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ORIGINAL ARTICLE

A Pharmacological Study on Antinociceptive and Anti-hyperglycemic Effects of Methanol Extract of Leaves of *Phyllanthus Reticulatus* Poir. In Swiss Albino Mice

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Mohammed Rahmatullah, Khokon Chandra Ghosh, Abdullah Al Mamun, Md. Tozammal Hossain, Salman Ahmed, Md. Ashikur Rahman, Bipasha Eva, Shahnaz Rahman, Majeedul H. Chowdhury; A Pharmacological Study on Antinociceptive and Anti-hyperglycemic Effects of Methanol Extract of Leaves of *Phyllanthus Reticulatus* Poir. In Swiss Albino Mice

ABSTRACT

Phyllanthus reticulatus Poir. (Euphorbiaceae) is used in folk medicinal practices of Bangladesh as an antinociceptive (reducing sensitivity to painful stimuli) and for treatment of kidney, gall bladder, liver, and gastrointestinal disorders of diabetic patients. The objective of the present study was to investigate the antinociceptive and anti-hyperglycemic activity of methanolic leaf extract of *Phyllanthus reticulatus* in Swiss albino mice. A model of acetic acid-induced gastric pain in mice was utilized to determine the antinociceptive effects, and anti-hyperglycemic activity was determined through glucose tolerance test using glucose-loaded mice. In writhing assays induced by acetic acid, the methanolic leaf extract showed significant inhibition compared to control. The maximum writhing inhibition (39.1%) was found at a dose of 200 mg extract/kg body weight which, however, was lesser than that of the antinociceptive drug, aspirin (50.4%), when used at a dose of 200 mg/kg body weight. Dose-dependent and significant anti-hyperglycemic activity of the extract was found in mice loaded with glucose at extract doses of 100, 200 and 400 mg extract/kg body weight. Maximum tolerance (35.0%) was showed at 400 mg extract/kg body weight, compared to that of the standard drug, glibenclamide at 10 mg/kg body weight (57.8%). In summary, the methanol extract of *Phyllanthus reticulatus* leaves had beneficial effects as a pain reliever and also in reducing the elevated blood glucose level of hyperglycemic mice, which validates the use of the plant in Bangladesh folk medicinal practices as a treatment for pain and diabetes-related disorders.

Key words: *Phyllanthus reticulatus, antinociceptive activity,* anti-hyperglycemic.

Introduction

Phyllanthus reticulatus Poir. (Family: Euphorbiaceae, local name: chitki, pan chitki) is a large climbing shrub growing from 8-10 feet in height. The plant is found in the wild as well as fallow lands of Bangladesh. It is also present in India, where it is used in the traditional medicinal system of the country for treatment of a variety of ailments including small pox, syphilis, asthma, diarrhea, and bleeding from gums (Kirtikar, K.R., and Basu, B.D., 2003; The Wealth of India, 2005). In Bangladesh, the traditional medicinal practitioners (Kavirajes) use various parts of the plant like leaf, bark, root, stem, and fruit for treatment of edema, constipation, helminthiasis, dysentery, diarrhea, pain, and kidney, gall bladder, liver and gastrointestinal

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disorders of diabetic patients.

The phytochemicals, lupeol, lupeol acetate, and stigmasterol have been reported to be present in the plant Jamal *et al.,* (2008) Dichloromethane extract of leaves of the plant revealed presence of three compounds, (5R*,6R*)-4,6-dimethoxycarbonyl-5-[2 ,3 ,4 -trihydroxy-6 -(methoxycarbonyl) phenyl]-5,6-dihydro-2H-pyran-2-one along with 3,4,3 -tri-*O*-methylellagic acid, and methyl gallate. The first compound reportedly demonstrated weak insecticidal activity against *Spodoptera frugiperda* Pojchaijongdee *et al.,* (2010).

