



Anti-tumor activity of 50% aqueous ethanol extracts of *Acanthospermum hispidum* DC in mice

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ABSTRACT

The study investigated the anti-tumor activity of 50% aqueous ethanol extract of *Acanthospermum hispidum* DC against Dalton's ascites lymphoma in mice. The extracts were prepared by cold maceration with 50% aqueous ethanol and evaporated in vacuum to dry. (Yield: 50% aqueous ethanol -8.62% w/w). The extract were suspended in water with tween 20 and used for the study. The 50% aqueous ethanol extract (300 mg/kg p.o) was administered to tumor bearing mice (DAL) and examined for changes in dead cell count, histopathology of tumor cells, haematological parameters and median survival time (MST) and the results compared with that of tumor control or 5-FU. The findings reveal that 50% aqueous ethanol extracts possess anti-tumor activity. Thus it is suggested that *Acanthospermum hispidum* DC appears promising for the development of phyto-medicine for the treatment of cancer.

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KEYWORDS

Acanthospermum hispidum DC;Anti-tumor activity;
Dalton's ascites lymphoma;
50% aqueous ethanol
extract.

INTRODUCTION

The availability of effective drugs for the treatment of cancer remains a challenge. Currently available anti-cancer drugs are highly reactive leading to indiscriminate reaction with a wide range of cell constituents. Hence herbal drugs are considered as an alternative in the management of cancer. Several plant products have been reported to possess anti-cancer property^[1,2,3,6,14,16]. *Acanthospermum hispidum* DC (Family: Asteraceae, N.O. Compositae) is a hispid herb; mainly distributed in South America, India and other tropical countries^[10,19]. The plant has been documented as diuretic, febrifuge, sudorific and in the treatment of gonorrhoea in some parts of South America. The plant also possess anti-viral activity against alpha herpes virus^[17] and anti-plasmodial activity against *Plasmodium falciparum* chloroquine resistant W₂ strains^[18], an *in-vitro* studies revealed

the immuno - modulatory capacity of the plant to enhance the proliferation of T-Lymphocytes after stimulation with ConA or allogenic stimulator cells in the mixed leucocyte culture^[18]. The plant has also been reported for the presence of terpenoid and phenolic constituents, with some of the former possessing *in-vitro* anti-neoplastic activity^[11]. Other species of this genus presenting cytotoxic and anti-cancer activity has been documented^[15]. Literature search indicated that no reports are available on the *in-vitro* anti-tumor property of *Acanthospermum hispidum* DC and hence the same investigated in the present study.

EXPERIMENTAL

Plant material collection and extraction

The whole plant excluding fruits of *Acanthospermum*

hispidum DC were collected from rain forest area, Thirunelveli district, Tamilnadu, India, in the month of May-2003 and authenticated by Survey of Medicinal Plants Unit-Siddha, C.C.R.A.S., Government of India, Thirunelveli, Tamil Nadu, India. The plant material was shade dried and pulverized. The powder was extracted by cold maceration in an aspirated bottle with 50% aqueous ethanol for 3-7 days. The extract was concentrated under vacuum and dried in a desiccator (Yield: 50% aqueous ethanol - 8.62 % w/w).

Animals

Adult male Swiss albino mice (20-25g) were procured from King Institute, Chennai, India. They were housed in polypropylene cages in groups of six and had free access to food (Pellets obtained from Lipton, India) and water prior to as well as during experimentation. They were maintained under normal room temperature (28-30°C) and acclimatized in the laboratory conditions for 3 days, prior to experimentation with 12/12 hour light/dark cycle. The experiments were conducted during the light period. The study was conducted after obtaining the institutional animal ethical committee clearance.

Tumor cell line used

Dalton's lymphoma in ascitic form was obtained through the courtesy of Amala Cancer Research Center, Thirussur, Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells/mouse^[8]. Tumor in mice was induced as follows:

The ascitic fluid of the DAL was drawn out from the donor mice carrying the tumor for 7-9 days. The freshly drawn ascitic fluid was diluted in phosphate buffer saline (pH 7.4) to a concentration of 10^6 cells/ml and aliquots of 0.3-0.5ml of the diluted solution was injected intra-peritoneally into the mice belonging to age group of 4-6 weeks. The development of tumor (7-9 days) was confirmed from the cells count of the ascitic fluid equivalent to 10^6 cells/ml.

