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Antitumor Potential of *Drosera Indica* L against Ehrlich Ascites Carcinoma (EAC) Tumor in Mice

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ABSTRACT

To study the antitumor effect of *Drosera indica* L against Ehrlich ascites carcinoma (EAC) tumor in mice. Antitumor activity of ethanol and aqueous extracts (250 and500 mg/kg) of *D. indica* L was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. Acute toxicity study was conducted to find out the safety of both extracts of *D. indica* L. After 24 h of tumor inoculation, the extracts were administered daily for 14 days. On day 15, mice were sacrificed for observation of antitumor activity. The effect of both extracts on the growth of transplantable murine tumor, life span of EAC bearing hosts and simultaneous alterations in the hematological profile and peritoneal fluid analysis (DNA, RNA, caspase-3 and total protein) were estimated. Both the extracts showed decrease in packed cell volume and viable cell count and increased in mean survival time thereby increasing life span of EAC tumor bearing mice. Hematological profile reverted to more or less normal levels in extracts treated mice. Treatment with extracts decreased the levels of RNA, DNA and increased level of caspase-3.The ethanol and aqueous extracts of *D. indica* L exhibited antitumor effect in EAC bearing mice.

Key words: *Drosera indica* L, Ehrlich ascites carcinoma, hematological profile, peritoneal fluid analysis

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INTRODUCTION

Cancer chemotherapy now plays an important role in the treatment of many malignancies, either the drug alone or as an adjuvant to surgery and/or radiation or palliative care, depending upon the specific tumor situation. Plants have a long history of use in the treatment of cancer. Plant-based systems continue to play an essential role in healthcare and it has been estimated by the WHO that approximately 80% of the world's population rely mainly on traditional medicine for their primary healthcare. Of the plant derived anticancer drugs in clinical use, the best known is vinca alkaloids, which include vinblastine and vincristine[1]. In these series one of the best plants is *Drosera indica* L. belongs to Droseraceae.

Drosera is a cosmopolitan genus of insectivorous plants and consists of approximately 170 species. *D. indica* L., *D. burmanii* and *D. peltata* J.E.Sm. ex Wild have been reported from different locations in India. These species are used as vital components in an Ayurvedic preparation called 'Swarnabhasma' (Golden ash). Macerated *D. indica* L is used to remove corns and this species has been categorized under the vulnerable medicinal plants list [2, 3]. The genus Drosera contains naphthoquinones such as plumbagin, 7-methyljuglone and Quercetin as a flavonoid, which have pharmacological value[4]. Evaluation of the antitumor activity of the plant has not been reported. This present study was carried out to evaluate the in vivo antitumor activity of ethanol and aqueous extracts of the plant of *D. indica* (L) against Ehrlich ascitescarcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant Material

The plants of *D. indica* L were collected from foot hills of Svanadurga, Karnataka, India in the month of December 2010 and it was identified and authenticated by Dr. S. N. Yoganarasimhan, Taxonomist and Research Co-ordinator at M. S.Ramaiah College of Pharmacy, Bangalore, Karnataka, India.

Extraction

The dried powder material of whole plant of *D. indica* L was extracted with ethanol in a soxhlet apparatus. The methanol extract was then distilled, evaporated, and dried in vacuum. The extract was suspended in water for further experiments. The plant material marc was soaked in the water- chloroform for 72 hr. The solvents were removed by distillation on a water bath at atmospheric pressure and the last traces were removed under reduced pressure using rotary evaporator. The ethanol (EEDI) and aqueous (AEDI) extracts were completely dried and used for the *in vivo* antitumor activities.

Tumor cell line

EAC cells were obtained from Amala Cancer Research Institute, Trissure, Kerala, India. The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells/mouse.

Animals

Studies were carried out using male Swiss albino mice weighing 20±2 g were obtained from KM College of Pharmacy, Madurai, Tamil Nadu, India. All procedures described were reviewed and approved by the Animals Ethical Committee (Protocol.No.A.Raju 0903PH2254/ JNTUH 2009).

