International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Original Article **CODEN: IJPNL6**

ANTIDIABETIC ACTIVITY OF ETHANOLIC & AQUEOUS EXTRACT OF AERIAL PART OF *THESPESIA LAMPAS* (CAV) DALZ AND GIBS ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The study evaluates antidiabetic activity of ethanolic & aqueous extract of aerial part of *Thespesia.lampas* on streptozotocin induced diabetes in rats. Ethanol & Aqueous extract were prepared by soxhlet & maceration process respectively. Antidiabetic activity of the aerial part of the extract at dose 200mg/kg in STZ (i.p 65mg/kg body weight) induced diabetic rats. The study also included the estimation of different biochemical parameters on STZ induced diabetes. The reduced blood glucose levels was statistically significant ($P<0.05$) in the dose of 200mg/kg of ethanol and aqueous leaf extract when compared with control. The results exhibited potent antihyperglycemic activity in normal and STZ induced diabetic rats so it might be useful in the treatment of diabetes.

Key Words: STZ induced diabetes, *Thespesia lampas* aerial part, ethanolic & aqueous extract.

INTRODUCTION

Diabetes is a state where homeostasis of metabolism of carbohydrate and lipid is unbalanced by the hormones of pancreas i.e. insulin, which results in elevated blood glucose level. It is the world's largest endocrine disorder and is one of the major causing deaths in the world [1]. According to World Health organization (WHO), the world wide global population is in the midst of a diabetes epidemic with people in Southeast Asia and Western pacific mostly at risk. The number of cases for diabetes which is currently at 171 million is predicted to reach 366 million by the end of 2030 [2] .Search for drugs that can manage metabolic disorders is very much important.

Thespesia lampas belongs to Malvaceae family with its vernacular name known as *"Ranbhendi"* a wild herb that grows during monsoon on the hills throughout the India and also in Eastern Tropical Africa [3] .Literature survey shows the root of the plant possesses antidiabetic [4], anti-hyperlipidemic [5], hepatoprotective [6] , antioxidant [7] activities. In folklore medicine several plants are used to treat diabetes mellitus, most of the plants are not scientifically validated for their therapeutic efficacy and safety. The aerial part of *Thespesia. lampas* are used traditionally for antidiabetic activity. There is scientific evidence on the roots and stems for antidiabetic activity. As per our knowledge there are no reports particularly on the ethanolic & aqueous extracts of *Thespesia lampas* on streptozotocin induced diabetes in rats.

MATERIALS AND METHODS

Plant material: The aerial part of *Thespesia lampas* was collected from chittoor district Andhra Pradesh in the month of september-october.The plant was authenticated by Dr.K.Madhavachetty, Department of Botany, Sri Venkateswara University Tiruphati, and Voucher specimen of the leaf of the plant was kept in museum of OPJS.

Preparation of ethanolic leaf extract: The aerial part of *Thespesia lampas* were shade dried for 3-5 days. Dried plant material was ground to coarse powder using a blender and stored at ambient temperature and passed through sieve and extracted in a soxhlet apparatus for two days using alcohol. The extract was concentrated under reduced pressure using a rotary evaporator. The yield of the extract was found to be 2.5 %. Extract was preserved in a desicator until further use.

Preparation of aqueous leaf extract: The aerial part of *Thespesia lampas* were shade dried and powdered. The aqueous extract was prepared by cold maceration for 7days. The powder were soaked in distilled water and stirred intermittently and then left overnight. Macerated aerial part of extract were filtered through coarse sieve and filtered. The filtrate was dried at reduced pressure in a rotary evaporator and freeze dried. The extracts were used for further studies. The yield of the extract was found to be 3.25%.

Preliminary Phytochemical screening [8]: The presence of various phytochemical constituents in the extract was determined using standard screening tests.

Animals: Male albino rats (150-175 g) were used for this study. Before and during the experiment the animals were maintained in well ventilated room at room temperature with natural day-night cycle in polypropylene cages lined with husk in standard environmental conditions temperature 25 ± 2º C, relative humidity 55 ± 10 % and 12:12 light: dark cycle. The rats were fed on standard pellet diet ad libium and had free access to water. The experiments were performed after the approval of protocol by the institution animal ethics committee (IAEC) and were carried out in accordance with current guidelines for the care of laboratory animals.

