academicJournals

Vol. 10(43), pp. 790-795, 17 November, 2016
DOI: 10.5897/JMPR2015.5806
Article Number: 5CD4FBE61808
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Pharmacological activities of methanol extract of Phyllanthus acidus pulp

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Received 8 April, 2015; Accepted 13 November, 2015

The methanolic extract of *Phyllanthus acidus* pulp was screened in the present study to explore pharmacological activities on laboratory animals. The hypoglycemic activity was assessed by glucose tolerance test and the antidiarrheal activity was evaluated by castor oil induced diarrhea inhibition method. The analgesic and anesthetic activity was determined by Tail immersion method and Thiopental sodium induced sleeping time method, respectively. The extract showed significant (P<0.05) blood glucose activity at 500 mg/kg of body weight. The extract at both doses showed pronounced antidiarrheal activity and inhibited the average number of defecation by 54.24% (P<0.05) and 10.17% at both doses, respectively. In Tail-immersion test, the extract showed significant (P<0.05) analgesic activity at both doses. The extract also showed good CNS anesthetic activity to the experimental animal. It was concluded that from this study, the methanolic extract of *P. acidus* have potential different pharmacological activities and justify its use in folkloric medicines.

Key words: *Phyllanthus acidus*, hypoglycemic, anti-diarrheal, analgesic, anesthetic.

INTRODUCTION

Plants have been used for mankind as remedies from the very beginning of civilization. To date, they play an important role in health care for about 80% of the world's population. They have long been a very important source of new drugs. Many plant species have been screened for substances with therapeutic activity. Bangladesh possesses a rich flora of medicinal plant. Out of the estimated 5000 species of phanerogams and pteridophytes growing in this country, more than a thousand are regarded as having medicinal properties (Mia and Rahman, 1990). A large number of plants included in Euphorbiaceae family have been investigated all over the world.

Euphorbiaceae plants, which are investigated in Bangladesh show wide range of secondary metabolites including antibacterial, hypoglycemic, antidiarrheal, anaesthetic and analgesic compounds.

Phyllanthus acidus, a plant belongs to the family Euphorbiaceae. It is commonly known as the Otaheite gooseberry, Malay gooseberry, Tahitian gooseberry, Country gooseberry, Star gooseberry, Starberry, West India gooseberry, Grosella (in Puerto Rico), Jimbilin (in Jamaica), damsel (in Grenada), karamay (in the Northern Philippines), or simply gooseberry tree. It is mostly cultivated for ornamentation and locally name as Arboroi (Bengali). This tropical or subtropical species is found

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throughout Asia and also has a home in the Caribbean region, Central and South America (Janick and Robert, 2008).

The plant is an intermediary between shrubs and tree with edible small yellow berries fruits. There are many important chemical constituents found in this plant. For example, fruits contain ascorbic and tartaric acids and tannin, root bark contains tannin (18%), gallic acid, saponin, lupeol and a crystalline substance (Chopra et al., 1992), stem bark includes a phytosterol, different from lupeol (Ghani, 2003). The plant is reported to have many uses. Latex is credited with emetic and purgative activity, bark is used to treat bronchial catarrh and is a popular local treatment. Roots are used to alleviate asthma and also used to treat psoriasis of the feet, leaf decoction is applied to treat urticarial. On the other hand, fruit is used as a laxative and the antioxidants present in fruit have a hepatoprotective effect on liver (Reddy, 2014; Jain et al., 2011). Extract has shown nematicidal activity against the pine wood nematode and have possibility to provide treatment of cystic fibrosis of the lungs (Sousa et al., 2007). Leaves are useful to treat blood vomiting, piles, small pox, fever, itching and gum infection (Subhadrabandhu, 2001). Furthermore, it is known to possess anticancer (Mahidol et al., 2002), antibacterial (Melendez and Capriles, 2006) and antiviral (Direkbusarakom et al., 1996) activities. The fruit is extensively used in Ayurvedic system as a liver tonic and blood purifier. It is also used in several vitiated conditions of jaundice, piles, constipation, vomiting, bronchitis, biliousness and urinary concretions (Kirtikar and Basu, 1987).

All these previous studies revealed that there are a lot of phytochemical constituents which may possess some effect on antidiarrheal, anesthetic or analgesic like action. Previous *in vitro* studies reveal the possibilities of such kind of *in vitro* model studies. However, the present study intended to investigate the pharmacological like hypoglycemic, antidiarrheal, analgesic and anesthetic activity of methanolic extract of the pulp of *P. acidus* in swiss-albino mice, which is abundantly growing in Bangladesh.

