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Research Article

CYTOTOXIC AND ANTITUMOR ACTIVITY OF METHONOLIC EXTRACTS *DESMODIUM TRIANGULARE* (RETZ) MERR. ROOT

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ABSTRACT

Cytotoxic and antitumor activities of methanolic extract Root of *Desmodium triangulare* were studied. The extract showed potent *in vitro* cytotoxic activity against DLA and EAC cell lines.IC_{50} value for DLA cell line was 99 μg and for EAC cell lines 89 μg. The antitumor activity of the extract was determined by using DLA cell line induced solid tumor and EAC cell lines induced ascites tumor model in mice and its comparison with standard anticancer drug cyclophosphamide. The treatment with methanolic extract Root of *Desmodium triangulare* (50 mg/kg and 250 mg/kg body weight) significantly reduced the volume of solid tumor development and the increase in body weight of ascites tumor model. The life span of treated animals was increased up to 74.47%, The results were more significant in mice treated with 250 mg/kg body weight. This indicates the *in vitro* cytotoxic and antitumor properties of *Desmodium triangulare* suggesting its potential use as an anticancer agent.

Keywords: *Desmodium triangulare,* Antitumor activity, In Vitro cytotoxicity

INTRODUCTION

Natural Products, especially plants, have been used for the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient time and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of plants appeared in about 2600 BC Cancer is a group of diseases in which cells are aggressive, invasive, and sometimes metastatic. Cancer may affect people at all ages. Apart from humans, forms of cancer may affect other animals and plants [1]Chemotherapy is one of the methods for the treatment of cancer. A major complication of chemotherapy is its toxicity to normal cells, which is due to the inability of drug to differentiate between normal cells and malignant cells. This often impacts the efficacy of the treatment and even makes it impossible to cure the patients. One of the requisites of cancer chemo preventive agent is elimination of damaged or malignant cell through cell cycle inhibition or induction of apoptosis with less or no toxicity to normal cells [2]Lymphoma is a disease of the lymphocytes (a type of white blood cell involved in immune responses) and the lymphatic system, which includes the spleen, thymus, and liver, as well as other lymphatic tissues. Dalton's ascites lymphoma is transplantable, poorly differentiated malignant tumor which appeared originally as lymphocytes in a mouse. It grows in both solid and ascitic forms[3]. *In vitro* cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work[4, 5] This present study was carried out to evaluate the *in vivo* anti-tumor activity of methanolic extract of the root of *Desmodium triangulare* belong to the family (Fabaceace) against Ehrlich ascites carcinoma (EAC) and Dalton lymphoma ascites (DLA) in mice.

MATERIALS AND METHOD

Animals

Female Swiss Albino (22-28 g size) was purchased from Small Animal Breeding Station, College of Veterinary, Agricultural University, Thrissur, and Kerala. The animals were maintained under standardized environmental conditions (22-28^{0}C, 60-70% relative humidity, 12 hr. dark/light cycle) and fed with standard rat feed (Sai Durga Feeds and Foods) and water *ad libitum*. All the animal experiments were carried out in Amala Cancer Research Centre by the prior permission of Institutional Animal Ethics Committee (IAEC)

Tumor Cell Lines

1. Dalton's Lymphoma Ascites (DLA) cells
2. Ehrlich ascites tumor (EAC) cells

The cell lines were maintained at Amala Cancer Research Centre, Amala Nagar, and Thrissur, India.

Preparation of plant extract

About 100g of air dried powdered material was taken in a soxhlet apparatus and extracted by using 70% methanol (Merck India) as solvent, till colour disappeared. After that extract was concentrated by distillation and solvent was recovered. The final solution was evaporated to dryness. The dried extract was redisolved in distilled water and used for further studies.

In Vitro Cytotoxicity Assay

The cytotoxicity was determined by Trypan blue exclusion method [6] It is based on the principle that live cells possess intact cell membranes that exclude the dye while the dead cells do not and have blue coloured cytoplasam under light microscope. For the assay DLA and EAC cells aspirated from mice intraperitoneal cavity and were counted to a density of 1 x 10^6 in 0.1 ml phosphate buffer saline (PBS, pH 7.4). To about 0.8 ml of PBS add 0.1 ml of cell suspension containing 1 x 10^6 cells and different concentration of extract ranging from 10 µg – 1000 µg/ml. These were incubated for 3 hours at 37^{o}C. After incubation, 0.1 ml trypan blue dyes was added and apply a drop of tryphan blue - cell mixture to a hemocytometer and count the stained (non-viable) and unstained (viable) cells separately under a microscopic field

Antitumour Activity of *Desmodium Triangulare*

For assessing the antitumor activity of *D.triangulare* methanol extract, Dalton's Lymphoma Ascites (DLA) cell induced solid tumor model and Ehrlich ascites Carcinoma (EAC) cell induced ascites tumor model were employed.

