

Antidiabetic activity of *Thespesia Populnea* bark and leaf extract against streptozotocin induced diabetic rats

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Abstract: *Thespesia Populnea* is a reputed ever green tree belonging to the family malvaceae; commonly known as Indian tulip tree. The plant is distributed in tropical regions and coastal forest in India. It is well known and all the parts are used in Indian system of medicine. The plant has been used as astringent, antibacterial, hepatoprotective, haemostatic, anti-diarroheal and anti-inflammatory. The ethanolic extract of the plant bark (TPBE) and leaf (TPLE) were evaluated for its effect on blood sugar, against the streptozotocin (STZ)-induced diabetic rats and compared it with standard drug glibenclamide. The result of this experimental study indicates that both the ethanolic extract possess anti-diabetic effect against STZ induced diabetic rats and also showed the possible mechanism due to inhibition of generation of free radical.

Key words: *Thespesia populnea*, Ethanol extract-antidiabetic.

Introduction

Diabetic mellitus (DM) is the condition arising due to abnormal metabolism of carbohydrate, proteins and fats. It is caused by insulin deficiency, often combined with insulin resistance¹. This disorder occurs world wide and its occurrence is increasing quickly in most of the countries. Various complications develop as a consequence of the metabolic derangement in diabetes². The treatment of DM is based on parenteral insulin and oral anti-diabetic drugs. Oral hypoglycemic agents, currently used have serious side effect hence there is a need to search a newer anti-diabetic agents that having high therapeutic efficacy with minimum side effect³. This may be fulfilled by treating DM with traditional medicine using as anti-diabetic agents from medicinal plants.

Thespesia populnea soland ex correa (family malvaceae) is a large tree found in the tropical regions and coastal forests in India and cultivated in the gardens. All the parts of the plant used in traditional system of medicine. The bark, leaves, flower and fruits are useful in cutaneous infection such as scabies, psoriasis, eczema, ringworm, and guinea worm. The decoction of the bark is commonly used for the treatment of skin and liver diseases. A compound oil of bark and capsules is useful in urethritis and gonorrhoea. The bark, root, fruits were used in dysentery, cholera and hemorrhoids⁴. The fruits of the plant are used in ayurveda for the control of diabetes⁵. An ayurvedic preparation contains *Thespesia populnea*, namely "panchvalkala" possess free radical

scavenging activity⁶. The barks and flowers possess astringent, hepatoprotective, antioxidant and anti-inflammatory activities in rats^{7,8,9,10}, and also supposed to improve the memory¹¹.

The leaves and bark of this tree are still used to mix with oil for the treatment of fracture wounds and as an anti-inflammatory poultice applied to ulcers and boils, as described in folk medicine. Gossypol was found to be the major component of *Thespesia populnea*¹² producing anti-fertility effects in rats^{13,14} as well as in human beings¹⁵. Four naturally occurring quinones viz thespone, thespesone, mansonone-D, and mansonone-H have been extracted from heart wood of the plant¹⁶. The phytochemical study of bark reveals the presence of gossypol, tannin and coloring matter¹⁷ and leaf extract indicates the presence of lupeol, lupenone, β -sistosterol¹⁸ and also acacetin, quercetin, vanillic, syringic, melilotic, and ferulic acid¹⁷.

The fruit extract of the plant has already shown a significant hypoglycemic effect. Hence in present study, the ethanolic extract of *Thespesia populnea* bark and leaf was investigated for hypoglycemic effect in streptozotocin induced diabetic rats and to compare this effect with glibenclamide, a standard hypoglycemic agent and also measure the lipid peroxide, superoxide dismutase and catalase enzyme level in the liver and kidney of the animal.

Materials and methods

Plant materials:

The fresh bark and leaves were collected from Selaiyur university campus in Chennai, Tamil nadu. The plant material was taxonomically identified and authenticated by Director, National institute of Herbal Sciences, Chennai. A voucher specimen (PARC/236/07) has been deposited in the herbarium of the same department.

