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ANTIHYPERGLYCEMIC ACTIVITY OF *THESPESIA POPULNEA* SEEDS METHANOL EXTRACT ON STZ INDUCED ALBINO RATS

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ABSTRACT

Thespesia populnea, an important medicinal plant, reported to possess to cure, various ailments in traditional medicine. In the present study, we evaluated Methanol seed extract of *T. populnea* for its antidiabetic and antihyperlipidemic activity in STZ induced diabetic rats. Methanol extract of *T. populnea* seeds (MTPSE) (200mg/kg, 400mg/kg B.W) was administered orally to diabetic rats for 15 days. Biochemical parameters, estimated to determine the antihyperglycemic activity. Oral administration of Methanol seed extract of *T. populnea*, showed significant decrease of blood glucose levels, along with the different lipid profile parameters in Diabetic treated rats. The results exhibited that *T. populnea* seeds methanol extract possess significant antihyperglycemic activity. Normalization of insulin levels, in diabetic treated rats, suggests *T. populnea* seeds might be useful for the management of diabetes.

INTRODUCTION

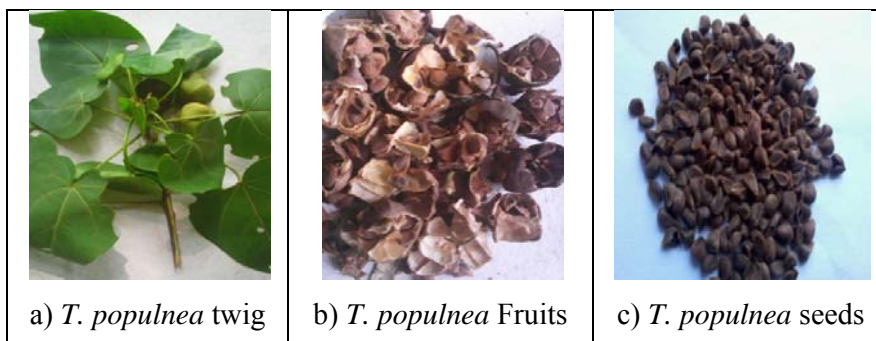
Diabetes mellitus is one of the most debilitating disease in the world. Diabetes mellitus is characterized by hyperglycemia, hypercholesterolemia, and hypertriglyceridemia, resulting from defects in insulin secretion or reduced sensitivity of the tissue to insulin (insulin resistance) and/or combination of both^{1, 2}. The worldwide survey reported that the diabetes is affecting nearly 10% of the population³. It is the third leading cause of death (after heart disease and cancer) in many developed countries. It is a serious endocrine syndrome with poor metabolic control and responsible for increased risk of cardiovascular diseases including atherosclerosis, renal failure, blindness or diabetic cataract worldwide^{4, 5}. The management of diabetes is considered a global problem and cure has yet to be discovered. The synthetic antidiabetic agents like sulfonylureas, biguanides, thiazolidinedione and α -glucosidase inhibitors reduce the blood glucose level but have different adverse effects thus limiting their use and made diabetes and the related complications continued to be a major medical problem. The limitation of the pharmaceutically available anti diabetic drugs paved the way is search for the alternative medicine. Use of medicinal plants to cure various ailments including diabetes is the age old tradition in Indian systems of traditional medicine, till it is practised successfully by the Ayurvedic medicinal practioners. Plants are the major source of pharmaceutical drugs, because of their diverse phytoconstituents. The searching for new antidiabetic drugs from natural plants is still attractive because, their usage was safe and minimal side effects. To date, however, only a few of these medicinal plants have received scientific scrutiny, despite the fact that the World Health Organization has recommended that medical and scientific examinations of such plants should be undertaken⁶.

Thespesia populnea soland ex correa is a large tree belongs to the family Malvaceae, found in tropical regions and coastal forests of India. The bark and flowers are useful in folk medicine to treat cutaneous infections such as scabies, psoriasis, eczema, ringworm, guinea worm while the leaves of this plant are used to reduce inflammation. Various parts of *T. populnea* are found to possess useful medicinal properties, such as antifertility, antimicrobial, anti-inflammatory, antioxidant, purgative and hepatoprotective activity⁷. The fruits of this plant are used in Ayurveda for the control of diabetes. Antidiabetic activity of bark, leaf and whole fruit extracts has already been reported earlier^{8, 9}. In the current study, we investigated the antihyperglycemic effect of methanol extract of *T. Populnea* seeds in STZ induced diabetic rats and to compare this effect with glibenclamide, a standard hypoglycaemic agent.

