Pharmacogn J. 2020; 12(2): 369-376
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Correlation between the *In-Vitro* and *In-Vivo* Antihyperglycemic Effect of Ocimum Sanctum, Trigonella Foenum Graecum and Curcuma Longa

Inbaraj SD*, Muniappan M*

ABSTRACT

This study is carried out to investigate the correlation between the *in-vitro* and *in-vivo* studies which demonstrates the antihyperglycemic effect of T*rigonella foenum graecum, Ocimum sanctum and* C*urcuma longa* extracts. Methanolic seed extract of *Trigonella foenum graecum,* methanolic leaf extract of *Ocimum sanctum*, ethyl acetate rhizomes extract of *Curcumina longa* are prepared and supplied by Sami labs, Bangalore, India on request. *In-vitro* studies such as alpha glucosidase inhibitory and DPP-IV inhibitory activity were done for all the three extracts as per previous studies. After Institutional animal ethical committee clearance male albino rats (155–215 g) were divided into 5 groups. Each group consists of randomly assigned 6 albino rats. The placebo (Normal saline) control group, Standard (Vildagliptin) group and 3 above mentioned extract groups. Oral glucose tolerance test (OGTT) was done. Blood samples were collected for blood sugar estimation at -30 (before extract),0, 15, 45 minutes and blood sugar levels were done by enzymatic assay. **Results**: The maximum alpha-glucosidase inhibitory activity at 100 µg/ml by *Trigonella foenum graecum* extract was 68% with IC 50 value of 57.25, *Ocimum sanctum* leaf extract was 65% with IC 50 value of 59.55, *Curcuma longa* was 72% with IC 50 value of 56.79 when compared to the Acarbose (STD) of 94% with IC 50 values of 42.78. The maximum % of DPP IV inhibition at 320 µg/ml of *Trigonella foenum graecum* extract was 77.84% with IC_{50} value of 52.26, *Ocimum sanctum* extract was 86.98% with IC_{50} value of 46.08 and *Curcuma longa* was 76.47% with IC 50 value of 55.06 when compared to the Vildagliptin (STD) was 80.15% with IC 50 value of 22.98. The OGTT results of *Ocimum sanctum* (200 mg/kg) shows reduction in blood glucose (p=<0.05) at 15 minutes and significant reduction (p=<0.001) at 45 minutes and *Trigonella foenum graecum* (2 gm/kg) showed significant reduction in blood glucose (p=<0.001) at 15 minutes when compared to control. Further clinical studies are necessary to establish the therapeutic potential of these extracts in the treatment of type 2 diabetes mellitus. **Conclusion**: *Ocimum sanctum* leaf extract, *Trigonella foenum graecum* seed extract shows significant alpha-glucosidase and DPP-IV inhibitory activity which correlates with the antihyperglycemic effects by *in-vivo* oral glucose tolerance test. Further clinical studies are necessary to establish the therapeutic potential of these extracts in the treatment of type 2 diabetes mellitus.

Key words: Type 2 diabetes mellitus; *Ocimum sanctum*; *Curcuma longa*; *Trigonella foenum graecum*; GLP-1; Glucagon; Insulin.

Inbaraj SD*, Muniappan M*

Department of Pharmacology, Sree Balaji Medical College and Hospital, Bharath Institute of Higher Education and Research [BIHER]. No. 7 Works Road, Chromepet, Chennai-600044, INDIA.

Correspondence

Dr. Inbaraj SD

Research Scholar & Professor, Department of Pharmacology, Sree Balaji Medical college and Hospital, Bharath Institute of Higher Education and Research [BIHER], No. 7 Works Road, Chromepet, Chennai-600 044, INDIA.

Phone no: 9444413424;

E-mail: inbaraj4@yahoo.co.in

Dr. Muniappan M

Professor of Pharmacology (Rtd), Department of Pharmacology, Sree Balaji Medical college and Hospital, Bharath Institute of Higher Education and Research [BIHER], No. 7 works road, Chromepet, Chennai- 600 044, INDIA.

Phone no: 9444151662;

Email: mmuniappan@yahoo.com

History

- Submission Date: 12-12-2019;
- Review completed: 02-01-2020;
- Accepted Date: 13-01-2020.

