ISSN: 2231 3788 (Print)
Journal of PharmaSciTech
2012; 2(1):31-33
Research Article

Studies on Antitumor Activity of *Bryophyllum calycinum* Salisb. against Ehrlich Ascites Carcinoma in Swiss Albino Mice

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Abstract

The aim of the present study is to evaluate the antitumor effect of *Bryophyllum calycinum* Salisb. (Family: Crassulaceae) against Ehrlich Ascites Carcinoma (EAC) bearing Swiss albino mice. The effect of methanol and aqueous extracts of *Bryophyllum calycinum* Salisb. on tumor growth was studied by the following parameters: percentage inhibition of ascetic cells and percentage inhibition of tumor weight. Methanol and aqueous extracts were administered at doses of 100,200 and 400 mg/kg body weight intraperitonially once a day for 7 days, after 24h of tumor inoculation. Decrease in tumor cell count and tumor weight were observed in extract treated animals when compared to EAC treated animals. The results were dose dependent and promising in case of methanol extract. The results suggest that both the extracts of the stem of Bryophyllum calycinum Salisb. exhibit significant antitumor effects in EAC bearing mice.

***Keywords*:** *Bryophyllum calycinum* Salisb., Ehrlich Ascites Carcinoma (EAC), Antitumor activity.

Introduction

The plant *Bryophyllum calycinum* Salisb. (Crassulaceae) is a shrub mainly found in the tropical parts of Bengal and in southern Africa and American continents. It is locally known as "patharkuchi' and has long been used in Ayurvedic medicine. As per traditional use, the leaves and leaf juice are used as antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumorous, hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, antidiabetic, astringent, antiseptic, antilithic and cough suppressant.[1-9] Some activities of some plants of this genus have been reported earlier.[10-12]

Materials and methods

Plant material

The plant was identified (Ref. No. CNH/I-I(53)/2004-Tech-I/885) by the taxonomists of Botanical Survey of India, Shibpur, Howrah. After authentication, the fresh leaves were collected in bulk from young matured plants at the rural areas of Howrah during August-September 2005 and washed, shade dried and milled into coarse powder by a mechanical grinder. The powder was passed through sieve number 40 (B.P standard) and used for further studies.

Preparation of extracts

The powdered plant material (1000g) was extracted with 850 ml of redistilled petroleum ether (40-60°C) followed by redistilled methanol at 40°C for 72 h by hot continuous extraction method. The solvent was evaporated under reduced pressure at 50oC and dried in vacuum (Yield of methanol extract: 5.7% w/w on dried plant material basis). The aqueous extract was then prepared by decoction process using double distilled water , filtered, evaporated and dried under reduced pressure (yield: 21% w/w on dried plant material basis). The dried extracts thus obtained were dissolved in phosphate buffer (pH 7.2) solution and used directly for the assessment of antitumor activity.

Ethical clearance

Protocol used in this study for the use of mice as an animal model for cancer was approved by the University Animal Ethical Committee.

Experimental animals

Female Swiss albino mice of about 10 weeks of age with an average body weight of 18-20g were used for the experiment. The animals were bred and brought up in our laboratory facility with 12 h cycles of light and dark at 24°C. They were kept on basal metabolic diet with water ad libitum.

Tumor cells

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Chittaranjan National Cancer Institute, Kolkata, India. The EAC

cells were maintained *in vivo* in Swiss albino mice by intraperitoneal (i.p.) transplantation of 2 x 10^6 cells/mouse after every 10 days. EAC cells of 9 days old were used for the screening of the extracts.

Experimental protocol

Standard experimental protocol was followed for the evaluation of antitumor activity of the extracts.[13-15] Female Swiss albino mice were divided into five groups of five animals each. The methanol and aqueous extracts were dissolved in phosphate buffer solution and used directly in the assay. EAC cells were collected from donor mouse and were suspended in sterile isotonic saline. The viable EAC cells were counted (Trypan blue indicator) under the microscope and were adjusted at 10 x 10^6 cells/ml. 0.1ml of EAC cells per 10g body weight of the animals was injected (i.p.) on day zero. A day of incubation was allowed for multiplication of the cells. Seven doses of the extracts (100mg, 200mg and 400mg/kg, 0.1ml per 10g body weight) and mitomycin-C (1mg/kg) were injected i.p. from the first day up to the seventh day with 24 h intervals. Control animals received only vehicle (phosphate buffer solution: pH 7.2). Food and water were withheld 6 h before sacrificing the animals. On day 8, all the animals were sacrificed. Some amount of the fluid of the peritoneal cavity was kept aside for counting of cells and rest of the fluid was wiped off with absorbent cotton. Weight of the animals was taken before sacrificing and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor weight. Mitomycin-C at a dose level of 1mg/kg body weight was used as standard, which showed 100% inhibition at all times which is shown in the table.

