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Antihyperglycaemic effect of flower of *Phlogacanthus Thyrsiflorus* Nees on streptozotocin induced diabetic mice

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ABSTRACT

Objective: To study the anti hyperglycaemic effect of aqueous extract of the flower of *Phlogacanthus thyrsiflorus* Nees in streptozotocin (STZ) induced diabetic mice. **Method:** The flower extract of *Phlogacanthus thyrsiflorus* in doses 100 and 200 mg/kg b.w was administered for 21 days and blood glucose level, serum cholesterol, liver glycogen was estimated. **Results:** Treatment of the streptozotocin induced diabetic mice with the flower extract resulted in significant reduction of blood glucose level ($P< 0.0001$), serum cholesterol ($P< 0.01$) and increase in liver glycogen ($P< 0.0001$). **Conclusions:** The results suggest that the flower extract of *P.thyrsiflorus* possess anti hyperglycaemic effect in streptozotocin induced diabetic mice which justifies the traditional use of this plant as ethnomedicine in treatment of diabetes.

1. Introduction

Diabetes mellitus is a complex and a multifarious group of disorders that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin[1]. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically[2].A variety of ingredients present in medicinal plants are thought to act on variety of targets by various modes and mechanisms. They have a potential to impart therapeutic effect in complicated disorders like Diabetes and its complications[3]. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand of antidiabetic medicinal plant which has comparatively less side effects. Indian traditional medicines belong to one of the richest medicinal systems are among those available in the world. Especially North Eastern part of India is blessed with a very rich biodiversity with a rich wealth of traditional knowledge which is yet to be explored. So more and more research is required to explore the traditional knowledge of this region. According to the recommendation of the WHO expert committee on Diabetes mellitus (WHO, 1980), an investigation of hypoglycaemic agents of plant origin used in traditional medicine have become more important[4].

Phlogacanthus thyrsiflorus Nees is found in the sub tropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam[5]. *Phlogacanthus thyrsiflorus* Nees is a medicinal herb which belongs to Acanthaceae family. It is known as Vasaka in Hindi. An evergreen shrub upto 2.4 m high, branchlets quadrangular, leaves are 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Flowers are in terminal elongated, thyrsoid panicles, upto 30cm long. Capsule is 3.8 cm long, linear clavate. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety flower. Calyx lobe is 6.8 mm, bristly haired. Bracts are 6 to 12 mm long. Seeds are disc like. Flowering occurs in the month of February to April[6]. Whole plant is used like Adhatoda vasica in Whooping cough and Menorrhagia. Fruits and leaves are burnt and it is prescribed for fever. The leaves are reported to contain diterpene lactone, Phlogantholide A. A decoction of leaves is also beneficial in liver and spleen diseases[5]. Jaintia tribe of Meghalaya uses fruit and leaf ash of *Phlogacanthus thyrsiflorus* Nees and use it to treat fever[7]. Ethanolic extract of *Phlogacanthus thyrsiflorus* Nees has analgesic activity on experimental mice[8]. *Phlogacanthus thyrsiflorus* Nees has

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antimicrobial activity also[9].The generation of free radicals has been implicated in the causation of several diseases of known and unknown etiologies such as Rheumatoid Arthritis, Cancer, Diabetes etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Phlogacanthus thyrsiflora Nees has prominent free radical scavenging property so it may prove as a very good medicinal herb[10].

2. Materials and Method

2.1. Chemicals

Streptozotocin and Glibenclamide was purchased from Sigma Chemical Co, St Louis, MO, USA. All other chemicals and reagents used were of analytical grade.

2.2. Plant material

The flowers of *Phlogacanthus thyrsiflorus* Nees were collected from local market in April 2011 and herbarium was prepared. The herbarium was identified for authenticity by the experts of Dept of Botany, Gauhati University, Assam. The flowers were thoroughly washed and shade dried.

2.3. Preparation of Plant extract

After shade drying the dried flowers were powdered in mixture grinder. The powdered flower was macerated with distilled water for 72 hrs at room temperature with occasional stirring. It was then filtered through Whatman filter paper. The filtrate was air dried and stored in refrigerator for further use as PTAE (*Phlogacanthus thyrsiflorus* aqueous extract). The yield of the extract was 10% (w/w). During experiment the crude extract was diluted with distilled water just before administration to animals.

