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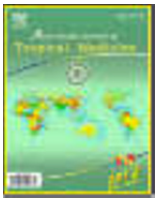

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Evaluation of antihyperglycemic activity of methanolic *Tecomaria capensis* Thunb. (Bignoniaceae) leaves extract in alloxan induced hyperglycemic rats

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ABSTRACT

Objective: To evaluate the antihyperglycemic activity of *Tecomaria capensis* (*T. capensis*) Thunb. (Bignoniaceae) methanolic leaves extract (TCLE) using blood glucose level in normal fasted rats, glucose tolerance test and alloxan induced hyperglycemia models. **Methods:** TCLE (100, 300, 1 000 and 2 000 mg/kg body wt.) was given to rats orally to observe acute toxicity, and observed for 14 d. TCLE 200 and 400 mg/kg, and glibenclamide 0.6 mg/kg were given orally in all models. **Results:** Results demonstrated that the no mortality was reported even after 14 d. This indicates that the methanol extract is safe up to a single dose of 2 000 mg/kg body weight. TCLE (200 and 400 mg/kg *p.o.*) exhibited remarkable blood glucose lowering effect in blood glucose level in normal fasted rats, glucose tolerance and alloxan induced hyperglycemia model. Cholesterol and triglyceride also decreased in alloxan induced hyperglycemia model. **Conclusions:** The results of this study exhibites that methanol extract of *T. capensis* possesses antihypergycemic activity and it may prove to be effective for the treatment of hyperglycemia.

1. Introduction

Tecomaria capensis (*T. capensis*) Thunb. (Bignoniaceae), also known as Cape-honeysuckle a fast growing, scrambling shrub which may grow up to 2-3 m high and spread more than 2.5 m. *T. capensis* is an evergreen plant in warm climate areas but loses its leaves in colder areas. It has pinnately compound leaves that have oval leaflets with blunt teeth. Flowering time for this shrub is very erratic and often it flowers all year round. Flowers are orange in color. Flowers are tubular and bird pollinated, attracting nectar-feeding birds, especially sunbirds. The powdered bark of this plant is used as a traditional medicine to relieve pain and sleeplessness[1]. Dried powdered bark infusions are taken for sleeplessness[2] and are reported to induce sleep[3]. It is included in the list of African plants evaluated for in vitro antiplasmodial activity against *Plasmodium falciparum* (*P. falciparum*)[4].

The present study was carried out to determine the effect of methanolic extract of *T. capensis* leaves (TCLE) on wound healing based on two evidence, (1) Previously methanol extract of *T. capensis* leaves reported as antimicrobial[5] and antioxidant[6]; (2) Phytochemical investigation of methanol extract of *T. capensis* leaves revealed the presence of triterpenoids, phenolics, glycosides, flavonoids.

It is well known that certain flavonoids exhibit hypoglycemic activity[7,8] and are also known for their ability of beta cell regeneration of pancreas[9,10].

2. Materials and methods

2.1. Plant material

The leaves of *T. capensis* Thunb. (Bignoniaceae) were collected from Jaipur National University, Jaipur, Rajasthan, India on 1 July, 2010. The plant was identified by the Mr. Vinod Sharma, Herbarium Head, Department of Botany, Rajasthan University, Jaipur. A voucher specimen (RUBL 20847) for this plant material was preserved in the herbarium of Department of Botany, Rajasthan University, Jaipur, Rajasthan, India. The leaves, dried in shade were powdered and subjected to soxhlet extraction with methanol at 40-60 ℃ for 72 h. The extract collected was evaporated (yield 26.7%. w/w), and stored in a vacuum desiccator. The preliminary phytochemical investigations with the methanolic extract revealed the presence of flavonoids, flavones, phenolic compound, tannins, volatile oil, fixed oil, steroids, saponins, glycosides[11-13].

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2.2. Drugs and chemical

The following drugs and chemical namely, glibenclamide, Alloxan, glucose and EDTA were used during the experimental study.

