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Research Article

Effect of *Ocimum sanctum* Leaf Extract on Entero-Insular Axis by Oral Glucose Tolerance Test in Albino Rats

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ABSTRACT

This research study evaluates the effect of *Ocimum sanctum* leaf extract on the entero-insular axis and the hyperglycemia due to oral glucose tolerance test in albino rats. Methanolic leaf extract of *Ocimum sanctum* was prepared by Sami labs, Bangalore and used for the study. After obtaining Institutional Animal ethical committee clearance male albino rats (155–215 g) were divided into 4 groups. 6 albino rats were randomly assigned to Control (Normal saline) group, Standard (Vildagliptin 50 mg/kg) group, *Ocimum sanctum* leaf extract (100 mg/kg) and (200 mg/kg) groups. After overnight fasting the baseline biochemical evaluation in the fasting state blood glucose, Insulin level, Glucagon level, GLP-1 (Glucagon like peptide-1), GIP (Glucose dependent Insulinotropic peptide) level were measured for all the above groups. Control, Vildagliptin (STD) and the extracts were orally administered by using rat oral feeding tube. 30 minutes later oral glucose tolerance test (OGTT) was done. Blood samples were evaluated for blood sugar, Insulin, Glucagon, GLP-1 and GIP at 0, 15 and 45 minutes after oral glucose load. *Ocimum sanctum* (200 mg/kg) showed significant ($p \leq 0.05$) reduction in blood glucose at 45 minutes when compared to control. *Ocimum sanctum* (100 mg/kg & 200 mg/kg) showed significant ($p \leq 0.001$) increase in GIP levels at 45 minutes of OGTT. *Ocimum sanctum* leaf extract showed significant antihyperglycemic and incretin enhancing effects. Further in-depth animal and clinical studies are necessary to demonstrate the therapeutic potential of this extract for the treatment of type 2 diabetes mellitus.

Key words: *Ocimum sanctum*, Entero-Insular axis, Antihyperglycemic, Insulin, Glucagon.

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INTRODUCTION

Incidence of diabetes is increasing day by day in India and around the world. Hence most of the research studies are directed towards diabetes especially the pathophysiology and treatment aspects of type 2 diabetes mellitus. International Diabetes federation data shows currently 366 million people are suffering from diabetes and this may increase twice in number by 2030.[1] In India type 2 diabetes mellitus presentation is postprandial hyperglycemia attributed to high carbohydrate consumption. Medical nutrition therapy along with herbals ingredients may control hyperglycemia, hyperlipidemia and arrest the micro and macro vascular complications of diabetes mellitus.[2] Dietary polyphenols has anti-hyperglycemic activity by acting on the beta cells and insulin resistance.[3]

In India more than 75% of the diet consists of carbohydrates. [4] To tide over this problem inclusion of edible herbs in diet may reduce carbohydrate absorption from gut and act in the entero-insular axis by enhancing incretins and Insulin.[5] This is an interesting area of research which may be explored in future. In the current study we evaluated the anti hyperglycemic and incretin enhancing effect of commonly used edible herbal *Ocimum sanctum* leaf extract on the entero-insular axis in albino rats. *Ocimum sanctum* is a common herb belongs to family- Labiatae locally known as Tulsi and Holy basil is widely used in the Indian system of medicine. Each and every part of the plant has enormous medicinal property.[5] It has diaphoretic and expectorant effect and used to relieve headache, dermatological diseases. The antidiabetic property of *Ocimum sanctum* leaves has been demonstrated in diabetic rats.[6,7] *Ocimum sanctum* leaf extracts also has α-glucosidase inhibitory activity and antidiabetic activity in in-vitro and *in-vivo* studies.[8,9] This research is undertaken to through some light on the role of *Ocimum sanctum* on emerging pharmacotherapy of type 2 diabetes.

MATERIALS AND METHODS

This is a randomized controlled study with animals (albino rats) to evaluate the antihyperglycemic effect of leaf extract of *Ocimum sanctum* by using approved Oral glucose tolerance test (OGTT). It evaluates the hyperglycemic and consequent hormonal influences of OGTT on the entero-insular axis.

