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Evaluation of Antihyperglycemic and other Complication Effects of Extracts of *Thespesia lampas* Dalz and Gibs on Streptozotocin Induced Diabetic Rats

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Abstract

The methanol and aqueous extracts of *Thespesia lampas* Dalz and Gibs were tested for anti-hyperglycemic, anti-hyperlipidaemic and spermatogenetic effects of streptozotocin (STZ) induced diabetic rats. Diabetes was induced in adult male albino Wister rats by intra peritoneal (i.p) injection of streptozotocin at a dose of 150 mg/kg. Methanol and aqueous extracts of *T. lampas* (METL and AETL) at doses of 300 and 600 mg/kg b. wt were administered as a single dose per day to diabetes rats for the period of 15 days, respectively. The blood glucose levels and serum lipid profiles like total cholesterol, triglycerides, phospholipids, low density, very low density and high density lipoprotein and serum enzymes like ALT, ASP, ALP were measured in the diabetic and non diabetic rats. An oral glucose tolerance test (OGTT) was also performed, in which there was a significant improvement in glucose tolerance in rats treated with extracts. A comparison was made between the extracts and standard anti-diabetic drug glibenclamide. The extracts exhibited significant anti-hyperglycemic and anti-hyperlipidaemic effects on STZ induced diabetic rats when compared to that of standard drug (Glibenclamide 500µg/kg). The present investigation of this plant established pharmacological evidence to support the folklore claim that it is an anti-diabetic agent.

1. Introduction

Diabetes is a syndrome characterized by deranged carbohydrate metabolism resulting in abnormally high blood sugar level (hyperglycemia). It is caused by hereditary, increasing age, poor diet, imperfect digestion, obesity, sedentary lifestyle, stress, drug-induced, infection in pancreas, hypertension, high serum lipid and lipoproteins, less glucose utilization and other factors. It is estimated that the diabetic patients in India will increase by 195% in the near future**.** The treatment of diabetes with synthetic drugs is costly and chances of side effects are high. For example, long-term use of *Exenetide* (Byetta) has lead to side effects such as nausea, vomiting, diarrhea, dizziness, headache, jittery feeling and acidity (Sarita singh, 2009).

Thespesia lampas Dalz and Gibs (Nadkarni, 1954; Gamble, 1984) belongs to the family Malvaceae and its roots and fruits are used for treating gonorrhea, jaundices, syphilis (The wealth of India, 1976) anti-microbial (Vasaraj, 1997) and earlier report like hepatoprotective activity, antihyperlipidaemic activity and *In-vitro* antioxidant and antihyperglycemic studies were carried out (Sangameswaran, 2008).

2. Materials and Methods

2.1. Plant material

The plant part was collected from the foot hill of Yercaud, Salem, in the month of September 2005. The plant was identified and authenticated by a Botanist and a voucher specimen (TL-12) has been kept in our museum for future reference. The plant material was collected and shade dried at room temperature for 10 d and coarsely powdered and the powder was passed through sieve No.60.

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2.2. Preparation of the Extract

The powdered material of roots of *T. lampas* was extracted with methanol by soxhlet apparatus and water by cold maceration, separately. After extraction, the extracts were concentrated under reduced pressure. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

2.3. Preliminary Phytochemical Screening

The methanol and aqueous extracts were subjected to preliminary screening for various active phytochemical constituents (Kokate, 2005).

2.4. Preparation of extracts and standard drug

The extracts were administered orally to rats at various doses, as a suspension with Tween 80. Glibenclamide (500 µg/kg) was made suspension with Tween 80 and administered orally.

2.5. Animals

Male albino rats, 8-12 weeks old with an average weight of 200-250 g were purchased from M/S Venkateshwara enterprises (P) Ltd, Bangalore and used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum.* The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12h. The experimental protocols were approved by the Institutional Animal Ethics Committee and were cleared by the same (IAEC NO: P.Cog-1/06).

2.6. Toxicity Evaluation in Mice

To determine acute toxicity of a single oral administration of the methanol and aqueous extracts (Separately) of *T. lampas* different doses (200, 300, 400, 500 and 600 mg/kg) were administrated to different groups of mice. The animals were observed continuously for the initial 4 h and intermittently for the next 6 h and then again at 24 h and 48 h following drug administration. Mortality and general behavior of the animals were observed periodically for 48 h (Ghosh, 2007).

2.7. Drugs and Chemicals Used

Glibenclamide was procured from Aventis Pharma, Mumbai. India. Streptozotocin (STZ), hematoxylin, eosin and assay kits for Serum alanine aminotransferase (ALAT), aspartate aminotranferase (ASAT) and alkaline phosphates (ALP) were purchased from sigma chemicals Co Bangalore.

