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

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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 lazar 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. 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]

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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 nicotimanide (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]×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 ± 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.

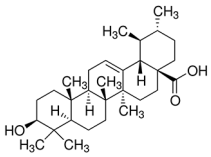
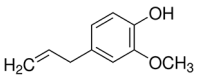
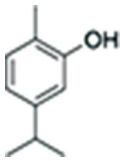
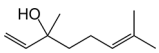
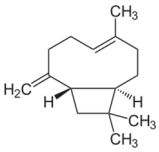
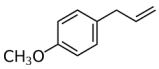
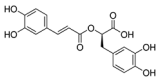
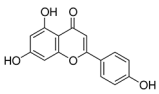
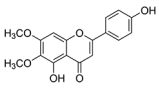
Photochemistry

The leaves of the Tulsi contain essential oils such as benzaldehyde, borneol, n-butylbenzoate, caryophyllene oxide, carvacrol, cis-α-terpineol, cubenol, eugenol, frualdeheyde, limonene, linalool, methyl carvicol (estragol: 1-allyl-4-methoxybenzene), ocimene, oleic acid, sebinene, phytol, veidifloro, β-pinene, α-thujjene, methyl chavicol and ursolic acid. The seeds of the Tulsi contain fixed oils such as oinoleic acid, oleic acid, palmitic acid, and stearic acid.[1,16] *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 vicenin, 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, caryphylline, 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 lazar toxicity prediction tools (http://lazar.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 oleanoic acid and pentacyclic triterpenoid carboxylic acid, present in *Calluna vulgaris*, *Eribotrya japonica, Eucaluputs hybrid, Glechoma hederacea, Lelaleuca lecadendron, Ocimum sanctum, Rosmarinus officinalis, Pyrola rotundifolia, Psychotria serpens, Sambucus chinesis, Solanum incanum and Tripterospermum taiwanense*. 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 *Syzygium aromaticum* (clove) and widely used as anesthetic and analgesic in dentistry.[22]
Carvacrol		Carvacrol is a monoterpenoid phenol isolated form the plant parts of *Shigella sonnei, Shigella flexneri, Origanum vulgare* and *Ocimum sanctum*.[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 is *Aniba rosaeodora*, *Cinamomon camphora* and *Ocimun sanctum*.[27] Commonly reported pharmacological actions of linalool are antileishmanial, anticonvulsant and anti-infl ammatory properties.[28,29]
Caryophyllene		Caryophyllene is a natural bicyclic sesquiterpene isolated from *Didymocarpus tomentosa, Syzygium aromaticum, Cannabis sativa* and *Ocimum sanctum*.[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 *Foeniculum vulgare,*[34] *Ocimum basilicum,*[35] *Agastache rugosa*[36] and *Clausena suffruticosa*.[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 *Rosmarinus officinalis, Mellisa officinalis, Mentha piperita, Mentha spicata, Perovskia artemisoides* and other Labiatae and Boraginaceae family plants. This compound has anti-inflammatory, antiallergic, antidepressant, antimicrobial, antihyperglcemic, antimicrobial, antidiabetic and antiviral properties.[38-40]
Apigenin		It is a natural plant flavone commonly present in *Achillea millefolium, Apium graveolens*, *Artemisia dracunculus, Camellia sinensis, Chamaemelum nobile, Coriandrum sativum, Digitalis purpurea, Echinacea spp, Gingko biloba, Glycyrrhiza glabra, Linum usitatissimum, Marrubium vulgare, Matricaria retcutita, Mentha spicata, Ocimum basilicum* and *Origanum vulgare*. Apigenin has anti-inflammatory, anti-oxidant and anti-carcinogenic properties.[41,42]
Cirsimaritin		Cirsimaritin is a flavonoid of *Microtea debilis, Stizolophus balsamita, Artemisia judaica, Salvia officinalis* and *Ocimum sanctum*.[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, caryphylline, 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 lazar toxicity prediction tools. The lazar 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 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	7th day	14th day	21st 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***
O. tenuiflorum (125 mg/kg)	228.20±8.58	220.80±6.28	194.00±10.47	198.00±10.45
O. tenuiflorum (250 mg/kg)	224.60±8.49	190.80±7.00*	160.00±6.93***	150.00±3.90***
O. tenuiflorum (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].

