

Anthelmintic and Antiproliferative activity of aerial parts of *Persicaria hydropiper*

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ABSTRACT

A study was conducted to investigate aerial parts of *Persicaria hydropiper* (*P. hydropiper*) as an effective remedy for gastrointestinal helminthes and proliferative disease. *In vitro* anthelmintic activity was carried out against *Pheretima posthuma*. The parameters like: time of paralysis and time of death were determined by using the extract at the concentrations of 10, 25 and 50 mg/ml. The extract exhibited significant anthelmintic activity at highest concentration of 50 mg/ml as compared with piperazine citrate (10 mg/ml) as standard reference and distilled water as control. *In vivo* antiproliferative activity of the same extract at the dose of 30, 40 and 50 mg/kg/day (i.p.) were evaluated against Ehrlich Ascites Carcinoma (EAC) cells in Swiss albino mice. Experimental parameters like tumor cell count, mean survival time and life span enhancement were evaluated to assess antiproliferative activity. We found that the extract at the dose of 50 mg/kg/day (i.p) significantly ($P < 0.05$) decreases tumor weight, increases life span and reduces tumor cell growth rate in comparison to those of EAC bearing mice receiving no extract (negative control) in a dose-dependant manner.

Key words: Anthelmintic, Antiproliferative activity, *Persicaria hydropiper*, EAC cell, *Pheretima posthuma*.

INTRODUCTION

Helminth infections in human are one of the most common parasitic infections disturbing a huge population around the world. During the past decade there have been major efforts to plan, implement, and sustain measures for reducing the burden of human disease that accompanies helminth infections [1]. A wide variety of anthelmintics are used for the treatment of helminths in animals and in human. However, majority of gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs. Moreover synthetic drugs used in helminthiasis treatment have some potential side effects. Hence there is an increasing demand towards natural anthelmintics [2-4]. One the other hand cancer is the second leading cause of death in developed countries after cardiovascular diseases [5]. Three basic strategies are used in treatment of cancer: surgery, radiotherapy and chemotherapy, which may be

employed alone or in combination with the other two methods. About 65% of drug used in chemotherapy are of natural origin [6]. Although chemotherapy is effective in preventing cancer at a very early stage, the side effects and resistance toward drugs are a problem. Hence new drug(s) or treatment(s) is needed.

Bangladesh has an immense wealth of plant species, both endemic and non-endemic. *Persicaria hydropiper* (Family: Polygonaceae) is an important medicinal plant grown everywhere in Bangladesh. Previous phytochemical screening with Polygonaceae family members have led to the isolation of variety of flavonoids [7] terpenoids [8, 9] anthraquinones [10] apianen lactones [11] and steroids [12]. A number of bioactive constituents have also been reported from various *Persicaria* (Syn: Polugonum) species with anticancer [13], antioxidant [14], antileukaemic [15], antimicrobial [16] and tyrosinase-inhibitory activities [17]. *P.hydropiper* whole plant possesses bitter, stimulant, tonic, diuretic, carminative, emmeragogue, haemostatic and lithotripter properties [18]. The plant, either on its own or mixed with other herbs, is decocted and used in the treatment of a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and hemorrhoids [19]. Plant root is used as anthelmintic in traditional Chinese folk medicine [20]. The aim of our work was to carry out a detailed phytochemical investigation of the aerial part of *P. hydropiper* to justify its folkloric use as a potent anthelmintic and antiproliferative agents.

MATERIALS AND METHODS

Cell lines and Chemicals

Ehrlich Ascites Carcinoma (EAC) cells were obtained by the courtesy of the Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh and were maintained by weekly intraperitoneal (i.p.) inoculation of 10^5 cells/mouse in the laboratory. Piperazine Citrate was purchased from GlaxoSmithKline (BD) Limited. Unless stated otherwise, all other reagents were from Sigma Chemicals limited.

Worm Collection and Authentication

Earthworm's *pheretima posthuma* (Annelida) were collected from moist soil at Jahangirnagar University, Savar, Dhaka and washed with normal saline to remove soil and fecal matter. Earthworms were identified by Zoology Dept. Jahangirnagar University. The earthworms of 4-6 cm in length and 0.2-0.3 cm in width were used for the experimental protocol.

