

## ANTI-DIABETIC EFFECT OF METHANOLIC EXTRACT OF *THESPESIA POPULNEA* FLOWER AND LEAF IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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### ABSTRACT

Methanolic extract of *Thespesia populnea* flower and leaf exhibited significant Anti hyperglycaemic activity in Alloxan-induced diabetic rats. The flower extract of *Thespesia populnea* has potent anti hyperglycaemic property compared to standard Glibenclamide. Alloxan induced diabetic rats showed significant increase in the level of blood sugar. Oral administration of flower extract and leaf extract at the dose of 400 mg/kg body weight showed the significant decrease ( $P < 0.01$ ) on blood sugar level in 10 to 15 days of treatment. 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  $\beta$ -cells of islets of langerhans or by increasing the peripheral glucose uptake.

**KEYWORDS:** *Thespesia populnea*; Glibenclamide; Alloxan;

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### INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by high blood sugar (glucose) levels that result from defects in insulin secretion, or action, or both. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycaemia. Diabetes affects approximately 17 million people (about 8% of the population) in the United States. In addition, an estimated additional 12 million people in the United States have diabetes and don't even know it. Diabetes is the third leading cause of death in the United States after heart disease and cancer<sup>1</sup>.

*Thespesia populnea* (L.) Sol. ex Correa belongs to the family Malvaceae, commonly known as umbrella tree in English, porush in Hindi and poovarasu in Tamil<sup>2</sup>. It Distributed Coastal areas of the Indian and Pacific Oceans. It a Small tree typically 6–10 m (20–33 ft) at maturity. 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, ring worm, 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

haemorrhoids<sup>3</sup>. The phytochemical study of bark reveals the presence of gossypol, tannin and coloring matter and leaf extract indicates the presence of lupeol, lupenone,  $\beta$ -sistosterol and also acacetin, quercetin, vanillic, syringic, melilotic, and ferulic acid<sup>4</sup>. The fruit extract of the plant has already shown a significant hypoglycemic effect. Since no previous attempts have been made to examine the Anti-diabetic effect of methanolic extract of the *Thespesia populnea* flower and leaf on experimental animal.

### MATERIALS AND METHODS

#### Plant material

The fresh flowers and leaves were collected from Guntur (Andhra Pradesh). The plant material was taxonomically identified and authenticated by The Head, Pharmacognosy Department, Nandha College of Pharmacy, Erode, Tamilnadu. A voucher specimen (NCP/PCOL/08/04) has been deposited at the herbarium of Nandha College of Pharmacy, Erode, Tamilnadu, for ready reference.

#### Preparation of the extract

The freshly collected flowers 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 flowers and leaves were

extracted separately by maceration at room temperature with methanol. The solvent was prepared by using methanol: water (9:1) ratio. The methanolic extracts were collected and filtered. The extracts were concentrated under reduced pressure and dried in vacuum desiccator. A brownish red residue was obtained from flower (yield 18.07% w/w) and greenish brown residue was obtained from leaves (yield 18.26% w/w), which were kept in a desiccator. The methanolic extract of *Thespesia populnea* flower (TPFE) and leaf (TPLE) were suspended in 0.5% CMC and used for the experiment.

#### Animals

All the experiments were carried out using male Wistar rats (150–200 g) procured from the animal house, Nandha college of Pharmacy, Erode, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Reg no: 688/2/C-CPCSEA) and were in accordance with the guidelines of the CPCSEA.

#### Vehicle

A plant extracts (TPLE and TPFE), was suspended in 0.5% CMC and was administered orally to animals. Alloxan monohydrate was dissolved in 0.9% Normal saline and injected intraperitoneally.

#### Acute oral toxicity studies

Acute oral toxicity studies will be performed (Ecobichon, 1997) according to OECD-423 guidelines (acute toxic class method). Swiss mice (n=3) of either sex select by random sampling technique are employed in this study. The animals are fasted for 4 hrs with free access to water only. The *Thespesia populnea* leaves and flower extracts was administered orally at a dose of 5 mg/kg initially and mortality is observed for 3 days. If mortality is observed in 2/3 or 3/3 animals, then the dose administered is considered as toxic dose. However, if the mortality is observed in only one mouse out of three animals then the same dose is repeated again to confirm the toxic effect. If mortality is not observed, the procedure is then repeated with higher doses such as 50, 300 and 2000 mg/kg.

#### Preliminary chemical screening

The extract was subjected to preliminary screening, for various active phytochemical constituents such as

alkaloids, carbohydrates, glycosides, protein, flavonoids, tannins, phenols, fixed oils, saponins and terpenoids.

#### Experimental induction of Diabetes

After fasting for 18hrs 30 rats were injected (except group I) by intraperitoneally with a single dose of 150 mg/kg Alloxan after dissolving it in 0.9% normal saline. After the injection they had free access to feed and water and were given 5% glucose solution to drink over night to counter the hypoglycaemic shock. The development of diabetes was confirmed after 48hrs of the Alloxan injection. The animal having fasting blood glucose levels more than 200mg/dl were selected for the experimentation.

