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A preliminary evaluation of cytotoxicity, antihyperglycemic and antinociceptive activity of *Polygonum hydropiper* L. ethanolic leaf extract

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Abstract

Background: *Polygonum hydropiper* L. is used by folk medicinal practitioners of Bangladesh. Juice of leaves is used in headache, pain, toothache, liver enlargement, gastric ulcer, dysentery, loss of appetite and dysmenorrhea; roots are used as stimulant; juice is applied to wounds, skin. In this study, we analyze the cytotoxicity property of aerial parts of the plant along with antihyperglycemic and antinociceptive activity.

Methods: Cytoxicity was determined by brine shrimp lethality assay and antihyperglycemic activity was measured by oral glucose tolerance tests. Antinociceptive activity was determined by observing decreases in abdominal writhings in intraperitoneally administered acetic acid-induced pain model in mice.

Results: Administration of ethanol extract of leaf led to dose-dependent and significant reductions in blood glucose levels in glucose-loaded mice. Blood sugar levels of the tested mice were reduced significantly by 48.8, 51.5, 54.1, 58.2 % ($p < 0.05$) compared to control mice with the increasing dosage of the extract such as 50, 100, 200 and 400 mg/kg of body weight respectively. In contrast, when glibenclamide, a standard antihyperglycemic drug was administered at a dose of 10 mg/kg body weight, it reduced blood glucose level by 42.1 %. On the other hand stem extract only reduced 1.5 % of blood sugar level of the tested mice which was not significant. In the case of antinociceptive activity tests, the extract at the above four doses reduced the number of abdominal writhings by 14.10, 17.95, 29.49 and 41.02 % respectively in comparison with a standard drug Aspirin. In cytotoxicity tests, nauplli were treated with a gradually increased concentration of the extract, ranging from 10 to 45 µg/ml. The LC_{50} was found at a concentration of 16.22 µg/ml, compared with control.

Conclusion: The results of the present study showed that *Polygonum hydropiper* L. ethanolic leaf extract possess antihyperglycemic and antinociceptive activity. All these effects could be due to the bioactive components of this plant and need to be identified for further research about this plant. This could justify its ethnomedical use.

Keywords: Antihyperglycemic, Antinociceptive, Cytotoxicity, LC_{50}

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Background

Medicinal plants are the gift from nature. Until recently, medicinal plants have been employed for the treatment to the people in diverse complaints. The availability of these plants make easier to people to use the different parts which offers the medicinal activity to cure the illness of human beings. It is estimated that about 80 % of the world population depends on medicinal plants for their primary health care [1].

Bangladesh is enriched with different types of medicinal plants which belong to various families. *Polygonum hydropiper* L., locally called Bishkatali in Bangladesh, and distributed worldwide mostly in Southeast Asia [2–4]. It has a long history of herbal use and belongs to the family of Polygonaceae [5]. Either the whole plant or mixed with other herbs is used in the treatment of various types of infirmities including diarrhea, dyspepsia, itching skin, excessive menstrual bleeding and hemorrhoids [6]. Different types of antioxidant flavonoids are isolated from this plant like quercetin, kaempherol, catechin, 6-hydroxyapigenin and some others [7–9]. It has also the antibacterial, antifungal and anti-inflammatory activities [10, 11]. Besides, the antifertility activity showed on albino rats in various chromatographic fractions of this plant [12]. This plant also plays an important role in antifeedant [13], antiadipogenic [14] and anticholinesterase [15, 16] activities. Antinociceptive activity of the whole plant of *Polygonum hydropiper* L. was inspected with the hexane, ethylacetate and methanol extracts in mice model [17]. Further, some inhibition of the bovine lens aldose reductase by various sulfated flavonoid of this plant suggests that the plant might have some antidiabetic activity [18, 19]. Recently, the phytochemical, phytotoxic and antihelmintic activity of crude methanolic extract of this plant has been investigated [20].

Till to date, any research yet not has been carried out on the antihyperglycemic activity of *P. hydropiper* L. plant. The antinociceptive and brine shrimp cytotoxicity ethanolic leaf extract is also unreported till now. In the present study, we have investigated the brine shrimp cytotoxicity, antihyperglycemic and antinociceptive activity of *Polygonum hydropiper* L. ethanolic leaf extract, to uphold the medicational satisfactory against diabetes, pain and other diseases.

Methods

Plant material collection

Aerial parts (leaves and stems) of *Polygonum hydropiper* L were collected from the Santosh, Tangail district of Bangladesh in 2015. Botanical identification was carried out at the National Herbarium, Mirpur, Bangladesh where an accession No. 40213 has been deposited.

