Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 699 - 706

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CLINICAL EVALUATION OF ANTI-HYPERGLYCEMIC ACTIVITY OF *OCIMUM SANCTUM* IN COMPARISON WITH GLIBENCLAMIDE IN THE RAT MODEL OF T2DM

DWIVIDENDRA KUMAR NIM*, PRATAP SHANKAR, RAKESH CHAURASIA***, BHAUMIC GOEL* AND RAKESH KUMAR DIXIT******

**Junior Resident Pharmacology, Moti Lal Nehru Medical College Allahabad*
***Ph.D Scholar Pharmacology, King George's Medical University Lucknow*
**** Assistant Professor Pharmacology, Moti Lal Nehru Medical College Allahabad*
*****Professor Pharmacology, King George's Medical University Lucknow*

ABSTRACT

India leads the world in total number of diabetic cases with the estimate of 50.8 million adult populations in 2010. Overall predicted increase in number from 2010 to 2030 is 54% at an annual growth of 2.2%, which is nearly twice the annual growth of total world population. Hyperglycemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood. Diabetes Mellitus is one such important disease and characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. *Ocimum sanctum* is a herb and found throughout the India, with several medicinal and religious purposes and for its essential oils and also possesses anti-hyperglycemic activity. Only a very few studies have been done on this plant to establish their anti-hyperglycemic property. The purpose of this study is to evaluate the anti-hyperglycemic activity of *Ocimum sanctum* in the rat model of Diabetes Mellitus Type-2.

KEYWORDS: **Anti-hyperglycemic,*Ocimum sanctum,* Glibenclamide, T2DM**

PRATAP SHANKAR
Ph.D Scholar Pharmacology, King George's Medical University Lucknow

Corresponding author

INTRODUCTION

India leads the world in total number of diabetic cases with the estimate of 50.8 million adult populations in 2010. Overall predicted increase in number from 2010 to 2030 is 54% at an annual growth of 2.2%, which is nearly twice the annual growth of total world population[1]. Adding to the problem is increased susceptibility of the Indian population to diabetes and increasing urbanisation[2]. Hyperglycemia or high blood sugar is a condition in which an excessive amount of glucose circulates in the blood. In the last century, advancement of science successfully developed treatment for several diseases. But the developments of human beings also led to the adoption of a life style that flourished some common diseases of the past to the most important causes of morbidity and mortality in present. Diabetes Mellitus is one such important disease.Diabetes Mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. Diabetes Mellitus usually presents with its characteristic symptoms such as thirst, polyuria, blurring of vision, weight loss and polyphagia. Sometimes its acute complications like keto-acidosis or non-ketotic hyperosmolarity may be the first presentation. These complications in the absence of timely treatment may lead to death[1].*Ocimum sanctum* is an aromatic plant in the family *Lamiaceae*. It is native throughout the world tropics and widespread as a cultivated plant and an escaped weed. This herb is found throughout the India, up to an altitude of 1,800 m in the Himalayas. It is cultivated for several medicinal and religious purposes and for its essential oils. It has been used in the “Ayurvedic system of medicine” since ancient times to treat wide variety of ailments. Previous studies suggest that this plant possesses anti-hyperglycemic activity. But only a very few studies have been done on this plant to establish their anti-hyperglycemic property. The purpose of this study is to evaluate the anti-hyperglycemic activity of plant in the rat model of Diabetes Mellitus Type-2.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology and the Department of Biochemistry, Moti Lal Nehru Medical College, Allahabad and albino rats of both sexes (male and female) weighing between 100 - 150 gm. were used and obtained from registered sellers (Reg. No.- B-37/0605003769) and kept in animal house under the supervision of veterinary doctor. All rats were housed at an ambient temperature of 25^{o}C± 2°C with a 12 hour light/dark cycle, and provided with standard pellet diet/high fat diet and water *ad libitum.* The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of Institutional Animal Ethics committee and the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH Publication. No. 85-23 revised 1996, Latest revision in 2011).