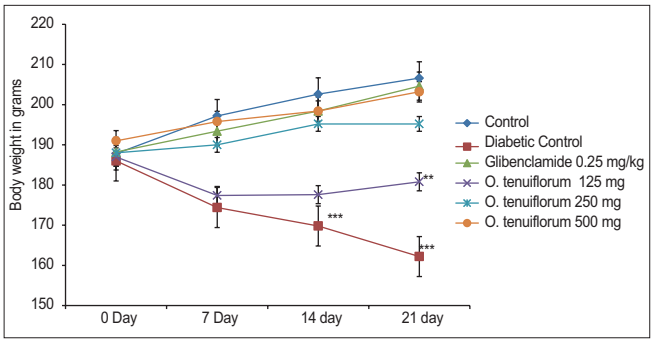


Figure 1: The effect of hydroalcoholic extract of leaves of *O. tenuiflorum* on body weight in streptozotocin-and nicotimanide induced diabetic rats (*O. tenuiflorum*: Hydroalcoholic extract of leaves of *O. tenuiflorum*. All the values are mean ± standard error of the mean [$N = 5$]. $^{*}P < 0.05$,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

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

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

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean±SEM (n=5). *P<0.05, **P<0.01, ***P<0.001as 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±7.42	48.60±5.03	7.58±0.29	4.80±0.29	0.76±0.02	22.40±1.63	6.36±0.48
Diabetic control	165.20±7.22**	116.60±6.98***	4.38±0.62**	2.80±0.21**	1.38±0.06***	52.20±4.00***	2.03±0.15***
Glibenclamide (0.25 mg/kg)	131.40±5.38	50.40±5.54$^{\$\$\$}$	8.32±0.76$^{\$\$\$}$	4.68±0.34$^{\$\$}$	1.18±0.09*	28.80±2.71$^{\$\$}$	4.04±0.42
Ocimum tenuiflorum (125 mg/kg)	157.20±7.42	110.40±10.01***	5.62±0.57	2.88±0.30**	1.32±0.08***	44.40±6.49**	2.25±0.34***
Ocimum tenuiflorum (250 mg/kg)	132.40±7.09	59.20±6.58$^{\$\$\$}$	7.12±0.42$^{\$}$	4.34±0.35$^{\$}$	0.98±0.10$^{\$}$	29.20±3.62$^{\$\$}$	4.48±0.21$^{\$}$
Ocimum tenuiflorum (500 mg/kg)	117.00±9.55$^{\$\$}$	42.40±4.08$^{\$\$\$}$	8.96±0.53$^{\$\$\$}$	4.98±0.31$^{\$\$\$}$	0.80±0.09$^{\$\$\$}$	23.60±2.44$^{\$\$\$}$	6.68±1.00$^{\$\$\$}$

Ocimum tenuiflorum: Hydroalcoholic extract of leaves of *Ocimum tenuiflorum*. All the values are mean±SEM (n=5). **P<0.01, ***P<0.001 as compared to control; $^{\$}P$<0.05, $^{\$\$}P$<0.01, $^{\$\$\$}P$<0.01 compare to diabetic control, One-way ANOVA followed by Bonferroni *post-hoc* test. SEM=Standard error of mean; STZ=Streptozotocin; NIC=Nicotimanide; 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
O. tenuiflorum (125 mg/kg)	5.82±0.21	0.74±0.02	3.23±0.16	0.41±0.02	4.09±0.16
O. tenuiflorum (250 mg/kg)	6.24±0.10	0.82±0.03	3.20±0.04	0.42±0.01	4.18±0.10
O. tenuiflorum (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=Nicotimanide; *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=Dihydrouracil 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 form 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.