Animals

White albino male mice (Swiss-webstar strain, 20-25 g body weight) were collected from the animal research branch of Pharmacy Department, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. The animals were provided with standard laboratory food and tap water ad libitum and maintained at natural day night cycle. The animals were acclimatized to laboratory condition for one week prior to experimentation

Ethical clearance

Protocol used in this study for the use of mice as animal model for cancer research was approved by the Rajshahi University Animal Ethical committee (27/08/RUBCMB).

This research work was approved by Ethical Review Committee of Research cell of Rajshahi Medical College, Bangladesh (ref. RMC/ER/2010-2013/01).

Preparation of plant Extract

The dried aerial parts were coarsely powdered and about 1000 g of powdered material was macerated with 99% methanol at room temperature for a period of 7 days accompanying occasional shaking and stirring. The whole mixture was then filtered and the filtrate thus obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was then kept at room temperature under a ceiling fan to get a dried extract (about 10%). The extract thus prepared was used for study.

Preparation of test sample

Sample for anthelmintic assay was prepared by dissolving extract in Saline water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25, 50 mg/ml extract solutions.

Anthelmintic assay

The anthelmintic activity was evaluated according to the method of Ajayieoba E. O. et al. [21] with minor modifications. In this study adult Earthworm (*Pheretima posthuma*) was used due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human being [22]. Three groups of equal sized earthworms consisting of six earthworms in each group were released in 50ml of sample with desired concentrations, (10, 25 and 50 mg/ml). Group of earthworms in saline solution was used as Control group and Group of earthworms in Piperazine citrate (10mg/ml) was used as Reference. Observations were made for the time taken to paralysis and death of individual worms. Paralysis as said to occur when no movement of any sort could be observed except the worms was shaken vigorously. Time for death of worms was recorded after death and was conformed when the worms neither moved when shaken vigorously nor when dipped in warm water at 50 °C.

Acute toxicity study (LD₅₀)

The LD₅₀ value was determined following conventional methods (Litchfield JT et al, 1949). The test compound was dissolved in distilled water and injected intraperitoneally to six groups of mice (each group containing 5 mice) at different doses (20, 50, 100, 200, 400 and 600 mg/kg). LD₅₀ was evaluated by recording mortality after 24 hours.

Evaluation of Anticancer potentiality

Anticancer potentiality of methanol extract of the aerial parts of *P.hydro Piper* was evaluated by measuring tumor cell growth inhibition, regression of tumor size and increase of survival time.

EAC Cell growth inhibition

In vivo tumor cell growth inhibition was carried out by the method as described by Sur et al. [23]. For this study, 5 groups of mice (5 in each group) were used. For therapeutic evaluation 14 × 10⁵ cells/mouse were inoculated into each group of mice on the first day. Treatment was started after 24 hours of tumor inoculation and continued for 5 days. Group 1 to 3 received the test compound (effective dose selected on the basis of 1/10 of LD₅₀ value) at the doses of 30 mg/kg (i.p.), 40 mg/kg (i.p.) and 50 mg/kg (i.p.) respectively per day per mouse. In each case the volume of the test solution injected (i.p.) were 0.2ml/day per mouse. Group 4 received bleomycin (0.3 mg/kg, i.p.) and finally group 5 was treated with the vehicle (normal saline) and was considered as untreated control. The mice were sacrificed on the 6th day after transplantation and tumor cells were collected by repeated intraperitoneal wash with 0.9% saline. The ascitic fluid was taken in a hematocrit (micro) tube and diluted 1000 times. Then a drop of the diluted cell suspension was placed on the Neubauer counting chamber and the cells in 64 small squares were counted. Viable tumor cells per mouse of the treated group were compared with those of control.

The cell growth inhibition was calculated by using the formula,

$$\% \text{ Cell growth inhibition} = \left(1 - \frac{T_w}{C_w}\right) \times 100$$

Where, Tw = Mean of number of tumor cells of the treated group of mice and
Cw = Mean of number of tumor cells of the control group of mice.