2.8. Oral Glucose Tolerance Test

After overnight fasting, a 0 min blood sample was taken from the rats in normal control (Group I), diabetic control (Group II), diabetic + glibenclamide (Group III), diabetic + methanol extract (300 and 600 mg/kg) (Groups IV and V) and diabetic + aqueous extract (300 and 600 mg/kg) (Groups VI and VII). Glucose solution (2 g/kg) was administered orally immediately (Sweety Lanjhiyana, *etal.,* 2011). Four more samples were taken at 30, 60, 90 and 120 min after glucose administration. All blood samples were collected in potassium oxalate and sodium fluoride containing tubes and used for the estimation of blood glucose.

2.9. Streptozotocin-Induced Diabetic Rats

Streptozotocin (STZ) was dissolved in 0.9% ice-cold saline immediately before use. Diabetes was induced in rats by intra peritoneal (i.p) injection of streptozotocin at a dose of 150 mg/kg, dissolved in saline (Pulok K Mukarjee, 2012). Forty eight hours after streptozotocin administration, blood samples were drawn from tail and glucose levels determined to confirm diabetes. The diabetic rats exhibiting blood glucose levels higher than 200 mg/dl were selected for the studies.

2.10. Experimental Procedure

In experiment, a total of 42 rats were used (36 diabetic surviving rats, 6 control rats). The rats were divided into seven groups

Group I   Control rats (Vehicle treated).

Group II   Diabetic control (Received 0.5 ml of 5% Tween 80).

Group III   Diabetic rats given Glibenclamide 500 µg/kg (Received 0.5 ml of 5% Tween 80).

Group IV and V Diabetic rats given METL 300 and 600 mg/kg b. wt,.

Group VI and VII   Diabetic rats given AETL 300 and 600 mg/kg b. wt, respectively.

Blood samples were collected from the tail for glucose estimation just before drug administration on 1st day and 1 h after drug administration on days 4, 7, 10 and 15. Blood samples were collected and centrifuged to separate serum for estimation of lipid profile and other biochemical parameters.

2.11. Determination of Serum Insulin

To determine the effect of extracts of *T. lampas* on serum insulin levels, same group of animals were used (Procedure described earlier). Blood samples were collected from experimental animals on first day and end of experiment. Serum was separated from the samples and insulin levels were determined. Insulin levels were measured by radio-immuno-assay method using kits obtained from Board of Radiation and Isotope Technology, Mumbai, India.

2.12. Anti-Hyperlipidaemic activity

Total cholesterol, HDL- C, LDL-C, VLDL-C, phospholipids, triglycerides and total cholesterol were analyzed from serum. (Vinoth kumar, 2010)

2.13. Determination of Biological Assay

Serum was separated by centrifugation at 3000 rpm at 25^0C for 15 min and analyzed for assorted biochemical parameters. The serum ALT, AST and ALP levels were measured using the respective spectrophotometer diagnostic kit obtained from Biosino Biotechnology (ompany Ltd (Beijing, PR China). (Sayed M. Rawi, 2011)

2.14. Statistical Evaluation

All the data are presented as mean ± SEM. The differences between group were evaluated by one-way analysis of variance (ANOVA) followed by the Dunnette multiple comparisons test. *P*<0.01 were considered to be significant.

3. Results and Discussion

3.1. Phytochemical Screening

Preliminary phytochemical screening revealed that the presence of triterpenoids, carbohydrates, vitamins, amino acids, proteins, tannins, saponin glycosides, phyotsterol and steroids.

3.2. Oral Glucose Tolerance Test

Table 1 shows the changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (3 g/kg). The diabetic rats showed that significant increase in the blood glucose at 30 and 120 min. In extracts and glibenclamide treated animals blood glucose concentration was significantly decreased after 60 min.

The hyperglycemic animals showed significant decrease in the glucose level on long term treatment for 15 days model at the doses of 300 and 600mg/kg of methanol and aqueous extracts of T. lampas (Group-IV-VII).

Oral administration of methanol and aqueous extracts of T. lampas to STZ induced diabetic rats significantly reduced blood glucose levels. Diabetic rats treated with methanol extract (300 and 600 mg/kg) showed significant reduction in blood glucose from 348.32 ± 7.81 to 190.77 ± 6.31 and 352.33 ± 3.41 to 188.6 ± 2.2 mg/dl, and aqueous extract (300 and 600 mg/kg) from 358.26 ± 5.2 to 187.6 ± 2.2 and 346.2 ± 4.8 to 174.33 ± 2.97 mg/dl, in comparison to untreated diabetic control, whereas standard drug (Glibenclamide 500µg/kg) treated rats also showed significant reduction in blood glucose concentration from 346.8 ± 8.2 to 123.3 ± 4.8 mg/dl, respectively. The results were recorded in table 2.

