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Evaluation of anti-diabetic efficacy of the leaves and flower of *Phlogacanthus thyrsoiflorus* Nees

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Abstract

Diabetes mellitus (DM) is a metabolic disorder in which blood glucose level is abnormally high resulting from defects in insulin production, insulin action or both. The number of individual diagnosed by this disease is increasing worldwide. It is diagnosed with a global incidence of 463 million and more than 62 million individuals in India. *Phlogacanthus thyrsoiflorus* Nees is a traditional plant with medicinal property which is used in the treatment of diabetes, wounds, whooping cough, menorrhagia, tumours and as a blood purifier. This study compares the antidiabetic property of leaves and flowers for establishing qualitative and quantitative standards on *Phlogacanthus thyrsoiflorus*'s leaves and flowers. The antidiabetic study was done in-vivo in albino rats using streptozocin as the toxicant. The rats were treated for twenty-one days. The data obtained from the ethanolic extracts of leaves and flowers shows the effect in lowering the blood glucose level and maintains other biochemical parameters in the test group which was compared with the group treated with standard drug metformin and negative control group. The leaf and flower of *Phlogacanthus thyrsoiflorus* produced significant antidiabetic effects in comparison to standard and diabetic control.

Keywords: *Phlogacanthus thyrsoiflorus* Nees, pharmacognosy, biochemical investigation, antidiabetic activity

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, insulin action or both. With the help of the hormone insulin, cells throughout the body absorb glucose and use it for energy. Diabetes develops when the body doesn't make enough insulin or is not able to use insulin effectively, or both.

Phlogacanthus thyrsoiflorus Nees. is a medicinal plant belonging to the family Acanthaceae. In Assam, this plant is known as Titaaphul. The plants can be seen growing mostly during Dec-April and is distributed throughout the tropics and in the entire North East Region of India. Titaaphul is a found in subtropical Himalayas, from Garhwal to Bhutan, and North Bengal, at altitudes up to 1000m. Whole plant is used in whooping cough and menorrhagia. Fruits and leaves are used in burnt and prescribed for fevers. In North Eastern India, flowers are used for treating wounds, tumours growth and as a blood purifier. From the ethnobotanical study of Singh *et al.* the folk medicinal uses of *Phlogacanthus thyrsoiformis* Nees are cold, cough, influenza, easy deliver of child birth, abortion, irregular menstruation, diarrhea, dysentery, cholera, high blood pressure control, boils, small pox, skin problems, sprains, body ache, constipation and burns. In Assam, this plant is used in herbal recipe or bitter vegetable during 'Bohag Bihu', the main festival of Assam.

Materials and Methods**Plant material**

The leaves and flower of *Phlogacanthus thyrsoiflorus* Nees were collected from Dibrugarh University Campus, Assam, India in the month of July, 2016. The plant was identified and authenticated taxonomically by Botanical Survey of India, Shillong (No-BSI/ERC/Tech/2017/710 Dated: 27/3/2017). The leaves of the plant were cut into pieces. Both the leaves and flowers were washed thoroughly with water and then dried partially under sunlight and partially under the shade for a week. The dried leaves and flowers were then pulverized in a mechanical grinder to coarse powder and then stored in air tight containers free from moisture for further use.

Chemicals and diagnostic kit

Streptozotocin (Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, India), metformin (Sigma-Aldrich Chemical Company), one touch glucometer accurure (Microgene Diagnostic Systems Pvt. Ltd.) were used in this study.

Preparation of plant extract

The powdered crude drug of *Phlogacanthus thyrsoiflorus* Nees, leaves and flowers were extracted by Soxhlet extraction (Continuous hot extraction) with petroleum ether and ethanol subsequently for 18 hours at 40-60 °C to defat the material and 65 °C respectively. The ethanol extract was concentrated and dried and stored in a desiccator for use in animal study experiments.

Anti-Diabetic Activity

Animals and experimental design

Male albino rats of Wistar strain and weighing about 80-200gm were obtained from Sh. Sanjay Saha, Proprietor, Saha Enterprise, Kolkata and used for experimental study. All the protocols were approved by the Institutional Animal Ethics Committee (IAEC), Dibrugarh University, Assam and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals); vide approval number 1576/GO/Ere/S/11/CPCSEA, dated: 30/3/2015. The animal house was well ventilated and maintained at room temperature of 20-25 ± 2 °C, 30-35% of relative humidity and 12 hours dark/light cycle. The animals were housed in large spacious hygienic cages during the course of the experimental period and were provided with pellet diet and water ad libitum. Prior to the experimental study, animals were fasted by depriving them of food for 16 hr but allowing free access of water. Animals were kept for 1 week to acclimatize to laboratory conditions before starting the experiment, they were allowed to free access for water and standard rat feed.

Induction of experimental diabetes

Ethanollic extracts of *Phlogacanthus thyrsoiflorus* were assessed by induced Streptozotocin in diabetic mice. Streptozotocin (65mg/kg i.p.) treated rats were provided with 10% glucose solution after 3 hours for the next 24 hours to prevent fatal hypoglycemia.