MATERIALS AND METHODS

Chemicals and drugs

Glibenclamide (Dibenol), Loperamide hydrochloride (Imotil) are brand products of Square Pharmaceuticals Ltd, Morphine Sulphate (Morphinex®), Diazepam (Easium) and Thiopental sodium (Anestho) product of Popular Pharmaceuticals, Opsonin Pharma and Incepta Pharmaceuticals Ltd, respectively were purchased from the local market of Bangladesh. All other chemicals and reagents used throughout the whole study were of analytical grade.

Collection of plant

The fresh fruits of *P. acidus* were collected form comilla, Bangladesh in August 2012. It was identified and authenticated by National Herbarium of Bangladesh, Mirpur, Dhaka (voucher specimens of the collection DACB Accession no. 38200) and have been deposited in Bangladesh National Herbarium (BNH) for future references.

Preparation of methanol extracts

The collected plant parts (fruits-pulp) were separated from seed. They were air-dried for one week. Then, the plant parts were pulverized into a coarse powder with the help of a suitable grinder. About 110 g of powered material was taken in a clean glass container and soaked in 300 ml of 80% methanol (Merck, Germany) and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then filtrated by a piece of clean and white cotton material. Then, it was filtered using Whatman No. 42 filter paper. After obtaining clear filtrates, they were then evaporated by using traditional spontaneous natural vaporization method at room temperature. It rendered an oily concentrate of yellowish black color. The crude extract was stored at 4°C until analysis.

Experimental animals

Swiss-albino mice of either sex, aged 4 to 5 weeks, obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiments. They were housed in standard polypropylene cages and kept under controlled at room temperature (24 ± 2°C; relative humidity 60 to 70%) in a 12 h light-dark cycle and fed ICDDR, B formulated rodent food and water (*ad-libitum*). They were kept before the test for at least 3 to 4 days in the environment where the experiment took place, due to their sensitivity with the environmental changes.

Hypoglycemic activity

A Glucose Tolerance Test (GTT) is the most commonly used method to evaluate the hypoglycemic activity in which glucose is given in the systemic circulation. Blood sample were taken afterward to determine how quickly it is cleared from the blood. In the present study, hypoglycemic effect of methanolic extract of *P. acidus* at 250, 500, and 1000 mg/kg doses were examined and compared relative to that of control and standard group. Glibenclamide was used as a standard drug in this experiment (Pari and Venkateswaran, 2002). Ten experimental animals were randomly selected and divided into five groups consisting of 2 mice in each group. Each group received an individual treatment. In order to administer the extract at doses of 250, 500, and 1000 mg/kg body weight of mice, the exactly weighed extracts were measured, respectively and triturated in unidirectional way by adding small amount of Tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added with it. The ultimate or final volume of the suspension was made up to 3 ml. To stabilize the suspension, it was stirred well by using vortex mixture. For the preparation of standard (Glibenclamide) at the dose of 10-mg/kg body weight, 10 mg tablet was dissolved into 3 ml normal saline (0.9% NaCl) (Kasiviswanath et al., 2005). At zero hour test samples, control (1% Tween-80 solution in saline) and Glibenclamide (in standard groups) were administered orally by means of a long needle with a ball shaped end. After 60 min, all groups were treated with 10% glucose solution (2 g/kg body weight). Then, after 30, 60 and 120 min of glucose loading, blood samples were collected from tail vein. By using glucometer blood glucose level was measured (Kamaeswara

Table 1. Hypoglycemic activity of *P. acidus* pulp in experimental animals.

Test group	Dose (mg/kg)	Plasma glucose level (mmol)			
		0 h	0.5 h	1 h	2 h
Control	1% tween 80	6.2±0.23	10.5±0.42	7.3±0.23	5.7±0.23
Standard	10	5.45±0.33	4.8±0.21*	3.5±0.23*	1.7±0.23*
P. acidus	250	4.05±0.31	5.0±0.26	5.55±0.23	4.7±0.23
P. acidus	500	3.6±0.22	4.8±0.28*	4.6±0.33*	4.0±0.27*
P. acidus	1000	5.85±0.23	4.45±0.20	4.15±0.23	5.55±0.23

Values are expressed as mean ± SEM. *Significant as compared to standard (Glibenclamide) and control p<0.05.

et al., 2001).

Antidiarrheal activity

The anti-diarrheal activity of the methanolic extract of *P. acidus* was evaluated using the method of castor oil induced diarrhea in mice (Shoba and Thomas, 2001). According to this method, each mouse was fed with 1 ml of highly pure analytical grade of castor oil which would induce diarrhea. The numbers of fecal stools were recorded for each individual mouse. The observations of the experimental groups were compared against that of the control to evaluate the anti-diarrheal activity of the samples. The experimental animals were divided into control, positive and test groups, with two mice in each group. The control group received vehicle (1% tween in normal saline) at dose 10 ml/kg body weight orally. Loperamide was given to the positive control group at the dose of 50 mg/kg orally. The test group received methanolic extract of *P. acidus* at the doses of 200 and 400 mg/kg body weight. Each animal was placed in an individual cage; the floor lining was changed at every hour. Diarrhea was induced by oral administration of castor oil to each mouse after the above treatment. During an observation period of 5 h; the number of diarrhoeic feces excreted by the animals was recorded (Maikere et al., 1989).