2.4. Phytochemical screening

Phytochemical screening of the crude plant material was carried on using standard protocols for detection of flavonoid, phenol, tannin, saponin, steroid, alkaloid, carbohydrate.[11-15]

2.5. Experimental Animals

Healthy adult albino mice of both sexes (20-25 g) in house bred at the Animal house of Gauhati University, Assam, India were used for the study. Mice were housed in polypropylene cages lined with husk in standard environmental conditions and 12:12 light:dark cycle. The animals were fed on a standard pellet diet ad libitum and had free access to water. The experiments were performed after approval of the protocol by the Institutional Animal Ethics Committee (IAEC) and were carried out in accordance with the current guidelines for the care of laboratory animals.

2.6. Experimental Design

Antidiabetic activity of *Phlogacanthus thyrsiflorus* aqueous extract was assessed in normal, glucose loaded hyperglycaemic and streptozotocin induced diabetic mice. In all studies, the animals were fasted overnight for 16h with free access to water throughout the duration of the experiment.

2.7. Evaluation of extract on normal healthy mice

At the end of the fasting period taken as zero time (0 h), blood was withdrawn from the tail vein. Serum was separated by centrifugation and glucose was estimated. The animals were randomly divided into four groups of six animals each. Group 1 served as control and received only distilled water. Group II, III and IV received *P.thyrsiflorus* orally at the dose of 50, 100, 200 mg/kg. Blood glucose levels were determined in 1, 2, 3h following treatment[16].

2.8. Evaluation of extract in Oral glucose tolerance test

Healthy mice were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water) and Groups II, III and IV received *P.thyrsiflorus* orally at the dose level of 50, 100, 200 mg/kg, respectively. All the animals were given glucose (2g/kg) 60 min after dosing. Blood samples were collected from tail vein just prior to (0h) and at 30, 60, 90 and 120 min after glucose loading and blood glucose levels were estimated[16].

2.9. Evaluation of extract in streptozotocin induced diabetic mice

Experimental diabetes was induced by single intraperitoneal injection of 55mg/kg of Streptozotocin (STZ) freshly dissolved in distilled water. Control animals received only distilled water. After 48 hrs of Streptozotocin injection animals with fasting blood glucose above 200mg/dl were considered as diabetic and included in the study. The animals were randomly assigned into five groups of six animals each and received the following treatments: Group I: Normal control + distilled water, Group II: Diabetic control + distilled water, Group III: Diabetic + *P.thyrsiflorus* (100mg/kg), Group IV: Diabetic + *P.thyrsiflorus* (200mg/kg), Group V: Diabetic+ Glibenclamide (10mg/kg) [17].

The freshly prepared solutions were orally administered daily for 21 days. Body weights and blood glucose analysis (with the help of Glucometer) was done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Liver was dissected out, immediately rinsed in ice cold saline and stored for further biochemical analysis.

2.10. Biochemical analysis

Serum glucose analysis was done by GOD-POD method using Glucose Estimation kit (Crest Biosystems). Serum

Cholesterol was estimated spectrophotometrically (CHOP-PAP method, Crest Biosystems). Liver glycogen was estimated by the method of Seifter Sam et al (1950)[18].

2.11. Acute oral toxicity study

Acute oral toxicity of *P.thyrsiflorus* was performed on Swiss albino mice, according to OECD Guidelines 423. Two groups of three animals in each were used for the study. Group I received distilled water. Group II received oral dose of 1000mg/kg for 3 days. The animals were observed for gross behavioural, neural, autonomic and toxic effects at short intervals of time for 24 hrs and then daily for 7 days. Food consumption and body weights was monitored daily.

2.12. Statistical analysis

All results were expressed as Mean ± SEM. The significance of the difference between the means of test and control studies was established by student's t-test. P value less than 0.01, 0.001, 0.0001 were considered significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening of flower of *P.thyrsiflorus* showed the presence of flavonoid, phenol, tannin, saponin, steroid and trace amount of alkaloid.