DOI : 10.5530/pj.2020.12.58

Article Available online

http://www.phcogj.com/v12/i2

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease, characterized by hyperglycemia and derangement of carbohydrate, protein and fat metabolism due to relative or absolute insulin deficiency. International Diabetes federation data reveals currently 366 million people are suffering from diabetes and this rate will double by 2030. In India currently have 40 million people living with diabetes. India is predicted to have highest number of diabetic patients of 60 million by 2025.[1] Indian type 2 diabetes commonly presents with high post prandial hyperglycemia mainly due to increased carbohydrate consumption in diet. This plays an important role in the progression of the disease resulting in microvascular and macrovascular complications. Apart from the dietary management of type 2 diabetes drugs which inhibit α-glucosidase enzyme responsible for absorption of glucose from gut plays a vital role in controlling post prandial hyperglycemia.[2]

Indian diet constitutes more than 75% of carbohydrates, hence changing the dietary pattern exclusively is not possible.[3] We can reduce the carbohydrate absorption from gut by using appropriate herbal products with less adverse effects and compliance of the patients. This is an interesting area of research which needs to be explored.[4] The current study is designed to evaluate the correlation between *in-vitro* studies (alpha glucosidase inhibition and DPP-IV inhibition) and *in-vivo* study of antihyperglycemic effect of commonly used edible herbals like C*urcuma longa*, *Ocimum sanctum* and T*rigonella foenum graecum* in albino rat animal model. This study will pave the way for newer herbal drug development for type 2 diabetes mellitus in future.

Cite this article: Inbaraj SD, Muniappan M. Correlation between the *In-Vitro* and *In-Vivo* Antihyperglycemic Effect of Ocimum Sanctum, Trigonella Foenum Graecum and Curcuma Longa. Pharmacog J. 2020;12(2):369-76.

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.[5] The important effect of *Ocimum santum* leaves in decreasing blood glucose level and antidiabetic property has been reported in diabetic rats.[6] *Ocimum santum* leaf powder supplementation at 1 to 2% dose levels showed significant hypolipidemic effect in rabbits.

Curuma longa

Curcuma longa (Turmeric) (Family Zingeberaceae), commonly known as Haldi in Hindi has been used as spice and coloring agent. It is a well-known household herbal remedy used for healing of wounds, allergy, digestive disorders, hepatic disorders, sinusitis, rheumatism and cosmetics etc., Many experimental studies showed its anti-inflammatory, antioxidant, anticarcinogenic effect, antibacterial, anti-fungal, antiviral and anticoagulant effects.[7] Curcumin is known for its therapeutic potential as anti-oxidant, anti-inflammatory and diabetes related liver disorders, adipocyte dysfunction, neuropathy, nephropathy, vascular diseases, pancreatic disorders.[8] *C. longa* rhizomes have been reported to possess antidiabetic properties in alloxan induced diabetic rats. Antidiabetic effect was established by many animal studies. Curcumin reduced advanced glycation end products and its complications.[9]

***Trigonella foenum graecum* (Fenugreek)**

Trigonella foenum graecum (also known as fenugreek, locally called as methi), is widely used as spice and household medicine in India. Experimental studies showed it has got hypolipidemic, anti-inflammatory, and reducing gastrointestinal diseases, anti-inflammatory activity as local application in the form of gel.[10] Earlier animal studies suggested hypoglycemic and anti-hyperglycemic action of oral fenugreek seed powder in diabetic rat models.[11]

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 evaluate and correlate the *in-vitro* and *in-vivo* studies in establishing the antihyperglycemic effect of the above herbs. This may significantly contribute for the new drug development of type 2 diabetes mellitus.

MATERIALS AND METHODS

In-vitro studies

Determination of alpha – glucosidase inhibitory activity:

Materials required

Phosphate buffer: 50 mM, pH 6.8

Sodium carbonate: (0.1 M).

PNPG: 1 mM

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

Alpha- glucosidase: 1 u/ml-SRL

Procedure

Alpha-glucosidase inhibitory activity of extracts was carried out according to method of Bachhawat *et al.* with slight modification.[12] Reaction mixture containing 50 µl phosphate buffer, 10 µl alpha-glucosidase and 20 µl of varying concentrations of extracts was pre-incubated at 37°C for 15 min. Then 20 µl 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 50 µl sodium carbonate. The yellow color produced was read at 405 nm. 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 *in-vitro* 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. DPP4 plays 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.[13] 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 m mole of AMC per minute at 37°C.[14]

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 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 DPP4positive control solution to wells and adjust to 50 mLwith 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.

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 pmole/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 todetermine 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 pmole/min/mL =microunit/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. The statistical analysis was done using Microsoft Excel program 2013.

