

## Antitumor Effect of *Dregea volubilis* Fruit in Ehrlich Ascites Carcinoma Bearing Mice

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**Abstract:** *Dregea volubilis* Benth (Asclepiadaceae), commonly known as *Jukti* in Bengali is a tall woody climber grown throughout warmer regions of India. The present study was undertaken to evaluate the use of petroleum ether extract of *Dregea volubilis* fruits (PEDV) for antitumor effect in Ehrlich ascites carcinoma (EAC) bearing Swiss albino mice. 24 h after intraperitoneal inoculation of tumor (EAC) cells in mice, PEDV was administered at 100 and 200 mg/kg body weight for 9 consecutive days. On 10<sup>th</sup> day half of the mice were sacrificed and rest were kept alive for assessment of increase in life span. The antitumor effect of PEDV was assessed by evaluating tumor volume, tumor weight, viable and non-viable tumor cell count, median survival time and increase in life span of EAC bearing hosts. Hematological profiles were investigated. PEDV showed significant ( $p < 0.001$ ) decrease in tumor volume, tumor weight and viable cell count and increased the life span of EAC bearing mice. Hematological profiles were significantly ( $p < 0.001$ ) restored to normal levels in PEDV treated mice as compared to EAC control. Therefore, from the present study it can be concluded that *Dregea volubilis* fruit exhibited remarkable antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss mice.

**Key words:** Antitumor · *Dregea volubilis* · Ehrlich ascites carcinoma

### INTRODUCTION

Several methods exist for the treatment of cancer in modern medicine. These include chemotherapy, radiotherapy and surgery. Chemotherapy is now considered as the most effective method of cancer treatment. The aim of the current research has been based on the identification of natural and synthetic compounds that can be used in the prevention and/or treatment of cancer. An ideal anticancer agent should be both tissue and cell specific i.e. it should kill or incapacitate cancer cells without causing excessive damage to normal host cells. Unfortunately, currently available cancer chemotherapeutic agents insidiously affect the host cells, especially bone marrow, epithelial tissues, reticulo-endothelial system and gonads [1]. Hence, the natural products now have been contemplated of exceptional value in the development of effective anticancer agents with minimum host cell toxicity.

*Dregea volubilis* Benth (Asclepiadaceae), commonly known as *Jukti* in Bengali is a tall woody climber with densely lenticulate branches, occurring throughout the warmer regions of India and Car Nicobar Islands ascending to an altitude of 1500 m. The parts of the plant have been traditionally used for medicinal purposes. The juice of the plant is used as a sternutatory and leaves are employed in application for boils and abscesses. The roots and tender stalks are used as emetic and expectorant. It is reported that an alcohol (50%) extract of the plant showed activity on the central nervous system as well as anti-cancer activity against Sarcoma 180 in mice. Two pregnane glycosides dregeosides were isolated from this plant collected from Thailand showed antitumor activities against melanoma B-16 in mice [2]. The isolation and characterization of twelve polyhydroxy C/D cis-pregnane glycosides were reported from the same plant collected from Thailand [3,4]. Isolation of  $\beta$ -sitosterol, kaempferol-3-galactoside, a 2- deoxy sugar, drevogenin

A, drevogenin P, D-cymarose and L-olendrose from the plant was also reported [5]. The authors reported the isolation and characterization of a novel pentacyclic triterpenoid designated as taraxerone having anti-leishmanial and anti-cancer activity on K562 leukemic cell line [6]. The present study was carried out to investigate the antitumor effect of petroleum ether extract of *Dregea volubilis* fruit (PEDV) against Ehrlich ascites carcinoma (EAC) in Swiss albino mice.

## MATERIALS AND METHODS

**Plant Material:** The fruits of *D. volubilis* were collected during August 2008 from South 24-Paraganas, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Howrah, West Bengal, India. The voucher specimen [CNH/I-(267)/2008/Tech.II/267] was maintained in our laboratory for future reference. The fruits were shade-dried with occasional shifting and then powdered with mechanical grinder passing through sieve no. 40 and stored in an air-tight container.

**Drugs and Chemicals:** 5-fluorouracil (5-FU) from Sigma Chemical Co., St. Louis, Mo, USA. All the other reagents used were of analytical reagent grade obtained commercially.

**Preparation of Extract:** The powdered plant material (450 g) was extracted with petroleum ether (60-80°C) for 72 h in the cone shaped percolator at 33°C. The solvent was distilled in reduced pressure and resulting semisolid mass was vacuum dried using rotary flash evaporator to yield a solid residue (PEDV, 5.33 % w/w). Preliminary phytochemical studies on PEDV revealed the presence of alkaloids, triterpenoids and steroids [7].

**Animals:** Adult male Swiss albino mice weighing  $20 \pm 2$  g were obtained from Laboratory Animal Centre, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India. The mice were grouped and housed in polyacrylic cages (38×23×10 cm) with not more than four animals per cage and maintained under standard laboratory conditions (temperature  $25 \pm 2^\circ\text{C}$  with dark/light cycle 12/12 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before commencement of the experiment. All experimental procedures were reviewed and approved by University Animal Ethics Committee, Jadavpur University.