Average tumor weight and survival time

These parameters were measured under similar experimental conditions as stated in the previous experiment. Tumor growth was monitored daily by measuring weight change. The host survival time was recorded and expressed as mean survival time in days and percent increase of life span was calculated [24] as follows:

$$\text{Mean survival time (MST)} = \frac{\sum \text{Survival time in days of each mouse group}}{\text{Total number}}$$

$$\text{Percent increase of life span (ILS) \%} = \left(\frac{\text{MST of treated group}}{\text{MST of control group}} - 1 \right) \times 100$$

RESULTS

Anthelmintic activity evaluation

The methanolic extract of the aerial parts of *P.hydro Piper* showed significant (Maximum activity given at the concentration of 50 mg/ml) anthelmintic activity. The activity increased with the increase of concentration (10 to 50mg/ml) of the test sample. Observations of the experiment are given in the table 1.

Acute Toxicity study (LD₅₀)

No mortality was noticed up to 400 mg/kg body weight (i.p.), whereas, 100% mortality was noticed at the dose of 600 mg/kg (i.p.). The LD₅₀ of the extracts was found to be 500 mg/kg body weight (i.p.). One-tenth of this dose was selected as the therapeutic dose [25] for the evaluation of antiproliferative activity.

Cell growth inhibition

The effects of the different doses of methanol extract of the aerial parts of *P.hydro Piper* at the dose of 30 mg/kg (i.p), 40 mg/kg (i.p), and 50 mg/kg (i.p) and bleomycin at 0.3mg/kg (i.p) per mouse per day on EAC cell growth inhibition (*In vivo*) was observed. Methanol extract at the dose of 50mg/kg body weight showed maximum antiproliferative activity with 84.54% inhibition of EAC cell growth. The activity was comparable to that of standard drug bleomycin, which showed 98.55% cell growth inhibition when administered similarly at a dose of 0.3 mg/kg (i.p). The same extract at the dose of 30mg/kg and 40 mg/kg body weight also showed significant activity in a dose dependent manner with 74.15% and 78.74% cell growth inhibition respectively (Figure 1).

Average tumor weight and survival time

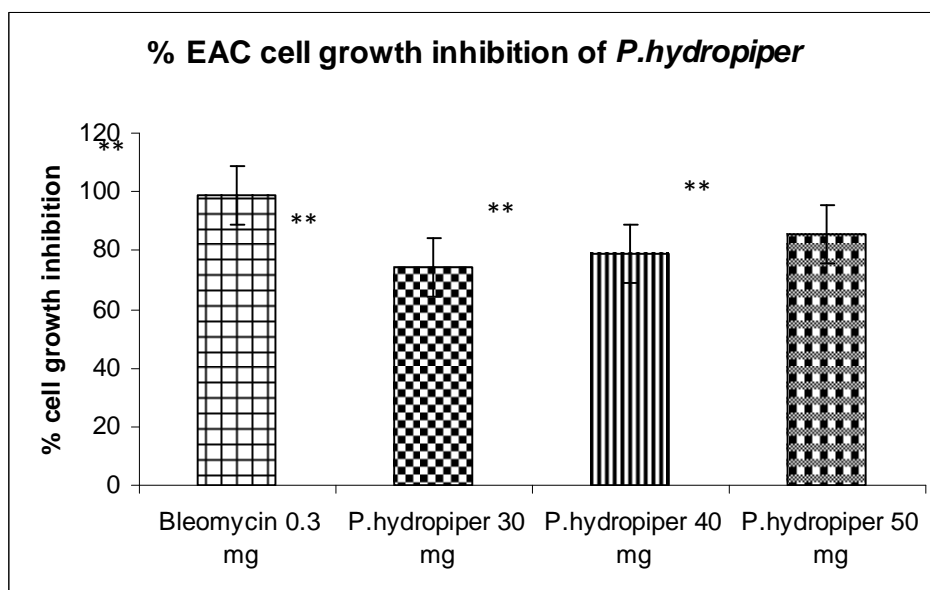
Tumor weight of EAC cell bearing mice after treatment with methanol extract of *P.hydro Piper* at the dose of 30mg/kg, 40mg/kg and 50 mg/kg for 20 days was calculated. It was found that tumor weight decreases approximately in a similar manner with bleomycin (0.3 mg/kg). Highest tumor weight reduction (7.85g) was observed at the dose of 50 mg/kg (i.p) compared to the standard drug bleomycin (7.05g). Whereas same extract at the dose of 30 and 40 mg/kg body weight showed moderate reduction of tumor cell weight (Figure 2).