Experimental design

Animals with blood glucose level above 250 mg/dl were selected and divided in to five groups comprising five animals in each group. Group I: Normal control+ distilled water, Group II: Diabetic control+ distilled water, Group III: Diabetic+ Standard drug (10mg/kg), Group IV: Diabetic+ *P. thyrsoiflorus* (leaves extract 200mg/kg), Group V: Diabetic + *P. thyrsoiflorus* (flower extract 200mg/kg).

Blood samples were collected from the tail vein of the overnight (12-15 hr) fasted rats and blood glucose level was determined on 0th, 5th, 10th and 15th day. On the 15th day all the animals were sacrificed and blood were collected from the animals and centrifuged at 4000 rpm for 5minutes to separate the serum for the biochemical parameters as well as histopathology of the pancreas tissue.

Biochemical investigation

At 15th day of treatment blood were collected from the animals and centrifuged at 4000 rpm for 5minutes to separate the serum. From the serum different biochemical parameters like Total cholesterol (TC), High Density Lipoprotein (HDL), Triglycerides (TG), Aspartate transaminase (SGOT), Alanine transaminase (SGPT), Alkaline phosphatase (ALP) and Total Protein levels (TP) in serum were measured colorimetrically.

Statistical analysis

The datas obtained from the experiment 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, 0.05 were considered significant.

Results

Effect of ethanolic extract of *Phlogacanthus thyrsoiflorus* Nees. (EEPT) in diabetic mice

The effect of Test drug (200mg/kg of bodyweight) i.e. EEPT (leaves and flower) on fasting blood level were investigated in the Streptozotocin- induced diabetic Wister albino rat; using metformin hydrochloride as standard drug (10 mg/kg of bodyweight). The divergence of body weights of the animals and the mean blood glucose levels of controlled groups and test groups were noted as shown in the Fig. 1 on 0th, 5th, 10th and 15th day of treatment. From the various articles OGTT shows that dose 200 mg/kg showed maximum improvement in glucose tolerance. Hypoglycemic effect was observed in animals treated with ethanolic leaves and flower extract of *Phlogacanthus thyrsoiflorus* Nees.

Effect of ethanolic extract of *Phlogacanthus thyrsoiflorus* Nees on the lipid profile of STZ induced diabetic mice

Alanine transaminase (SGPT), aspartate transaminase (SGOT), alkaline phosphate (ALP), cholesterol and triglyceride were significantly lower in STZ treated mice compared to the normal mice except total protein value which was lower in diabetic control. The continuous treatment with the leaves and flowers extract of *Phlogacanthus thyrsoiflorus* Nees. for 21 days brought the lipid parameters in the diabetic mice to almost normal levels, but the leaves extract of ethanol shows better result compared to the ethanolic extract of flower. The treatment of leaves extract reversed the increased levels of these lipid ratios and normalized soon after.

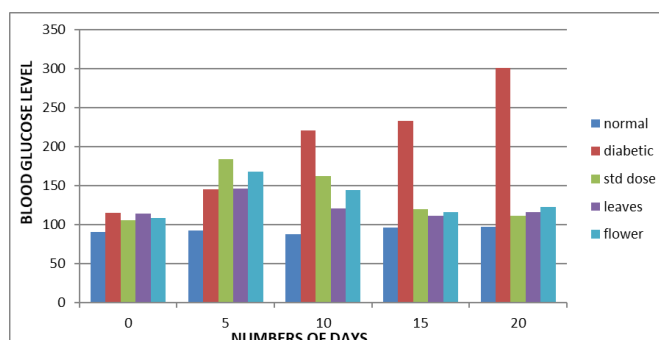


Fig 1: Fasting blood glucose level

Table 1: Biochemical parameters levels at 21st day (Values are mean \pm SEM (n=5), statistical significance: # P > 0.05, **P < 0.01, *P < 0.05; compared with diabetic control group II)

Groups	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Triglyceride (mg/dl)
G-I Normal control	29.25 \pm 3.326 [#]	20.0 \pm 0.4082 ^{**}	11.5 \pm 0.6455 ^{**}	7.45 \pm 0.2630 ^{**}	69.05 \pm 0.5694 ^{**}	72.125 \pm 0.4270 ^{**}
G-II Diabetic control	40.75 \pm 2.955	48.25 \pm 3.119	19.0 \pm 1.871	5.25 \pm 0.4787	93.5 \pm 1.190	196.95 \pm 2.823
G-III Standard control	21.75 \pm 3.198 ^{**}	21.0 \pm 1.291 ^{**}	11.25 \pm 0.6292 ^{**}	6.45 \pm 0.2398 [*]	79.5 \pm 0.6455 ^{**}	74.25 \pm 1.315 ^{**}
G-IV EEPT of leaves (200mg/kg)	25.25 \pm 1.702 ^{**}	23.75 \pm 2.529 ^{**}	12.0 \pm 0.9129 ^{**}	7.1 \pm 0.1472 ^{**}	82.0 \pm 1.354 ^{**}	76.25 \pm 1.315 ^{**}
G-V EEPT of flower (200mg/kg)	31.25 \pm 3.497 [#]	26.25 \pm 1.750 ^{**}	16.0 \pm 0.7071 [#]	6.2 \pm 0.2041 [#]	84.0 \pm 0.4082 ^{**}	78.75 \pm 0.4787 ^{**}