Acute toxicity studies [9]: Acute oral toxicity studies were performed in rats according to OECD guidelines 425.The dose selected were 200 mg/kg b.wt

Experimental induction of diabetes: Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution (65mg/kg dissolved in citrate buffer 0.1 M, pH 4.5) to overnight fasted rats. Control rats received only the buffer. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 h.The animals with blood glucose concentration more than 250mg/dl were considered to be diabetic and used for the experiment. Blood samples were collected by retro orbital sinus puncture.

Oral glucose tolerance test [10]: The test were performed according to the reported method

Experimental procedure: In this experiment the rats were divided in to following groups
Group I: Administered vehicle as normal control
Group II: Administered Streptozotocin (65mg/kg, i.p)
Group III: Diabetic rats treated with *T.lampas* ethanolic extract (200mg/kg p.o. once daily)
Group IV: Diabetic rat treated with *T.lampas* aqueous extract (200mg/kg p.o. once daily)
Group V: Diabetic rats treated with glibenclamide (2.5 mg/kg b.w p.o. once daily)

Biochemical tests: After 21-days treatment, blood from all the groups were collected by retro-orbital puncture under mild anesthesia for estimating blood glucose levels, total cholesterol levels, total triglyceride levels, serum urea, serum creatinine, serum insulin, HDL, LDL & VLDL levels.

Statistical analysis: The results were expressed as mean ± SEM for six rats in each group. Data were analyzed by one way of analysis of variance followed by Student newman-keuls using graph prism software.

RESULTS

Phytochemical screening: The ethanolic & aqueous extract of *T.lampas* showed the presence of saponin glycosides, triterpenoids, carbohydrates, phytosterols, flavanoids, carbohydrates, proteins and aminoacids.

Oral Glucose tolerance test: Table 1 represents changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (3g/kg). In single oral treatment rats showed significant increase in the blood glucose at 30 and 120 min.When compared to diabetic control significant reduction of blood glucose levels were seen at 120 min, blood glucose levels were significantly decreased from 330.8 ± 1.28 (diabetic control) to 227.6 ±1.55 (*T.lampas* ethanolic extract) , 218.53 ±2.45 (*T.lampas* aqueous extract).

Table 1: Effect of *T.lampas* ethanolic & aqueous extract on oral glucose tolerance test in Streptozotocin induced diabetes

GROUPS	0 min	30 min	60 min	120 min
Normal control	96.13±1.45	164.83±1.30	134.33±1.49	112.08±1.71
Diabetic Control	320.68 ± 2.65	325.13±3.26	322.10±1.30	330.8 ±1.28
Diabetic + *T.Lampas* ethanolic extract (200mg/kg)	245.11 ± 4.33^{ns}	232.75±2.30**	230.13 ± 5.31*	227.6±1.55**
Diabetic ++*T.lampas* Aqueous extract(200mg/kg)	242.73±6.31^{ns}	227.25 ± 4.60**	231.15±6.30**	218.53 ± 2.45**

Values are mean±SEM (n=6) when compared with diabetic control * P<0.05, **P<0.01, ***P<0.001 were considered significant compared to diabetic control

Table-2: Effect of *T.lampas* ethanolic & aqueous aerial part of extract on Streptozotocin induced diabetes

Groups (mg/kg)	1st day	4th day	7th day	10th day	21st day
Normal control	93.36 ±0.20	93.61 ± 0.29	93.86 ± 0.19	93.41 ± 0.10	93.48 ±0.24
Diabetic control	320.33 ±7.24	340.5 ± 7.29	352.33 ±10.7	354± 12.7	357.5 ±6.16
Diabetic control +T.lampas ethanolic leaf extract	347.50±7.62^{ns}	342.5±5.59**	242.66±7.92^{ns}	213.33±9.01*	121.5±8.35*
Diabetic control +T.lampas aqueous leaf extract	348.91±3.67**	336.1±8.51^{ns}	238±8.32*	205.66±11.2^{ns}	113±3.62**
Diabetic control + Glibenclamide	316.33±8.96**	335.16±5.50**	238.33±6.53**	210±6.05**	111.16±5.15***

Values are mean±SEM (n=6) when compared with diabetic control * P<0.05, **P<0.01, ***P<0.001 were considered significant compared to diabetic control

Table 3: Effect of *T.lampas* ethanolic & aqueous aerial part of extract on serum insulin, serum urea, serum creatinine after 3 weeks treatment

GROUPS	SERUM INSULIN	SERUM UREA	SERUM CREATININE
Normal control	12.97±0.72	33.65±0.75	0.58±0.11
Diabetic control	3.78±0.13	75.13±0.70	1.43±0.11
Diabetic control +T.lampas ethanolic leaf extract	10.45±0.81**	38.38±0.47*	0.856±0.16**
Diabetic control +T.lampas aqueous leaf extract	10.81±0.59**	34.51±0.54**	0.64±0.13**
Diabetic control + Glibenclamide	14.98±0.36***	23.0±0.73***	0.55±0.26***