Analgesic activity

Evaluation of central analgesic activity was carried by tail immersion method using morphine as a positive control. The changes in sensitivity of test animal due to analgesic activity of drugs are measured in this method. A constant heat stress is applied to rat tail, which acts as pain stimulus. When the stimulus exceeds the threshold, rat shows a quick withdrawal of its tail. Time taken by the rat to withdraw the tail is termed as tail immersion time (Upudha et al., 2007). Analgesic compound elongates this responding time. The test rats were orally fed with test materials whereas the positive control received morphine subcutaneously. From 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C .The reaction time is the time required by the mice to deflect their tails. The time required to withdraw the tail was recorded. The experimental animals were divided into control, positive and test groups, with two mice in each group. Control and test samples (at doses of 200 and 400 mg/kg body weight of mice) were given orally. At zero hour, 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time is the time required by the mice to deflect their tails. The first reading is rejected and the reaction time is recorded as a mean of the next three reading. A latency period of 20 s was defined as complete analgesia. The latent period of tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs. A 30 min interval was given to ensure proper absorption of the administered substances. Then morphine solution (a dose of 2 mg/kg for 25 mg mice) was administered subcutaneously to the mice. After 30, 60 and 90 min, the tail immersion time was measured.

Anesthetic activity

The method described by Williamson et al. (1996) was followed for this study. The test animals were divided in four groups consisting of two mice each group. Group I was the control group, Group II was the standard group, whereas group III (A) and III (B) were experimental groups. The experimental groups were treated with test samples prepared with normal saline water and tween-80 at doses of 200 and 400 mg/kg body weight, standard group was administered intraperitonially with Diazepam (1 mg/kg) containing normal saline water, while the control group was administered normal saline water containing 1% Tween 80 solution. 30 min later thiopental sodium (40 mg/kg body weight) was administered intraperitonially to all the groups to induce sleep. The onset of sleep and total sleeping time were recorded.

Statistical analysis

Descriptive statistics were calculated for all variables by using SPSS software package (version 19.0). All of the values were expressed as mean ± standard error of mean (SEM). Data analysis among the groups was compared using one-way ANOVA analysis. P value of <0.05 was considered as significant.

RESULTS

Hypoglycemic activity

The result of hypoglycemic screening of pulp extract of *P. acidus* shows effective blood glucose lowing activity at dose of 250, 500, and 1000 mg/kg, but more significant at 500 mg/kg dose in comparison with standard (Glibenclamide) and control group in statistical analysis greater than dose effect in Table 1.

Antidiarrheal activity

The methanolic extract of pulp of *P. acidus* was subjected

Table 2. Effect of methanol extract of *P. acidus* pulp and Loperamide on castor oil induced diarrhea in mice.

Test group	Dose (mg/kg)	No. of diarrheal faeces in 5 h	% Inhibition
Control	10 ml/kg	29.5	
Loperamide	50	9.0	69.49*
P. acidus	200	13.5	54.24*
P. acidus	400	26.5	10.17

*****Significant as compared to control p<0.05.

Table 3. Analgesic activity of *P. acidus* pulp by tail immersion method.

Test group	Dose (mg/kg)	Reaction time (min)			
		0	30 m	60	90
Control	Vehicle	3.45 ± 0.33	4.35 ± 0.23*	2.41 ± 0.19*	4.23 ± 0.31*
Morphine	2	5.14 ± 0.33	19.17 ± 0.43*	20.0 ± 0.21*	16.0 ± 0.21*
P. acidus	200	3.42 ± 0.33	3.30 ± 0.13*	13.9 ± 0.31*	6.62 ± 0.32*
P. acidus	400	4.13 ± 0.33	11.11 ± 0.25*	5.90 ± 0.11*	15.50 ± 0.44*

Values are expressed as mean ± SEM. *All data were found to be significant at 5% level of significance where p<0.05

Table 4. Effect of methanol extract of *P. acidus* pulp on thiopental sodium-induced sleep.

