

Full Length Research Paper

Antitumor and antioxidant potential of *Tragia Plukenetii* R.Smith on Ehrlich ascites carcinoma in mice

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This investigation aims to evaluate the antitumour and antioxidant potential of the ethanol extract of *Tragia Plukenetii* R.Smith (ETP) on Ehrlich ascites carcinoma (EAC) tumor model. Tumor was induced in mice by intraperitoneal injection of EAC cells (2×10^6 cells/mouse). Ethanol extract of *T. Plukenetii* (ETP) was administered to the experimental animals at the dose levels of 100, 200 and 300 mg/kg/day after 24 h of tumour inoculation. The antitumour effect of ETP was evaluated by assessing *in vitro* cytotoxicity, survival time, hematological and antioxidant parameters. Oral administration of ETP increased the survival time of the EAC bearing mice. The ETP brought back the altered levels of the hematological and antioxidant parameters in a dose dependent manner in EAC bearing mice. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-fluorouracil (20 mg/kg.bw). Thus present study revealed that ETP possessed significant antitumor and antioxidant activity.

Key words: *Tragia Plukenetii*, Ehrlich ascites carcinoma, hematological parameters, antioxidant, tumour growth response.

INTRODUCTION

Drug discovery from the medicinal plants has played an important role in the treatment of cancer and indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half-century have been made towards combating cancer (Newman et al., 2003). *Tragia Plukenetii* R.Smith (Euphorbiaceae) is an erect or climbing shrub distributed widely throughout India. The plant is traditionally used for the treatment of sore tongue and it also has diaphoretic action. This study was undertaken to evaluate the antitumor and antioxidant potential of the ethanol extract of *T. Plukenetii* (ETP) against the Ehrlich ascites carcinoma (EAC) tumor model.

MATERIALS AND METHODS

Plant collection

T. plukenetii R.Smith (Euphorbiaceae) was collected in and around Trichy District, Tamil Nadu. Plant material was identified with the help of "The Flora of the Tamil Nadu Carnatic". A voucher specimen [No: RHT 8279] was deposited in the Rapinat Herbarium, St.Joseph's College, Trichy, Tamil Nadu.

Preparation of ethanol extract

The plant material was shade dried and pulverized. Alcohol extract of the coarsely powdered material was prepared employing Soxhlet method with 200 ml of ethanol for 7 h and the extract was concentrated to 5 g. The extract was dissolved in dimethylsulphoxide (<0.1%) before administration.

Animals

Adult Swiss male albino mice (20-25 g) were procured from Tamil-

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Table 1. *In vitro* cytotoxicity studies of *Tragia Plukenetii* R.Smith (ETP) on Ehrlich ascites carcinoma (EAC) cells.

Concentration of ETP ($\mu\text{g/ml}$)	No. of viable cells	Viable cells (%)	No. of dead cells	Dead cells (%)
control	93	96.87	3	3.13
150	60	68.18	28	31.82
300	43	45.74	51	54.26
500	26	25.74	75	74.26
1000	15	15.79	80	84.21

nadu Veterinary College, Chennai, Tamilnadu and used throughout the study. They were housed in microfan boxes in a controlled environment (temperature $25\pm 2^\circ\text{C}$ and 12 h dark and light cycle) with standard diet and water *ad libitum*. The study was conducted after obtaining institutional animal ethical committee clearance (Reg.No:790/03/ac/GPCSEA).

Cells

EAC cells were obtained through the courtesy of Amala Cancer Research Centre, Thrissur. They were maintained by weekly intraperitoneal inoculation of 10^6 cells/mouse (Ramakrishna et al., 1984).

Effect of ETP on *in vitro* cytotoxicity

Short-term cytotoxicity was assessed by incubating 1×10^6 EAC cells in 1 ml phosphate buffer saline with varying concentration of ETP at 37°C for 3 h. The viability of the cells was determined by the Trypan blue exclusion method.

Effect of ETP on tumor growth response

Animals were inoculated with 2×10^6 cells/mouse on day '0' and treatment with ETP started 24 h after inoculation, at a dose of 300 mg/kg/day, p.o. The control group was treated with same volume of 0.9% sodium chloride solution. All the treatments were given for 14 days. The mean survival time (MST) of each group, consisting of 10 mice was noted. The antitumor efficacy of ETP was compared with that of 5-fluorouracil (Dabur Pharmaceutical Ltd, India; 5-FU, 20 mg/kg/day, i.p for 14 days). Mean survival time and Increased Life Span (%ILS) was calculated using the following equation (Mazumder et al., 1997; Gupta et al., 2000):

$$\text{MST} = (\text{Day of First Death} + \text{Day of last death})/2$$

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

Antitumor activity

Male Swiss albino mice were divided into 6 groups ($n = 6$). All the groups were injected with EAC cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally (Gupta et al., 2004) except Group I. This was taken as day Zero.

