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Anti-tumor activity of hexane and chloroform extracts of *Acanthospermum hispidum* dc in mice

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ABSTRACT

The study investigated the anti-tumor activity of hexane and chloroform extract of *Acanthospermum hispidum* DC against Dalton's ascites lymphoma in mice. The extracts were prepared by successive extraction with hexane and chloroform and evaporated in vacuum to dry. (Yield: Hexane-2.9% w/w, chloroform-1.72% w/w). The extracts were suspended in water with tween 20 and used for the study. Hexane extract (50 mg/kg p.o) and chloroform 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 both hexane and chloroform extracts possess anti-tumor activity. In order to ensure the standards of the extract, finger printing of the extracts in the crude form is done using HPTLC technique, using hexane and benzene as solvent system and the peaks obtained is recorded. Thus it is suggested that *Acanthospermum hispidum DC* appears promising for the development of phyto-medicine for the treatment of cancer.

KEYWORDS

Acanthospermum hispidum DC;
Anti-tumor activity;
Dalton's ascites lymphoma;
Hexane extract;
Chloroform extract;
Diterpenes.

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[1-3,6,15]. *Acanthospermum hispidum DC* (Family: Asteraceae, N.O.Compositae) is a hispid herb; mainly distributed in South America, India and other tropical countries[10,18].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[16] and anti-plasmodial activity against Plasmodium falciparum chloroquine resistant W_2 strains[17], 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[17]. The plant has also been reported for the presence of terpenoid and phenolic constituents, with some of the former pos-

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sessing *in vitro* anti-neoplastic activity[11]. Other species of this genus presenting cytotoxic and anti-cancer activity has been documented[14] 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 successively extracted by cold maceration in an aspirated bottle with hexane and chloroform for 3-7 days. The extract was concentrated under vacuum and dried in a desiccator (Yield: Hexane-2.9% w/w, chloroform-1.72 % 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 (pH7.4) 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 hexane and chloroform extracts was suspended in distilled water with tween - 20 and used for the study. Four groups of tumor bearing mice (n=6) were used for the study. Group I was treated with 300mg/kg. p.o. of chloroform extract, Group-II received 50mg/kg.p.o. of hexane extract, Group-III was treated with 12.5mg/kg p.o. of 5-Fluorouracil-(Biochem Pharmaceuticals, Mumbai, India). The untreated Group IV was used as control. The dose for extracts 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.

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

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[4,5]. The

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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 = T-C×100/C

Where, T=Number of days the treated animals survived, C=Number of days tumor control animals survived. All the datas 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 extracts. One animal died on treatment with hexane extract (300 mg/kg p.o) and so the next lower dose (50 mg/kg p.o) was selected for the study. No animal died at 300 mg/kg p.o of chloroform extract and the same dose used for the study.

Both hexane and chloroform extract treatment showed significant increase in dead cell count as compared to tumor control on both 11th and 20th day. Hexane extract treatment produced 44% protection against tumor, where as it was 40% with that of chloroform extract on the 11th day. On 20th day, the percentage protection against tumor decreased to 17.1% and 8.6% for hexane and chloroform extract respectively. 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 hexane extract treatment showed both vacuolization and necrosis of tumor cells on 11th as well as 20th day. In contrast, chloroform extract treatment showed only vacuolization on 11th day, whereas vacuolization and necrosis of the tumor cells on 20th day. In comparison with 5-FU treatment, hexane 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.

Tumor bearing mice induced changes in haematological parameters. There was significant increase in WBC and neutophil and significant decrease in RBC, Hb and lymphocyte as compared to normal animals. Both hexane and chloroform extracts treatment significantly altered these changes and the same are comparable to that of 5-FU.

