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Antihyperglycemic activity of *Trichosanthes tricuspidata* root extract

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Abstract

To evaluate the anti-diabetic activity of ethanolic extract of *Trichosanthes tricuspidata* root in alloxan induced diabetic rats and to perform the phytochemical screening. The extraction, preliminary phytochemical screening, anti-diabetic activity in alloxan induced diabetic rats by oral administration of extract (200 and 400 mg/kg b.w.), the blood glucose level and biochemical parameters like cholesterol, triglyceride, serum protein, SGPT, SGOT, and ALP were estimated. Phytochemical studies shows the presence of carbohydrates, proteins, glycosides and terpenoids and the ethanolic extract of *T. tricuspidata* root significantly lowered the blood sugar level. The above findings justified the traditional claim of anti-diabetic activity in this plant.

Introduction

The use of natural products in primary healthcare has been interesting part since long. Numerous plants have been identified for their anti-diabetic activity. Several plants such as *Actinodaphne hookeri*, *Aegle marmelos*, *Bombax ceiba, Cajanus scarabaeoides*, *Eulophia herbacea, Gymnema lactiferum*, *Lagerstroemia speciosa*, *Mangifera indica*, *Meyna spinosa, Nigella sativa*, *Teucrium stocksianum* have shown antidiabetic effect (Prajapati et al., 2008; Ravi et al., 2009; Pattanayak et al., 2009; Tatiya et al., 2013; Bandara et al., 2009; Saha et al., 2009; Bhowmik et al., 2009; Sen et al., 2013; Khanam et al., 2009; Alamgeer et al., 2013). *Trichosanthes tricuspidata* (Family: Cucurbitaceae), a wild growing tropical plant possesses various medicinal properties. *T. tricuspidata* is considered to be medicinally important in several traditional systems. In *ayurvedic* medicines, the fruits are used in the treatment of asthma, earache and ozoena (intranasal crusting, atrophy and fetid odor). The seeds are emetic and a good purgative. In the Thai traditional system of medicine, the plant is used as an anti-fever remedy, a laxative, an anthelmintic as well as in migraine treatments (Kanchanapoom et al., 2002). The roots of the plant are used to treat lung diseases in cattle and for the treatment of diabetic carbuncles and headaches (Chopra et al., 1956). The use of this plant in curing bronchitis, and the application of seed paste for hoof and mouth disease in cattle was reported (Gaur, 1999).

Medicinal plants play an important role in the management of diabetes mellitus especially in developing countries where resources are meager. Several medicinal plants have been used as dietary adjunct and in the treatment of numerous diseases without proper knowledge of their function. Although phytotherapy continues to be used in several countries, few plants have received scientific or medical scrutiny. Moreover, a large number of medicinal plants possess some degree of toxicity. For example, it was reported that about one third of medicinal plants used in the treatment of diabetes are considered to be toxic (Marles and Farnsworth, 1994). A number of pharmacologically important phytochemicals, such as cucurbitacins and trichotetrol, have been isolated from this plant. This report has provided an introduction to the panoply of reported therapeutic uses of *T. tricuspidata*. Mohamed

(Mohamed et al., 1974) isolated a tetrahydroxy pentacyclic triterpene "trichotetrol" from the root extract of this vine. From the fruits of *T. tricuspidata*, 14 cucurbitane glycosides were isolated (Kasai et al., 1999). An extract of the fruits of this plant was found to be cytotoxic in KB cells, and two new cucurbitacins were reported: tricuspidatin and 2-O-glucocucurbitacin J (Maile et al., 2002). Kaneda and Uchikoba (Kaneda and Uchikoba, 1994) reported a protease from the sarcocarp of the fruits of this plant. The root contains methyl palmitate, palmitic acid, suberic acid, α-spinasterol, stigmast-7-en-3β-ol, α-spinasterol 3-o-β-D-glucopyranoside, stigmast-7-en-3β-ol-3-O-β-D-glucopyranoside, glyceryl 1-palmi-tate, glyceryl 1-stearate, bryonolic acid, cucurbitacin B, isocucurbitacin B, 3-epi-isocucurbitacin B, 23,24-dihy-drocucurbitacin D, isocucurbitacin D and D-glucose. The roots of *T. tricuspidata* contain more than 6 times more cucurbitacin than the roots of *T. kirilowii* Maxim. Var. *japonicum* Kitam (Kitajima et al., 1989). Kasai et al. (1999) isolated 3 new cycloartane glycosides, named cyclotricuspidosides A, B and C, from the leaf and stem parts. Randomized trials should ultimately be conducted to rigorously evaluate the safety and efficacy of this popular medicinal plant. The present study was taken up to evaluate the anti-diabetic effect of ethanolic root extract of *T. tricuspidata linn*.

