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Evaluation of Antidiabetic, Hypolipidemic and Hepatoprotective Activity of *Phlogacanthus thyrsiflorus* Nees in Streptozotocin induced Diabetic Mice: A 7 Days Intensive Study

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Abstract:

Objectives: To evaluate the antidiabetic, hypolipidemic and hepatoprotective activity in Streptozotocin (STZ) induced diabetic mice. **Method**: The flower extract of *Phlogacanthus thyrsiflorus* in doses 100 and 200 mg/kg b.w was orally administrated for 7 days and Glucose, Triglyceride, Cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosthatase (ALP), Urea, Creatinine was estimated. **Results**: Administration of the flower extract of *Phlogacanthus thyrsiflorus* Nees (100 and 200 mg/kg) for 7 days resulted in significant reduction in blood glucose level (P< 0.0001). Chronic effects of the extracts on serum biochemistry were also studied and it was found that there was significant reduction of serum cholesterol(P< 0.0001), triglycerides(P< 0.0001),Low density lipoprotein(LDL) (P< 0.0001), Very low density lipoprotein(VLDL) (P< 0.0001), Serum Glutamate Pyruvate Transaminase (SGPT) (P<0.0001) Serum Glutamate Oxaloacetate Transaminase (SGOT) (P<0.001), Alkaline Phosthatase (ALP) (P<0.0001), Urea (P<0.0001), Creatinine (P<0.01) but High density lipoprotein (HDL) level (P<0.0001) was found to be increased significantly after treatment. **Conclusion**: Thus this study shows that *Phlogacanthus thyrsiflorus* has antidiabetic, hypolipidemic, hepatoprotective activity and the extract should further be subjected to bioactivity guided drug discovery to isolate the active compound responsible for these activities.
Keywords: *Phlogacanthus thyrsiflorus*, Diabetes, Hypolipidemic, Hepatoprotective, Streptozotocin, Traditional medicine.

Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defect in insulin secretion, insulin action or both[1]. Globally the estimated incidence of diabetes and projection for the year 2030, as given by International Diabetes Federation(IDF) is 350 Million[2]. Synthetic antidiabetic agents can produce serious side effects. In view of the adverse effects associated with the synthetic drugs and considering natural medicine safer, cheaper and effective traditional antidiabetic plants can be explored[3]. Furthermore after the

recommendation made by WHO on diabetes mellitus, investigation on hypoglycemic agents from medicinal plants 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 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].In the current literature there is not much data concerning the effect of *Phlogacanthus thyrsiflorus* on the lipid parameters and hepatic enzymes which are abnormally altered in Diabetes.

Materials and Method

Chemicals:

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

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.

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.

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.

Induction of diabetes:

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.

Assessment of antidiabetic, hypolipidaemic response and effect on hepatic enzymes of extract in STZ induced diabetic mice:

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).

The freshly prepared solutions were orally administered daily for 7 days. At the end of the experimental period, the animals were fasted overnight and blood was collected for various biochemical estimations. Blood was collected in heparinized tubes, and the serum was separated by centrifugation. The animals were sacrificed by cervical decapitation.

Biochemical analysis:

Serum glucose was estimated by GOD-POD method using Glucose Estimation kit (Crest Biosystems), Serum Cholesterol was estimated spectrophotometrically (CHOP-PAP method,Crest. Biosystems). Triglyceride was estimated using diagnostic kit (GPO-PAP method, Crest Biosystems), HDL Cholesterol was also estimated using diagnostic kit (PEG ppt method, Crest Biosystems). The VLDL cholesterol was calculated using the formula (TG/5). The serum LDL cholesterol was estimated by the method of Friedwald *et al*. (1972)[11]. Serum Glutamate Pyruvate Transaminase (SGPT)[12], Serum Glutamate Oxaloacetate Transaminase (SGOT)[12], Alkaline Phosthatase (ALP)[13] were estimated by diagnostic kits of Crest Biosystems. Serum Creatinine (Alkaline picrate method)[14] and Serum Urea (Mod. Berthelot method)[15] was estimated by diagnostic kits of Crest Biosystems.

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.