In-vivo study

This is a randomized controlled study using animals (albino rats) to the correlation between the *in-vitro* and *in-vivo* studies demonstrating the antihyperglycemic effect of Curcuma *longa*, *Ocimum sanctum* and T*rigonella foenum graecum* extracts. Institutional research committee and animal ethical committee approval (Protocol No.002/09/2015/IAEC/SBMCH) obtained through proper channel. All the three extracts were prepared and supplied by Sami labs Limited, Bangalore on request. Product Code 2045, Batch No. C170698EM. Date of manufacture April 2017. Methanolic leaf done extract of *Ocimum sanctum* containing Ursolic acid by HPLC 2.53% w/w, Oleanolic acid by HPLC 1.95% w/w. Curcumin C3 Complex (*Curcuma longa* extract) Product Code 0330, Batch No. C161833. Date of manufacture Nov 2016. Ethyl acetate rhizomes extract consists of total curcuminoids by HPLC 95.78%. Fenugreek (*Trigonella foenum graecum*) seed methanolic extract (Fenusterols) Product Code 0566, Batch No. H170111. Date of manufacture January 2017. Content of steroidal saponins by gravimetry 52.04% w/w, Alkaloids 0.63% and Diosgenin by HPLC 1.51% w/w.

Physical, Chemical and Microbiological testing for all the above extracts and certificate of analysis was issued by the Sami Labs Limited (www.samilabs.com). These extracts were used for the study.

T. Vildagliptin 50 mg is procured from the local pharmacy. Manufactured by Novartis (LOT no. KA 395, MFD: 05-2017, EXD: 10 2018). Powdered and made into a suspension (10 mg/ml) and orally administered to rats by oral feeding tube. The dose of Vildagliptin 50 mg/kg was arrived based on the research article.[15]

Male & 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 water, and they were kept under conventional conditions of 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 & Animal ethical Committee's guideline.

Preliminary acute toxicity study

Done with single oral dose of 2000 mg/kg for 3 extract groups of 6 albino rats (3 males and 3 females in each group) weighing 155-215 gm.[16] Similarly, one control group of 6 rats administered orally distilled water (1 ml/100 g)

The animals were monitored for clinical symptoms and mortality intensively for first 6 hours and next 14 days. The results showed that all the three extracts at a single oral dose of 2000 mg/kg did not cause death or any abnormal clinical symptoms in male or female rats.

The effective dose is found out by referring to previous studies carried out in this direction. The following doses were used for the *in-vivo* study.

Ocimum sanctum extract 200 mg/kg.

Trigonella foenum graecum extract 2 gm/kg, *Curcuma longa* 200 mg/kg, Vildagliptin (Standard drug) (50/kg). The doses were determined by previous similar research studies.[17]

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test method is considered as the best physiological test to evaluate the effect of any substance on the gut secretary mechanism.[18] It matches the correct route of (i.e., orally) of administration of carbohydrates by human beings. The ingested glucose or starch administered into the stomach is absorbed in the intestinal tract and absorbed into the splanchnic circulation and then enters the systemic circulation. The high blood glucose concentration stimulates the pancreatic beta cells to release insulin, which in turn activates the glucose uptake by peripheral tissues and organs and controls blood sugar. The passage of the carbohydrates and nutrients through the early

part of the intestine stimulates the release of the gut hormones (e.g., Glucose dependent insulin tropic polypeptide- GIP, and Glucagon like peptide-1 GLP-1), which in turn physiologically stimulate the beta cells to synthesis and release insulin and thereby control the blood sugar.

Grouping of animals

Male albino rats (155–215 g) were randomly divided into 5 groups each with 6 albino rats.

- Group1: Placebo control group (N=6)
- Group 2: Standard (Vildagliptin 50 mg/kg) group (N=6)
- Group 3: *Ocimum sanctum* (dose 200 mg/kg) (N=6)
- Group 4: *Trigonella foenum graecum* (dose 2 gm/kg) (N=6)
- Group 5: *Curcuma longa* (dose 200 mg/kg) (N=6)

Procedure

The baseline fasting blood glucose (-30 minutes) was measured for all the above groups of animals.

Oral glucose tolerance test

After overnight fasting oral administration of the following extracts to 5 groups of rats were done separately using rat feeding tube. *Ocimum sanctum* (dose 200 mg/dl), *Trigonella foenum graecum* (dose 2 gm/kg), *Curcuma longa* (dose 200 mg/kg), Vildagliptin 50 mg/kg, and control (Normal saline). 30 minutes later oral glucose load (dose 2.2 gm/kg) is administered in few seconds by oral feeding tube.[19]

Blood samples were collected at 0,15, 45 minutes after oral glucose load from rats through the tail vein (0.2-0.25 ml) and preserved in heparinized tubes. Blood glucose test done by regular enzymatic assay kit.[20]

Blood glucose determination

Blood samples (0.2-0.25 ml) were collected from the rat tail vein at 0,15, and 45 mts after test drugs and standard drug and mixed with 140 µl of 0.6 M perchloric acid. After centrifugation, the supernatants were assayed for glucose using an enzymatic assay kit.

