



Antidiabetic activity of methanolic extract of *Operculina turpethum* (L.) *Silva manso* stem in streptozotocin induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia. the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 is rising much more rapidly because of increasing obesity and reduced activity levels as counties become more industrialized. The antidiabetic potential of the methanolic extract of *Operculina turpethum* stem (MEOTS) (Convolvulaceae), a medicinal plant widely used in the traditional Ayurveda and Siddha systems of medicine for the treatment of diabetes mellitus was evaluated in the Streptozotocin (STZ) - induced type 2 diabetic models. The doses of 50 mg/kg and 100 mg/kg of MEOTS were administered to normal, glucose loaded and experimental diabetic rats for 21 days. Significant ($p < 0.05$) reduction in fasting blood glucose levels were observed in the normal rats at 3hr as well as in the treated diabetic animals at 21 days Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the indigenous system of medicine.

Key words: Type 2 diabetes, STZ, *Operculina turpethum*, Glibenclamide

INTRODUCTION

Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease¹. The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000. Based on the current trends > 360 million individuals will have diabetes by the year 2030².

Operculina turpethum is a perennial with milky juice belongs to family Convolvulaceae. This plant was widespread in old tropics from E. Africa to N. Australia, this plant common in Godavari, Andhra Pradesh, India. It is widely distributed in tropical Africa and Asia. In India it is found in damp and it occurs almost throughout India up to an altitude of about 1000 m. It is some times grown in gardens for its beautiful flowers. It is rare on open sandy soils. It is occasionally cultivated in India³.

Traditionally, *Operculina turpethum* root is prescribed in the treatment of snake bite (sushruta and vrindamadhava) and scorpion sting (sushruta), but it is not an antidote to either snake-venom (Mhaskar and Caius) or scorpion-venom (Caius and Mhaskar). In constipation, it is an effective laxative. It is used in periodic fevers and in the treatment of anemia accompanied by splenomegaly. It is also used to relieve flatulence and colic and in the treatment of obesity to decrease fat. It is used to treat dropsy, dyspepsia with constipation and flatulence, gout and rheumatism, and other inflammations⁴. In the present study, stem methanolic extract was used to study hypoglycemic activity in normal and diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma Aldrich chemicals Pvt. Ltd. USA. Glibenclamide was obtained as a gift sample from Sanofi Aventis India Ltd. Glucometer (Bayer Health Care, Japan) purchased from local pharmacy. All other chemicals and reagents used were of analytical grade.

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Collection of plant material

Operculina turpethum (L.)*Silva Manso* plant material collected from local areas of Vijayawada, A.P. Its parts were botanically authenticated by Prof. S.V. Raju, Taxonomist, Department of Botany, Kakatiya University, Warangal, A.P, and India. The herbarium was maintained in the Department of Pharmacognosy and phytochemistry, Vaagdevi College of pharmacy, Hanamkonda. *Operculina turpethum* stem was washed under tap water and were efficiently dried under shade for about one week and protected from deterioration. The shade dried stem was grinded made into powder with the help of a laboratory mixer. These were efficiently dried under shade for about one week and protected from deterioration and then grinded and made into powder.

Preparation of extract

The chemical compounds were extracted from the stem using successive solvent extraction process (soxhlation apparatus). The stem powder (100 g) was extracted with methanol for 6 hours. After completion of soxhlation process the liquid extract was collected and concentrated under reduced pressure below 50°C, until a soft mass obtained it was dried and kept in a desiccator.

Preparation of standard drug

Glibenclamide was suspended in 0.5% Sodium Carboxy Methyl Cellulose (CMC). 5 mg/kg of glibenclamide was administered to each rat in standard group.

Experimental design

Antidiabetic activity of MEOTS was assessed in normal, glucose loaded and STZ - induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

Animals used

Experiments were performed with male wistar rats procured from Mahaveera enterprises (Hyderabad, A.P., India), weighing between 180 - 220 g. The animals were housed in individual polypropylene cages under standard laboratory conditions of light, temperature ($22 \pm 1^\circ\text{C}$) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water *ad libitum*. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The experiments were planned after the approval of Institutional Animal Ethical Committee (IEAC).