Preparation of the plant extract

Aerial parts were cut, air-dried and powdered in a grinding machine and stored in an airtight container until further analyzed. Powdered dried leaves (100 g) of the plant were extracted with ethanol (1.0 L) in flat bottom glass container, through occasional shaking and stirring for 7 days. For the stem we also used the same amount (100 g dissolved into 1.0 L ethanol). The whole mixture of both stem and leaf were then filtered separately by Whatman No 1 filter paper and the filtrates were dried at 40 °C in vacuum using a rotatory evaporator [21] to afford a blackish mass. Finally we got 4.80 g leaf and 2.27 g stem extract. All above extraction procedures are repeated thrice and finally selected the extract randomly for the experimental purposes. The crude extracts were then kept at 4 °C in sterile universal bottles.

Chemicals and drugs

Glibenclamide, aspirin, and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. Vincristine sulphate was collected from Beacon Pharmaceuticals Limited Bangladesh. All other chemicals were of analytical grade.

Model animals

Swiss albino mice (male), which weighed between 28–32 g were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of Mawlana Bhashani Science and Technology University, Tangail, Bangladesh.

Brine shrimp cytotoxicity assay

Brine shrimp (*Artemia salina*) cytotoxicity assay was carried out to check the cytotoxic activity of the plant extract. The assay was done according to Meyer's process with some modification [22]. Simply, Brine shrimp nauplii were obtained by hatching brine shrimp eggs (O.S.I. Brine Shrimp Egg Company Utah, U.S.A.) in artificial sea water (3.8 % sodium chloride solution) for 48 h.

After hatching, active nauplii were collected and 10 nauplii were drawn through a dropper and placed in each well of microtitre plate containing 200 μl of seawater. Then 100 μl of plant extract (leaf) solution (extract dissolved in 20 % ethanol) was added to make final concentration of plant extract as 10 μg/ml, 15 μg/ml, 20 μg/ml 25 μg/ml, 30 μg/ml, 35 μg/ml, 40 μg/ml and 45 μg/ml. Vincristine sulphate (Beacon Pharmaceuticals Limited, Bangladesh) was used as positive and 20 % ethanol was used as negative control respectively. After

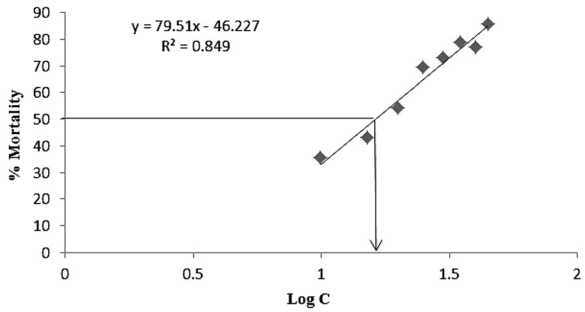

Fig. 1 Determination of lethal concentration 50 (LC_{50}) of *P. hydropipper* L. A straight line obtained by plotting percentage of mortality of brine shrimps nauplii against the logarithm of the concentration of leaf extract (from Table 1). From the graph, log LC_{50} was obtained at 50 % mortality. LC_{50} value obtained for *P. hydropipper* L. extract was 16.22 μg/ml

24 h incubation dead and live nauplii were counted under microscope. Each experiment was performed in three replicas. The percentage of mortality was then determined. Lethal Concentration 50 (LC_{50}) value was obtained from the best-fit line by plotting concentration versus percentage of mortality.

Oral glucose tolerance tests for evaluation of antihyperglycemic activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) [23] with minor modifications. Briefly, fasted mice were grouped into seven groups of five mice each. The various groups received different treatments such as group 1 received vehicle (1 % Tween 80 in water, 10 ml/kg body weight) and served as control, group 2 received standard drug (glibenclamide, 10 mg/kg body weight) and groups 3–6 received extract (EEPHL) at doses of 50, 100, 200 and 400 mg per kg body weight. For the stem extract (EEPHS) we only used 400 mg per kg body weight and that was treated for group seven. All substances were orally administered. Following a period of two hours, all

Table 1 Effect of *P. hydropipper* L. ethanolic leaf extract on brine shrimp nauplii

Concentration (μg/ml)	Log C	% Mortality	Log LC_{50} (μg/ml)	LC_{50} (μg/ml)
Negative control (ethanol)	—	0.0	1.21	16.22
10	1	35.71		
15	1.176	42.86		
20	1.301	54.55		
25	1.398	69.23		
30	1.478	73.33		
35	1.544	78.57		
40	1.602	76.92		
45	1.653	85.71		

Table 2 Effect of Vincristine sulphate on brine shrimp nauplii

Concentration (μg/ml)	Log C	% Mortality	Log LC_{50} (μg/ml)	LC_{50} (μg/ml)
Solvent	–	0.0	-0.539	0.288
1.25	0.096	70		
2.5	0.397	83.33		
5	0.698	90		

mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected two hours after the glucose administration through puncturing heart.

