Anti-tumor Activity of Tylophora Asthmatica
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Received: 22.5.2000                                                                 Accepted: 11.6.2000

**ABSTRACT:** T. asthmatica, which belongs to the family Asclepiadaceae is a small twining 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.

**INTRODUCTION**

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) 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 catarr.

**MATERIALS AND METHODS**

**PREPARATION OF THE DRUG**

The entire plant of tylophora asthamatica was used. The plant material was collected from 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 Lymphoma ascites and EAC (Ehrlich ascites Cells). *In vivo* study was carried out only on DLA cells.

Cell lines were obtained from 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 $1 \times 10^6$ 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, 0.2, 0.5, 0.75 and 1 mg) in PBS per animal and 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 with an average life span of 27 days. With 0.5 mg the life span of 70% animals were found to be increased to over 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 exclusion method$^4$ tumor cells were aspirated from ascites tumor bearing mice and washed three times with PBS. $1 \times 10^6$ 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 $1 \times 10^6$ cells were given intraperitoneally to develop ascites tumor. After 24 hrs (preventive dose) and from 11$^{th}$ day onwards after tumor transplantation (curative dose) five doses of drug (0.5 mg/ml PBS/animal) were given intraperitoneally on alternate days. The controls were left untreated. The mortality of animals were noted and the percentage increase in the life span was calculated from the formula $5$

\[
\% \text{ ILS} = \left( \frac{T-C}{C} \right) \times 100
\]

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 while 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.

**ACKNOWLEDGEMENT**

Thanks are due to Mr. Devasia Joseph, Menacheril for giving us information about the plant . asthmatic and to Mrs. Latha, Ethanopharmacology division TBGRI, TVM for providing EAC and DLA cells.

**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 Life span | Increased Life span $\{(T-C)/C\} \times 100$ |
|----------------|---------------------------|-------------------|-----------------------------------------------|
|                | 10 days 20 days 30 days 40 days 50 days 60 days |                  |                                               |
| 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 doses of 0.5 mg of PE extract/ ml PBS from 2$^{nd}$ day of tumor transplantation.

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

![Graph 1](image-url)

Cytotoxic effect of different concentrations of PE extract against DLA and EAC.
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