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ANTI DIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF OCIMUM DRY LEAVES POWDER IN STZ INDUCED RATS

[1]Sreedhar Ganga
PhD Scholar
Santosh Medical College, Ghaziabad, NCR – Delhi, India

[2]Kumar Raja Madasu
K.M Medical college and Hospital
Sonkh Road, PaliDungra, Mathura 281123, India

[3]Dr. Lingala sai Samara simha reddy
Assistant Professor,
Mahavir institute of Medical Sciences and Research, Bhopal, Madhya Pradesh, India

[4]Dr. Jyotsna Sharma
(Professor of Pharmacology)
Santosh Medical College & Hospital
Ghaziabad, NCR – Delhi, India

[5]Dr. Shaktibala Dutta
(Prof. & Head of Pharmacology)
Santosh Medical College & Hospital
Ghaziabad, NCR – Delhi, India

Corresponding Author: Sreedhar Ganga

ABSTRACT

Ocimum sanctum has been used medicinally throughout history by many different cultures. Many compounds have been found in the exudates of the *Ocimum sanctum* plant that have been used medically by humans. We have examined the pharmacological hypoglycemic action of *Ocimum sanctum* in diabetic rats. *Ocimum sanctum* 250mg/kg (single dose study) reduced glucose, cholesterol, triglycerides, urea, creatinine, and lipids after treatment for 24 hrs. In chronic study (multiple dose study) also *Ocimum sanctum* reduced creatinine, urea, lipids, triglycerides and glucose after 15days and significantly reduced glucose levels at 15th day in diabetic rats. In glucose tolerance test in diabetic rats with *Ocimum sanctum* 250 mg/kg demonstrated glucose levels were found significantly less compared to the control group. *Ocimum sanctum* serves as an important alternative source in the management of diabetes

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mellitus involved in reducing increased blood glucose during diabetes which should be examined further by oral hypoglycemic therapy.

Key words: *Diabetes, glucose, Ocimum sanctum, Cholesterol, Urea, Creatinine, Triglycerides.*

1.INTRODUCTION

Diabetes is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of Polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Based on the WHO recommendations hypoglycemic agents of plant origin used in traditional medicines are important [1]. Plant drugs and formulations are frequently considered to be less toxic and more free side effects than synthetic one [2].

Several research offers evidence that Tulsi is useful against stress; it enhances stamina and increases efficient use of oxygen by body; strengthens immune system; reduces inflammation; protects from radiation; reduces aging; supports the lungs, liver and heart; it exhibits antibiotic, antiviral and antifungal, antioxidant properties. Different parts of plant have been used in Ayurvedic ancient Medicine to cure an array of ailments including common cold, cough, headache, flu, asthma, fever, colic pain, sore throat, bronchitis, hepatic diseases, malaria fever, as an antidote for snake bite, flatulence headaches, fatigue, skin diseases, wound, insomnia, arthritis, influenza, digestive disorders, night blindness, diarrhea. Tulsi acts as an adaptogen that helps the body and mind to encounter different physical, chemical emotional and infectious stresses, and restore physiological and psychological functions. Such significant and health promising potential, in addition to its highly specific therapeutic actions, paved way for the broad range of Tulsa's traditional medical uses, and also contributes for its mythological importance and religious sanctity.[3-5]

2.MATERIALS AND METHODS

Drugs and Chemicals

Test drug (OS, ethanolic extract, used at doses of 1.75, 4.25, and 8.5 mg/kg procured from Himalaya Health Centre, Bengaluru. Glibenclamide (2.5 mg/kg) dose was obtained from Sun Pharma Laboratories Ltd., Mumbai. Drugs and vehicles were administered by intraperitoneal (IP) route. [6]

Animals

Wistar rats of weight between 150 to 200 g obtained from NIN, Hyderabad, India, were used in the study. The animals were maintained under standard conditions in animal house. The rats were males 8-10 weeks old with average weight of 150-200g. Animals were housed 3-4 per cage in a temperature-controlled (22±1) AC room, with a light/dark cycle of 12hr for a week

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following their arrival; the animals were allowed free access to the standard rat chow diet and tap water they were acclimating to the environment. Rats were also monitored daily and cages cleaned thrice weekly. At the start of the experiment animals were randomly distributed so that body weights, initial triglycerides (TG), total cholesterol (TC), other parameters in all the experimental groups were similar.

Experimental protocol [7]

The test samples were suspended in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route.