Preparation of the plant extract:

The freshly collected bark and leaves were dried under shade, cut in small pieces and made into coarsely powder using mechanical grinder and preserved in air tight container. The powdered bark and leaves were extracted separately by percolation at room temperature with 95% ethanol. The ethanolic extracts were collected and filtered. The extract were concentrated under reduced pressure and dried in vacuum desiccator. A brownish black colored residue was obtained from bark (Yield 16.5%w/w) and greenish brown residue was obtained from leaves (Yield 21.26%), which were kept in a desiccator. This ethanolic extracts of *Thespesia populnea* bark (TPBE) and leaf (TPLE) were suspended in 1% SCMC and used for the experiments.

Preliminary phytochemical screening:

The extracts were preliminary investigated for various phytochemical constituents such as alkaloids, Carbohydrates, Steroids, Proteins, Phenols, Tannins, Flavonoids, Glycosides, Gums, Saponins and terpenes¹⁹.

Animals:

Wistar rats (150-200gm) were purchased from King Institute, Chennai for experimental study. They were acclimated to animal house condition feed with commercial pellets Rats chon (Hindustan Lever Ltd, Bangalore, India) and had free access to water. The experimental protocol was approved by the IAEC (Institute Animal Ethical Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal).

Acute toxicity studies:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (bark and leaves) were administered orally at the dose of 2000 mg/kg by intragastric tube and observed for 2 days for the gross behavioral changes and mortality.

Anti-diabetic activity:

Experimental Induction of diabetes:

After fasting for 18hrs 40 rats were injected by intraperitoneally with a single dose of 50 mg/kg streptozotocin after dissolving it in freshly prepared ice cold citrate buffer (PH 4.5). After the injection they had free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycemic shock. The development of diabetes was confirmed after 48hrs of the streptozotocin injection. The animal having fasting blood glucose levels more than 200mg/dl were

selected for the experimentation. Out of 40 animals 3 were died before grouping and one was omitted from the study because of mild hyperglycemic. Remaining 36 diabetic animals were divided into 6 groups each having 6 rats²⁰.

Experimental protocol:

Group I – streptozotocin induced diabetic animals received 1% SCMC 5ml/kg PO for 15 days.

Group II – Streptozotocin induced diabetic animals received TPLE extract 200mg/kg PO for 15 days.

Group III – Streptozotocin induced diabetic animals received TPLE extract 400mg/kg PO for 15 days.

Group IV – Streptozotocin induced diabetic animals received TPBE extract 200mg/kg PO for 15 days.

Group V – Streptozotocin induced diabetic animals received TPBE extract 400mg/kg PO for 15 days.

Group VI – Streptozotocin induced diabetic animals received the standard drug glibenclamide 1.25/kg PO for 15 days.

All the group of animals received the treatment for 15 days. Blood samples were collected one hr after the drug administration and the day 5th, 10th, 15th to determine the blood glucose level by electronic glucometer²¹. The organ like liver and kidney were removed on 15th day and 10% tissue homogenate were prepared with 0.025 M Tris-Hcl buffer (pH 7.5). After centrifugation at 10,000 rpm for 10 minutes, the clear supernatant was used to measure thiobarbituric acid reactive substances (TBARS) and hydroperoxides. For the estimation of non-enzymic and enzymic antioxidants, tissue was minced and homogenized (10% w/v) in 0.1 M phosphate buffer (ph 7.0) and centrifuged for 10 minutes and the resultant supernatant was used for estimation such as catalase, superoxide dismutase and glutathione-S-transferase²².

Statistically 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.

Result and Discussion

The preliminary phytochemical studies indicated the presence of carbohydrate, protein, tannins, phenol, flavonoids, terpenes, saponins and gums in the ethanolic extract of the bark and leaf. In acute toxicity study, the ethanolic extract of *Thespesia populnea* bark and leaf did not produced lethality up to the dose level of 2000 mg/kg.

In the antidiabetic activity, the blood sugar levels were measured in First to Six groups of experimental rats in initial and at the 5, 10 and 15 days of treatments are given in the Table 1. streptozotocin induced diabetic rats showed significant increase in the level of blood sugar. Oral administration of bark extract and leaf extract at the dose of 400 mg/kg body weight showed the significant

decrease ($P < 0.05$) on blood sugar level in 10 to 15 days of treatment.

