HOSTED BY
ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.10.002

Hypoglycemic and anti-hyperglycemic study of *Ocimum tenuiflorum* L. leaves extract in normal and streptozotocin-induced diabetic rats

Leila Mousavi[1], Rabeta Mohd Salleh[1*], Vikneswaran Murugaiyah[2], Mohd Zaini Asmawi[2]

[1]*Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*

[2]*Discipline of Pharmacology, School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*

ARTICLE INFO

Article history:
Received 6 Feb 2016
Received in revised form 1 Mar, 2nd revised form 11 Mar, 3rd revised form 16 Mar, 4th revised form 24 Mar 2016
Accepted 10 Aug 2016
Available online 15 Oct 2016

Keywords:
Ocimum tenuiflorum L.
Streptozotocin-induced diabetes
Antidiabetic
Fasting blood glucose
Subcutaneous glucose tolerance test
Phytochemical analysis

ABSTRACT

Objective: To investigate the antidiabetic activity of *Ocimum tenuiflorum* L. (*O. tenuiflorum*) leaves used in the traditional medicine management of diabetes in Malaysia.
Methods: *O. tenuiflorum* leaves were extracted sequentially with hexane, chloroform, ethyl acetate, methanol, and water. The extracts were evaluated in terms of antidiabetic activity by using acute, subcutaneous glucose tolerance, and sub-chronic tests in streptozotocin-induced diabetic rats. The extracts were also subjected to phytochemical analyses.
Results: With an acute dose (1 g/kg), the methanol extracts showed significant reduction (31%) in fasting blood glucose (FBG) of the streptozotocin-induced diabetic rats. The FBG-decreasing effect of ethyl acetate extract was more rapid than that of the other extracts; the decreasing rates were 20% after 2 h, 21% after 3 h, and 8% after 5 and 7 h. After 7 h (31%), the effect of methanol extract on FBG was significantly lower than that of metformin. In the subcutaneous glucose tolerance test, only methanol and hexane extracts showed the similarity of metformin in diabetic rats. After 14 days, the effects of these extracts were similar to those of metformin (63.33%). The total flavonoid and phenolic contents of extracts decreased as the polarity of the extraction solvent increased.
Conclusions: The results obtained provide support for a possible use of *O. tenuiflorum* leaves in managing hyperglycemia and preventing the complications associated with it in type 2 diabetic.

1. Introduction

Dietary substrates play a key role in physiological mechanisms controlling intermediary metabolism and various abnormalities in different human diseases, including cardiovascular and other metabolic diseases, hyperlipidemia, thrombosis, hypertension, and diabetes [1]. Diabetes mellitus is caused by several abnormalities in intermediary metabolism, such as disarrangements in carbohydrates, proteins, and fat metabolism resulting from the complete or relatively insufficient insulin secretion and activity [2]. Diabetes is categorized into two types: types 1 and 2. Type 1 diabetes is also called insulin-dependent diabetes mellitus. In this condition, the body does not produce insulin; therefore, patients with type 1 diabetes must receive daily insulin injections to remain alive. Type 1 diabetes also accounts for 5%–10% of diabetes cases. Type 2 diabetes is also known as non-insulin-dependent diabetes mellitus; in this condition, the body does not produce enough amounts of insulin or does not properly use this substance. Type 2 diabetes is the most common type of this disease. In adults, this type of diabetic accounts for about 90%–95% of all diagnosed. However, 9 out of 10 people of this group don't know that they have pre-diabetes [3,4]. Reasons for this rise include the increase in sedentary lifestyle, consumption of energy-rich diet, obesity, higher life span, *etc.* [5]. Type 2 diabetes is widely distributed in Asia and

*Corresponding author: Rabeta Mohd Salleh, Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia.
Tel: +60 04 653 5211, ext. 5211
Fax: +60 04 653 6375
E-mail: rabeta@usm.my

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals, as approved by the Animal Ethical Committee, Universiti Sains Malaysia Penang, Malaysia (USM/Animal Ethics Approval/2013/(89) (479) and Animal Research and Service Center, USM (Main Campus).