Test Drugs and Chemicals

All the drugs were administered orally with the help of feeding cannula after preparing suspension in distilled water (vehicle).

Ocimum sanctum extract

It was procured as commercially available crude extract in dry powder form, from The Himalaya Drug Co., Banglore, India. It was given in doses of 200 mg/kg and 400 mg/kg[3].

Glibenclamide

It was given in a dose of 0.6 mg/kg[2].It was procured from USV Pharma Ltd, India.

Streptozocin

(minimum assay 97%) was procured from Spectrochem Pvt. Ltd., Mumbai. Glucose estimation kit used for estimation of plasma glucose was purchased from Span Diagnostic limited, Surat, India. All the chemicals and reagents used were of analytical grade.

Study Design

The study was started with 24 rats. Baseline fasting plasma glucose (FPG) levels of all the

rats were determined. All the animals were fed on high fat diet (58% energy as fat) for two weeks. After two weeks fasting plasma glucose levels were taken and all the rats (24) were injected intraperitonially with 35 mg/kg of streptozocin in citrate buffer (single dose)[4]. The FPG levels were estimated in all the rats after 1 week. The rats with plasma glucose level > 200 mg/dl were considered to be diabetic[5] and were included in the study. They were randomly (by using a random number table) divided into 4 groups of 6 rats each, so that a total of 4 groups were formed. The drugs were administered orally once daily after preparing suspension in distilled water for further 10 weeks. Fasting plasma glucose of all the rats was taken every two weeks. Blood samples were drawn from the tail vein and plasma glucose estimation was done by the Glucose-Oxidase method. The observations of the test groups (2 & 3) were compared with that of the standard (glibenclamide) and the diabetic control (vehicle) as shown in Table 1. All the study was designed as per the diagrammatic representation as shown in Fig. 1.

Table 1
Grouping of study animals

Group No.	Group name (n=6)	Drug administered	Dose
1	Diabetic Control (DC)	Vehicle only (distilled water)	0.5 ml/day
2	Low dose *Ocimum sanctum* crude extract (OSL)	*Ocimum sanctum* crude extract	200 mg/kg/day
3	High dose *Ocimum sanctum* crude extract (OSH)	*Ocimum sanctum* crude extract	400 mg/kg/day
4	Standard drug (SD)	Glibenclamide	0.6 mg/kg/day

Statistical Analysis

The observations were analyzed using one way “ANOVA” and “student t test” where ever needed.