Evaluation of MEOTS on normal healthy rats⁵

At the end of the fasting period, taken as zero time (0 h), blood was withdrawn

from the tail vein. Blood glucose was estimated with glucometer. The animals were then randomly divided into four groups of six animals each. Group I served as control and received 0.5% sodium CMC. Groups II treated with glibenclamide (5 mg/kg), group III and IV received MEOTS orally at the dose of 50 and 100 mg/kg. Blood glucose levels were determined 1, 2, 3 and 4 h following treatment with glucometer.

Evaluation of MEOTS on oral glucose tolerance test⁶

Healthy rats were divided into four groups of six animals each. Group I served as control received 0.5% sodium CMC. Groups II treated with glibenclamide (5 mg/kg), group III and IV received MEOTS orally at the dose of 50 and 100 mg/kg. All the animals were given glucose (2 g/kg) 60 min after dosing. Blood samples were collected from the tail vein just prior to (0 h) and at 30, 60, 90 and 120 min after the glucose loading, and blood glucose levels were estimated by glucometer.

Evaluation of MEOTS in STZ - induced diabetic rats⁷

Experimental diabetes was induced by single intraperitoneal injection of 55 mg/kg of STZ, freshly dissolved in cold citrate buffer, pH 4.5. Control animals received only citrate buffer. After 5 days of STZ injection, animals with fasting blood glucose above 300 mg/dl were considered as diabetic and included in the study. The animals were randomly divided into four groups of six animals each and received the following treatments: Group I received 0.5% sodium CMC, group II received glibenclamide (5 mg/kg), group III and IV received MEOTS orally at the dose of 50 and 100 mg/kg. The freshly prepared solutions were orally administered daily for 21 days. Blood glucose analysis was done weekly on overnight fasted animals using glucometer. All data expressed as mean + S.D. Statistical analysis was performed by ANOVA.

RESULTS

Effect of MEOTS on normoglycemic rats

Results of the effect of graded doses of MEOTS on blood glucose level of normal healthy rats were presented in table 1 and figure 1. MEOTS produced peak hypoglycemia at 3 h. Dose dependent blood glucose reduction was observed in animals treated with 50 and 100 mg/kg (12.73% and 20.62%, respectively). Blood glucose levels were restored in all treatment groups by 4h.

Table 1: Effect of MEOTS on serum glucose level in normal rats

Time (hr)	Group I	Group II	Group III	Group IV
0	76.5 ± 3.3	74.6 ± 2.3	78.5 ± 2.6	76.2 ± 1.9
1	74.5 ± 2.1	72.1 ± 3.8	76.2 ± 4.5	73.8 ± 2.5
2	73.7 ± 3.5	65.2 ± 3.2	74.8 ± 3.2*	70.7 ± 3.4*
3	72.3 ± 3.6	60.6 ± 4.5	68.5 ± 2.6**	60.4 ± 5.4**
4	73.6 ± 2.4	77.5 ± 2.9	76.3 ± 3.5	77.5 ± 4.3

*P < 0.01 when compared to glibenclamide treated group

**P < 0.001 when compared to glibenclamide treated group

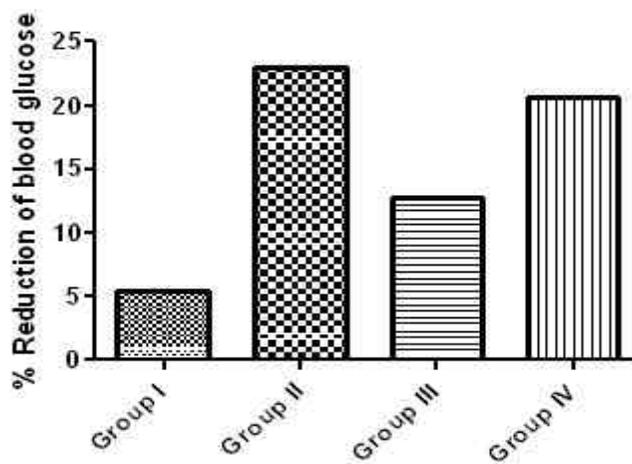


Fig 1: Effect of MEOTS on serum glucose level in normal rats

Effect of MEOTS on oral glucose tolerance in normal rats

MEOTS, when administered 60 min. prior to glucose loading produced significant reduction (P < 0.05) in the rise in blood glucose levels at 60 min. after glucose administration. MEOTS at doses of 50 and 100 mg/kg produced 14.13% and 19.89% reduction in blood glucose respectively when compared to vehicle treated group at 120 min.

