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EVALUATION OF ANTICANCER POTENTIAL OF PLANT: *ADIANTUM VENUSTUM DON*

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ABSTRACT

Cancer is a malignant disease that is characterized by rapid and uncontrolled formation of abnormal cells, which may mass together to form a growth or tumour, or proliferate throughout the body. Next to heart disease, cancer is a major killer of mankind. Present study aims at a preliminary phytochemical screening and anticancer evaluation of *Adiantum venustum* Don against Ehrlich Ascites Carcinoma in animal model. Results indicate that ethanolic extract of *Adiantum venustum* Don possess significant anticancer activity and also reduce elevated level of lipid peroxidation due to higher contents of terpenoids and flavonoids. Thus, ethanolic extract of Adiantum *venustum* Don could have wide therapeutic application against cancer.

Key words: Cancer, *Adiantum venustum,* Lipid peroxidation, Flavonoids, Terpenoids

INTRODUCTION

The chemotherapy of neoplastic disease has become increasingly important in recent years. The relatively high toxicity of most anticancer drugs has fostered the development of supplementary drugs that may alleviate this toxic effect or stimulate the regrowth of depleted normal cells. Plants have a long history of use in the treatment of cancer. Plants have played an important role as a source of effective anticancer agent, and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural sources, including plants, marine organism and microorganisms[1]. It was also observed from Ayurvedic literature and ethanobotanical studies that the plant is very useful in treating tumour, prevention of hair from falling and as a diuretics but no scientific investigation has

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been done in such direction[2-4]. Therefore, it was thought worth while to carry out preliminary phytochemical screening and screening of *Adiantum venustum* Don for anticancer activity against Ehrlich Ascites Carcinoma in animal model.

EXPERIMENTAL

Plant source

The plant leaves and stem of *Adiantum venustum* Don were collected from Kolli Hills, Namakkal District, Tamilnadu, India, and were authenticated. Reference number of the authentication report is BSI/SC/5/23/05.06/Tech/603.

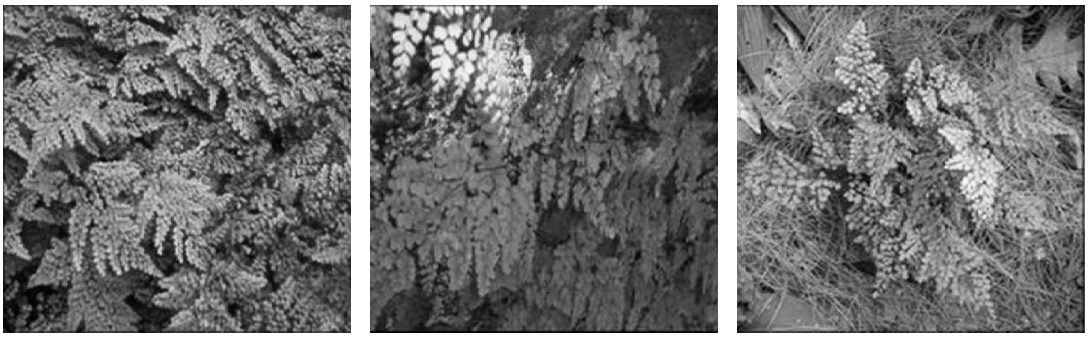

Fig. 1: Plant image (*Adiantum venustum* Don)

Extraction procedure

The leaves and stem of *Adiantum venustum* were dried under shade, mixed together and then made into a coarse powder with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use. The dried powder material (150 g) was defatted with petroleum ether (60-80^{o}) to remove waxy substances and chlorophyll, which usually interfere in the isolation of phytoconstituents. The marc after defatting with petroleum ether was dried and extracted with ethanol (99.9 % v/v) in a Soxhlet extractor for 72 hr. The solvent was then distilled off and the resulting semisolid mass was dried in a vacuum evaporator to get a yield of 14 % w/w[5,6].

