Research Article

Effect of *Ocimum sanctum* Leaf Extract on Alpha Glucosidase Inhibition and DPP- IV Inhibition Activity – an *in vitro* Study

S.D.Inbaraj*, M.Muniappan
Department of Pharmacology, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research [BIHER].
No.7 works road, Chromepet, Chennai, Tamilnadu, India.
***Corresponding author's E-mail:** inbaraj4@yahoo.co.in

Received: 07-08-2018; **Revised:** 28-08-2018; **Accepted:** 09-09-2018.

ABSTRACT

The objective of the study is to find out the effect of *Ocimum sanctum* leaf extract on the inhibition of α-glucosidase and DPP IV enzyme activity by in-vitro method. Methanolic extract of *Ocimum sanctum* leaf is prepared and supplied by Sami labs, Bangalore. Different concentrations (0,29,38,52,63,72 µg/ml) of *Ocimum sanctum* leaf extracts were subjected to α-glucosidase Inhibitory assay and (0,5,10,20,40,80,160,320 µg/ml) for DPP IV enzyme inhibitory assay respectively. The absorbance was measured at 540 and 405 nm using multiplate reader and the percentage of α-glucosidase and DPP IV enzyme inhibitory activity were determined. Acarbose for alpha glucosidase inhibition and Vildagliptin for DDP IV inhibition were used as standard drugs. The IC_{50} value for alpha glucosidase inhibition and DPP IV inhibition were determined. The maximum alpha-glucosidase inhibitory activity of by *Ocimum sanctum* leaf extract at at 100 µg/ml was 65% with IC 50 value of 59.55 when compared to the Acarbose (STD) of 94% with IC 50 values of 42.78. The maximum % of DPP IV inhibition of *Ocimum sanctum* extract at 320 µg/ml is 86.98% with IC_{50} value of 46.08 when compared to the Vildagliptin (STD) is 80.15% with IC 50 value of 22.98. The results of the in vitro studies showed *Ocimum sanctum* leaf extract has significant alpha glucosidase and very significant DPP IV inhibition activity. Further in vivo and clinical studies are necessary to establish the antihyperglycemic and antidiabetic potential of *Ocimum sanctum* leaf extract for the treatment of type 2 diabetes mellitus.

Keywords: α-glucosidase, DPP IV enzyme, *Ocimum sanctum*, type 2 diabetes.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome, characterized by hyperglycemia and alteration of carbohydrate, protein and fat metabolism due to relative or absolute insulin deficiency. As per the International Diabetes federation 366 million people are suffering from diabetes and this may double by 2030. In India alone 40 million people are living with diabetes. India is going to have the highest number of diabetic patients around 60 million by 2025.[1] In type 2 diabetes the more common presentation is post prandial hyperglycemia which plays an important role in the progression of the disease leading to complications.[2] Even though dietary management plays a vital role in the management of type 2 diabetes. The drugs such as Acarbose, Voglibose which inhibits α-glucosidase enzyme responsible for absorption of glucose from gut plays a vital role in controlling post prandial hyperglycemia.[3]

In India our dietary pattern constitutes more than 75 % of carbohydrates; hence changing the dietary pattern completely is not practically possible. Instead we can restrict the carbohydrate absorption from gut using tolerable natural products which is an important area of research interest. This study is designed to evaluate the inhibitory role of edible herbal plants like *Ocimum sanctum* on α-glucosidase and DPP IV enzyme. This action will help in reducing the carbohydrate absorption and probably enhancing the incretins like GLP 1 and GIP in the gut and play a vital role in the drug therapy of type 2 diabetes.

Ocimum sanctum

Ocimum sanctum (Family- Labiatae) known as Tulsi in Hindi and Holy basil in English is widely used in the Indian system of medicine. Every part of the plant has medicinal value. The extract of the stem and leaves has diaphoretic and expectorant effect. It relieves headache, earache and is used to treat dermatological disorders.[4] The important efficacy of *Ocimum sanctum* leaves in decreasing blood glucose level and antidiabetic property has been reported in diabetic rats.[5, 6] *Ocimum sanctum* leaf powder supplementation at 1 to 2% dose levels showed significant hypolipidemic effect in rabbits.[7].

Most of the studies done previously showed antidiabetic activity and prevention of complications but exact mechanism of action is yet to be explored. Hence this study is conducted to explore the important mechanism of *Ocimum sanctum* leaf extract in control of hyperglycemia. Two mechanisms were evaluated by in-vitro methods i.e., inhibition of gut absorption of glucose by alpha glucosidase inhibition and increase of the gut hormones (Incretins viz., GIP,GLP -1) essential for the blood sugar control after oral intake of carbohydrates by DPP-IV inhibition.