Materials and Methods

Plant material

The roots of *T. tricuspidata* (Family: Cucurbitaceae) were collected at Yercaud in Salem District, Tamil Nadu, India and was authenticated by the Botanist Dr. A. Balasubramanian, Joint Director, ABS Botanical Conservation Research and Training, Southern circle, Kaaripatti, Salem-638 106 T.N. India. The voucher specimen has been deposited in the herbarium of ABS BCRT.

Preparation of extract

The roots were washed with distilled water in order to remove soil material, shade dried and grinded to course powder. The powdered root material was evenly packed in the soxlet apparatus and continuous hot extraction was carried out using solvents like petroleum ether, chloroform, and ethanol individually. The solvent used were purified after each extraction. The extract was filtered through Whatman filter paper to remove any impurities if present. The extracts were concentrated by vacuum distillation. Then the concentrated extracts were placed in desiccators to remove the excess moisture. The dried extract were kept in airtight container and used for further studies.

Phytochemical screening

To identify the phytoconstituents, ethanolic extract of *T. tricuspidata* roots were subjected to preliminary phytochemical screening (Kokate, 1994; Harborne et al., 2005; Persinos et al., 1967; Peach, 1955), which was carried out using standard procedure.

Animals

The healthy male Wister albino rats of weighing about 150-200 g were procured from the Animal House of Nandha College of Pharmacy and maintained the temperature at 22 ± 2°C with the 12 hours light/dark cycle. Feed and water were provided *ad libitum*. Animals were deprived of food and allowed free access to water.

Evaluation of anti-diabetic activity

The animals were allowed to fast for 12 hours prior to the induction of diabetes. Diabetes was induced by single dose of intraperitoneal injection of freshly prepared alloxan monohydrate, 150 mg/kg (Sigma-Aldrich, Bangalore) in normal saline. After 1 hour of alloxan administration, the animals were fed standard pellets and water *ad libitum*. The animals were stabilized in the diabetic state over a period of 21 days; the blood glucose level was estimated (by GOD-POD method) and rats with blood glucose level of 250 mg/dL or higher were considered to be diabetic and selected for the experiment. There was no mortality rate of the animals.

Diabetic animals were randomly assigned to groups. The experimental rats were divided into five groups (n = 5). Group I animals served as normal control. Group II served as diabetic control. Group III animals of diabetic rats were received the reference standard drug glibenclamide (5 mg/kg, i.p.) at a single dose per day (for 21 days), as positive control and groups of IV and V received the ethanolic extracts of *T. tricuspidata* root at the doses of 200 and 400 mg/kg, respectively.

Biochemical Estimation

Determination of blood glucose

A small amount of blood was collected from the tail vein of animal by snipping off the tip of the tail end used to determine the blood glucose level. As bleeding starts, the animal were held close to the Pulsated blood glucose test strip and allowed the drop of blood to fall on the strip. The pulsatum glucometer was switched on and test was allowed to react with the blood. After few seconds the blood glucose level was displayed on screen.

Estimation of biochemical parameters in blood serum

After the completion of experiment, the blood were collected through the retro orbital puncture of eye of animal under mild anesthesia in Eppendorff's tube (1 mL) containing 50 µL of anticoagulant (10%trisodium citrate) and the serum was separated by centrifuging at 6,000 rpm for 15 min. The biochemical parameters

cholesterol, triglyceride, serum protein, SGPT, SGOT, and ALP are determined by using the Ecoline kit.

Statistical analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Dunnet's t-test. Statistical significance was set at $p<0.001$, $p<0.01$, $p<0.0$

Results

The preliminary phytochemical screening of the ethanolic extract of *T. tricuspidata* roots revealed that the presence of carbohydrates, proteins, glycosides and terpenoids.

Analysis of data shows a decrease in the blood glucose level on treatment with the ethanolic extract of roots (200 and 400 mg/kg orally for 21 days). The blood glucose level was significantly ($p<0.01$) high in alloxan control rats compared with normal control. But the level of blood glucose was significantly decreased ($p<0.01$) in diabetic rats treated with ethanolic extract as compared with diabetic control rats (Suresh et al., 2009). The 400 mg/kg dose was found better than 200 mg/kg when compared with diabetic control (Table I).