**Tumor Cells:** The transplantable murine tumor cells namely Ehrlich ascites carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute (CNCI), Kolkata, India. The EAC cells were maintained in the ascitic form *in vivo* by sequential passages in Swiss mice, by means of intraperitoneal transplantation of  $2 \times 10^6$  cells/mouse after every 10 days. Ascitic fluid was drawn out from EAC bearing mouse 8 days after transplantation. The freshly drawn fluid was diluted with ice-cold sterile normal saline and the tumor cell count was adjusted to  $2 \times 10^7$  cells/ml by sterile normal saline.

**Acute Toxicity:** The acute oral toxicity of PEDV in male Swiss albino mice was studied as per reported method [8].

**Treatment Schedule:** The animals were divided into five groups ( $n = 12$ ). All groups except first group received 0.1 ml of EAC cell suspension ( $2 \times 10^6$  cells/mouse, i.p.). This was taken as day '0'. The first group served as normal saline control (5 ml/kg body weight, i.p.). The second group served as EAC control. After 24 h of tumor inoculation the third and fourth group received PEDV at the doses of 100 and 200 mg/kg body weight, i.p. respectively and the fifth group received reference drug 5-fluorouracil (20 mg/kg body weight, i.p) for 9 consecutive days. 24 h after the last dose and after 18 h of fasting, blood was collected from six mice of each group, by cardiac puncture for the estimation of hematological parameters and then sacrificed by cervical dislocation for the study of antitumor parameters. The rest six mice of each group were kept alive with food and water *ad libitum* to check the increase in the life span of the tumor bearing hosts [9]. The effect of PEDV on tumor growth and host's survival time was assessed by observation of tumor volume, packed cell volume, viable and non-viable cell count, median survival time (MST) and percentage increase in life span (% ILS).

**Determination of Tumor Volume and Weight:** The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weighed immediately.

**Estimation of Viable and Non-viable Tumor Cell Count:** The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted suspension was placed on the Neubauer counting chamber and the cells were then stained with Trypan blue (0.4 % in normal saline) dye. The cells that did not take up the dye were viable and those that took the stain were non-viable. These viable and non-viable cells were counted.

Cell count =  $\frac{\text{No. of cells} \times \text{dilution factor}}{\text{area} \times \text{thickness of liquid film}}$ .

**Determination of Median Survival Time and Percentage Increase in Life Span:** The mortality was monitored by recording percentage increase in life span (% ILS) and median survival time (MST) as per the following formulae.

$$\% \text{ ILS} = \frac{[\text{Mean survival time of treated group} / \text{Mean survival time of control group} - 1]}{\times 100}$$

$$\text{Median survival time (MST)}^* = [\text{First death} + \text{Last death}] / 2$$

\*Time denoted by number of days.

**Estimation of Hematological Parameters:** Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) count and white blood cell (WBC) count [10,11].

**Statistical Analysis:** The experimental results were expressed as mean  $\pm$  standard error of mean (SEM). Statistical significance was analyzed by one-way ANOVA followed by Dunnett's *post hoc* test of significance. *p* values of  $< 0.001$  were considered as statistically significant.

## RESULTS

**Acute Toxicity:** The oral LD<sub>50</sub> value of the methanol extract of *Dregea volubilis* fruits (PEDV) in mice was 900 mg/kg body weight.

**Tumor Growth and Survival Parameters:** PEDV at 100 and 200 mg/kg body weight significantly reduced the tumor volume, tumor weight and viable tumor cell count in dose dependent manner as compared to EAC control ( $p < 0.001$ ). Furthermore, PEDV increased non-viable tumor cell counts and decreased viable tumor cell counts as compared with the EAC control ( $p < 0.001$ ). In EAC control group, the median survival time (MST) was 20.40  $\pm$  0.05 days, whereas in PEDV treated groups these were 29.71  $\pm$  0.10 (100 mg/kg) and 40.53  $\pm$  0.18 (200 mg/kg) days, respectively. The reference drug 5-fluorouracil (20 mg/kg) showed MST 41.52  $\pm$  0.21 days (Tables 1 and 2).

**Hematological Parameters:** Hematological parameters of tumor bearing mice were found to be significantly altered compared to those of normal saline group. The WBC count was found to be increased and RBC and hemoglobin decreased in EAC control animals significantly ( $p < 0.001$ ) when compared with the normal

Table 1: Effect of PEDV on tumor volume, tumor weight, median survival time (MST), percentage increase in life span (% ILS) in EAC bearing mice

Treatment	Tumor volume (mL)	Tumor weight (g)	Median survival time (days)	% ILS
EAC control	2.91 $\pm$ 0.17	3.41 $\pm$ 0.24	20.40 $\pm$ 0.05	-
EAC + PEDV (100 mg/kg)	1.67 $\pm$ 0.22*	1.35 $\pm$ 0.24*	29.71 $\pm$ 0.10*	45.63 $\pm$ 0.45*
EAC + PEDV (200 mg/kg)	0.75 $\pm$ 0.24*	0.71 $\pm$ 0.27*	40.53 $\pm$ 0.18*	98.67 $\pm$ 0.63*
EAC + 5-FU (20 mg/kg)	0.51 $\pm$ 0.21*	0.59 $\pm$ 0.12*	41.52 $\pm$ 0.21*	103.52 $\pm$ 1.8*