3.2. Effect of P.thyrsiflorus aqueous extract on normoglycaemic mice

Results of the effect of graded doses of *P.thyrsiflorus* on blood glucose level in normal healthy mice are presented

Table 1.
Effect of phlogacanthus thyrsiflorus aqueous extract in normoglycaemic mice (Mean±SEM)(n=6)

SL NO	GROUPS	DOSES(mg/kg)	Blood glucose levels(mg/dl)			
			0hr	1hr	2hr	3hr
1.	I(control)	Distilled water	71±.58	74.6±.33	71±.58	71±.58
2.	II	50	74.5±.5	70.33±.33b	64±.58a	74.5±.5
3.	III	100	74.5±.5	70.33±.33b	62.6±.33b	74.5±.5
4.	IV	200	80.5±.5	74.6±.33	62.6±.33b	80.5±.5

aP< 0.01 when compared with corresponding values of control group
bP< 0.001 when compared with corresponding values of control group

Table 2.
Effect of p.thyrsiflorus on oral glucose tolerance in normal mice (Mean±SEM)(n=6)

Sl no	Groups	doses(Mg/kg)	Blood glucose levels(mg/dl)				
			0hr	30min	60min	90min	120min
1.	I(control)	Distilled water	80.5±.5	140.3±.33	170.3±.33	140.3±.33	130.3±.33
2.	II	50	74.5±.5	134.6±.33a	140.3±.33b	130.3±.33b	121±.58b
3.	III	100	74.5±.5	130.33±.33b	140.3±.33b	130.3±.33b	121±.58b
4.	IV	200	83.5±.5	124.3±.33b	134.3±.33b	126±.58b	116.3±.33b

aP< 0.001 when compared with corresponding values of control group
bP< 0.001 when compared with corresponding values of control group

Table 3.
Effect of p.thyrsiflorus on blood glucose in stz induced diabetic mice (Mean±SEM)(n=6)

Animal grouping	Blood glucose levels(mg/dl)			
Group	1th day	7th day	14th day	21st day
Control	94.7 ±5.64	96.7±5.70	94.5±5.61	95.1±5.62
Diabetic control	209.5±8.35	209.8±8.37	209.5±8.35	210.06±8.36
Treated 100mg/kg	204.06±8.24	194.6±8.05 a,b	164.2±7.39a,b	150.3±7.08a,b
Treated 200mg/kg	207.6±8.31	191.3±7.99a,b	160±7.30a,b	144±6.92a,b
Glibenclamide(10mg/kg)	207.6±8.31	189.6±7.94a,b	160±7.30a,b	140.3±6.84a,b

a P< 0.0001 compared to diabetic control
b p< 0.0001 compared to day 1of same group

Table 4.
Effect of p.thyrsiflorus on body weight of stz induced diabetic mice

Body weight (gm)				
Group	1st day	7th day	14th day	21st day
Control	25.06±2.88	25.06±2.88	25.6±2.91	25.6±2.91
Diabetic control	25.06±2.88	23.6±2.80	21.06±2.64	16.6±2.34
Treated 100mg/kg	25.6±2.91	23.6±2.80	25.6±2.91	26.6±2.97
Treated200mg/kg	26.2±2.94	25.7±2.97	25.8±2.97	26.6±2.97
Glibenclamide(10mg/kg)	25.8±2.97	23.6±2.80	24.9±2.91	27.2±3.00

in Table 1. *P.thyrsiflorus* produced peak hypoglycaemia at 2h. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. *P.thyrsiflorus* at dose 200mg/kg showed significant reduction in blood glucose ($P< 0.001$) when compared to control. Blood glucose levels were restored in all treatment group in 3h.

3.3. Effect of P.thyrsiflorus aqueous extract on oral glucose tolerance in normal mice

P.thyrsiflorus when administered 60 min prior to glucose loading produced significant reduction in the rise in blood glucose levels at 60 min after glucose administration which is shown in Table 2. Dose dependent blood glucose reduction was observed in animals treated with 50, 100, 200 mg/kg. All the doses showed significant reduction in blood glucose ($P< 0.001$) when compared to control.

Table 5.
Effect of p.thyrsiflorus on serum cholesterol and liver glycogen in stz induced diabetic mice

Group	Serum cholesterol(mg/dl)	Liver Glycogen(mg/g)
Control	41.6±.33	38.5±.35
Diabetic Control	82.4± 3.4207a	11.86±.338c
Treated 100mg/kg	55.6± .50b	29.6±.29d
Treated 200mg/kg	53.2± 1.41b	30.7±.87d
Glibenclamide(10mg/kg)	48.8± 2.83b	31.6±.27d

a $P< 0.001$ Compared to normal control
b$P< 0.01$ Compared to diabetic Control
c$P< 0.0001$ compared to the corresponding values of normal control
d$P< 0.0001$ compared to the corresponding values of diabetic control

