

ANTINOCICEPTIVE AND HYPOGLYCEMIC ACTIVITY OF METHANOLIC EXTRACT OF *Phlogacanthus thyrsoiflorus* NEES

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Received: 6 September 2011, Revised and Accepted: 5 October 2011

ABSTRACT

The aim of the present study was to evaluate the antinociceptive and hypoglycemic potential of the stem bark and leaf of the plant *Phlogacanthus thyrsoiflorus* Nees. The stem and leaf powders were extracted with methanol. The crude methanolic extract of stem bark was further partitioned with chloroform and petroleum ether respectively. The crude extracts of leaf and stem bark, chloroform and petroleum ether fractions of stem bark were subjected to acetic acid induced writhing and oral glucose tolerance test. The leaf and stem bark crude extract along with solvent fractions of stem bark of *Phlogacanthus thyrsoiflorus* Nees at a dose of 100 and 200mg/kg body weight were administered orally to the different groups of *swiss albino* mice and the percent inhibition of writhing was calculated. Reduction of blood glucose level was calculated using the same doses in hyperglycemic mice loaded with 2g/kg body weight of glucose after administration of extracts. Diclofenac Na and Glibenclamide were used as reference standards at a dose of 10mg/kg body weight respectively for evaluation of antinociceptive and hypoglycemic activity. The antinociceptive effect was dose dependent and all the extracts and fractions showed highly significant ($P < 0.05$) antinociceptive activity at a dose of 200mg/kg body weight. On the other hand, the methanolic crude extract of stem bark and leaf showed significant ($P < 0.05$) hypoglycemic activity at a dose of 200mg/kg body weight. The present study suggests highly significant antinociceptive and hypoglycemic activity of the plant which could be attributed to the presence of potential bioactive compounds.

Keywords: antinociceptive, hypoglycemic, *Phlogacanthus thyrsoiflorus* Nees, acetic acid induced writhing method, oral glucose tolerance test, Diclofenac Na, Glibenclamide.

INTRODUCTION

Pain may be defined as a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia) or can persist long after the precipitating injury has been healed (e.g. phantom limb pain)¹. An analgesic only relieve pain as symptoms, having no effects on its cause². Due to having adverse side effects, like gastric lesions, caused by NSAIDs and lesions and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects³.

Diabetes mellitus is a multifactorial disease which is characterized by hyperglycemia⁴, lipoprotein abnormalities⁴, raised basal metabolic rate⁵, defect in reactive oxygen species scavenging enzymes⁶ and altered intermediary metabolism of major food substances. Yet with the tremendous scientific advances witnessed in this century medical science cannot claim it knows all that needs to be known about this disease, including its management. At present, the oral anti-diabetic agents belong to sulphonylureas, biguanides, α -glucosidase inhibitors, thiazolidinediones and meglitinide derivatives. The major limitations of these drugs are their side effects. The high cost of modern treatment of diabetes indicates a great need for the development of alternative strategies for prevention and treatment of diabetes and since plants are the basic source of modern medicine, recently the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine and so far a number of hypoglycemic agents have been derived from different plant origin.

Phlogacanthus thyrsoiflorus Nees (Acanthaceae) locally known as rambasak is a large shrub found usually in the sub tropical Himalayas, Bihar, North Bengal, Assam and Bangladesh⁷. The leaves are 0.15-0.2m long. They are unequal and sword shaped. The flowers are orange or red. They occur in compact, compound flower arrangements at the end of branches⁸. The whole plant is used in whooping cough and menorrhagia. A decoction of leaves is given for diseases of spleen and liver and for fever⁷. Flowers are used as

antidote to pox, prevents skin diseases like sores, scabies⁹. Recent pharmacological studies on the leaves of this plant ascertained its central and peripheral analgesic activity¹⁰ and antimicrobial activity¹¹. The leaf of the plant also yielded a number of bioactive compounds e.g. β -sitosterol¹², lupeol¹², betulin¹², phloganthoside¹³, phlogantholide-A¹⁴ upon phytochemical investigation.

To our knowledge, no pharmacological investigation has been conducted so far on the stem bark of this plant. Previous study claimed antinociceptive activity of leaf part. The family Acanthaceae is well known for producing pharmacologically active compounds and also for its use in the oriental traditional medicine systems, particularly in the treatment of pain, inflammatory conditions, fever, infection and as a hypoglycemic agent e.g. *Andrographis paniculata* (Burm. f.) Wall. ex Nees¹⁵, *Adhatoda zeylanica* Medic¹⁶. This observation has driven the interest towards the investigation of the antinociceptive and hypoglycemic properties of the methanolic extracts of *Phlogacanthus thyrsoiflorus* Nees (Acanthaceae) in mice model.

