

Evaluation of Antidiabetic and Antihyperlipidemic Effects of Hydroalcoholic Extract of Leaves of *Ocimum tenuiflorum* (Lamiaceae) and Prediction of Biological Activity of its Phytoconstituents

Subramani Parasuraman, Subramani Balamurugan¹, Parayil Varghese Christapher, Rajendran Ramesh Petchi², Wong Yeng Yeng, Jeyabalan Sujithra, Chockalingam Vijaya²

Unit of Pharmacology, Faculty of Pharmacy, AIMST University, Bedong 08100, Kedah, Malaysia, ¹Department of Pharmacology, College of Pharmacy, Madras Medical College, Chennai, ²Department of Pharmacology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India

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ABSTRACT

Objective: The aim was to evaluate the anti-diabetic and anti-hyperlipidemic effects of hydroalcoholic extract of leaves of *Ocimum tenuiflorum* (Lamiaceae) and prediction of biological activities of its phytoconstituents using *in vivo* anti-diabetic model and *in silico* analysis respectively. **Materials and Methods:** The leaves of *O. tenuiflorum* were extracted with 60% ethanol, and the extract was used for further pharmacological screening. The acute toxicity of the extract was evaluated as per the guidelines set by the Organization for Economic Co-operation and Development, revised draft guidelines 423. The oral anti-diabetic activity of the hydroalcoholic extract of *O. tenuiflorum* (125, 250 and 500 mg/kg) was studied against streptozotocin (STZ) (50 mg/kg; i.p.) + nicotinamide (120 mg/kg; i.p.) induced diabetes mellitus. The animals were treated with the investigational plant extract and standard drug (glibenclamide) for 21 consecutive days and the effect of hydroalcoholic extract of *O. tenuiflorum* on blood glucose levels was measured at regular intervals. At the end of the study, blood samples were collected from all the animals for biochemical estimation, then the animals were sacrificed and the liver and kidney were collected for organ weight analysis. Prediction for pharmacological and toxicological properties of phytoconstituents of *O. tenuiflorum* was carried out using online web tools such as online pass prediction and lazax toxicity prediction. **Results:** The hydroalcoholic extract of *O. tenuiflorum* showed significant anti-diabetic and anti-hyperlipidemic activity at 250 and 500 mg/kg, and this effect was comparable with that of glibenclamide. Predicted biological activities of phytoconstituents of *O. tenuiflorum* showed presence of various pharmacological actions, which includes anti-diabetic and anti-hyperlipidemic activities. Prediction of toxicological properties of phytoconstituents of *O. tenuiflorum* did not show any major toxic effects. **Conclusion:** The hydroalcoholic extract of *O. tenuiflorum* showed significant anti-diabetic and anti-hyperlipidemic activity against STZ + nicotinamide induced diabetes mellitus in rats. Further studies are required to confirm the anti-diabetic and anti-hyperlipidemic activities of individual phytoconstituents of *O. tenuiflorum*.

Key words: Anti-diabetic activity, Anti-hyperlipidemic activity, *In silico* analysis, *Ocimum tenuiflorum*, Phytoconstituents

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INTRODUCTION

Ocimum tenuiflorum (*Ocimum sanctum*) belongs to the family of Lamiaceae and is commonly known as Thulasi/Tulsi in India.

Address for correspondence:

Dr. Subramani Parasuraman, Unit of Pharmacology, Faculty of Pharmacy, AIMST University, Bedong 08100, Kedah, Malaysia.
E-mail: parasuraman@aimst.edu.my/parasuphd@gmail.com

It is widely grown in all regions and is considered as the sacred plant of India. Tulsi or Holy Basil is a sacred plant used by Vaishnavas for thousands of years. In Indian sub-continent, fresh leaves of this plant are most commonly used for the treatment of cough, cold, abdominal pain, skin diseases, arthritis, painful eye diseases, measles, and diarrhea. The preclinical evaluation on various extracts of different parts of *O. tenuiflorum* showed anti-fertility, anti-cancer, anti-diabetic, anti-fungal, hepatoprotective and cardioprotective actions.^[1]

Mixture of Tulsi leaves and black pepper seeds are used for the treatment of fever and malaria as a traditional medicine.^[2] In Ayurveda, the therapeutic effect of Tulsi is well-described as Dashemani Shwasaharni (anti-asthmatic) and anti-kaphic drugs (Kaphaghna).^[1] The leaves of the Tulsi contain essential oils including carvacrol, ursolic acid, eugenol and the seeds contain fixed oils, including oinoleic acid, oleic acid, palmitic acid, and stearic acid.^[3] The reported activities are determined using the crude extract of either the whole plant or parts of the plant and only a few studies are available with the individual phytoconstituent's effects. Ethanolic extract of *O. Sanctum* at 400 mg/kg showed significant anti-diabetic effect in alloxan induced diabetes mellitus in rats, and the fixed oil of *O. sanctum* significantly reduced hyperlipidemia induced by high fat diet fed Wistar rats.^[4,5] The effect of *O. tenuiflorum* on streptozotocin (STZ) induced diabetes mellitus and hyperlipidemia remains unclear. Hence, this study was planned to evaluate the anti-diabetic and anti-hyperlipidemic effects of hydroalcoholic extract of leaves of *O. tenuiflorum* (Lamiaceae) using STZ induced diabetes mellitus in rats and prediction of biological activities of its phytoconstituents using *in silico* analysis, respectively.

