

Hypolipidaemic and Hepatoprotective activity of *Phlogacanthus thyrsoiflorus* Nees Flower extract in Streptozotocin induced Diabetic Mice

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Abstract: In this study, the hypolipidaemic and hepatoprotective effect of aqueous extract of *Phlogacanthus thyrsoiflorus* Nees was investigated in streptozotocin induced diabetic mice. Method: The flower extract of *Phlogacanthus thyrsoiflorus* in doses 100 and 200 mg/kg b.w was administered for 21 days and 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 Phosphatase (ALP). Results: Administration of the flower extract of *Phlogacanthus thyrsoiflorus* Nees (100 and 200 mg/kg) for 21 days resulted in significant reduction in serum cholesterol ($P < 0.0001$), Triglyceride ($p < 0.0001$). In addition to that significant decrease in high density lipoprotein (HDL) ($P < 0.0001$) whereas significant increase in low density lipoprotein (LDL) ($P < 0.0001$), very low density lipoprotein (VLDL) were observed in diabetic mice, which were normalized after 21 days of flower treatment. In addition to that there was a significant increase in hepatic enzymes Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) ($P < 0.0001$) in diabetic mice but after the administration of the flower extract the enzymes levels reduced significantly. Conclusion: The flower extract at both the doses 200mg/kg and 100mg/kg have significant hypolipidaemic and hepatoprotective effect in streptozotocin induced diabetic mice.

Keywords: Diabetes mellitus, Lipoproteins, Hepatic enzyme, Hypolipidaemic effect, Hepatoprotective effect, *Phlogacanthus thyrsoiflorus*.

Introduction:

Diabetes mellitus is a metabolic disorder resulting from defect in insulin secretion, insulin action or both^[1]. It represents a heterogeneous group of disorders having hyperglycaemia which is due to impaired carbohydrate utilization resulting from a defective or deficient insulin secretory response. Along with hyperglycaemia there are also abnormalities in serum lipids, Carbohydrate, Protein metabolism which increases the risk of cardiovascular disease complications^[2]. The two forms of Diabetes, type 1 and 2 differ in their basic mechanisms of development and in physiologic characteristics such as association with obesity, age, and insulin. Both types of diabetes share the common characteristics of hyperglycaemia, micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects. Moreover alteration of lipoproteins metabolism are involved in the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way^[3]. Alteration of hepatic enzymes are also common in diabetes. To date there are different groups of oral hypoglycaemic agents for clinical use having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural product with antidiabetic activity and less side effects. Traditionally many herbal medicines and

medicinal plants have been used for the treatment of diabetes as an alternative medicine^[3]. 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 seems important.

Phlogacanthus thyrsoiflorus Nees is found in the sub tropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam^[4]. *Phlogacanthus thyrsoiflorus* 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^[5]. 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^[4]. Jaintia tribe of Meghalaya uses fruit and leaf ash of *Phlogacanthus thyrsoiflorus* Nees and use it to treat fever^[6]. Ethanolic extract of *Phlogacanthus thyrsoiflorus* Nees has analgesic activity on experimental mice^[7]. *Phlogacanthus thyrsoiflorus* Nees has antimicrobial activity also^[8]. 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 thyrsoiflora* Nees has prominent free radical scavenging property so it may prove as a very good medicinal herb^[9]. In the current literature there is not much data concerning the effect of *Phlogacanthus thyrsoiflorus* on the lipid parameters and hepatic enzymes which are abnormally altered in Diabetes.

Therefore the present study has been planned to investigate how the flower extract of *Phlogacanthus thyrsoiflorus* Nees influences lipid parameters and hepatic enzymes in streptozotocin induced diabetic mice and it was compared with Glibenclamide as a reference standard.

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 thyrsoiflorus* 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 thyrsoiflorus* 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 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 21 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 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)^[10]. Serum Glutamate Pyruvate Transaminase (SGPT)^[11], Serum Glutamate Oxaloacetate Transaminase (SGOT)^[11], Alkaline Phosthatase (ALP)^[12] were estimated by diagnostic kits of Crest Biosystems. Serum Creatinine (Alkaline picrate method)^[13] and Serum Urea (Mod. Berthelot method)^[14] was estimated by diagnostic kits of Crest Biosystems.

Statistical analysis

All results were expressed as mean \pm 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 0.001, 0.0001 were considered significant.

Results

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

Serum 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 21 days brought down the above lipid parameters in the diabetic mice to almost normal levels 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 treatment of flower extract reversed the increased levels of these lipid ratios and normalized soon after.

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 Phosthatase(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 21 days brought down the above hepatic enzymes in the diabetic mice to almost normal levels which is shown in Table 2. The *Phlogacanthus thyrsiflorus* extract in both the doses ie 100mg/kg and 200 mg/kg reduced the SGPT, SGOT significantly ($P < 0.0001$) when compared to diabetic control. *P.thyrsiflorus* in 200mg/kg reduced the ALP level significantly ($P < 0.0001$) *P.thyrsiflorus* in 100mg/kg also

reduced the ALP level significantly ($P < 0.001$) when compared to diabetic control. The treatment of the flower extract normalizes the hepatic enzymes thus have hepatoprotective activity.

Effect of *Phlogacanthus thyriflorus* on 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**.

