

## ANTIHYPERGLYCEMIC AND HYPOGLYCEMIC EFFECT OF *THESPESIA POPULNEA* FRUIT IN NORMAL AND ALLOXAN-INDUCED DIABETES IN RABBITS

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تمت دراسة التأثيرات الخافضة لنسبة السكر في الدم وكذلك التأثيرات المضادة لارتفاع نسبة السكر في الدم للمستخلص الكحولي لثمرة نبات *Thespesia populnea* في كل من الأرناب الطبيعية والأرناب المصابة بمرض السكر المستحث بالألوكسان (110 مغ/كغ، عن طريق البريتون). وقد تم سحب عينات دم من الوريد الأذني الخافوي قبل وعند 0.5، 1، 2، 4، 6، 8، و12 ساعة بعد إعطاء جرعات فموية مقدارها 100، 200 و 300 مغ/كغ. وقد تم تحليل نسبة جلوكوز الدم بطريقة جلوكوز أو أكسيداز باستخدام مقياس الطيف الضوئي المرئي. وقد نتج عن إعطاء جرعات متدرجة من المستخلص لكل من الأرناب الطبيعية والأرناب المصابة بالسكر انخفاضاً معنوياً في نسبة الجلوكوز في الدم وذلك بعد 6 ساعات من تعاطي المستخلص ( $p < 0.001$ ). وتبين أن هذا التأثير يعتمد على الجرعة في جميع المعالجات بالجرعات المعطاة. تدل هذه الدراسة بوضوح على التأثير المعنوي للنشاط الخافض للسكر في الدم لثمرة نبات *Thespesia populnea* مما يدعم الاستخدام التقليدي لهذه الثمار من قبل أطباء الأيورفيديا الشعبيين للتحكم في مرض السكر.

The hypoglycemic and antihyperglycemic effects of an alcoholic extract of the fruit of *Thespesia populnea* was investigated in both normal and alloxan-induced diabetes in rabbits. Blood samples were collected from marginal ear vein before and at 0.5, 1, 2, 4, 6, 8 and 12 h after the oral administration of 100, 200 and 300/kg doses. Blood glucose was analysed by glucose-oxidase method using a visible spectrophotometer. Graded doses of the extract when given to both normal and diabetic rabbits produced significant reductions in blood glucose at the 6 h after extract administration ( $P < 0.001$ ). The effect was found to be dose dependent with all treatments at the doses administered. The present study clearly indicated a significant antidiabetic activity of the fruit of *T. populnea* and supports the traditional usage of fruits by the Ayurvedic physicians for the control of diabetes.

**Keywords:** *Thespesia populnea*, blood glucose, alloxan, diabetes mellitus, rabbits

### Introduction

Diabetes mellitus is a major disease affecting nearly 10% of the population (1). In spite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem. Many indigenous Indian medicinal plants have been found to be successfully used to manage diabetes (2-7) and some of them

have been tested and the active principles isolated. The available literature shows that there are more than 400 plant species having hypoglycemic activity (8-10). However, search for new antidiabetic drugs continues.

*Thespesia populnea* Soland ex Correa (Family : Malvaceae) is a large avenue tree found in the tropical regions and coastal forests in India. The bark, leaves, flowers and fruits are useful in cutaneous infections, such as scabies, psoriasis, eczema, ringworm and guinea worm (11). A decoction of the bark is commonly used for the treatment of skin and liver diseases. Oil of bark mixed with vegetable oil is useful in urethritis and

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gonorrhoea. The astringent bark, roots and fruits were used in dysentery, cholera and hemorrhoids; the mashed bark is employed as a poultice for wounds. The leaves were reported to be employed locally as anti-inflammatory in swollen joints (12). The fruits of the plant are used in Ayurveda for the control of diabetes (13). Hence, in the present study the alcoholic extract of *T. populnea* has been evaluated for hypoglycemic and antihyperglycemic activities in both normal and alloxan induced diabetic rabbits and to compare these effects with tolbutamide, a standard hypoglycemic agent.

### Materials and Methods

#### *Plant material*

Fresh Fruits of the plant were collected from our University campus, Andhra University, Visakhapatnam, India. It was authenticated by the noted Botanist Dr. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam. A voucher specimen (PC-09) has been kept in our laboratory for future reference. Samples were dried under mild sun. The completely dried fruits were powdered with an electric grinder and used for extraction.

Since ethanol is the common solvent for extracting most of the constituents present in herbal material, the powdered fruits were extracted by percolation at room temperature with 80% ethyl alcohol. The extract was concentrated under reduced pressure (bath, temperature 50°C) and dried in a vacuum desiccator (Yield 14% w/w). Samples were then suspended in 5% gum acacia at a concentration of 100 mg/ml and used in all experiments.