MATERIALS AND METHODS

Evaluation of anti-diabetic and anti-hyperlipidemic effects of hydroalcoholic extract of leaves of *Ocimum tenuiflorum*

Plant profile

Ocimum is a genus of about 68 different species of aromatic annual and perennial herbs and shrubs in the family of Lamiaceae, native of a tropical region. *O. tenuiflorum* is 30–70 cm height erect herb, which grows in semitropical and tropical parts of India. Leaves have aromatic taste and are 2.5–5 cm long and 1.6–3.2 cm simple, opposite, elliptic, oblong or acute, with entire or sub-serrate or dentate margins, pubescent on both sides, minutely gland-dotted, with slender, hairy petioles. Inflorescence is verticillate and flowers are in racemes 15–20 cm long in close whorls.^[6,7]

Collection of the plant

Taxonomically identified *O. tenuiflorum* (Lamiaceae) plant was collected from rural parts of Vellore, Tamil Nadu in December 2013. Plant was identified and authenticated by Botanist of the Agricultural Research Station, Vellore, Tamil Nadu. The plant leaves were dried under the shade for a week and grounded using an electrical grinder to a coarse powder.

Extraction of leaves

The powdered leaves of *O. tenuiflorum* was packed in a Soxhlet apparatus and extracted with 60% ethanol. The extraction was carried out for 24 h at about 55–60°C; the extract was filtered through muslin cloth. The filtrate

was concentrated to a dry mass by evaporation under reduced pressure. The yield was found to be 7% w/v. The hydroalcoholic extract of leaves of *O. tenuiflorum* was stored in a desiccator at room temperature until further analysis.

Chemicals

Streptozotocin was purchased from Avra Synthesis Pvt Ltd., Hyderabad. Glibenclamide was received as a gift drug from Aurobindo Pharma Ltd., Hyderabad. Biochemical assay kits for glucose, serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), total cholesterol, total protein, triglyceride, and high-density lipoprotein (HDL) cholesterol kits were procured from Coral diagnostics Ltd., Mumbai. All other chemicals used were of analytical grade and purchased from SD Fine Chemicals Limited, India.

Animals

The male Wistar albino rats, (180 ± 20 g body weight [BW]), were obtained from Sainath Enterprises, Hyderabad, India. The animals were housed in large, spacious polyacrylic cages at an ambient room temperature with 12 h-light/12 h-dark cycle. Rats have free access to water and rat pellets (VRK Nutritional Solution, Sangli, Maharashtra). The study was approved by the Institute Animal Ethics Committee of Ultra College of Pharmacy, Madurai, India. All the animal experiments were carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

Acute oral toxicity studies

Acute oral toxicity of the hydroalcoholic extract of *O. tenuiflorum* was carried out as per the guidelines set by the Organization for Economic Co-operation and Development, revised draft guidelines 423. The principle involves a step-wise procedure with the use of the minimum number of animals per step to obtain sufficient information on the acute toxicity of the test substance to enable its classification. Healthy Wistar rats (3 animals/dose) of either sex were used for the experiment. Overnight fasted rats were orally fed with the hydroalcoholic extract of *O. tenuiflorum* in increasing dose levels of 5, 50, 300, and 2000 mg/kg BW, respectively. The rats were observed closely for their behavioral, neurological and autonomic profiles continuously for 24 h after dosing. After a period of 24 h, animals were observed (at least two times a day) for 14 days to evaluate the changes on behavioral, neurological, autonomic profiles and mortality.^[8]

Anti-diabetic effect of hydroalcoholic extract of *Ocimum tenuiflorum*

Healthy, adult male Wistar albino rats weighing between 180 ± 20 g were used for the experiment. Diabetes mellitus was induced in overnight-fasted rats by single

intraperitoneal injection of freshly prepared 50 mg/kg BW STZ, followed by 120 mg/kg of nicotimamide (NIC) in 0.1 M citrate buffer (pH 4.5). After 24 h of diabetes mellitus induction, the rats were given 5% w/v of glucose solution (2 ml/kg BW) to prevent hypoglycemic mortality. Diabetes mellitus was confirmed after 48 h of induction by measuring fasting blood glucose level using tail vein blood sample. Rats with fasting blood glucose of more than 200 mg/dl were considered as diabetics and used for further experiment.^[8,9] Diabetic animals were randomly divided into four groups (Group II–VI) as follows.

Group I: Normal control.

Group II: Diabetic control.

Group III: Diabetic animals treated with glibenclamide (0.25 mg/kg).

Group IV: Diabetic animals treated with hydroalcoholic extract of *O. tenuiflorum* (125 mg/kg).

Group V: Diabetic animals treated with hydroalcoholic extract of *O. tenuiflorum* (250 mg/kg).

Group VI: Diabetic animals treated with hydroalcoholic extract of *O. tenuiflorum* (500 mg/kg).

Group I (normal control) and group II (diabetic control) animals received 0.5% w/v carboxymethyl cellulose (CMC). Animals in group III were treated with 0.25 mg/kg BW of glibenclamide and animals in group IV–VI were treated with hydroalcoholic extract of *O. tenuiflorum* at dose levels of 125, 250 and 500 mg/kg BW. The doses of hydroalcoholic extract of *O. tenuiflorum* were selected from toxicology study. The standard and test drugs were suspended in 0.5% w/v CMC and administered once daily through oral gavage for 21 consecutive days. Few drops of venous blood were collected on 7th and 14th day of the experiment and immediately used for the estimation of blood glucose (whole blood) with glucometer.^[10] Throughout the study, experiment animals' BW variations were monitored at regular intervals. At end of the study (i.e. 21st day), blood sample was withdrawn from all the experimental animals through retro-orbital plexus puncture, and the serum was separated and used for biochemical analysis.