MATERIALS AND METHODS

All chemicals used were of analytical grade and were obtained from BDH Chemicals, Ltd. Diclofenac Na (Voltalin), Glibenclamide (Dionil) were obtained from Novartis Pharma (BD) and Sanofi Aventis Pharma (BD) Limited. Instruments, Glass wares were procured from BDH, Germany. Sterile syringe (1ml, 100 divisions) (CHPL, India), Tuberculin syringe with ball shaped end (Merck, Germany), were obtained from a local pharmacy.

Collection of plant material

Plant sample (stem bark and leaf) of *Phlogacanthus thyrsoiflorus* Nees were collected from Sylhet, Bangladesh, in September 2009 and authentication of the plant sample was confirmed by Mr. Nasiruddin, the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka. A voucher specimen has been deposited (accession number is 35388) in the Herbarium for further reference.

Preparation and extraction procedure

The stem bark and the leaves after cutting into small pieces were sun dried for several days. The plant materials were further oven dried for 24 hours at considerably low temperature (40°C) for better

grinding. The dried samples were then ground in coarse powder using high capacity grinding machine. About 500g of stem bark and 1kg of leaf powdered material were taken in separate clean, round bottomed flask and dissolved with 3 liters and 5 liters of methanol respectively and kept for 5 days. The whole mixture was then filtered through cotton followed by Whatman No.1 filter paper and the filtrate thus obtained was concentrated at 40°C by rotary evaporation. The concentrated extract was then air dried to solid residue (yield 1.6% for stem bark and 2% for leaf). The stem bark crude extract was then partitioned between chloroform and petroleum ether and the stem bark, leaf crude extract along with solvent fractions were then subjected to pharmacological investigation.

Collection and maintenance of experimental animals

Swiss-albino mice of either sex, aged 4-5 weeks, weighing 20-25g each obtained from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B) were used for the experiment. The animals were acclimatized for 10 days before being used for the experiment. They were kept in clean and only dry polypropylene cages with 12:12 hour, light: dark cycle at 25±2°C and 45-55% relative humidity. The animals were fed with pelletized mice feed supplied from ICDDR,B and tap water. The experimental protocol was approved by the Institutional Ethical Review Committee.

Evaluation of antinociceptive activity

Preparation of the extracts

Required amount of extracts were measured and triturated unidirectional way by the addition of small amount of suspending agents Tween-80 and Dimethylsulphoxide. For proper mixing it was stirred well by vortex mixture. After proper mixing of Tween 80 and Dimethylsulphoxide with the extract, the volume was adjusted with the slow addition normal saline. To stabilize the suspension, it was stirred well by vortex mixture.

Experimental design

Fifty experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III, group-IV, group-V, group-VI, group-VII, group-VIII, group-IX, group-X consisting of five mice in each group. Group -I received 1% Tween-80 and DMSO(dimethylsulphoxide) in normal saline, termed as control group, Group-II received Diclofenac Na at a dose of 10mg/kg body weight, group-III to VI received the bark crude extract, its chloroform fraction, petroleum ether fraction, leaf crude extract respectively at a dose of 100mg/kg body weight. Group-VII to X received the same treatment as before at a dose of 200mg/kg body weight. Prior to any treatment, each mouse was weighed properly and the doses of the test samples, standard and control materials were adjusted accordingly.

Procedure

The antinociceptive activity of the extracts was assessed by the acetic acid induced writhing method¹⁷. At zero hour, test samples, control (1% Tween-80 solution in saline) and Diclofenac Na were administered orally by means of a long needle with a ball-shaped end. After 40 minutes, Acetic acid (0.7%) was administered intraperitoneally to each of the animals of all the groups. The 40 minutes interval between the oral administration of test materials and intraperitoneal administration of acetic acid was given to assure proper absorption of the administered samples. Five minutes after the administration of acetic acid, number of squirms or writhing were counted for each mouse for 15 minutes. Then the number of

writhing of the test samples were compared in relative to control and standard groups.

Evaluation of hypoglycemic activity

Preparation of the extracts

Required amount of extracts were measured and triturated unidirectional way by the addition of small amount of suspending agents Tween-80 and Dimethylsulphoxide. For proper mixing it was stirred well by vortex mixture. After proper mixing of Tween 80 and Dimethylsulphoxide with the extract, the volume was adjusted with the slow addition normal saline. To stabilize the suspension, it was stirred well by vortex mixture.