Foundation Project: Funded by a grant of USM (101/PTEKIND/812176).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Africa, where diabetes mellitus rates increase by 2-fold to 3-fold higher than the present rates [2]. Synthetic hypoglycemic agents and insulin can produce serious side effects. They are also inappropriate for use during pregnancy. As traditional medicine, plants represent a valuable alternative to control and treat diabetes mellitus with minimal or no side effects in clinical settings; plants also entail relatively lower costs than oral synthetic hypoglycemic agents [6,7]. According to ethnobotanical reports, approximately 800 plants may exhibit antidiabetic properties [8]. As such, many plants have been recommended to treat diabetes [5,6]. The principal chemical compounds isolated and identified from plants are glycans, proteins, and mucilages. Other compounds, such as phenolics, flavonoids, steroids, triterpenoids, and alkaloids, with hypoglycemic activity, have been extracted from traditional medicinal plants by using different organic solvents [9]. *Ocimum sanctum* (*O. sanctum*) as one of the main genus of *Ocimum* (Lamiaceae) is medicinally used to treat diuresis, cardiopathy, hemopathy, leucoderma, asthma, bronchitis, catarrhal fever, otalgia, hepatopathy, lumbago, ophthalmia, and gastropathy in children, as well as gastrointestinal disorders, ringworm, verminosis, and skin diseases [10]. Previous studies indicated that the hydroalcoholic and chloroform extracts of *O. sanctum* aerial part are effectively used to treat diabetes. Hydroalcoholic extracts are more potent than chloroform extracts [11]. *Ocimum tenuiflorum* L. (Lamiaceae) (*O. tenuiflorum*), called "ruku" in Malaysia, is usually cultivated as an ornamental plant because of its yellowish flowers.

In Malaysia, young *O. tenuiflorum* leaves are used to make Nasi Ulam. *O. tenuiflorum* is classified as a herb because of its medicinal properties [12]. Its leaves contain essential oil with various compounds exhibiting medicinal values [13].

In India, plants are highly sacred because of their numerous curative uses [12]. Notwithstanding the usage of this plant, it has not been attending to make the study of the effects of *O. tenuiflorum* leaves extract by non-polar to polar solvent with antidiabetic potential. These studies were thus initiated with the aim of evaluating the effect of *n*-hexane, chloroform, ethyl acetate, and methanol and aqueous extracts of *O. tenuiflorum* leaves on blood glucose level, short-term treatment, sub-chronic toxicity, single oral glucose tolerance test, and the toxic effect and measuring the total phenolics and total flavonoids of different crude extracts.

2. Materials and methods

2.1. Collection of plant materials

Fresh *O. tenuiflorum* (Lamiaceae) was collected in Perak, Malaysia. A voucher specimen (Voucher No: 11400) was deposited in the herbarium of the School of Biological Sciences, Universiti Sains Malaysia (USM). The leaves were separated from stems and washed with tap water. Visibly damaged leaves were removed, rinsed with distilled water, stripped, and freeze-dried for 3 days in a freeze dryer (Millrok Technology, LD53, Kingston, USA), as described in a previous study [11,12]. The fully dried *O. tenuiflorum* leaves were ground into powder by using a dry blender (Panasonic, MX 335, Malaysia). The obtained leaf powder was vacuum-packed and stored at 4 °C (Toshiba, GR-M48MP, Minato-ku, Japan) until further analysis [14].

2.2. Preparation of plant extracts

The powdered leaves (200 g) were placed in an extraction chamber and soaked in 800 mL of each of the non-polar and polar solvents for 7 days at room temperature. The solvents were then filtered using Whatman No. 1 filter paper [15]. The filtrate was concentrated in vacuum in a rotary evaporator (EYELA, N1200B, Ser: 1101348) at 30 °C. The residue of the plant material from the above was dried and re-extracted with next solvent using the same procedure until the last solvent re-extracted.