Phytochemical identification tests

Various chemical tests were performed for the phytochemical identification of the

ether and ethanolic extracts of the plant leaves and stem of *Adiantum venustum* as peer standard procedure[7, 8].

Anticancer activity

Toxicity evaluation (LD_{50}): (Karber's methods)

Thirty mice including both male and female weighing 20–25 g were selected for the study. LD_{50} was measured by Karber's methods

Animals

Male Swiss Albino mice weighing between 18-25 g were used for present study. They were maintained under standard environmental conditions and were fed with standard pellet diet of water and *ad libitum*. The mice were acclimatized and laboratory condition for 10 days before commencement of experiment. All procedure described were reviewed and approved by the Institutional Animal Ethical Committee of J.K.K. Nataraja College of Pharmacy, Komarapalayam.

Cancer cell line

EAC cells were obtained from Amala Cancer Research Center, Thrissur, Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells/mouse.

Preparation of extract drug and mode of administration

In the present anticancer study, ethanolic extract of *Adiantum venustum* (EEAV) in the dose of 100 mg/kg and 200 mg/kg were prepared as suspension by dissolving the ethanolic extract in propylene glycol and sterile physiological saline containing Tween 20 to get the desired concentration[9, 10].

Tumor transplantation

Ehrlich's Ascites Carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. Ascetic fluid was drawn out from tumor bearing mice at the log phase (day 78 of tumor bearing) of the tumor cells. The tumor cell number was adjusted to 2 x 10^6 tumor cells/mL. Sample showing more than 90% viability was used for transplantation. Each animal received 0.2 mL of tumor cell suspension containing 2 x 10^6 cells/mL intraperitoneally[11].

Drug treatment schedule

Male Swiss Albino mice were divided into 5 groups (n = 8). All the groups were injected with EAC cells (0.2 mL of 2 x 10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero. From the first day, normal saline 5 mL/kg/mouse/day and propylene glycol 5 mL/kg /mouse/day was administered to normal and EAC control groups, respectively for 14 days intraperitoneally. Similarly EEAV at different doses (100 mg and 200 mg/kg/mouse/day) were administered in groups 3, 4 and 5, respectively after the administration of last dose followed by 18 hrs. Fasting 4 mice form each group were sacrificed for the study of antitumor activity, hematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival lime (MST) and percent increase in life span of the tumor bearing hosts.[12-14] Various parameters like body weight of animals, life span of animals, cytological studies on cell lines, hematological parameter, RBC, WBC, hemoglobin, differential count and biochemical parameters evaluated in the present study.

Anticancer effect of EEAV was assayed by observation of change with respect of body weight, ascitic tumor volume, packed cell volume, viable and non viable tumor cell count, mean survived time (MST) and percentage increase in life span (%ILS) [12].

Tumor cell volume and packed cell volume

The mice were dissected to collect ascitic fluid from peritoneal cavity and centrifuged to determine packed cell volume at 1000 rpm for 5 min.[12] The transplantable murrane tumor was carefully collected to measured the tumor volume.

Viable and non viable cell count

Viable and non viable cell counting of the ascetic cell was done by staining with tryphan blue (0.4% in normal saline), dye exclusion test and count was determined in a neubauer counting chamber. The cells that did not take up the dye were viable and those that took the stain were not viable[12].

Mean survival time and percent increase in life span

The effect of EEAV on tumor growth was observed by MST and % ILS. MST of each group continuing 4 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation[11,12].

$$MST = (\text{Day of first death} + \text{Day of last death})/2 \quad ...(1)$$

$$\% \text{ ILS} = \left\{ \frac{\text{MST of treated group}}{\text{MST of control group}} \right\} - 1 \times 100 \quad \ldots(2)$$

Effect of EEAV on hematological parameters

Blood was collected from each mice by intracardial puncture with blood anticoagulant (Heparin) and while blood cells (WBC), red blood cells (RBC); hemoglobin and differential counts were determined in group comprise of (i) Tumor bearing mice (control), (ii) Tumor bearing mice treated with EEAV (100 mg/kg/mice/day), (iii) Tumor bearing mice treated with EEAV (200 mg/kg/mice/day) and (iv) Normal group[15].