Both hexane and chloroform extract treatment increased the median survival time (MST) of mice as compared to tumor controls. There was 5% and 15% increase in life span with hexane and chloroform extracts respectively. However, the increase in life span observed in the extracts treated groups is less than that of 5- FU treated groups. The HPTLC finger print of the chloroform and hexane extracts showed multiple peaks with various R_f values. The results of the present study documented the anti-tumor property of hexane and chloroform extracts of *Acanthospermum hispidum DC*. The major criteria for the anti-tumor activity are reduction in WBC and increase in life span. A small reduction in WBC and increase in life span observed in the extract treated groups represent the anti-tumor property of the extracts, which is further supported by significant changes in RBC, Hb, lymphocyte and neutrophils as compared to tumor control. Additionally, significant increase in dead cell count and changes in histopathology of tumor cells marked by vacuolization and necrosis also supported the anti-tumor property of the extracts. Phyto-chemical investigation revealed the presence of mono, di and triterpenoids and sesquiterpene lactones. These constituents which were identified in other plants have been documented to possess anti-tumor property[9]. The anti-tumor activity of the extracts observed in the present study may be attributed to the presence of these compounds in the extracts. The role of diterpenes in the anti-tumor activity and their mecha-

TABLE 1: Effect of hexane and chloroform extract of *Acanthospermum hispidum* DC on dead cell count of DAL in mice

S. 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.	Hexane extract	36	41	44	17.14
4.	Chloroform extract	35	38	40	8.57

Each value represents the mean of six experiments

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TABLE 2: Effect of hexane and chloroform extract of *Acanthospermum hispidum* DC on changes in haematological parameters induced by DAL in mice

Description	Day	WBC±SEM	RBC×10^6±SEM	Hb g/dl±SEM	Lymphocytes ±SEM	Neutrophils± SEM	Monocytes
Normal mice	-	5000-7000	8.7-12.5	10.2-16.2	70-75	23-28	0.1-3.5
Tumor Control	11	15,175±1.86*	3,495±0.01*	6.9±0.97*	57.10±0.86*	38.25±1.13*	2
(DAL)	20	16,633±2.04*	2.153±0.02*	6.46±0.08*	28.66±0.81*	58.66±0.81*	2
5-FU (Standard	11	12,7375±2.05	2.40 ±0.08	5.8±0.98	48.06±0.41	56±0.82	2
12.5 mg/kg.p.o)	20	11,600±2.03	2.61±0.03	7.2±0.04	54.98±0.81	34.98±0.03	2
Hexane extract (50	11	13,133.33± 2.04**	2.22±0.02**	6.05±0.02**	34.33±1.63**	42.01±1.63**	2
mg/kg.p.o)	20	12,650±1.85**	2.65±0.08**	7.95±0.13**	54.46±0.40**	37.52±1.22**	2
Chloroform extract	11	11,266±2.36**	2.06±0.04**	5.63±0.29**	41.66±0.41**	52.2±0.81**	2
(300 mg/kg.p.o)	20	10,250±2.05**	3.05±0.12**	9.16±0.11**	55.51±2.04**	36.49±2.06**	2

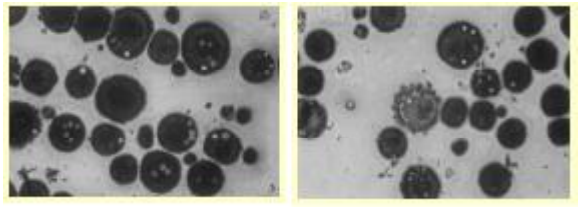
n = 6 animals in each groups, *P<0.001 vs normal mice, **P<0.001 vs tumor control. Value are expressed as mean ±SEM.

TABLE 3: Effect of hexane and chloroform extract of *Acanthospermum hispidum* DC on median survival time (MST) in mice

Groups	No. of days alive±SEM	Mean survival time (%)
Tumor control (DAL)	20±0.35	100
5-FU (Standard)	24±0.24*	120
Hexane extract	21 ± 0.22*	105
Chloroform extract	23 ± 0.28*	115

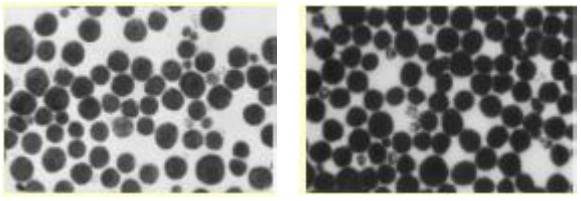
n= 6 animals in each group, *P<0.001 vs control, Values are expressed as mean ±SEM