3.4. Effect of P.thyrsiflorus aqueous extract on fasting blood glucose and body weight in STZ induced diabetic mice

The effect of repeated oral administration of *P.thyrsiflorus* on blood glucose levels in Streptozotocin induced diabetic mice and body weight is given in Table 3 and Table 4. *P.thyrsiflorus* administered in two different doses to Streptozotocin treated diabetic mice showed significant reduction of blood glucose levels which was related to dose and duration of the treatment. Maximum reduction was observed on day 21. *P.thyrsiflorus* in both doses 200mg/kg, 100mg/kg exhibited significant glucose lowering effect in diabetic mice ($P< 0.0001$) as compared to the control. Streptozotocin produced significant loss of body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while *P.thyrsiflorus* treated group at all the two doses showed improvement in body weight compared to diabetic control.

3.5. Effect of P.thyrsiflorus aqueous extract on serum cholesterol and Liver glycogen in STZ induced diabetic mice

P.thyrsiflorus treated group showed reduction in serum cholesterol compared to the diabetic control which is shown in Table 5. *P.thyrsiflorus* in both the doses 200mg/kg, 100mg/kg were effective in reducing the cholesterol levels ($P< 0.01$). Glycogen content in liver decreased in diabetic control compared to normal control. Administration of *P.thyrsiflorus* at the doses of 100 and 200 mg/kg for 21 days resulted in significant increase in the glycogen levels in liver ($P< 0.0001$) which is shown in Table 5.

3.6. Acute Oral Toxicity Study

P.thyrsiflorus showed no mortality or behavioural change upto 1000mg/kg in the animals.

4. Discussion

The study was undertaken to evaluate the hypoglycaemic activity of *P.thyrsiflorus* in normal, glucose loaded hyperglycaemic and streptozotocin induced diabetic mice. In normoglycaemic mice *P.thyrsiflorus* showed dose dependent hypoglycaemic effect in 2 h. From OGTT it could be concluded that dose 200mg/kg showed maximum improvement in glucose tolerance.

Streptozotocin significantly induced hyperglycaemia. Oral administration of *P.thyrsiflorus* for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which *P.thyrsiflorus* mediated its antidiabetic effect could be by improvement of pancreatic secretion of insulin from existing β cells of islets. The hypoglycaemic effect of *P.thyrsiflorus* was compared with Glibenclamide, a standard hypoglycaemic drug. From the present study it may be suggested that the mechanism of action of *P.thyrsiflorus* may be similar to glibenclamide action. So oral administration of *P.thyrsiflorus* has prominent hypoglycaemic effect.

Hypercholesteremia is one of the primary factor involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes[19]. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus; this is found in about 40% of diabetics[20].This abnormal increase of serum cholesterol is mainly due to uninhibited action of lipolytic hormones on the fat depots[21]. Earlier reports suggest that hypercholestrolaemia occurs in streptozotocin induced diabetic rats[21]. *P.thyrsiflorus* significantly reduced serum cholesterol in STZ diabetic mice. Thus it is reasonable to conclude that *Phlogacanthus thyrsiflorus* Nees could modulate blood cholesterol abnormalities.

Diabetes mellitus impairs the normal capacity of the liver to synthesise glycogen. Glycogen is the primary intracellular storable form of glucose and its level in various tissues especially in hepatic tissues are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthatase and inhibiting glycogen phosphorylase. Since destruction of β cells of islets of Langerhans results in marked decrease in insulin levels it is rational that glycogen level in liver tissue decrease as they depend on insulin for influx of glucose. A normal level of glycogen level reflects the normalization of insulin levels[21]. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and its activation appears to be defective in diabetes. It supports the findings of Grover et al[22]. Decrease

in hepatic glycogen was observed in this study. Treatment with *Phlogacanthus thyrsiflorus* Nees (100 and 200mg/kg) for 21 days significantly increased liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract.

Thus the significant antidiabetic effect of *Phlogacanthus thyrsiflorus* Nees could be due to the presence of various phytoconstituents detected in the phytochemical screening which alone can impart therapeutic effect. From this study we can conclude that aqueous extract of *Phlogacanthus thyrsiflorus* Nees flower has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes. Further studies are necessary to elucidate in detail the mechanism of action of the medicinal plant at the cellular and molecular levels. The studies on the effect of *P.thyrsiflorus* aqueous extract on lipid profiles and liver enzymes in Streptozotocin induced diabetic mice is going on in our laboratory.

Conflict of interest statement

We declare that we have no conflict of interest.

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