Test group	Treatment	Dose (mg/kg)	Time of onset of sleep (min)	Total sleeping time (min)
I (Control)	1% tween 80	Vehicle	16	107.8
II (Standard)	Diazepam	1	9	158
III	Test drug A	200	27	155
IV	Test drug B	400	20	139

to castor oil induced diarrhea experiment at doses 200 and 400 mg/kg and compared with relative to that of control and standard (loperamide) group. The obtained data showed that the pulp extract exhibited significant (p<0.05) anti-diarrheal activity at dose 200 mg/kg compared to control group (Table 2).

Analgesic activity

In the tail immersion method of analgesic study, the methanolic extract showed significant analgesic activity at both doses of 200 and 400 mg/kg (Table 3) when compared with relative to that of control and standard (morphine) group.

Anesthetic activity

Table 4 presented the values of anesthetic activity of the methanolic extract of pulp of *P. acidus* was tested at 200 and 400 mg/kg doses and compared with relative to that of control and standard (Diazepam) group.

DISCUSSION

Diabetes, this chronic metabolic disorder, is a major public concern in the whole world characterized by high blood glucose concentration due to insulin deficiency or frequently combined with insulin resistance. In recent years, its increasing rate is very high in developing countries likes Bangladesh. Traditionally, many plants are used for treatment and management of diabetes mellitus throughout the world (Marles and Farnsworth, 1995). In the present study, we explore the hypoglycemic activity of methanolic extract of *P. accidus* pulp at different doses level, 250, 500 and 1000 mg/kg and the finding of the study showed the effective hypoglycemic activity which is more significant (4.8±0.28, 4.6±0.33 and 4.0±0.27) at 500 mg/kg dose in comparison with standard (Glibenclamide). Obviously, there is less dose effect on hypoglycemic activities which may be overdose saturation of receptor activities. But according to the statistical data, dose at 500 mg/kg is significant against the control values. Recinolic acid, an active metabolite of castor oil, which causes diarrhea due to increase in peristaltic movement of the small intestine leading to alteration in electrolyte

permeability in the intestinal mucosa and it is a hypersecretory response. Since the methanolic extract of pulp of *P. acidus* fruitfully inhibited castor oil induced diarrhea and assume that this action was mediated by an antisecretory mechanism (Stewart et al., 1975).

The crude methanolic extract of *P. acidus* pulp exhibited significant ($p<0.05$) anti-diarrheal activity at dose 200 mg/kg (54.24%) compared to control group. Here also the low dose effect, which reveals that, this crude extract has more effect at lower dose instead of high dose. In this experiment, the crude methanolic extract of *P. acidus* pulp showed significant analgesia (3.30 ± 0.13, 13.9 ± 0.31, and 6.62 ± 0.32) at different time interval (30, 60 and 90 min) in the dose of 200 mg/kg body weight compared to standard (19.17 ± 0.43, 20.0 ± 0.21, and 16.0 ± 0.21) at the same time intervals. A relevant phenomenon involved for the effect of analgesic against thermal noxious stimuli may be produced through opoid receptors or through modulation of several neurotransmitters. The activities of analgesic and anti-diarrheal have been attributed due to their phytochemical compounds of saponin, terphenoids, flavoids and phenolic contents. The phytochemical screening of this plant by other researchers (Sukanya et al., 2013; Manjulatha et al., 2014) revealed that *P. accidus* possessed the maximum phytochemicals like alkaloids, flavonoids, saponins, tannins and phenolic compounds. CNS anesthetic activity was performed for the pulp of *P. acidus* at 200 and 400 mg/kg doses and was administered to the test animal (swiss-albino) for thiopental sodium induced sleeping time test.

The pulp extract slightly increase the thiopental sodium induced sleeping time shown in Table 4. The time of onset of sleep was 16 min in control group, whereas in experimental group, it was 27 and 20 min at doses of 200 and 400 mg/kg body weight, respectively. The total sleeping time was about 155 and 139 min at the doses of 200 and 400 mg/kg body weight, respectively, while it was 107.8 min in control group. So, it is clear from the observation that the methanolic extract at 200 mg/kg body weight showed better CNS anesthetic activity than 400 mg/kg body weight dose. All the experimental values are effective at lower dose and it may be due to the effectiveness of the active phytoconstituents at low dose and further investigations are required to establish the plant's activates over a wide range.

Conclusion

The results of the present study led us to the inference that the pulp extract possess significant hypoglycemic, anti-diarrheal, analgesic, and anesthetic properties. Since this plant is used in traditional medicine, the extracts should be further explored for its phytochemical profile to identify active constituent responsible for hypoglycemic, anti-diarrheal, analgesic, and anesthetic activities, especially at the lower dose.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors thank the Department of Pharmacy, Noakhali Science and Technology University for providing necessary logistic support to carry out the research. They would also like to thank Md. Hasan Kawsar, Associate Professor, Department of Pharmacy, State University of Bangladesh for his support to conduct different pharmacological tests.

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