Mean survival time (MST) of the untreated tumor bearing mice was 15.0 days. With the treatment of the three different doses of methanol extract of *P.hydro Piper*, this value increased remarkably. Maximum of 68.0% enhancement of life span was found at the dose of 50 mg/kg (i.p.) while at the dose of 30 mg/kg (i.p) and 40 mg/kg (i.p.) the plant extract showed moderate effect having enhancement of life span of 25.33% and 45.33% respectively (Figure 3). Under the same experimental condition, bleomycin at the dose of 0.3 mg/kg (i.p.) increased the life span value to 94.66 % (p<0.01).

DISCUSSION

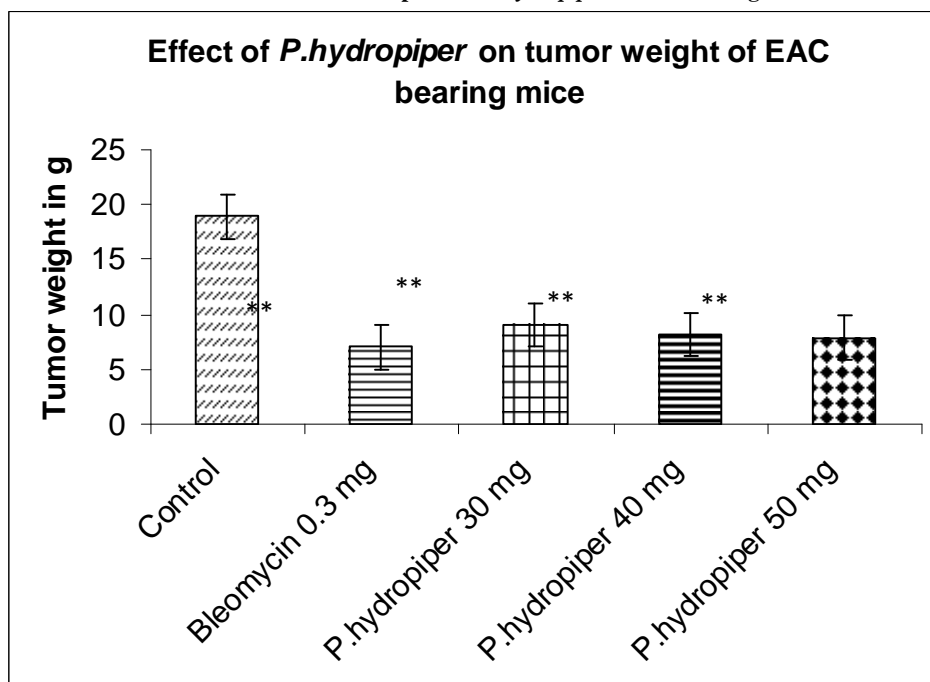
Our investigational result presented above demonstrated that methanol extracts of the aerial parts of *P.hydro Piper* can cause paralysis as well as death of worms at a time comparable to reference standard drug especially at the concentration of 50mg/ml in dose dependent manner. Standard drug Piperazine citrate cause a flaccid paralysis on the worm that result in expulsion of the worm by peristalsis. Piperazine citrate, by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [26]. Literature data [27-30] revealed the presence of antioxidant flavonoids and phenolic compound as one of the major chemical constituents (whole plant contain 46.68% antioxidant) of the selected plant parts. Polyphenolic compounds shown anthelmintic activity; chemically tannins are polyphenolic compounds [31]. Some synthetic phenolic anthelmintics e.g., niclosamide, oxyclozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation [32]. Another possible anthelmintic effect of tannins is that they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [33]. So, strong anthelmintic activity of our extract under study is rationale with regards to its phenol and flavonoid content. Our study results also proved that the methanol extract of the aerial parts of *P.hydro Piper* at its different doses (maximum effect at the dose of 50 mg/kg) can slow down the growth of tumor satisfactorily ($P<0.05$), reduce tumor weight markedly ($P<0.05$) and increase life span considerably ($P<0.05$). All these are measured as very important aspects in justifying the effectiveness of a compound in cancer chemotherapy [34] Traditional screening models for antiproliferative drugs are geared toward the selection of antioxidant and cytotoxic drugs.

Figure 1: Effect of methanol extract of the aerial parts of *P.hydro Piper* on cell growth inhibition of EAC cell bearing mice. (*in vivo*)



Values are mean \pm SEM, (n = 5); where significant values are, * $p < 0.01$ and ** $p < 0.001$, Dunnet test as compared to control.

