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ANTI CANCER ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF WHOLE PLANT OF *DIPTERACANTHUS PROSTRATUS NEES*

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ABSTRACT

Despite the great development of organic synthesis, currently 25% of prescribed drugs worldwide are still derived from plant source, showing that plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer. The present study was carried out to evaluate the anticancer activity of aqueous and ethanolic extract of the whole plant of *Dipteracanthus prostratus* (AEDP, EEDP respectively) in mice. The anticancer activities of AEDP, EEDP were studied against Ehrlich ascities carcinoma (EAC) in mice. It was revealed that administration of AEDP, EEDP of whole plant of *Dipteracanthus prostratus* exhibited ability on reduction significantly in tumor volume, viable cell count, while increasing significantly the non viable tumor cells count compared to EAC control group. It was found that the mean survival time and percentage of life span in mice were increased in extracts treated groups. These results suggest that the extract of whole plant of *Dipteracanthus prostratus* exhibits potential anticancer activities.

KEYWORDS

Ehrlich Ascites Carcinoma, Dipteracanthus Prostratus Nees, 5-fluorouracil, Tumor volume, Viable cell count and Non viable cell count.

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INTRODUCTION

A number of natural products have been studied for anticancer activity on various animal models[1]. This has resulted in availability of nearly 30 effective anti cancer drugs. The plant *Dipteracanthus prostratus* (Family:-*Acanthaceae)* is widely available in Tamil Nadu. It is a prostrate perennial herb, ovate green leaves 2-10 cm long, have lower surface conspicuously paler. The leaves are ovate or elliptic acute hairy, entire with narrow base. The leaf stalk 5-30 mm long. Almost 8 species *Dipteracanthus prostratus Nees* occurs in India. The leaves are

useful for treatment of inflammation, anti-fungal, anti-bacterial, fungicide and cancer[2,3]. The aim of the present study was to evaluate anticancer activity of aqueous and ethanolic extract of whole plant of *Dipteracanthus prostratus* (EEDP, AEDP) on Ehrlich Ascites Carcinoma (EAC) in Swiss Albino mice and to evaluate its *in-vivo* anticancer activity.

MATERIALS AND METHOD

Plant material and extraction

The whole plant of *Dipteracanthus prostratus* were collected from foot hills of Yercaud, Tamil Nadu, India in the month of September 2010 and identified and authenticated by Dr. A Balasubramaniam (ABS garden, Karripatti, Salem, Tamil Nadu). The air dried and coarsely powdered whole plant material (500 g) extracted with 1.5 L of petroleum ether (60-80^{o}C), chloroform, acetone and ethanol in a soxhlet extractor for 72 h. The aqueous extract was prepared by powdered the whole plant of *Dipteracanthus Prostratus* were extracted with distilled water by stirring overnight and then centrifuged at room temperature. The supernatant was collected and evaporated to dryness. The petroleum ether extract yielded a dark green color residue weighing 2g, ethanol extracts yielded dark brown and semi solid residues weighing 3g and aqueous extract yielded brown color residues weighing 2.5 g. The extract was preserved in a refrigerator at 4^{0}C until further use.

Chemicals

5-Flurouracil (5-FU) was obtained from Ranbaxy, Ltd., Gurgaon, India. Trypan blue was obtained from Hi-Media Laboratories, Ltd., Mumbai, India. All other chemicals were used analytical grade.

Experimental animals

Studies were carried out by using adult male Swiss Albino mice 180-230 g. They were obtained from venkateswara enterprises, Bangalore. They were kept in quarantine for 10 days under standard laboratory conditions (25±2^{o}C relative humidity) for 12 h dark and light cycle respectively and were given standard food and water *ad libitium*. The study was permitted by the Institution of Animal ethical committee with registered no. Ph/Chem/25/2010/IAEC/VMCP.

Phytochemical screening

Freshly prepared AEDP, EEDP were tested for the alkaloid, steroids, terpenoids, flavonoids, and glycosides using standard procedure.

Acute oral toxicity study

Acute oral toxicity was performed by following Guideline-423 fixed dose procedure for aqueous and ethanolic extract of *Dipteracanthus Prostratus* and it was found that dose increasing up to 2000 mg/kg body weight shown number of mortality in experimental rats. The LD_{50} of the aqueous and ethanolic extract of *Dipteracanthus prostratus* as per OECD Guideline-423 is greater than 2000 mg/kg[4,5].