Experimental design

Fifty experimental animals were randomly selected and divided into ten groups denoted as group-I, group-II, group-III, group-IV, group-V, group-VI, group-VII, group-VIII, group-IX, group-X consisting of five mice in each group. Group -I received 1% Tween-80 and DMSO(dimethylsulphoxide) in normal saline, termed as control group, Group-II received Glibenclamide at a dose of 10mg/kg body weight, group-III to VI received the bark crude extract, its chloroform fraction, petroleum ether fraction, leaf crude extract respectively at dose of 100mg/kg body weight. Group-VII to X received the same treatment as before at a dose of 200mg/kg body weight. Prior to any treatment, each mouse was weighed properly and the doses of the test samples, standard and control materials were adjusted accordingly.

Procedure

The hypoglycemic effect was assessed by oral glucose tolerance test¹⁸. To perform the glucose tolerance test, mice were kept fasting overnight. Fasting blood glucose level of each mice was measured using glucometer and glucose oxidase-peroxidase reactive strips and it was denoted as 0 minute record. Blood sample was collected by cutting the tail tips with a sharp blade. After collection of blood, the tail tips were exposed to povidone iodine ointment to counteract the possibility of infection and inflammation. The extracts, standard and control samples were given orally with the help of feeding needle. All the groups were administered (2g/kg body weight) glucose solution orally 30 minutes after receiving the treatment. Blood glucose levels were measured 30 minutes, 90 minutes and 150 minutes after glucose administration.

Statistical Analysis

Statistical analysis was performed using SPSS-11.5 statistical Software for Windows. All values were expressed as mean± standard error of mean. One way ANOVA followed by Dunnett's test was used for statistical comparison. Statistical significance was considered to be indicated by $P < 0.05$.

RESULTS

All the extracts showed highly significant antinociceptive activity at both of the doses. But percent inhibition of writhing was higher at the dose of 200 mg/kg body weight [Table 1]. The most potent antinociceptive activity was obtained with the leaf crude extract and the petroleum ether fraction with an inhibition of writhing response 78.87% and 77.46% respectively at a dose of 200mg/kg body weight. The bark crude extract and the chloroform fraction also showed significant antinociceptive activity with inhibition of 64.97% and 67.6%. The order of antinociceptive activity at a dose of 100mg/kg body weight of the extracts was as follows: leaf crude extract > petroleum ether > chloroform > bark crude extract.

Table 1: Analgesic effect of extracts and different fractions of *Phlogacanthus thyriflorus* Nees

Group	Treatment	Acetic acid induced writhing response in mice	
		Mean	%inhibition
Control	1% Tween 80 and DMSO in 0.9% NS	42.6±0.68	0
Diclofenac Na	10 mg/kg	16.8±0.58*	60.56
Bark extract	100 mg/kg	32±1.5*	24.88
	200 mg/kg	15±0.54*	64.97
Chloroform fraction	100 mg/kg	31.8±1.5*	25.35
	200 mg/kg	13.8±0.98*	67.6
Petroleum ether fraction	100 mg/kg	24.4±1.2*	42.72
	200 mg/kg	9.6±1.1*	77.46
Leaf crude extract	100 mg/kg	20.6±1.02*	51.64
	200 mg/kg	9±0.89*	78.87

Values are expressed as mean ± standard error of mean.

*P<0.05 vs control

The bark crude extract exhibited significant hypoglycemic activity compared to control and maximum reduction was observed at a dose of 200 mg/kg body weight (29.6% reduction for bark crude extract after 90 minutes of glucose administration. The hypoglycemic effect persisted after 150 minutes of glucose administration for bark crude extract (31% reduction) compared to control.

Blood glucose level was increased for petroleum ether fraction and leaf crude extract by 18.57% and 22.24% after 90 minutes of glucose

administration compared to control. Glucose level was reduced by 4.5% for petroleum ether fraction after 150 minutes whereas leaf crude extract lowered the blood glucose level by 40.2% compared to control.

At a dose of 100 mg/kg body weight, the bark crude extract showed moderate reduction (13.2%) at 90 minutes interval. The leaf crude extract and the petroleum ether fraction did not show any hypoglycemic effect after 90 minutes, but after 150 minutes significant reduction was observed for leaf extract. [Table 2].

