

Anticancer activity of ethanol extract of *Asystasia travancorica* Bedd (Acanthaceae) whole plant against Dalton Ascites Lymphoma

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ABSTRACT

The present study aims to evaluate the antitumor activity of ethanol extract of whole plant of *Asystasia travancorica* Bedd on DAL induced tumor in Swiss Albino mice. Evaluation of the antitumor effect of ethanol extract of whole plant of *Asystasia travancorica* on tumor growth and host survival time was made by the study of the following parameters: tumor volume, viable and non viable cell count and life span of host. The results showed decrease in tumor volume and viable cell count. Hematological studies revealed that, the Hb count decreased in DAL treated mice, whereas it was induced by the drug treated animals and showed an increase in Hb near to normal levels. The results suggested that the extract of whole plant of *Asystasia travancorica* exhibited significant antitumor activity on DAL bearing mice.

Keywords: *Asystasia travancorica*, antitumor, lifespan, WBC, DAL, HB.

INTRODUCTION

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth.^[1] Cancer may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. It is caused by a complex, poorly understood interplay of genetic and environmental factors. It continues to represent the largest cause of mortality in the world and claims over 6 million. Cancer kills annually about 3500 per million populations around the world.^[2] Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind.^[3]

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The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity.^[4] Over 3000 species of plants with antitumour properties have been reported.^[5] Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources.^[6]

Chemoprevention is recognized as an important approach to control growth and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents.^[7] In any cancer drug discovery program, a theory based on ethnobotanical and ethnopharmacological data would be more economical and beneficial in identifying potential anticancer molecules than mass screening of plant species.^[8] Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources.^[9]

According to Cragg and Newman [10] over 50 % of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them. Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine and camptothecin.[11] The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery.

Asystasia includes approximately 70 species of perennial herbs and subshrubs from tropical Africa, India and Asia. *Asystasia* belongs to the family Acanthaceae. Paste of leaves and flowers of *Asystasia travancorica* mixed with honey is taken orally, twice a day, for three weeks for the treatment of rheumatism.[12] Taking into consideration of the medicinal importance of *A. travancorica*, the ethanol extract of the whole plant *A. travancorica* were analyzed for their anticancer activity against Dalton Ascites Lymphoma (DAL) tumor model.

MATERIALS AND METHODS

Collection of plant sample

Whole plant of *Asystasia travancorica* Bedd was collected from Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, the specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for anticancer activity

The whole plants of *Asystasia travancorica* were cut into small pieces, washed dried at room temperature; the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered whole plant was separately packed in a Soxhlet apparatus and extracted with ethanol. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract of whole plant was used for anticancer activity.

Animals

Healthy male adult Swiss Albino mice (20-25gm) were used for the study. The animals were housed in microlon boxes in a controlled environment (temperature 25±20c) and 12 hr dark/eight cycle) with standard laboratory diet (Sai Durga feeds and foods, Bangalore) and water *ad libitum*. The mice well segregated based on their gender and quarantined for 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygienic environment in our animal house.

Tumor cells

Dalton Ascites Lymphoma (DAL) cells were obtained from Division of Oncology Department of Biotechnology, Tamil Nadu, Veterinary and Animal Husbandry, Chennai, Tamil Nadu, India. The DAL cells were maintained *in vivo* in Swiss albino mice by weekly intra peritoneal (i. p) inoculation of 10⁶cells / mouse after every ten days. DAL cells 9 days old were used for the screening of the anticancer activity.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline – 420 [13] fixed dose procedure for ethanol extract of whole plant of *A. travancorica* and it was found that dose increasing up to 2000 mg / kg body weight, shown no toxicity or mortality in experimental mice.