Biochemical assay

After the collection of blood samples, the mice were sacrificed and their liver was excised. The isolated liver was rinsed in ice cold normal saline followed by cold phosphate buffer having pH 7.4, and blotted dry and weighed. A 10% w/v homogenate of liver was prepared in ice cold phosphate buffer (pH 7.4) and a portion was utilized for estimation of lipid peroxidation and other portion of the same after precipitation of proteins with TCA was used for estimation of glutathione. Remaining homogenate was centrifuged at 1500 rpm at 4^{o}C for 15 min. The supernatant thus obtained was used for the estimation of superoxide dismutase, catalase and protein contents[14].

Statistical analysis

The experimental results were expressed as mean ± SEM. Data were assessed by the student t-test $P < 0.05$ was considered as statistically significant.

RESULT AND DISCUSSION

Phytochemical screenings suggest that ethanolic extract of plant contain terpenoid, phytosterols, flavanoid and saponin (Table 1).

Table 1: Result of phytoconstituent identification tests of ethanol extract of *Adiantum venustum Don*

Phytoconstituent	Phytosterol	Flavonoids	Triterpenoids	Saponin
Ethanol Extract	+	+	+	+

Anticancer activity

Toxicity evaluation (LD_{50}): In acute toxicity study, the given extract of *Adiantum venustum* did not show any mortality up to the dose of 2000 mg/kg. The extract shows sedation, hypnosis and mild muscle relaxant property.

Effect of EEAV on mean survival time and percent increase in life span, tumor cell volume, packed cell volume, viable and non viable cell counts

Administration of EEAV reduces the tumour volume, packed cell volume and viable tumour cell count in a dose dependant manner, when compared to EAC control mice. In EAC control mice, the median survival time was 22 ± 0.25 days. Whereas, it was significantly increased; median survival time (24 ± 0.33, 29 ± 0.49) with different doses (100 and 200 mg/kg) of EEAV and standard drug, respectively. The mean survival time and effect of EEAV (100 mg/kg, 200 mg/kg) at different doses on tumour volume, viable and non viable cell counts, are shown in Tables 2 and 3 and graphically represented in Figure 2.

Table 2: Effect of ethanol extract of *Adiantum venustum* on survival time on EAC bearing mice

Experimental groups	Mean survival time (MST) days	Increase in life span (%)
Normal control (normal saline 5 mL/kg b.w.)	-	-
EAC control	22 ± 0.25	-
EAC + EEAV (100 mg /kg)	24 ± 0.33	9.09
EAC + EEAV (200 mg/kg)	29 ± 0.49	31.81
EAC + Vincrystine (0.8 mg/kg) std	31 ± 0.55	40.90

Values are mean ± SEM (Standard error of mean), Number of mice in each group (n = 4), $P < 0.001$, Experimental group was compared with EAC control.

Table 3: Effect of ethanol extract of *Adiantum venustum* on tumor volume, packed cell volume, viable and non viable tumour cell counts of EAC bearing mice

Parameters	EAC control	EEAV 100 (mg/kg)	EEAV 200 (mg/kg)	Standard vincristine 0.8 (mg/kg)
Body weight	26.11 ± 0.12	24.34 ± 0.16	23.28 ± 0.13	23.9 ± 0.02
Tumour volume (mL)	5.82 ± 0.042	4.22 ± 0.051	3.42 ± 0.082	2.42 ± 0.13
Packed cell volume (mL)	2.12 ± 0.104	1.75 ± 0.043	1.05 ± 0.092	1.15 ± 0.03
Viable tumour cell count % 10^7 cells /mL	11.25 ± 0.098	7.78 ± 0.18	4.85 ± 0.23	4.90 ± 0.015
Non viable tumour cell count x 10^7 cells/mL	0.5 ± 0.017	0.92 ± 0.023	1.47 ± 0.021	1.23 ± 0.81