Institutional research committee and animal ethical committee approval (Protocol No.002/09/2015/IAEC/SBMCH) obtained through proper channel. The extract was manufactured and supplied by Sami labs Limited, Bangalore. Product Code 2045, Batch No. C170698EM. Date of manufacture April 2017. *Ocimum sanctum* (Tulsi) methanolic leaf extract was used for this study. Physical, Chemical and Microbiological testing for the above extract was done and certificate of analysis was issued by

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the Sami Labs Limited. T. Vildagliptin 50 mg was obtained from the local pharmacy, powdered and made into a suspension (10 mg/ml) and administered to rats by oral feeding tube.[10]

Male and female albino rats (weighing 150-250 grams) total 70 numbers were purchased from King Institute, Guindy, Chennai. The rats were provided with a commercial diet and maintained under controlled temperature, humidity, and lighting (22 ± 2°C, 55 ± 5%) and a 12-hr light/dark cycle with lights on at 7:00 AM. All procedures were conducted according to the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines and as per the Animal ethical Committee regulations.[11]

Preliminary acute toxicity

Single oral dose of 2000 mg/kg for the extract groups of 6 albino rats (3 males and 3 females in each group) weighing 155-215 gm.[12] Similarly, one control group of 6 rats was administered with distilled water (1 ml/100 g) orally. The animals were observed for clinical manifestations and mortality for first 6 hours and then for next 14 days. The results and conclusion is that the single oral dose of 2000 mg/kg *Ocimum santum* leaf extract did not cause death or any abnormal clinical manifestations in male or female rats.

The effective dose was arrived at by studying previous similar research articles. 2 dose levels (low and high) were chosen for the extract. *Ocimum sanctum* leaf extract 100 mg/kg (low dose) and 200 mg/kg (High dose). Vildagliptin (Standard drug) (50/kg).[13,14]

Grouping of animals

Male albino rats (155–215 g) were randomly divided into 4 groups each having 6 albino rats as follows:

- Group1: control (Normal saline 1 ml/100 g) group (n = 6)
- Group 2: Standard (Vildagliptin 50 mg/kg) group (n = 6)
- Group 3: *Ocimum sanctum* (Low dose 100 mg/kg) (n = 6)
- Group 4: *Ocimum sanctum* (High dose 200/kg) (n = 6)

Procedure

After overnight fasting the baseline biochemical evaluation such as fasting blood glucose, Insulin, Glucagon, GLP-1 and GIP blood levels were estimated for all the 4 groups.

The extract of Ocimun *sanctum* (Low dose 100 mg/kg) and (High dose 200 mg/kg), Vildagliptin 50 mg/kg, and control (Normal saline 1 ml/100 gm) were administered orally to the respective groups of rats using rat feeding tube. 30 minutes later oral glucose load (dose 2.2 gm/kg) was administered by oral feeding tube to all animals as per the PTGO protocol. [15] Blood samples were collected at 0, 15, 45 minutes after oral glucose load from the tail vein of the rat (0.2-0.25 ml) and transferred into heparinized tubes. Blood glucose test and hormonal assays (Insulin, Glucagon, GLP-1 and GIP) were performed as per the procedure.[16]

The Biochemical and hormonal assays were carried out in Aaranya Biosciences Pvt. Ltd, SIPCOT, Siruseri, Navalur. Hormonal assay kits were purchased from Hysel India Pvt. Ltd. (Manufacturer Raybiotech Inc, USA. Insulin- ELR-Insulin-2 Ray Bio® rat Insulin ELISA Kit. Glucagon-EIAR-GLU-2 Ray Bio® rat Glucagon EIA kit. GLP-1 : EIAR –GLP-1 Ray Bio® rat GLP-1 EIA kit. GIP- EIAR-GIP-1 –Ray Bio® rat GIP –EIA Kit.

Blood glucose determination

Blood samples were collected as per the approved blood collection techniques for laboratory animals. Institutional animal ethical committee permission obtained to collect blood with 7 appropriate technique.[17] Blood samples (0.2-0.25 ml) were collected from the rat tail vein at 0, 15 and 45 minutes. It was mixed with 140 µl of 0.6 M perchloric acid. After centrifugation, the supernatants were assayed for glucose using an enzymatic assay kit.

Plasma insulin, GLP-1, GIP determination:

Approximately 250 µl of blood samples were collected from the tail vein[18] at 0, 15 and 45 minutes of the test in the heparinized capillary tubes. After centrifugation, supernatants were assayed for plasma insulin**,** glucagon, active GLP-1 and GIP levels.[19]

Plasma insulin and glucagon levels were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Plasma active GLP-1& GIP levels were determined using an ELISA kit [GLP-1) Active ELISA kit.[20]

Statistical analysis

Biochemical and hormonal test result data are expressed as the mean ± S.E.M. Differences in the values of blood glucose in an OGTT between the groups treated with control, standard drug and the extract of 2 doses were determined by one-way ANOVA, followed by Dunnett's multiple comparison test.