Biochemical analysis

During the experiment, blood glucose levels were estimated using animals' whole blood sample with the help of One-Touch Horizon Glucometer; Ortho-Clinical Diagnostics, Johnson and Johnson Company, USA. At the end of the experiment, few milliliter of the blood sample was collected in plain glass tube through retro-orbital plexus and the serum was separated by centrifuging at 3000 RPM for 20 min.^[11] The serum sample was used for estimation of biochemical markers such as total serum cholesterol, serum triglyceride, HDL cholesterol, SGOT, SGPT, creatinine, urea, total protein, and albumin.

The calculated low-density lipoprotein (LDL) cholesterol, HDL ratio, and atherogenic index were determined. The LDL was calculated using Iranian formula ($LDL = TC/1.19 + TG/1.9 - HDL/1.1 - 38$ [mg/dL]); HDL ratio was calculated using formula ($[HDL - cholesterol / TC - HDL - cholesterol] \times 100$ [%]); very low-density lipoprotein (VLDL) was calculated using formula $LDL/5$; albumin: Creatinine ratio was calculated using formula (albumin/creatinine [g/mg]) and kidney: BW ratio was calculated using formula (weight of both kidney [mg]/BW of the animal [g]).^[12-15]

Statistical analysis

All the data were expressed as mean $\pm$ standard error of the mean and the statistical significance between the groups were tested using one-way analysis of variance followed by Bonferroni *post-hoc* test. The statistical analysis was calculated using GraphPad Instat 3.06 (GraphPad Software, CA). $P < 0.05$ was considered as significant.

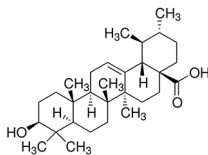
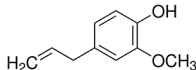
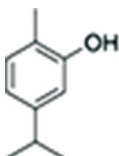
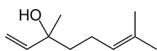
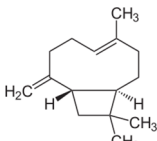
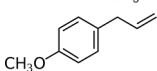
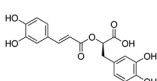
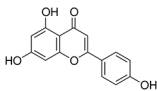
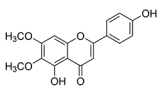
Photochemistry

The leaves of the Tulsi contain essential oils such as benzaldehyde, borneol, n-butylbenzoate, caryophyllene oxide, carvacrol, cis- α -terpineol, cubenol, eugenol, furaldehyde, limonene, linalool, methyl carvicol (estragol: 1-allyl-4-methoxybenzene), ocimene, oleic acid, sebinene, phytol, veidifloro, β -pinene, α -thujene, methyl chavicol and ursolic acid. The seeds of the Tulsi contain fixed oils such as oinoleic acid, oleic acid, palmitic acid, and stearic acid.^[14] *O. tenuiflorum* leaves contain 0.7% of volatile oil including 71% of eugenol and 20% of methyl eugenol. The leaves also contain flavonoids such as orientin and vicerin, and few phenolic compounds namely apigenin, cirsilineol, cirsimaritin, isothymusin, isothymonin, apogenin, rosmarinic acid and eugenol.^[7,17] The main phytoconstituents present in the plant leaves are eugenol and ursolic acid. The pharmacological screening of whole plant extract or plant part extract showed the presence of various activities, including anti-microbial, hypolipidemic, anti-diabetic, analgesic, anti-inflammatory, cardioprotective and anti-asthmatic activities.^[18-20] Important phytoconstituents of *O. tenuiflorum* and its phytochemical importance were summarized in Table 1.^[21-47]

Prediction of biological activities of constituents of *Ocimum tenuiflorum*

Ursolic acid, eugenol, carvacrol, linalol, caryophylline, estragole, rosmarinic acid, apigenin and cirsimaritin are the important compound present in the leaves *O. tenuiflorum*.^[48] The canonical simplified molecular-input line-entry system (SMILES) format of phytoconstituents of *O. tenuiflorum* were obtained from PubChem [<https://pubchem.ncbi.nlm.nih.gov/>] and used for biological activity prediction. Prediction of biological activity spectra and toxicity profile were carried out with online prediction of activity spectra of substances (PASS) prediction tools (www.pharmaexpert.ru/passonline/), and lazarus toxicity prediction tools (<http://lazarus.in-silico.de/>)

