was allowed for multiplication of the cells. Seven doses of the extracts (100 mg, 200 mg and 400 mg/kg, 0.1 mL per 10g body weight) and mitomycin-C (1 mg/kg) were injected i.p. from the first day up to the seventh day with 24 hours intervals. Control animals received only vehicle (phosphate buffer solution: pH 7.2). Food and water were withheld 6 hours before sacrificing the animals. On day 8, all the animals were sacrificed. Some amount of the fluid of the peritoneal cavity was kept aside for counting of cells and rest of the fluid was wiped off with absorbent cotton. Weights of the animals were taken before sacrificing and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor (ascetic fluid) weight. Mitomycin-C at a dose level of 1 mg/kg body weight was used as standard, which showed 100% inhibition at all times which is shown in the Table 2.

The antitumor activity of the extracts were measured in EAC animals with respect to the following two parameters:

Tumor cell count

The ascetic fluid was collected in a graduated centrifuge tube and diluted 100 times with sterile isotonic saline solution. Then the diluted fluid was taken in a WBC pipette and diluted 100 times again with sterile isotonic saline. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted. Then the percentage inhibition of ascetic cells was

calculated as $(1-T/C) \times 100$, where C and T were the average number of ascetic cells per mL of fluid in the control and test groups, respectively.

Viable/non-viable tumor cell count

The cells were then stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non-viable. These viable and non-viable cells were counted.

Tumor (ascetic fluid) weight

The mice were dissected and some amount of the ascetic fluid was collected from the peritoneal cavity. The rest of the fluid was wiped off with absorbent cotton.

The weight of animals were taken before sacrificing and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor weight. Then the percentage inhibition of tumor (ascetic fluid) weight was calculated as $(1 - T / C) \times 100$, where C and T were the average tumor (ascetic fluid) weights of control and test groups, respectively.

Statistical analysis

The experimental results were expressed as mean \pm SEM (standard error of mean). Data was assessed by the Student's t- Test, $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The results of the preliminary phytochemical characterization of extracts and antitumor activity are shown in Tables 1 and 2, respectively. Antitumor activity of the extracts against EAC tumor bearing mice was assessed by the parameters such as cell count (viable and non-viable) and tumor (ascetic fluid) weight.

Methanol extract of the plant at a dose of 200 and 400 mg/kg showed significant ($p < 0.05$) result in both percentage inhibition of ascetic cells (54.54 and 59.74, respectively) and percentage inhibition of tumor (ascetic fluid) weight (41.62 and 58.37, respectively). Aqueous extract at a dose of 400 mg/kg also showed significant ($p < 0.05$) antitumor activity. Other doses of methanol and aqueous extracts showed some activity. Treatment with the above doses of extracts significantly reduce viable cell count and tumor (ascetic fluid) weight, when compared to that of EAC control group. Further, non-viable

tumor cell counts were increased as compared to that of EAC control group. The results clearly indicate that the extracts of the plant *Tinospora tomentosa* Miers. has a capacity to inhibit the growth of tumor induced by EAC cell line in a dose dependent manner in experimental animals.

The present investigation was carried out to evaluate the antitumor activity of methanol and aqueous extracts of the plant *Tinospora tomentosa* Miers. in EAC tumor bearing mice. The methanol extract at the doses of 200 and 400 mg/kg and aqueous extract at a dose of 400 mg/kg significantly inhibited the viable cell count and tumor (ascetic fluid) weight. From the preliminary phytochemical investigations, it was observed that methanol extract contains alkaloids, flavonoids, tannins and sugars while protein, saponins, tannins and sugars were present in aqueous extract. From the above results, We can conclude that in case of methanol extract, it could be the alkaloids or flavonoids while for aqueous extract, it could be the protein or saponins, which are responsible for their antitumor activity. Isolation and characterization of individual compounds are presently under progress in our laboratory.

Table 1 : Preliminary phytochemical characterization of extracts of *Tinospora tomentosa* Miers

Constituents	Observations			
	PE	CE	ME	AE
Alkaloids	-	+	+	-
Flavonoids	-	-	+	-
Tannins	-	+	+	+
Saponins	-	-	-	+
Sugars	+	+	+	+
Protein	+	-	-	+
Organic acids	+	-	-	-
Glycoside	-	-	-	+

PE = Petroleum ether extract; CE = Chloroform extract;
ME = Methanol extract; AE = Aqueous extract

**Table 2. Effect of the methanol and aqueous extracts of *Tinospora tomentosa* Miers. on viable and non-viable tumor (ascetic fluid) cell count and tumor (ascetic fluid) weight of EAC bearing mice. n = 5. Mean ± SEM.
* p < 0.05 : Statistically significant, when compared with EAC control group**

Treatment	Dose (mg/kg)	Avg. no. of ascetic cells/mL in control gr. 'C' (x10 ⁶ cells/mL)		Avg. no. of ascetic cells/mL in test gr. 'T' (x10 ⁶ cells/mL)		% Inhibition of viable ascetic cells (1-T/C) x 100	Avg. weight of ascetic fluid in control gr. 'C' (g)	Avg. weight of ascetic fluid in test gr. 'T' (g)	% inhibition of ascetic cells x /C(1- T 100
		Viable	Non-viable	Viable	Non-viable				
Methanol extract	100			117.5 ± 2.45*	16.20 ± 0.12*	38.96		1.74 ± 0.24*	21.25
	200	192.5 ± 2.88	12.8 ± 0.07	87.5 ± 2.69*	19.44 ± 0.08*	54.54	2.21 ± 0.36	1.29 ± 0.17*	41.62
	400			77.5 ± 3.58*	24.79 ± 0.06*	59.74		0.92 ± 0.18*	58.37
Aqueous extract	100			170.5 ± 9.89	14.21 ± 0.15*	11.42		1.86 ± 0.28	15.83
	200	192.5 ± 2.88	12.8 ± 0.07	168.0 ± 9.92*	15.94 ± 0.17*	12.72	2.21 ± 0.36	1.76 ± 0.23	20.36
	400			112.5 ± 4.71*	18.30 ± 0.04*	41.55		1.32 ± 0.11*	40.27
Mytomicin-C	1	192.5 ± 2.88	12.8 ± 0.07	0	--	100	2.21 ± 0.36	0	100

In case of control group, a regular rapid increase in ascetic tumor volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells²². Treatment with extracts inhibited the tumor cell count and tumor (ascetic fluid) weight. It may be concluded that the methanol and aqueous extracts of the plant decreases the nutritional fluid volume and arrests the tumor growth. Thus, the extracts possess antitumor activity against EAC bearing mice.

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