MATERIALS AND METHODS

Methanolic extract of *Ocimum sanctum* leaves were prepared and supplied by Sami labs Limited, Bangalore on request. Product Code 2045, Batch No. C170698EM. Date of manufacture April 2017. Methanol was used for extraction and DM water was used for manufacture .The active principle by chemical assay showed Ursolic acid by HPLC 2.53 % w/w, and Oleanolic acid by HPLC 1.95% w/w. Physical, Chemical and Microbiological testing done and certificate of analysis was issued by the Sami Labs Limited (www.samilabs.com). This extract was used for the two in-vitro tests.

Determination of alpha – Glucosidase inhibitory activity

Materials required

Phosphate buffer : 50mM, pH 6.8

Sodium carbonate : (0.1M).

PNPG : 1mM

Sample : extract with range of concentrations 0-100 µg /ml

Alpha- glucosidase : 1u /ml-SRL

Procedure

Alpha-glucosidase inhibitory activity of extracts were carried out according to method of Bachhawat *et al* with slight modification.[7] Reaction mixture containing 50µl phosphate buffer, 10µl alpha-glucosidase and 20µl of varying concentrations of extracts was pre-incubated at 37˚C for 15 min. Then 20µl p-nitrophenyl-α-D-Glucopyranoside (PNPG) was added as a substrate and incubated further at 37˚ C for 30 min. The reaction was stopped by adding 50µl sodium carbonate .The yellow color produced was read at 405nm. Each experiment was performed along with appropriate blanks. Acarbose at various concentrations (0-100 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition.

Calculation

Inhibition (%) = Abs.control – Abs.sample/Abs.control X 100,

DPP4 Inhibitory Invitro Assay

Dipeptidyl peptidase-4 (DPP4), also known as CD26 and adenosine deaminase complexing protein 2, is a serine exopeptidase that cleaves X-Proline and X-Alanine residues from the N-termini of polypeptides. DPP4 is a transmembrane glycoprotein whose activity regulates the bioactivity of multiple peptides such as growth factors, chemokines, and neuropeptides. DPP4plays a major role in glucose metabolism via the regulation of glucagon-like peptide-1 and inhibitors of DPP4 are commonly used for the treatment of type 2 diabetes.[8] DPP4 also plays an important role in immune regulation and may play a role in tumor suppression. In this assay, DPP4 cleaves anon-fluorescent substrate, H-Gly-Pro-AMC, to release a fluorescent product, 7-Amino-4-Methyl Coumarin (AMC) (lex = 360/lem = 460 nm). One unit of DPP4 is the amount of enzyme that will hydrolyze the DPP4 substrate to yield 1.0 mmole of AMC per minute at 37 °C.[9]

Methods and Materials

- DPP4 Assay Buffer 25 mL(Catalog Number MAK088A)
- DPP4 Substrate, H-Gly-Pro-AMC 0.2 mL(Catalog Number MAK088B)
- DPP4 Positive Control 20 mL(Catalog Number MAK088C)
- AMC Standard, 1 mM 0.1 mLCatalog Number MAK088D
- DPP4 Inhibitor, Vildagliptin 1 mL.

Procedure

All samples and standards should be run in duplicate. AMC Standards for fluorometric detection. Dilute 10 mL of the 1 mM AMC Standard solution with990 ml of water to prepare a 10 mM (10 pmole/mL) standard solution. Add 0, 2, 4, 6, 8, and 10 mL of the10 mM standard solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add DPP4 Assay Buffer to each well to bring the volume to 100 mL.

Sample Preparation

Samples can be directly added to the wells. A sample blank is required for each test sample. Prepare a duplicate well for each sample to be used as the sample blank Bring test samples and sample blanks to a final volume of 50 mL with DPP4 Assay Buffer. For the positive control, add 1–2 mL of the DPP4 positive control solution to wells and adjust to 50 mL with the DPP4 Assay Buffer.

Assay Reaction

1. Add 10 mL of the DPP4 Assay Buffer to each of the sample wells. Add 10 mL of the DPP4 inhibitor to each of the sample blank wells. Mix well by pipetting, and incubate for10 minutes at 37 °C.
2. Set up the Master Reaction Mix according to the scheme in Table 1. 40 mL of the Master Reaction Mix is required for each sample and sample blank well. Do not add the Master Reaction Mix to the Standard Curve wells.