Statistical analysis

Data are expressed as the mean ± S.E.M. Differences in the values of blood glucose in the Oral glucose tolerance test between the groups treated with vehicle, standard drug, and 3 extracts were determined by 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

The following are the results of *in-vitro* and *in-vivo* tests

Alpha glucosidase enzyme inhibitory action

This *in-vitro* study showed maximum alpha glucosidase inhibition of *Trigonella foenum graecum* 68%, *Ocimum sanctum* 65% and *Curcuma longa* 72% at 100 µg/ml, where as the standard drug Acarbose at 100 µg/ml was 94% (Table 1 and Figure 1).

Dipeptidyl peptidase -IV enzyme inhibitory action

The DPP-IV inhibition of *Ocimum sanctum* extract is 86.97%, *Trigonella foenum graecum* extract is 77.84% and *Curcuma longa* 76.47% at 320 µg/ml of concentration whereas the standard drug Vildagliptin at same concentration shows 80.15% inhibition at 320 µg/ml of concentration (Table 2 and Figure 2).

Results of *in-vivo* test

Oral glucose Tolerance test

Graph shows effect of Vidagliptin 50 mg/kg, *Ocimum sanctum* 200 mg/kg, *Trigonella foenum graecum* 2 g/kg, *Curcuma longa* 200 mg/kg on blood glucose (Tables 3 and 4).

VG (STD 50 mg/kg) and FG (2 gm/kg) shows significant reduction in blood glucose (p=<0.001) at 15 minutes when compared to control. OS (200 mg/kg) shows reduction in blood glucose (p=<0.05) at 15 minutes when compared to control (Table 5 and Figure 3).

Vildagliptin shows significant reduction in plasma glucose at 15 minutes of OGTT

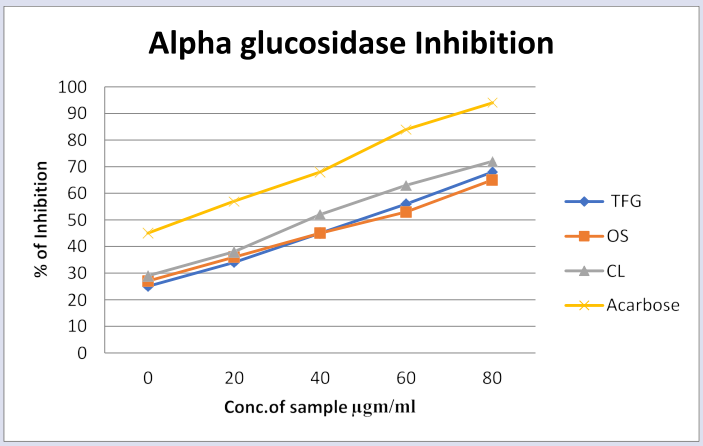


Figure 1: Percentage inhibitory activity of *Trigonella foenum graecum*, *Ocimum sanctum*, *Curcuma longa* on alpha-glucosidase enzyme activity in comparison with acarbose.

TFG: *Trigonella foenum graecum*; OS: *Ocimum sanctum;* CL: *Curcuma longa*

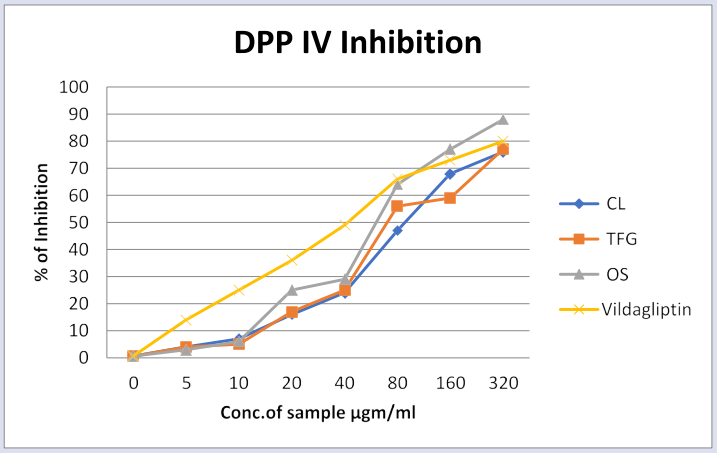


Figure 2: Percentage inhibitory activity of *Curcuma longa*, *Trigonella foenum graecum* and *Curcuma longa* on Dipeptidyl Peptidase-IV enzyme in comparison with vildagliptin.