Values are mean ± SEM., No. of mice in each group (n = 4), $P < 0.01$, experimental groups was compared with EAC control, Weight of normal mice = 20 ± 0.15

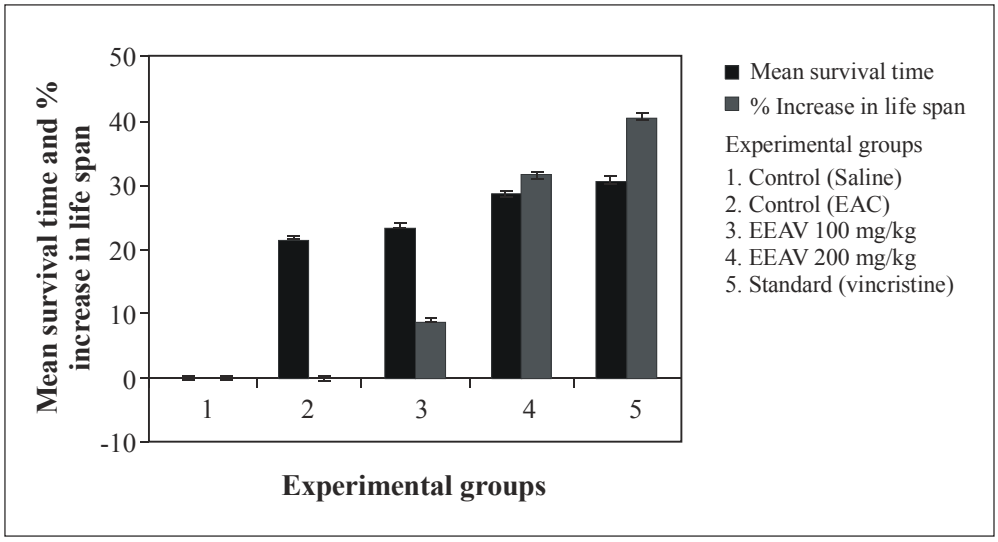

Fig. 2: Mean survival time and % increase in life span of mice

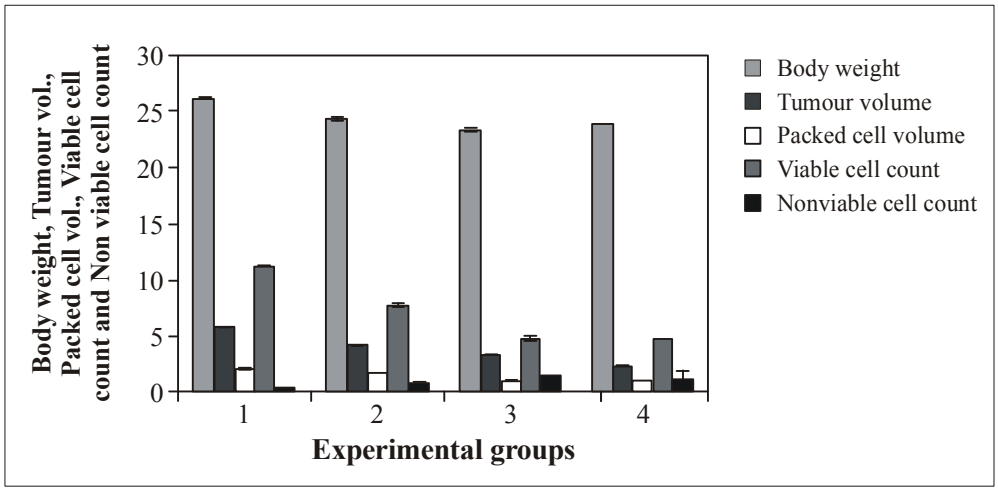

Fig. 3: Effect of EEAV on tumour volume and packed cell volume viable cell count and non viable cell counts of mice

Effect of EEAV on haematological parameters

For EEAV at the dose of 100 and 200 mg/kg, the haemoglobin contents in EAC bearing mice were increased to 10.6 ± 0.057 and 11.45 ± 0.057. The haemoglobin contents in the EAC control mice (9.8 ± 0.02) was significantly decreased as compared to normal mice (12.85 ± 0.25) (Table 4). The total WBC count was significantly higher in the EAC treated mice, when compared with normal mice. Whereas EEAV treated mice show significant reduction in the WBC counts as compared to that of control mice. Significant changes were observed on differential count, when extract treated mice were compared with EAC control mice (Table 4).

