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Der Pharmacia Lettre, 2010, 2(2): 10-15
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Antitumor activity of the ethanol extract of *Amaranthus spinosus* leaves against EAC bearing swiss albino mice

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Abstract

The present study was carried out to evaluate the antitumor potentials of Amaranthus spinosus against EAC bearing Swiss albino mice. The ethanol extract of its leaves given orally to mice at the dose of 100 and 200 mg/kg body weight for 16 days. It was observed that decrease in tumor volume and viable cell count, while increase in mean survival time and non viable tumor cell count, when compared to the mice of the EAC control group. Restoration of hematological and biochemical parameters towards normal was also observed. The results suggest that the ethanol extract of Amaranthus spinosus leaves exhibits significant antitumor effects in EAC bearing mice.

Key Words: Amaranthus spinosus, EAC, 5-Fluorouracil, tumor volume, viable cell count.

INTRODUCTION

For many centuries, plants have provided a rich source of therapeutic agents and bases for synthetic drugs. Despite the great development of organic synthesis, currently 25% of prescribed drugs worldwide are still derived from plant sources, showing that plant species are still an important source of new drugs for diseases that continue to lack a cure, such as cancer[1].

Amaranthus spinosus (Family Amaranthaceae) known in Hindi as Cauleyi or Kateli, is an erect, spinous, annual or perennial herb found through out India. The plant is a constituent of an herbal

drug LEUCOSOL-H which is found to be effective in Leucorrhoea. The grain was found to be a nutritious pseudo cereal, yielding high energy, starch and protein. It can be utilized in the development of acceptable value added products with high nutritive value. The leaves contain high amount of Oxalic acid, 1161.4 mg/100 g. In ancient and medieval India, a paste of the roots prepared in rice-wash was used by women for sterilization after menstruation. The seed oil contains a relatively high concentration (2.4-8.0%) of squalene. The oil also contains relatively high concentration of tocotrienols, a rare form of Vitamin E, which is reported to inhibit 3-hydroxy-3-methyl glutaryl coenzyme A reductase, the key regulatory enzyme in cholesterol biosynthesis. In folk medicine, this plant is used for the treatment of a variety of diseases such as malaria, hepatic disorders, fever, inflammation, leprosy, eczema, leucorrhoea and bronchitis.

In the present study we have investigated the antitumor effects of ethanol extract of *Amaranthus spinosus* leaves against EAC bearing Swiss albino mice[3-4].

MATERIAL AND METHODS

Plant materials

The leaves of *Amaranthus spinosus* was collected from Dharmapuri, Tamil Nadu in the month of June. The collected material was authenticated by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai. Dried powder of leaves was exhaustively extracted in soxhlet apparatus, using ethanol. Solution of ethanol extract was prepared and was divided in two doses 100 mg/kg and 200 mg/ kg and subjected for antitumor activity.

Experimental animals

Swiss albino mice having weight 180-230gm were kept in quarantine for 10 days under standard husbandry conditions (27.3^{o}C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad. libitum*. The study was permitted by the Institution Animal Ethical Committee with Reg. No. CPCSEA/1230/a.

Acute oral toxicity study

Acute oral toxicity was performed by following OECD guideline – 420 fixed dose procedure for ethanol extract and it was found that dose increasing up to 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The LD_{50} of the ethanol bark extract as per OECD guidelines – 420 is greater then 2000 mg/kg[5,6].

Tumor Cells

Ehrlich ascites carcinoma (EAC) cells were obtained from Amala Cancer Research Institute, Thirussur, India. The EAC cells were maintained *in vivo* in Swiss albino mice, by intraperitoneal (i.p.) transplantation of 1×10^{6} cells/mouse after every 10 days.

Experimental Protocol

Swiss albino mice were divided in to four groups of sixteen animals (n=16) each. The ethanol extract was dissolved in isotonic saline (0.9% NaCl w/v) solution and used directly in the assay. EAC cells were collected from the donor mice and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted

at 10^6 cells/mL. 0.1 mL of EAC cells per 10g body weight of the animals was injected (i.p.) to whole animals on day zero. A day of incubation was allowed for multiplication of the cells. Group I served as EAC control mice administered 0.9% w/v sodium chloride solution for 16 days. Group II EAC mice administered orally ethanol extract (100 mg/kg). Group III EAC mice administered orally ethanol extract (200 mg/kg). The extract was administered for 16 days. Group IV EAC mice administered standard drug 5-Fluorouracil (20 mg/kg body weight) were injected i.p. for 16 days. On day 21, six animals in each cage were sacrificed and the remaining animals were kept to observe the life span of the hosts.

The antitumor activity of the ethanol extract was measured in EAC animals with respect to the following parameters:

(a) 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 and packed cell volume was determined by centrifuging at 1000 rpm for 5 min.

(b) Viable/non-viable tumor cell count: 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 nonviable. These viable and nonviable cells were counted.

$$\text{Cell Count} = \frac{\text{No. of Cell X Dilution}}{\text{Area X Thickness of liquid film}}$$

(c) Percentage increase life span. The effect of extract on tumor growth was monitored by recording the mortality daily for a period of 6 weeks and percentage increase in life span (%ILS) was calculated.