TFG: *Trigonella foenum graecum*; OS: *Ocimum sanctum;* CL: *Curcuma longa*

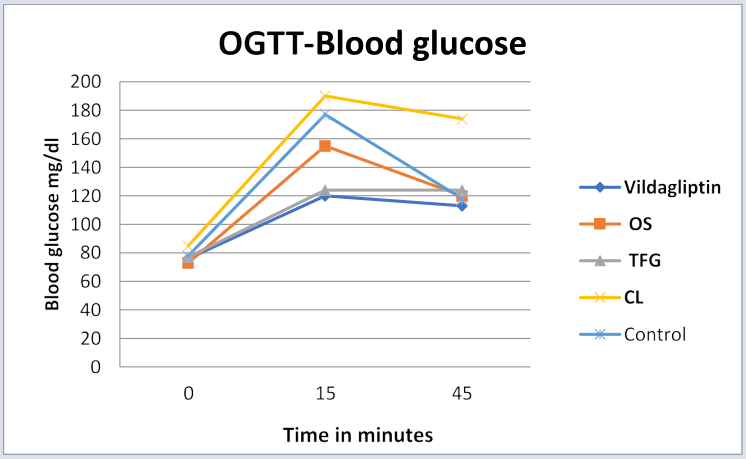


Figure 3: Effect of *Trigonella foenum graecum*, *Ocimum sanctum* and *Curcuma longa* on glucose tolerance test in albino rats.

OS: *Ocimum sanctum*; TFG: *Trigonella foenum graecum*; CL: *Curcuma longa*

Table 1: Percentage inhibitory activity of *Trigonella foenum graecum*, *Ocimum sanctum*, *Curcuma longa* on alpha-glucosidase enzyme activity in comparison with Acarbose (n=6).

Conc. Of sample (µg/ml)	% Inhibition of Trigonella foenum graecum	% Inhibition of Ocimum Sanctum	% Inhibition of Curcuma longa	% Inhibition of Acarbose
0	0.21	0.22	0.20	0.22
20	25	27	29	45
40	34	36	38	57
60	45	45	52	68
80	56	53	63*	84
100	68 *	65*	72*	94

Maximum % of inhibition by *Trigonella foenum graecum*, *Ocimum sanctum*, *Curcuma longa* *P<0.05 when compared to Acarbose (n=6)

Table 2: Inhibitory concentration IC 50 of *Trigonella foenum graecum*, *Ocimum sanctum*, *Curcuma longa* on alpha-glucosidase enzyme activity in comparison with Acarbose.

IC50 (in-vitro Alpha glucosidase inhibition)	Microgram/ml
Curcuma longa	56.79
Trigonella foenum graecum	57.25
Ocimum sanctum	59.55
Acarbose	42.78

Table 3: Percentage inhibitory activity of *Curcuma longa*, *Trigonella foenum graecum* and *Curcuma longa* on Dipeptidyl Peptidase-IV enzyme in comparison with Vildagliptin (n=8).

Conc. of sample (µg/ml	% Inhibition of TFG	% Inhibition of OS	% inhibition of CL	% Inhibition of VL
0	0.66	0.66	0.66	0.66
5	4.52	3.26	4.2	13.66
10	4.41	5.78	6.93	25.32
20	17.44	24.58	15.63	36.13
40	25.21	29.1	24.16	48.63
80	56.83	63.76*	46.15	66.1
160	59.85	76.68***	68.67*	73.32
320	77.84 **	86.97***	76.47**	80.15

Values are mean ±SD of the % of inhibition (n=8) *P<0.05, **P<0.01 and ***P<0.001 when compared to Vildagliptin.

Table 4: Inhibitory concentration (IC50) of *Curcuma longa*, *Trigonella foenum graecum* and *Curcuma longa* on Dipeptidyl Peptidase-IV enzyme in comparison with Vildagliptin.

IC50 (in-vitro DPP-IV inhibition)	Microgram/ml
Curcuma longa	55.06
Trigonella foenum graecum	52.26
Ocimum sanctum	46.03
Vildagliptin	22.98

Table 5: Effect of *Trigonella foenum graecum*, *Ocimum sanctum* and *Curcuma longa* on Glucose tolerance test in albino rats.

Groups (n=6)	Blood glucose level		
	0 mts ± SD(n=6)	15 mts ± SD (n=6)	45 mts ± SD (n=6)
Groups:			
Control	77.5±9.1	176.7 ± 9.6	118.2 ± 8.8
VG (50 mg/kg)	76 ±6.0	119±4.5***	113± 11.8
OS (200 mg/kg)	72.5 ±17.1	155.5 ±.77*	120.5± 7.3
TFG (2 gm/kg)	76 ±16.2	124 ±4.7***	123.7±3.7
CL (200 mg/kg)	76 ±14.8	171±5.8	129 ±8.8

Values are mean ± SD; (n=6) *p<0.05, ***p<0.001. VG-Vildagliptin, OS-*Ocimum sanctum*, TFG- *Trigonella foenum graecum*, CL- *Curcuma longa*.