#### *Animals*

Adult albino rabbits (1.5 – 2 Kg) of either sex obtained from Ghosh Enterprises, Calcutta were used in the study. They were divided into 10 groups of five each and were provided with standard diet and water *ad libitum*. All the rabbits were kept in cages with wide square mesh at the bottom to avoid coprophagy and maintained in a well ventilated animal house with 12 h light and dark cycle. They were fasted for 18 h prior to the experiment, allowing access to water only, and were deprived of both food and water during the 12 h monitoring period of the experiment after the treatment either with the drug (or) vehicle. The experimental protocol has been approved by the institutional

animal ethics committee and by the animal regulatory body of the Indian Government (Regd. No. 516/01/A/CPCSEA).

#### *Chemicals used*

Tolbutamide was a generous gift sample from Hoechst Pharmaceuticals, Bombay and alloxan monohydrate was purchased from Sigma-Aldrich, St. Louis, USA. The glucose oxidase reagents kits were purchased from Dr. Reddy's Laboratories, Hyderabad.

#### *Effect on normal rabbits*

Groups I, II, III were given the alcoholic extract of *Thespesia populnea* fruit (suspended in 5% gum acacia) orally following a standard procedure (14) at doses of 100, 200, 300 mg/kg body weight, respectively. Animals in Group IV received tolbutamide at a dose of 250 mg/kg body weight and served as standard. Group V served as a normal control and received appropriate volumes of vehicle orally.

#### *Induction of diabetes*

Groups VI – X were rendered diabetic by injecting a freshly prepared aqueous solution of alloxan monohydrate (110 mg/kg, i.p.) after a baseline blood glucose estimation was done. After two weeks when the condition of diabetes was stabilized, animals with blood glucose levels above 250 mg/dl were selected for the study.

#### *Effect on diabetic rabbits*

Groups VI – VIII were treated with alcoholic extract of *T. populnea* fruit (Suspended in 5% gum acacia) in the form of mucilage orally by gavage at doses of 100, 200 and 300 mg/kg body weight, respectively. Group X served as a diabetic control and received appropriate volume of the vehicle orally while group IX received tolbutamide at a dose of 250 mg/kg body weight and served as a standard.

#### *Collection of Blood and determination of blood glucose:*

Blood samples were collected from the marginal ear vein of each rabbit before and at 0.5, 1, 2, 4, 6, 8, & 12 h after drug administration. The samples were analyzed for blood glucose content by using glucose-oxidase method(15) with optical density measured by visible spectrophotometer at 520nm.

*Statistical Analysis*

Data were expressed as means  $\pm$  standard error of means. Statistical comparisons were made by

analysis of variance (ANOVA) and post-hoc comparisons were done by using Dunnett's t-test (16). P values  $p < 0.05$  were considered as significant.

**Table 1.** Effect of *T. populnea* fruit extract on blood glucose levels after oral administration in normal rabbits.

Group (n=5)	Dose	Blood glucose levels at different hours after the treatment							
		0	0.5	1	2	4	6	8	12
Control	---	110.84 $\pm$ 4.10	109.72 $\pm$ 3.10	108.06 $\pm$ 3.01	108.77 $\pm$ 2.40	108.67 $\pm$ 1.92	109.58 $\pm$ 0.98	108.53 $\pm$ 1.92	109.23 $\pm$ 2.56
<i>T. populnea</i>	100 mg/kg	109.56 $\pm$ 2.10	108.31 $\pm$ 3.42	104.92 $\pm$ 3.56	99.27 $\pm$ 2.59*	99.33 $\pm$ 1.19**	86.74 $\pm$ 5.26**	86.90 $\pm$ 3.26***	97.65 $\pm$ 4.12*
<i>T. populnea</i>	200 mg/kg	105.72 $\pm$ 5.29	104.07 $\pm$ 6.10	97.05 $\pm$ 4.20	94.30 $\pm$ 5.19	85.67 $\pm$ 3.10*	80.15 $\pm$ 2.95***	82.18 $\pm$ 3.25***	90.72 $\pm$ 1.92*
<i>T. populnea</i>	300 mg/kg	98.56 $\pm$ 4.20	95.82 $\pm$ 3.26	89.64 $\pm$ 1.98	86.12 $\pm$ 3.26*	77.80 $\pm$ 4.92*	72.61 $\pm$ 2.62***	74.39 $\pm$ 0.45***	86.55 $\pm$ 3.20*
<i>Tolbutamide</i>	250 mg/kg	100.19 $\pm$ 1.92	94.79 $\pm$ 2.65	88.65 $\pm$ 3.12*	85.55 $\pm$ 2.94**	75.72 $\pm$ 3.12***	70.45 $\pm$ 4.05***	77.02 $\pm$ 2.12***	89.20 $\pm$ 3.54*

Values are mean blood glucose levels ( $\pm$  S.E.M.) of five animals.

Significant difference from control at corresponding intervals: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

**Table 2.** Effect of *T. populnea* fruit extract on blood glucose levels after oral administration in diabetic rabbits.