Table 1: Important phytoconstituents of *O. tenuiflorum*

Phytoconstituents	Chemical structure	Phytochemical importance and pharmacological use
Ursolic acid		Ursolic acid is an isomer of oleanolic acid and pentacyclic triterpenoid carboxylic acid, present in <i>Calluna vulgaris</i> , <i>Eribotrya japonica</i> , <i>Eucalyptus hybrid</i> , <i>Glechoma hederacea</i> , <i>Lelaleuca lecadendron</i> , <i>Ocimum sanctum</i> , <i>Rosmarinus officinalis</i> , <i>Pyrola rotundifolia</i> , <i>Psychotria serpens</i> , <i>Sambucus chinesis</i> , <i>Solanum incanum</i> and <i>Tripterospermum taiwanense</i> . Commonly reported pharmacological actions of ursolic acid are hepatoprotective, anti-inflammatory, cytotoxic to leukemia cells and antimicrobial activities. ^[21]
Eugenol		Eugenol is a major constituent of <i>Syzygium aromaticum</i> (clove) and widely used as anesthetic and analgesic in dentistry. ^[22]
Carvacrol		Carvacrol is a monoterpenoid phenol isolated from the plant parts of <i>Shigella sonnei</i> , <i>Shigella flexneri</i> , <i>Origanum vulgare</i> and <i>Ocimum sanctum</i> . ^[23,24] Commonly reported pharmacological actions of carvacrol are hepatoprotective, anti-inflammatory, cytotoxic, antiviral and antioxidant activities. ^[25,26]
Linalool		Linalool, is a terpenic alcohol and principle compound present in <i>Aniba rosaeodora</i> , <i>Cinamomon camphora</i> and <i>Ocimum sanctum</i> . ^[27] Commonly reported pharmacological actions of linalool are antileishmanial, anticonvulsant and anti-inflammatory properties. ^[28,29]
Caryophyllene		Caryophyllene is a natural bicyclic sesquiterpene isolated from <i>Didymocarpus tomentosa</i> , <i>Syzygium aromaticum</i> , <i>Cannabis sativa</i> and <i>Ocimum sanctum</i> . ^[30-32] Commonly reported pharmacological actions of caryophyllene are local anesthetic, anti-inflammatory, cytotoxic and anticancer activities. ^[31-33]
Estragole		Estragole is a phenylpropanoids present in <i>Foeniculum vulgare</i> , ^[34] <i>Ocimum basilicum</i> , ^[35] <i>Agastache rugosa</i> ^[36] and <i>Clausena suffruticosa</i> . ^[37] The laboratory investigation show estragole has antimicrobial and cytotoxic properties. ^[37]
Rosmarinic acid		His an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid commonly found in <i>Rosmarinus officinalis</i> , <i>Mellisa officinalis</i> , <i>Mentha piperita</i> , <i>Mentha spicata</i> , <i>Perovskia artemisoides</i> and other Labiatae and Boraginaceae family plants. This compound has anti-inflammatory, antiallergic, antidepressant, antimicrobial, antihyperglycemic, antimicrobial, antidiabetic and antiviral properties. ^[38-40]
Apigenin		It is a natural plant flavone commonly present in <i>Achillea millefolium</i> , <i>Apium graveolens</i> , <i>Artemisia dracunculus</i> , <i>Camellia sinensis</i> , <i>Chamaemelum nobile</i> , <i>Coriandrum sativum</i> , <i>Digitalis purpurea</i> , <i>Echinacea spp</i> , <i>Ginkgo biloba</i> , <i>Glycyrrhiza glabra</i> , <i>Linum usitatissimum</i> , <i>Marrubium vulgare</i> , <i>Matricaria retcutita</i> , <i>Mentha spicata</i> , <i>Ocimum basilicum</i> and <i>Origanum vulgare</i> . Apigenin has anti-inflammatory, anti-oxidant and anti-carcinogenic properties. ^[41,42]
Cirsimaritin		Cirsimaritin is a flavonoid of <i>Microtea debilis</i> , <i>Stizolophus balsamita</i> , <i>Artemisia judaica</i> , <i>Salvia officinalis</i> and <i>Ocimum sanctum</i> . ^[17,43-45] Preclinical experiments cirsimaritin showed presence of adenosine antagonistic property, which may be helpful in management of acute renal failure. ^[47]

O. tenuiflorum=*Ocimum tenuiflorum*

respectively in the period between March and May 2014. The input canonical SMILES format of ursolic acid, eugenol, carvacrol, linalol, caryophylline, estragole, rosmarinic acid, apigenin and cirsimaritin were used for prediction of biological activity spectra and toxicity profile [Table 2].

Prediction of biological activity spectra

The PASS internet tools were used for prediction of biological activity of constituents of *O. tenuiflorum*. The software provided Pa and Pi ratio (active and inactive ratio) in Pa > 30%, Pa > 50% and Pa > 70% levels.

Prediction of toxicity profile

The toxicity of the constituents of *O. tenuiflorum* was predicted with the help of online lazarus toxicity prediction tools. The lazarus online toxicity prediction calculated the measured activity based on the comparison of new structure

with similar existing structure available in the database. Lazar prediction indicated the active groups in red color and inactive groups in green colour with confidence index.

RESULTS

Acute oral toxicity studies

Hydroalcoholic extract of *O. tenuiflorum* did not show any mortality up to 2000 mg/kg when given as single oral administration. Hence, the study was carried out at the dose levels of 125, 250 and 500 mg/kg BW.

Effect of hydroalcoholic extract of *Ocimum tenuiflorum* on blood glucose in streptozotocin and nicotimanide induced diabetic rats

Throughout the study, a significant reduction in BW was observed with diabetic control and *O. tenuiflorum* 125 mg/

kg treated animals when compared with that of control animals. However, the diabetes mellitus induced BW reduction was inhibited by the glibenclamide and dose of *O. tenuiflorum* at 250 and 500 mg/kg [Figure 1]. The increase in blood glucose level was observed with diabetic control animals and *O. tenuiflorum* 125 mg/kg treated animals when compared with that of control animals. The mean blood glucose level in the diabetic control group on day 0 was 227.20 ± 8.55 mg/dl and on day 21 was 284.40 ± 6.82 mg/dl. Whereas significant reduction in blood glucose level was observed with glibenclamide and *O. tenuiflorum* 250 and 500 mg/kg treated animals when compared with that of diabetic control animals. The effects of *O. tenuiflorum* 250 and 500 mg/kg on STZ- and NIC- induced diabetic in rats were variable, but the efficacy of *O. tenuiflorum* 500 mg/kg is comparatively better than *O. tenuiflorum* 250 mg/kg at the end of the study. The effect of *O. tenuiflorum* on blood glucose in STZ- and NIC- induced diabetic rats were summarized in Table 3.

Effect of hydroalcoholic extract of *Ocimum tenuiflorum* on lipid profile in streptozotocin and nicotimanide-induced diabetic rats

Diabetic control and *O. tenuiflorum* 125 mg/kg treated animals showed significant increase of total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol levels, and significant reduction in HDL level and HDL ratio when compared with that of control animals. Whereas significant reduction in serum total cholesterol, triglyceride, LDL cholesterol, VLDL cholesterol levels and significant

increase in HDL ratio were observed with glibenclamide and dose of *O. tenuiflorum* at 250 and 500 mg/kg treated animals when compared with that of diabetic control animals. The effect of *O. tenuiflorum* on lipid profile in STZ- and NIC- induced diabetic rats were summarized in Table 4.