2.3. Chemicals

As a standard anti-diabetic drug, metformin (500 mg/kg; Glucophage®, Lipha Pharma Ltd., United Kingdom) was used as positive control. Streptozotocin (STZ; Sigma–Aldrich Chemical Co., USA) was used to induce diabetes. Other chemicals and reagents were of analytical grade and were procured from approved organizations.

2.4. Induction of diabetes

Diabetes was induced by intraperitoneally injecting 55 mg/kg body weight of STZ (Sigma–Aldrich Chemical Co., USA) reconstituted in 0.1 mol/L cold citrate buffer (pH 4.5) after the rats were subjected to fasting overnight. After 3 days of STZ administration, glucose level was measured in the blood collected from the tail vein punctured by using an Accu-check Advantage II clinical glucose meter (Roche Diagnostics Co., USA). In this study, the rats with fasting blood glucose (FBG) > 15 mmol/L (270 mg/dL) were considered diabetic and induced in the study. The percentage change in blood glucose was calculated thus:

$$\text{Percentage of glycemic change} = (G_x - G_i)/G_x \times 100$$

where G_x is the glycemia at time x and G_i is the glycemia at initial time (i).

2.5. Experimental design

2.5.1. Animals

Male Sprague–Dawley rats weighing 250–300 g were procured from Animal Research and Service Center, USM. The rats were kept in polypropylene cages under standard conditions: (22 ± 3) °C and a 12 h/12 h light/dark cycle. The rats were fed with a commercial diet (Gold Moher, Lipton India Ltd.) and allowed free access to water *ad libitum*. All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals, as approved by the Animal Ethical Committee, USM, Penang, Malaysia [USM/Animal Ethics Approval/2013/(89) (479) and Animal Research and Service Center, USM (Main Campus)].

2.5.2. Assessment of acute/single dose glucose response test in non-diabetic and STZ–induced diabetic rats

The rats were divided into fourteen groups. Each group contained six rats. Groups 1 and 2 were treated as normal positive and negative control, respectively; Groups 3, 4, 5, 6, and 7 were normally treated with 1 g/kg body weight of chloroform, *n*-hexane,

ethyl acetate, methanol, and water extracts of *O. tenuiflorum* leaves, respectively; Groups 8 and 9 were treated as diabetic positive and negative control, respectively; Groups 10, 11, 12, 13, and 14 were treated with 1 g/kg body weight of chloroform, n-hexane, ethyl acetate, methanol, and water extracts of *O. tenuiflorum* leaves, respectively. Oral hypoglycemic agents were dissolved in distilled water. Negative and positive normal rats and diabetic control rats were fed with 10 mL/kg body weight of 1% carboxymethyl cellulose (vehicle) and 500 mg/kg body weight of metformin. After the rats fasted overnight, the plant extract suspended in distilled water was fed to the rats through gastric intubation by using a force-feeding needle. Blood samples were then collected to measure blood glucose level from the tail vein at 0 min and at 1, 2, 3, 5, and 7 h after the plant extracts were administered; blood glucose levels were determined [14].

2.5.3. Assessment of subcutaneous glucose tolerance test (SGTT) in normal rats

The overnight fasted normal rats were divided into seven groups, with six rats in each group. They were orally administered with 1 g/kg body weight of vehicle, chloroform, *n*-hexane, ethyl acetate, methanol, and water extracts of *O. tenuiflorum* leaves and with 500 mg/kg body weight of metformin. Glucose (50 mg/kg body weight) was fed within 30 min after the extracts were administered [16]. Blood was taken through the tail vein at 0, 30, 60, and 120 min of glucose administration; glucose levels were measured by using a glucometer (Roche Diagnostics Co., USA).

2.5.4. Assessment of SGTT in STZ-induced diabetic rats

In this test, the procedure including doses of extraction used, animal grouping, duration and glucose measurement and glucose loading was the same in the section above, except animal grouping that was considered as STZ-induced diabetic rats and control groups as well.