Ocimum sanctum (200 mg/kg) shows reduction in blood sugar level (p=<0.01) at 15 minutes of OGTT.

Trigonella foenum graecum (2 gm/kg) showed significant (p=<0.001) reduction in blood glucose level at 15 minutes of OGTT.

DISCUSSION

This study evaluated the correlation between the *in-vitro* that is alpha glucosidase inhibition, DPP IV inhibition and the *in-vivo* Oral glucose tolerance test in albino rats. Similar studies were done to review the *in-vitro* and *in-vivo* studies.[22] There are many separate *in-vitro* and *in-vivo* studies done to establish the different mechanisms of antidiabetic drugs and antidiabetic phytochemicals.[23] However, most of them are not having correlation when compared to the *in-vivo* studies hence this study is done to evaluate the correlation that will help in the development of new drug for the treatment of diabetes mellitus.[24] *Trigonella foenum graecum* shows significant alpha glucosidase inhibition (p< 0.05) when compared to Acarbose and significant DPP IV inhibition (p<0,05) when compare to Vildagliptin. This finding confirms the similar study results done with the plants in Indonesia.[25] These findings correlate with the results of Oral glucose tolerance test at 15 minutes with significant reduction in blood glucose (p<0.001) when compared to the control. An *in-vivo* alloxan induced diabetes in rats showed hypoglycaemic effect.[26] *Ocimum sanctum* shows significant alpha glucosidase inhibition (p< 0.05) when compared to Acarbose and very significant DPP IV inhibition (p<0.001) when compared to the Vildagliptin. Though the *in-vitro* studies show significant results the OGTT study shows less significance (p< 0.05) in blood sugar reduction. Though previous studies show significant hypoglycaemic effect in alloxan induced diabetic rats[27] the current study shows less significant antihyperglycemic effect.

Curcuma longa shows significant alpha glucosidase inhibition (p< 0.05) and significant DPP IV inhibition (p<0.05) when compared to the control but there was no effect on OGTT in terms of blood sugar reduction. This may be due to the well-established role of curcumin in reducing the insulin resistance than hypoglycaemia.[28]

There is definite correlation between *in-vitro* and *in-vivo* studies as for as *Trigonella foenum graecum* is concerned. Further in depth *in-vivo* studies are required to establish the mechanism of action of these herbal extracts and development of new drug for the treatment of diabetes mellitus.

CONCLUSION

Among the three herbal extracts evaluated *Trigonella foenum graecum* shows correlation between alpha glucosidase inhibition, DPP IV inhibition *in-vitro* studies and OGTT *in-vivo* studies with antihyperglycemic activity. Further in-depth animal and human studies are needed to develop this molecule as an antidiabetic agent.

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.

AUTHORS CONTRIBUTIONS

Dr. S. D. Inbaraj conducted the experiment and prepared the manuscript. Prof. M. Muniappan designed and supervised the experiment.

