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Phytochemical Evaluation and Antidiabetic Potential of Trichosanthes Dioica Roxb. in Streptozotocin Induced Diabetic Rats

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Abstracts

Ethan Pharmacological Relevance: *Trichosanthes diocia* Roxb. (Family: Cucurbitaceae) has been long used as a folk medicine to treat anti-inflammatory, antihelmintic, skin eruptions, liver congestion, antidiabetic activity of the plant extract.

Materials and Methods: The phytochemical tests to detect the presence of different compounds were based on the visual observation of color change or formation precipitate after the addition of specific reagents. Diabetes was induced in rats by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 55mg/kg bw. Administration of extract of *Trichosanthes diocia* (800mg/kg/p.o) were studied for their effect on blood glucose level in streptozotocin induced(55mg/kg/i.p) diabetic rats. The blood glucose levels were estimated by glucometer.

Result: *Trichosanthes diocia* extract reduced the levels of blood glucose; the study scientifically validates the traditional use of *T. diocia* in diabetes management and could be developed as an effective oral agent for treating diabetes mellitus.

Conclusions: *Trichosanthes diocia* exhibits considerable antidiabetic activity. Our study suggests that further detailed studies and mechanism of action of *T. dioica* would be useful for undertaking human trials.

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Introduction

Diabetes Mellitus (DM), characterized by hyperglycemia and carbohydrate, protein and fat metabolism disturbances, is a wide-spread metabolic disease [1]. Knowledge about DM existed in ancient Egypt and Greece. The word 'diabetes' is derived from the Greek word "Diab" (meaning to pass through, referring to the cycle of heavy thirst and frequent urination); 'mellitus' is the Latin word for "sweetened with honey" (refers to the presence of sugar in the urine). Earliest reference about a disease with 'polyurea' was made in "Ebers Papyrus" (Egypt), a document outlining clinical symptoms of the disease (1550 BC). Greeks had a knowledge of a disease (Celsus, 30-38 AD) accompanied by polyurea and wasting of body, whereas Aretaeus of Cappadocia (150 AD) mentioned a disease characterized by thirst and polyurea which was christened as Diabetes. Subsequently, the knowledge permeated to Chinese (Tehang Tehong King, 200 AD), Iranians (Rhazes (860- 932 AD) and Arabians (Avicena, 980-1037 AD). [2].

Diabetes mellitus and its complications are becoming a global burden and have to be deal with firmly. Hypercholesterolemia associated with this dreaded disease [3,4]. Has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases [5]. World Health Organization (WHO) has recommended the development of oral hypoglycemic agents from medicinal plants [6] as herbal natural remedies to treat diabetes mellitus being cost-effective and safe [7]. Many plants have been explored scientifically and systematically and proved to be beneficial for the treatment of diabetes mellitus. The present study is a further effort in the direction of developing a novel, oral antidiabetic agent with high potential with minimal or no side effects.

Trichosanthes dioica (T. dioica) Roxb. (Family: Cucurbitaceae) is commonly known as "Sespadula" in English and "Parwal" in Hindi and is widely grown throughout India [8]. Its fruits are used as vegetable from the time immemorial and have also been proved as hypocholesterolemic and hypoglyceridimic in case of normal animals after shade drying and mixing in the food [9].

Table 1: Preliminary phytochemical screening of root extract of *Trichosanthes dioica*.

Chemical compound	Ethanolic root extract of *T.diocia*	Ethylacetate root extract of *T.diocia*
Alkaloids	+ + +	+ + +
Flavonoids	+ + +	+ +
Proteins	–	–
Reducing sugar	+ + +	+ + +
Saponin	+	+
Steroids	+ +	++
Tanin	–	–

Table 2: Preliminary phytochemical screening of stem extract of *Trichosanthes dioica*.

Chemical compound	Ethanolic stem extract of *T.diocia*	Ethylacetate stem extract of *T.diocia*
Alkaloids	+ + +	+ + +
Flavonoids	+ + +	+ + +
Proteins	+	+
Reducing sugar	+ + +	+ + +
Saponin	+	+
Steroids	++	+ +
Tanin	–	–

High concentration+++, Moderate concentration++, Less concentration +, Absent –

Phytochemical investigation of its fruits and seeds reveal the presence of kall those classes of compounds which are responsible either for managing diabetes or its complications, namely, flavonoids [10,11], alkaloids [12-17], glycosides [18,19], terpenes, and sterols [20-22]. This plant also serves as a rich source of minerals such as Mg, Na, K, Cu, and S [23] whose significant role in controlling and managing diabetes is well known and cannot be ignored as specific concentration of these minerals have been reported to take part in carbohydrate metabolism as well as insulin release [24-26]. The present study was designed to scientifically validate the use of *T. dioica* in folklore medicines for treating diabetes by evaluating its glycemic potential. The effect was observed on blood glucose level (BGL) of normal and streptozotocin- (STZ-) induced diabetic rats. Biochemical parameters such as level of blood glucose (BGL) were considered. Results of all valuable scientific measures taken into consideration reveal that along with anti diabetic activities.

Materials and Methods

Animals

Healthy adult male and female Wistar rats of body weight 150–200 g were employed in present study. Animals procured from the disease free small animal house, Lala Lajpat Rai university of veterinary and animal sciences, hissar, all animals were housed under laboratory conditions with alternating light and dark cycles of 12h each. They had free access to food and water. The experimental protocols were approved by institutional animal ethics committee, M.D University, Rohtak.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, MO, USA. The chemicals were of analytical grade.