There was a significant ($p<0.01$) increases in serum cholesterol, serum triglycerides, serum VLDL, serum alkaline phosphatase, SGOT and SGPT in diabetic control rats when compared to the normal control. The above parameters were decreased significantly ($p<0.01$) by Glibenclamide and the ethanolic extract of *T. tricuspidata* roots due to 21 days of treatment (Table II and III).

Table I

Effect of ethanolic extract of *T. tricuspidata* root on blood glucose and total protein

Group	Blood glucose (mg/dL)	Total protein (g/dL)
Normal control (2 mL/kg)	115.1 ± 1.4	6.5 ± 0.8
Diabetic control (alloxan monohydrate 150 mg/kg)	337.8 ± 2.4	2.3 ± 0.6
Extract 200 mg/kg	233.7 ± 1.2	4.0 ± 1.1
Extract 400 mg/kg	196.9 ± 1.2	6.1 ± 0.9
Glibenclamide 5 mg/kg	188.1 ± 5.6	7.2 ± 0.8

Data represents mean ± SD; n=5

Discussion

The present study investigates the anti-diabetic and hypolipidemic effect of roots of *T. tricuspidata* on alloxan-monohydrate induced diabetic rats. Insulin is a naturally occurring hormone in the blood which helps

Table II

Effect of ethanolic extract of *Trichosanthes tricuspidata* root on serum cholesterol, triglycerides and VLDL of control and experimental animals

Group	Cholesterol (mg/dL)	Triglyceride (g/dL)	VLDL (mg/dL)
Normal control (2 mL/kg b.w.)	180.2 ± 1.2	127.0 ± 0.9	25.5 ± 1.5
Diabetic control (alloxan monohydrate 150 mg/kg)	238.7 ± 2.9	152.4 ± 2.5	30.5 ± 1.4
Extract 200 mg/kg	215.2 ± 1.5	142.9 ± 1.3	25.6 ± 1.8
Extract 400 mg/kg	190.6 ± 1.0	130.4 ± 0.9	26.1 ± 2.5
Glibenclamide 5 mg/kg	185.1 ± 1.8	126.2 ± 1.9	24.2 ± 2.6

Data represents mean ± S.D; n = 5

Table III

Effect of ethanolic extract of *Trichosanthes tricuspidata* root on SALP, SGOT and SGPT of control and experimental animals

Group	Alkaline phosphatase (IU/L)	SGOT (IU/L)	SGPT (IU/L)
Normal control (2 mL/kg)	285.3 ± 12.6	34.2 ± 1.8	41.8 ± 0.5
Diabetic control (alloxan 150 mg/kg)	552.8 ± 11.7	71.5 ± 3.5	88.5 ± 1.9
Extract 200 mg/kg	371.0 ± 12.2	62.4 ± 2.9	73.0 ± 1.6
Extract 400 mg/kg	283.6 ± 12.2	35.1 ± 2.9	40.3 ± 1.5
Glibenclamide 5 mg/kg	358.1 ± 10.8	56.4 ± 3.6	68.5 ± 1.4

Data represents mean ± S.D; n=5

sugar to move through the bloodstream into the cells. When glucose cannot enter our cells, it builds up in the blood (hyperglycemia) which damages organs like eyes, kidneys, blood vessels and nerves. This is caused by deficiency, inherited and/or acquired, in production of insulin by the pancreas (type I), or by resistance to the insulin produced (type II). The basic mechanism underlying hyperglycemia in diabetes mellitus involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased consumption of glucose by the tissues (Halberstam et al., 1996).

Medicinal plants could be considered as potential sources for providing a reasonable amount of the required elements other than diet to the patients of diabetes mellitus (Subbiah et al., 2006). In alloxan monohydrate induced diabetic rats there was a significant increase in lipids, total cholesterol, triglycerides ($p<0.01$). In *T. tricuspidata* root treated rats, there was a reduction in cholesterol, triglycerides, lipids, which shows the hypolipidemic effect of this plant. The hypolipidemic effect may be due to the inhibition of fatty acid synthesis.