2.3. Animals

Albino rats of either sex (150-200 g) were used for the experimental study. The animals were maintained under standard husbandry conditions in polypropylene cages and provided with food and water *ad libitum*. The animals were kept on fasting overnight prior to the experimentation and all the procedures used in these studies were approved by the Institutional Animal Ethics Committee.

2.4. Acute toxicity studies

The acute toxicity was performed according to OECD guideline[14]. The selected female albino rats were used for toxicity studies. The animals were divided into four groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 100, 300, 1 000 and 2 000 mg/kg body wt. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24 h, and daily for 14 d for any behavioural change or mortality.

2.5. Blood glucose level in normal fasted rats

Four randomized groups six in each of normal fasted rats were administered 200 and 400 mg/kg of the TCLE, 0.6 mg/kg of glibenclamide and vehicle orally. Subsequently, blood sugar levels were assessed at 1, 2 and 3 h intervals[15].

2.6. Glucose tolerance test

Four groups of 6 rats each were used for the study. Group 1 served as normal Vehicle, Group 2 animals were administered with 0.6 mg/kg of glibenclamide orally, Group 3 animals were administered with TCLE 200 mg/kg orally and Group 4 animals were administered with TCLE 400 mg/kg orally. The rats of all the groups were loaded with 60% glucose (3 g/kg *p.o.*) 30 min after extract administration. Blood sugar levels were assessed at 1, 2 and 3 h intervals[16].

2.7. Alloxan induced hyperglycemia

Induction of hyperglycemia: alloxan 150 mg/kg was administered i.p. following which glucose was added to the drinking water of the animals to prevent hypoglycemic crisis. Fasting blood sugar (FBS) for the animals was measured after 72 h. Animals with FBS level $\geqslant$ 300 mg/dL were considered hyperglycemic[17].

The animals showing hyperglycemia were then grouped in 4 groups of 6 animals each. Group 1 received vehicle. Group 2 were administered with glibenclamide 0.6 mg/kg orally, Group 3 animals were administered with TCLE 200 mg/kg orally and Group 4 animals were administered with TCLE 400 mg/kg orally. Blood samples were obtained at 2, 4 and 6 h after treatment[18].

For repeated dose treatment animals were treated once a day for 14 d and were given free access to food and water ad libitum. On the 15th day animals were killed by decapitation and blood was collected from the arterial jugular and serum was separated. The serum was used for the estimation of various biochemical parameters including blood glucose level, cholesterol level and triglyceride level[19].

2.8. Method of blood collection and glucose, cholesterol and triglyceride estimation

Blood samples were collected from the retro-orbital puncture. Blood glucose level was determined by using glucometer (one touch ultra). Cholesterol and triglyceride level were determined by using commercial kit for cholesterol and triglyceride estimation.

2.9. Statistical analysis

Results are expressed as mean±S.E.M. Statistical significance was determined by using the one way ANOVA followed by Dunnett's multiple comparison test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acute toxicity studies

In toxicity study four groups of rats were administered with methanolic *T. capensis* leaves extract in graded doses of 100 mg/kg, 300 mg/kg, 1 000 mg/kg and 2 000 mg/kg *p.o.*, respectively. The animals were kept under observation for the change in behavior or death up to 14 d following the plant extract administration. The extract administration neither caused any significant change in the behaviors nor the death of animal(s) in all the test groups. This indicates that the methanol extract is safe up to a single dose of 2 000 mg/kg body weight. Hence we have selected 200 to 400 mg/kg oral doses of methanolic *T. capensis* leaves extract to evaluate different pharmacological activity in rats.

Table 1
Effect of TCLE on fasting blood glucose level in normal rats.

Treatment groups	Glucose concentration at different time interval (mg/dL)			
	0 h	1 h	2 h	3 h
Control	60.33±1.10	63.50±2.14	62.67±2.65	61.50±1.71
Glibenclamide	64.33±2.75	56.67±2.01	51.83±1.68**	46.67±2.01***
TCLE 200 mg/kg	65.17±3.12	58.50±2.71	55.50±1.86	53.17±0.91*
TCLE 400 mg/kg	70.17±2.47	63.00±2.54	58.83±2.43	55.83±2.37*

Values are expressed as mean±S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way anova followed by Dunnett's multiple comparison test.