The data of plasma insulin, Glucagon, GLP-1 and GIP values at 0, 15 and 45 min were compared with the standard drug Vildagliptin. The data were analyzed using one-way ANOVA, followed by Dunnett's multiple comparison test. *p value of < 0.05 (two-sided) was considered statistically significant.* Statistical analyses were performed using Graph Pad software (Prism Windows 5).[21]

RESULTS AND DISCUSSION

Tables 1-3.

Table 1: Effect of Ocimum sanctum leaf extract on Glucose tolerance test in albino rats.

Groups (n = 6)	Blood glucose level			
Groups	-30 mts ± SD (n = 6)	0 mts ± SD (n = 6)	15 mts ± SD (n = 6)	45 mts ± SD (n = 6)
Control	76.4 ± 6	77.5 ± 9.1	176.7 ± 9.6	118.2 ± 8.8
VG (50 mg/kg)	77.1 ± 2	76 ± 6.0	119 ± 4.5***	113 ± 11.8
OS (100 mg/kg)	72.2 ± 15	72.5 ± 17.4	168 ± 11.9	142 ± 4.3
OS (200 mg/kg)	73.3 ± 16	72.5 ± 17.1	155.5 ± 7.7*	120.5 ± 7.3

Vildagliptin VG (STD 50 mg/kg) shows significant reduction in blood glucose ($p \leq 0.001$) at 45 min and Ocimum sanctum (OS 200 mg/kg) shows significant reduction in blood glucose ($p \leq 0.05$) at 15 minutes of OGTT when compared to the control. (Refer Table -1 and Graph -1) .

Table 2: Effect of Ocimum sanctum on GLP-1, GIP on Glucose tolerance test in albino rats.

Parameter STD (mean n = 6)	GLP-1 (pg/ml) Time (minutes)				GIP (pM) Time (minutes)			
Groups	-30	0	15	45	-30	0	15	45
Control	14 ± 1	15 ± 0.2	31 ± 0.4	16 ± 0.4	10 ± 1	10 ± 0.5	63 ± 2.4	15 ± 0.7
VG(50 mg/kg)	15 ± 0.5	16 ± 0.5	45 ± 2.0*	26 ± 18.3	11 ± 1	10 ± 0.2	60 ± 0.7	24 ± 1.4***
OS (100 mg/kg)	15 ± 1	15 ± 0.6	39 ± 0.4	21 ± 13	9 ± 0.1	9 ± 0.3	60 ± 3.5	42 ± 2.1***
OS (200 mg/kg)	14 ± 0.1	13 ± 0.2	27 ± 2.9	22 ± 16	8 ± 0.2	8 ± 0.4	55 ± 4.6	30 ± 2.8***

***p ≤ 0.001; *p = 0.05; Values are STD mean (n = 6) VG- Vildagliptin , OS- *Ocimum sanctum*.
Vildagliptin (50 mg/kg) shows significant increase in GIP (p ≤ 0.001) at 45 minutes and increase in GLP-1(p ≤ 0.05) when compared to the control. *Ocimum sanctum* (100 mg/kg & 200 mg/kg) shows significant (p ≤ 0.001) increase in GIP levels at 45 minutes of OGTT.

Table 3: Effect of Ocimum sanctum on Insulin and Glucagon during Glucose tolerance test in albino rats.

Parameter STD (mean n = 6)	Insulin (ng/ml) Time (minutes)				Glucagon (pmol/L) Time (minutes)			
Groups	-30	0	15	45	-30	0	15	45
Control	0.2 ± 1	0.2 ± 0.01	3 ± 0.1	1 ± 0.04	6.3 ± 1	6.4 ± 0.07	3.7 ± 0.1	2.4 ± 0.07
VG(50 mg/kg)	0.21 ± 1	0.2 ± 0.0	4 ± 0.2***	3 ± 0.07***	5.8 ± 1	5.9 ± 0.1	4.3 ± 0.3	3 ± 0.2
OS (100 mg/kg)	0.2 ± 0.2	0.2 ± 0.03	0.6 ± 0.1	1 ± 0.3	6.2 ± 0.1	6.1 ± 0.4	3.3 ± 0.07	2.4 ± 0.04
OS (200 mg/kg)	0.20 ± 1	0.20 ± 0.1	1 ± 0.08	1..3 ± 0.1	5.8 ± 1	5.9 ± 3	3 ± 0.3	2.5 ± 0.6

Values are STD mean (n = 6) VG-Vildagliptin , OS-Ocimum sanctum.
Vildagliptin (50 mg/kg) shows significant increase in Insulin level (p ≤ 0.001) at 15 and 45 minutes when compared to control. Ocimum sanctum does not show any increase in insulin or glucagon level when compared to the control.