Group (n=5)	Dose	Blood glucose levels at different hours after the treatment							
		0	0.5	1	2	4	6	8	12
Control	---	260.19 $\pm$ 3.20	252.90 $\pm$ 3.06	252.12 $\pm$ 4.12	255.11 $\pm$ 2.64	253.42 $\pm$ 2.95	256.41 $\pm$ 1.25	254.85 $\pm$ 3.60	258.44 $\pm$ 3.00
<i>T. populnea</i>	100 mg/kg	300.16 $\pm$ 2.56	293.67 $\pm$ 3.42	288.18 $\pm$ 4.29*	272.27 $\pm$ 2.19**	243.54 $\pm$ 1.26***	194.92 $\pm$ 1.99***	211.61 $\pm$ 2.54***	206.87 $\pm$ 2.20***
<i>T. populnea</i>	200 mg/kg	270.86 $\pm$ 1.98	261.37 $\pm$ 2.64*	250.65 $\pm$ 2.98***	243.34 $\pm$ 3.56***	219.34 $\pm$ 1.64***	167.87 $\pm$ 1.52***	176.05 $\pm$ 0.98***	242.64 $\pm$ 2.40***
<i>T. populnea</i>	300 mg/kg	290.54 $\pm$ 1.66	277.29 $\pm$ 2.50***	266.74 $\pm$ 3.40***	251.92 $\pm$ 3.02***	227.14 $\pm$ 2.40***	151.34 $\pm$ 2.56***	174.84 $\pm$ 1.96***	200.21 $\pm$ 2.98***
<i>Tolbutamide</i>	250 mg/kg	288.19 $\pm$ 2.48	274.35 $\pm$ 2.12**	258.62 $\pm$ 1.88***	247.03 $\pm$ 3.01***	218.50 $\pm$ 2.98***	138.10 $\pm$ 1.96***	164.03 $\pm$ 1.86***	189.71 $\pm$ 1.98***

Values are mean blood glucose levels ( $\pm$  S.E.M.) of five animals.

Significant difference from control at corresponding intervals: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$

## Results

### *Effect of fruit extract on blood glucose levels in normal rabbits*

The mean blood glucose concentration of control and drug-treated animals (after oral administration of different doses of *T.populnea* fruit extract) at various time intervals are shown in Table.1 A dose-dependent hypoglycemia was observed in animals treated with *T.populnea* fruit extract. A significant reduction ( $p<0.001$ ) in blood glucose of 20.82%, 24.18%, and 26.32% was observed at the 6 h with doses of 100, 200 and 300 mg/kg body weight respectively. There were also significant reductions starting 1hr following treatment . The maximum reduction was observed 6 hr after treatment.

### *Effect of fruit extract on blood glucose in diabetic rabbits*

The mean blood glucose concentrations of control, *T.populnea*-treated (100, 200 and 300 mg/kg, p.o.) and tolbutamide treated (250 mg/kg, p.o.) rabbits are shown in Table. 2. Dose dependent antihyperglycemic activity was also observed with *T.populnea* in alloxan-induced diabetic rabbits. The percentage reduction of blood glucose was higher in the diabetic state compared to the normal state by the three doses of *T.populnea*. A significant reduction ( $p<0.001$ ) in blood glucose of 35.06%, 38.02%, and 47.92% was observed at 6 h with the doses of 100, 200 and 300 mg/kg body weight, respectively. There were also significant reductions starting 2 h following treatment . The maximum reduction was observed 6 h after treatment. Tolbutamide produced a significant reduction ( $p<0.001$ ) in blood glucose compared to diabetic control at the 6 h (53.56%).

## Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (17). Traditional plant medicines are used throughout the World for a range of diabetic presentations. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

In the present study ethanolic extract of the *T.populnea* fruit suppressed blood glucose levels in normal and alloxan induced diabetic rabbits, when

compared to control animals. The hypoglycemic potential of the extract was comparable with that of tolbutamide in normal and diabetic rabbits. On the other hand, tolbutamide caused significantly ( $p<0.001$ ) more hypoglycemia in comparison with the plant extract at 300 mg /kg body weight( $p<0.001$ ) . An emphasis is laid on glucose homeostasis as severe hypoglycemia can result in life threatening situation. The mechanism of this hypoglycemic effect of the extract is not elucidated in this study. Some medicinal plants with hypoglycemic properties are known to increase circulating insulin level in normoglycemic rats (18). The antidiabetic action of *T.populnea* is probably due to enhanced insulin secretion or due to increase in peripheral glucose uptake.

In conclusion our results have showed that fruits of *T.populnea* possess blood glucose lowering effect in normoglycemic and in alloxan induced hyperglycemic rabbits. Thus the folk use of this plant may be validated by this study. The fruits seem to have a promising value for the development of potent phytomedicine for diabetes. Further investigation is expected to characterize the active hypoglycemic principle and to elucidate the mechanism of action.

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