Table 1: Effect of *Phlogacanthus thyriflorus* Nees on the Lipid Profile in STZ induced Diabetic Mice

GROUPS	TOTAL CHOLESTEROL (mg/dl)	TRIGLYCERIDE (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	91.06±.58	72.8±.21	40.6±.34	36.0±.65	14.5±.06
Diabetic Control	201.7±1.15 ^a	204.5±.50 ^a	27.1±.10 ^a	132.6±.40 ^a	40.9±.10 ^a
Treated 100mg/kg	122.06±1.50 ^b	72.5±.50 ^b	44.5±.50 ^b	63.5±1.90 ^b	14.5±.10 ^b
Treated 200mg/kg	121±.57 ^b	74.5±.50 ^b	46.5±.50 ^b	59.0±1.0 ^b	14.9±.01 ^b
Glibenclamide(10mg/kg)	95.7±.93 ^b	70.5±.50 ^b	47.5±.50 ^b	35.0±1.00 ^b	14.1±.10 ^b

^a $P < 0.0001$ Compared to normal control

^b $P < 0.0001$ Compared to diabetic Control

Table 2: Effect of *Phlogacanthus thyriflorus* Nees on the Hepatic enzymes in STZ induced Diabetic Mice

GROUPS	SGPT (u/ml)	SGOT (u/ml)	ALKALINE PHOSPHATASE (KA Unit)
Control	33.0±.57	21.4±.30	10.5±.28
Diabetic Control	44.4±.30 ^a	50.6±.34 ^a	20.3±.33 ^a
Treated 100mg/kg	28.3±.31 ^b	26.2±.11 ^b	14.6±.33 ^c
Treated 200 mg/kg	30.3±.17 ^b	23.1±.05 ^b	11.7±.37 ^b
Glibenclamide (10mg/kg)	28.4±.30 ^b	24.06±.06 ^b	11.1±.16 ^b

^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 thyriflorus* Nees 21 days treatment on Renal parameters in STZ induced Diabetic Mice

GROUPS	UREA (mg/dl)	CREATININE(mg/dl)
Control	21.4±.30	.68±.04
Diabetic Control	59.7±.25 ^a	2.45±.05 ^c
Treated 100mg/kg	24.2±.25 ^b	.94±.04 ^d
Treated 200 mg/kg	21.7±.25 ^b	.80±.00 ^d
Glibenclamide (10mg/kg)	20.7±.75 ^b	.72±.02 ^d

^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:

Hyperglycaemia a primary clinical manifestation of diabetes contributes to diabetic complications by altering vascular matrix and circulating lipoproteins is associated with an increased risk of coronary heart disease^[15]. High level of total cholesterol is one of the major factors of coronary heart diseases and it is well known that hyperlipidemia and the incidence of atherosclerosis is increased in diabetes. The liver and some

other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of lipoproteins. Lowering of serum lipid levels through drug therapy seems to be associated with a lower risk of vascular diseases and related complications^[16]. The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia^[17,18] and contribute to coronary heart disease^[19]. Many herbs and plant products have been shown to have antihyperglycaemic and antihyperlipidaemic properties^[20]. The elevated triglyceride in diabetic animals might be due to the consequence of increased synthesis of triglycerides rich lipoprotein particles (very low density lipoprotein VLDL) in liver and diminished catabolism^[21]. The hypolipidemic effect may be due to inhibition of fatty acid synthesis^[22,23]. This abnormal increase of serum lipid is mainly due to the decrease in the action of lipolytic hormones on the fat depots mainly due to the action of insulin. Under normal circumstances insulin activates the enzyme lipoprotein lipase which hydrolyses triglyceride and insulin deficiency resulting in hypertriglyceridemia^[24,25]. In the present study also the diabetic mice showed hypercholesterolemia and hypertriglyceridemia and treatment with flower extract for 21 days significantly reduced both cholesterol ($P < 0.0001$) and triglyceride ($P < 0.0001$) which shows that the flower extract possesses definite hypotriglyceridemic properties in STZ diabetic mice shown in Table 1. Moreover there is also abnormal increase in LDL, VLDL levels and decrease in HDL levels in diabetic mice but the treatment with *Phlogacanthus thyrsoiflorus* extract reduced the LDL, VLDL significantly ($P < 0.0001$) and increased the HDL level significantly ($P < 0.0001$) in diabetic mice shown in Table 1.

Table 2 summarised the effect of streptozotocin on the activity of the hepatic marker enzymes in serum. In the present study the levels of SGPT, SGOT, ALP in streptozotocin induced diabetic mice were elevated. It may be due to STZ mediated liver damages which may cause leaking of enzymes into blood^[26]. Aspartate amino transaminases and Alanine transaminases were used as markers to assess the extent of liver damage in streptozotocin induced diabetic rats^[27]. Treatment STZ induced diabetic mice with plant extract caused reduction in the activity of these enzymes compared to the diabetic control and consequently may alleviate liver damage caused by STZ induced diabetes. These results are in agreement with those obtained by El Demerdash et al 2005^[28]. In this study the aqueous extract of *P.thyrsoiflorus* decreased the activity of SGPT ($P < 0.0001$), SGOT ($P < 0.0001$), ALP ($P < 0.0001$) significantly in liver of mice intoxicated with streptozotocin. The effect of glibenclamide on the recovery of hepatic enzyme activity in serum was very similar to that of the earlier study^[29]. The restoration of SGPT, SGOT, ALP to their respective normal levels after treatment with both glibenclamide and *P.thyrsoiflorus* extract strengthen the antidiabetic effect of these extract. Moreover SGPT, SGOT, ALP levels also acts as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver since streptozotocin can also affect the liver by free radical mechanism.

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^[30]. 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^[31]. Administration of *P.thyrsoiflorus* decreased the urea and creatinine level significantly in extract treated group in comparison to diabetic control.

From this study we can conclude that *Phlogacanthus thyrsoiflorus* flower extract has beneficial effects on hyperlipidemia due to diabetes, hepatoprotective and also kidney protective effect. Further pharmacological and biochemical investigation are underway to elucidate the mechanism of the antidiabetic, hypolipidemic, hepatoprotective effect of *Phlogacanthus thyrsoiflorus*.

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