Table 1. Effect of oral glucose tolerance test

Treatment/ mg/kg	Blood glucose levels (mg/dL) after different times			
	0 min	30 min	60 min	120 min
Normal control	95.16 ± 2.90	169.17 ± 3.06	134.00 ± 2.59	108.5 ± 1.99
Diabetic control	412.33 ± 4.83	418.00 ± 4.53	427.50 ± 4.68	420.50 ± 4.63
Glibenclamide 500 µg/kg	200.00 ± 2.53	$213.33 \pm 2.29^{**}$	$202.0 \pm 2.04^{**}$	$213.50 \pm 2.36^{**}$
METL 300	225.83 ± 2.54	$230.75 \pm 2.44^{*}$	$238.4 \pm 6.09^{*}$	$235.2 \pm 1.94^{*}$
METL 600	236.41 ± 1.62	$242.2 \pm 5.01^{*}$	$239.83 \pm 2.25^{**}$	$234.75 \pm 0.93^{**}$
AETL 300	227.83 ± 4.82	$236.50 \pm 4.53^{*}$	$233.16 \pm 3.18^{**}$	$228.33 \pm 4.31^{**}$
AETL 600	$236.16 \pm 4.13^{*}$	$240.16 \pm 4.67^{*}$	$232.16 \pm 3.18^{**}$	$234.50 \pm 3.82^{**}$

METL- Methanolic extract of T. lampas, AETL- Aqueous extract T. lampasValues are mean ±SEM, n= 6. *,**, statistically significance of *P*<0.05, *P*<0.001, when compared with respective diabetic control. (One way ANOVA Followed by Dunnette multiple comparison test)*Anti*-Hyperglycemic Activity..\

Table 2. Anti-hyperglycemic effects of methanol and aqueous extracts of T. lampas on STZ induced rats

Treatment/ mg/kg	Blood glucose level mg/dl					Serum insulin level IU	
	1st Day	4th Day	7th Day	10th Day	15th Day	1st Day	15th Day
Norma control	94.7 ± 2.3	95.2 ± 2.5	94.8 ± 2.3	94.3 ± 1.9	94.5 ± 2.3	17.2 ± 3.0	18.0 ± 2.9
Diabetic control	352.0 ± 7.4	363.5 ± 6.1	373.3 ± 7.0	400.3 ± 8.5	434.3 ± 10.5	17.4 ± 2.8	14.3 ± 2.8
Glibenclamide 500 µg/kg	346.8 ± 8.2	$333.7 \pm 9.6^{*}$	$248.3 \pm 8.10^{*}$	$193.5 \pm 6.3^{**}$	$123.3 \pm 4.8^{**}$	14.3 ± 2.8	$16.4 \pm 2.9^{**}$
METL 300	348.32 ± 7.81	$330.83 \pm 3.98^{*}$	$264.50 \pm 3.60^{*}$	$218.33 \pm 2.98^{*}$	$190.77 \pm 6.31^{*}$	14.8 ± 4.8	$18.8 \pm 0.2^{*}$
METL 600	352.33 ± 3.41	$334.2 \pm 3.2^{**}$	$250.0 \pm 6.2^{**}$	$202.8 \pm 4.8^{**}$	$188.6 \pm 2.2^{**}$	15.2 ± 2.6	$19.3 \pm 3.0^{**}$
AETL 300	358.26 ± 5.2	$332.2 \pm 5.2^{*}$	$268.2 \pm 4.2^{*}$	$200.2 \pm 4.6^{*}$	$187.6 \pm 2.2^{*}$	15.4 ± 4.8	$19.8 \pm 2.2^{**}$
AETL 600	328.00 ± 4.63	$328.00 \pm 4.63^{**}$	$246.50 \pm 6.29^{**}$	$193.00 \pm 8.04^{**}$	$174.33 \pm 2.97^{**}$	14.6 ± 3.2	$21.2 \pm 2.6^{**}$

METL- Methanolic extract of *T. lampas,* AETL- Aqueous extract *T. lampas*Values are mean ±SEM, n= 6. *, **, statistically significance of *P*<0.05, *P*<0.001, when compared with respective diabetic control. (One way ANOVA Followed by Dunnette multiple comparison test).

3.3. Determination of Serum Insulin Level

The serum insulin levels in all groups of animals were estimated at first and 15th day after extract administration and the values were recorded.

The methanol and aqueous extracts of *T. lampas* (300 and 600 mg/kg) treated rats were significantly influence serum insulin levels from 14.8 ± 4.8 to 18.8 ± 0.2 and 15.2 ± 2.6 to 19.3 ± 3.0, and 15.4 ± 4.8 to 19.8 ± 2.2 and 14.6 ± 3.2 to 21.2 ± 2.6 IU in STZ induced diabetic animals, respectively. In diabetic control (untreated group) group of rats, decreases in the serum insulin level (Table 2).

