Antidiabetic and Antihyperlipidemic Effects of *Thespesia populnea* Fruit Pulp Extracts on Alloxan-induced Diabetic Rats

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Present study was carried to find out the antihyperglycemic and antihyperlipidemic activity of ethanol and aqueous extract of *Thespesia populnea* fruit pulp on alloxan-induced diabetic rats. Diabetes was induced in rats by administration of alloxan (150 mg/kg, i.p.). After the successful induction of experimental diabetes, the rats were divided into five groups each comprising a minimum of six rats. Phytochemical analysis and acute toxicity study of extracts was also done. The effects of extracts and metformin on fasting blood glucose and plasma lipid were examined for 28 days. Statistical analysis was carried out by using analysis of variance followed by Dunnet's multiple comparison test and paired *t*-test were done as the test of significance using GraphPad Prism. $P \leq 0.05$ was considered as the minimal level of statistical significance. Therapeutic dose of extract was found to be 200 mg/kg on the basis of acute toxicity study. Aqueous and alcoholic extract showed a significant reduction in blood glucose levels as well as a lipid profile of diabetic rats at the end of 28th day of treatment. However, in groups treated with plant extract the reduction in the blood glucose and improvement in lipid profile was slightly less than that achieved with the standard group (metformin). From this study, it can be concluded that ethanol and aqueous extract of *Thespesia populnea* exhibited significant antihyperglycemic and antihyperlipidemic effects on alloxan-induced diabetic rats.

Key words: Alloxan, antihyperglycemic, antihyperlipidemic, glucose level, *Thespesia populnea*

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action[1]. DM is also associated with an increased risk for developing premature atherosclerosis due to independent risk factors such as hypercholesterolemia and hypertriglyceridemia[2]. Atherosclerosis is characterized by the deposition of cholesterol and cholesterol ester of lipoproteins in the connective tissue of the arterioles[3].

The pathogenesis of DM is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents have been successful in maintaining euglycaemia and controlling long-term microvascular and macrovascular complications[4]. The number of patients with DM is predicted to increase globally to approximately 200 million within the next few years[5]. Therefore, there is growing need of exploring medicinal plants, which are having the advantages of little or no side effect. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been reported in the literature[6,7]. Some of them are being used in traditional systems of medicine from hundreds of years in many countries of the world[8].

Thespesia populnea (Linn) Sol. ex Correa (*Malvaceae*) is a large tree found in tropical regions and coastal forests of India. The bark and flowers are useful in folk medicine to treat cutaneous infections

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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, antiinflammatory, antioxidant, purgative and hepatoprotective activity[9]. Antidiabetic activity of bark, leaf and whole fruit extracts has already been reported earlier[10,11]. In view of this, only fruit pulp of *T. populnea* was studied separately evaluating the antihyperglycemic and antihyperlipidemic activity of ethanol and aqueous extracts in alloxan-induced diabetic rats.

T. populnea fruits were collected from the local area of Newasa, Ahmednagar district (Maharashtra) and authenticated by expert taxonomist of Botanical Survey of India, Pune, (Voucher No. RBPST 1, Ref. No. BSI/WC/Tech/126). Relevant voucher specimen is kept for reference in the Department Herbarium. The seeds were separated; fruit pulp was shade-dried and powdered in a grinder. The powder was extracted successively with petroleum ether (60-80°) and absolute alcohol (ethanol) using Soxhlet apparatus to get ethanol extract of *Thespesia populnea* (EETP). Whereas, aqueous extract of *Thespesia populnea* (AETP) was prepared by cold maceration method (water and 5% chloroform). The extracts were concentrated under reduced pressure at a low temperature (40-50°)[12]. The ethanol and aqueous extracts were subjected to various preliminary phytochemical analysis tests. The test were carried out for alkaloids, flavonoids, tannins, triterpenes, glycosides, saponins, proteins, steroids, carbohydrates and phenolic compounds, gums and mucilage for the presence or absence of phytoconstituents in both the plant extract[13].

Swiss albino mice weighing between 25 and 30 g of both sex and Wistar rats weighing between 175 and 200 g of either sex (Sri Venkateshwara Enterprises, Bangalore, India) were used. Animals were housed under standard conditions of temperature (24±2°) and relative humidity (50-70%) with a 12:12 light: Dark cycle. The animals were fed with standard pellet diet (Hindustan lever Ltd., Bangalore) and water *ad libitum*. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The experimental protocols were approved by The Institutional Animal Ethic Committee (IAEC No. P'col/18/2006) of the Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu.