Group I - Normal control.

Group II - Disease Control, EAC cell line (2×10^6 cell mouse).

Group III - EAC cell line (2×10^6 cells) treated with 100mg/kg p.o. of ETP.

Group IV - EAC cell line (2×10^6 cells) treated with 200mg/kg p.o. of ETP.

Group V - EAC cell line (2×10^6 cells) treated with 300mg /kg p.o. of ETP.

Group VI - EAC cell line (2×10^6 cells) treated with standard [5-fluorouracil (20 mg/kg i.p.)]

After 14 days of treatment, animals from each group were sacrificed by retro orbital plexus method to evaluate the antitumor and antioxidant potential of ETP (Kavimani and Manisenthil kumar, 2000).

Hematological studies

Hemoglobin content, red blood cells count (RBC) and white blood cells count (WBC) were measured from freely flowing tail vein blood from all the groups.

Antioxidant studies

The liver was excised out from the sacrificed animals rinsed in ice-cold normal saline followed by cold 0.15 M Tris-HCl (pH 7.4) and dried. A 10% (w/v) homogenate was prepared in 0.15 M Tris-HCl buffer, a portion was utilized for the estimation of lipid peroxidation and a second portion after precipitating proteins with TCA, was used for the estimation of glutathione (Ohkawa et al., 1979). The rest of the homogenate was centrifuged at 1500 rpm for 15 min at 4°C . The supernatant that obtained was used for the estimation of super oxide dismutase (SOD) and catalase (Kakkar et al., 1984 and Aebi and Burgmeyer, 1983).

Statistical analysis

Values were recorded as mean \pm S.E.M. The data were analyzed by student's t test; P values less than 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

It is evident from Table 1 that the death rate of EAC cells increases with increase in the concentration of the ETP. ETP was found to be cytotoxic to EAC cells. The effect of ETP on the survival of tumor bearing mice is shown in Table 2. The MST for the control group was 17.42 ± 0.17 days, whereas it was 31.33 ± 0.13 days and 33.60 ± 0.12 days for the groups treated with ETP (300 mg/kg/day p.o.) and 5-FU (20 mg/kg/day i.p), respectively. The percentage increase in the lifespan of tumor bearing mice treated with ETP (300 mg/kg/day p.o.) and 5-FU (20 mg/kg/day i.p) was found to be 82.52 and 92.88 respectively as compared to the disease control group. Hemato-

Table 2. Effect of *Tragia Plukenetii* R.Smith (ETP) treatment on the survival of Ehrlich ascites carcinoma (EAC) bearing mice.

Parameter	Body weight (g)	MST (Days)	%ILS
Group II (Disease control)	28.1±0.14	17.42±0.17	-
Group III ETP (100 mg/Kg p.o.)	27.32±0.12*	22.51±0.16*	29.41
Group IV ETP (200 mg/Kg p.o.)	25.93±0.14**	25.13±0.17**	47.05
Group V ETP (300 mg/Kg p.o.)	24.7±0.16***	31.33±0.13***	82.52
Group VI 5-FU (20 mg/Kg i.p.)	22.81±0.17***	33.60±0.12***	92.88

MST = mean survival time; ILS = increased life span.

Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

Table 3. Effect of *Tragia Plukenetii* R.Smith (ETP) on hematological parameters of Ehrlich ascites carcinoma (EAC) bearing mice.

Group	Hemoglobin (%)	RBC (1x10 ⁶ cells/mm ³)	WBC (1x10 ³ cells/mm ³)	Packed cell volume (ml)
Group I (Normal Control)	13.2 ± 1.52	6.4 ± 5.61	9.4 ± 1.02	0.2 ± 0.86
Group II (Disease Control)	10.3 ± 1.39 ^{^^^}	2.9 ± 9.36 ^{^^^}	18.0 ± 3.46 ^{^^^}	2.7 ± 0.80 ^{^^^}
Group III ETP (100mg/Kg p.o.)	11.1 ± 1.05*	3.8 ± 1.42*	15.9 ± 0.97*	1.4 ± 0.24*
Group IV ETP (200 mg/Kg p.o.)	11.9 ± 1.88**	5.0 ± 2.98**	12.1 ± 2.11**	0.9 ± 0.77**
Group V ETP (300 mg/Kg p.o.)	12.6 ± 1.74***	5.7 ± 2.57***	10.5 ± 1.51***	0.3 ± 0.65***
Group VI 5-FU (20 mg/Kg i.p.)	13.0 ± 0.95***	6.2 ± 3.32***	11.1 ± 0.88***	0.3 ± 0.91***

RBC = Red blood cells; WBC = white blood cells.