Table 4: Effect of ethanol extract of *Adiantum venustum* on haematological parameters of EAC treated mice

Parameter	Normal saline 0.5 mL/kg	EAC control 2 x 10^6 cells/mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg/kg	EAC Cell + Vincristine 0.8 mg/kg
Haemoglobin (g)	12.85 ± 0.25	9.8 ± 0.02	10.6 ± 0.057	11.45 ± 0.18*	11.7 ± 0.045*
Total RBC million/mmcu	6.65 ± 0.18	3.8 ± 0.035	4.75 ± 0.032	5.42 ± 0.22*	5.8 ± 0.054

Cont…

Parameter	Normal saline 0.5 mL/kg	EAC control 2 X 10^6 cells/mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg/kg	EAC Cell + Vincristine 0.8 mg/kg
Total WBC Million/mmcu	7.8 ± 0.045	20.07 ± 0.068*	11.92 ± 0.042	8.85 ± 0.059	9.12 ± 0.055
Lymphocyte	77.75 ± 0.19	33.37 ± 0.56*	52.7 ± 0.50*	60.72 ± 0.36*	59.12 ± 0.30
Monocyte	1.7 ± 0.035	0.82 ± 0.024	1.15 ± 0.014*	1.2 ± 0.045	1.32 ± 0.024
Granulocyte	29.97 ± 0.46	52.6 ± 0.37*	40.87 ± 0.2	31.72 ± 0.63*	41.65 ± 0.29

Values are mean ± SEM, (n = 4), EAC control group compared with normal group, Experimental group compared with EAC control. P < 0.01, *P < 0.05

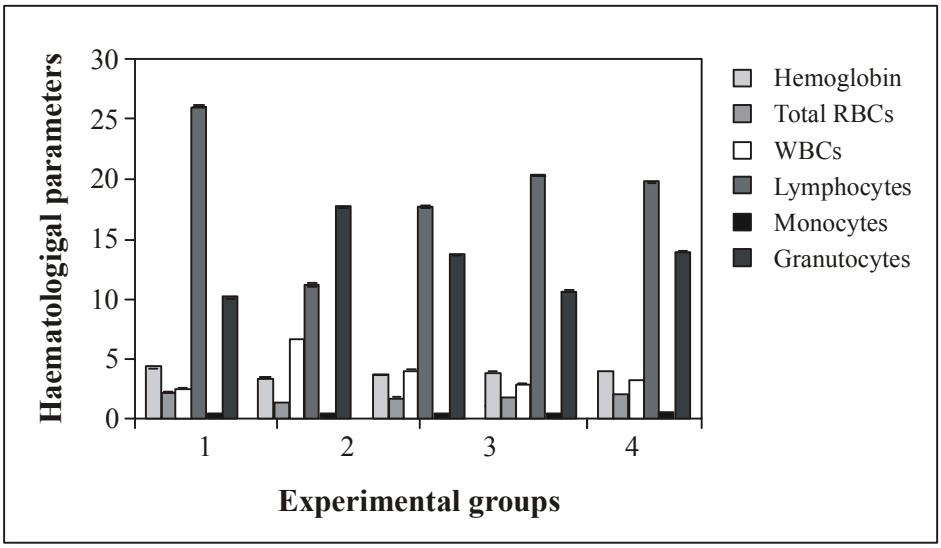

Fig. 4: Effect of EEAV on haematological parameters

Biochemical assay

Biochemical assay indicated that EEAV significantly reduced the elevated levels of lipid peroxidation and thereby, it may act as an antitumor agent. The level of lipid peroxidation, catalase and protein contents are summarized in Table 5 and graphically represented in Figure 5.