For acute oral toxicity and LD_{50} determination, the Organisation for Economic Co-operation and Development guidelines 425 were followed[14].

DM was induced in a batch of normoglycaemic albino rats starved for 16 h, by injecting intraperitoneally 150 mg/kg body weight of alloxan monohydrate dissolved in physiological saline. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution intraperitoneally after 6 h. For the next 24 h, the rats were kept on 5% glucose solution in their cages to prevent hypoglycemia as revealed by the determination of blood glucose level (BGL)[1]. Diabetes in experimental animals was confirmed a week after alloxan injection by determining the blood glucose concentration. Animals with blood glucose of 300-350 mg/dl were used for the experiment.

The rats were divided into six groups each carrying six animals. Group I: Normal control rats administered vehicle (suspension of 5% tween 80 in distilled water, 5 ml/kg, p.o.). Group II: Diabetic control rats administered vehicle (suspension of 5% tween 80 in distilled water, 5 ml/kg, p.o.). Group III: Diabetic rats administered EETP, 200 mg/kg, p.o. Groups IV: Diabetic rats administered AETP, 200 mg/kg, p.o. Group V: Diabetic rats administered metformin (250 mg/kg, p.o.). The quantities of the both the extracts and standard drug, metformin were administered according to their body weight. For acute study one single dose was given, whereas for subacute study daily administration was done for 28 days.

Blood samples for glucose determination were obtained from the retro orbital puncture of fasting animals and BGLs were determined by using a glucometer[5]. The acute study involved withdrawal of blood at 0, 1, 3, 5, 7 and 24 h after administration of vehicle, extracts and metformin while the sub-acute study involved estimation of blood glucose at 0, 7, 14, 21 and 28 day after administration of vehicle, extracts and metformin.

Approximately 1.5 ml of blood was collected from each animal at 0 day and 28 day for the analysis of plasma lipids. Blood was taken by retro-orbital puncture method and collected into ethylenediaminetetraacetic acid coated tubes. The plasma was immediately separated by centrifugation at 3000 rpm for 10 min and analyzed.

Total cholesterol (TC) and high-density lipoprotein (HDL)-cholesterol estimation was done by cholesterol

oxidase-phenol+aminophenazone (PAP) method while plasma triglyceride was estimated by glycerol-3-phosphate oxidase-PAP method using Erba diagnostic kit. Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were calculated as follows[7], VLDL=Triglycerides (TG)/5 and LDL=TC−(HDL+VLDL).

The results were expressed as mean±SE. Statistical analysis was carried out by using analysis of variance followed by Dunnet's multiple comparison test and paired *t*-test were performed as the test of significance, using GraphPad Prism. $P \leq 0.05$ was considered as the minimal level of statistical significance.

The preliminary phytochemical screening of ethanol and aqueous extract revealed the presence of alkaloids, flavonoids, tannins, triterpenes, glycosides, saponins, proteins, steroids and phenolic compounds, gums and mucilage. The extractive values of extracts of ethanol and water were found to be 7.8 and 9.7%, respectively.

Acute toxicity studies revealed that *T. Populnea* extracts did not produce any toxic symptoms when administered orally to mice. The LD_{50} value of extract was 2000 mg/kg of body weight. As effective dose 50 (ED_{50}) is considered 10 times less than of LD_{50}, therefore its ED_{50} was found to be 200 mg/kg.

The effects of both extracts on the BGL in alloxan-induced diabetic rats were determined at various time intervals for 24 h after oral administration at 200 mg/kg. There was a significant elevation in the BGL by 3-4 times in alloxan-induced diabetic rats, when compared to normal rats. Administration of a single dose of EETP and AETP in diabetic rats, showed significant ($P<0.05$) reduction in BGL at all intervals. The maximum reduction in BGL for EETP was found to be 62.97% and for AETP was found to be 59.31% at the end of 5 h after the treatment. Metformin (250 mg/kg) showed maximum reduction of 65.42% at the end of 3 h after the treatment (Table 1).

Effect of daily dose of EETP, AETP and metformin on BGLs in alloxan-induced diabetic rats is given in Table 2. When data were compared with initial values, BGL was significantly ($P<0.05$) decreased by 63.15% for EETP, 54.84% for AETP and 64.89% for metformin.