Statistically significant compared to Group I; ^{^^^}P<0.001.

Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

Table 4. Effect of *Tragia Plukenetii* R.Smith (ETP) on antioxidant parameters of Ehrlich ascites carcinoma (EAC) bearing mice.

Group	LPO (nmol MDA/Mg protein)	Glutathione (mg/g wet tissue)	Catalase (U/mg protein)	SOD (U/mg protein)
Group I (Normal Control)	0.96 ± 0.031	2.35 ± 1.532	26.4 ± 0.023	4.49 ± 3.74
Group II (Disease Control)	3.26 ± 0.039 ^{^^^}	0.91 ± 1.333 ^{^^^}	9.67 ± 0.114 ^{^^^}	1.56 ± 1.01 ^{^^^}
Group III ETP (100mg/Kg p.o.)	2.59 ± 0.031*	1.28 ± 2.056*	13.7 ± 0.009*	2.28 ± 3.39*
Group IV ETP (200mg/Kg p.o.)	2.42 ± 0.075**	1.77 ± 1.872**	19.4 ± 0.055**	2.47 ± 4.00**
Group V ETP (300mg/Kg p.o.)	1.33 ± 0.015***	2.11 ± 0.931***	21.3 ± 0.063***	3.78 ± 8.32***
Group VI 5-FU(20mg/Kg i.p.)	1.27 ± 0.041***	2.09 ± 0.977***	21.9 ± 0.011***	3.62 ± 3.17***

LPO = lipid peroxide; SOD = super oxide dismutase.

Statistically significant compared to Group I; ^{^^^}P<0.001.

Statistically significant compared to Group II; *P<0.05. **P<0.01. ***P<0.001.

logical parameters of tumor bearing mice on day 14 showed significant changes when compared with normal control (Group I). The total WBC count and PCV were found to increase with a reduction in the hemoglobin content of RBC (Table 3). At the same time interval, ETP (300 mg/kg/day p.o.) treatment changed these altered parameters to near normal. Antioxidant parameters of tumor bearing mice were altered during diseased condition and brought back to normal level after the treatment with ETP (300 mg/kg/day p.o.) (Table 4). The antioxidant potential of ETP was evaluated by estimating

the amount of parameters like super oxide dismutase (SOD), lipid peroxide (LPO), reduced glutathione, catalase from the liver tissues of EAC bearing mice. Generally, the result obtained at the dose level of 300 mg/kg.bw was highly significant and comparable to that of Standard drug.

The present study was planned to evaluate the anti-tumor potential and antioxidant status of ETP in EAC bearing mice. The *in vitro* cytotoxicity study revealed that the ETP is toxic to the EAC cells as there is an increase in the number of cells stained with trypan blue dye with

the increase in concentration of ETP (Clarkson and Burchenal, 1965). The reliable criterion for assessing the value of any anticancer drug is the prolongation of the life span of animals. It is observed that ETP increased the life span of the EAC bearing mice by inhibiting the activity of the EAC cells. The hematological parameters revealed considerable changes leading to toxic effect in mice treated with ETP. After 14 days of inoculation, ETP was able to reverse these changes in hematological parameters and could reduce the toxic effects (Price et al., 1958; Hogland, 1982).

The implication of free radicals in tumor is well documented (Ravid and Korean, 2003; Feng et al., 2001). Lipid peroxide, an autocatalytic free radical chain propagation reaction, is known to be associated with pathological condition of a cell. The presence of tumor is known to affect many functions of the vital organs, especially the liver. This leads to an increase in the level of MDA (malondialdehyde), end product of lipid peroxide, in disease control group (Sinclair et al., 1990). It has been reported that a decrease in SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver of EAC bearing mice (Sun et al., 1989). Glutathione and catalase were also involved in the free radical scavenging activity. There is a reduction in the levels of the scavengers as a result of tumour growth in disease control animals. Treatment with ETP brought back the levels of these scavengers in a dose dependant manner and reduced the level of LPO. The findings were compared to that of the standard drug 5-fluorouracil. The free radical hypothesis also supported the fact that the ETP possesses significant antitumour and antioxidant potential against EAC bearing mice.

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