Hematological Parameters

At the end of the experimental period, all mice were by cervical dislocation. Blood was collected from freely flowing tail vein and used for the estimation Hemoglobin (Hb) content, red blood cell count (RBC) and white blood cell count (WBC). WBC differential count was carried out from Leishman stained blood smears[7-11].

Statistical analysis

Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Sigma stat 3.5). P values <0.001 were considered statistically significant.

Table 1 Antitumor activity of ethanol extract of *A. spinosus* leaves on tumor volume, Viable tumor cells count and Non viable tumor cells count

Treatment	Tumor volume (ml)	Viable tumor cells count (10^6 cells/mouse)	Non viable tumor cells count (10^6 cells/mouse)
EAC Control	4.9±0.91	10.2±0.31	0.4±0.03
EAC+Ethanol Extract (100 mg/kg)	3.8±0.83	7.3±0.03	0.7±0.05
EAC+Ethanol Extract (200 mg/kg)	2.8±0.53*	4.8±0.09*	0.5±0.03
EAC+5FU 20 mg/kg	1.9±0.48*	3.1±0.17*	0.9±0.01

*Values are expressed as mean ± SEM, n = 6 in each group. *P<0.001 compared to EAC control group*

Table 2 Antitumor activity of ethanol extract of *A. spinosus* leaves on median survival time and percentage increase in life span

Treatment	Median Survival time (days)	Percentage Increase in Life Span
EAC Control	23.2±1.32	100
EAC+Ethanol Extract (100 mg/kg)	33.8±2.5	143
EAC+Ethanol Extract (200 mg/kg)	40.3±2.2	175
EAC+5FU 20 mg/kg	48.4±1.5	209

Values are expressed as mean ± SEM, n = 10 in each group.

Table 3 Antitumor activity of ethanol extract of *A. spinosus* leaves on hematological parameters

Treatment	Hb content	Total RBC cells/ml×10^6	Total WBC cells/ml×10^6	Differential count		
				Lymphocyte (%)	Neutrophils (%)	Momnocytes (%)
EAC Control	13.2±0.9	1.34±0.2	15±1.0	24.0±8.9	73.0±9.8	3±0.9
EAC+Ethanol Extract (100 mg/kg)	14.8±0.6	1.39±1.2	10.3±0.3	42±5.2	51±4.6	3±0.5
EAC+Ethanol Extract (200 mg/kg)	16.4±1*	1.45±0.2*	6.9±1.0*	62±4.6	33±2.1	2±0.8
EAC+5FU 20 mg/kg	16.3±0.7*	1.40±0.09*	6.7±0.5*	68±3.0	30±2.0	2±0.1*

*Values are expressed as mean ± SEM, n = 6 in each group. *P<0.001 compared to EAC control group.*

RESULTS AND DISCUSSION

From table 1, it was revealed that administration of ethanol extract of leaves as well as the standard drug 5-Fluorouracil exhibited an ability to reduction significantly in tumor volume and viable cell count, while increase significantly in non viable tumor cell count, compared to EAC control group. From table 2 it was found that the median survival time and percentage of life span in mice was increased in extract treated group.

From table 3, it revealed that hematological parameters of tumor bearing mice on day 16 were found to be significantly differences compared to the extract treated group. In tumor bearing mice it was found that increased in WBC count and decreased in HB content with RBC count. In differential count of WBC, the percent of neutrophils and momnocytes increased while the lymphocyte count decreased. The extract treated group at dose of 100 and 200 mg/kg body weight restored all the altered hematological parameters to almost near normal. All these results suggest the anticancer nature of the extract and among the two doses, the higher dose of 200 mg/kg body weight was found to be more potent than 100 mg/kg body weight. However, the standard 5-FU at the dose of 20 mg/kg body weight produced better results in all these parameters.

The present investigation was carried out to evaluate the antitumor activity of the ethanol extract of *Amaranthus spinosus* leaves in EAC bearing mice. The reliable criteria for judging the value of an anticancer drug is the prolongation of the life span and median survival time of animals. In tumor bearing mice, 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 The EAC bearing mice orally administered ethanol extract of *A. spinosus* leaves at 100 or 200 mg/kg body weight showed no significant change in the average life span compared to animals of the tumor control group. However, increase in packed tumor cell volume, and number of viable tumor cells were found to be significantly less than the tumor control animals, mean while increase significantly in non viable tumor cell count, indicating the anticancer nature of the extract. It may be concluded that ethanol extract by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC bearing mice.

The reversal of Hb content, RBC, WBC and differential count of WBC by the ethanol extract treatment towards the normal values clearly indicates that ethanol extract of *A. spinosus* leaves possessed protective action on the haemopoietic system.

In conclusion, the present study demonstrates that the ethanol extract of *A. spinosus* leaves increased the life span of EAC tumor bearing mice and was effective in inhibiting the tumor growth in ascitic tumor models. All these parameters suggest that the ethanol extract of *A. spinosus* leaves exhibits potential antitumor activities.

CONCLUSION

The present study revealed that the ethanol extracts of both doses possess potent antitumor effect against EAC bearing mice.

Acknowledgements
The authors are thankful to Prof. A. Jaswanth, Principal, Srikrupa Institute of pharmaceutical sciences, Medak, Andhra Pradesh for providing the entire lab facilities timely and guidance for carrying out pharmacological studies.

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