Administration of the vehicle to alloxan-induced diabetic rats resulted in a gradual increase in the level of TC, TG, LDL and VLDL and gradual decrease in HDL at 28 days of study. In contrast to this, continuous administration of EETP and AETP (200 mg/kg) in the diabetic rats, levels of TC, TG, LDL and VLDL were decreased significantly ($P<0.05$) while the value of HDL was increased significantly ($P<0.05$) (Table 3).

Present study reports that the EETP and AETP effectively decrease the BGL in alloxan-induced

TABLE 1: EFFECT OF SINGLE DOSE ON BLOOD GLUCOSE LEVELS IN ALLOXAN- INDUCED DIABETIC RATS

Name of group	Blood glucose level in mg/dl at different hours after the treatment					
	Initial	1 h	3 h	5 h	7 h	24 h
Normal control	89.2±2.892*	91.83±2.892*	91.17±2.315*	91.83±3.46*	91.83±2.272*	91.50±3.7*
Diabetic control	313.5±4.16	313.5±4.16	312.3±4.551	314.3±4.42	313.3±4.287	314.2±4.14
EETP	351±2.798	271.0±2.176*	173.8±2.315*	130.0±2.89*	134.3±3.827*	349.7±2.813
AETP	344±4.064	280.2±3.468*	197.5±2.553*	140.2±2.90*	159.8±2.315*	345.0±4.04
Positive control	347±2.798	255.0±3.661*	120.5±4.048*	125.2±2.63*	139.5±2.363*	346.0±3.44

Values are expressed in mean±SEM, each group comprises 6 animals, statistical analysis was done using one way ANOVA followed by Dunnette's, multiple comparisons test, Group II (diabetic control) was compared with normal control, EETP=ethanol extract of *Thespesia populnea* (200 mg/kg), AETP=aqueous extract of *Thespesia populnea* (200 mg/kg) and positive control (metformin 250 mg/kg), *$P<0.05$ was considered statistically significant

TABLE 2: EFFECT OF MULTIPLE DOSES ON BLOOD GLUCOSE LEVELS IN ALLOXAN-INDUCED DIABETIC RATS

Name of groups	Blood glucose level in mg/dl at different days after the treatment				
	Initial	7^{th}	14^{th}	21^{th}	28^{th}
Normal control	89.83±3.07*	88.83±2.84*	86.70±2.80*	93.50±1.77*	91.20±2.51*
Diabetic control	313.2±2.38	345.5±4.87	372.00±6.43	394.00±5.90	406.00±4.66
EETP	350.8±4.65*	266.8±3.88*	177.00±3.94*	152.00±3.73*	129.00±4.36*
AETP	329.0±2.60*	274.5±3.24*	190.00±2.60*	171.00±3.54*	142.00±4.74*
Positive control	336.5±3.96*	230.8±2.44*	142.00±2.59*	133.00±4.06*	118.00±5.66*

Values are expressed in mean±SEM, each group comprises 6 animals, statistical analysis was done using one way ANOVA followed by Dunnette's, multiple comparisons test, Group II (diabetic control) was compared with normal control, EETP=ethanol extract of *Thespesia populnea* (200 mg/kg), AETP=aqueous extract of *Thespesia populnea* (200 mg/kg) and positive control (metformin 250 mg/kg), *$P<0.05$ was considered statistically significant