Drug treatment

The 50% aqueous ethanol extract was suspended in distilled water with tween - 20 and used for the study. Three groups of tumor bearing mice (n=6) were used for the study. Group I was treated with 300mg/kg. p.o. of 50% aqueous ethanol extract, Group-II was treated with 12.5mg/kg p.o. of 5-Fluorouracil-(Biochem Pharmaceuticals, Mumbai, India). The untreated Group III was used as control. The dose for extract was selected

based on toxicity studies^[12], which showed no toxicity upto 5g/kg for both. Animal received drug treatments on 9th and 18th day and were examined for various parameters for anti tumor activity on 11th and 20th day post tumor inoculation.

Effect on dead cell count

0.1ml of the peritoneal fluid was aseptically withdrawn from the animal using a 1ml syringe and diluted approximately with tryphan blue solution and examined under microscope.

The number of dead cells (Stained cells) randomly in every 200 cells were counted and the results were recorded as percent protection against tumor growth using the following calculation.

Percent protection against tumor growth = $\frac{\text{No. of dead cells in treated groups} - \text{No. of dead cells in untreated groups}}{\text{No. of dead cells in untreated groups}} \times 100$

Effect on histopathology of tumor cells

A small volume of the peritoneal fluid from the treated as well as untreated animals was withdrawn aseptically and stained with Maygrunwald's reagent and examined for histo - pathological changes.

Effect on hematological parameters

Blood was drawn from each animal from the retro-orbital flexes and the white blood cell count (WBC), red blood cell count (RBC), hemoglobin and differential leukocyte count (DLC) were determined^[5]. The data were statistically analyzed by two way - ANOVA followed by Dunnett's 't' test. $P < 0.05$ was considered as statistically significant.

Effect on median survival time (MST)

The median survival time of the treated groups was determined and compared with that of the tumor control group using the following calculation.

Percent increase in life span = $\frac{T-C}{C} \times 100$

Where, T = Number of days the treated animals survived, C = Number of days tumor control animals survived. All the data were statistically analyzed by one way ANOVA by Dunnet's 't' tests. $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Acute toxicity study indicated no significant changes in the behavioral response of the animals at different doses of the extract. On the 11th day post tumor inoculation extract treated groups showed anti-tumor

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activity with varying degrees of protection against tumor. The 50% aqueous-ethanol extract gave 52% protection but higher than that of standard 5-Fluorouracil treated groups. On the 20th day post tumor inoculation 50% aqueous-ethanol extract produced 22.86% protection. On comparison of the anti-tumor activity of extract with standard 5-Fluorouracil between 11th day and 20th day post tumor inoculation extract treated groups gave better results. Extract showed increased anti-tumor activity on 20th day post tumor inoculation.

Histopathology of ascitic tumor

The histopathological changes like vacuolisation, necrosis, and apoptosis, in the tumor cells are indicative of anti-tumor activity.

On 11th day post tumor inoculation only vacuolisation was observed with 50% aqueous-ethanol extract (300 mg/kg.p.o) treatment on 11th day post tumor inoculation and there was more necrosis of the cells on the 20th day post tumor inoculation (Figure 1). The standard 5-Fluorouracil treated groups showed marked vacuolisation and necrosis of the tumor cells on 20th day as compared to 11th day post tumor inoculation (Figure 2).

Comparison between the standard 5-Fluorouracil treated animals and the extract treated animals, the extracts produced more or less similar intensity of vacuolisation and necrosis of the tumor cells on 20th day post tumor inoculation. In contrast 50% aqueous ethanol extract showed greater degree of vacuolisation and necrosis on the 20th day post tumor inoculation as compared to standard 5-Fluorouracil.

Haematological parameters

Tumor growth normally affects the haematological picture resulting in changes in various haematological parameters such as White Blood Cell count (WBC) Red Blood cell count (RBC), Haemoglobin content (Hb), lymphocyte, neutrophil and monocyte count. The anti-tumor activity is generally assessed by restoration of the changes in these parameters to normal and more significantly a significant reduction in WBC count, an increase in RBC, lymphocyte count, or haemoglobin content as compared to tumor control.

Extract / (vs) tumor control

The extract produced significant reduction in WBC count as compared to tumor control ($P < 0.001$) on both 11th and 20th day post tumor inoculation. The RBC