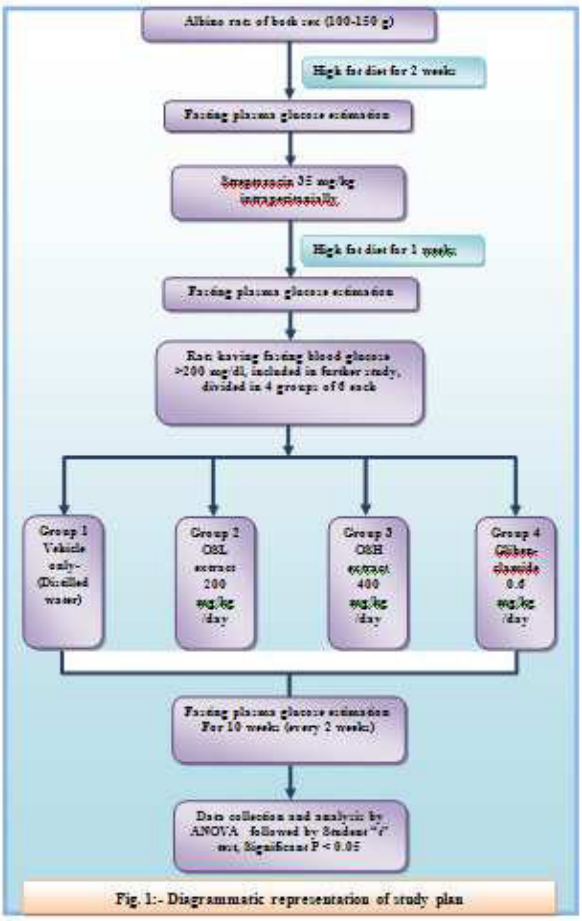


Fig. 1:- Diagrammatic representation of study plan

RESULTS

All the groups were observed during the study period as per the study requirements. Their basal fasting plasma glucose (FPG) levels were measured at the start of the study. After feeding rats with high fat diet for 2 weeks, FPG levels were again measured and they were injected with streptozocin in citrate buffer or plain citrate buffer depending upon the group as described above. One week after the injections, FPG levels were again measured and the diabetic status was ascertained. This reading was considered as that of zero weeks. After this, rats were continued on their respective diet and drugs. FPG levels were determined every 2 weeks till the end of the tenth week. The values of the test groups were compared with that of the control and standard groups.Baseline FPG levels in Diabetic Control (DC), Group 2 & 3 and standard group ranged from 76-87, 76-90, 77-88 and 78-91 respectively with mean ± S.D. being 82.33±3.78, 83.5±4.60, 82.67±3.89 and 84.17±4.96 respectively. On comparing the mean FPG together ANOVA revealed similar mean FPG among the groups ($F = 0.141$, $p>0.05$) i.e. mean baseline FPG levels did not differ significantly between the groups. FPG levels was again measured before administration of streptozocin. At this time, FPG in Diabetic Control, group 2 & 3 and standard group (SD) ranged from 80-91, 83-88, 83-92and 80-88 respectively with mean±S.D. being 86.17±3.97, 86±2.10, ±86.67±3.39 and 84.5±3.45. On comparing the mean FPG together, ANOVA again revealed similar mean FPG among the groups ($F=0.378$, $p>0.05$) i.e. mean FPG did not differ significantly between the groups. Mean fasting plasma glucose levels of diabetic control group did not varied much over the period of 10 weeks. Mean values at any point of the time did not varied more than 11.33 mg/dl from mean baseline value of 360.17 mg/dl. Group given Glibenclamide (Standard Drug), showed consistent improvement in FPG level over 10 weeks with the maximum improvement of 57% from baseline values at the end of 10 weeks. Maximum reduction in mean FPG level (34.11%) was seen in first two weeks. Effects of low dose *Ocimum sanctum* (200 mg/kg) is shown in *Ocimum sanctum* also decreased fasting plasma glucose levels consistently from 2 weeks (4.35%) to 10 weeks (24.36%). The maximum reduction in blood glucose level was seen at the end of 10 weeks (24.36%). At the high dose of 400 mg/kg *Ocimum sanctum* was able to decrease fasting plasma glucose levels by 30.35% by 10 weeks. This reduction in fasting plasma glucose level was 4.08% at 2 weeks and increased consistently throughout the study period. On further observation it was seen that at high dose of *Ocimum sanctum* reduction in plasma glucose levels were also significant from 2 weeks onwards ($p<0.05$ at weeks and $p<0.001$ at weeks 4, 6, 8 and 10). This reduction in Plasma glucose levels was less than that of glibenclamide at all the time and glibenclamide was significantly better than high dose *Ocimum sanctum* at all times. On further comparison of low and high doses of *Ocimum sanctum*, it was seen that at week 2 reduction in plasma glucose with low dose was slightly more but after 4 weeks reduction was more with high doses till the end of study. (Table 2) This difference was insignificant till 8 weeks but become significant at week 10 (Table 3). The effect of the low and high doses of *Ocimum* sanctum extract on FPG with standard and diabetic control group can be best to see in the Bar diagram of Fig. 2.