3.2. Blood glucose level in normal fasted rats

Fasting blood glucose test in normal rats revealed that TCLE given at 200 mg/kg and 400 mg/kg produced a significant fall in blood glucose (compared to the initial level) 3 h after administration (Table 1), though the effect was less than that produced by glibenclamide 0.6 mg/kg *p.o.*

3.3. Glucose tolerance test

The TCLE 200 mg/kg, TCLE 400 mg/kg and glibenclamide p.o. exhibited remarkable blood glucose lowering effect in the glucose tolerance test (Figure 1) compare to control. The TCLE 200 mg/kg and 400 mg/kg inhibit glucose increase 31% and 34.5% respectively, and glibenclamide 0.6 mg/kg p.o. inhibit glucose increase 34.18% compare to control at 1 h.

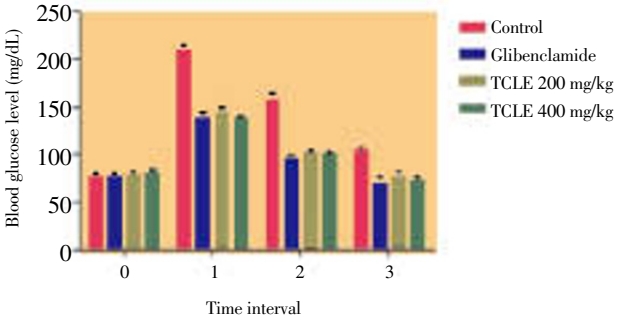

Figure 1. Effect of TCLE on blood glucose level in glucose tolerance test.
Values are expressed as mean±S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way anova followed by Dunnett's multiple comparison test.

3.4. Alloxan induced hyperglycemia

A single oral dose treatment of TCLE 200 mg/kg and TCLE 400 mg/kg in hyperglycemic rats after 4 h of treatment caused a significant (P<0.001) decrease in blood glucose from 302.33 mg/dL to 192 mg/dL, and 311.17 mg/dL to 160.33 mg/dL respectively, while glibenclamide 0.6 mg/kg p.o. decrease blood glucose from 313.83 mg/dL to 169.33 mg/dL (Figure 2).

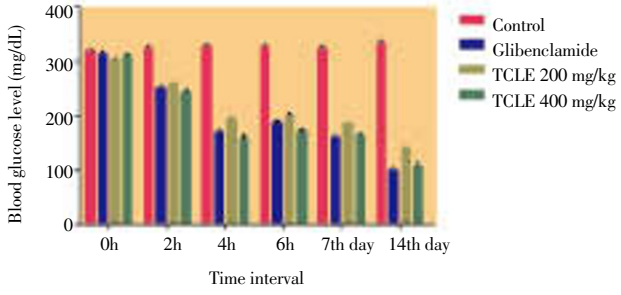

Figure 2. Effect of TCLE on blood glucose level in alloxan induced hyperglycemic rats.
Values are expressed as mean±S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way anova followed by Dunnett's multiple comparison test.

Repeated oral dose treatment of TCLE 200 mg/kg and TCLE 400 mg/kg in hyperglycemic rats at 7th day caused a significant (P<0.001) decrease in blood glucose from 302.33 mg/dL to 186.33 mg/dL and 311.17 mg/dL to 162.67 mg/dL respectively, while glibenclamide 0.6 mg/kg p.o. decrease blood glucose from 313.83 mg/dL to 159.5 mg/dL (Figure 2). TCLE 200 mg/kg and TCLE 400 mg/kg in hyperglycemic rats at 14th day caused a significant (P<0.001) decrease in blood glucose from 302.33 to 138.5 mg/dL and 311.17 mg/dL to 106.5 mg/dL respectively, while glibenclamide 0.6 mg/kg p.o. decrease blood glucose from 313.83 mg/dL to 100.17 mg/dL (Table 3).