Table 2: Hypoglycemic effect of extracts and different fractions of *Phlogacanthus thyriflorus* Nees

Group	Treatment	Blood glucose level millimole/liter			
		0 min	30 min	90 min	150 min
Control	1% Tween 80 and DMSO in normal saline	7.14±0.34	10.2±0.53	9.8±0.67	8.5±0.21
Glibenclamide	10 mg/kg	5.4±0.89	7.5±0.13	4.62±0.43	3.32±0.28
Bark extract	100 mg/kg	6.5±0.2	10.2±0.71	8.5±0.18	7.2±0.16
	200 mg/kg	6.8±0.33	9.4±0.67	6.9±0.64*	5.8±0.69*
Chloroform fraction	100 mg/kg	6.9±0.19	10.2±0.29	9.0±0.43	7.9±0.82
	200 mg/kg	6.5±0.17	9.3 ±0.5	8.1±0.46	6.8±0.29
Petroleum ether fraction	100 mg/kg	6.5±0.54	9.5±0.16	12.2±0.66	9.1±0.65
	200 mg/kg	6.1±0.21	8.7±0.55	11.6±1.3	8.1±0.53
Leaf crude extract	100 mg/kg	5.3±0.28	9.1±0.22	12.8±0.76	7.5±0.31
	200 mg/kg	4.8±0.37	7.3±0.33	11.9±0.88	5.1±0.43*

Values are expressed as mean ± standard error of mean.

*P<0.05 vs control

DISCUSSIONS

From the study, it is demonstrated that, the bark crude extract and its fractions, the leaf extract possessed strong antinociceptive activity in a dose dependent manner. This suggests the presence of highly potent bioactive antinociceptive constituent(s) in the extracts and the fractions.

Intraperitoneal administration of acetic acid (0.7%), a pain stimulus, produces localized inflammation by releasing the free arachidonic acid from tissue phospholipids through the action of phospholipase A₂ and other acyl hydrolases¹⁷. The synthesis of the eicosanoids from arachidonic acid incorporates the following pathways: eicosanoids with ring structures, e.g. prostaglandins, thromboxanes and prostracyclines are synthesized via the cyclooxygenase pathway and the hydroxylated derivatives of straight chain fatty acids e.g. leucotrienes, HPETE(hydroperoxy eicosatetraenoic acid) and HETE(hydroxy eicosatetraenoic acid) are synthesized via the lipoxygenase pathway. The released prostaglandins, mainly prostacyclines and prostaglandin-E have been reported to be responsible for pain sensation by exciting the Aδ-fibers. Activity in the Aδ-fibers causes a sensation of sharp localized pain. Antinociceptive activity was determined by measuring the writhing effect which was produced by administration of the acetic acid and the inhibition of writhing effect produced by the test samples. Any agent that lowers the number of writhing demonstrates antinociception by inhibiting prostaglandin synthesis, a peripheral mechanism of pain inhibition. This hypothesis is in consonance with those authors who postulated that acetic acid induced writhing test methods are useful technique for the evaluation of peripherally and centrally acting analgesic drug¹⁷. Therefore it is reasonable to

assume that the antinociceptive effect of the extracts of *Phlogacanthus thyriflorus* Nees might have been mediated both peripherally and centrally.

Another finding was that, the bark and leaf crude extract possessed hypoglycemic activity at a dose of 200mg/kg body weight. The effect of bark crude extract was observed both after 90 and 150 minutes of glucose administration. This suggests the hypoglycemic compound(s) present in this extract might have sustained action. No significant effect was observed for the chloroform fraction, petroleum ether fraction, and the leaf crude extract after 90 minutes at both of the doses used, but after 150 minutes of glucose administration the hypoglycemic effect was sufficiently significant for the leaf crude extract at a dose of 200mg/kg body weight. It suggests that the hypoglycemic compound(s) in leaf crude extract is/are absorbed slowly or the compound(s) is/are not hypoglycemic itself, is/are broken down into metabolites with hypoglycemic potential and hence give delayed action.

The bark crude extract was able to restore the blood glucose level to the baseline value which indicates that it might contain potential hypoglycemic compound(s).

Glibenclamide works by blocking the ATP sensitive K⁺ channel in pancreatic cell, which causes membrane depolarization, influx of Ca⁺⁺ ions through voltage sensitive Ca⁺⁺ channel, degranulates insulin from myosin filament and releases into blood. The hypoglycemic effect might be resemble to that of Glibenclamide or it could be due to any extrapancreatic mechanism. Mechanism based

in vitro or *in vivo* studies are necessary to understand the mode of action of the extracts of the plant.

ACKNOWLEDGMENTS

The authors wish to thank ICDDR,B (International Research for Diarrhoeal Disease Research, Bangladesh) to assist in conducting the animal research.

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