Effect of hydroalcoholic extract of *Ocimum tenuiflorum* on biochemical parameters in streptozotocin and nicotimanide induced diabetic rats

Diabetic animals and *O. tenuiflorum* 125 mg/kg treated animals showed significant increase in SGOT, SGPT, creatinine and urea levels and significant reduction in total protein, albumin and albumin: creatinine ratio when compared with that of control animals, whereas glibenclamide and *O. tenuiflorum* 250 and 500 mg/kg treated animals reversed the effect of STZ and NIC on biochemical parameters to normal levels. Effects of glibenclamide and *O. tenuiflorum* on the liver and renal markers of diabetic animals were presented in Table 5.

Effect of hydroalcoholic extract of *Ocimum tenuiflorum* on organ weight in streptozotocin and nicotimanide induced diabetic rats

At the end of the study, no significant changes in relative organ weight and kidney: BW ratio were observed with experimental animal groups, but only a significant increase on absolute organ weight of liver was observed when compared with that of control animals. Effects of glibenclamide and *O. tenuiflorum* on the absolute and relative

Table 2: Canonical SMILES format of phytoconstituents of *O. tenuiflorum*

Phytoconstituents	Molecular formula	Canonical SMILES format
Ursolic acid (CID 64945)	$C_{30}H_{48}O_3$	CC1CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1C)C)C(=O)O
Eugenol (CID 3314)	$C_{10}H_{12}O_2$	COC1=C(C=CC(=C1)CC=C)O
Carvacrol (CID 10364)	$C_{10}H_{14}O$	CC1=C(C=C(C=C1)C(C)C)O
Linalool (CID 6549)	$C_{10}H_{18}O$	CC(=CCCC(C)(C=C)O)C
Caryophyllene (CID 5281515)	$C_{15}H_{24}$	CC1=CCCC(=C)C2CC(C2CC1)(C)C
Estragole (CID 8815)	$C_{10}H_{12}O$	COC1=CC=C(C=C1)CC=C
Rosmarinic acid (CID 5315615)	$C_{18}H_{16}O_8$	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O
Apigenin (CID 5280704)	$C_{15}H_{10}O_5$	C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O
Cirsimaritin (CID 188323)	$C_{17}H_{14}O_6$	COC1=C(C(=C2C(=C1)OC(=CC2=O)C3=CC=C(C=C3)O)O)OC

SMILES=Simplified molecular-input line-entry system; *O. tenuiflorum*=*Ocimum tenuiflorum*

Table 3: The effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on fasting blood glucose levels (mg/dl) in STZ- and NIC- induced diabetic rats

Treatment	Blood glucose level in mg/dl			
	0 (prestudy) day	7 th day	14 th day	21 st day
Control	90.60±4.07	87.60±4.40	93.40±3.63	90.80±4.03
Diabetic control	227.20±8.55	257.20±12.83	272.40±7.57	284.40±6.82
Glibenclamide (0.25 mg/kg)	226.40±8.33	176.80±10.44**	138.40±7.88***	112.80±5.75***
<i>O. tenuiflorum</i> (125 mg/kg)	228.20±8.58	220.80±6.28	194.00±10.47	198.00±10.45
<i>O. tenuiflorum</i> (250 mg/kg)	224.60±8.49	190.80±7.00*	160.00±6.93***	150.00±3.90***
<i>O. tenuiflorum</i> (500 mg/kg)	229.80±10.00	170.00±11.21*	160.80±16.41*	129.00±13.20***

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean±SEM (n=5). *P<0.05, **P<0.01, ***P<0.001 compare to prestudy day, One-way ANOVA followed by Bonferroni post-hoc test. SEM=Standard error of mean; STZ=Streptozotocin; NIC=Nicotimanide; *O. tenuiflorum*=*Ocimum tenuiflorum*

organ weight of liver and kidney of diabetic animals were summarized in Table 6.

Prediction of biological activity and toxicity profile of constituents of *Ocimum tenuiflorum*

The biological activity prediction at 70% levels showed various biological actions which were summarized in Table 7 and some activities are scientifically proven for *O. tenuiflorum* phytoconstituents. Most of the *O. tenuiflorum* phytoconstituents showed anti-inflammatory and anti-diabetic activities at various Pa: Pi levels. Toxicity

prediction of the phytoconstituents of *O. tenuiflorum* did not show any major toxicity, carcinogenicity and mutagenicity [Table 8].

DISCUSSION

In the present study, an anti-diabetic and hyperlipidemic effects of *O. tenuiflorum* was studied against chemical (STZ- and NIC-) induced diabetes mellitus model. STZ is a glucosamine-nitrosourea derived from *Streptomyces achromogenes* (Gram-positive bacterium) and, it is used for the treatment of pancreatic beta cell carcinoma and to induce diabetes mellitus in rodents.^[10] NIC was administered, followed by STZ injection to produce stable, moderate hyperglycemia and to prevent early inhibition of beta cell function by STZ, which may be helpful to reduce/prevent the incidence of diabetic coma caused by STZ.^[48] STZ causes hyperglycemia after 2 h of injection, hypoglycemia in 6 h and finally hyperglycemia by decreasing the insulin levels through the inhibition/ destruction of pancreatic beta cell function.^[9,49]