Tumor cell lines

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Bhopal Cancer Research Centre Bhopal (M.P.), India. EAC cells were maintained *in-vivo* in Swiss albino mice by intra peritoneal (i.p.) transplantation.EAC cells aspirated from the peritoneal cavity of mice were washed with phosphate buffer solution (pH 7.4) and used for inducing ascetic tumor in mice under study and inoculation of 1x10^{6} cells/mouse after every 10 days.

In vivo anti cancer activity

After acclimatization, mature male Swiss albino mice divided into four groups of six animals (n=6) each. The test samples were dissolved in isotonic saline (0.9% NaCl w/v) solution. EAC cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue) under the microscope and were adjusted at 1x10^{6} cells/ml. 0.1 ml of EAC cells per 10 g body weight of the animals were injected (i.p.) on day zero (day 0).

A day of incubation was allowed for multiplication of the test sample (AEDP 200 mg/kg and EEDP 200 mg/kg), 0.1 ml/ 10 g body weight and 5-fluorouracil (5FU) at the dose level of 20 mg/kg body weight was used as standard. The anticancer activities of the test sample were measured in EAC animals with respect of following parameters. The effect of aqueous and ethanolic extract on tumor growth and

host's survival time were examined by studying parameters like tumor volume, tumor cell count, viable tumor cell count, non viable tumor cell count, mean survival time and increase in life span.

Tumor volume

The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 rpm for 5 minutes.

Tumor cell count

The ascitic fluid was taken in a RBC pipette and diluted 1000 times. Then a drop of diluted cell suspension was placed on a Neubauer counting chamber and the number of cells in 64 small squares was counted.

Estimation of viable and non viable tumor cell count

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

(Cell count = (Number of cells x Dilution)/ Area x Thickness of liquid film)

Percentage increase life span

Recording the mortality monitored the effect of the AEDP and the EEDP on tumor growth and the percentage increase in life span (ILS %) were calculated.

(ILS% = [(Mean survival of treated group/Mean survival of control group)-1x100]

Mean survival time = [1st death + last death]/2).

Haematological studies

At the end of the experimental period all mice were by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of hemoglobin (Hb) content, red blood cell count (RBC). WBC differential count was carried from Leishman stained blood smears[6,7].

Histopathological studies

The animals used in the curative study were sacrified the liver was examined grossly. A small portion of liver was fixed in 10% formalin solution processed and embedded in paraffin wax to obtain 5-6 µm and eosin stained sections[8].

Statistical analysis

The experimental results were expressed as the mean ±S.E.M. Data were accessed by the one way ANOVA followed by Dunnett's post hoc test. P value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results of the preliminary phytochemical analysis carried out on the crude aqueous and ethanol, extract indicated the presence of alkaloids, phenolic compounds, tannins, carbohydrates, flavonoids and glyocosides.

As shown in Table No.1 the average number of tumor volume EAC treated animals was found to be 4.9±0.91. AEDP and EEDP treatment at both dose levels significantly ($p<0.001$) reduced tumor volume which was found to be 2.8±0.53 and 3.8 ± 0.83 respectively. Viable cell count of the tumor bearing mice was significantly decreased non-viable cell count were increased in AEDP and EEDP treated groups in dose levels when compared with EAC treated group.

As shown in Table No.2 the effect of AEDP and EEDP on the survival time of tumor bearing mice showed MST for the tumor control group (EAC treated) to be 18 ± 1.32 days. While it was 29 ± 2.5 (61.11%) and 31 ± 0.92 (77.78%) for the group treated with *Dipteracanthus Prostratus* at the dose of 200 mg/kg of both aqueous and ethanolic extract respectively. The standard drug 5FU (20mg/kg) showed 35 ± 1.5 (94.44%) respectively.

As shown in Table No.3 hematological parameters of tumor bearing mice on 15th day were found to be significantly altered from normal groups. The total WBC count was found to be decreased with a reduction of the haemoglobin and RBC. In a differential count of WBC the percent of neutrophil increased while the lymphocyte count decreased. At the same time interval AEDP and EEDP treated could change altered parameters to near normal. The above results demonstrated the anti tumor effect of AEDP and EEDP against EAC in Swiss Albino

mice. A significant ($p<0.001$) enhancement of MST and non-viable cell count in peritoneal exudates was observed due to EEDP treatment. Estimated the EEDP treatment in inhibited tumor cell growth, the effect of EEDP treatment was examined on the viable and non viable cell counts against tumor bearing mice normally each mouse contains about $1x10^6$ intra peritoneal cells. AEDP and EEDP treatment was found to enhance non viable cell count peritoneal exudates and decreases the viable cells count. It may be due to the absorption AEDP and EEDP by viable cells which leads to lysis of cell through the activation of macrophages or cytokines production in peritoneal cavity. Analysis of other hematological parameters showed minimum toxic effect in the mice which were treated with AEDP and EEDP. After 14 days of transplantation, EEDP treated groups were able to reverse the changes in hematological parameters consequent to tumor inoculation.

