Anti diabetic effect of ethanolic extract of leaves of *Ocimum sanctum* in alloxan induced diabetes in rats**Abhilash Rao S*, Vijay Y, Deepthi T, Sri Lakshmi Ch, Vibha Rani, Swetha Rani, Bhuvaneswara Reddy Y, Ram Swaroop P, Sai Laxmi V, Nikhil Chakravarthy K, Arun P**

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Received: 20 July 2013**Accepted:** 4 August 2013***Correspondence to:**

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ABSTRACT

Background: Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycemia resulting from defects of reduced insulin secretion, decreased glucose utilization and increase in glucose production. It is estimated that there are currently 285 million people worldwide and this number is set to increase to 438 million by the year 2030. India has the highest number of patients with known diabetes worldwide, with a prevalence of 11.6%. The aim of the study was to evaluate the anti diabetic activity of ethanolic extract of leaves of plant *Ocimum sanctum* in alloxan induced diabetes in rats.

Methods: The study was conducted on 4 groups of 6 rats each to evaluate the hypoglycaemic effect of ethanolic extract of *Ocimum sanctum*. Glibenclamide was used as a standard drug and the results were compared in reference to it. Tween 80 was given for both normal and diabetic control groups. The fasting blood sugar levels were recorded on 1st, 3rd, 5th, 7th, 10th days by glucometer.

Results: The results indicate that the test compound ethanolic extract of *Ocimum sanctum* has significant and sustained oral hypoglycaemic activity, comparable with the hypoglycaemic effect of glibenclamide, a sulfonylurea.

Conclusion: The hypoglycaemic potential of the test compound is found to be comparable with that of the standard drug glibenclamide.

Keywords: Diabetes mellitus, Alloxan induced diabetes, Hypoglycemic drugs, Glibenclamide, *Ocimum sanctum*

INTRODUCTION

Diabetes is the most important non infective epidemic to hit the globe in the present millennium. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.^{1,2}

Currently anti diabetic drugs like sulfonylureas, meglitinides, the biguanide metformin, thiazolidinediones, alpha-glucosidase inhibitors, and the oral dipeptidyl-peptidase-4 inhibitor sitagliptin can only be used for patients with type 2 diabetes. They are mainly used to replace the insulin deficiency or to enhance the action of insulin or decrease the insulin resistance for the treatment of diabetes and management of its complications.

Current drugs used for diabetes therapy are not free from side effects and do not restore normal glucose homeostasis.

Glibenclamide is an antidiabetic drug in a class of sulfonylureas. The drug works by inhibiting the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (KATP)³ in pancreatic beta cells.

This inhibition causes cell membrane depolarization opening voltage-dependent calcium channel. This results in an increase in intracellular calcium in the beta cell and subsequent stimulation of insulin release.

Tulsi (*Ocimum sanctum* Linn.), commonly known as Holy Basil, is an herbaceous plant found throughout the

south Asian region. Different parts of plant are used in ayurveda and siddha systems of medicine for prevention and cure of many illnesses.⁴ Extract of *Ocimum sanctum* increases intra cellular calcium of beta islet cells of pancreas and causes insulin secretion.⁵

METHODS

Fresh leaves of *Ocimum sanctum* Linn were dried under shade for 10 days. The dried leaves were finely powdered & packed separately in air tight containers. Extract was prepared by using absolute ethanol with the help of Soxhlet apparatus.⁶

Male albino rats of Wistar strain weighing 150-200 grams and of more than 3 months of age were obtained. The animals were fed with grain and water ad libitum. They were maintained at a temperature of 24-27°C with relative humidity of 30-70 % with 12 hr light dark cycle. Freshly prepared 5% (dissolved in 0.9% W/V normal saline) solution of Alloxan monohydrate was injected intraperitoneally to overnight fasted rats to induce diabetes. After 6-8 hours of Alloxan injection the dextrose (5gm) mixed with water was given to the rats orally to prevent early hypoglycaemic condition of rats with alloxan. 72 hours later, blood glucose levels of all rats were determined by using glucometer. The rats with fasting blood glucose levels of 200 mg/dl were considered as diabetic and were employed for further study.