Acute toxicity studies

Acute toxicity study was carried out on EEDB and AEDB following OECD guidelines (OECD 423) [5].

Study of antitumor activity [6]

Healthy Swiss albino mice were divided into 7 groups of 10 animals in each. All the groups were injected with EAC cells (2×10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. Group 1 (vehicle control) received water once daily for 14 days. Group 2 (EAC control) received water once daily for 14 days. Group 3 (Standard) received 20mg/kg of 5Flurouracil, intraperitoneally for 14 days. Group 4, 5, 6 and 7 received EEDI and AEDI at the doses of 250 and 500 mg/kg orally once daily for 14 days.

After 24 h of the last dose including 18 h of fasting five mice in each group were sacrificed and rest of them were kept to check the mean survival time (MST) of the tumor bearing hosts.

Blood collected and hematological parameters were determined as described in hematological studies. Liver and other important internal organs were removed to know the antioxidant enzyme level.

Assay of DNA, RNA, caspase-3 and total protein

The peritoneal fluid was collected in heparinized tube, washed and suspended in saline. The total protein was assayed by the method of [7].Nucleic acid was extracted by the method of Schneider 1945. DNA and RNA were estimated by diphenylamine [8] using arsenal reaction for RNA[9]. Packed peritoneal cells lysate were used to measure capase-3 activity using sigma reagent kit. Total DNA was extracted and analyzed on agarose gel [10, 11].

Antitumor effect of EEDI and AEDI were assessed by observation of changes with respect to body weight, packed cell volume (PCV), viable tumor cell count, mean survival time (MST) and percentage increase in life span (% ILS). MST of each group containing six mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated using following equation.

MST= (Day of first death+ Day of last death)/2.

ILS (%) = [(Mean survival time of treated group/mean survival time of control group)–1] ×100.

Statistical analysis

The results are expressed as mean ± S.E.M. The evaluation of the data was performed using one way ANOVA followed by Newman-Keul's multiple comparison test; $p< 0.05$ implied significance.

RESULT AND DISCUSSION

Acute toxicity studies

Ethanol and aqueous extract of *D. indica* L were administered separately up to 3000 mg/kg body weight and since these extracts did not produce any toxic symptoms of mortality, they were considered safe for further pharmacological screening.

Reliable criteria for judging the value of any anticancer agents is the prolongation of life span of animals decrease in WBC count [12] and decreased in viable tumor cell count. The effect of EEDI and AEDI on mean survival time and tumor growth was shown in Table 1. In the EAC control group the mean survival time was 11.25±0.5 day, while it increased to 32.5±0.9 and 39.5±0.3 days for 250 and 500mg/kg of EEDI and 29.25±0.9, 31.5±0.7days for two doses of AEDI respectively. The group treated with the standard drug 5-fluoruracil (20 mg/kg) showed 32±1.2days. Treatment with EEDI and AEDI at the doses of 250 and 500 mg/kg reduced the body weight, packed cell volume, and viable tumor cell count in a dose-dependent manner as compared to that of EAC control group. Reduction in viable cell count reduced the tumor burden and increased in life span of EAC bearing mice under treatment with EEDI and AEDI at the dose of 250 and 500 mg/kg.

Table 1: Effect of EEDI and AEDI on mean survival time and tumor growth on EAC bearing mice

Parameters	Normal	EAC control	EAC+5FU (20mg/kg)	EAC+EEDI (250mg/kg)	EAC+EEDI (500mg/kg)	EAC+AEDI (250mg/kg)	EAC+AEDI (500mg/kg)
Body Weight (g)	22.93±1.3	37.1±1.5	22.65±1.35	23.78±1.0[a]	21.95±0.7[a]	29.98±1.3[a]	23.9±0.8[a]
MST (Days)	40	11.25±0.5	32±1.2	32.5±0.9[a]	39.5±0.3[a]	29.25±0.9[a]	31.5±0.7[a]
ILS (%)	100	45±1.0	77.5±2.7	79.38±2.1[a]	93.13±2.1[a]	73.13±2.8[a]	83.75±3.9[a]
PCV(ml)	-	27.7±0.8	19.33±1.8	18.58±2.4[a]	13.6±0.4[a]	22.03±0.9[a]	19.28±0.5[a]
Viable cell count	0	13.1±1.8	1.2±0.23	8.5±1.3[b]	1.2±0.3[a]	9.2±0.6[b]	6.9±0.7[a]