2.5.5. Assessment of the daily treatment of O. tenuiflorum leaf extracts on STZ-induced diabetic rats

The rats were divided into fourteen groups; seven groups with six rats in each group were used as normal treatment and seven groups with six rats in each group were set as diabetic treatment. Groups 1 and 2 were treated as normal positive and negative control, respectively; Groups 3, 4, 5, 6, and 7 were normally treated with 1 g/kg body weight of chloroform, *n*-hexane, ethyl acetate, methanol, and water extracts of *O. tenuiflorum* leaves, respectively; Groups 8 and 9 were treated as diabetic positive and negative control, respectively; Groups 10, 11, 12, 13, and 14 were treated daily with 1 g/kg body weight of chloroform, *n*-hexane, ethyl acetate, methanol, and water extracts of *O. tenuiflorum* leaves for fourteen days, respectively. FBG levels were determined on Days 0, 7, and 14 of the treatment. The body weight was monitored daily [14,17].

2.6. Phytochemical analysis of the crude O. tenuiflorum extracts

2.6.1. Determination of the total phenolic content

The total phenolic contents of the five different extracts of *O. tenuiflorum* leaves prepared through maceration were determined using Folin–Ciocalteu reagent method. In brief, 0.4 mL (1 mg/mL) of each extract was pipetted into a test tube, and 2 mL of (10% v/v) Folin–Ciocalteu reagent was added to the extract sample. After 5 min, 1.6 mL of (7.5%) sodium carbonate solution was added to the sample. The sample was then incubated for 1 h at room temperature. Absorbance was detected using a Perkins Elmer UV–visible spectrometer (USA) at 760 nm. A series of standard gallic acid solutions (20–200 μg/mL) was prepared. Absorbance was obtained at the same wavelength, and data were used to plot the calibration curve. The total phenolic content was calculated as μg/mL of the gallic acid equivalent of the extracts [18]. All of the samples were analyzed in triplicate.

2.6.2. Determination of the total flavonoid contents

The total flavonoid contents of the five different extracts of *O. tenuiflorum* leaves prepared through maceration were determined using an aluminum chloride colorimetric method [19]. In brief, 1.5 mL of the extract solution in the test tube was mixed with 1.5 mL of 2% aluminum chloride solution prepared in methanol. Absorbance was determined at 415 nm after the extract solution was incubated for 10 min at room temperature by using a double-beam Perkins Elmer UV–visible spectrophotometer (USA). The flavonoid content of the extracts was calculated in μg/mL as quercetin equivalent by using an equation obtained from the quercetin calibration curve. The calibration curve was constructed using six different concentrations (3.125–100.000 μg/mL) of the quercetin solution prepared in methanol. All of the samples were analyzed in triplicate.

2.7. Statistical analysis

Values were represented as mean ± SEM. Data were statistically analyzed with One-way ANOVA followed by Dunnett's honestly significant difference (HSD) test by using GraphPad Prism (version 6). P value < 0.05 was considered significant [15].

3. Results

3.1. Effect of the acute/single oral administration of extracts/metformin on normal and STZ-induced diabetic rats

The administration of 1 g/kg of hexane, ethyl acetate, and methanol extracts of *O. tenuiflorum* leaves showed 5%, 10%, and 13% decrease in blood glucose level 7 h after the treatment (Table 1). This decrease was also significantly lower than that observed in the normal control group ($P < 0.001$). Chloroform and aqueous extracts respectively increased the blood glucose level by 41% and 12% during observation. Therefore, the hexane and aqueous extract treatments in this test significantly increased the blood glucose level of the normal rats at the administered dose of 1 g/kg body weight ($P < 0.001$). The effects of the extracts on the diabetic rats were more remarkable (Table 2) than those on the diabetic control rats. The hexane extract significantly increased the blood glucose level by 15% and 8% ($P < 0.001$) after 5 h and 7 h consequently. The ethyl acetate extract induced an 8% decrease in blood glucose level relative to that observed in the control group after 7 h.