Table 1: Master Reaction Mix

Reagent	Volume
DPP4 Assay Buffer	38 mL
DPP4 Substrate	2 mL

3. Add 40 mL of the Master Reaction Mix to each of the wells. Mix well by pipetting. Cover the plate and protect from light during the incubation.
4. Incubate the plate at 37 °C. After 5 minutes, take the initial measurement (T initial). Measure the

fluorescence intensity (FLU) initial, lex = 360/lem = 460 nm).

Note: It is essential (FLU) initial is in the linear range of the standard curve.

5. Continue to incubate the plate at 37 °C taking measurements (FLU) every 5 minutes. Protect the plate from light during the incubation.
6. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (100 p mole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.
7. The final measurement [(FLU) final] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5.
   The time of the penultimate reading is T final.

Calculations

Correct for the background by subtracting the final measurement [(FLU) final] obtained for the 0 (blank) AMC standard from the final measurement [(FLU)final] of the standards and samples. Background values can be significant and must be subtracted from all readings. Plot the standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement from T initial to T final for the samples.

DFLU = (FLU) final – (FLU) initial

Also, subtract the Sample Blank D measurement value from the sample D measurement values. Compare the DFLU of each sample to the standard curve to determine the amount of AMC released by the DPP4assay between T initial and T final (B).

The DPP4 activity of a sample may be determined by the following equation:

DPP4 Activity = B * Sample Dilution Factor/(Reaction Time) * V

B = Amount (pmole) of AMC released between T initial and T final.

Reaction Time = T final – T initial (minutes)

V = sample volume (mL) added to well

DPP4 activity is reported as p mole/min/mL = micro unit/ mL

One unit of DPP4 is the amount of enzyme that will hydrolyze the DPP4 substrate to yield 1.0 mmole of AMC per minute at 37 °C.

Statistical Analysis

The collected data from the observations were assessed for descriptive statistics such as mean, Standard deviation, standard error and % of inhibition is calculated accordingly.[10] The statistical analysis was done using Microsoft Excel program 2013.

RESULTS AND DISCUSSION

Alpha glucosidase enzyme inhibitory action

This in-vitro study shows maximum alpha glucosidase inhibition of *Ocimum sanctum* leaf extract at 100µg/ml is 68% where as the standard drug Acarbose at 100µg/ml is 94% (Refer table 1 & Figure 1).

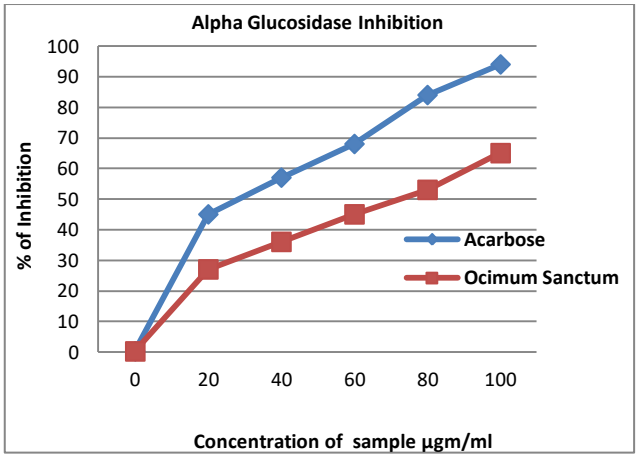

Figure 1: Percentage inhibitory activity of *Ocimum sanctum* leaf extract on alpha- glucosidase enzyme activity in comparison with Acarbose. (n=6)

There is significant alpha glucosidase inhibitory activity of *Ocimum sanctum* leaf extract at maximum concentration of 100 µg/ml when compared to Acarbose (p = < 0.05).

Review article on role of natural products elaborated and confirmed the alpha glucosidase inhibitory role of *Ocimum sanctum*.[11] Another in-vitro study revealed endophytic fungi isolated from *Ocimum sanctum* showed maximum inhibition of alpha glucosidase inhibition.[12] Where as in our study it shows moderate inhibition due to its active incredients only . An in-vivo rat study which demonstrated reduction of glucose absorption from the intestine by the ethanolic extract of *Ocimum sanctum* leaves.[13] probably due to alpha glucosidase inhibition. *Ocimum sanctum* an important herbal medicine which may be developed into a novel herbal antidiabetic medication to reduce carbohydrate absorption from diet and thereby reduce the post prandial hyperglycemia in diabetic patients.