#### Experimental protocol

- Group I - Normal control Received the 0.5% (CMC) carboxy methyl cellulose solution 1ml/kg
- Group II - Alloxan induced Diabetic animals received distilled water 5ml/kg PO for 15 days.
- Group III - Alloxan induced Diabetic animals received the Standard drug Glibenclamide 1.25/kg PO for 15 days.
- Group IV - Alloxan induced Diabetic animals received TPFE extract 200mg/kg PO for 15 days.
- Group V - Alloxan induced Diabetic animals received TPFE extract 400mg/kg PO for 15 days.
- Group VI - Alloxan induced Diabetic animals received TPLE extract 200mg/kg PO for 15 days.
- Group VII - Alloxan induced Diabetic animals received TPLE extract 400mg/kg PO for 15 days.

All the group of animals received the treatment for 15 days. Blood samples were collected 2 hrs after the drug administration and the day 5th, 10th, 15th to determine the blood glucose level by Glucometer (One-Touch).

#### Statistical analysis

All the results were expressed as mean ± standard error (S.E.M.). Data were analyzed using one-way ANOVA followed by Dunnett's t-test.

## RESULTS

#### Acute oral toxicity test

*Thespesia populnea* leaves and flower extract did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed. All the doses (5, 50 and 300 mg/kg, p.o.) of TPLE and TPFE were thus found to be non-toxic. Two doses (200 and 400 mg/kg, p.o.) of TPLE and TPFE were selected for further pharmacological studies.

#### Preliminary chemical tests

Our phytochemical studies indicate that methanolic extract of leaves and flowers contains alkaloids, carbohydrates, tannins, phenols, flavonoids, glycosides, saponins and terpenoids. Protein and fixed oil showed negative results (Table 1).

**Anti-diabetic activity**

Anti-diabetic activity of methanolic extract of *Thespesia populnea* flower and leaf were summarized in Table 2.

**DISCUSSION**

In the anti-diabetic activity, the blood sugar levels were measured in first to seven groups of experimental rats in initial and at the 5, 10 and 15 days of treatments are given in the Table 2. Alloxan induced diabetic rats showed significant increase in the level of blood sugar. Oral administration of flower extract and leaf extract at the dose of 400 mg/kg body weight showed the significant decrease ( $P < 0.01$ ) on blood sugar level in 10 to 15 days of treatment.

In the present study the hypoglycaemic activity of methanolic extract of flowers and leaves of *Thespesia populnea* was evaluated in alloxan induced diabetic rats. The standard drug glibenclamide has been used to treat diabetes, which stimulate insulin secretion from pancreatic  $\beta$ -cells, it may be suggested that the mechanism of action of methanolic 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  $\beta$ -cells of islets of langerhans or by increasing the peripheral glucose uptake.

**CONCLUSION**

In conclusion, the data obtained in this study demonstrated that the methanolic extract of *Thespesia populnea* flower and leaf exhibited significant Anti hyperglycaemic activity in Alloxan-induced diabetic rats. The flower extract of *Thespesia populnea* has potent anti hyperglycaemic property compared to standard Glibenclamide. Further studies are necessary to elucidate the mechanisms behind its traditional effects.

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**Table 1. Qualitative analysis of Phytochemicals in *Thespesia populnea* leaves and flower**

Phytochemical constituent	Presence of phytochemical constituents
Alkaloids	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Proteins	-
Carbohydrates	+
Tannins	+
Phenols	+
Fixed oils	-
Saponins	+

**Table 2. Anti-diabetic effect of *Thespesia populnea* in alloxan induced rats.**

Groups	Blood Sugar Level (mg/dl)			
	Initial	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day
<b>Group I</b> Normal control (0.5% CMC)	96.48±2.03***	103.84±1.66***	98.04±2.74***	101.98±2.69***
<b>Group II</b> -ve control (Alloxan)	266.21±4.58	279.84±4.92	285.91±4.64	312.86±5.02
<b>Group III</b> +ve control (Glibenclamide)	249.16±4.81	211.18±4.81*	116.18±4.72**	141.48±3.86**
<b>Group IV</b> Test 1 TPFE(200 mg/kg)	262.16±5.28	252.62±5.41	226.38±5.78*	212.46±4.98**
<b>Group V</b> Test 2 TPFE (400 mg/kg)	258.14±4.96	244.24±4.81	206.68±4.72**	169.91±4.18**
<b>Group VI</b> Test 3 TPLE (200 mg/kg)	264.21±5.12	241.53±5.19	212.39±3.98	178.48±4.81*
<b>Group VII</b> Test 4 TPLE (400 mg/kg)	262.41±5.16	224.16±4.63	173.87±4.22*	156.86±3.93**

Values are mean ± SEM; n=5 animals in each group; \*p<0.05, \*\*P<0.01, \*\*\*P<0.001 when compared to paracetamol control group

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