Antitumor activity

Healthy Swiss albino mice were divided in to five groups of five animals (n=5) each. The test samples were dissolved in isotonic saline (0.9% NaCl W/V) and used directly in the assay. DAL cells were collected from the donor mouse and were suspended in sterile isotonic saline. The viable DAL cells were counted (Trypan blue indicator) under the microscope and were adjusted at 1 X 10⁶ cells/ ml. 0.1 ml of DAL cells per 10g body weight of the animals were injected (i. p) to each mouse of each group except normal saline group (Group I). This was taken as Day 0. Group I served as a normal saline control (1mL/kg, p.o) and group II served as DAL bearing control. On day 1, the ethanol extract of *A. travancorica* at a dose of 100 and 200mg/kg each of the Group III, IV were

administrated orally and continued for 14 consecutive days respectively. Group V served as tumor induced animal administrated with vincristine (80mg/kg body weight) for 14 consecutive days. On day15, half of the animals (n=3) in each case were sacrificed and the remaining animals were kept to observe the life span study of the tumor hosts. The effect of ethanol extract of *A. travancorica* on tumor growth and host's survival time were monitored by studying parameters like tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span.^[14-15]

Determination of tumor volume

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. Packed cell volume was determined by centrifuging the ascitic fluid at 1000 rpm for 5min.

Determination of tumor cell count

The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension as placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted.

Estimation of viable and non viable tumor cell count (Tryphan blue dye assay)

The cells were then stained with tryphan blue (0.4% 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.

Percentage increase of life span (% ILS)

The percentage increase in life span (% ILS) was calculated from the following equation.

$$\text{Increase in life span} = \frac{T - C}{C} \times 100$$

Body weight

Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (zero day) and sequentially on every 5th day during the treatment period.

Haematological studies

At the end of the experimental period, all mice were sacrificed by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation of Hemoglobin content(Hb), Red blood cell count (RBC) and White blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears.^[16]

Statistical analysis

The data were analyzed using student's t- test statistical methods. For the statistical tests, *p* values of less than 0.01 and 0.05 were taken as significant.

RESULTS AND DISCUSSION

The acute toxicity study, ethanol extract of *A. travancorica* whole plant did not show any toxic effect upto the dose of 2000mg/kg body weight, according to 100mg/kg and 200mg/kg were taken as low and high dose of whole plant of *A. travancorica* for the experiment. The present investigation indicates that ethanol extract of whole plant of *A. travancorica* showed significant antitumor activity in DAL bearing mice.

Table-1 shows administration of ethanol extract of whole plant of *A. travancorica* to DAL bearing mice showed reduction in bodyweight (g), spleen, thymus, liver, kidney and lungs. The effects of ethanol extract of *A. travancorica* whole plant at the doses of 100 and 200mg/kg on solid tumor volume is shown in Table-2. Treatment with ethanol extract of whole plant of *A. travancorica* and vincristine at the dose of 100 and 200mg/kg significantly ($P < 0.01$) reduces the solid tumor volume in a dose dependent manner as compared to that of the DAL control group. The effects of ethanol extract of *A. travancorica* whole plant at the doses of 100 and 200mg/kg on Survival time (days), Life span (%), Packed cell volume, tumor cell count(viable and non viable cell) shown in Table-3. In the DAL control group, the mean survival time was 19.80 ± 0.11 days, while it increased 28.14 ± 0.21 (100mg/kg) and 35.65 ± 0.67 (200mg/kg) days respectively, in the ethanol extract of *A. travancorica*

treated groups, whereas the standard drug vincristine (80mg/kg) treated group had a mean survival time of 34.90 ± 0.55 days. The percentage increase in survivals, it was found to be 42.12%, 80.05% and 76.26% respectively as compared to DAL control group.

Treatment with ethanol extract of *A. travancorica* whole plant at the doses of 200mg/kg significantly ($P < 0.01$) reduced the packed cell volume and viable tumor cell count in a dose dependent manner as compared to that of the DAL control group. Furthermore, nonviable cell counts at different doses of ethanol extract of *A. travancorica* were increased in a dose dependent manner.

As shown in (Table-4) RBC, HB, lymphocytes were decreased and WBC count, Neutrophil, Eosinophil were significantly increased in the DAL control group compared to the normal control group. Treatment with ethanol extract of whole plant of *A. travancorica* at the dose of 100 and 200mg/kg significantly increases in the HB count and RBC significantly decreased the WBC count to about normal level. All these results suggest the anticancer nature of the extract. However, the standard vincristine at the dose of 80mg/kg body weight produced better result in all these parameters.