Table 1
Effect of a single/acute dose of extracts/metformin on blood glucose of non-diabetic rats.

Treatment groups	Baseline FBG	1 h	2 h	3 h	5 h	7 h
Control	6.4 ± 0.7	6.5 ± 0.4	7.3 ± 0.6	6.0 ± 0.6	8.0 ± 0.4	9.2 ± 0.6
Hexane	6.3 ± 0.1	7.0 ± 0.1	6.0 ± 0.2	6.5 ± 0.4	6.4 ± 0.3[b] (2%)	6.0 ± 0.4[a] (5%)
Chloroform	5.3 ± 1.2	10.0 ± 2.3	11.0 ± 2.0[a]	8.2 ± 1.3[a]	9.0 ± 1.2 (70%)	7.5 ± 1.2[a] (41%)
Ethyl acetate	7.0 ± 0.3	7.5 ± 0.4	8.0 ± 0.6	7.0 ± 0.3	6.3 ± 0.4[a]	6.3 ± 0.6[a] (10%)
Methanol	6.0 ± 0.1	6.0 ± 0.6	6.0 ± 0.6[b]	6.0 ± 0.2	6.0 ± 0.3[a]	5.2 ± 0.2[a] (13%)
Aqueous	8.0 ± 0.1	9.5 ± 0.3	10.0 ± 0.2[a]	9.0 ± 0.3[a]	9.0 ± 0.5	9.0 ± 0.4[a] (12%)
Metformin	7.6 ± 1.2	6.7 ± 0.4	6.0 ± 0.5[c]	5.0 ± 0.5	6.5 ± 0.6[a] (15%)	5.0 ± 0.6[a] (35%)

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test). Percentage in bracket showed the amount of decreasing or increasing of the level of blood glucose regarding to baseline of FBG.

Table 2
Effect of a single/acute dose of extracts/metformin on blood glucose of STZ-induced diabetic rats.

Treatment groups	Baseline FBG	1 h	2 h	3 h	5 h	7 h
Control	23.6 ± 0.4	21.3 ± 9.0	22.3 ± 4.0[a]	27.8 ± 3.3	25.1 ± 2.6	21.2 ± 2.5
Hexane	26.0 ± 4.0	33.5 ± 0.5	31.0 ± 2.2[a]	30.0 ± 2.0 (15%)	30.0 ± 2.0[a] (15%)	28.1 ± 2.0[a] (8%)
Chloroform	32.0 ± 1.1	31.0 ± 4.0	31.0 ± 3.0[a]	29.0 ± 2.0 (9%)	28.0 ± 1.2[c] (12%)	27.0 ± 1.3[a] (16%)
Ethyl acetate	24.0 ± 4.0	31.0 ± 4.0	29.0 ± 2.0[a] (20%)	29.0 ± 1.3 (21%)	26.0 ± 1.1 (8%)	22.0 ± 2.4 (8%)
Methanol	26.0 ± 4.4	29.5 ± 1.0	29.0 ± 1.1[a] (11%)	25.0 ± 1.2 (4%)	23.0 ± 1.5 (11%)	18.0 ± 2.0[b] (31%)
Aqueous	31.0 ± 2.0	28.0 ± 6.0	28.0 ± 2.5[a]	28.0 ± 1.3 (10%)	28.0 ± 1.3[c] (10%)	26.0 ± 1.4[a] (16%)
Metformin	22.2 ± 1.7	18.0 ± 2.1	15.0 ± 0.15[a] (32%)	12.5 ± 1.3 (44%)	10.1 ± 0.7 (55%)	8.7 ± 0.3[a] (61%)

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test). Percentage in bracket showed the amount of decreasing or increasing of the level of blood glucose regarding to baseline of FBG.