Figure 2: Effect of methanol extract of the aerial parts of *P.hydro Piper* on tumor weight of EAC cell bearing mice.



Values are mean \pm SEM, (n = 5); where significant values are, * $p < 0.01$ and ** $p < 0.001$, Dunnet test as compared to control.

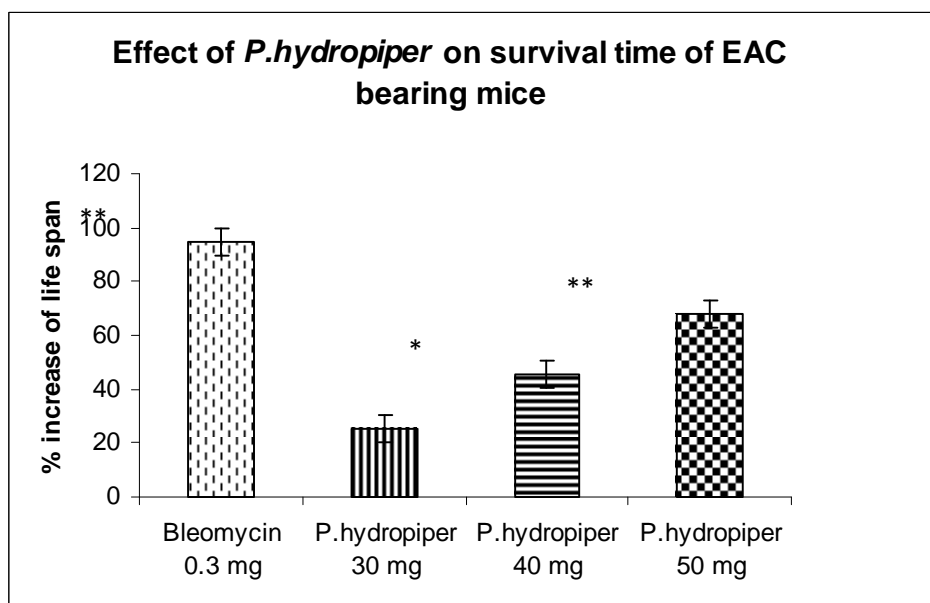
Antioxidants have been extensively studied for their ability to prevent cancer in human [35]. Several plant species rich in antioxidant flavonoids are reported to reduce the risk of disease and have high therapeutic values for the treatment of cancer [36, 37]. In addition, plant phenolics have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases such

as cancer [38]. Notable cytotoxicity to EAC cell of the methanol extract of *P.hdropiper* could be attributed mainly to phenol, flavones and flavonoid glycosides compound [39]. Numerous animal studies have been published demonstrating decreased tumor size and/or increased longevity with the combination of chemotherapy and antioxidants [40](Chinery R et al, 1997).

Table 1: Anthelmintic activity of aerial parts of *P.hdropiper* against *Pheretima posthuma*.

| Treatment | Concentration (mg/ml) | Time for paralysis (minute) | Time for death (minute) |
|-------------------------------|-----------------------|-----------------------------|-------------------------|
| Control (Saline water) | 10 | No paralysis | No death observed |
| Piperazine citrate (Standard) | 10 | 24 ± 0.87 | 38 ± 0.63 |
| Methanolic extract | 10 | 58.46 ± 0.05 | > 90 |
| | 25 | 26.32 ± 0.26 | 55.17 ± 0.41 |
| | 50 | 12.44 ± 0.32 | 18.19 ± 0.31 |

Figure 3: Effect of methanol extract of the aerial parts of *P.hdropiper* on survival time of EAC cell bearing mice



Values are mean ± SEM, (n = 5); where significant values are, *p<0.01 and **p<0.001, Dunnet test as compared to control.

CONCLUSION

In conclusion, the present study results provide strong evidence that the methanol extracts from the aerial parts of *P.hdropiper* have shown active *in vitro* anthelmintic effect against adult stage of *Pheretima posthuma* and active *in vivo* antiproliferative activity against EAC cell line. However much more investigation (including toxicological and hematological studies) with this plant extract has to be carried out using higher animal models, in order to authenticate it as a potent anthelmintic and antiproliferative agent.

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