Histology

Normal group: The slide shows of cell compactness, and there is no cell integrity. The islet boundaries are clear and the profiles of the islet cells are clearly visible. The duct area is prominent no necrosis or fatty degeneration observed. The acinar cells are stained strongly and found to arranged in lobules with prominent nuclei. The islet cells were found to be embedded in between the acinar cells. The overall architecture was found to normal and healthy.

Diabetic Control group: The slide shows of cell compactness, and there is no cell integrity. The islet boundaries are clear and the profiles of the islet cells are clearly visible. The duct area is prominent no necrosis or fatty degeneration observed. The acinar cells are stained strongly and found to arranged in lobules with prominent nuclei. The islet cells were found to be embedded in between the acinar cells. The overall architecture was found to normal and healthy.

Standard drug treated group: The slide shows of cell compactness, and there is no cell integrity. The islet

boundaries are clear and the profiles of the islet cells are clearly visible. The duct area is prominent no necrosis or fatty degeneration observed. The acinar cells are stained strongly and found to arranged in lobules with prominent nuclei. The islet cells were found to be embedded in between the acinar cells. The overall architecture was found to normal and healthy.

EEPT of Leaves (200 mg/kg): The architecture of cells were reverted back to normal with a large proportion of islets cells observed in good health. The acinar cell arrangements were found to be normalized sufficiently as compared to positive control group.

EEPT of Flower (200 mg/kg): Fatty layers degradation has noticed. The duct area is prominent and ballonic present. The compactness of cells is comparatively better than diabetic control. In most portions cellular integrity is normal with slight fatty degeneration. Disarrangement of architecture was observed with no islets cells around along with some artifacts of fixation. The remaining acinar cells were observed to be normal as compared positive control group.

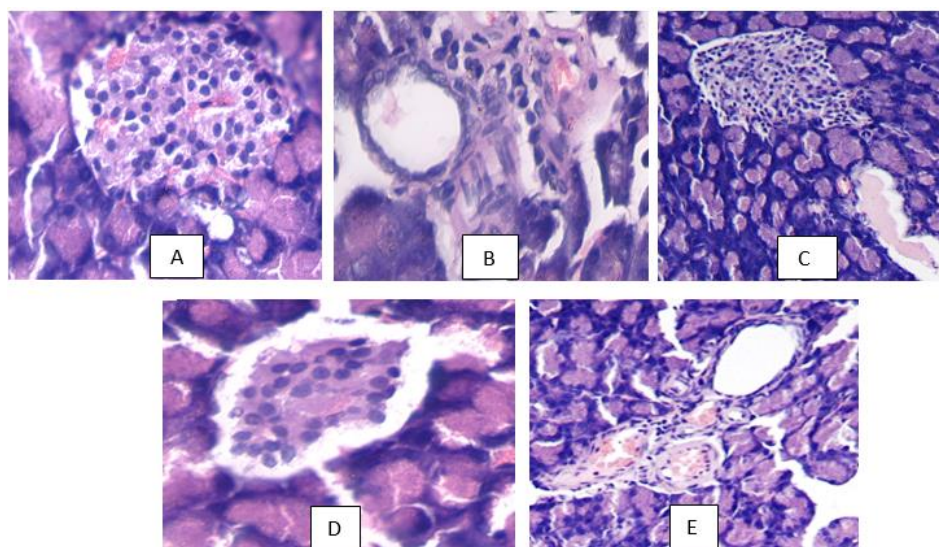


Fig 2: Histopathology of the pancreas of the rats in Anti-diabetic study (Where A= Normal group pancreas, B= Diabetic control group pancreas, C= Standard drug treated group pancreas, D= EEPT of leaves dose (200mg/kg BW) group pancreas, E= EEPT of flower dose (200mg/kg BW) group pancreas.)

Discussion

Natural product research is the work horse behind the discoveries of bioactive compounds which serve as a framework for the development of newer drugs with improved features including the inseparable safety and efficacy criteria. In-vivo evaluation of the antidiabetic activity of ethanolic extract of the leaves and flower of *Phlogacanthus thyrsoiflorus* Nees. was carried out for 14 day animal study protocol and measurement of different biochemical parameters, and histopathological study against

Streptozotocin induced diabetes mellitus. From the histopathological studies it was seen that in EEPT of leaves (200 mg/kg b.w.) the overall architecture of the cells seemed to be reverted back to normal and the integrity of the cells were also very much better as compared with the EEPT of flower (200 mg/kg b.w.).

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