Figure No.1 Microscopical examination of liver section of normal control group showed normal architecture, tumor control group showed neoplastic proliferation of hepatocytes and standard drug treated showed normal hepatocytes with mild congestion. Microscopical examination of liver section of extracts treated showed almost normal architecture of hepatic parenchyma with focal collection of mononuclear cells and normal hepatocytes with mild congestion.

Table No.1: Anticancer activity of Aqueous and Ethanol extracts of *Dipteracanthus prostratus Nees* on tumor volume, viable tumor cells count and non viable tumor cells count

S.No	Treatment group	Tumor volume (ml)	Viable tumor cells count (10^6 cells/mouse)	Non viable tumor cells(10^6cells/mouse)
1	EAC Control	4.9±0.91	10.2±0.31	0.4±0.03
2	EAC +Ethanolic extract(200mg/kg)	2.8±0.53*	4.8±0.09*	0.5±0.03
3	EAC+Aqueous extract(200mg/kg)	3.8±0.83	7.3±0.03	0.7±0.05
4	EAC + 5FU (20mg/kg)	1.9±0.48*	3.1±0.17*	0.9±0.01

Values are expressed as mean ± SEM,***p<0.001,p<0.01,p<0.05, Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test., *p<0.01 calculated by comparing treated groups with EAC control group. n=6.

Table No.2: Effect of Aqueous and Ethanolic extracts of *Dipteracanthus prostratus Nees* on Mean survival time and Percentage increase in life span

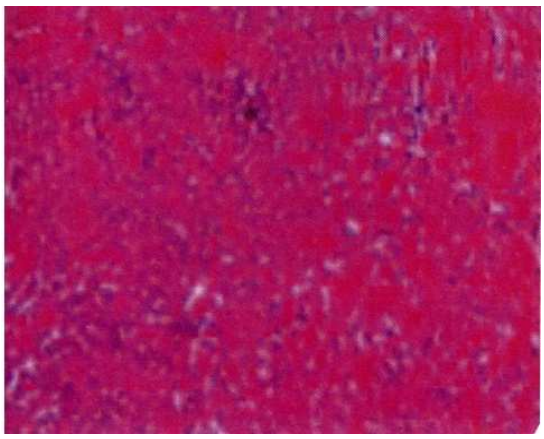
S.No	Treatment group	Mean survival time (in days)	% Increase in life span
1	EAC control	18 ± 1.32	-
2	EAC+Aqueous extract (200 mg/kg)	29 ± 0.86*	61.11
3	EAC+Ethanolic extract (200 mg/kg)	32 ± 0.92*	77.78
4	EAC + 5 FU (20 mg/kg)	35 ± 1.5**	94.44

Values are expressed as mean ± SEM,***p<0.001,p<0.01,p<0.05, Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test, *p<0.01 calculated by comparing treated groups with EAC control group. n=6.

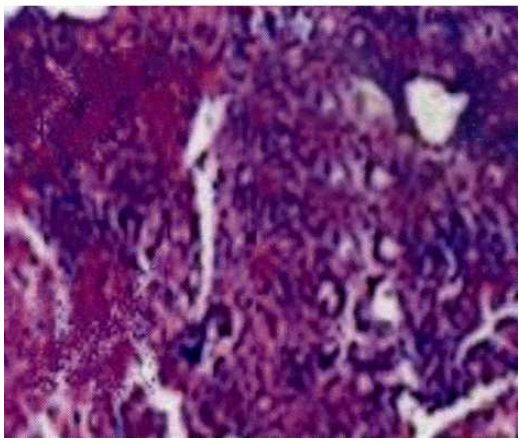
Table No.3: Haematological parameters of AEDP and EEDP treated mice bearing EAC cell line

S.No	Treatment Groups	Hb(g%)	RBC (Million/mm.cu)	WBC (Million/mm.cu)	Differential count (%)		
					Lymphocytes	Neutrophils	Monocytes
1	Normal control	13.5±0.32	4.82±0.13	7.86±0.06	76.80±0.47	29.51±0.27	2±0
2	EAC+Control	8.5±0.95	1.15±0.21	15.1±1.24	44.0 ± 1.82	53.0 ± 9.9	3±0.1
3	EAC+Aqeuous extract(200 mg/kg)	10.2±0.22	4.22±0.06	12.01±0.26	51.23±0.28	42.3 ± 0.31	2±1
4	EAC+Ethanolic extract(200 mg/kg)	11.4±0.63*	4.65±0.52	11.21±0.36	62.42 ± 0.36	32.52±0.35	2±1.1
5	EAC + 5 FU (20 mg/kg)	12.2±0.62*	5.22±0.42*	8.6±0.42	60.24 ± 0.42	26.2±0.42	1±0