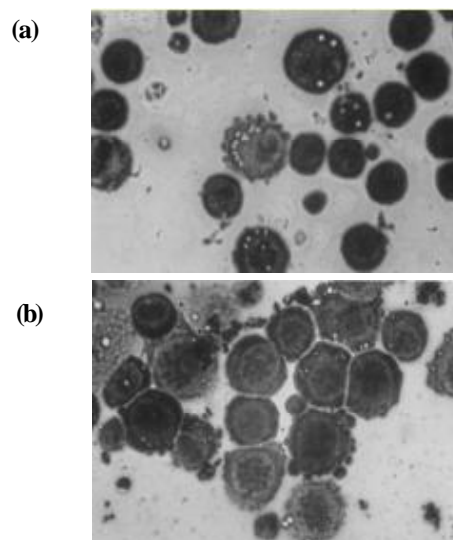


Figure 1: (a) 50% Aqueous - ethanol extract treated animals on 11th day post tumor inoculation; (b) 50% Aqueous - ethanol extract treated animals on 20th day post tumor inoculation

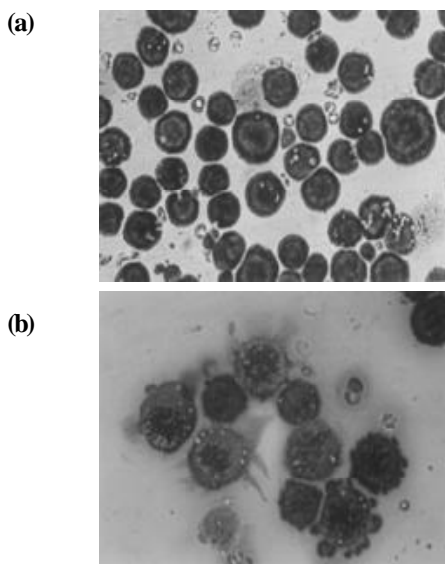


Figure 2: (a) 5-FU treated animals on 11th day post tumor inoculation; (b) 5-FU treated animals on 20th day post tumor inoculation

count was significantly lowered by the extract on 11th day post tumor inoculation ($P < 0.001$). On 20th day post tumor inoculation 50% aqueous-ethanol extract did not show any significant change in RBC count as compared to tumor control. The haemoglobin content was not affected 50% aqueous-ethanol extract.

Extracts / (vs) standard 5-fluorouracil

Further analysis into comparison between various extract / and the standard 5-Fluorouracil on changes in

TABLE 1: Effect of 50% aqueous-ethanol extract of *Acanthospermum hispidum* DC on dead cell count of DAL in mice

Sl. no.	Group	% of dead cells		% protection against tumor	
		11th day	20th day	11th day	20th day
1.	Control (DAL)	25	35	-	-
2.	5-FU (Standard)	35	49	40	40
3.	50% Aqueous - ethanol extract	38	43	52	22.86

Each value represents the mean of six experiments

TABLE 2: Effect of 50% aqueous-ethanol extract of *Acanthospermum hispidum* DC on changes in haematological parameters induced by DAL in mice

Description	Day	WBC \pm Sem	RBCx10 ⁶ \pm Sem	Hb g/dl \pm Sem	Lymphocytes \pm Sem	Neutrophils \pm Sem	Monocytes
Normal mice	-	5000-7000	8.7-12.5	10.2-16.2	70-75	23-28	0.1-3.5
Tumor control (DAL)	11	15,175 \pm 1.86*	3,495 \pm 0.01*	6.9 \pm 0.97*	57.10 \pm 0.86*	38.25 \pm 1.13*	2
	20	16,633 \pm 2.04*	2.153 \pm 0.02*	6.46 \pm 0.08*	28.66 \pm 0.81*	58.66 \pm 0.81*	2
5-FU (Standard 12.5 mg/kg.p.o)	11	12,7375 \pm 2.05	2.40 \pm 0.08	5.8 \pm 0.98	48.06 \pm 0.41	56 \pm 0.82	2
	20	11,600 \pm 2.03	2.61 \pm 0.03	7.2 \pm 0.04	54.98 \pm 0.81	34.98 \pm 0.03	2
50% Aqueous – ethanol extract (300 mg/kg.p.o)	11	11,966.66 \pm 0.82	2.37 \pm 0.82	6.5 \pm 0.12	42.38 \pm 0.41	53.66 \pm 0.50	1
	20	11,600 \pm 2.21	2.42 \pm 0.05	7.2 \pm 0.28	52.93 \pm 0.83	35 \pm 0.41	2

n = 6 animals in each groups; * P<0.001 vs normal mice; ** P<0.001 vs tumor control; Value are expressed as mean \pm SEM

