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Anti-tumor Activity of Tylophora Asthmatica

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ABSTRACT: *T. asthmatica, which belongs to the family Asclepiadaceae is a small twining a plant with long fleshy roots. The plant shows broad activity against EA and DLA cells. The intraperitoneal injection of PE extract obtained from the powdered entire plant material to the tumor cell transplanted animals arrests the tumor growth and prevents the formation of the tumor. A significant increase in the life span of the drug treated tumor bearing mice were found.*

INTRODUCTIION

Tylophora asthmatica (Mal. Vallippala, Tam: Nanjaruppan, San Lataksiri, Eng; Emetic swallow wory) is a small twining plant with long fleshy roots common in the forests throughout Eastern India, Bengal and Assam. Ratnagiriswaran are venkatachalam (1935)[1]investigated the plant and isolated two crystalline alkaloids named tylophorinine ($C_{24}H_{27}NO_4$) and tylophorinine ($C_{23}H_{27}NO_4$). Apart from alkaloids the plant also contains cetyl alcohol, a phytosterol, a neutral substance of an alcoholic nature, a wax, a resin, chlorophyll, colouring matter, tannin, glucose, calcium salts, potassium chloride etc. This plant is extensively used in traditional ayurvedic medicines for dysentery and catrarr2.

MATERIALS AND METHODS
PREPARATION OF THE DRUG

The entire plant of tylophora asthamatica was used. The plant material was collected form market, washed. Shade dried and powdered. Aqueous extract of the drug was prepared in different concentrations (20%,10%,5%,2%, and 1%) by adding to the plant material trice the amount of water that was required and reduced the column to one third by heating at low temperature. This was used to test cytotoxicity. Since this aqueous extract of T. asthmatica exhibited cytotoxicity, different solvents like petroleum ether, methanol and chloroform were used to extract the active principle. The petroleum ether (PE) extract exhibited maximum cytotoxicity and hence it was used for further study.

COLLECTION OF PE EXTRACT

50g of powdered entire plant (T. asthmatica) was subjected to extraction with PE in a soxhlet's apparatus for 8 hrs. The extract was evaporated to dryness and dissolved in PBS at appropriate concentrations and tested for cytotoxicity From 50g of powdered plant material about 10g of PE extract was obtained.

ANIMALS AND DIETS

Inbred strains of swiss albino mice having an average weight of 30gms which were supplied from our animal house, Department of Biochemistry were used for all the experiments. They were housed six per cage and maintained on standard pellet diet (Hindustan Lever Ltd., India) and water. No

special arrangements were made for the maintenance of temperature and lights so that animals have a natural environment.

Cell line and maintenance of tumour cell line in mice

For in vitro cytotoxicity study of PE extract the cell lines used were DLE(Dalton's Lmphoma ascites and EAC (Ehrlich ascites Cells). *In vivo* stud was carried out only on DLA cells.

Cell lines were obtained form Tropical Botanical garden and research Institute (TBGRI), Palode, Thiruvananthapuram.

Tumor was maintained in inbred swiss albino whit mice of 9-10 weeks age by serial intraperitoneal injection of $1x10^6$ cells in PBS, which were aspirated from the peritoneal cavity of tumor bearing mice. Palpable tumors appeared in a span of 7-12 days and the life span of the mice were found to be 25-27 days[3].

DRUG DOSAGE

For selecting the drug dose , a preliminary experiment was done using different doses of PE extract (0.1,o.2,0.5,0.75 and 1 mg) in PBS per animal and b noting their life span. Drug was injected intraperitoneally for five alternate days starting from 24 hrs after tumor transplantation.

Doses below 0.5 mg did not produce any significant response wit an average life span of 27 days. With 0.5 mg the life span of 70% animals were found to be increased to ore tan 60 days. By increasing the dose to 0.75 mg and 1mg no further response was found and hence 0.5 mg per animal was used for further study.

In vitro cytotoxicity determination of PE extract against DLA and EAC cells and their LD^{50} value determination.

Short term cytotoxicity of the drug was tested by trypan blue exclusionmethod4 tumor cells were aspirated from ascites tumor bearing mice and washed trice wit PBS. $1x10^6$ DLA and EAC cells were incubated with different concentration of the drug extract (50,40,25,20,17.5,12.5, and 10 µg PE extract in PBS) at 37^0C for 3hrs. After incubation the percentage of dead cells were determined using trypan blue exclusion method. From the graph plotted the in vitro LD_{50} value of the drug for DLA and EAC were determined.

In vivo studies: Dalton's Lymphoma Ascites development and survival of animals

Animals were divided into 3 groups of six mice each. DLA cells were aspirated from the peritoneal cavity of mice, washed with PBS and $1x10^6$ cells were given intrperitoneally to develop ascites tumor. After 24 hrs (Preventive dose) and fro 11^{th} day onwards after tumor transplantation (curative dose) five doss of drug (0.5 mg/ml PBS/animal) were given intraperitoneally on alternate days. Te controls were left untreated. The mortality of animals were noted and the percentage increase in the life span was calculated from the formula 5

% ILS = (T-C/C) x100

where T is the average no. of days the treated animals survived

and C is the average no. of days the control animals survived

RESULTS

In the cytotoxicity determination of PE extract in vitro against DLA and EAC it was found that 17.5 µg produced 25% cell death wile 50 µg caused 100% cell death in the case of DLA cells and in the case of EAC it was found to be 7% and 100% respectively. (Table 1) from the graph it was observed that for 50% cell death a concentration of 22.5 µg was required for DLA cells and 30 µg for EAC. The comparative effect of different concentrations of PE extract against DLA and EAC are represented in the graph.