Table 2
Comparison (in mean % reduction) of fasting plasma glucose levels in study groups receiving low and high doses of Ocimum sanctum extract

Groups	Time in Weeks				
	Week 2	Week 4	Week 6	Week 8	Week 10
***Ocimum sanctum* extract 200 mg/kg**	4.35%	13.46%	18.61%	23.05%	24.36%
***Ocimum sanctum* extract 400 mg/kg**	4.08%	15.55%	23.06%	27.47%	30.53%

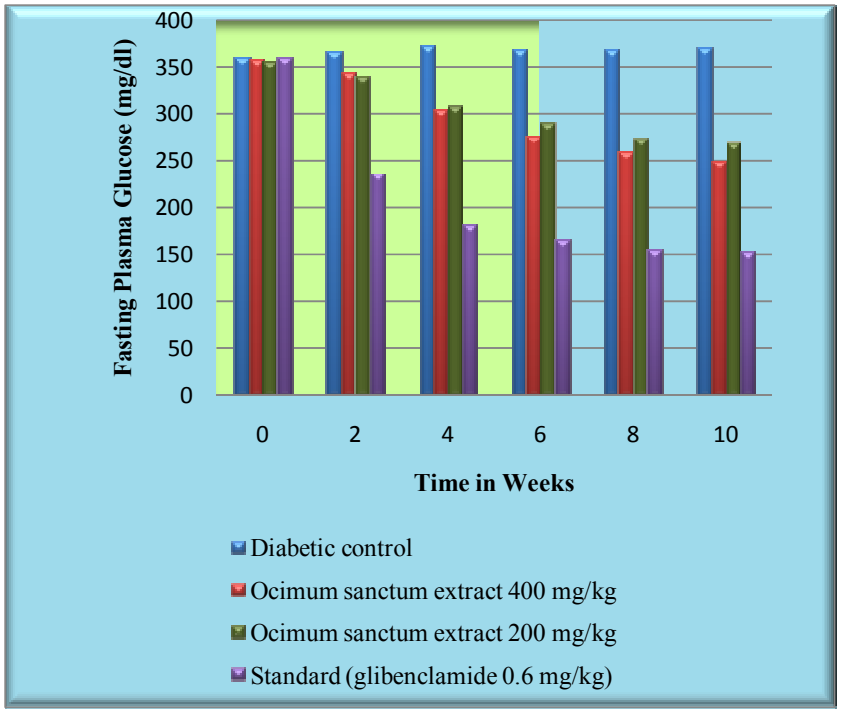
Table-3

Comparison (in terms of p and t value) of fasting plasma glucose levels in study groups receiving low and high hoses of Ocimum sanctum extract

Groups	Time in Weeks				
	Week 2	Week 4	Week 6	Week 8	Week 10
t- statistic	-0.38	0.658	1.904	1.96	3.113
p value	>0.05	>0.05	>0.05	>0.05	<0.05

Figure-2

Bar diagram showing effect of low and high doses of Ocimum sanctum extract on FPG with standard and diabetic control group



On simultaneous comparison of all the groups the maximum mean % reduction in fasting plasma glucose of all the groups, the order of their effects is-
Glibenclamide >*Ocimum sanctum* (400 mg/kg) >*Ocimum sanctum* (200 mg/kg).
This trend in reducing plasma glucose levels can be better visualized in the line diagram of fig. 3 and in table 4.

Table-4

Comparison of effect of all the drugs in decreasing fasting plasma glucose levels

Groups	Time in Weeks				
	Week 2	Week 4	Week 6	Week 8	Week 10
Diabetic control (vehicle only)	-1.67%	-3.15%	-2.31%	-2.04%	-2.96%
Ocimum sanctum extract 200 mg/kg	4.35%	13.46%	18.61%	23.05%	24.36%
Ocimum sanctum extract 400 mg/kg	4.08%	15.55%	23.06%	27.47%	30.53%
Standard (glibenclamide 0.6 mg/kg)	34.11%	49.31%	53.75%	56.44%	57%