***Single dose study (Acute study)*:**

In normoglycemic rats:

The rats were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the retro orbital route of each rat under mild ether anesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from 'One touch ultra', Horizon. U.S.A. The normal rats were then divided into five groups of five rats each. Group I and II were noted as normal control and diabetic control. Groups III and IV received the test extract at a dose of 100 and 250 mg/kg, respectively, through oral route. Group V (standard) received glibenclamide (2.5 mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 1, 2, 4, 6, 8, 12 and 24 hrs of administration of single dose of test samples.

In STZ induced diabetic rats:

The acclimatized rats were kept fasting for 24 hrs with water *ad libitum* and injected intraperitoneally a dose of 60 mg/kg of STZ in normal saline. After 1 hr, the rats were provided feed *ad libitum*. The blood glucose level was checked before STZ and 24 h after STZ as above

Experimental Design

Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition *was* observed at the end of 48 hrs after STZ. The rats were segregated into five groups of five rats in each

Group I – Normal Control and rats received only vehicle that is distilled water.

Group II – Diabetic control and rats received only vehicle that is distilled water.

Group III – Rats received Ethanol Extract of *Ocimum sanctum* (100 mg/kg/day p.o) suspended in distilled water.

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Group IV - Rats received Ethanol Extract of *Ocimum sanctum* (250 mg/kg/day p.o) suspended in distilled water.

Group V – Rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80 solution.

***Multidose study (Chronic Study)*:**

In STZ induced diabetic rats

The selected rats were treated with similar test samples as above, but the blood glucose level was measured on 1, 3, 5, 7, 9 and 14 days of treatment. Glucose testing kit utilized for the measuring of plasma glucose levels was manufactured by Excel Diagnostic Pvt. Ltd.

Estimation of Lipid Profile:

Estimation of Lipid profile such as Total Cholesterol, Triglycerides, HDL, LDL, VLDL and serum glucose level was conducted appropriately as per specifications. Cholesterol- EGD test kit manufactured by Excel Diagnostics Pvt. Ltd. was used for this purpose. The test kit utilizes CHOD/ POD method for cholesterol analysis. Triglycerides testing kit utilized for measuring the triglycerides in the plasma was also manufacture by Excel Diagnostics Pvt. Ltd.

Estimation of Urea and Creatinine: Urea and Creatinine levels were also checked using the respective kits that were both manufactured by Excel Diagnostics Pvt. Ltd.

***Statistical Analysis*:**

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

DATA ANALYSIS

All data are expressed as the standard error of the mean. Comparisons among the control and treatment groups were made using analysis of variance followed by a Student- Newman-Keuls t-test using the Graph pad instat statistical program. With all analyses, an associated probability (p value) of less than 5% ($P<0.05$) was considered significant.

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3.RESULTS

Table .No.1.Effect of ethanolic extract of *Ocimum sanctum* on serum glucose levels in STZ induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	115.0 ±3.53	114.4 ±7.41	113.0 ±8.84	111.8 ±4.95	111.1 ±7.72	110 ±8.29	111.1 ±2.41	111 ±4.71
II	Diabetic Control	5% w/v Tween 80	283.4 ±30.1	281.4 ±95.20	282.3 ±88.74	277 ±40.8	285.3 ±37.1	283.1 ±42.3	285.2 ±38.2	282.1 ±35.40
III	Diabetic Control+ os.	100 mg/Kg	28**1**.1 ±**24**.1	281 ±18.**0**	26**1**.5 ±20.4	23**1**.4 ±24.3	19**1**.7 ±29.3	131.1 ±14.5	102.4 ±8.71	86.4 ±10.3
IV	Diabetic Control+ os.	250 mg/Kg	286 ±66.1	277 ±68.1	258 ±78.6	232.5 ±86.2	186.6 ±75.2	128.1 ±75.1	99.1 ±48.4	84.3 ±68.41
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	288.6 ±3.7	281.8 ±4.4	272 ±5.51	240.1 ±.96	191.6 ±5.4	131 ±4.89	101.8 ±.59	85.65 ±7.24