The hydroalcoholic extract of *O. tenuiflorum* exhibited significant anti-diabetic and anti-hyperlipidemic activities against STZ- and NIC- induced diabetic rats at the dose levels of 250 and 500 mg/kg BW. The effect was comparable with glibenclamide but not superior to it. At the end of this study, glibenclamide reduced the glucose

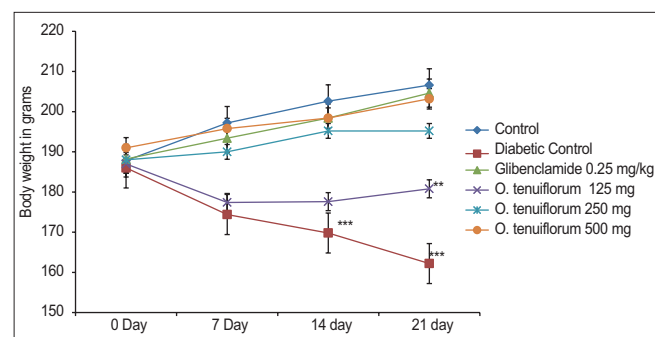


Figure 1: The effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on body weight in streptozotocin- and nicotimanide induced diabetic rats (*O. tenuiflorum*: Hydroalcoholic extract of leaves of *O. tenuiflorum*. All the values are mean $\pm$ standard error of the mean [$N = 5$]. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ when compared with control; one-way analysis of variance, followed by Bonferroni post-hoc test) *O. tenuiflorum* = *Ocimum tenuiflorum*

Table 4: Effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on lipid profile in STZ induced diabetes

Treatment	TCs (mg/dl)	TG (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	HDL ratio
Control	91.60 $\pm$ 4.45	66.20 $\pm$ 4.57	23.60 $\pm$ 3.31	52.36 $\pm$ 4.89	10.47 $\pm$ 0.98	39.61 $\pm$ 11.82
Diabetic control	178.60 $\pm$ 8.55***	154.40 $\pm$ 10.87***	13.20 $\pm$ 2.42*	181.35 $\pm$ 11.94***	36.27 $\pm$ 2.39***	8.00 $\pm$ 1.40**
Glibenclamide (0.25 mg/kg)	98.00 $\pm$ 6.32 ^{sss}	74.40 $\pm$ 4.12 ^{sss}	20.00 $\pm$ 2.00	65.33 $\pm$ 5.86 ^{sss}	13.07 $\pm$ 1.17 ^{sss}	25.74 $\pm$ 2.29
<i>O. tenuiflorum</i> (125 mg/kg)	166.00 $\pm$ 9.12***	134.80 $\pm$ 8.80***	10.80 $\pm$ 1.62**	162.62 $\pm$ 7.35***	32.52 $\pm$ 1.47***	7.12 $\pm$ 1.24**
<i>O. tenuiflorum</i> (250 mg/kg)	138.00 $\pm$ 10.08 ^s	100.00 $\pm$ 4.38 ^{sss}	17.80 $\pm$ 1.28	114.42 $\pm$ 7.94 ^{sss}	22.88 $\pm$ 1.59 ^{sss}	15.54 $\pm$ 2.34
<i>O. tenuiflorum</i> (500 mg/kg)	101.20 $\pm$ 6.28 ^{sss}	77.20 $\pm$ 4.50 ^{sss}	22.80 $\pm$ 1.85	66.95 $\pm$ 7.12 ^{sss}	13.39 $\pm$ 1.42 ^{sss}	29.88 $\pm$ 3.37

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean $\pm$ SEM ($n=5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to control; * $P < 0.05$, ** $P < 0.01$ compare to diabetic control, One-way ANOVA followed by Bonferroni post-hoc test. SEM=Standard error of mean; *O. tenuiflorum*=*Ocimum tenuiflorum* STZ=Streptozotocin; TG=Triglyceride; TC=Total cholesterol; HDL=High-density lipoprotein; LDL=Low-density lipoprotein; VLDL=Very-low-density lipoprotein

Table 5: The effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on biochemical parameters in STZ- and NIC- induced diabetic rats

Treatment	SGOT (IU/l)	SGPT (IU/l)	Total protein (g/dl)	Albumin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Serum albumin: Creatinine ratio (g/mg)
Control	123.60 $\pm$ 7.42	48.60 $\pm$ 5.03	7.58 $\pm$ 0.29	4.80 $\pm$ 0.29	0.76 $\pm$ 0.02	22.40 $\pm$ 1.63	6.36 $\pm$ 0.48
Diabetic control	165.20 $\pm$ 7.22**	116.60 $\pm$ 6.98***	4.38 $\pm$ 0.62**	2.80 $\pm$ 0.21**	1.38 $\pm$ 0.06***	52.20 $\pm$ 4.00***	2.03 $\pm$ 0.15***
Glibenclamide (0.25 mg/kg)	131.40 $\pm$ 5.38	50.40 $\pm$ 5.54 ^{sss}	8.32 $\pm$ 0.76 ^{sss}	4.68 $\pm$ 0.34 ^{ss}	1.18 $\pm$ 0.09*	28.80 $\pm$ 2.71 ^{ss}	4.04 $\pm$ 0.42
<i>Ocimum tenuiflorum</i> (125 mg/kg)	157.20 $\pm$ 7.42	110.40 $\pm$ 10.01***	5.62 $\pm$ 0.57	2.88 $\pm$ 0.30**	1.32 $\pm$ 0.08***	44.40 $\pm$ 6.49**	2.25 $\pm$ 0.34***
<i>Ocimum tenuiflorum</i> (250 mg/kg)	132.40 $\pm$ 7.09	59.20 $\pm$ 6.58 ^{sss}	7.12 $\pm$ 0.42 ^s	4.34 $\pm$ 0.35 ^s	0.98 $\pm$ 0.10 ^s	29.20 $\pm$ 3.62 ^{ss}	4.48 $\pm$ 0.21 ^s
<i>Ocimum tenuiflorum</i> (500 mg/kg)	117.00 $\pm$ 9.55 ^{ss}	42.40 $\pm$ 4.08 ^{sss}	8.96 $\pm$ 0.53 ^{sss}	4.98 $\pm$ 0.31 ^{sss}	0.80 $\pm$ 0.09 ^{sss}	23.60 $\pm$ 2.44 ^{sss}	6.68 $\pm$ 1.00 ^{sss}