From in vivo studies the average life span of tumor control mice were found to be 25 days. But the animals that were given preventive dose survived for months without any tumor development . In the case of animals which were given curative dose, the further growth of tumor was found to be arrested. This group of animals had an average life span of 50 days.

DISCUSSION

The results of in vitro and in vivo studies carried out with T. asthmatica extract shows that the plant has antitumor activity. Since the PE extract showed a significant cytotoxicity compared to the aqueous extract of the powdered plant material and the other solvent extracts it can be neutral fat.

When the cytotoxic effect of different concentration of PE extract was tested against DLA and EAC cells by trypan blue exclusion method a better effect was found in the case of DLA cells compared to EAC, Probable because of te specific nature of the drug towa4rds the cell ie., each drug ma have specific effects to different cell lines probably due to te difference in their mechanism of action or inability to penetrate the cell membrane6.

The studies carried out in vivo with PE extract sowed a significant reduction in tumor volume and an increase in the life span of tumor bearing animals.

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Table 1: Cytotoxic effect of different concentrations of PE extract against DLA and EAC

Con. Of PE extract/0.1 ml PBS (ug)	% cell death of DLA cells)(1x106 cells /ml PBS)	% cell death of EAC cells)(1x106 cells /ml PBS)
50.00	100	100
40.00	90	73
25.00	78	38
17.50	25	7
12.50	5	-
10.00	-	-

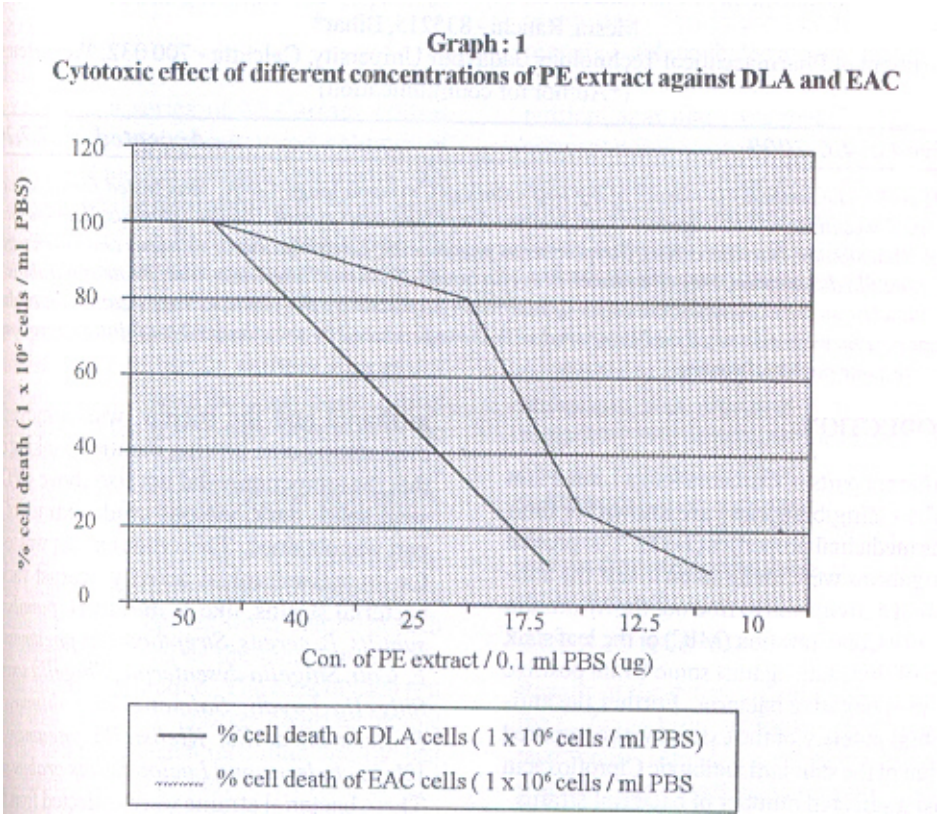
Table 2: Dalton's Lymphoma Ascites development and survival of animals

	No.of animals alive after						Average	Increased
	10 days	20 days	30 days	40 days	50 days	60 days	Life span	Life span {(T-C)/C}x100
Group I (C)	6/6	4/6	1/6	0/6	0/6	0/6	25 days	
Group II (T)	6/6	6/6	6/6	6/6	6/6	6/6		The were leading normal life for months without any problem
Group III (T)	6/6	6/6	5/6	5/6	3/6	3/6	25 days	

Group I : Tumour control

Group II: Animals treated with 5 doss of 0.5 mg of PE extract/ ml PBS from 2nd day of tumor transplantation.

Group III: Animals treated with 5 doses of 0.5 mg of PE extract/ ml PBS from 11th day of tumor transplantation.



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