Results:

Effect of *Phlogacanthus thyrsiflorus* on the lipid profile of STZ induced diabetic mice:

Serum glucose, cholesterol, triglyceride, LDL, VLDL were significantly higher in STZ treated mice ($P<0.0001$) compared to the normal mice except HDL which was lower in diabetic control ($P<0.0001$). The continuous treatment with the flower extract of *Phlogacanthus thyrsiflorus* for 7 days brought down the above lipid parameters in the diabetic mice which is shown in Table 1. The *Phlogacanthus thyrsiflorus* extract in both the doses ie 100mg/kg and 200 mg/kg reduced the cholesterol, triglyceride, LDL, VLDL level significantly ($P<0.0001$) but increased the HDL level significantly ($P<0.0001$) in diabetic mice when compared to diabetic control. The glucose level also decreased significantly in extract treated group ($P<0.0001$) when compared to diabetic control. The treatment of flower extract reversed the increased levels of these lipid ratios. It is evident from the study that *Phlogacanthus thyrsiflorus* has antidiabetic and hypolipidemic effect.

Effect of *Phlogacanthus thyrsiflorus* on the hepatic enzymes of STZ induced diabetic mice:

The levels of Serum Glutamate Pyruvate Transaminase (SGPT) Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase(ALP) were significantly higher in STZ induced diabetic mice ($P<0.0001$) when compared to normal control. The continuous treatment with the flower extract of *Phlogacanthus thyrsiflorus* for 7 days brought down the above hepatic enzymes in the diabetic mice which is shown in Table 2. The *Phlogacanthus thyrsiflorus* extract in both the doses ie 100mg/kg and 200 mg/kg reduced the SGPT($P<0.0001$), SGOT ($P<0.001$),ALP ($P<0.0001$) significantly when compared to diabetic control.

Effect of *Phlogacanthus thyrsiflorus* on the renal parameters of STZ induced diabetic mice:

Serum Urea($P< 0.0001$) and Serum Creatinine ($P<0.001$) increased significantly in diabetic control in comparison to normal control. Serum Urea ($P< 0.0001$) and Creatinine level ($P<0.01$) decreased significantly in

extract treated group when compared to diabetic control which is shown in Table 3.The treatment of the flower extract decreased the hepatic enzymes and also urea, creatinine levels thus have hepatoprotective and kidney protective activity.

Table 1: Effect of *Phlogacanthus thyrsiflorus* Nees on Glucose Level and Lipid Profile in STZ induced Diabetic Mice

Groups	Glucose (mg/dl)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	96.7±5.70	114.4±.30	101.2±.67	60.5±.34	33.5±.66	20.2±.13
Diabetic Control	209.8±8.37[a]	262.5±2.5[a]	201.2±1.0[a]	21.1±.10[a]	201.1±2.20[a]	40.2±.21[a]
Treated 100mg/kg	194.6±8.05 [b]	150.7±.55[b]	135.6±.60[b]	32.2±.25[b]	91.4±.40[b]	27.1±.10[b]
Treated 200mg/kg	191.3±7.9[b]	141.3±1.0[b]	131.1±1.05[b]	35.0±.00[b]	80.05±.85[b]	26.2±.19[b]
Glibenclamide (10mg/kg)	189.6±7.94[b]	131.05±.95[b]	120.2±.25[b]	37.2±.25[b]	69.7±.75[b]	24.05±.05[b]

Blood Glucose, Cholesterol, Triglyceride, HDL, LDL, VLDL values are expressed as mg/dl and each value is mean± SEM of 6 observation, [a] P< 0.0001 Compared to normal control, [b]P< 0.0001 Compared to diabetic Control

Table 2: Effect of *Phlogacanthus thyrsiflorus* Nees 7 Days treatment on the Hepatic Enzymes in Stz induced Diabetic Mice

Groups	SGPT (u/ml)	SGOT(u/ml)	Alkaline Phosphatase (KA Unit)
Control	27.5±.29	23.5±.28	12.2±.15
Diabetic Control	59.6±.40[a]	61.2±.25[a]	25.2±.25[a]
Treated 100mg/kg	45.1±.10[b]	55.2±.25[c]	17.2±.25[b]
Treated 200 mg/kg	42.2±.25[b]	51.7±.75[c]	15.2±.25[b]
Glibenclamide (10mg/kg)	40.2±.25[b]	50.2±.25[c]	15.3±.30[b]