MATERIALS AND METHODS

Plant Collection

The matured fruits of *Thespesia populnea* (Fig. 1) were collected from Tirupati surrounding areas Chittoor District of Andhra Pradesh, India during November 2014, and authenticated by taxonomist Dr. K. Madhava chetty, The voucher specimen (SVU 0579/02,) has been deposited in the herbarium Department of Botany S.V. University, Tirupati, A.P. India.



Preparation of plant extract

The fruits of *Thespesia populnea* were washed thoroughly and remove debris and then allowed to drip off water and air dried under room temperature. The dried seeds were pulverised into fine granules using electric blender. The powder was extracted successfully with methanol solvent, using soxhlet apparatus. Then the extract, was evaporated to dryness and the final crude extract was stored at -20°C until, used for further experimental studies.

Experimental animals

Thirty male albino rats weighing between 180 to 220g (six to eight weeks old) purchased from (Sri Raghavendra Enterprises, Bangalore, India) were used for this experimental study. They were kept in polycarbonate cage, acclimated to animal house, with 12h light/12h dark cycle at $25 \pm 2^{\circ}\text{C}$. The rats were fed with commercial Rat pellet diet (Hindustan Lever Ltd, Bangalore, India) and given water ad libitum. Handling management and use of animals for the experiment were as such that allowed minimal stress. The experimental procedures involving the treatment and care of animals were conducted in conformity with the approved guidelines by the Institutional Animal Ethics Committee (ResolutionNo:49/2012-13 i)/a/CPCSEA/IAEC/SVU/JKS-SVR).

Anti-diabetic activity:

Chemicals

Streptozotocin was obtained from Sigma chemicals, Bangalore. Glibenclamide used was purchased from Aventis Pharma Ltd., Goa, India. The other chemicals and reagents used were of analytical grade.

Experimental Induction of diabetes:

After fasting for 18hrs rats were injected intraperitoneally with a single dose of 50 mg/kg B.W, streptozotocin after dissolving it in freshly prepared citrate buffer (0.1M, PH 4.5). After the injection they had free access to feed and water. The state of diabetes was observed after 48 hours of STZ induction, by the symptoms of polyuria and glucosuria and this state was confirmed using uristic test strip (Bayer Health Care LLC, USA. The animal having fasting blood glucose levels more than 250mg/dl were selected for the experimentation.). The blood glucose level was tested 1 week after induction and at the end of the experiment using a Glucometer.

Experimental design

In this present experiment 30 rats were used, divided into five groups, each group carries 6 animals. Group 1 served as normal control group receiving 0.1ml/kg B.W normal saline solution. Diabetes was induced by using STZ to, Group II, III, IV, V animals. Group II as diabetic control rats did not receive any extract. While Group III, IV, corresponding to 200, and 400 mg/kg body weight doses of the plant extract and Group V was treated with Glibenclamide 10 mg/ kg body weight. The *T. Populnea* seeds methanol extract were dissolved in distilled water and administered orally for 15 days.

Determination of blood glucose and urine sugar

Blood glucose was determined by the O-toluidine method¹⁰. 0.1 ml of blood was precipitated with 1.9 ml of 10% TCA and the precipitate was removed after centrifugation. 1 ml of supernatant was mixed with 4 ml of O-toluidine reagent and kept in a boiling water bath for 15 min and cooled. The absorbance was read at 620 nm. Glucose was expressed as mg/dL of blood. Urine glucose was assessed in fresh urine using glucose indicator sticks (Boehringer Mannheim, Germany).

Oral glucose tolerance test

OGTT was performed at the end of the experimental period. Prior to OGTT rats were fasted overnight (at least 12 h). 30 min following the various treatment schedules, each rat was given an oral glucose load, 2 g/kg body weight^{11, 12}. Blood was withdrawn from the retro orbital sinus at -30 min (just before the administration of the extract), time 0 (prior to the glucose load), 30, 60 and 120 min after the glucose load. Blood glucose concentrations were estimated using a glucose oxidase-peroxidase reactive strips and a glucometer (Accu-chek, Roche Diagnostics, USA).