The alternative system of medicines like Ayurvedic, Siddha, Unani and other tribal folklore medicines have significantly contributed to the health care of the population of India. Today these systems are not only complementary but also competitive in the treatment of various diseases. Plants have served as a good source of antitumor agents. Several studies have been conducted on herbs under a multitude of ethanobotanical grounds. A large number of plants possessing anticancer properties have been documented.^[17-22] The present investigation was carried out to evaluate the antitumor activity of ethanol extract of *A. travancorica* in DAL tumor bearing mice. The ethanol extract of *A. travancorica* treated animals at the doses of 100 and 200 mg/kg significantly decreased the tumor volume, packed cell volume, tumor (viable) cell count and brought back

the hematological parameters to more or less normal levels.

In DAL tumor bearing animals a regular rapid increase in ascitic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells.^[23]

Treatment with ethanol extract of *A. travancorica* inhibited the tumor volume, viable tumor cell count and increased the life span of the tumor bearing mice. The reliable criteria for judging the value of any anticancer drug are the prolongation of the lifespan of animals. ^[24] It may be concluded that ethanol extract of *A. travancorica* by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of DAL bearing mice. Thus ethanol extract of *A. travancorica* have antitumor activity against DAL bearing mice.

Usually, in cancer chemotherapy the major problems that are being encountered are myelosuppression and anemia. ^[25-26] The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions.^[27] In DAL control group, a differential count the presence of neutrophils increased, while the lymphocyte count decreased, the observed leucocytopenia indicates a common symptom of immunosuppression in many types of cancer^[28-29] and one of the causes of neutrophilia is myeloid growth factors which are produced in malignant process as part of a paraneoplastic syndrome. In addition to this another factor granulocyte colony stimulating factor produced by the malignant cells has also been attributed to be the cause of neutrophilia because of its action on bone marrow granulocytic cells in cancer. After the repeated, ethanol extract of *A. travancorica* able to reverse the changes in altered neutrophils and lymphocytes count.^[30-31] Treatment with both doses of ethanol extract of *A. travancorica* brought back the Hb content, RBC and WBC count more or less to normal

levels significantly. This clearly indicates that ethanol extracts of *A. travancorica* possess protective action on the haemopoietic system.

Plant derived compounds have played an important role in the development of several clinical useful anticancer agents.^[32] Phytol, Tetrahydrospirilloxanthin and levo- α -Elemene were reported in the ethanol extract of *A. travancorica* whole plant by GC-MS analysis. These compounds may play a role in anticancer activity.^[33]

CONCLUSION

Table 1: Effect of ATW on relative organ weights of tumor induced (DAL) and drug treated mice

Treatment	Relative Organ Weight (g/100g body wt.)					
	Body weight (g)	Spleen	Thymus	Liver	Kidney	Lungs
Normal control(Group I)(Saline)	19.28±0.56	0.39±0.014	0.21±0.013	3.18±0.64	0.91±0.04	0.54±0.011
Tumor induced (GroupII) control(Saline)	41.64±1.93**	0.58±0.026*	0.39±0.026*	4.93±0.29ns	1.74±0.05*	0.87±0.053 ^{ns}
ATW extract (100 mg/kg)+(GroupIII) DAL	30.26±1.42 ^{aa}	0.38±0.023 ^a	0.29±0.012 ^a	3.92±0.20	1.21±0.04 ^{ns}	0.55±0.021 ^a
ATW extract(200mg/kg)+(GroupIV)DAL	18.91±0.65 ^{aa}	0.29±0.022 ^{aa}	0.24±0.037 ^a	3.24±0.25	0.89±0.03 ^{ns}	0.47±0.018 ^a
Vincristine (80 mg/kg)+ (GroupV)DAL	20.43±0.22 ^{aa}	0.28±0.019	0.23±0.014 ^a	3.81±0.18	0.98±0.07	0.54±0.027 ^a