Table. No.2. Effect of ethanolic extract of *Ocimum sanctum* on serum glucose levels in STZ induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14day
I	Normal control	5% w/v Tween 80	111.5 ±6.31	113.1 ±7.41	114.3 ±8.81	111±7.1	163 ±3.1
II	Diabetic Control	5% w/v Tween 80	284.7 ±93.53	283.4 ±91.21	279.2 ±88.73	281 ±43.91	281.3 ±37.41
III	Diabetic Control+ os.	100 mg/Kg	287.1 ±13.1	263.1 ±18.3	198.2 ±20.4	152.4 ±24.51	131.5 ±29.3
IV	Diabetic Control+ os.	250 mg/Kg	288 ±66.1	261.1 ± 68.2	187.3 ±78.72	104.3 ±86.1	128.6 ±75.2
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	289.4 ±3.8	258.6 ±4.9	163.1 ± 6.51	137.1 ± 3.96	125.9 ±5.4

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Table.No.3: Effect of ethanolic extract of *Ocimum sanctum* on triglyceride levels in serum in STZ induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	91.2 ±2.3	91.31± 2.4	91.21 ±8.6	92.6± 3.0	91.2± 3.1	91.13± 2.2	93.61± 2.0	92.92± 3.4
II	Diabetic Control	5%w/v Tween 80	180.1± 4.3	180.3± 4.2	179.3 ±4.2	179.3± 4.2	177.03 ±4.1	178.6± 4.1	182.92± 6.3	172.1± 4.3
III	Diabetic Control+ os.	100 mg/Kg	169.21± 4.1	166.13± 4.1	163.5 ±4.2	159.4± 4.1	156.45± 4.1	152.7± 4.0	148.01± 3.2	140.2± 2.6
IV	Diabetic Control+ os.	250 mg/Kg	170.3± 1.6	168.1± 1.3	164.52± 3.1	161.1± 2.3	152.61± 3.2	151.71± 3.2	145.62± 2.1	143.42± 2.4
V	Diabetic Control+ Glibenclamide	1.4 mg/kg	171± 3.4	168.4± 3.3	164.2 ±3.3	162.1± 3.2	151.86 ±5.4	149.1± 2.8	147.41± 3.4	137.41± 3.21

Table.No.4: Effect of ethanolic extract of *Ocimum sanctum* on total cholesterol in STZ induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5%w/v Tween 80	71.10 ±1.38	70.87 ±1.0	76 ±1.3	70.02 ±0.8	78 ±1.2	69.04 ±1.3	70.84 ±0.6	70 ±0.8
II	Diabetic Control	5%w/v Tween 80	298.2 ±3.9	296.0 ±3.2	297.9 ±3.2	297.5 ±2.7	293.6 ±2.7	296.5 ±0.14	294.8 ±1.8	298.4 ±1.76
III	Diabetic Control+ caps..	100 mg/Kg	302	292	276.2 ±4.8	261	243	197	160.6 ±3.7	131
IV	Diabetic Control+ caps.	250 mg/Kg	302.1 ±5.7	287.1 ±7.7	266	258	247.4 ±8.87	193	158.1	127.4 ±8.2
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	299.5 ±3.2	278.0 ±1	237.1 ±3	217.1 ±3.4	196.8 ±2.5	172.5 ±2.7	141.2 ±1.8	121.1 ±1.5

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Table.No.5: Effect of ethanolic extract of *Ocimum sanctum* on serum triglycerides levels in STZ induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	91.4 ±2.8	91.51 ±2.8	91.66 ±2.1	92.73 ±3.04	91.32 ±2.4
II	Diabetic Control	5% w/v Tween 80	180.1 ±4.4	181.3 ±4.4	179.2 ±4.2	179.3 ±4.8	177.01 ±4.7
III	Diabetic Control+ os.	100 mg/Kg	178.8 ±4.5	157.14 ±4.2	149.3 ±4.4	145.3 ±4.2	133.41 ±4.2
IV	Diabetic Control+ os.	250 mg/Kg	177.3 ±1.5	151.21 ±1.9	147.3 ±2.7	141.4 ±2.8	131.61 ±2.9
V	Diabetic Control+Glib-enclamide	1.4 mg/kg	175.21 ±3.4	154.61 ±3.3	141.21 ±3.2	138.3 ±3.1	129.81 ±5.3