Quantitative determination of plasma insulin

Insulin level was estimated in Plasma of normal and STZ induced diabetic rats by ELISA method.

Table-1: Effect of *T. populnea* seed Methanol extract (MTPSE) on change in the levels of blood glucose in experimental rats.

Group	Blood glucose (mg/dL)			
	0days	5 days	10 days	15 days
Normal	70.84±6.15	69.31±9.09	71.05±3.47	72.48±6.84
Diabetic	267.54±6.88	273.71±8.19	272.13±9.24	271.49±8.24
Diabetic+ MTPSE (200mg/kg)	270.73±8.09	251.60±7.35	249.54±14.4	240.90±10.54
Diabetic+ MTPSE (400mg/kg)	268.05±7.80	162.24±10.50	143.52±6.65	96.41±11.8
Diabetic+ Glibenclamide (600 µg/kg)	277.57±7.08	135.51±11.9	115.16±5.90	91.56±6.52

All the values are (mg/dL) mean ± SEM for six rats. Values deviate very significantly from diabetic control group ($P \leq 0.05$).

Table -2: Effect of MTPSE on Oral glucose tolerance test in normal and experimental rats

Group	Blood glucose (mg/dL)				
	0h	1h	3h	5h	7h
Normal	72.93±4.94	68.42±6.03	69.09±3.21	72.40 ±7.16	69.33±4.50
Diabetic	274.04±4.63	278.44±8.70	274.55±4.29	272.1±4.41	275.51±5.35
Diabetic+MTPSE (200mg/kg)	276.77±4.89	256.86±6.64	250.35±13.2	228.62±9.68	226.53±13.2
Diabetic+MTPSE (400mg/kg)	279.37±4.39	252.57±11.08	217.87±16.15	122.11±5.75	114.70±7.88
Diabetic+ Glibenclamide (600 µ g/kg)	277.68±4.57	252.28±8.80	211.51±10.79	145.48±6.99	115.93±6.98

All the values are (mg/dL) mean ± SEM for six rats. Values deviate very significantly from diabetic control group ($P \leq 0.05$).

Table - 3: Effect of MTPSE on change in plasma insulin and body weight of experimental rats.

Group	Plasma Insulin(µU/mL)	Change in body weight(g)		Urine sugars
		Initial	Final	
Normal	126.47 ± 1.34	190.67 ± 1.15	215.67±3.44	Nil
Diabetic Control	52.43 ± 2.78	220.83 ± 2.32	145.00±2.85	+
Diabetic + MTPSE (200mg/kg)	108. 64 ± 3.17	180.43 ± 2.18	143.26 ±1.72	+
Diabetic + MTPSE (400mg/kg)	117.02 ± 1.54	200.53 ± 4.12	159.50±2.12	Nil
Diabetic+ Glibenclamide (600 µ g /kg)	124. 15 ± 0.97	190.04±3.11	127.40±1.71	Nil

All the values are (mg/dL) mean ± SEM for six rats. Values deviate very significantly from diabetic control group ($P \leq 0.05$).

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean \pm SD (Difference between the treatments in this experiment was tested for significance using Paired *t*-test). P value < 0.05 considered as significant

RESULTS

In the antidiabetic activity, the blood sugar levels were measured in all groups of experimental rats in initial and at the 5, 10 and 15 days of treatments are represented in Table 1. The level of blood glucose was significantly increased in diabetic rats when compared to normal rats. Oral administration of MTPSE (400mg/kg B.W), showed significant decrease (96.4%) and standard control glibenclamide (600 μ g /kg B. W) to diabetic rats showed significantly decrease (91.5%) in the blood glucose levels. In the MTPSE treated groups, although a significant antihyperglycemic effect was evident from day 5 onwards, decrease in blood glucose was maximum at the end of the 10th day. The study was extended further and more significant decrease in blood glucose was observed on the 15th day. The effect exerted by the extract was greater than that of the standard control glibenclamide. The continuous treatment of the Methanol seed extract for a period of 15 days produced a significant decrease in the blood sugar levels of diabetic rats, which is dose dependant and also comparable to that of the standard glibenclamide. The standard drug glibenclamide has been used to treat diabetes, which stimulate insulin secretion from pancreatic β -cells, it may be suggested that the mechanism of action of methanol seed extract of *T. populnea* is similar to glibenclamide. The possible mechanism by which the plant extract decreases the blood sugar level may be by potentiation of insulin effect either by increasing the pancreatic secretion of insulin from β -cells of islets of langerhans or by increasing the peripheral glucose uptake.