Values are expressed as mean  $\pm$  SEM ( $n = 6$ ); \* $p < 0.001$  compared with EAC control group

Table 2: Effect of PEDV on viable and non-viable tumor cell count in EAC bearing mice

Treatment	Total cells ( $\times 10^7$ cell/ml)	Viable cells ( $\times 10^7$ cell/ml)	Non-viable cells ( $\times 10^7$ cell/ml)	Viable cell (%)	Non-viable cell (%)
EAC control	8.4 $\pm$ 1.8	8.1 $\pm$ 0.18	0.3 $\pm$ 0.11	96.4 $\pm$ 7.8	3.5 $\pm$ 0.15
EAC + PEDV (100 mg/kg)	4.6 $\pm$ 1.5*	3.7 $\pm$ 0.24*	1.8 $\pm$ 0.15*	80.4 $\pm$ 7.1*	39.1 $\pm$ 0.45*
EAC + PEDV (200 mg/kg)	4.1 $\pm$ 2.4*	1.3 $\pm$ 0.54*	3.3 $\pm$ 0.18*	31.7 $\pm$ 5.4*	80.5 $\pm$ 1.8*
EAC + 5-FU (20 mg/kg)	3.8 $\pm$ 1.3*	0.81 $\pm$ 0.63*	3.5 $\pm$ 0.54*	21.3 $\pm$ 3.3*	92.1 $\pm$ 4.5*

Values are expressed as mean  $\pm$  SEM ( $n = 6$ ); \* $p < 0.001$  compared with EAC control group

Table 3: Effect of PEDV on hematological parameters in EAC bearing mice

Treatment	WBC (cells $\times 10^3/\text{mm}^3$ )	RBC (cells $\times 10^6/\text{mm}^3$ )	Hemoglobin (g %)
Normal saline (5 ml/kg)	3.83 $\pm$ 0.8	4.20 $\pm$ 1.5	13.20 $\pm$ 3.1
EAC control	5.66 $\pm$ 1.4*	2.83 $\pm$ 1.8*	5.40 $\pm$ 1.9*
EAC + PEDV (100 mg/kg)	4.55 $\pm$ 0.9**	3.66 $\pm$ 1.1**	8.90 $\pm$ 2.9**
EAC + PEDV (200 mg/kg)	2.36 $\pm$ 1.3**	4.07 $\pm$ 1.4**	11.9 $\pm$ 3.3**
EAC + 5-FU (20 mg/kg)	3.95 $\pm$ 1.5**	4.10 $\pm$ 1.3**	12.8 $\pm$ 3.6**

Values are expressed as mean  $\pm$  SEM ( $n = 6$ ); \* $p < 0.001$  compared with normal control and \*\* $p < 0.001$  compared with EAC control group

control group. Treatment with PEDV at both test doses significantly ( $p < 0.001$ ) increased the hemoglobin content and RBC count towards the normal levels and decreased WBC count towards the normal counts when compared to EAC control group (Table 3).

## DISCUSSION

The present work aimed to study the antitumor activity of petroleum ether extract of *Dregea volubilis* fruits (PEDV) in EAC tumor bearing mice. The results of this study revealed that PEDV at the doses of 100 and 200 mg/kg significantly reduced the tumor volume, tumor weight, tumor cell count (viable and non-viable) and restored the hematological parameters towards normal values.

The Ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascitic form it has been used as a transplantable tumor model to investigate the antitumor effects of several substances [12, 13].

In EAC tumor bearing hosts, a drastic increase in ascitic fluid volume was observed. Treatment with PEDV reduced intraperitoneal tumor burden, thereby reducing the tumor volume, tumor weight, viable tumor cell count and increased the life span of the tumor bearing mice in a dose dependent manner. The results demonstrated that the viable cell count decreased with increased count of non-viable cells. This implies that the antitumor action of PEDV had a direct relationship with the tumor cells, indicating loss of viability of the PEDV treated cells.

The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the tumor bearing animal [14]. It can therefore be inferred that HASR increased the life span of EAC bearing mice may be due to the prevention of tumor progression. Usually in cancer, chemotherapy the major problems that are being encountered of myelosuppression and anemia [15]. Results of present study indicate that PEDV dose dependently and significantly increased the erythrocyte count and hemoglobin level when compared to those of EAC control mice. The WBC count was reduced as compared with that of EAC control mice. These indicating parameters revealed that PEDV exerted less toxic effect to the haemopoietic system and plausibly had selective affinity to the tumor cell and thereby it could maintain the normal hematological profile.

In present study, it was noted that PEDV significantly reduced tumor growth, viability of tumor cells, normalized the hematological profiles, raising life span as compared with those of EAC control mice. Therefore, it can be concluded that the petroleum ether extract of *Dregea volubilis* fruit demonstrated remarkable antitumor effect in Ehrlich ascites carcinoma bearing Swiss mice.

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