Table.No.6: Effect of ethanolic extract of *Ocimum sanctum* on total cholesterol in STZ induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	71.15 ±1.39	70.85 ±1.0	70.75 ±1.4	70.91 ±0.8	70.75 ±1.2
II	Diabetic Control	5% w/v Tween 80	298.1 ±3.9	297.0 ±3.2	297.8 ±3.2	297.46 ±2.7	293.5 ±2.7
III	Diabetic Control+ os.	100 mg/Kg	300 ±3.5	245 ±4.3	192.2 ±4.8	143 ±4.0	125.4 ±4.91
IV	Diabetic Control+ os.	250 mg/Kg	302.2 ±5.4	238.2 ±7.3	187 ±8.1	139 ±9.51	121.3 ±8.87
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	299.74 ±3.2	228.0 ±2	174.2 ±3	125.2 ±3.4	116.81 ±2.5

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Table.No.7: Effect of ethanolic extract of *Ocimum sanctum* on serum creatinine levels in alloxan induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	0.384 ±0.02	0.384 ±0.02	0.373 ±0.02	0.368 ±0.01	0.375 ±0.02	0.386 ±0.02	0.388 ±0.02	0.371 ±0.02
II	Diabetic Control	5% w/v Tween 80	6.21 ±0.31	6.38 ±0.32	6.26 ±0.30	6.32 ±0.33	6.28 ±0.3	6.32 ±0.3	6.36 ±0.23	6.24 ±0.19
III	Diabetic Control+ os.	100 mg/Kg	6.24 ±0.35	6.15 ±0.17	5.94 ±0.19	5.64 ±0.27	5.22 ±0.24	4.76 ±0.11	3.56 ±0.20	2.61 ±0.19
IV	Diabetic Control+ os.	250 mg/Kg	6.36 ±0.24	6.29 ±0.21	6.12 ±0.25	5.82 ±0.33	5.48 ±0.32	4.62 ±0.41	3.34 ±0.26	2.49 ±0.18
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	6.32 ±0.14	6.24 ±0.15	6.02 ±0.02	5.68 ±0.2	5.32 ±0.21	4.53 ±0.19	3.12 ±0.29	2.14 ±0.33

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Table.No.8: Effect of ethanolic extract of *Ocimum sanctum* on serum creatinine levels in STZ induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	0.384 ±0.02	0.383 ±0.02	0.372 ±0.02	0.363 ±0.01	0.373 ±0.02
II	Diabetic Control	5% w/v Tween 80	6.23 ±0.31	6.34 ±0.32	6.23 ±0.30	6.32 ±0.33	6.34 ±0.2
III	Diabetic Control+ c.p.	100 mg/Kg	6.34 ±0.34	5.32 ±0.17	4.94 ±0.15	3.64 ±0.27	2.23 ±0.24
IV	Diabetic Control+ c.p.	250 mg/Kg	6.36 ±0.23	5.28 ±0.22	4.64 ±0.25	3.58 ±0.31	2.18 ±0.31
V	Diabetic Control+ Glibenclamide	1.4 mg/kg	6.32 ±0.13	5.18 ±0.17	3.74 ±0.01	2.94 ±0.20	2.12 ±0.23

Table.No.9: Effect of ethanolic extract of *Ocimum sanctum* on urea levels in serum in STZ induced diabetic rats after single dose administration

GROUPS	DRUG	DOSE	0 Hr	1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	24 Hr
I	Normal control	5% w/v Tween 80	29.1 ± 1.0	28.4 ±1.6	28.6 ±6.81	28.9 ±1.3	29.4 ±1.4	30.1 ±1.3	29.2 ±1.3	29.3 ±1.5
II	Diabetic Control	5% w/v Tween 80	147.1 ±1.3	144.6 ±3.22	145.0 ±1.4	144.8 ±2.3	143.6 ±2.1	144.56 ±2.1	144.3 ±2.7	145.2 ±2.8
III	Diabetic Control+ o s.	100 mg/Kg	146.1 ±4.9	144.9 ±5.2	134.5 ±4.9	125.1 ±7.1	117.8 ±6.8	108.72 ±5.06	95.6 ±6.6	85.9 ±7.2
IV	Diabetic Control+ . o s	250 mg/Kg	147.3 ±13.2	143.0 ±14.1	130.8 ±40.6	120.9 ±11.6	115.2 ±7.1	106.9 ±7.06	93.1 ±7.2	82.4 ±7.1

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V	Diabetic Control+Glibenclamide	1.40 mg/kg	148.1 ±8.6	142.4 ±9.5	123.5 ±12.9	117.3 ±12.4	111.1 ±12.44	102.4 ±9.4	92.4 ±9.3	78.5 ±7.3