The chloroform and aqueous extracts also exhibited the same pattern. In particular, these extracts decreased the blood glucose level by 16% after 7 h. The effect of the methanol extract was very different. In particular, the methanol extract significantly decreased the blood glucose level by 31% after 7 h ($P < 0.01$) relative to its effect on the diabetic control rats. This effect was similar to that of metformin. Among the extracts used in this study, the methanol extract was the most effective.

3.2. Effect of extracts/metformin on the SGTT of normal and STZ-induced diabetic rats

Table 3 shows the effect of extracts on blood glucose of non-diabetic rats after subcutaneous glucose load. Compared to the control, drug administration and the hexane, chloroform, methanol and aqueous extracts significantly suppressed the peak blood glucose after 15 min ($P < 0.001$) while ethyl acetate extract at the same time displayed the significance ($P < 0.01$) compared to control. However, only chloroform extract showed significant ($P < 0.01$) decrease until the 90th min. While other extracts showed significance at the same level ($P < 0.001$). Table 4 shows the effect of extracts of leaves of *O. tenuiflorum* and metformin on SGTT. The hexane extract exerted significant effect on blood glucose at the 90th min until the 120th min ($P < 0.05$) compared to control group at the same time. Whereas the other extracts except hexane extract did not show any significant effects during the study. Metformin demonstrated a significant effect all the time, with the higher reduction in glucose level compared to treatment groups with crude extracts ($P < 0.05$).

3.3. Effect of extracts/metformin on the body weight and blood glucose of STZ-induced diabetic rats

Table 5 lists the changes in the body weight of the control and experimental rats treated with the extracts and metformin. The body weight (4.13%) of the STZ-induced diabetic rats was significantly lower than that of the normal control groups after 14 days. In addition, 14 daily administrations of ethyl acetate, aqueous, and methanol extracts (1 g/kg) decreased the body weight by 22.09%, 14.87%, and 7.52%, respectively. Furthermore, the daily administration of the extracts for 14 days caused a significant reduction compared with the diabetic control group (Table 6). Almost the test extracts showed a significant reduction in blood glucose level at Day 14 except aqueous extract. The

Table 3
Effect of the extracts/metformin on blood glucose level after subcutaneous loading of 50 mg/kg glucose in non-diabetic rats.

Treatment groups	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control	6.4 ± 0.2	10.0 ± 1.2	7.0 ± 0.3	7.0 ± 0.4	8.5 ± 0.1	6.0 ± 0.9	7.0 ± 0.5
Metformin	6.2 ± 0.7	6.3 ± 0.1[a]	7.0 ± 0.1	6.1 ± 0.7	7.3 ± 0.2[c]	7.0 ± 0.1[a]	6.2 ± 0.1
Hexane	7.0 ± 0.2	9.2 ± 1.2	8.0 ± 0.4	8.0 ± 0.3[b]	9.0 ± 0.3	8.0 ± 0.4[a]	7.0 ± 0.5
Chloroform	7.0 ± 0.6	15.1 ± 0.9[a]	15.1 ± 0.5	11.0 ± 0.4[a]	9.0 ± 1.2	6.4 ± 0.7[b]	8.0 ± 1.5
Ethyl acetate	7.0 ± 0.1	8.0 ± 1.0[b]	8.4 ± 0.1	10.2 ± 0.7[a]	8.4 ± 0.4	6.3 ± 0.3[a]	7.0 ± 0.2
Methanol	7.0 ± 0.1	7.0 ± 0.4[a]	8.5 ± 0.4	9.0 ± 0.4[a]	8.2 ± 1.0	7.0 ± 0.0[a]	6.5 ± 0.5
Aqueous	7.2 ± 0.8	7.0 ± 0.1[a]	9.0 ± 0.1	8.2 ± 0.4[a]	8.0 ± 0.1	9.0 ± 0.1[a]	9.0 ± 0.2[a]

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test).

Table 4

Effect of the extracts/metformin on blood glucose level after subcutaneous loading in STZ-induced diabetic rats.