Diabetic animals were randomly divided into three groups, six animals in each group, and non diabetic animals 6 in number in one group subsequently named as normal control group, which were kept separately in different cages.

Individual animals were identified by a mark on the tail with a permanent marker and the cages are identified with label pasted on the cages with groupings written on them.

They are named as

S. No.	Group	Treatment
1	Normal control	1% Tween 80
2	Diabetic control	Alloxan 130mg/kg b.w.+1ml 1% Tween 80
3	Standard group	Alloxan 130mg/kg b.w. + 0.5 mg/kg b.w. Glibenclamide
4	Test group	Alloxan 130mg/kg b.w. +400 mg/kg ethanolic extract of tulasi).

The animals were allowed free access to food and water but were withheld food and only given water 12 hours prior to estimation of fasting blood glucose.

6 non diabetic animals were taken as normal control for study. They were given 1ml of 1% Tween 80. The diabetic controls were given with 1ml of 1% Tween 80. The diabetic standard were given glibenclamide suspended in 1% Tween 80, in a dose of 0.5mg/kg body weight. The test group were given ethanolic extract in a dose of 400 mg/kg body weight. The animals were given the drugs and suspensions with the help of feeding tube once daily for 10 days.

Method of blood collection

Blood was collected from the lateral tail vein. The animal was placed in a suitable restrainer and the tail veins in the rat were made prominent by tying a tourniquet at the base of the tail and dipping the tail in water at 40-50°C. It was then cleaned with an antiseptic. The tail was held between the thumb and the index finger and at the base of the tail a gentle prick with the needle was made.⁷ Blood drop which was formed was used for blood glucose estimation.

Estimation of blood glucose levels

Blood samples were collected from dorsal/lateral tail vein of rats for the estimation of fasting blood sugars (12 hr overnight fasting), on 1st, 3rd, 5th, 7th and 10th days. Blood glucose levels were estimated using ONE TOUCH UltraTM Glucometer.

Statistical analysis

The results were analyzed using one-way ANOVA with post hoc Dunnett's multiple comparisons test where the test group and the standard were compared with diabetic control group. The P values obtained were significant.

RESULTS

Effect of ethanolic extract of tulasi on mean fasting blood glucose levels in rats for 10 days study period

With 10 days of administration of ethanolic extract of tulasi, there seemed to be maximum reduction in blood glucose levels.

On day 1 percentage of reduction in glucose levels was 33%, increased to 51.0% on 3rd day. Sustained hypoglycaemic effect was seen after 3rd day (nearly 45%).

Comparison of effect of ethanolic extract of tulasi with glibenclamide on mean fasting blood glucose levels in rats for 10 days study period

With 10 days of administration of test drug & standard drug, there seemed to be maximum reduction in blood glucose levels. On 3rd day, the percentage of reduction of blood glucose levels being 51.5% & 51.0% with standard & test respectively. On 10th day, the percentage of

reduction of blood glucose levels being 57.1% & 49.3% with standard & test respectively (Table 3).

Mean Percentage change of reduction of fasting blood glucose levels in 10 days study were 52% and 45% for standard & test drug respectively.

The hypoglycaemic effect of ethanolic extract of *Ocimum sanctum* was comparable with that of glibenclamide in hyperglycaemic rats in 10 days of study.

DISCUSSION

Several medicinal properties have been attributed to *Ocimum sanctum*. Different parts of the plant such as leaves, flowers, root, seeds, stem are known to possess therapeutic activities and have been used by traditional medical practitioners.⁸⁻¹⁰

Extract of *Ocimum sanctum* increases intra cellular calcium of beta islet cells of pancreas and causes insulin secretion.

The study was conducted on 4 groups of 6 rats each to evaluate the hypoglycaemic effect of ethanolic extract of *Ocimum sanctum*. Glibenclamide was used as a standard drug and the results were compared in reference to it. Tween 80 was given for both normal and diabetic control groups. The fasting blood sugar levels were recorded on 1st, 3rd, 5th, 7th, 10th days by glucometer (Table 1, Figure 1).