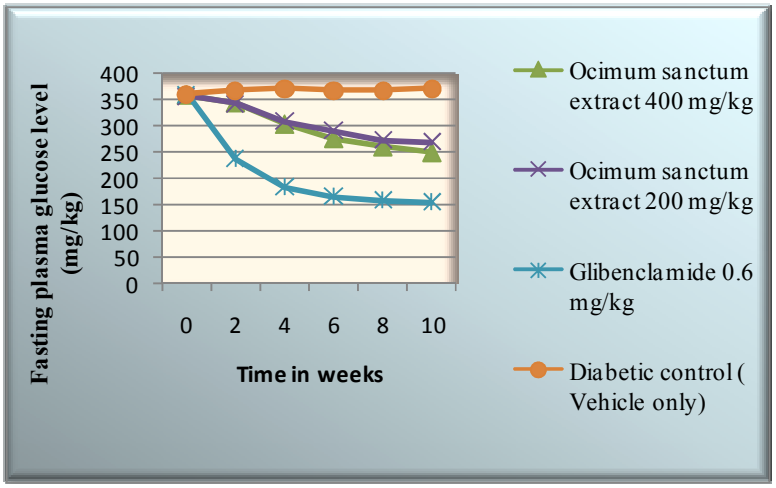


Figure-3
Line diagram showing fasting plasma glucose levels over the period of 10 weeks in all 6 study groups

DISCUSSION

The present study was conducted to evaluate the anti-hyperglycemic activity of extracts of *Ocimum sanctum* in rats as well as to provide an introductory approach for the evaluation of their traditional preparations in order to scientifically validate the therapeutic preparation of these plants in the control of hyperglycemia in Diabetes mellitus type-2. Insulin secretion is a tightly regulated process designed to provide stable concentrations of glucose in blood during both fasting and feeding. This regulation is achieved by the coordinated interplay of various nutrients, GI hormones, pancreatic hormones, and autonomic neurotransmitters. Glucose, amino acids, fatty acids and ketone bodies promote the secretion of insulin. There are some drug groups that can cause increases in insulin secretion and thus called insulin secretogogues. These groups are – sulfonylureas, meglitinides, GLP-1 agonists and Dipeptidyl Peptidase-4 (DPP-4) inhibitors. Sulfonylureas are further divided in two groups according to generation of agents. The first generation sulfonylureas (Tolbutamide, Tolazamide and Chlorpropamide) are very rare now days for treatment of Diabetes Mellitus type-2. The second generation sulfonylureas (Glibenclamide, Glipizide and Glimepiride) are more potent anti-hyperglycemic and hypoglycemic agents than first generation sulfonylureas[6]. As most of the anti-diabetic drugs possess some serious side effects, this group of drug is also not free of such side effects. On administration acute and chronic complication may develop if the therapy is not monitored regularly, this can lead to noncompliance and worsening of disease condition. There are many known plants that possess anti-hyperglycemic activity. *Ocimum sanctum* is such a plant which shown to possess anti-hyperglycemic activities by a few studies. The present study was conducted to evaluate its anti-hyperglycemic activity in high fat diet and streptozocin induced diabetic rats. This effect was compared with the anti-hyperglycemic activity of glibenclamide. *Ocimum sanctum* is considered to act mainly by increasing insulin secretion from pancreatic β-cells. Most of the studies conducted to evaluate anti-hyperglycemic activities of this plant were of relatively short duration. Moreover, to our knowledge none of the studies tried to took over the trend of plasma glucose reduction over time. In this study, we tried to address this issue by monitoring the plasma glucose level every two weeks for a duration of 10 weeks so that the anti-hyperglycemic effect of these drugs could be evaluated for a longer duration of treatment.