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

Tumor cell count

The ascetic fluid was collected in a graduated centrifuge tube and diluted 100 times with sterile isotonic saline solution. Then the diluted fluid was taken in a WBC pipette and diluted 100 times again with sterile isotonic saline. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the number of cells in the 64 small squares was counted. Then the percentage inhibition of ascetic cells was calculated as (1-T/C) x 100, where C, T were the average number of ascetic cells per ml of fluid in the control and test groups respectively.

Tumor weight

The mice were dissected and some amount of the ascetic fluid was collected from the peritoneal cavity. The rest of the fluid was wiped off with absorbent cotton. The weight of animals was taken before sacrificing and after removing the fluid from the peritoneal cavity. The difference in weight was considered as tumor weight. Then the percentage inhibition of tumor weight was calculated as (1- T / C) x 100, where C, T were the average tumor weights of control and test groups respectively.

Statistical analysis

The experimental results were expressed as mean ± SEM (standard error of mean).Data was assessed by the Student's t-Test, p- value < 0.05 was considered as statistically significant.

Results and Discussion

The result of the above study is shown in the following table. Antitumor activity of the extracts against EAC tumor bearing mice was assessed by the parameters such as tumor cell count and tumor weight. Methanol extract of the plant at a dose of 200 and 400 mg/kg showed promising result in both percentage inhibition of ascetic cells (54.54 and 59.74, respectively) and percentage inhibition of tumor weight (32.57 and 58.37, respectively). Aqueous extract at a dose of 400 mg/kg also showed good antitumor activity. Other doses of methanol and aqueous extracts showed some activity. The results clearly indicate that the extracts of the plant *Bryophyllum calycinum* Salisb. has a capacity to inhibit the growth of tumor induced by EAC cell line in a dose dependent manner in experimental animals.

Conclusion

The present investigation was carried out to evaluate the antitumor activity of methanol and aqueous extracts of the plant *Bryophyllum calycinum* Salisb. in EAC tumor bearing mice. The extracts at the doses of 200 and 400 mg/kg significantly inhibited the tumor cell count and tumor weight.

In case of control group, a regular rapid increase in ascetic tumor volume was observed. Ascitic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells.[16] Treatment with extracts inhibited the tumor cell count and tumor weight. It may be concluded that the methanol and aqueous extracts of the plant decreases the nutritional fluid volume and arresting the tumor growth. Thus the extracts possess antitumor activity against EAC bearing mice.

Acknowledgement

The authors are thankful to the authority of Jadavpur University, Kolkata-700032 for providing all necessary facilities required to conduct the present study.

Table 1. Antitumor activity of the methanol and aqueous extracts of Bryophyllum calycinum Salisb.

Treatment	Dose (mg/kg)	No. of animals in each group	Avg. no. of ascitic cells/ml in control gr. 'C' ($x10^6$ cells/ml)	Avg. no. of ascitic cells/ml in test gr. 'T' ($x10^6$ cells/ml)	% inhibition of ascitic cells (1-T/C) x100	Avg. weight of ascitic fluid in control gr. ' (g)'C	Avg. weight of ascitic fluid in test gr. ''T (g)	% inhibition of ascitic cells (1- T /C) x 100
Methanol extract	100	5	192.5±0.88	127.5±0.79	33.76	2.21±0.56	1.74± 0.24	21.25
	200	5		95.0± 0.55	50.64		1.49± 0.37	32.57
	400	5		85.0± 0.78	55.84		0.92± 0.18	58.37
Aqueous extract	100	5	192.5±0.88	165.0± 0.82	14.28	2.21±0.56	1.94± 0.39	12.21
	200	5		152.5± 0.96	20.77		1.85± 0.45	16.28
	400	5		140.0± 0.60	27.27		1.78± 0.38	19.45
Mytomycin -C	1	5	192.5±0.88	0.00	100.0	2.21±0.56	0.00	100.0

Counts are average of 5 animals ± SEM, P< 0.01 (Student's't' Test).

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