Histopathology of tumor cells



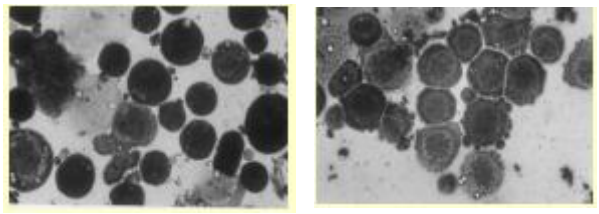
(a) (b)

Figure 1(a): Hexane extract treated animals on 11th day post tumor inoculation, (b): Hexane extract treated animals on 20th day post tumor inoculation



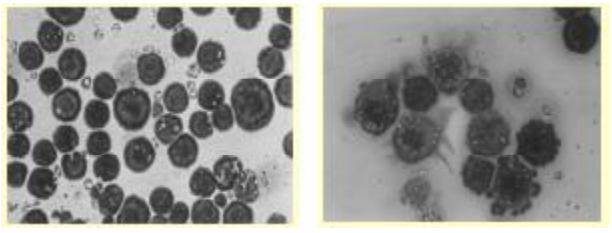
(a) (b)

Figure 2(a): Tumor control animals on 11th day post tumor inoculation, (b): Tumor control animals on 20th day post tumor inoculation



(a) (b)

Figure 3(a): Chloroform extract treated animals on 11th day post tumor inoculation, (b): Chloroform extract treated animals on 20th day post tumor inoculation



(a) (b)

Figure 4(a): 5-FU treated animals on 11th day post tumor inoculation, (b): 5-FU treated animals on 20th day post tumor inoculation

nisms has not been reported[4]. Diterpenes inhibit the activation of transcription factor NF kappa B, the central mediator of apoptosis and immune response by directly targeting DNA binding activity of gene P50[9]. Diterpenes also possess non cytotoxic anticancer property through farnesyl protein transferase inhibition[7], besides inhibition of histone deacetylase. One or more of these mechanisms may be contributing to the anti-tumor activity of the hexane and chloroform extracts of *Acanthospermum hispidum DC*. However, the phyto-constituent responsible for the activity is to be investigated in detail. Thus it can be concluded that *Acanthospermum hispidum DC* has anti-tumor property and this property may be attributed to the presence of mono, di and triterpenes and sesquiterpene lactones. HPTLC finger printing revealed the presence of various phyto-constituents with various Rf values and

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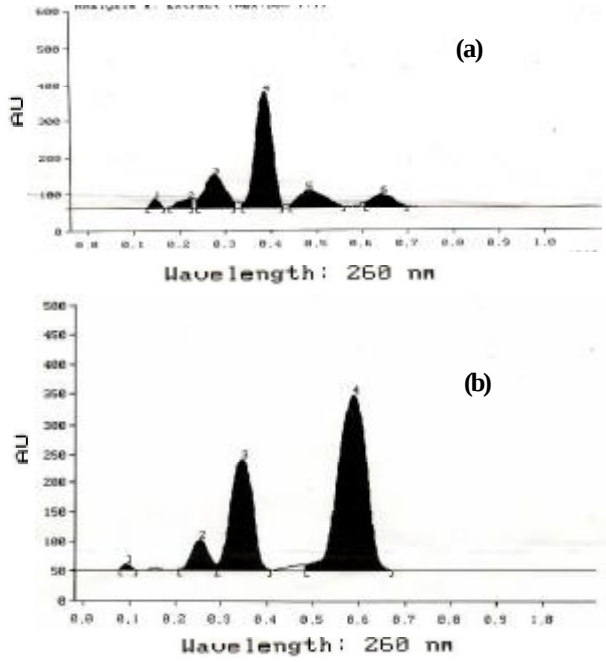

Figure 5(a): HPTLC finger printing-Chloroform extract, (b): HPTLC finger printing-Hexane extract

the same may be useful in setting standards in the method of preparation of extracts for further investigation of phyto-chemical characterization and biological activities. 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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