Table 1: Percentage inhibitory activity of *Ocimum sanctum* leaf extract on alpha- glucosidase enzyme activity in comparison with Acarbose. (n=6)

Conc. of sample (µg/ml)	Mean±SD(n=6)	% Inhibition of *Ocimum sanctum*	Mean±SD (n=6)	% Inhibition of Acarbose
0	0.713±0.0024	0.22	0.73± 0.0024	0.22
20	0.716±0.007	27	0.510± 0.006	45
40	0.604±0.011	36	0.410± 0.002	57
60	0.516 ±0.006	45	0.301± 0.001	68
80	0.402 ±0.002	53	0.135± 0.009	84
100	0.314±0.001	65*	0.124± 0.020	94

Values are mean ± SD; (n=6) *P <0.05 when compared to Acarbose

Dipeptidyl peptidase -IV enzyme inhibitory action:

Ocimum sanctum leaf extract shows significant DPP4 inhibition by in-vitro method. At 320µg/ml of concentration it shows 86.97 % inhibition with IC $_{50}$ value of 46.03 whereas the standard drug Vildagliptin at same concentration shows only 80.15% inhibition with IC_{50} value of 22.98 (Refer table 2 & Figure 2).

Table 2: Percentage inhibitory activity of *Ocimum sanctum* on Dipeptidyl Peptidase –IV enzyme in comparison with Vildagliptin. (n= 8)

Conc. of Sample (µg/ml)	Mean± SD (n=8)	% Inhibition of Oci.Santum	Mean± SD (n=8)	% Inhibition of Vildagliptin
0	0.715 ±0.005	0.66	0.952 ±0.0058	0.66
5	0.936 ±0.172	3.26	0.826±0.0052	13.66
10	0.879 ±0.015	5.78	0.710± 0.0030	25.32
20	0.723±0.005	24.58	0.602±0.0119	36.13
40	0.655± 0.019	29.1	0.480±0.0072	48.63
80	0.344±0.006	63.76**	0.326±0.0096	66.18
160	0.255±0.029	76.68***	0.260±0.0070	73.32
320	0.137±0.011	86.97 ***	0.179±0.0087	80.15

Values are mean ± SD; (n=8) *** P < 0.001,**P < 0.01 when compared to Vildagliptin

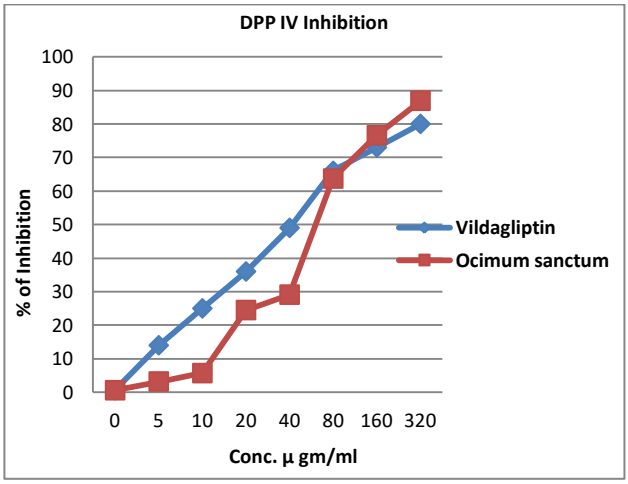

Figure 2: Percentage inhibitory activity of *Ocimum sanctum* on Dipeptidyl Peptidase-IV enzyme in comparison with Vildagliptin

A research study evaluated the DPP –IV inhibitory role of *Ocimum sanctum* and Momordia charantia extract.[14] In that study *Ocimum sanctum* shows significant (P<0.001) DDP-IV inhibition when compared to the standard Diprotein A. In our study we used Vildagliptin as standard drug and *Ocimum sanctum* showed very significant DPP-IV inhibition (P<0.0001) probably Vildagliptin is not a potent DPP-IV inhibitor when compared to Diprotein A.

Because of high DPP-IV inhibitory effect, *Ocimum sanctum* extract may have potent Insulin releasing action or enhance glucose utilization by peripheral tissues and controls the blood sugar level.[15] The phyto chemical analysis of *Ocimum sanctum* leaf extract shows Ursolic acid and Oleanolic acid (Triterpenoids) which may be having DPP-IV inhibitory action and potentiation of insulin release . A study done to evaluate antihyperglycemic activity of *Ocimum sanctum* in comparison with glibenclamide in rat model.[16] which showed significant antihyper glycemic effect of *Ocimum sanctum*. Further in-vivo animal studies and human studies are required to demonstrate the mechanism of action of *Ocimum sanctum* for developing it as a natural, holy herbal remedy for the treatment of Diabetes mellitus.