Dalton's Lymphoma Ascites (DLA) cell induced solid tumor model

DLA cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1 ml containing 10^6 cells was injected intramuscularly into the right hind limb of Swiss albino mice. Four groups (6 animals per each group) were used. Group 1 served as untreated control, group 2 received cyclophosphamide 10mg/kg, group3 received *D. triangular*e methanol extract (50 mg/kg), group 4 received

D.triangulare methanol extract (250 mg/kg). The drug administration was continued for 10 consecutive days and diameter of the tumor was measured using a vernier caliper at fixed intervals (on each 3rd day) and the volume was calculated using the formula,

$$\text{Tumor volume} = 4/3\ \pi\ r_1^2 x\ r_2$$

And the percentage of inhibition of tumor volume in animals was calculated by,

% of Inhibition = [(Tumor volume of Control on 30th Day – Tumor Volume of Treated on 30thday) / (Tumor volume of Control on 30th Day)] x 100

Ehrlich ascites Carcinoma (EAC) cell induced ascites tumor model

EAC cells were aspirated from peritoneal cavity of the tumor bearing mice and 0.1 ml containing 10^6 cells was injected intraperitoneally into the Swiss albino mice. Four groups (10 animals per each group) were used. Group 1 served as untreated control, group 2 received cyclophosphamide 10 mg/kg, group3 received *D.triangulare* methanol extract (50 mg/kg), group 4 received *D.triangulare* methanol extract (100 mg/kg). The drug administration was continued for 10 consecutive days. The animals were observed for the development of ascites tumor and death due to tumor burden was recorded for 30 consecutive days. The life span of animals was calculated using the formula,

$$\textbf{\% ILS= (T-C)/C x 100}$$

Where, T and C are mean survival of treated and control mice respectively [7].

Statistical Analysis

The values are presented as mean ± SD. Differences between group's means were estimated using a one way analysis of variance followed by Dunnett test, using GraphPad Instat Software. The results were considered statically significant when *P<0.05*.

RESULTS

***In Vitro* cytotoxic activity**

The effect of extract on cancer cell lines, DLA and EAC after trypan blue exclusion assay showed 100% cell death within a small concentration of drug. The IC_{50} concentration (i.e.), the amount of the extract required to cause the death of 50% cancer cells was found to be 99µg/ml and 89 µg/ml for DLA(Tab:1) and EAC cells, (Tab:2) respectively.

Antitumor Activity

Solid tumor activity *of D.triandulare* extract

Administration of methanolic extract (50mg/kg body weight and 250mg/kg body weight) inhibited DLA induced solid tumor in a dose defendant manner when compared to control group The tumor volume in untreated control mice was 3.24 ± 01.11 on day 30 and this was reduced to 1.64 ± 0.72 with(49.38%) reduction in tumor volume in 50mg/kg extract treated animals. With 250mg/kg extract administration the tumor volume was reduced to maximum of 1.15 ± 0.47 with a percentage reduction of (64.50%) This reduction in tumor volume with 250mg/kg extract was greater than that of the standard drug cyclophosphamide, which showed only an inhibition percentage of (58.02%) (Table: 3, 4).

Ascites tumor activity of *D.triangulare* root extract

The ascites tumor study employed for studying the increase in life span of tumor bearing animals for 30 days revealed that, the extract treated animals showed a dose dependened increase (Table:5,6). The lower drug dosage 50mg/kg (25.16±1.47) made the animal to live long as within % ILS of (48.00%) The higher drug dosage 250 mg/kg (29.66 ± 0.1516) made the animals to live long as with an % ILS of(74.47%), when compared to control animals (17±1.47196) and this increase was quiet satisfactory when compared to standard drug cyclophosphamide (28.83±1.47196), with %ILS of (69%.)