Figure 3 & 4 reports the effect on biochemical parameters such as serum cholesterol and triglyceride on hyperglycemic rats treated with TCLE once a day for 2 weeks. TCLE 200 mg/kg decreases cholesterol and triglyceride level 35.56% and 21.43% respectively. TCLE 400 mg/kg decreases cholesterol and triglyceride level 41.76% and 28.04% respectively. Glibenclamide 0.6 mg/kg p.o. decreases cholesterol and triglyceride level 45.15% and 29.73% respectively.

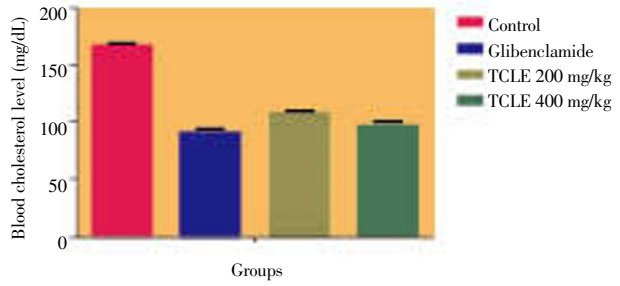

Figure 3. Effect of TCLE on blood cholesterol level in alloxan induced hyperglycemic rats after 14 d treatment.
Values are expressed as mean±S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way anova followed by Dunnett's multiple comparison test.

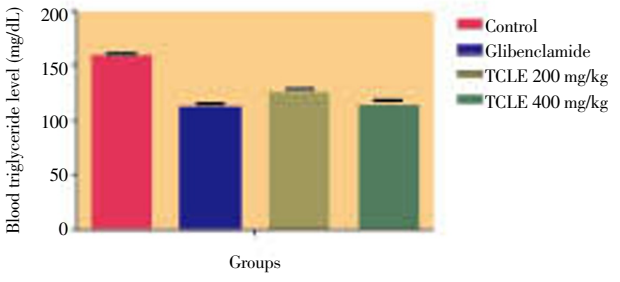

Figure 4. Effect of TCLE on blood triglyceride level in alloxan induced hyperglycemic rats after 14 d treatment.
Values are expressed as mean±S.E.M. (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared to control. One way anova followed by Dunnett's multiple comparison test.

4. Discussion

Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive antihyperglycemic principles[20–23]. Flavonoids are known to regenerate the damaged beta cells in the alloxan hyperglycemic rats[9]. Phenolics are found to be effective antihyperglycemic agents[24–28]. Our previous study, we found that TCLE have very good antimicrobial and antioxidant activity. TCLE revealed much amount of flavonoids and phenolics[6].

The present study is the preliminary assessment of the antihyperglycemic activity of the methanolic extract of

T. capensis leaves. The extracts showed a dose-dependent fall in fasting blood sugar in alloxan induced hyperglycemic rats. Alloxan induces hyperglycemia by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation[29]. The antihyperglycemic activity of TCLE may be due to its stimulating effect on the remnant cells or improvement in insulin action at cellular level or it could also be due to the insulin like effect of the active principle(s) present in the extract.

When TCLE were administered to glucose loaded normal rats (OGTT) fasted for 18 h, reduction in blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h compare to control. This result indicates that the extracts have sound capacity to block glucose absorption[28].

In the alloxan-induced hyperglycemia in rats, the rise in blood glucose is accompanied by an increase in the serum cholesterol and triglyceride. The treatment with TCLE reduced cholesterol significantly in hyperglycemic rats. It is well known that the level of glycemic control is the major determinant of serum level of triglycerides[31].

It is concluded from the data that TCLE possesses significant antihyperglycemic activity and it may prove to be effective for the treatment of hyperglycemia. However, longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antihyperglycemic drug.

Conflict of interest statement

We declare that we have no conflict of interest.

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