TABLE 3: EFFECT ON LIPID PROFILE IN ALLOXAN-INDUCED DIABETIC RATS

Parameter	Treatment	Blood lipid level in mg/dl at initial and after treatment	
		0 day	28 days
TC	Normal control	109.2±3.91	107.2±4.89
	Diabetic control	103.0±2.19	167.2±3.81*
	EETP (200 mg/kg)	102.8±2.77	107.3±3.88
	AETP (200 mg/kg)	107.3±2.53	121.7±3.72*
	Positive control	109.2±3.91	115.8±4.18
TG	Normal control	114.2±2.82	114.7±3.85
	Diabetic control	116.0±3.33	145.0±2.78*
	EETP (200 mg/kg)	118.3±1.60	122.2±2.56
	AETP (200 mg/kg)	110.7±3.63	125.8±2.15*
	Positive control	112.7±3.67	114.5±4.47
HDL	Normal control	32.83±1.40	32.50±1.40
	Diabetic control	33.67±1.30	19.67±1.25*
	EETP (200 mg/kg)	34.00±1.06	32.17±1.24
	AETP (200 mg/kg)	34.33±1.11	30.83±1.79*
	Positive control	37.00±1.46	36.67±1.80
LDL	Normal control	53.33±2.29	52.00±1.57
	Diabetic control	46.33±2.89	116.67±2.72*
	EETP (200 mg/kg)	45.83±1.90	50.83±1.92
	AETP (200 mg/kg)	49.33±1.64	65.50±1.52*
	Positive control	50.33±2.29	54.00±1.57
VLDL	Normal control	23.00±1.94	23.50±1.68
	Diabetic control	23.17±1.74	30.00±1.59*
	EETP (200 mg/kg)	23.33±1.02	24.33±1.47
	AETP (200 mg/kg)	22.17±1.01	25.33±1.70
	Positive control	22.37±1.11	23.00±1.65

Values are expressed in mean±SEM; each group comprises 6 animals, statistical analysis was done by pair *t*-test of significance using GraphPad Prism; EETP=ethanol extract of *thespesia populnea* (200 mg/kg), AETP=aqueous extract of *thespesia populnea* (200 mg/kg) and positive control (metformin 250 mg/kg), *P<0.05 was considered statistical significance, TC=total cholesterol, TG=triglycerides, HDL=high-density lipoprotein, VLDL=very low-density lipoprotein, LDL=low-density lipoprotein, *T. Populnea=Thespesia Populnea*

diabetic rats. Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans and thereby induces hyperglycemia[15,16]. *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, causing cell necrosis. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to rapid destruction of beta cell[12]. Excess of fatty acid in plasma produced by the alloxan-induced diabetes promotes the liver conversion of some fatty acids into phospholipids and cholesterol. These two substances along with excess TG formed at the same time in the liver may be discharged into the blood in the lipoproteins[17].

However, the mechanism of these plants used has not been clearly defined. Hyperglycemia increases the generation of free radicals by glucose autooxidation and the increment of free radicals may lead to liver cell damage. The increase in oxygen free radicals in diabetes could be primarily due to the increase in BGLs and secondarily due to the effects of the diabetogenic agent alloxan[6]. In this study we suggest that the possible mechanism of action by extracts could be related to antioxidants activity that aid to recover from impaired metabolism of glucose. Previous studies have demonstrated that *T. populnea* have potential antioxidant activity[4,18].

Prolonged administration of EETP and AETP leads to a significant reduction in plasma glucose and lipids level. Diabetic rats were observed to have increased plasma glucose and lipids, which are responsible for several cardiovascular disorders[19]. The higher lipid levels seen in diabetic rats were due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones[20]. The EETP and AETP leads to regeneration of the β-cells of the pancreas and potentiation of insulin secretion from surviving β-cells; the increase in insulin secretion and the consequent decrease in BGL may lead to inhibition of lipid peroxidation and control of lipolytic hormones. In this context, a number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects[21].

In the present study, the antihyperglycemic activity of EETP and AETP was evaluated in alloxan-induced diabetic rats. Single-dose study with 200 mg/kg showed significant (P<0.05) decrease in serum glucose level at 1, 3, 5 and 7 h. Continuous treatment with the EETP and AETP at dose 200 mg/kg for a period of 28 days showed a significant decrease (P<0.05) in the serum glucose level in diabetic rats. Maximum reduction of serum glucose level occurred at 5 h. We also found that oral administration of EETP and AETP for 28 days produced a significant decrease in blood glucose. Oral administration of metformin (250 mg/kg p.o.) showed the maximum reduction (69.65%) in BGL. Moreover, *T. populnea* produced significant beneficial effects in the lipid profile in diabetic rats, reducing TC, TG, LDL and VLDL and increasing HDL, significantly. The ethanol extract stimulates fatty acid biosynthesis and also the incorporation of fatty acids into TG in the liver and adipose tissue.

On the basis of the aforementioned results, we concluded that *T. Populnea* fruit pulp shows a significant antihyperglycemic and antihyperlipidemic effect in diabetic rats and when its effect is comparable

to that of metformin. Therefore, this medicinal plant is considered to be effective and alternative treatment for diabetes with cardiovascular disease.

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