DISCUSSION

Since olden days herbs play an important role in the treatment of diabetic patients. Even the modern allopathic drugs are derived from plant sources. Most of the research studies are done to evaluate the antidiabetic activity of herbals. Our study is carried out with the aim of evaluating the effect of edible herbals on entero-insular axis. Edible herbs are wonderful agents which has the potential to stimulate the incretins and control the blood sugar through the entero-insular axis which is clearly demonstrated in our study.[22] Vildagliptin (STD 50 mg/kg) and *Ocimum sanctum* (200 mg/kg) showed significant reduction in blood glucose (p ≤ 0.001) and (p ≤ 0.05) respectively at 15 minutes of OGTT (Table 1) when compared to control. This reference study also demonstrates the antihyperglycemic effect of *Ocimum sanctum* on oral glucose as well as oral sucrose tolerance test. The antihyperglycemic effect of *Ocimum sanctum* was demonstrated also in type 2 diabetic rat model in comparison with Glibenclamid.[23] Probably this antihyperglycemic action of *Ocimum sanctum* is mediated through entero insular axis.

Vildagliptin (50 mg/kg) showed incretin enhancing effect by increasing GLP-1 (p ≤ 0.05) after 15 minutes and GIP (p ≤ 0.001) at 45 minutes of OGTT (Table 2). A similar study showed increase in GLP-1 level at 5 minutes after OGTT.[24] In our study the Vildagliptin shows increase in GLP-1 level at 15 minutes of OGTT and there is significant increase in GIP levels (p ≤ 0.001) at 45 minutes of OGTT for *Ocimum sanctum* (100 mg/kg and 200 mg/kg), Vildagliptin group when compared to the control (Table 2). The *Ocimum sanctum* leaf extract at low and high doses showed significant increase in GIP levels (p ≤ 0.001) at 45 minutes of OGTT (Table 2). This is an important observation in our study to prove that it is having effect on the entero-insular axis. It may be due to direct stimulatory effect on the G cells of intestine to secret GIP or it may be due to inhibition of DPP IV enzyme inhibition and enhancing the GIP level.[25] An *in-vitro* study done on the pancreatic beta cells showed that GIP has mitogenic and anti-apoptotic factor effect on beta cells.[26] Hence *Ocimum sanctum* leaf extract may also have the mitogenic and beta cell proliferating action through GIP. However further in depth research will through light on this effect. Though many studies demonstrated insulin secretary action of *Ocimum sanctum,*[27] this study could not demonstrate any effect on insulin or glucagon secretion.

CONCLUSION

Ocimum sanctum leaf extract shows significant antihyperglycemic and incretin enhancing effects through entero-insular axis. Further scientific and clinical studies are necessary to establish the therapeutic potential of the extract in the treatment of type 2 diabetes mellitus.

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CONFLICTS OF INTEREST

Conflicts of interest declared none.

AUTHORS CONTRIBUTION STATEMENT

Prof. M. Muniappan conceived the idea and guided me in conducting this research study and also reviewed themanuscript. Dr. S. D. Inbaraj myself carried out the research study, evaluated the results and written the manuscript.

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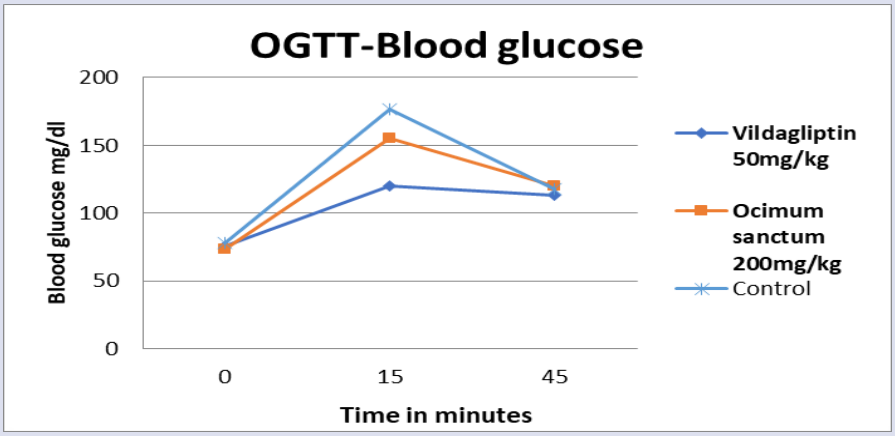
GRAPHICAL ABSTRACT



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