REFERENCES

1. Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review. Indian J Physiol Pharmacol 2005;49:125-31.
2. Sharma MP, Ahmad J, Hussain A, Khan S. Folklore medicinal plants of Mewat (Gurgaon District), Haryana, India. Pharm Biol 1992;30:129-34.
3. Narwal S, Rana AC, Tiwari V, Gangwani S, Sharma R. Review on chemical constituents and pharmacological action of *Ocimum kilimandscharicum*. Indo Glob J Pharm Sci 2011;1:287-93.
4. Rao SA, Vijay Y, Deepthi T, Lakshmi CS, Rani V, Rani S, *et al*. Antidiabetic effect of ethanolic extract of leaves of *Ocimum sanctum* in alloxan induced diabetes in rats. Int J Basic Clin Pharmacol 2013;2:613-6.
5. Suanarunsawat T, Boonnak T, Na Ayutthaya WD, Thirawarapan S. Anti-hyperlipidemic and cardioprotective effects of *Ocimum sanctum* L. fixed oil in rats fed a high fat diet. J Basic Clin Physiol Pharmacol 2010;21:387-400.
6. Devendran G, Balasubramanian U. Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves. Asian J Plant Sci 2011;1:44-8.
7. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian J Exp Biol 2002;40:765-73.
8. Petchi RR, Vijaya C, Parasuraman S. Antidiabetic activity of polyherbal formulation in streptozotocin – Nicotinamide induced diabetic Wistar rats. J Tradit Complement Med 2014;4:108-17.
9. Rabbani SI, Devi K, Khanam S. Protective role of glibenclamide against nicotinamide-streptozotocin induced nuclear damage in diabetic Wistar rats. J Pharmacol Pharmacother 2010;1:18-23.
10. Petchi RR, Parasuraman S, Vijaya C. Antidiabetic and antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Linn.) in streptozotocin-induced

diabetic rats. J Basic Clin Pharm 2013;4:88-92.

11. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother 2010;1:87-93.
12. Ahmadi SA, Boroumand MA, Gohari-Moghaddam K, Tajik P, Dibaj SM. The impact of low serum triglyceride on LDL-cholesterol estimation. Arch Iran Med 2008;11:318-21.
13. Egbuonu AC, Ezeanyika LU. L-arginine exposure improves renal function markers of metabolic syndrome in female rats Am J Biochem Mol Biol 2013;3:50-60.
14. Cachat F, Lange-Sperandio B, Chang AY, Kiley SC, Thornhill BA, Forbes MS, *et al.* Ureteral obstruction in neonatal mice elicits segment-specific tubular cell responses leading to nephron loss. Kidney Int 2003;63:564-75.
15. Warner EA, Herold AH. Interpreting laboraotory Report. In: Rakel RE, Rakel DP, editors. Textbook of Family Medicine. 8th ed. Philadelphia, PA: Elsevier Saunders; 2011. p. 194.
16. Triveni, Kumar K, Singh AK, Kumar R, Gupta V, Tripathi K. *Ocimum sanctum* Linn: A review on phytopharmacology and therapeutic potential of Tulsi. Int J Pharm Phytopharmacol Res 2013;3:148-51.
17. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev 2010;4:95-105.
18. Mali RG, Dhake AS. A review on herbal antiasthmatics. Orient Pharm Exp Med 2011;11:77-90.
19. Singh S, Taneja M, Majumdar DK. Biological activities of *Ocimum sanctum* L. fixed oil – an overview. Indian J Exp Biol 2007;45:403-12.
20. Fathiazad F, Matlobi A, Khorrami A, Hamedeyazdan S, Soraya H, Hammami M, *et al.* Phytochemical screening and evaluation of cardioprotective activity of ethanolic extract of *Ocimum basilicum* L. (basil) against isoproterenol induced myocardial infarction in rats. Daru 2012;20:87.
21. Liu J. Pharmacology of oleanolic acid and ursolic acid. J Ethnopharmacol 1995;49:57-68.
22. Pramod K, Ansari SH, Ali J. Eugenol: A natural compound with versatile pharmacological actions. Nat Prod Commun 2010;5:1999-2006.
23. Bagamboula CF, Uyttendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiol 2004;21:33-42.
24. De Falco E, Mancini E, Roscigno G, Mignola E, Taglialatela-Scafati O, Senatore F. Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. Molecules 2013;18:14948-60.
25. Suganthi RU, Manpal S. Biological and pharmacological of actions carvacrol and its effects on poultry: An updated review. World J Pharm Pharm Sci 2013;2:3581-95.
26. Gilling DH, Kitajima M, Torrey JR, Bright KR. Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. J Appl Microbiol 2014;116:1149-63.
27. do Socorro S Rosa Mdo S, Mendonça-Filho RR, Bizzo HR, de Almeida Rodrigues I, Soares RM, Souto-Padrón T, *et al.* Antileishmanial activity of a linalool-rich essential oil from *Croton cajucara*. Antimicrob Agents Chemother 2003;47:1895-901.
28. Elisabetsky E, Brum LF, Souza DO. Anticonvulsant properties of linalool in glutamate-related seizure models. Phytomedicine 1999;6:107-13.
29. Peana AT, D'Aquila PS, Panin F, Serra G, Pippia P, Moretti MD. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 2002;9:721-6.
30. Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, *et al.* Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A 2008;105:9099-104.
31. Gowda PJ, Ramakrishnaiah H, Krishna V, Narra S, Jagannath N. Caryophyllene-rich essential oil of *Didymocarpus tomentosa*: Chemical composition and cytotoxic activity. Nat Prod Commun 2012;7:1535-8.
32. Ghelardini C, Galeotti N, Di Cesare Mannelli L, Mazzanti G, Bartolini A. Local anaesthetic activity of beta-caryophyllene. Farmaco 2001;56:387-9.
33. Legault J, Pichette A. Potentiating effect of beta-caryophyllene on anticancer activity of alpha-humulene, isocaryophyllene and paclitaxel. J Pharm Pharmacol 2007;59:1643-7.
34. Afify AE, El-Beltagi HS, Hammama AA, Sidky MM, Mostafa OF. Distribution of trans-anethole and estragole in fennel (*Foeniculum vulgare* Mill) of callus induced from different seedling parts and fruits. Not Sci Biol 2011;3:79-86.
35. Hassanpouraghdam MB, Hassani A, Shalamzari MS. Menthone-and estragole-rich essential oil of cultivated *Ocimum basilicum* L. from Northwest Iran. Chemija 2010;21:59-62.
36. Li HQ, Liu QZ, Liu ZL, Du SS, Deng ZW. Chemical composition and nematicidal activity of essential oil of *Agastache rugosa* against *Meloidogyne incognita*. Molecules 2013;18:4170-80.
37. Rahman MA, Chakma JS, Bhuiyan NI, Islam MS. Composition of the essential oil of *Clausena suffruticosa* Leaf and evaluation of its antimicrobial and cytotoxic activities. Trop J Pharm Res 2012;11:739-46.
38. Shekarchi M, Hajimehdipoor H, Saeidnia S, Gohari AR, Hamedani MP. Comparative study of rosmarinic acid content in some plants of Labiatae family. Pharmacogn Mag 2012;8:37-41.
39. Petersen M, Simmonds MS. Rosmarinic acid. Phytochemistry 2003;62:121-5.
40. Hasanein P, Mohammad Zaheri L. Effects of rosmarinic acid on an experimental model of painful diabetic neuropathy in rats. Pharm Biol 2014;52:1398-402.
41. Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: Progress, potential and promise (review). Int J Oncol 2007;30:233-45.
42. Shukla S, Gupta S. Apigenin: A promising molecule for cancer prevention. Pharm Res 2010;27:962-78.
43. Kavvadias D, Monschein V, Sand P, Riederer P, Schreier P. Constituents of sage (*Salvia officinalis*) with *in vitro* affinity to human brain benzodiazepine receptor. Planta Med 2003;69:113-7.
44. Suleimenov EM, Raldugin VA, Adekenov SM. Cirsimaritin from *Stizolophus balsamita*. Chem Nat Compd 2008;44:398.
45. Abdalla SS, Abu Zarga MH. Effects of cirsimaritin, a flavone isolated from *Artemisia judaica*, on isolated guinea-pig ileum. Planta Med 1987;53:322-4.
46. Hasrat JA, De Bruyne T, De Backer JP, Vauquelin G, Vlietinck AJ. Cirsimarin and cirsimaritin, flavonoids of *Microtea debilis* (Phytolaccaceae) with adenosine antagonistic properties in rats: Leads for new therapeutics in acute renal failure. J Pharm Pharmacol 1997;49:1150-6.
47. Rahman S, Islam R, Kamruzzaman M, Alam K, Jamol AH. *Ocimun sanctum* L.: A review of phytochemical and pharmacological profile. Am J Drug Discov Devel 2011:1-15.
48. Lenzen S. Alloxan and Streptozotocin Diabetes Mellitus. Available from: http://www.saw-leipzig.de/forschung/projekte/zeitstrukturen-endokriner-systeme/endokrinologieiii/endo_07-lenzen.pdf. [Last accessed on 2014 Aug 06].
49. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.