Table-2 represents the blood glucose levels of normal, diabetic control, MTPSE and glibenclamide treated animals after oral administration of glucose. In diabetic animals, blood glucose levels reached peak at 1h after glucose administration. Although the glucose levels started to decline, they remained high after 3h. MTPSE and glibenclamide treated animals showed a significant decrease at 3h and 5h after oral glucose administration when compared with diabetic control animals. At the end of 7h the blood glucose reached to near normal levels in diabetic rats treated with MTPSE. The effect of MTPSE at 400mg/kg B.W was more pronounced when compared with glibenclamide.

Table-3 illustrates the effect of MTPSE on plasma insulin, urine sugar and change in body weight of normal and experimental animals. STZ caused a significant decrease in plasma insulin. Administration of *T. Populnea* seed methanol extract, at conc. 400mg/kg B. W caused significant increase in plasma insulin levels at the end (15 days) of the study. The increase in the plasma insulin levels by MTPSE was comparable to the increment of plasma insulin by glibenclamide. The level of urine sugar was significantly increased in diabetic rats when compared with normal rats. Administration of MTPSE and glibenclamide to diabetic rats significantly reversed all these changes to near normal levels. Body weights were also significantly reduced in diabetic rats when compared to normal rats while the extract significantly prevented a decrease in the MTPSE treated animals.

DISCUSSION

Diabetes mellitus, a metabolic disorder is characterized with increase in blood glucose level. The main objective of the drugs acting as anti-hyperglycaemic is to reduce the blood glucose level to normal so as to reduce the diabetes related complications. The world is facing an explosive increase in the incidence of diabetes mellitus and cost effective complementary therapies are needed. Although insulin has become one of the most important therapeutic agents known to medicine, there is a continuing effort to find insulin substitutes, secretagogues, or sensitizers from synthetic or plant sources for the treatment of diabetes¹³.

In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat the diabetes¹⁴.

Induction of diabetes with streptozotocin is associated with the characteristic loss of body weight, which is due to increased muscle wasting and due to loss of tissue proteins¹⁵. Diabetic rats treated with the MTPSE showed significant gain in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting (i.e. reversal of gluconeogenesis and glycogenolysis) and may also be due to the improvement of insulin secretion and glycaemic control. Estimation of the blood glucose levels of control, diabetic (DM) and Diabetic treated (DMT) and standard control experimental groups confirmed hyperglycemia in the test groups (i.e. DM and DMT), thus suggesting that the insulin producing pancreatic beta cells were destroyed by streptozotocin (STZ) administered for the induction of DM in these groups. Treatment with the Methanol extract of TPSE normalized the blood glucose level compared to the diabetic control group, the level of reduction was significant when compared with the diabetic control group.

This study indicated that diabetes caused a significant decrease in body weight; however, there were significant effect of MTPSE on body weight loss (Table 3). These findings are consistent with the studies of Mellert *et al*¹⁷, Sindhu *et al*¹⁸, and Duzguner *et al*¹⁹. Lipolysis and gluconeogenesis are the 2 main reasons for weight loss during diabetes²⁰. Diabetes-increased blood glucose levels were suppressed by the MTPSE during the 15 days period (Table 1). Diabetes caused a significant decrease in the plasma insulin concentration due to the damage caused by the cytotoxic effects of STZ in the pancreatic β cells. This was reversed by the oral administration of MTPSE. *T. Populnea* fruit pulp aqueous and ethanol extracts at conc. 200mg/kg B.W²¹, Bark and leaf ethanol extracts²², Flower and leaf methanol extracts at 400 mg/kg B.W conc. exhibited significant antihyperglycemic activity²³, similar antihyperglycemic response exhibited by *T. Populnea* seed methanol extract (400mg/kg B.W).

CONCLUSION

In conclusion, the findings in this study showed that, the methanol extract of *T. populnea* seeds normalized the hyperglycemic condition in STZ induced diabetic rats, because of its potent antihyperglycemic activity. This study reports the preliminary antidiabetic potential of *T. Populnea* seeds. Further investigation is needed to identify, the particular bioactive compounds responsible for its promising antidiabetic activity

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