Table 5: Effect of different doses of ethanolic extract of *Adiantum venustum* on different biochemical parameters in EAC bearing mice

Parameter	Normal saline 0.5 ml/kg	EAC control 2 x 10^6 cells/mice	EAC + EEAV 100 mg/kg	EAC + EEAV 200 mg/kg
Lipid peroxidation n mole MDA/g of tissue	0.92 ± 0.02	1.36 ± 0.09*	1.27 ± 0.04*	1.13 ± 0.02
Catalase (units /mg tissues)	2.51 ± 0.72	1.71 ± 0.15*	1.75 ± 0.13	2.34 ± 0.23*
Protein content (gm/100 mL)	12.66 ± 0.69*	17.25 ± 0.76	16.50 ± 0.70	16.10 ± 0.55

Values are mean ± SEM, (n = 4) EAC control group compared with normal group, Experimental group compared with EAC control. P < 0.05, *P< 0.05

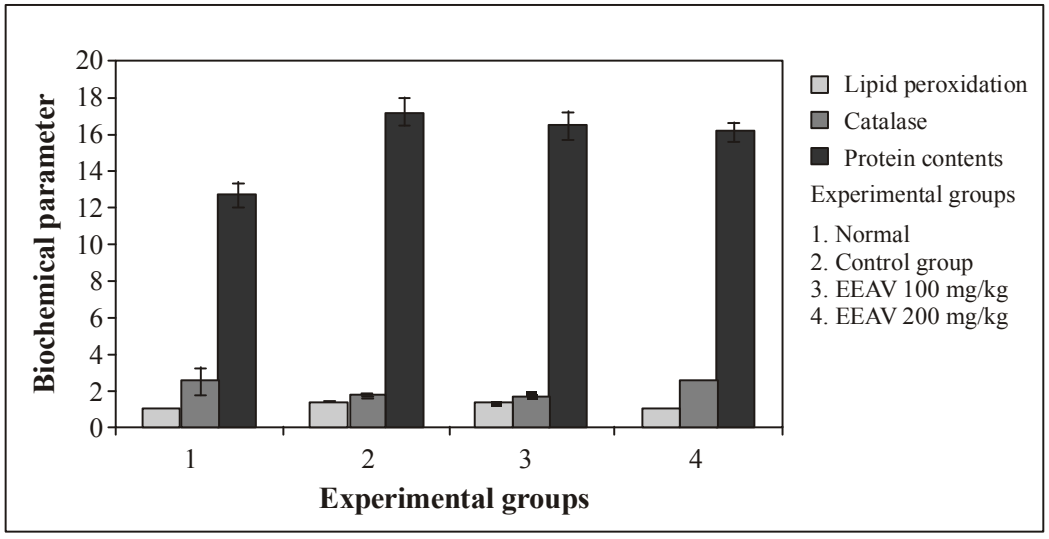

Fig. 5: Effect of EEAV on different biochemical parameters

The plant leaves and stem of *Adiantum venustum* Don were found to contain higher amounts of triterpenoids. In present study, anticancer potential of the plant was estimated in EAC bearing carcinoma cell. Ethanolic extract of *Adiantum venustum* Don has considerably reduced tumour volume and increased the life span of the test animals. It was also found that

EEAV significantly reduced the elevated levels of lipid peroxidation and thereby, it may act as an antitumour agent.

CONCLUSION

The ethanolic extract of *Adiantum venustum* Don possessed significant anticancer and antioxidant activity due to its higher terpenoid and flavonoid contents. Further investigation on different biological activities of this plant with different modes will not only validate the types of activities claimed by Ayurvedic, Siddha and traditional practitioners, but will also bring out innovation in the field of therapeutics.

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