Table 1: Effect of methalonic extract of *D.triangulare* root of cytoxicity of DLA cells

Concentration µg /ml	Percentage of cell death	IC_{50}*
40	22.72	
80	45.67	99.00µg/ml
120	55.32	
160	63.33	
200	66.00	
240	73.5	

*IC50 value is the amount of extract needed to cause 50 % of cell death

Table 2: Effect of methonolic extract *of D. triangulare* root on cytotoxicity in EAC cells

Concentration µg /ml	Percentage of cell death	IC_{50}*
40	27.32	
80	48.75	89 µg/ml
120	57.32	
160	67.32	
200	70.32	
240	75.11	

*IC50 value is the amount of extract needed to cause 50 %

Table 3: Antitumor effect of *D.triangulare* Root of methanolic extract in reducing solid tumor volume induced by DLA Cell in Swiss albino Mice

Groups	Initial	0th day	5th day	10th day	15th day	20th day	25th day	30th day
Control	0.68±0.16	0.88 ± 0.18	0.97 ± 0.07	1.29 ± 0.23	1.75 ± 0.19	2.33 ± 1.22	2.75 ± 0.18	3.24 ± 01.11
Cyclophosphamide	0.67±0.21	0.89±0.19	0.98±0.08	1.20±0.14	1.51±0.08*	1.45±0.08	1.43±0.17**	1.36±0.008**
D.*triangulare*	0.68±0.24	0.88±0.18	0.98±0.10	1.24±0.07	1.60±0.17	1.88±0.94	1.75±0.74**	1.64±0.72**
D.triangulare	0.69±0.13	0.88±0.08	0.96±0.09	1.17±0.07	1.39±0.05**	1.36±0.44	1.26±0.39**	1.15±0.47**

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

Table 4: Inhibition of solid tumor induced tumor volume in mice by *D. triangulare* treatment on 30th day.

Groups	Doses	Tumor volume in cm^3 (30 days after tumor inoculation)	Percentage of inhibition of tumor volume after 30 days
Control	___	3.24 ± 01.11	……
Cyclophosphamide	10 mg/kg body weight	1.36 ±0.008**	58.02
D.triangulare	50 mg/kg body weight	1.64±0.72**	49.38
D.triangulare	250 mg/kg body weight	1.15±0.47**	64.50

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control

Table 5: Antitumor effect of *D.triangulare* root of methanolic extract increasing the life span of EAC Induced ascites tumor bearing Swiss albino mice.

Groups	Doses	No: of animals developed tumor	No. of days survived	% increase in life span (% ILS)
Control	___	10/10	17±1.41421	……
cyclophosphamide	10 mg/kg body weight	10/10	28.83±1.1667	69
D.triangulare	50 mg/kg body weight	10/10	25.16±1.47196	48
D.triangulare	250 mg/kg body weight	10/10	29.66±0.1516398	74.47

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control

DISCUSSION

The results of present investigation reveals that *D. triangulare* with wide spectrum of medicinal properties have cytotoxicity against DLA and EAC cell lines. Cytotoxicity is one of the chemotherapeutic targets of antitumor activity [8] Most of the clinically used antitumor agents possess significant cytotoxic activity in cell culture systems. The cytotoxic activity of *D .triangulare* against DLA and EAC cell lines partially explains its significant anti tumor activity against solid and ascites tumor.

The antitumor activity was evaluated in solid tumor model. Methanolic extract of *D.triangulare* reduced the tumor burden effectively. The antitumor activity of *D.triangulare* extract is in a dose dependent manner. In the solid tumor model, the standard reference drug cyclophosphamide at a dose of 10 mg/kg body weight for ten consecutive days were used which was significantly effective to inhibit solid tumor. The antitumor activity of *D.triangulare* extract 250mg/kg body weight is more effective than the standard drug suggesting its potent activity as an antitumor agent.

Ehrlich ascites tumor is a rapidly growing carcinoma with very aggressive behaviour [9] It is able to grow in almost all strains of mice. The Ehrlich ascitic tumor implantation induces a local inflammatory reaction, with increasing vascular permeability, which results in an intense edema formation, cellular migration, and a progressive ascitic fluid formation [10] The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells [11]The results of present study proved that *D.triangulare* extract can reduce the ascites tumor burden in a dose depended manner.

D.triangulare. extract 250 mg/kg body weight is more effective than the standard drug cyclophosphamide at a dose of 10 mg/kg body weight. This could indicate either a direct cytotoxic effect of *D.triangulare* extract on tumor cells or an indirect local effect, which may involve macrophage activation and vascular permeability inhibition. The percentage of increased in life span at the 250 mg/kg body weight dose of the *D. triangulare.* Extract was found to be the highest among the two doses tested, indicating its potent anticancer nature.

CONCLUSION

The present study provides strong evidence suggesting that *D.triangulare* root have *in vitro* cytotoxicity against DLA and EAC cell lines. The extract was found to be effective against DLA induced solid tumor and EAC induced ascites tumor. The 250 mg/kg body weight was more effective than the standard drug cyclophosphamide at a dose of 10 mg/kg body weight. No toxic symptoms were observed for all two doses during the period of study. Potent medicinal properties of *D.triangulare* root. This may be used to development of effective therapeutic approaches towards the prevention or treatments of various immune conditions and different types of cancer.

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