The data were expressed as the mean± S.E.M. n = 10. Analysis of the data was performed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test

a. $p<0.001$, compared to the EAC control group
b. $p<0.01$, compared to the EAC control group

Treatment with EEDI and AEDI altered the changed hematological parameters more or less normal when compare with EAC control mice , which were shown in Table 2.Hemoglobin content, RBC count, monocyte and lymphocyte in the EAC control group was decreased when compared to normal group. Hematocrit (%) also reduced in EAC control mice when compared with normal mice. Treatment with EEDI and AEDI at the dose of 250 and 500 mg/kg increased the hemoglobin content, RBC count, monocyte and lymphocyte count to more or less normal levels. The total WBC count and neutrophil count were found to be increased in EAC control group when compared with normal group. Administration of EEDI and AEDI at the dose of 250 and 500 mg/kg in EAC bearing mice reduced significantly ($p<0.001$) WBC count and neutrophil count compared with EAC control.

Table 2: Effect of EEDI and AEDI on Hematological parameters in EAC bearing mice

Parameters	Normal	EAC control	EAC+5FU (20mg/kg)	EAC +EEDI (250mg/kg)	EAC+ EEDI (500mg/kg)	EAC+AEDI (250mg/kg)	EAC+AEDI (500mg/kg)
Hemoglobin (g%)	13.65±0.3	9.4±0.6	12.25±0.5	13.3±0.1[a]	14.15±0.3[a]	11.58±0.3[a]	13.25±0.4[a]
Hematocrit(%)	41.22±0.6	22.78±1.0	40.03±0.9	38.1±0.9[a]	39.78±0.5[a]	34.48±0.3[a]	35.25±0.9[a]
RBC Count (10^6/mm^3)	5.88±0.3	3.15±0.2	5.3±0.3	4.28±0.2[c]	5.62±0.3[a]	4±0.04[c]	4.33±0.1[c]
WBC Count (10^3/mm^3)	4±0.1	9.65±0.2	6.15±0.9	5.45±0.3[a]	4.45±0.4[a]	6.97±0.1[a]	5.37±0.3[a]
Neutrophil(%)	17.12±0.5	64.7±0.8	24.97±1.4	25.7±0.7[a]	18.3±0.9[a]	35.72±1.8[a]	23.12±0.9[a]
Monocyte (%)	1.57±0.04	0.62±0.09	1.2±0.1	1.03±0.1[b]	1.37±0.1[a]	1.1±0.2[c]	1.07±0.1[b]
Lymphocyte (%)	78.7±0.5	32.72±1.1	74.27±1.04	50.85±5.7[a]	71.62±1.02[a]	44.3±2.4[b]	56.35±3.9[a]

The data were expressed as the mean± S.E.M. n = 10. Analysis of the data was performed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test

a- $p<0.001$, compared to the EAC control group

b- $p<0.01$, compared to the EAC control group

c- $p<0.05$, compared to the EAC control group

In cancer chemotherapy the major problem are of myelo suppression and anemia[6]. The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin or hematocrit percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions [13]. Treatment with EEDI and AEDI brought back the hemoglobin content, RBC, hematocrit and WBC cell count near to normal values. This indicates that EEDI and AEDI posse's protective action on the heamotopoietic system.

