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Evaluation of Antitumor Effects of the Aerial Parts of *Polygonum viscosum* Linn.

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Abstract: The present study is aimed to evaluate the methanolic fractions of the aerial parts of *Polygonum viscosum* (MAPV) for antitumor activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Twenty four hours after intraperitoneal inoculation of tumor (EAC) cells in mice, MAPV was administered at 50, 100 and 200 mg/kg body weight for nine consecutive days. On day 10 half of the mice were sacrificed and rest were kept alive for assessment of increase in life span. The antitumor effect of MAPV was assessed by evaluating tumor volume, packed cell count, viable and non-viable tumor cell count, median survival time and increase in life span of EAC bearing mice. Hematological profiles and serum biochemical parameters were estimated. MAPV showed a significant (p<0.05) decrease in tumor volume, packed cell volume and viable cell count and increased the life span of EAC bearing mice. Hematological and serum biochemical profiles were restored to normal levels in MAPV treated mice as compared to EAC control mice. The present study demonstrates that ethylacetate fraction of *Polygonum viscosum* leaves exhibited remarkable antitumor activity that is plausibly attributable to its augmenting endogenous antioxidant mechanisms.

Key words: Antitumor · Antioxidant · Ehrlich Ascites Carcinoma · *Polygonum viscosum*

INTRODUCTION

Polygonum viscosum Linn. is an important medicinal plant belongs to the family of polygonaceae. The genus Polygonum is well known for producing a number of pharmacologically active compounds and also for its use in oriental traditional medicine [1]. Antiinflammatory activity of the aqueous ethanolic extract of *Polygonum bistorta* [2] and antipyretic and antiinflammatory activities of the aqueous and the hydroalcoholic extracts of *Polygonum punctatum* [3] have been reported. Compounds having antiinflammatory and antiallergic activities and tumour cell growth inhibitory activity have previously been isolated, respectively, from *Polygonum chinensis* [4] and Polygonum hypoleucum [5]. *Polygonum viscosum* is used for the treatment of wide range diseases including diarrhea, dyspepsia, itching skin and excessive menstrual bleeding etc. [6]. It possesses stimulant, tonic, diuretics, anthelmintic and carminative properties. *P. viscosum* also have remarkable antibacterial, antifungal properties [7]. The whole plant has been found to contain flavones and flavonoids glycosides such as quercetin galactosides, a sesquiterpene acid, viscosumic acid, oxymethyl anthraquinones and polygonic acid [8]. From the aerial part, many drimane-type sesquiterpenes such as viscozulenic acid, viscoazucine and viscoazulone were isolated which possesses anti-cholinergic, cytotoxic and anti-HIV-1 activities [9]. However, no studies to date have been conducted to demonstrate the antitumor activity of *Polygonum viscosum*. The present study was carried out to evaluate the *in vivo* antitumor activity of the methanolic extract of *Polygonum viscosum* leaves (MAPV) against Ehrlich ascites carcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant Materials: *Polygonum viscosum* plants were collected from the adjoining area of Jahangirnagar University Campus, Bangladesh during August 2012. The plant material was taxonomically identified by the National Herbarium of Bangladesh whose voucher specimen no. DACB-3324 is maintained in our laboratory for future reference.

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Chemicals and Reagents: Bovine serum albumin and Bleomycin from Sigma (St. Louis, MO); trichloroacetic acid (TCA) from Merck (Mumbai, India); thiobarbituric acid (TBA), nitroblue tetrazolium chloride (NBT) from Loba Chemie (Mumbai, India); 5,5'-dithio *bis*-2-nitro benzoic acid (DTNB), phenazonium methosulfate (PMS), nicotinamide adenine dinucleotide (NADH) and reduced glutathione (GSH) from SISCO (Mumbai, India). All other chemicals and reagents used were of highest analytical grade.

Preparation of Plant Extract: The plant material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in an air tight container. The dried powder material (1.0 kg) was refluxed with MeOH for three hours. The total filtrate was concentrated to dryness, *in vacuo* at 40°C to render the MeOH extract (60 g).

Animals: Albino mice (25-30g) were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum.* The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. Animal treatment and maintenance for acute toxicity and anticancer effects were conducted in accordance with the Principle of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and the Animal Care and Use Guidelines of Atish Dipankar University of Science & Technology, Dhaka, Bangladesh.