Table 2: Effect of MEOTS on oral glucose tolerance test

Time (Min)	Group I	Group II	Group III	Group IV
0	88.6 ± 6.8	92.1 ± 6.1	94.1 ± 4.5	93.5 ± 8.7
30	85.8 ± 3.3	91.7 ± 5.1	91.5 ± 6.5	90.1 ± 5.9
60	82.6 ± 9.8	88.9 ± 3.7	90.1 ± 8.4*	85.2 ± 8.1*
90	79.5 ± 10.4	76.5 ± 6.2	86.4 ± 6.5*	80.5 ± 5.7*
120	84.5 ± 4.2	72.8 ± 4.2	80.8 ± 5.3*	74.9 ± 9.8*

*P < 0.05 when compared to glibenclamide treated group

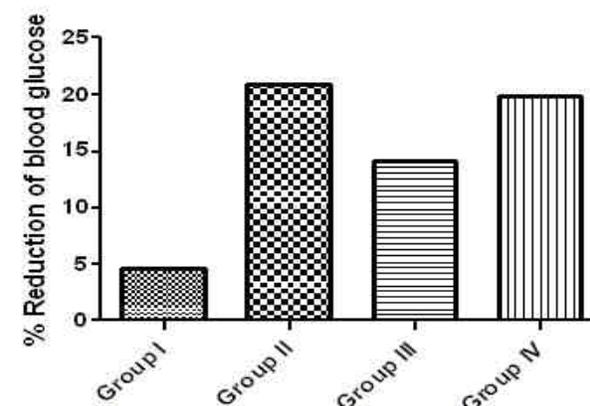


Fig 2: Table 2: Effect of MEOTS on oral glucose tolerance test

Effect of MEOTS on STZ - induced diabetic rats

The effect of repeated oral administration of stem methanolic extract on blood glucose levels in STZ-diabetic rats were presented in table 3 and figure 3. MEOTS, administered at three different doses of 50, 100 mg/kg to STZ-treated diabetic rats caused significant (P < 0.001) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 21 (30.5% and 43.66%, respectively). MEOTS 100 mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to the other dose. Glibenclamide exhibited a 47.28% reduction in blood glucose levels at the end of the study when compared to diabetic control.

Table 3: Effect of MEOTS on serum glucose level in STZ induced diabetic rats

Day	Group I	Group II	Group III	Group IV
1	271.1±11.3	242.9±10.6	218.6±6.8	222.6±18.8
7	269.0±6.8	207.5±11.6	157.0±2.3**	194.4±16.0**
14	263.8±12.7	181.4±4.8	149.4±8.4**	144.5±10.9**
21	265.3±12.5	128.0±6.4	151.9±7.9**	125.4±8.6**

**P < 0.001 when compared to glibenclamide treated group

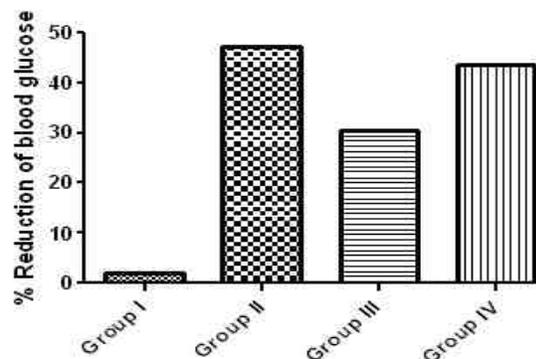


Fig 3: Effect of MEOTS on serum glucose level in STZ induced diabetic rats

DISCUSSION

This study was undertaken to evaluate the hypoglycemic activity of MEOTS in normal, glucose loaded and STZ - induced diabetic rats. In normoglycemic rats, MEOTS showed dose dependent hypoglycemic effect at 3 h. From oral glucose tolerance test (OGTT) it could be concluded that dose of 100 mg/kg showed the maximum improvement in glucose tolerance. STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of MEOTS for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which MEOTS mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β -cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. The hypoglycemic activity of MEOTS was compared with glibenclamide, a standard hypoglycemic drug. From the results of the present study, it may be suggested that the mechanism of action of MEOTS may be similar to glibenclamide action.

CONCLUSION

From this study, we can conclude that MEOTS has beneficial effects on blood glucose level. It has the potential to impart therapeutic effect in diabetes.

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