SGPT , SGOT values expressed as u/ml and ALP value expressed as KA Unit and each value is mean± SEM of 6 observation, [a] P< 0.0001 Compared to normal control, [b]P< 0.0001 Compared to diabetic Control,[c]P<0.001 Compared to diabetic Control

Table 3: Effect of *Phlogacanthus thyrsiflorus* Nees 7 Days treatment on Renal Parameters in Stz induced Diabetic Mice

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	21.4±.30	1.1±.05
Diabetic Control	55.1±.10[a]	2.1±.10[c]
Treated 100mg/kg	44.3±.10[b]	.92±.025[d]
Treated 200 mg/kg	40.7±.75[b]	.96±.06[d]
Glibenclamide (10mg/kg)	39.7±.25[b]	.80±.00[d]

Serum Urea and Creatinine values expressed as mg/dl and each value is mean± SEM of 6 observation [a] P< 0.0001 Compared to normal control, [b]P< 0.0001 Compared to diabetic Control,[c] P<0.001 Compared to normal control, [d] P<0.01 Compared to diabetic control

Discussion

Lipid Profile has been shown to be an important predictor for metabolic disturbances during diabetes. Higher concentration of Serum TC and TG in diabetes may be attributed to inhibition of cholesterol catabolism or may be due to insulin deficiency and mobilization of fatty acids from adipose tissues by lipolysis. Administration of PTAE showed a significant decrease in TC and TG with increased HDL Cholesterol significantly as compared to diabetic control mice. The increase of HDL Cholesterol is accompanied by increased catabolism of VLDL and replacement of TG in the core of HDL with Cholesterol[16,17]. This hypolidemic effect of extract has prognostic significance as free fatty acid are atherogenic in diabetes[18].

The elevation of biomarker enzymes such as SGPT, SGOT and ALP indicates hepatic damage[19]. The diabetic complication such as gluconeogenesis and ketogenesis may be due to increased transaminase activity[20]. It may be due to STZ mediated liver damages which may cause leaking of enzymes into blood[21]. Asparate amino transaminases and Alanine transaminases were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats[22].The elevated enzymatic activity of SGOT with only moderately increase of SGPT activity suggests cardiac damage while elevated activity of SGPT with only moderate increase in SGOT activity suggests liver damage[23].In the present study SGPT, SGOT, ALP decreased significantly in extract treated group in comparison to diabetic contol.

Kidney plays an important role to remove the metabolic waste such as creatinine and urea from the body, thereby helping to maintain body homeostasis of above substances. The persistant hyperglycaemia, haemodynamic changes within the kidney tissue and free radical generation mediated stress in diabetes produce renal dysfunction which results in elevation of urea, creatinine in blood[24-26]. Diabetic animals manifest negative nitrogen balance due to enhanced activity of proteases enzymes responsible for breakdown of proteins into amino acids in the muscles and other tissues leading to increased production of urea in the body[27]. Thus hyperuremia in blood reflects either increased synthesis of urea or its increased excretion. Creatinine is the breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body. Creatinine is chiefly filtered out of the blood by the kidneys. If the filtering ability of the kidney is deficient, blood creatinine levels arise. Therefore level in blood and urine may be used to assess the renal function. Oxidative stress in diabetes cause renal dysfunction[28].Administration of *P.thyrsiflorus* decreased the urea and creatinine level significantly in extract treated group in comparison to diabetic control. In the present study elevated level of SGPT, SGOT, ALP, Urea and Creatinine suggested liver and kidney damage after the administration of STZ to the mice compared to normal control mice. Administration of PTAE and Glibenclamide to the diabetic mice significantly reduced SGPT, SGOT, ALP, Urea and Creatinine which represents the protective action of PTAE on liver and kidney in diabetic condition.

In conclusion, the present study indicates that aqueous extract of *Phlogacanthus thyrsiflorus* possess significant antihyperglycaemic, hypolipidemic, hepatoprotective activity. Continuous treatment of diabetic animals with the plant extract for one week improved the lipid, hepatic enzymes, renal parameters. From this study it can be concluded that *P.thyrsiflorus* has antidiabetic activity which shows its effect in even 7 days treatment. Further studies of long duration is required in this field.

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