Our study showed that the test drug *Ocimum sanctum* extract is capable of decreasing plasma glucose level in type-2 diabetic rats. These reductions in plasma glucose levels were consistent throughout the 10 weeks duration of therapy. *Ocimum sanctum* extract in both low and high doses (200 mg/kg and 400 mg/kg) decreased plasma glucose levels significantly from 2 weeks onwards. This reduction was consistent throughout the study duration in both doses with the maximum reduction in plasma glucose being 30.53% in high dose group. There was no significant difference in anti-hyperglycemic activity of *Ocimum sanctum* extract in both doses up to 8 weeks, but the difference became significant at the end of study period i.e. 10 weeks. This anti-hyperglycemic effect of *Ocimum sanctum* is supported by few other studies which were done earlier[3,7]. But a study differs from our results. In this study researchers found no antidiabetic activity of *Ocimum sanctum* seed oil (0.8 g/kg/day) on hypercholesterolaemic rabbit model. This may be due to use of seed oil of *Ocimum sanctum* rather than plant extract, the possibility behind this can be the absence of active ingredient in seed oil which is responsible for its antidiabetic activity. Based on previous studies, following mechanism of *Ocimum sanctum* extract may account for its anti-hyperglycemic activity in our study-

1. *Alteration in activity of enzymes of glucose metabolism*

Ocimum sanctum shown to alter the key enzymes of glucose metabolism which may be responsible for its anti-hyperglycemic activities. In a study on streptozocin induced diabetic rats there was seen a significant increase in levels of enzymes glucokinase, phosphofructokinase and hexokinase[8].

2. *Inactivation of K_{ATP} channels*

In a study on perfused pancreas, isolated islets and clonal pancreatic β-cells there was seen the involvement of ion channels in the stimulatory action of *Ocimum sanctum* extract. Diazoxide, a K_{ATP} channel opener, inhibited the insulin releasing effect of *the Ocimum sanctum* extract. This study suggests that closure of K_{ATP} channels participates in the overall anti-hyperglycemic mechanism of action of *Ocimum sanctum*[9,10].

3. *Increased scavenging of reactive oxygen species (anti-oxidant effect)*

The relation between scavenging of free radicals and their positive effect on insulin release has been discussed earlier. There are few studies that evaluated the significant antioxidant effect of *Ocimum sanctum*. Which may be an important reason of anti-hyperglycemic activity of the drug[7,11,12]. These effects of *Ocimum sanctum* justify future investigation into their role in Diabetes and its complications in spite of the less effect which they have shown as compared to Glibenclamide. Although the drug shown to possess significant anti-hyperglycemic activity but in our study we did not included to evaluate the hypoglycemic activity, which is one of the most important adverse effect of our standard drug. As our study suggests that drug possess partial similarity in mechanism of anti-hyperglycemic activity between the test and standard drug, future studies can be done to evaluate the hypoglycemic activity of *Ocimum sanctum.* As our study revealed that *Ocimum sanctum* produce dose and time dependent anti-hyperglycemic effect. Future studies can be done taking different doses and durations of study in account.

CONCLUSION

The present study was carried out in albino rats of either sex, weighing 100-150 g. The aim of the study was to evaluate the anti-hyperglycemic activity of herb namely *Ocimum sanctum* and to compare them in high fat diet and streptozocin induced diabetic rats.The following conclusions was drawn after the completion of study-

- *Ocimum sanctum* found to possess anti-hyperglycemic activity.
- The group of rats that received *Ocimum sanctum* at low dose of 200 mg/kg/day showed significant reduction in plasma glucose levels from second week onwards.
- At high dose of 400 mg/kg/day *Ocimum sanctum* was able to significantly reduce plasma glucose levels from second week onwards.

- The improvement in plasma glucose levels was consistent from second to tenth weeks in both low and high dose *Ocimum sanctum* groups. Suggesting a time dependent effect.
- The overall reduction in plasma glucose level was greater in high dose *Ocimum sanctum* group suggesting a dose dependent effect.
- Our standard drug glibenclamide showed better anti-hyperglycemic effect than *Ocimum sanctum* at all the doses which we used.
- On comparing the maximum mean % reduction in fasting plasma glucose levels of all the groups, the order of their anti-hyperglycemic effect is – Glibenclamide>*Ocimum sanctum* (400 mg/kg/day) >*Ocimum sanctum* (200 mg/kg/day).
- More studies are needed to elucidate their optimum dose and exact mechanism of action.

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