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean $\pm$ SEM ($n=5$). ** $P < 0.01$, *** $P < 0.001$ as compared to control; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compare to diabetic control, One-way ANOVA followed by Bonferroni post-hoc test. SEM=Standard error of mean; STZ=Streptozotocin; NIC=Nicotinamide; SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamic pyruvate transaminase; *O. tenuiflorum*=*Ocimum tenuiflorum*

Table 6: The effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on relative, absolute organ weight and kidney weight: Body ratio in STZ– and NIC– induced diabetic rats

Treatment	Relative organ weight		Absolute organ weight		Kidney weight: Body weight ratio (mg/g)
	Liver (g)	Kidney (g)	Liver (g)	Kidney (g)	
Control	6.20±0.26	0.84±0.02	3.00±0.10	0.41±0.01	4.09±0.13
Diabetic control	5.76±0.13	0.72±0.03	3.56±0.11*	0.44±0.01	4.41±0.15
Glibenclamide (0.25 mg/kg)	6.12±0.12	0.80±0.03	3.00±0.12	0.39±0.02	3.94±0.18
<i>O. tenuiflorum</i> (125 mg/kg)	5.82±0.21	0.74±0.02	3.23±0.16	0.41±0.02	4.09±0.16
<i>O. tenuiflorum</i> (250 mg/kg)	6.24±0.10	0.82±0.03	3.20±0.04	0.42±0.01	4.18±0.10
<i>O. tenuiflorum</i> (500 mg/kg)	6.30±0.13	0.81±0.04	3.10±0.06	0.40±0.03	4.01±0.26

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean±SEM (n=5). *P<0.05 as compared to control; One-way ANOVA followed by Bonferroni post-hoc test. SEM=Standard error of mean; STZ=Streptozotocin; NIC=Nicotinamide; *O. tenuiflorum*=*Ocimum tenuiflorum*

Table 7: Predicted biological activity of phytoconstituents of *O. tenuiflorum*

Compound	Selective predicted activity with (Pa: Pi)
Ursolic acid	Insulin promoting, hepatoprotecting, chemoprotecting, antiprotozoal, hypoglycemic, anti-inflammatory, wound healing, antiulcer, nitric oxide antagonistic, antinociceptive and analeptic properties.
Eugenol	Antimutagenic, mucomembranous protecting, beta-adrenergic receptor kinase inhibiting, general anesthetic, cardiovascular and analeptic properties.
Carvacrol	Antiseptic, mucomembranous protecting, membrane permeability inhibiting, anti-infective, anthelmintic, beta-adrenergic receptor kinase inhibiting properties and used in phobic disorders treatment.
Linalool	Mucomembranous protector, fatty-acyl-coenzyme synthase inhibitor, beta-adrenergic receptor kinase inhibitor, G-protein-coupled receptor kinase inhibitor, lipid metabolism regulator, antisecretoric, anti-inflammatory, gastrin inhibitor, membrane permeability inhibitor, sugar-phosphatase inhibitor and antiviral properties.
Caryophyllene	Antineoplastic, antieczematic, antineoplastic, anti-inflammatory and antipsoriatic properties.
Estragole	Gluconate 2-dehydrogenase inhibitor, beta-adrenergic receptor kinase inhibitor, G-protein-coupled receptor kinase inhibitor, mucomembranous protector, antimutagenic, general anesthetic, fatty-acyl-coenzyme synthase inhibitor, saccharopepsin inhibitor, polyporopepsin inhibitor, nicotinic receptor antagonist and membrane permeability inhibitor properties.
Rosmarinic acid	Antidiabetic, membrane permeability inhibitor, mucomembranous protector, free radical scavenger and lipid peroxidase inhibitor properties.
Apigenin	Membrane permeability inhibitor, NADP+ inhibitor, aldehyde oxidase inhibitor, anaphylatoxin receptor antagonist, vasoprotector, antihemorrhagic, leukotriene-B420-monooxygenase inhibitor, histamine release inhibitor, mucomembranous protector, antineoplastic, alcohol dehydrogenase inhibitor, free radical scavenger, thioredoxin inhibitor and sugar-phosphatase inhibitor properties.
Cirsimaritin	Membrane permeability inhibitor, anaphylatoxin receptor antagonist, apoptosis agonist, peroxidase inhibitor, vasoprotector, antineoplastic NADP+inhibitor, cytoprotectant, free radical scavenger, lipid peroxidase inhibitor, antineoplastic and histamine release inhibitor properties.