Values are mean± SEM (n=6) when compared with diabetic control * P<0.05, **P<0.01, ***P<0.001 were considered significant compared to diabetic control

Table 4: Effect of *T.lampas* ethanolic & aqueous aerial part of extract on serum total cholesterol, serum total triglycerides after 3 weeks treatment

GROUPS	SERUM TOTAL CHOLESTEROL	SERUM TOTAL TRIGLYCERIDES
Normal control	113.5±1.30	77.66±3.04
Diabetic control	224.5±1.36	138.16±2.02
Diabetic control +T.lampas ethanolic leaf extract	136.16±1.90**	97.66±2.17**
Diabetic control +T.lampas aqueous leaf extract	119.5±1.11**	96.83±2.89**
Diabetic control + Glibenclamide	95.0±0.945***	88.33±1.94***

Values are MEAN ± SEM (n=6) when compared with diabetic control *P<0.05, **P<0.01, ***P<0.001 were considered significant compared to diabetic control.

Table 5: Effect of *T.lampas* ethanolic & aqueous aerial part of extract on serum HDL, LDL, VLDL levels after 3 weeks treatment

GROUPS	SERUM HDL LEVELS	SERUM LDL LEVELS	SERUM VLDL LEVELS
Normal control	61.95±2.02	71.33±1.70	14.13±1.41
Diabetic control	23.49±1.42	179.71±1.76	25.69±1.41
Diabetic control +T.lampas ethanolic leaf extract	34.28±1.73	111.53±2.08	23.77±1.26
Diabetic control +T.lampas aqueous leaf extract	35.19±1.79	110.03±2.56	21.53±1.33
Diabetic control + Glibenclamide	66.07±1.40	94.73±1.44	15.78±0.73

Values are MEAN ± SEM (n=6) when compared with diabetic control *P<0.05, **P<0.01, ***P<0.001 were considered significant compared to diabetic control.

Effect of *T.lampas* extract on blood glucose levels in diabetic rats: The effect of oral administration of *T.lampas* ethanolic & aqueous aerial part of extract on blood glucose levels in STZ diabetic rats is presented in Table-2. At dose level 200mg/kg b.wt both the extract showed a significant reduction in blood glucose levels on 21st day. Glibenclamide showed a significant reduction in blood glucose levels at 21st day when compared to control (Table-2).

Effect of *T.Lampas* extracts on serum insulin, serum urea and serum creatinine in diabetic rats: Streptozotocin caused significant reduction in insulin levels, increase in urea and creatinine levels. Administration of *T.lampas* aerial part of extract showed significant increase in serum insulin levels, significant decrease in serum urea and serum creatinine levels; was comparable to that of glibenclamide (Table-3).

Effect of *T.Lampas* extracts on lipid profile in diabetic rats: Administration of *T.lampas* aerial part of extract showed significant reduction in serum total cholesterol and serum total triglycerides when compared to diabetic control. When compared to diabetic control serum HDL levels were increased and serum VLDL was decreased by administration of *T.lampas* aerial part of extract (Table 4, 5).

DISCUSSION

There is increase in the number of diabetic patients there is need to find alternatives for screening of antidiabetic drugs. There are many different drugs for treatment of diabetes in the pharmaceutical market, but there is increase in demand to find for alternatives as allopathic medicines are having side effects, as an alternative to allopathic medicinal plants are used with success for the treatment of diabetes. In the present work antidiabetic activity of the ethanolic and aqueous leaf extract of the Thespesia.lampas in STZ

induced diabetes. From oral glucose tolerance test Thespesia.lampas leaf extract at dose 200mg/kg showed maximum improvement in glucose tolerance. When Thespesia.lampas leaf extract were administered to glucose loaded normal rats reduction in blood glucose levels was observed. In STZ induced model significant reduction in blood glucose levels were observed after 21 days. The data reveals that *Thespesia.lampas* aerial part of extract showed significant reduction in blood glucose levels.

CONCLUSION

Thespesia lampas ethanolic & aqueous extract showed significant hypoglycemic activity in streptozotocin induced diabetic rats. The extract showed improved in various biochemical parameters. However further Phytochemical investigations are required to isolate the active principle responsible for hypoglycemic activity and to know the mechanism of action for antidiabetic activity

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