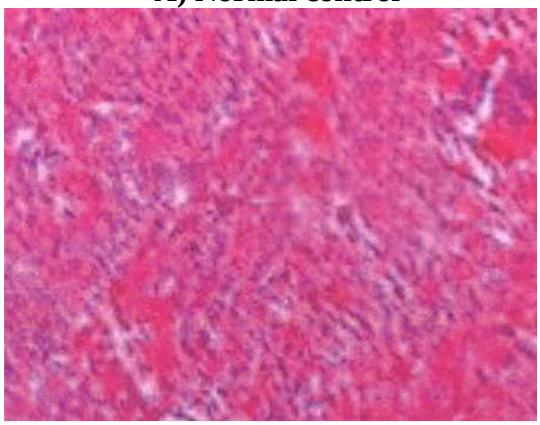
Values are expressed as mean ± SEM, n=6***p<0.001,p<0.01,p<0.05, Statistical significance (p) calculated by one way ANOVA followed by Dunnett's test, *p<0.01 calculated by comparing treated groups with EAC control group.



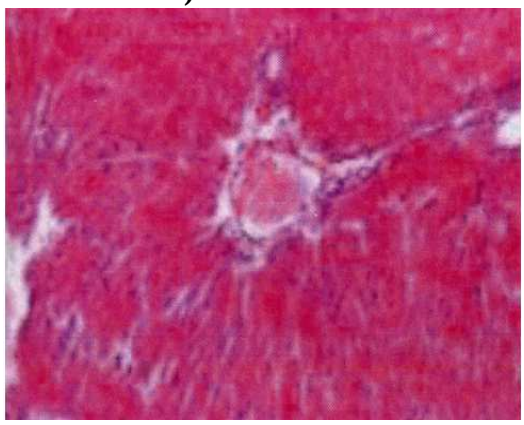
A) Normal control



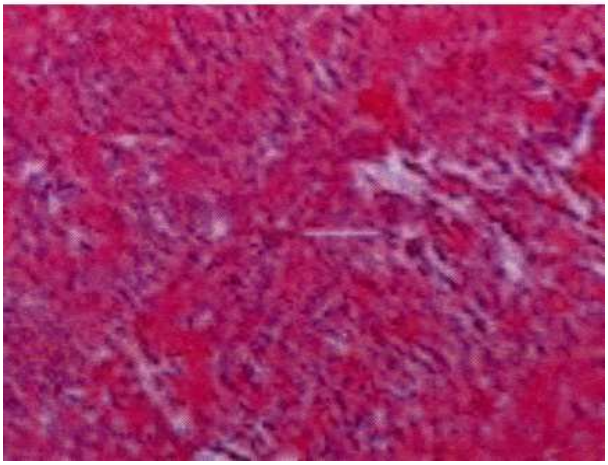
B) EAC control



C) Standard 5-FU 20mg/kg



D) AEDP 200mg/kg



E) EEDP 200mg/kg

Figure No.1: Histopathology of liver tissues of EAC bearing mice

CONCLUSION

The ethanolic extract of the whole plant of *Dipteracanthus prostratus Nees* as a novel, potent agent in the area of cancer treatment. The phytochemical studies indicated the presence of flavonoids, alkaloids and terpenoids in AEDP and EEDP. Flavonoids shown to possess anti-mutagenic and anti-malignant activity. Furthermor, flavonoids have a chemo preventive role in cancer through their effect on single transduction in cell proliferation and angiogenesis. The anticancer effect produced by the AEDP and EEDP may be due to its flavonoid as well as anti oxidant potential. The aqueous and ethanolic extract of *Dipteracanthus prostratus Nees* restore the mean survival time, decreased tumor volume count in treated mice. Analysis of the haematological parameters showed a minimum toxic effect in the mice which were cured by extract treated groups. Microscopical examination of liver section showed regeneration of hepatic parenchyma. The histopathological studies showed recovery and regeneration of damaged liver cells in extract treated groups. Thus a present study suggests the ethanolic extract of *Dipteracanthus Prostratus* possess anticancer activity and increase the life span. It was observed that the ethanolic extract is more potent than the aqueous extract eventhough both the extracts are endowed with the significant anticancer property.

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