Fasting blood sugar levels from Day0 to Day10 in all 4 groups of Rats

Figure 1: Fasting blood sugar levels from Day0 to Day 10 in all 4 groups of rats.

Table 2: Percentage reductions on mean blood glucose levels for 10 days for standard and test groups, p value<0.001%.

Group	Day1	Day3	Day5	Day7	Day10
Standard group	46.75	51.51	49.35	57.57	57.14
Test group	33.03	51.10	44.34	48.8	49.3

The hypoglycemic effect of ethanolic extract of *Ocimum sanctum* was comparable with that of glibenclamide in hyperglycemic rats in 10 days of study (Table 3, Figure 2). This may be due to the presence of hypoglycemic flavonoids, triterpenes or saponin glycosides that also requiring further investigation.

Table 1: Fasting blood sugar levels in all 4 groups of rats on day 0, 1, 3, 5, 7, 10.

Group	Treatment	Blood Glucose Concentration(mg/dl)					
		Day - 0	Day - 1	Day - 3	Day - 5	Day - 7	Day - 10
I	Normal Control	81.70 ± 2.92	80.0 ± 4.51	76.73 ± 2.82	85.83 ± 3.12	91.61 ± 3.74	84.73 ± 4.6
II	Diabetic Control (Alloxan 130mg/Kg IP + 1% tween80)	242.0 ± 22.78	231.0 ± 24.63	251.3 ± 14.32	223.3 ± 17.8	241.3 ± 14.68	224 ± 16.32
III	Standard Group (Alloxan 130mg/Kg IP + Glibenclamide 0.5 mg/Kg)	231.3 ± 20.34	123.5 ± 2.61	112.61 ± 4.06	117.8 ± 3.28	98.83 ± 4.83	99.3 ± 3.12
IV	Test Group (Alloxan 130mg/Kg IP + Ethanolic extract 400 mg/Kg)	221.3 ± 18.93	148.3 ± 3.81	114.32 ± 2.61	123.7 ± 1.83	113.42 ± 5.73	112.41 ± 3.35
P Value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Remarks		S	S	S	S	S	S

(Expressed in Mean ± SEM)

Note: S-Significant. All values expressed in Mean±SEM for 4 groups of 6 animals in each group. Group I, III, IV are compared with II.

Table 3: Mean percentage change in fasting blood sugar levels of 10 days study in standard and test group.

Group	% Change
Standard group	52%
Test group	45%

Figure 2: Percentage reductions on mean blood glucose levels for 10 days for standard and test groups.

CONCLUSION

The results indicate that the test compound ethanolic extract of *Ocimum sanctum* has significant and sustained oral hypoglycemic activity, comparable with the hypoglycemic effect of glibenclamide, a sulfonylurea. The anti diabetic effect may be due to increased insulin secretion. By utilizing the vast reserves of phytotherapy we can reduce the economic burden, especially in poor & developing countries.

ACKNOWLEDGEMENTS

We are very thankful to the staff of the Pharmacology Department, Osmania Medical College for their immense help and guidance in conducting the study. The present study had been approved by the Institutional Animal Ethics Committee formed by Dept. of Pharmacology, Osmania Medical College, Koti, Hyderabad and we are grateful to the committee for having given the permission.

Funding: None

Conflict of interest: None declared

Ethics approval: Approved by the IAEC/Pharma/OMC

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doi:10.5455/2319-2003.ijbcp20131018

Cite this article as: Rao SA, Y Vijay, T Deepthi, Lakshmi CS, Rani V, Rani S, Reddy YB, Swaroop PR, Laxmi VS, Chakravarthy KN, P Arun. Anti diabetic effect of ethanolic extract of leaves of *Ocimum sanctum* in alloxan induced diabetes in rats. *Int J Basic Clin Pharmacol* 2013;2:613-6.