3.4. Anti-Hyperlipidaemic Activity

The lipid profiles in control and experimental rats are depicted in Table 3 in STZ induced diabetic rats, there was a significant (*P*<0.001) increase of total cholesterol, triglycerides, phospholipids, and low density lipoproteins

(LDL) and very low density lipoprotein (VLDL) cholesterol and significant ($p<0.001$) decreases in high density lipoprotein (HDL) cholesterol in serum compared with normal control. The extracts treated rats were significantly ($p<0.001$) decreased the total cholesterol, triglycerides, phospholipids and LDL and VLDL cholesterol and significantly ($p<0.001$) increased HDL cholesterol.

Table 3. Anti-hyperlipidaemic effects of extracts of T. lampas on STZ induced diabetic rats.

Treatment/ mg/kg	Changes in mg/dl level					
	Serum total Cholestrol	Triglycerides	Serum HDL	Serum LDL	Serum VLDL	Serum Phospholipids
Normal control	81.00±7.21	87.50± 7.99	23.16 ± 2.00	95.33 ± 5.92	15.50 ± 1.23	48.50 ± 7.85
Diabetic control	196.83±9.58[*]	168.00± 5.72[*]	13.50 ± 1.50[*]	186.83 ± 5.05[*]	30.66 ± 1.83[*]	252.50 ± 9.91[*]
Glibenclamide 500 µg/kg	128.67±8.53[**]	116.50± 2.89[**]	21.33 ± 1.43[*]	123.00 ± 3.23[**]	17.83± 2.0[**]	177.83 ± 7.6[*]
METL 300	152.42±4.8[*]	132.02±4.2[**]	20.22 ± 0.82[**]	139.52 ±8.22[**]	22.62 ±2.20[**]	192.42 ±6.6[**]
METL 600	145.26±2.2[**]	126.24±2.4[**]	22.24 ± 0.84[**]	136.24 ±4.2[**]	18.20 ±1.2[**]	192.26 ±2.8[**]
AETL 300	153.50±10.25[*]	128.00±4.38[**]	18.33 ± 0.98[**]	137.50 ± 5.32[**]	21.66 ± 1.92[**]	190.62 ± 9.52[**]
AETL 600	142.67±6.26[**]	121.33±3.65[**]	19.16 ± 0.94[**]	134.17 ± 9.38[**]	17.66 ±1.30[**]	186.23 ± 8.46[**]

METL- Methanolic extract of *T. lampas,* AETL- Aqueous extract *T. lampas* Values are mean ±SEM, n= 6. *, **, statistically significance of *P*<0.05, *P*<0.001, when compared with respective diabetic control. (One way ANOVA Followed by Dunnette multiple comparison test) .

3.5. Determination Biochemical Parameters

Serum enzymes (ALP, AST and ASP) total protein levels are shown in Table 4. ALP, ASP levels were increased significantly ($p<0.001$) in STZ treated diabetic rats in comparison with normal animals.

The extracts significantly ($p<0.001$) decreased the elevated ALP, AST and ASP levels in treated rats. A significant ($p<0.05$) decrease in total protein level was observed with control rats.

Table 4. Biochemical parameters of methanol and aqueous extracts of *T. lampas* treated in STZ induced diabetic rats on 15th Day

Treatment /mg/kg	Changes in biochemical parameters			
	ALT U/L	AST U/L	ALP U/L	T. Bilirubin
Normal control	54.2 ± 1.02	34.4 ± 3.26	89.2 ± 3.16	0.20± 0.02
Diabetic control	82.59 ± 1.90	48.2± 6.4	92.4 ± 0.02	0.26± 0.06
Glibenclamide 500 µg/kg	58.26± 4.6[**]	39.0± 4.6[**]	98.20± 0.04[**]	0.32±0.06[**]
METL 300	53.2 ± 1.22[*]	36.4 ± 2.20[*]	91.2 ± 3.12[*]	0.18 ± 0.06[*]
METL 600	52.6 ± 2.26[**]	33.4 ± 2.2[**]	92.4 ± 2.4[**]	0.19 ± 0.04[**]
AETL 300	52.8 ±2.2[*]	38.2 ±3.22[*]	90.6 ±2.2[*]	0.20 ± 0.02[*]
AETL 600	53.2± 0.22[**]	34.6± 2.2[**]	89.4± 0.4[**]	0.19± 0.04[**]

METL- Methanolic extract of *T. lampas,* AETL- Aqueous extract *T. lampas* Values are mean ±SEM, n= 6. *, **, statistically significance of *P*<0.05, *P*<0.001, when compared with respective diabetic control. (One way ANOVA Followed by Dunnette multiple comparison test) .

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