50. Wu PP, Zhang K, Lu YJ, He P, Zhao SQ. *In vitro* and *in vivo* evaluation of the antidiabetic activity of ursolic acid derivatives. Eur J Med Chem 2014;80:502-8.
51. Kusunoki J, Aragane K, Kitamine T, Kozono H, Kano K, Fujinami K, *et al.* Postprandial hyperlipidemia in streptozotocin-induced diabetic rats is due to abnormal increase in intestinal acyl coenzyme A: Cholesterol acyltransferase activity. Arterioscler Thromb Vasc Biol 2000;20:171-8.
52. Jiao S, Matsuzawa Y, Matsubara K, Kihara S, Nakamura T, Tokunaga K, *et al.* Increased activity of intestinal acyl-CoA: Cholesterol acyltransferase in rats with streptozocin-induced diabetes and restoration by insulin supplementation. Diabetes 1988;37:342-6.
53. Zafar M, Naqvi SN, Ahmed M, Kaimkhani ZA. Altered liver morphology and enzymes in streptozotocin induced diabetic rats. Int J Morphol 2009;27:719-25.
54. Viswanathan V, Snehalatha C, Kumutha R, Jayaraman M, Ramachandran A. Serum albumin levels in different stages of type 2 diabetic nephropathy patients. Indian J Nephrol 2004;14:89-92.
55. Park KT, Yun CH, Bae CS, Ahn T. Decreased level of albumin in peripheral blood mononuclear cells of streptozotocin-induced diabetic rats. J Vet Med Sci 2014;76:1087-92.
56. Kohl T, Gehrke N, Schad A, Nagel M, Wörns MA, Sprinzl MF, *et al.* Diabetic liver injury from streptozotocin is regulated through the caspase-8 homolog cFLIP involving activation of JNK2 and intrahepatic immunocompetent cells. Cell Death Dis 2013;4:e712.
57. Ma G, Allen TJ, Cooper ME, Cao Z. Calcium channel blockers, either amlodipine or mibefradil, ameliorate renal injury in experimental diabetes. Kidney Int 2004;66:1090-8.
58. Kakoki M, Takahashi N, Jennette JC, Smithies O. Diabetic nephropathy is markedly enhanced in mice lacking the bradykinin B2 receptor. Proc Natl Acad Sci U S A 2004;101:13302-5.

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