CONCLUSION

The results of the in- vitro studies showed leaf extract of *Ocimum sanctum* has significant alpha glucosidase and highly significant DPP IV inhibition. Further in vivo and clinical studies are necessary to establish the antihyperglycemic and antidiabetic potential of *Ocimum sanctum* leaves extract for the treatment of type 2 diabetes mellitus.

Acknowledgement: I thank Prof. M.Muniappan, Professor of Pharmacology, Sree Balaji Medical College and Hospital, BIHER for guiding me to undertake the research. I gratefully acknowledge Sami labs limited, Bangalore for supplying the extracts free of cost and encouraged me to do research.

REFERENCES

1. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J Diabetes. 3, 2012, 201–7.
2. Hilary King, Ronald E. Aubert and William H. Herman, 1995. Global Burden of Diabetes, 2025. Diabetes care, 21(9), Sept 1998, 1414-1431.
3. Antonio Ceriello. Post prandial glucose levels clinically important treatment target. Diabetes care, 33, No: 8, 2010, 1905-1907.
4. Satyavati G V, Gupta,A.K. & Bhatla. N. *Ocimum sanctum* Linn (Lamiaceae: Labiateae), in Medicinal plants of India (Indian Council of Medical Research, New Delhi). Vol. 2 (1987) 354.
5. Chattopadhyay.R.R, Hypoglycemic effect of Ocium sanctum leaf extract in normal and streptozotocin diabetic rats. Indian journal of Experimental Biology 31 (11), 1993, 891-893.
6. Sarkar. A & Pant M.C. A comparative study of hypoglycemic action of seeds and fresh leaves of *Ocimum sanctum* (Tulsi). Indian journal of Physiology and Pharmacology; 33 (3), 1989, 197.
7. Sekar A, Lavania C, Pandey D.N. & Pant M.C. Changes in the blood lipid profile after administration of Ocium santum (Tulsi) leaves in the normal albino rabbits. Indian journal of Physiology and Pharmacology, 38, 1994, 311.
8. Avogaro. A.et al., Dipeptidyl peptidase-4 inhibitors can minimize the hypoglycemic burden and enhance safety in elderly people with diabetes. Diabetes Obesity Metabolism., 2014, doi:10.111/dom.12319 (2014).
9. Ghate, M., and Jain,S.V., Structure based lead optimization approach in discovery of selective DPP4 inhibitors. Mini Reviews in Medicinal Chemistry, Vol.13, (6), 2013, 888-914.
10. P.C.Lekshmi et al., In vitro antidiabetic and inhibitory potential of turmeric (Curcuma longaL) rhizome against cellular and LDL oxidation and angiotensin converting enzyme. Journal of Food Science Technology. 51(12), 2013, 3910-17
11. Javed Ahamad, Kamran J.Naquvi, Showkat R , Mir Mohd.Ali, Mohd.Shuaib. Review on role of natural alpha-glucosidase inhibitors for management of Diabetes mellitus. International Journal of Biomedical Research, 2 (6), 2011, 374-380.
12. Pavitra et al. In-vitro studies on α – Amylase and α-Glucosidase and Aldose reductase inhibitors found in endophytic fungi isolated from Ocium sanctum. Current Enzyme Inhibition, 10, 2014, 129-136.
13. Hannan et al; Actions underlying antidiabetic effects of *Ocimum sanctum* leaf extracts in animal models of type1 and type2 Diabetes. European Journal of Medicinal Plants, 5(1), 2015, 1-12.
14. Anand Krishna Singh, Rameshwar Jatwa, Jaya Joshi; Cytoprotective and Dipeptidyl peptidase IV (DPP-IV/CD26) Inhibitory roles of *Ocimum sanctum* and Momordica charantia extract. Asian Journal of Pharmaceutical and Clinical Research. Vo.7, Suppl 1, 2014, 115-120.
15. Raju Patil et al.; Isolation and characterization of anti-diabetic component (bioactivity-guided fractionation) from *Ocimum sanctum* L (Lamiacea) aerial part. Asian Pacific Journal of Tropical Medicine, 2011, 278-282.
16. Dwividendra kumar Nim et al.; Clinical evaluation of antihyperglycemic activity of *Ocimum sanctum* in comparision with Glibenclamide in the rat model of T2DM. International Journal of Pharma and Bio Sciences, 4(2), 2013, 699-706.

Source of Support: Nil, **Conflict of Interest:** None.