White blood cells are the infection-fighting cells in the blood and are distinct from the red (oxygen-carrying) blood cells known as erythrocytes. There are different types of white blood cells, including neutrophils (polymorphonuclear leukocytes; PMNs), band cells (slightly

immature neutrophils), Ttype lymphocytes (T cells), B-type lymphocytes (B cells), monocytes, eosinophils, and basophils. All the types of white blood cells are reflected in the white blood cell count. Treatment with 250 and 500 mg/kg of EEDI and AEDI nearly brought back the normal value when compared with EAC control mice. These indicating parameters reveal that the plant extracts possess less toxicity on hematological system.

Effect of EEDB and AEDB on Assay of DNA, RNA, caspase-3 and total protein in peritoneal fluid of EAC -bearing mice was shown in Table 3. The level of DNA, RNA and total protein level increased significantly in EAC control mice to 6.53±0.06, 10.83±0.2, and 118.03±0.92 respectively and after 14 days treatment with doses of 250,500 mg/kg of EEDB and AEDB reduced significantly whereas caspase-3 level increased significantly (($p<0.001$) to 1.3±0.2, 1.7±0.06, 0.48±0.05 and 0.8±0.09 in 250 and 500 mg/kg treatment of EEDI and AEDI respectively.

Table 3:Effect of EEDI and AEDI on peritoneal fluid analysis

Parameters	**DNA(mcg 10^{-6} cells)**	**RNA (mcg 10^{-6} cells)**	**Caspase-3(µmol pNA min^{-1} mL^{-1})**	**Total protein (mcg 10^{-6} cells)**
EAC control	6.53±0.06	10.83±0.2	0.17±0.01	118.03±0.92
EAC+5FU (20mg/kg)	2.35±0.16	4.25±0.36	0.85±0.13	38.73±0.92
EAC+EEDI (250mg/kg)	2.38±0.08[a]	4.2±0.11[a]	1.3±0.2[a]	37.4±0.92[a]
EAC+EEDI (500mg/kg)	1.85±0.07[a]	3.1±0.13[a]	1.7±0.06[a]	33.38±0.29[a]
EAC+AEDI (250mg/kg)	3.55±0.1[a]	5.75±0.2[a]	0.48±0.05[c]	51.15±4.73[a]
EAC+AEDI (500mg/kg)	2.75±0.2[a]	4.28±0.25[a]	0.8±0.09[a]	39±0.29[a]

The data were expressed as the mean± S.E.M. n = 10. Analysis of the data was performed by one-way analysis of variance (ANOVA) followed by Newman-Keul's multiple comparison test

a- $p<0.001$, compared to the EAC control group

c - $p<0.05$, compared to the EAC control group

In order to know the mechanism of antitumor effect of EEDI and AEDI, the main apoptotic marker, caspase-3 was estimated in the peritoneal cells. Caspases are the central executioners of the apoptotic pathway [14]. They bring about most of the visible changes like cell shrinkage, condensation, margination and fragmentation of chromatin [15]. Caspase is particularly activated during apoptosis and its activity was higher in extract treatment groups when compared with EAC control mice. According to willey, 1980 [14] during apoptosis a specific nuclease cuts the genomic DNA between nucleosomes to generate DNA fragments and the presence of this ladder

has been extensively used as marker of apoptotic cell death. It is also assumed that the DNA fragmentation is the hallmark of apoptosis. Several studies have demonstrated a positive association between DNA fragmentation and apoptosis [15]. The present study showed increased caspase-3 activity, decreased DNA, RNA, protein content in the extracts treated groups. It's strongly proposed that extracts activate apoptotic pathways and implement the antitumor activity of EAC cells.

CONCLUSION

The present study provides scientific evidence that the *D.indica* L can used for the treatment of cancer due to the presence of plumbagin. Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells[16] and antitumor activity in experimental animals[17].We propose that the additive and synergistic antioxidant activity of phytochemicals such as flavonoids, triterpenoids, steroids, etc, present in EEDI and AEDI are responsible for the its potent antitumor activity which can be inferred from the increased the life span of EAC tumor bearing mice. Further investigations are in progress in our laboratory to identity the antioxidant enzyme level in different organ and isolation and characterization of active principles involved in this antitumor activity.

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