Blood glucose levels were measured by standard glucose oxidase method [24]. The percent lowering of blood glucose levels were calculated according to the formula described below.

$$\text{Percent lowering of blood glucose level} = (1 - W_e/W_c) \times 100;$$

Where *We* and *Wc* represents the blood glucose concentration in glibenclamide, EEPHL or EEPHS administered mice (Groups 2–7), and control mice (Group 1), respectively.

Antinociceptive activity evaluation through abdominal writhing test

Antinociceptive activity of EEPHL was examined as previously described [25]. Briefly, mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard non-narcotic analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4–7 were administered EEPHL at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or EEPHL, all mice were intraperitoneally injected with 1 % acetic acid at a dose of 10 ml per kg body weight. A period of 5 min was given to each animal to ensure onset of chemically induced irritation of acetic acid, following the period, the number of abdominal writhings was counted for 10 min. The percent inhibitions of abdominal writhings were calculated according to the formula given below.

$$\text{Percent inhibition} = (1 - W_e/W_c) \times 100;$$

Where *We* and *Wc* represents the number of writhings in aspirin or EEPHL administered mice (Groups 2–7), and control mice (Group 1), respectively.

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical

Table 3 Effect of crude ethanolic extract of *P. hydropipper* L. aerial parts (EEPHL and EEPHS) on blood glucose level in hyperglycemic mice following two hours of glucose loading. All administrations were made orally

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.32 ± 0.32	–
Glibenclamide	10 mg	3.08 ± 0.08	42.1*
EEPHL	50 mg	2.72 ± 0.03	48.8*
EEPHL	100 mg	2.58 ± 0.06	51.5*
EEPHL	200 mg	2.44 ± 0.05	54.1*
EEPHL	400 mg	2.22 ± 0.07	58.2*
EEPHS	400 mg	5.24 ± 0.07	1.5

Values represented as mean ± SEM, ($n = 5$); *$P < 0.05$; significant compared to hyperglycemic control animals

comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results

Brine shrimp cytotoxicity bioassay

The ethanolic leaf extract of *P. hydropiper L* was evaluated for brine shrimp cytotoxicity with different concentrations. Vincristine sulphate and the solvent (ethanol) were used as positive and negative control respectively. All experiments were done in triplicate and the mean of the triplicate taken as final result. The lethal concentration LC_{50} of the test samples after 24 h was obtained by a plot of percentage of the dead nauplii against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis (Fig. 1). From the assay, LC_{50} value of the leaf extract was determined and it was 16.22 μg/ml (Table 1). The positive control was used here to check the validity of the test and LC_{50} of the control was 0.288 mg/ml (Table 2).

Antihyperglycemic activity

The blood glucose levels of the glucose loaded mice were reduced significantly when administered at doses of 50, 100, 200 and 400 mg/kg body weight of EEPHL. At these four doses, the percent lowering of blood glucose levels were 48.8, 51.5, 54.1 and 58.2 % respectively. For the case of stem extract (EEPHS) which administered at the dose of 400 mg per kg body weight and we found the blood glucose lowering level was only 1.5 % in the group seven mice which is not significant.

By comparison when a standard antihyperglycemic drug, glibenclamide, administered to mice at a dose of 10 mg/kg body weight it reduced blood glucose levels by 42.1 %. The results are shown in Table 3.

Antinociceptive activity

Dose-dependent and significant reductions in the number of abdominal writhings induced by intraperitoneal administration of acetic acid were observed with EEPHL. The reductions of the number of abdominal writhings were 14.10, 17.95, 29.49 and 41.02 % respectively at doses of 50, 100, 200 and 400 mg/kg body weight. The reductions of the abdominal writhings for the standard antinociceptive drug, aspirin, were 41.02 and 69.23 % for the concentration of 200 and 400 mg per kg body weight respectively (Table 4).

Discussion

Plants are important sources of novel therapeutics for human welfare from the beginning of the world. As a reservoir of traditional medicine, plants are encompassed with wide array of secondary metabolites, which can be used to treat chronic and even infectious diseases [26]. The cytoxic properties of different plant extract are

Table 4 Antinociceptive effect of crude ethanolic leaf extract of *P. hydropipper* L. in acetic acid-induced pain model mice. All administrations (aspirin and extract) were made orally

Treatment	Dose (mg/kg body weight)	Mean number of abdominal writhings	% inhibition
Control	10 ml	15.6 ± 0.75	–
Aspirin	200 mg	9.2 ± 0.66	41.02*
Aspirin	400 mg	4.8 ± 0.37	69.23*
EEPHL	50 mg	13.4 ± 0.51	14.10*
EEPHL	100 mg	12.8 ± 0.37	17.95*
EEPHL	200 mg	11 ± 0.71	29.49*
EEPHL	400 mg	9.2 ± 0.75	41.02*

Values represented as mean ± SEM, ($n = 5$); *$P < 0.05$; significant compared to control

also widely implemented to treat various cancerous cell lines in recent time [27, 28].