Ethanol extract of the plant has been observed to demonstrate hepatoprotective activity against carbon tetrachloride-induced liver damages in rats Dae *et al.,* (2008). Petroleum ether and ethanol extracts of leaves of the plant reportedly showed hypoglycemic effect in alloxan-induced diabetic mice, albeit at a high dose of 1000 mg/kg body weight. Phytochemical contents of the leaves included presence of terpenoid glycosides, protein, carbohydrates but absence of alkaloids and steroids Kumar *et al.,* (2008). Anti-plasmodial activity has been reported for extracts of the plant against both chloroquine-sensitive (K67) and chloroquine-resistant (ENT 36) strains of *Plasmodium falciparum* Omulokoli *et al.,* (1997).

Considering the folk medicinal uses of *Phyllanthus reticulatus* in Bangladesh, the objective of the present study was to evaluate the antinociceptive and anti-hyperglycemic effects of methanol extract of the leaves, respectively, in acetic acid-induced gastric pain writhing model, and oral glucose tolerance tests in glucose-loaded mice.

Materials and Methods

Plant material and extraction

The leaves of *Phyllanthus reticulatus* Poir. were collected from Gazipur, Bangladesh in December, 2009. The plant was taxonomically identified by Mr. Manzur-ul-Kadir Mia, ex-Principal Scientific Officer and Curator of Bangladesh National Herbarium at Dhaka. The leaves of *Phyllanthus reticulatus* were air-dried in the shade for 120 hours, grounded into a fine powder, and were extracted with methanol at a ratio of 1:3 (w/v). After 24 hrs, the mixture was filtered; filtrate was collected and the residue was again extracted with methanol at a ratio of 1:2 (w/v) for 24 hrs. Filtrates were combined and evaporated to dryness.

Chemicals and Drugs

Glacial acetic acid was obtained from Sigma Chemicals, USA; aspirin, glibenclamide and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

In the present study, Swiss albino mice (male), which weighed between 20-25g were used. The animals were obtained from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). All animals were kept under ambient temperature with 12h light followed by a 12h dark cycle. The animals were acclimatized for one week prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Acetic acid-induced writhing method

Antinociceptive activity of methanol extract of *Phyllanthus reticulatus* leaves was examined using previously described procedures of Deb *et al.,* (2010) with minor modifications. Pain was induced in mice in the writhing test through intraperitoneal administration of 1% acetic acid at a dose of 10 ml/kg body weight. Mice were separated into five groups of six mice each. Group-I served as control and was administered vehicle (1% Tween 80 in water, 10 ml/kg body weight). The standard drug, aspirin was administered to Group-II mice at a dose of 200 mg/kg body weight. Groups-III to V received extract, respectively at 50, 100 and 200 mg extract/kg body weight orally 30 min before acetic acid injection. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhings was counted for 10 min.

Anti-hyperglycemic activity

Glucose tolerance property of *Phyllanthus reticulatus* leaves was determined as per the procedure previously described by Joy and Kuttan, (1999) with minor modifications. In brief, fasted mice were grouped

into five groups of eight mice each. The various groups received different treatments like Group-I received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, group-II received standard drug (glibenclamide, 10 mg/kg body weight) and the other three groups (III-V) received the methanol extract of *Phyllanthus reticulatus* leaves at three different doses of 100, 200 and 400 mg/kg body weight. Each mouse was weighed and doses adjusted accordingly prior to administration of vehicle, standard drug, and test samples. All substances were orally administered. Following a period of one hour, all mice were orally administered 2 g glucose/kg of body weight. Blood samples were collected two hours after the glucose administration through puncturing heart. Serum glucose levels were measured by glucose oxidase method Venkatesh *et al.*, (2004).

Statistical analysis

Experimental values are expressed as mean ± SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.

Results and Discussion

Antinociceptive activity

The methanol extract of leaves of *Phyllanthus reticulatus* showed significant antinociceptive activity when administered to mice in acetic acid-induced gastric pain writhing tests. Maximum inhibition of writhing (39.1%) was observed at an extract dose of 200 mg/kg body weight. The standard drug, aspirin, when administered at a dose of 200 mg/kg body weight inhibited writhings by 50.4%. The results, shown in Table 1 demonstrate that the methanolic extract of leaves of *Phyllanthus reticulatus* could be used as a treatment for pain.