The plant extracts showed significant improvement in glucose tolerance in glucose fed hyperglycemic normal rats. Such an effect may be accounted for, in part, by a decrease in the rate of intestinal glucose absorption, achieved by an extra pancreatic action including the stimulation of peripheral glucose utilization or enhancing glycol tic and glycogenic process with concomitant decrease in glycogenolysis and glyconeogenesis (Porchezhian et al., 2000).

However, the effect was less significant when compared to standard drug glibenclamide. Alloxan is the most commonly employed agent for the induction of experimental diabetic animal models of human insulin-dependent diabetes mellitus. There is increasing evidence that alloxan caused diabetes by rapid depletion of a cells, by DNA alkylation and accumulation of cytotoxic free radicals that is suggested to result from initial islet inflammation, followed by infiltration of activated macrophages and lymphocyte in the inflammatory focus. It leads to a reduction in insulin release there by a drastic reduction in plasma insulin concentration leading to stable hyperglycemic states (Szkudelski, 2001).

In this study significant hyperglycemia was achieved within 48 hours after alloxan (150 mg/kg, i.p.) injection. Alloxan induced diabetic rats with more than 250 mg/dl of blood glucose were considered to be diabetic and used for the study. It is now established that there is a gradual decrease in β-cell function and mass that may occur in individuals at high risk of developing type II diabetes. To prevent the loss of β-cell function and mass, β-cell stabilization or regeneration must occur (Henry, 2006). The renewal of β-cells in diabetes has been studied in several animal models. For example epicatechin has been shown to act by β-cell regeneration (Chakravarthy et al., 1982). Similarly *Vinca rosea* extracts also cause regeneration of β-cell in alloxan - induced diabetic rats (Ghosh et al., 2001).

The studies on anti-diabetic activity in alloxanised rats, significant reduction of blood glucose was observed. The comparable effect of the extract with glibenclamide may suggest similar mode of action since alloxan permanently destroys the pancreatic β-cells and the extract lowered blood sugar level in alloxanised rats, indicating that the extent possesses extra pancreatic effect. In diabetic rats, the administration of ethanolic extract of *T. tricuspidata* root significantly reduced the fasting blood glucose level. And there was a significant elevation of blood glucose level in alloxan control rats compared with normal control.

In diabetic animals the change in the levels of serum enzymes are directly related to changes in the metabolism in which the enzymes are involved. Many workers have reported increase in transaminase activities in the liver and serum of diabetic animals. The increased activity of transaminases which are active in the absence of insulin because of increased availability of aminoacids in diabetic are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes (Felig et al., 1970).

The animals treated with alloxan developed hepatic damage which was evident from the increase in the enzyme activities. Treatment with ethanolic extract of *T. tricuspidata* and glibenclamide resulted in a decrease of transaminase activities in alloxan treated animals. In this study, it was observed that the levels of ALP, SGPT and SGOT in alloxan-induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely et al., 2000). In present study, the ethanolic extract of *T. tricuspidata* regulated the activity of SGOT, SGPT and ALP in liver of animals intoxicated with alloxan. The effect of Glibenclamide on the recovery of hepatic enzyme activity in serum was similar to that of the earlier study (Hakkim et al., 2007).

In the present study also indicated that the ethanolic extract significantly decreased the enzyme activities. Hence the improvements noticed in the levels of the enzymes are as a consequence of improvement in carbohydrates, fats and protein metabolism. The lowering the value of SGOT and SGPT from higher value after the treatment indicated the revival of insulin secretion. The increased level of ALP in diabetic rats found to be significantly reversed by the fraction. SGOT and SGPT levels are indicators of liver function, hence, restoration of normal levels indicate normal function of liver (Sumana et al., 2001). The results indicates that the ethanolic extract of *Trichosanthes tricuspidata* can reduce

the level of liver marker enzymes and confirms the possibility that the major function of the extract are on the protection of liver tissues, there by improves the liver function.

It is well-known that in uncontrolled diabetes mellitus, there is an increase in total cholesterol in blood, which may contribute to coronary artery diseases (Arvind et al., 2002). Protein levels and lipid profile were increased in diabetic rats except HDL which was decreased. This may be due to excessive catabolism of protein and the released amino acids are used for gluconeogenesis and lipolysis in adipose tissue which give rise to hyperlipidemia respectively.

Conclusion

The elevated serum cholesterol and triglyceride levels in diabetic rats are low down by the ethanolic extract of *T. tricuspidata* treatment.

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