Plant material

Trichosanthes dioica roots and stem collected freshly from the adjacent areas of Maharshi dayanand University. The plant was identified at the Herbarium of Botany Department in kurukshetra University. A voucher specimen (No. kuk/mdu/ips/41) was deposited in the Botany Department of kurukshetra University.

Preparation of the ethanolic and ethyl acetate extract

Plant material were drying and size reduction in to coarse powdered. The powdered material was charged into soxhlet apparatus and successive hot continuous extraction was carried out by using different solvents. Each time before extraction with the next solvent the powdered material was air dried. Each extract was concentrated by distilling off the solvent to obtain the crude extract. The drug was extracted with each solvent till complete extraction is affected (about 40 cycles). The color, consistency and percentage extractive values will be calculated.

Phytochemical screening of extract

Qualitative testing of the extract for alkaloids, tannin, flavonoids, steroids, saponins and carbohydrates, sugar and protein was carried out according to the method described by [27].

Induction of experimental diabetes

Diabetes was induced by a single intraperetonial injection of freshly prepared Streptozotocin (STZ) (purchased from Sigma Aldrich Chem. Co., St. Louis, USA) at a standard dose of 55mg/ kg^{-1} bw [28] in 0.1Mcitrate buffer (pH4.5) to a group of overnight fasted rats. After 3 days of STZ administration, the rats with serum glucose level above 250 mg/kg were selected for the experiment.

Experimental procedure

In the experiment a total of 18 rats (15 diabetic surviving rats, 3 normal rats) were used. The rats were divided into 6 groups of 3 rats each.

Group 1: Normal rats.

Group 2: Diabetic control.

Group 3: Diabetic rats were treated with Metformin orally (500mg/kg/day) in distilled water using an intragastic tube for 14 days.

Table 3: Effect of ethanolic extract of *T.diocia* root on blood glucose in experimental groups.

Group	Blood glucose(mg/dl) Single administration	4 days	8 days	12 days	14 days
Normal saline	90.2±2.4	91±2.5	93 ±1.8	95 ±2.3	92 ±2.5
Diabetic control	269±2.5	272±1.9	295 ±1.4	302±2.6	300 ±2.8
Diabetic + metformin (500mg/kg)	270 ±1.5	250 ±1.7	228 ±1.2	193 ± 2.9	176 ±1.5
Diabetic + *T.diocia* root ethnolic extract (800mg/kg)	287 ±2.1	260 ±1.2	233 ±1.3	202 ±1.5	193±1.9
Diabetic + *T.diocia* stem ethnolic extract (800mg/kg)	281±1.2	258±1.3	230 ±1.2	198 ±1.3	189±1.5
Diabetic +*T.diocia* stem	270 ±1.5	260 ±1.6	248 ±1.8	220 ±1.9	220±1.7

Table 3 demonstrate the level of blood glucose and experimental animals after single day and at the end of 5, 10, and 14 days of treatment. There was a significant increase in blood glucose in diabetic rats. The administration of *T.diocia* to diabetic rats resulted in a significant decrease in the level of blood glucose. The administration of *T.diocia* ethanolic extract of root at the dose of 800 mg/kg body weight showed a highly significant effect. In addition, the effect of *T.diocia* after single administration and that of after 5 days treatment are not significant. In the *T.diocia* treated groups, although a significant anti hyperglycemic effect was evident from the 10th day onwards, the decrease in blood glucose was highly significant on 14th day in the group treated with 800 mg/kg body weight. The study was extended further and a significant decrease in blood glucose was obtained on 14th day of treatment. As the effect of *T.diocia* at a dose of 800 mg/kg body weight was more effective in 14 days treatment, on repeated administration of ethanolic and ethyl acetate extract *T.diocia* stem daily up to 14 days exhibited significant antidiabtic activity in Streptozotocin induced diabetic rats. However, at the end of 14 day of treatment,there was decrease the blood glucose level. Compared with diabetic control.

Group 4: Diabetic rats given *T.diocia* root suspension of ethanolic extract orally (100 mg/kg body weight) using an intragastric tube for 14 days.

Group 5: Diabetic rats given *T.diocia* stem suspension of ethyl acetate extract orally (800 mg/kg body weight) using an intragastric tube for 14 days.

Group 6: Diabetic rats given *T.*diocia stem suspension of ethanolic extract orally (800 mg/kg body Weight) using an intragastric tube for 14 days.

The suspension of each extract was prepared in distilled water with help of CMC (0.5% w/v).

Estimation

Fasting blood samples were collected from the tail vein from the afore mentioned over night fasted rats, and the fasting glucose level estimated by glucometer , the treatment was continue for 14 days of drug admistration, daily estimation of blood glucose level determined using glucometer 14 days.

Result

The result of preliminary phytochemical screening of ethanolic and ethyl acetate extract of *Trichosanthase diocia* root and stem for the detection of various chemical constitutes are given in (Table 1 and 2).

Discussion

Currently-available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective anti diabetic drugs [29-31]. Overall result of the present investigation demonstrate that the *T.diocia* stem and root can significantly reduce the blood glucose levels of Streptozotocin induced diabetic rats. These results provide scientific evidence in support of the anti diabetic potential of *T.diocia* .There are various types of phytoconstituent present in the plant material belonging to different chemical classes Table 3. Phytoconstituents like alkaloids inhibits alpha glucosidase and decrease glucose transport through the intestinal epithelium. Imidzoline compounds stimulate insulin secretion in a glucose dependent manner. Polysaccharides increase the level of serum insulin, reduced the blood glucose level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose. Flavonoids suppress the glucose level, saponin stimulates the release of insulin and blocks the formation of glucose in the bloodstream [32].

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