Table.No.10: Effect of ethanolic extract of *Ocimum sanctum* on urea levels in serum in STZ induced diabetic rats after prolonged treatment

GROUPS	DRUG	DOSE	1 day	3 day	5 day	7 day	14 day
I	Normal control	5% w/v Tween 80	29.1 ± 1	28.3 ±1.6	28.6 ±6.89	28.9 ±1.2	29.4 ±1.1
II	Diabetic Control	5% w/v Tween 80	147.1 ± 1.3	145.6 ±3.21	145.3 ±1.6	146.8 ±2.6	144.6 ±2.5
III	Diabetic Control+ o s	100 mg/Kg	146.1 ±4.6	141.94 ±5.2	134.5 ±4.5	125.1 ±7.4	82.5 ±6.8
IV	Diabetic Control+ o s	250 mg/Kg	147.3 ±13.1	138.06 ±14.1	130.8 ±40.5	120.9 ±11.2	79.2 ±7.1
V	Diabetic Control+ Glibenclamide	1.40 mg/kg	148 ±8.3	134.4 ±9.8	123.5 ±12.1	117.3 ±12.6	72.1 ± 12.41

Upon administration of ethanolic extract of *Ocimum sanctum* significant changes were recorded in blood glucose levels, triglycerides, total cholesterol levels, urea and creatinine levels both in acute as well as in chronic study groups. It was observed that the higher dosage of *Ocimum sanctum* exhibited increased reduction in the values of parameters compared to low dosage administration. The values of the blood glucose levels observed by treating diabetes induced rats with ethanolic *Ocimum sanctum* was comparable to the values obtained by treating with glibenclamide. Recorded values showed a dose dependant reduction of blood glucose levels, total cholesterol, triglycerides and urea levels in the STZ induced diabetic rats treated with ethanolic extract of *Ocimum sanctum.*

SINGLE DOSE STUDY:

Administration of single dose of *Ocimum sanctum* 100 mg/Kg and 250 mg/Kg, oral, each to two study groups which are diabetes induced by STZ, significant reduction ($P>0.05$) in blood glucose levels was observed. The study period encompassed 24hrs. The results were significantly

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comparable to the standard drug glibenclamide. *Ocimum sanctum* at 250 mg/Kg exhibited better blood glucose level reduction compared to *Ocimum sanctum* administered at 100 mg/Kg and results shown in Table No.1,3,4,7,9.

CHRONIC STUDY:

During chronic study which encompassed a period of 15 days, the *Ocimum sanctum* (100 and 250 mg/kg, oral) produced a significant (P>0.05) in BGL of the diabetic rats compared to control. *Ocimum sanctum* at the dose of 250 mg/kg body weight exhibited better BGL reduction than 100 mg/kg body weight and results shown in Table. No. 2, 5, 6,8,10.

4.DISCUSSION*:*

Ocimum sanctum is one of the oldest herbs known for their beneficial uses. *Ocimum sanctum* have been shown to have multiple benefits in patients with diabetes such as reduction of blood sugar and its complications. Many earlier studies whether using the whole seeds or extracts showed that *Ocimum sanctum* decreased fasting blood sugar levels in animals.[8]

At present, the treatment of diabetes mainly involves a sustained reduction in hyperglycemia by the use of Biguanides, thiazolidinediones, sulphonylureas in addition to insulin[9]. However, due to unwanted side effects there is a demand for new compounds for the treatment of diabetes Hence; plants have been suggested as a rich source of potentially useful antidiabetic drugs.[10]

Our results showed that oral administration of fenugreek alkaloid for 24 hrs effectively controlled hyperglycemia. Maintenance of normoglycemia, normalization of serum lipid profile was maintained through leaves extract of *Ocimum sanctum*. The *Ocimum sanctum* maintains the blood glucose to normoglycemia during diabetes, which acts as an essential trigger for both liver and kidney to revert to their normal metabolic homeostasis.

5.CONCLUSION:

The mode of action of *Ocimum sanctum* is reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and also reducing the lipid profile (cholesterol, triglycerides) which protects from the risk factor of coronary heart disease. The further studies are carrying out in isolation of the active constituents involved in *Ocimum sanctum* for obtaining the more valuable results in treatment of the disorders facing by the mankind. Therefore, *Ocimum sanctum* serves as an important alternative source in the medicinal study. In conclusion, the leaves of *Ocimum sanctum* acts as for alternative or complementary medicine in the management of diabetes mellitus.

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