Acute Toxicity Study: Acute oral toxicity assay was performed in healthy nulliparous and non pregnant adult female albino Swiss mice (25-30g) divided into different groups. The test was performed using increasing oral dose of the MAPV in water (50, 100, 200, 500, 1000 mg/kg body weight), in 20 ml/kg volume to different test groups. Normal group received water. The mice were allowed to feed *ad libitum,* kept under regular observation for 48 hrs, for any mortality or behavioral changes [10].

Transplantation of Tumor: Ehrlich ascites carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascetic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment Schedule: The animals were divided into five groups (n = 12) and given food and water *ad libitum*. All the animals in each group except Group-I received EAC cells (2×10^6 cells/mouse i.p.). This was taken as day '0'. Group-I served as normal saline control (5 ml/kg body weight i.p.) and Group-II served as EAC control. 24-h after EAC transplantation, Group-III, IV and V received MAPV at a dose of 50, 100 and 200 mg/kg i.p. respectively and Group-VI received reference drug Bleomycin (0.3 mg/kg i.p) for nine consecutive days [11]. Twenty-four hours of last dose and 18 hrs of fasting, 6 animals of each group were sacrificed by cardiac puncture for the estimation of hematological and serum biochemical parameters and then sacrificed by cervical dislocation to measure antitumor and liver biochemical parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host. The antitumor activity of MAPV was measured in EAC animals with respect to the following parameters.

Determination of Tumor and Packed Cell Volume: The mice were dissected and the ascetic fluid was collected from the peritoneal cavity. The volume was measured using a graduated centrifuge tube and packed cell volume was determined by centrifuging at 1000 g for 5 min.

Viable and Nonviable Tumor Cell Count: The ascetic fluid was taken in a white blood cell (WBC) pipette and diluted 100 times. Then a drop of the diluted suspension was paled on the Neubauer counting chamber and 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 took the stain were non-viable. These viable and non-viable cells were counted.

Cell count = (Number of cells × dilution factor)/ (Area × thickness of liquid film)

Determination of Median Survival Time and Percentage Increase in Life Span: The mortality was monitored by recording percentage increase in life span (% ILS) and median survival time (MST) as per the following formula [12].

$$\% \text{ ILS} = \left(\frac{\text{Mean survival time of the treated group}}{\text{Mean survival time of the control group}} \right) \text{-1x } 100$$

Mean survival time* = (Day of First death + Day of last death)/2

(*Time denoted by number of days)

Estimation of Hematological and Serum Biochemical Parameters: Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count [13]. Serum biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT); serum glutamate pyruvate transaminase (SGPT) [14], serum alkaline phosphatase (SALP), were also estimated.

Statistical Analysis: All values were expressed as the mean ± SEM of three replicate experiments. The analysis was performed by using SPSS statistical package for WINDOWS (version 16.0; SPSS Inc, Chicago). All *in vivo* data are subjected to ANOVA followed by Dunnett's test and $p<0.05$ were considered to be statistically significant.

RESULTS

The acute toxicity studies mainly aims at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. MAPV was safe up to a dose of 1000 mg/kg (p.o.) body weight. Behavior of the animals was closely observed for the first 3hrs then at an interval of every 4hrs during the next 48hrs.The extract did not cause mortality in mice and rats during 48hrs observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Table 1: Effect of MAPV on tumor volume, packed cell volume, mean survival time (MST), percentage increase life span (% ILS), viable and non-viable tumor cell count in EAC bearing mice