In the present study the hypoglycemic activity of ethanolic extract of bark and leaves of *Thespesia populnea* was evaluated in STZ induced diabetic rats. For enzymatic anti oxidant studies the enzyme such as Catalase, Superoxide dismutase and Glutathione γ -S-transferase in the tissue of liver and kidney were also evaluated (Table 2). The continuous treatment of the 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 ethanolic extract of *Thespesia 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.

Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. In-vivo studies of the effect of various herbal drugs on diabetes mellitus with the aim of establishing the relationship between the free radicals, diabetes and its complication. An increase in hepatic and renal MDA (Malonyl dialdehyde) concentrations in STZ induced diabetic rats when compared with the normal rat was observed. In

diabetes hypoinsulinemia increases the activity of the enzymes which initiate oxidation of fatty acids, results in lipid peroxidation. Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity that leads to cell injury and damage²³.

Ethanolic extract of *Thespesia populnea* in diabetic rats showed a significant reduction in lipid peroxidase in hepatic and renal tissues as compared to diabetic group. Superoxide dismutase and catalase are the two major scavenging enzymes that remove toxic free radicals in-vivo. In our findings we have formed the reduced activities of superoxide dismutase and catalase. Removal of oxygen and OH \cdot is probably are of the most effective defense mechanism against various disorders. The result of this enzymatic analysis clearly shows that the leaves and bark extract of *Thespesia populnea* contains a free radical scavenging activity which could exert a beneficial action against diabetes mellitus induced by STZ.

Conclusion

The ethanolic extract of *Thespesia populnea* bark and leaf exhibited significant antihyperglycemic activity in streptozotocin (STZ)-induced diabetic rats. This extracts has also showed the effect due to enhancing effect on cellular antioxidant defenses to protect against oxidative damage. Present efforts are directed to isolate active constituent from the plant extract and confirmation of mechanism of action.

Table 1: Antidiabetic activity of Thespesia Populnea bark and leaf extract against streptozocin induced diabetic rats:

Sr. No:	Groups	Blood Sugar level in mg/dl (Mean \pm SD)			
		Initial	5 th Day	10 th Day	15 th Day
1	STZ	244.83 \pm 7.41	291.83 \pm 7.47	326.83 \pm 12.14	325.50 \pm 11.75
2	TPLE 200	241.63 \pm 6.09	246.33 \pm 5.60 NS	225.16 \pm 7.38 α	208.66 \pm 9.11 α
3	TPLE 400	244.66 \pm 6.37	227.66 \pm 8.45 α	214.00 \pm 3.89 α	196.83 \pm 7.70 α
4	TPBE 200	246.50 \pm 6.53	234.50 \pm 10.59NS	222.66 \pm 6.77 α	214.16 \pm 6.55 α
5	TPBE 400	242.16 \pm 9.28	217.50 \pm 3.93 α	206.33 \pm 5.81 α	189.50 \pm 6.92 α
6	Std Glibenclamide	242.66 \pm 12.54	205.50 \pm 8.73 α	149.33 \pm 6.50 α	123.66 \pm 9.11 α

The values are mean \pm SD of 6 animals in each group. Statistical analysis was carried out by paired sample t-test α P α 0.05, N.S. non significance.

Table 2: Effect of *Thespesia populnea* leaf and bark extract on antioxidant enzyme level.

Group and Treatment	Lipid peroxidase (1)		Catalase (2)		Superoxide dismutase (2)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
STZ	43±2.0	20.5±2.0	2.2±0.12	7.1±0.4	4.6±0.3	3.6±0.6
TPLE 200	35±2.6	19±1.8	3.8±0.22	10±0.32	7±0.38	5±0.52
TPLE 400	32±3.1	19±1.2	3.2±0.24	11±0.36	8±0.4	5±0.56
TPBE 200	38±3.4	17±1.0	4.0±0.41	10±3.8	8±0.32	4.8±0.42
TPBE 400	34±2.8	18±1.2	4.2±0.36	10±3.2	9±0.42	3.9±0.38
Glibenclamide	30±3.2	15±1.6	5.8±0.46	12±0.4	8.2±0.38	5.4±0.40

(1) mM of MDA liberated / 100gm of tissue / minutes. (2) Unit / mg of protein.

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