Acetic acid-induced writhing test is a suitable detector for both central and peripheral analgesia Shanmugasundaram and Venkataraman, (2005). Intraperitoneal administration of acetic acid (1%) leads to pain and inflammation involving a mechanism in which prostaglandins, mainly prostacyclines (PGI_2) and prostaglandin-E (PG-E) are produced, which have been reported to be responsible for excitation of the Ad-nerve fibers, leading to sensation of pain (Reynolds, 1982; Rang and Dale, 1993). Analgesia will be demonstrated then by any agent that lowers the number of writhing by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. Leaf extract of *Phyllanthus reticulatus* significantly caused reduction in the number of abdominal constrictions as well as stretching of hind limbs induced by the intraperitoneal injection of acetic acid. The results suggest that the methanolic extract of leaves may contain components, which when administered leads to inhibition of prostaglandin synthesis. The responsible component has not been identified in the present study and further laboratory investigations are on-going to identify the responsible components.

Table 1: Antinociceptive effect of crude methanol extract of *Phyllanthus reticulatus* leaves in the acetic acid-induced gastric pain model mice.

Groups	Dose (mg/kg body weight)	Mean no. of writhing	Inhibition (%)
Control (vehicle)	10 ml	11.5 ± 1.3	-
Aspirin	200 mg	5.7 ± 1.1	50.4*
Phyllanthus reticulatus	50 mg	7.7 ± 1.0	33.0*
Phyllanthus reticulatus	100 mg	7.8 ± 1.0	32.2*
Phyllanthus reticulatus	200 mg	7.0 ± 0.7	39.1*

All administrations were made orally. Values represented as mean ± SEM, (n=6); *$P < 0.05$; *significant compared to control.

Table 2: Effect of methanol extract of *Phyllanthus reticulatus* leaves on serum glucose level in hyperglycemic mice.

Treatment	Dose (mg/kg body weight)	Serum glucose level (mg/dl)	% lowering of serum glucose level
Control	10 ml	100.3 ± 4.9	-
Glibenclamide	10 mg	42.3 ± 5.2	57.8*
Phyllanthus reticulatus	100 mg	81.8 ± 6.4	18.4*
Phyllanthus reticulatus	200 mg	78.6 ± 7.8	21.6*
Phyllanthus reticulatus	400 mg	65.2 ± 2.8	35.0*

All administrations were made orally. Values represented as mean ± SEM, (n=8);*$P < 0.05$; significant compared to hyperglycemic control animals.

Anti-hyperglycemic effect

The results from the present study showed that the methanol extract of *Phyllanthus reticulatus* leaves exhibited dose-dependent and significant anti-hyperglycemic activity in glucose-induced hyperglycemic mice. Even at the lowest dose of the extract tested (100 mg/kg body weight) serum glucose levels were lowered by

18.4%. The maximum serum glucose lowering effect was found with the dose of 400 mg extract/kg body weight (35.0%). The standard drug, glibenclamide at a dose of 10 mg/kg body weight lowered serum glucose level by 57.8% (Table 2). The anti-hyperglycemic effect observed following administration of methanolic leaf extract suggests that the extract may potentiate pancreatic secretion of insulin or increase the glucose uptake (Nyunai *et al.,* 2009; Farjou *et al.,* 1987), or inhibit glucose absorption in gut Bhowmik *et al.,* (2009). The exact mechanism of action needs to be elucidated and can form the basis for further experiments.

The results of the present study demonstrate that the leaves of *Phyllanthus reticulatus* contains phytocomponents, which when administered, can lead to antinociceptive and anti-hyperglycemic effects in mice models. Overall, the results validate the folk medicinal use of the plant in Bangladesh as treatment of pain, or various organ complications in diabetic patients.

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