	EAC control ($2x10^6$ cell/mouse)	EAC+MAPV (50 mg/kg)	EAC+ MAPV (100 mg/kg)	EAC+ MAPV (200 mg/kg)	Bleomycin (0.3 mg/kg)
Tumor volume (ml)	5.34 ± 0.21	2.97 ± 0.11*	1.88 ± 0.25*	1.51 ± 0.18*	0.62± 0.21*
Packed cell volume (ml)	3.29 ± 0.24	1.99 ± 0.29*	1.14 ± 0.14*	0.82 ± 0.19*	-
MST (days)	20.17 ± 0.12	22.41 ± 0.11	30.09 ± 0.17*	35.22 ± 0.11*	38.50± 0.11*
% ILS	-	11.10	49.18	74.61	90.87
Viable cell (x 10^6 cell/ml)	10.21 ± 0.22	5.41 ± 0.12*	3.21 ± 0.02*	1.01 ± 0.22*	0.52 ± 0.15*
Non-viable cell (x 10^6 cell/ml)	0.84 ± 0.24	2.82 ± 0.74*	2.02 ± 0.24*	3.98 ± 0.64*	3.32 ± 0.05*
Total cell (x 10^6 cell/ml)	11.05 ± 0.46	8.23 ± 0.86	5.23 ± 0.26	4.99 ± 0.86	3.84 ± 0.15
Viable %	92. 39	65.73	61. 37	20.24	13.14
Non-viable %	7.61	34.27	38.63	79.76	86.86

Each point represent the mean ± SEM. (n = 6 mice per group), *$p<0.05$ statistically significant when compared with EAC control group.

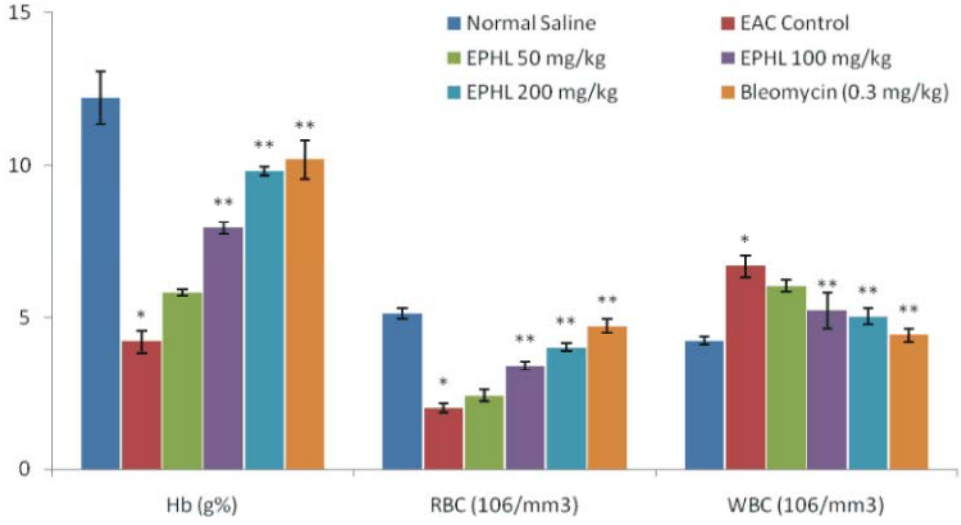

Fig. 1: Effect of MAPV on hematological parameters in EAC bearing mice. Each point represent the mean ± SEM. (n = 6 mice per group), *$p<0.05$ statistically significant when compared with normal saline group.**$p<0.05$ statistically significant when compared with EAC control group

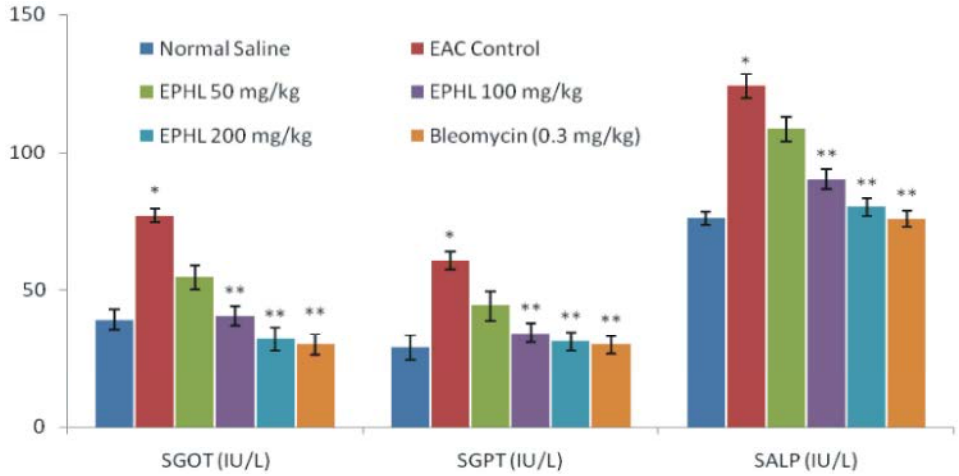

Fig. 2: Effect of MAPV on serum biochemical parameters in EAC bearing mice. Each point represent the mean ± SEM. (n = 6 mice per group), $^{*}p<0.05$ statistically significant when compared with normal saline group.* $p^{*}<0.05$ statistically significant when compared with EAC control group