Each Value is SEM of 6 animals Significance between normal control, tumor induced control vs drug treated group * P <0.05; ** P < 0.01; NS :Not significant : a P <0.05 ; aa P < 0.01- Significance between tumor induced control vs drug treated group

Table 2: Antitumor activity of ATW extracts on solid tumor volume in tumor(DAL)induced mice

Treatment	Solid Tumor Volume			
	15 th day	20 th day	25 th day	30 th day
Normal control(Group I)(Saline)	-	-	-	-
Tumor induced (Group II) control(Saline)	4.22±0.15	4.81±0.28	5.64±0.20	6.93±0.15
ALW extract (100mg/kg) + (Group III) DAL	3.24±0.21*	3.14±0.16**	4.36±0.18***	4.98±0.14***
ATW extract(200mg/kg)+(Group IV) DAL	2.96±0.34*	2.66±0.31**	2.41±0.17***	2.18±0.16***
Vincristine (80 mg/kg)+ (Group V) DAL	2.73±0.15**	2.57±0.16**	2.26±0.13***	2.07±0.24***

Each Value is SEM of 6 animals. Significance between tumor induced control vs drug treated group * P < 0.05 ;** P < 0.01 ;*** P < 0.001. NS :Not significant

The present study concluded that the ethanol extract has shown a remarkable anticancer activity against the experimental cells namely Dalton's Ascitic Lymphoma (DAL). This holds great promise for future research in human beings.

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Table 3: Antitumor activities of ATW on the survival time, life span, tumor volume and viable and non-viable cell count in tumor Induced mice

Treatment	Mean Survival time (Days)	Increase of life span (%)	Packed cell volume	Viable cell count X 10 ⁶ cells/ml	Non-viable tumor cells count X 10 ⁶ cells/ ml
Normal control(Group I)(Saline)	-	-	-	-	-
Tumor induced (Group II) control(Saline)	19.80±0.11	-	3.83±0.016	16.91±0.93	0.93±0.016
ATW extract (100mg/kg)+(Group III) DAL	28.14±0.21**	42.12	2.45±0.014**	9.28±0.18**	1.42±0.021**
ATW extract(200mg/kg)+(Group IV)DAL	35.65±0.47**	80.05	1.45±0.023**	6.19±0.25**	2.18±0.034**
Vincristine (80 mg/kg)+(Group V)DAL	34.90±0.55**	76.26	1.64±0.018**	6.26±0.13**	2.26±0.011**

Each Value is SEM of 6 animals Significance between tumor induced control vs drug treated group * P < 0.05 ;** P < 0.01 ; NS :Not significant

Table 4: Anticancer activity of ATW extracts on hematological parameters in tumor (DAL) bearing mice

Parameter	Hb (gm%)	RBC (million/mm ³)	WBC (10 ³ cells/mm ³)	Differential count		
				Lymphocytes	Neutrophils	Eosinophil
Normal control(Group I) (Saline)	11.96±0.12	3.13±0.27	9.16±0.88	52.12±2.11	43.16±1.13	4.72±0.14
Tumor induced (Group II) control (Saline)	8.12±0.16*	2.57±0.12*	13.86±0.36*	43.84±1.26	50.18±1.12*	5.98±0.26
ATW extract (100mg/kg)+(Group III) DAL	12.14±0.12 ^a	3.84±0.06 ^a	11.32±0.24 ^a	48.24±1.14	46.24±1.08	4.76±0.12
ATW extract (200mg/kg)+(Group IV) DAL	14.06±0.21 ^a	4.14±0.46 ^a	8.31±0.13 ^a	55.28±1.16	41.13±0.62	3.59±0.26
Vincristine (80 mg/kg)+(Group V) DAL	13.76±0.36 ^a	4.05±0.74 ^a	7.84±0.17 ^a	53.13±0.91	44.28±0.76	2.59±0.15 ^a

Each Value is SEM of 6 animals Significance between normal control, tumor induced control vs drug treated group * P < 0.05 ;** P < 0.01;

NS: Not significant: a P < 0.05 ; aa P < 0.01- Significance between tumor induced control vs drug treated group

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