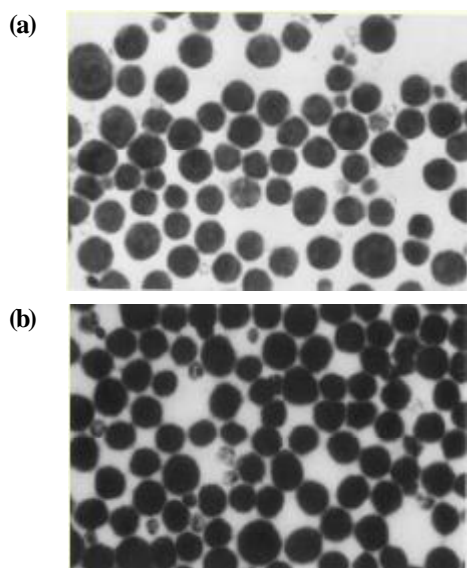


Figure 3: (a) Tumor control animals on 11th day post tumor inoculation; (b) Tumor control animals on 20th day post tumor inoculation

haematological parameters indicates that the results of 50% aqueous-ethanol extract is comparable with that of standard 5-Fluorouracil on 11th day post tumor inoculation (P<0.001).

50% Aqueous-ethanol extract gave comparable results with that of standard on WBC count and Hb content on 11th day post tumor inoculation and on RBC and lymphocyte count on 20th day post tumor inocula-

TABLE 3: Effect of 50% aqueous - ethanol extract of *Acanthospermum hispidum* DC on median survival time (MST) in mice

Groups	No. of days alive \pm SEM	Mean survival time (%)
Tumor control (DAL)	20 \pm 0.35	100
5-FU (Standard)	24 \pm 0.24*	120
50% Aqueous - ethanol extract	22	110

n = 6 animals in each group, *P<0.001 vs control. Values are expressed as mean \pm SEM

tion (P<0.001). While RBC count was not significantly influenced on 11th day post tumor inoculation, the effects was comparable with that of standard 5-Fluorouracil on 20th day post tumor inoculation (P<0.001). Lymphocyte count was not significantly altered on 11th day post tumor inoculation, however comparable results were observed on 20th day post tumor inoculation (P<0.001). Neutrophil count was unchanged on both 11th and 20th day post tumor inoculation in the 50% aqueous- ethanol treated groups (TABLES 1-3).

Mean survival time (MST)

The anti-tumor activity of a drug is best assessed by its effect on the life span prolongation of the animal (MST).

The average life span of the tumor inoculated animals (tumor control) was 20 days. The extract and standard 5-Fluorouracil extended life span of the animal to varying degree. The MST observed with 50% aqueous-ethanol extract was 110%.

50% Aqueous-ethanol extract increased the MST and the values are more or less close to that of the standard 5-Fluorouracil., which is more or less similar and near to that observed with the standard 5-Fluorouracil (120%) and tumor control.

Body weight

Tumor normally presents an increase in body weight and so the reduction in weight gain is considered as

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anti-tumor activity.

The tumor control animals on 20th day post tumor inoculation showed an increase in body weight. The extract showed significant reduction in weight gain as compared to tumor control thus showing the anti-tumor effect of the sample tested.

5-FU treatment produced 40% protection on both 11th and 20th day. However, both extracts treatments showed almost similar results on 11th day, did not show enhanced protection on 20th day as compared to 5-FU treatment.

Histopathological study reveals that 50% aqueous-ethanol extract treatment showed both vacuolization and necrosis of tumor cells on 11th as well as 20th day. In comparison with 5-FU treatment, 50% aqueous-ethanol extract treatment produced similar degree of vacuolization and necrosis of tumor cells to that of 5-FU on both 11th day and 20th day as compared to 5-FU treatment.

Prolongation of the mean survival time (MST) is a critical factor in determining the anti-tumor activity of a drug. The MST was extended to a greater extent (similar to standard) by 50% aqueous ethanol extract treatment. The mechanism for extended life span by the extract treatment may be the influence on changes in the histopathology or haematology of the tumor cells.

Tumor results in increase in body weight due to various factors discussed above. The results on body weight indicate that the extract significantly reduced the tumor volume as comparable to control or standard 5-Fluorouracil treated groups. However, 50% aqueous ethanol extract produced greater effect. This finding leads to support that 50% aqueous ethanol extract appears to be more promising for anti-tumor activity.

A comparative study of the effect of the 50% aqueous ethanol extract on dead cell count, histopathology, haematology, MST and body weight indicate that the findings observed in all these parameters agree with each other. An increase in dead cell count, or reduction in body weight representing anti-tumor activity is well reflected by the changes in histopathology or haematology of the tumor cells as seen with increased vacuolisation and shrinkage of the cells followed by necrosis or with decreased WBC count, increased RBC count, Hb content and lymphocyte count and reduced neutrophil count. Further investigation on the relationship between phyto-constituents and the anti-tumor property of the plant and the mechanisms of anti-tumor activity is in progress.

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