The predicted activities are listed based on descending order of its Pa: Pi ratio at 70% levels. NADP=Dehydrogenase; *O. tenuiflorum*=*Ocimum tenuiflorum*

levels from 226.40 ± 8.33 to 112.80 ± 5.75 , whereas *O. tenuiflorum* 500 mg/kg reduced the glucose levels from 229.80 ± 10.00 to 129.00 ± 13.20 . *O. tenuiflorum* exhibited significant anti-diabetic effect but the effect was not superior than glibenclamide. This may be due to the amount of active phytoconstituents present in the plant. However, the individual phytoconstituents of *O. tenuiflorum* such as ursolic acid (derivatives) and rosmarinic acid are known to have anti-diabetic activities.^[40,50]

Severe hyperlipidemia was observed with STZ– and NIC– induced diabetic animals, and this may be due to exogenous fat loading, an abnormal increase in small intestinal acyl-coenzyme A: Cholesterol acyltransferase activity and enhancement of intestinal CoA-dependent esterification.^[51,52] Both glibenclamide and *O. tenuiflorum* (at 250 and 500 mg/kg) reversed the STZ– and NIC– induced hyperlipidemia. However, the exact mechanism of action of anti-hyperlipidemic effect of *O. tenuiflorum* is unclear.

In diabetes mellitus control animals, liver and renal dysfunctions were observed. The increase in aminotransferase level may be due to the destruction of hepatocytes caused by STZ.^[53] Decrease in serum albumin levels was observed in diabetes mellitus animals and this may be due to deterioration of kidney function.^[54] Park *et al.* also reported that decreased levels of albumin in peripheral blood of STZ-induced diabetic rats.^[55] Alteration in serum albumin: creatinine ratio was observed in diabetes mellitus control animal and *O. tenuiflorum* 150 mg/kg treated animals, and this may be due to the alteration in renal functions.

The increased absolute organ weight of liver was observed in diabetic animals, and this may be due to cellular damage in the liver because of increasing resistance to insulin signaling pathways in hepatocytes.^[56] There was increased kidney weight: BW ratio (results were not significant) found in diabetes mellitus animals. This may be due to glomerular damage, changes in bradykinin system and increased

Table 8: Predicted toxicological properties of phytoconstituents of *O. tenuiflorum*

Compound	Prediction (confidence)				
	EPA v4b Fathead Minnow acute toxicity LC ₅₀ _mmol (fist lethality)	Carcinogenic potency in DBS hamster	Carcinogenic potency in DBS mouse	Kazius-Bursi Salmonella mutagenicity	FDA v3b maximum recommended daily dose_mmol
Ursolic acid	Not predicted	Noncarcinogen	Carcinogen (0.699)	Nonmutagenic	0.0050 (0.276)
Eugenol	0.1411 (0.381)	Noncarcinogen	Noncarcinogen	Nonmutagenic	0.0092 (0.212)
Carvacrol	0.0145 (0.332)	Noncarcinogen	Noncarcinogen	Nonmutagenic	0.0231 (0.156)
Linalool	0.04988 (0.129)	Noncarcinogen	Noncarcinogen	Nonmutagenic	0.1118 (0.129)
Caryophyllene	0.01247 (0.106)	Noncarcinogen	Noncarcinogen	Nonmutagenic	0.00645 (0.205)
Estragole	0.075259 (0.431)	Noncarcinogen	Carcinogen (measured activity)	Nonmutagenic	0.020585 (0.218)
Rosmarinic acid	0.00436 (0.173)	Noncarcinogen	Noncarcinogen	Nonmutagenic	0.012936 (0.109)
Apigenin	0.005571 (0.235)	Noncarcinogen	Noncarcinogen	Nonmutagenic	Not predicted
Cirsimaritin	0.006595 (0.23)	Noncarcinogen	Noncarcinogen	Mutagenic (0.0235)	Not predicted

DBS=Deep brain stimulation; FDA=Food and Drug Administration; EPA=Environmental Protection Agency; *O. tenuiflorum*=*Ocimum tenuiflorum*

gene expression of fibronectin and collagen I.^[57,58] *In vivo* study revealed that hydroalcoholic extract of *O. tenuiflorum* possesses the anti-diabetic and anti-hyperlipidemic activities but not superior to it., but the effect was not dose dependently. This may be due to the time of collection of the plant parts, and the amount of phytoconstituents present in the plant.

Ocimum tenuiflorum is known to have many pharmacological activities and it is traditionally used as an anti-tussive agent. In this present investigation, we predicted the biological activities of phytoconstituents of *O. tenuiflorum* which indicated pharmacological actions as insulin promotor activity, anti-oxidant activity, free radical scavenging property, anti-neoplastic effect, hypolipidemic effect, etc., This plant is known to have anti-diabetic, cardioprotective, wound healing, anti-oxidant, hypolipidemic, anti-microbial, gastroprotective, immunomodulatory, anti-nociceptive and anti-cancer effects.^[17] The whole plant may have different pharmacological effects at different doses, due to the variation in phytoconstituents and plant geographical location. Some of the individual phytoconstituents of *O. tenuiflorum* have anti-diabetic, anti-microbial, anti-cancer, gastroprotective, mucoprotective effects. Many of the listed predicted activities for the various phytoconstituents are under investigation. Hence further *in silico*, *in vitro* and *in vivo* pharmacological studies on *O. tenuiflorum* phytoconstituents may give new lead to the biomedical researchers.

CONCLUSION

Hydroalcoholic extract of leaves of *O. tenuiflorum* has significant anti-diabetic and anti-hyperlipidemic activities at 250 and 500 mg/kg BW against STZ and NIC – induced diabetes mellitus in rats. The anti-diabetic effect of hydroalcoholic extract of leaves of *O. tenuiflorum* is not dose dependent. The biological activity prediction of phytoconstituents of *O. tenuiflorum* showed “n” of biological

activities which include anti-diabetic and anti-hyperlipidemic properties at 70% Pa: Pi level and toxicological effect prediction did not show any major harmful effects. Further studies are required to confirm the anti-diabetic and anti-hyperlipidemic activities of individual phytoconstituents of *O. tenuiflorum*, which showed the mentioned properties in computer aided prediction.

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