MAPV at 50, 100 and 200 mg/kg body weight significantly ($p<0.05$) reduced the body weight, tumor volume, packed cell volume and viable tumor cell count whereas increased non-viable tumor cell count in a dose dependent manner as compared to EAC control group. Furthermore, the median survival time was increase to 22.41 ± 0.11 (% ILS = 11.10), 30.09 ± 0.17 (% ILS = 49.18) and 35.22 ± 0.11 (% ILS = 74.61) on administration of MAPV at a dose of 50, 100 and 200 mg/kg body weight respectively. The reference drug bleomycin showed 38.50±0.11 (% ILS = 90.87).

Hematological parameters (Figure 1) of tumor bearing mice were found to be significantly altered compared to the normal group. The total WBC count was found to be increased ($p<0.05$) with a reduction of Hb content and RBC count in EAC control animals when compared with the normal saline group. Treatment with MAPV at 50, 100 and 200 mg/kg body weight significantly ($p<0.05$) increased Hb content and RBC count towards the normal levels. In the differential count, lymphocytes and monocytes were found to be decreased and the neutrophils were increased in the EAC control group when compared with the normal group. MAPV treatment at different doses significantly ($p<0.05$) changes these altered parameters approximately to the normal values.

Figure 2 demonstrated that the biochemical parameters like SGOT, SGPT and SALP in the EAC control group were significantly ($p<0.05$) increased as compared to the normal group. Treatment with MAPV at doses of 50, 100 and 200 mg/kg significantly decreased ($p<0.05$) the SGOT, SGPT and SALP to approximately normal levels in a dose dependent manner.

DISCUSSION

In EAC tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. A rapid increase in ascetic fluid, the direct nutritional source for tumor cells, with tumor growth would be a means to meet the nutritional requirement of tumor cells [15]. Treatment with MAPV reduced the intraperitonial tumor burden, thereby reducing the tumor volume, tumor weight, viable tumor cell count and increased the life span of the tumor bearing mice. Prolongation of life span of animals is the steadfast criteria for judging the potency of any anticancer drug [16]. It can therefore be inferred that MAPV increased the life span of EAC bearing mice may be due to decrease the nutritional fluid volume and delay the cell division [17]. Anemia and myelosuppression have been frequently observed in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number [18]. Treatment with MAPV brought back the hemoglobin content, RBC and WBC count more or less to normal levels, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Preliminary phytochemical study indicated the presence of steroids, tannins, phenolic and flavonoid compounds and glycosides in crude extract of *Polygonum viscosum*. A number of scientific reports indicate certain steroids and phenolic compounds such as tannins, caumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis [19].

Furthermore, flavonoids such as quercetin, kaemferol and their glycosides have been shown to possess antimutagenic and antimalignant effect [20]. They also have been shown to enhance *in vitro* human peripheral blood lymphocyte and T-cell proliferation, which suggests a possible stimulation of the immune system function [21]. The anticancer activities of MAPV are probably due to the presence of phenolic compounds, as well as flavonoids and their synergistic effects.

Plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells [22] and antitumor activity in experimental animals [23]. The cytotoxic and antitumor activity of plant derived product is either through induction of apoptosis or inhibition of neovascularization [24]. In the present study it was noted that MAPV significantly reduced tumor growth and viability of tumor cells and normalized the hematological and serum biochemical profiles, raising life span as compared with those of EAC control mice.

It can be concluded that the methanolic extract of the aerial part of *Polygonum viscosum* demonstrated remarkable antitumor activity against Ehrlich ascites carcinoma in mice. Now our next aim is to explore the isolation and characterization of lead compound liable for aforementioned activity from this plant.

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