For the cytotoxicity assay, the brine shrimp lethality bioassay is now adopted everywhere. This assay embodies a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases allies reasonably well with pesticidal, antiplasmodial, antifilarial, antimalarial and anti-tumor properties [29, 30].

The brine shrimp cytotoxicity of the *P. hydropiper* L. was previously reported for the stem extracts [31]. In this study, ethanolic leaf extract of *P.hydropiper* L. was investigated for its potential bioactivity. The LC_{50} values of the plant extracts were obtained by a plot of percentage of the shrimp nauplii killed against the concentrations of the extracts and the best-fit line data by means of regression analysis. The assay plot suggests, the prepared extract was rich in bioactive compounds by obtaining a notable LC_{50} value of 16.22 μg/ml for the brine shrimp.

Diabetes mellitus is group of common metabolic disorder with projected population coverage of 387 million throughout the world in recent time [32]. Despite having some good treatments for the diabetes, scientists are looking for more potential therapeutics for it and medicinal plants are involved in this way for the healing of this disease. Crude extract from different plants might act upon on diabetes through engaging different mechanisms to lower the blood glucose level. Such types of mechanisms have been anticipated previously for extracts of *Picrorrhiza kurroa* [23] and *Helicteres isora* [24] and many others. It may facilitate to increase the glucose uptake or uphold to surge the pancreatic secretion [33] or may initiate the inhibitory role of glucose absorption in gut [34].

In the present study, the antihyperglycemic activity might be first reported for the *P. hydropiper* L. Here, we tested both the leaf and stem extracts' effect on glucose loaded mice in order to observe the antihyperglycemic activity. Significant amount of blood glucose lowering activity was observed for the ethanolic leaf extract. On the other hand, the result is insignificant for the stem extract in comparison with the standard drug, glibenclamide. The crude leaf extract of different concentrations showed remarkable effect over the drug, glibenclamide.

The acetic acid-induced abdominal writhing test has been reported to be useful methods to investigate peripheral, abdominal and central antinociceptive activity [35]. Intraperitoneal administration of acetic acid can accelerate pain, symptomically (consequent abdominal writhings) by inducing the release of mediators like prostaglandin E2 and lipooxygenase in the peritoneal cavity that eventually stimulate local peritoneal nociceptors [36–38]. Prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin- (PG-E), play a leading role for the excitation of Aδ- nerve fiber that has been shown to be sensation of pain [39].

The potential antinociceptive activity of the ethanol extract of *P.hydropiper* leaves was investigated using acetic acid-induced abdominal writhing test. The ethanol extract of *P. hydropiper* significantly and dose dependently suppressed the number of writhings induced by acetic acid in mice. At a concentration of 400 mg per kg body weight of the leaf extract, the reduction of the abdominal writhings was just equal of 42.1 % to the standard drug, aspirin at a concentration of 200 mg per kg body weight. As a crude extract the overall potentiality of reducing the writhing as a means of pain, is quite obvious than the previous report of the different fractionation of this plant [17].

P. hydropiper L. was extensively studied in innumerable experiments because of its potential sources of bioactive compounds. In current study, especially the first reported antihyperglycemic activity analysis is also on this way to introduce some important characteristics of this plant, which may contribute in the improved medication systems for diabetes.

Conclusion

From the results obtained, it might be concluded that the ethanolic leaf extract of *Polygonum hydropiper* L. has profound cytotoxic, antihyperglycemic and antinociceptive activities. In future extensive studies through further isolation and identification of the bioactive component(s) might produce some breakthrough leads for future biomedical and biopharmaceutical research.

Abbreviations

P.hydropiper: *Polygonum hydropiper*; (LC_{50}): Lethal concentration 50.

Competing interest

The authors' declare that they have no compete of interest.

Authors' contributions

ARO has made substantial contributions to design of the study, did the extraction, carried out experiments, wrote the manuscript draft and carried out statistical analysis. MAAS collected the plant and did the extraction, carried out experiments. MUH carried out experiments and helped to draft the manuscript. MRI participated in the design of the study and performed the statistical analysis. AAE conceived of the study, and participated in its design and coordination helped to draft the manuscript. All authors read and approved the final manuscript.

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