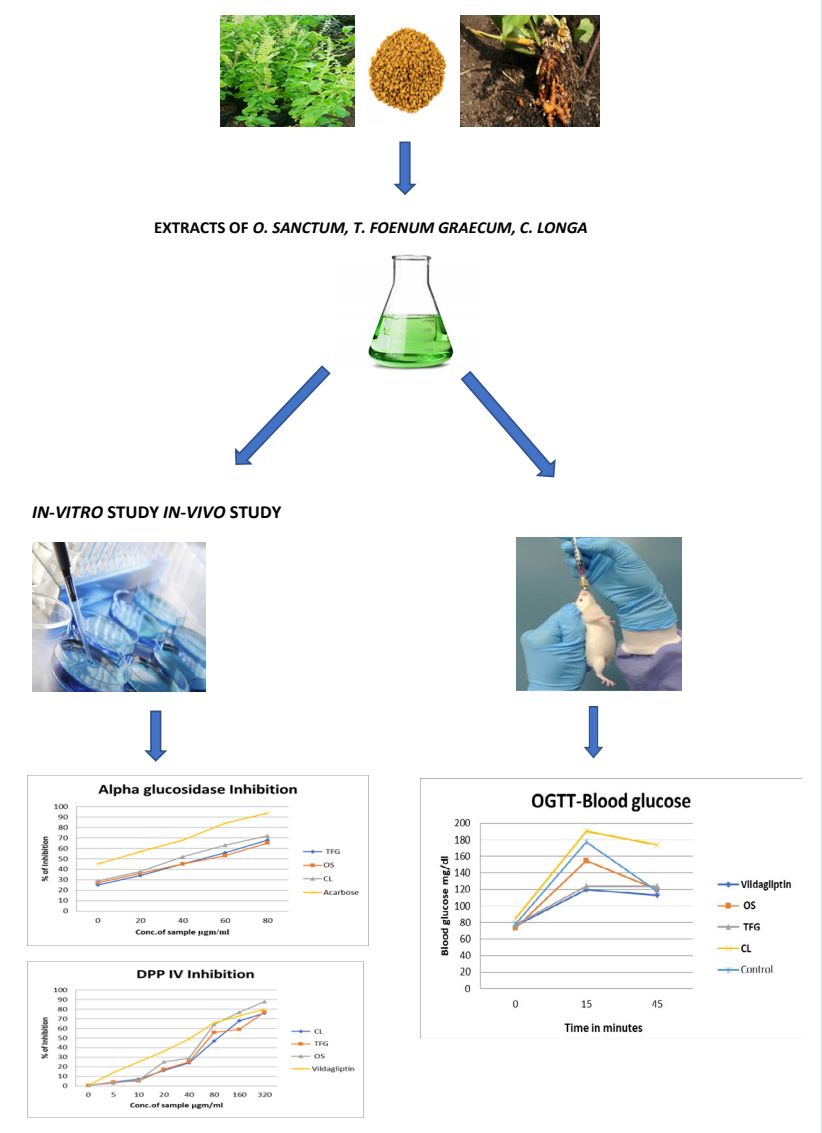
CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCES

1. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J Diabetes. 2012;3:201-7.
2. DiNicolantonio JJ, Bhutani J, O'Keefe JH. Acarbose: Safe and effective for lowering postprandial hyperglycemia and improving cardiovascular outcomes. Open Heart 2015;2:e000327.
3. Mohan V, Radhika G, Sathya RM, Tamil SR, GanesanA, Sudha V. Dietary carbohydrates, glycemic load, food groups and newly detected type 2 diabetes among urban Asian in Chennai, India (Chennai Urban Rural Epidemiology Study 59). Br J Nutr. 2009;102(10):1498-506.
4. AL-Azzawi AM, Mehdi N, AL-Juboori AG, Ejaz A, Ali H, Sarheed O. Herbal medicines questionnaire and evaluation of attitude, perceptions and self-use among health care professionals in RAK, UAE: Pilot study. Int J Pharm Pharm Sci. 2019;11(3):86-90.
5. Satyavati GV, Gupta AK, Bhatla N. Ocium santum Linn (Lamiaceae: Labiateae), in Medicinal plants of India (Indian Council of Medical Research, New Delhi). 1987;2:354.
6. Sarkar A, Pant MC. A comparative study of hypoglycemic action of seeds and fresh leaves of Ocimum sanctum (Tulsi). Indian journal of Physiology and Pharmacology. 1989;33(3):197.
7. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and Curcumin: Biological actions and medicinal applications. Current Science. 2002;87(1).
8. Aggarwal BB, Sundaram C, Malani A, Ichikawa H. Curcumin: the Indian solid gold. Advances in Experimental Medicine and Biology. 2007;595:1-75.
9. Sajithlal GB, Chittra P, Chandrakasam G. Effect of curcumin on advanced glycation and cross-linking of collagen in biabetic rats. Biochem Pharmacol. 1998;56:1607-14.
10. Jyothi D, Koland M. Formulation and evaluation of an herbal anti-inflammatory gel containing *Trigonella foenum greacum* seed extract. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8(1):41-4
11. Hannan JMA, Rokeya B, Faruque O, Nahar N, Moshiuzzaman M, Azad Khan AK, et al. Effect of soluble dietary fraction of Trigonella foenum graecum regulation status of type 2 diabetic model rats. Journal of Ethnopharmacology. 2003;88:73-7.
12. Bachhawat JA, Shihabudeen MS, Thirumurugan K. Screening of fifteen Indian Ayurvedic plants for Alpha-glucosidase inhibitory activity and enzyme kinetics. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;3(4):267-74.
13. Avogaro A, Dardano A, de Kreutzenberg SV, Del Prato S. Dipeptidyl peptidase-4 inhibitors can minimize the hypoglycemic burden and enhance safety in elderly people with diabetes. Diabetes Obesity Metabolism. 2014.
14. Ghate M, Jain SV. Structure based lead optimization approach in discovery of selective DPP4 inhibitors. Mini Reviews in Medicinal Chemistry. 2013;13(6):888-914.
15. Wang X, Ahang D, Xu W, Liu H, Wang W. Pharmacokinetics of lipoyl vildagliptin a novel dipeptidyl peptidase IV inhibitor after oral administration in rats. Xenobiotica. 2010;40(10):707-12.
16. Gopi S. Acute and sub chronic oral toxicity studies of hydrogenated curcuminoid formulation Curo White in rats. Toxicology Reports. 2016;3:817-25.
17. Jarald E, Bangar O, Edwin S, Ahmad S, Jammalludin S. Antidiabetic activity of few Indian medicinal plants vs their combination in alloxan induced diabetic rats. J Pharm Res. 2009;2(11):1760-3.
18. Pacini G, Omar B, Ahren B. Methods and models for metabolic assessment in mice. J Diabetes Res. 2013.
19. Yamazaki K, Yasuda N, Inoue T, Nagakura T, Kira K, Shinoda M, et al. 7-But-2-ynyl-9-(6-methoxy-pyridin-3-yl)-6-piperazin-1-yl-7,9-dihydro-purin-8-one is a novel competitive and selective inhibitor of dipeptidyl peptidase IV with an antihyperglycemic activity. J Pharmacol Ex Ther. 2006;319(3):1253.
20. Mogale MA, Lebelo SL, Thovhogi N, de Fretas AN, Shai L. α-amylase and α-glucosidase inhibitory effects of Sclerocarya birrea [A. Rich. Hochst] subspecies caffra (sond) Kokwaro (Anacardiaceae) stem-bark extracts. African Journal of Biotechnology. 2011;10(66):15033-9.
21. Dean R. Appling, software review of Prism 5. J Am Chem Soc 20018;130(18):6056.
22. De B, Bhandari K, Chakravorty N, Mukherjee R, Gundamaraju R, Singla RK, et al. Computational Pharmacokinetics and *in vitro-in vivo* correlation of anti-diabetic synergistic phyto-composite blend. World Journal of Diabetes. 2015;6(11):1179 -85.
23. Hansen L. *In Vitro* and *In vivo* studies of a naturally occurring variant of the human p85α regulatory subunit of the phosphoinositide 3-Kinase. Diabetes. 2001;50(3):690-3.
24. Karthikeyan M, Balasubramanian T, Kumar P. *In-vivo* Animal Models and *In-vitro* Techniques for Screening Antidiabetic Activity. J Develop Drugs 2016;5:153.
25. Riyanti S, Suganda AG, Sukandar EY. Dipeptidyl peptidase-IV inhibitory activity of some Indonesian medicinal plants. Asian J Pharm Clin Res. 2016;9(2):375-7.
26. Mowla A, Alauddin M, Atiar Rahman Md, Ahmed K. Antihyperglycemic effect of Trigonella foenum graecum (Fenugreek) seed extract in alloxan-induced diabetic rats and its use in diabetes mellitus: A brief qualitative phytochemical and acute toxicity test on the extract. Afr J Trad Cam 2009;6(3):255-61.
27. Jarald E. Antidiabetic activity of few Indian medicinal plants Vs their combination in alloxan induced diabetic rats. J Pharm Res. 2009;2(11):1760-3.
28. Ghorbani Z, Hekmatdoost A, Mirmiran P. Anti-Hyperglycemic and insulin sensitizer effects of turmeric and its principle constituent Curcumin. Int J Endocrinol Metb. 2014:12(4):e18081.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Dr. S. D. Inbaraj, MBBS, MD (Pharmacology) is a Research Scholar and Professor in the department of Pharmacology, Sree Balaji Medical college and Hospital, Bharath Institute of Higher Education and Research [BIHER]. No.7 works road, Chromepet, Chennai- 600 044. Having more than 10 years of teaching experience in Pharmacology for undergraduate and post graduate medical students. Having vast experience in treating general as well as Diabetic patients. His research interest is in the field of edible herbs and their effect on incretins and diabetes mellitus.

Dr. M. Muniappan, MSc, PhD., retired Professor of Pharmacology, Sree Balaji Medical college and Hospital, Bharath Institute of Higher Education and Research [BIHER]. No.7 works road, Chromepet, Chennai- 600 044. Having more than 25 years of teaching experience in Pharmacology for undergraduate and post graduate medical students. He guided many PhD scholars. His field of research is herbal medicines.

Cite this article: Inbaraj SD, Muniappan M. Correlation between the *In-Vitro* and *In-Vivo* Antihyperglycemic Effect of Ocimum Sanctum, Trigonella Foenum Graecum and Curcuma Longa. Pharmacog J. 2020;12(2):369-76.