Treatment groups	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control	26.9 ± 5.0	25.5 ± 11.3	28.2 ± 3.3	22.4 ± 10.0	25.5 ± 4.5	28.1 ± 3.5	30.5 ± 1.3
Metformin	17.5 ± 2.1	21.0 ± 2.5	19.0 ± 3.1[b]	17.0 ± 3.0	17.0 ± 3.0[b]	14.1 ± 3.0[b]	16.4 ± 3.6[c]
Hexane	28.0 ± 6.1	27.0 ± 3.1	27.0 ± 2.0	27.3 ± 3.0	26.0 ± 2.1	24.1 ± 2.3[c]	24.2 ± 5.4[c]
Chloroform	24.0 ± 6.1	28.0 ± 6.0	29.5 ± 3.0	26.3 ± 2.4	27.5 ± 2.4	27.5 ± 2.1	25.0 ± 6.0
Ethyl acetate	26.0 ± 3.2	28.0 ± 3.0	28.0 ± 2.3	29.0 ± 3.0	28.0 ± 2.4	26.0 ± 2.3	29.0 ± 1.1
Methanol	29.4 ± 3.0	30.0 ± 0.7	29.4 ± 0.9	28.0 ± 1.2	28.0 ± 1.2	28.0 ± 1.1	28.0 ± 4.4
Aqueous	30.0 ± 2.0	30.0 ± 1.2	29.0 ± 1.3	28.0 ± 0.1	30.0 ± 0.1[c]	30.0 ± 1.2	31.5 ± 1.2

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test).

Table 5

Effect of oral administration of extracts (1 g/kg) or metformin (500 mg/kg) on body weight (g) of STZ-induced diabetic rats.

Treatment groups	Day 0	Day 7	Day 14
Normal control	268.10 ± 30.40	255.50 ± 18.40 (+4.7%)	284.00 ± 14.80[c] (+5.93%)
Diabetic control	266.00 ± 15.50	234.00 ± 4.40 (−11.9%)	225.00 ± 8.40 (−4.13%)
Hexane	246.00 ± 52.33	216.33 ± 4.72 (−12.06%)	–
Chloroform	270.00 ± 39.22	254.20 ± 47.01 (−5.85%)	274.00 ± 41.90[c] (+1.48%)
Ethyl acetate	253.20 ± 7.62	207.00 ± 14.31 (−18.24%)	197.25 ± 9.00 (−22.09%)
Methanol	241.50 ± 22.43	228.33 ± 37.23 (−5.45%)	223.33 ± 40.76 (−7.52%)
Aqueous	260.00 ± 20.60	243.66 ± 24.11 (−6.28%)	221.33 ± 26.50 (−14.87%)
Metformin	266.50 ± 13.30	278.00 ± 1.60[c] (+4.31%)	267.70 ± 2.73[c] (+0.45%)

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test). − and + before the number of percentage, it means decrease and increase of the level of weight and blood glucose compared to the baseline.

Table 6

Effect of daily oral administration of extracts (1 g/kg) or metformin (500 mg/kg) on blood glucose level of STZ-induced diabetic rats.

Treatment groups	Day 0	Day 7	Day 14
Normal control	6.33 ± 0.60	5.00 ± 0.24	6.23 ± 0.16 (−1.57%)
Diabetic control	20.40 ± 1.31	22.03 ± 0.70	26.50 ± 1.50 (+29.9%)
Hexane	24.85 ± 3.70	22.75 ± 1.20	–
Chloroform	31.20 ± 1.08[c]	28.02 ± 8.50[b]	17.00 ± 13.30 (−45.51%)
Ethyl acetate	24.30 ± 2.50	19.45 ± 10.50	11.10 ± 2.50 (−54.32%)
Methanol	30.73 ± 1.00	14.40 ± 0.92[c] (−53.14%)	11.00 ± 1.08[a] (−64.2%)
Aqueous	21.50 ± 9.50	23.00 ± 5.20	24.00 ± 7.00 (+11.62%)
Metformin	19.50 ± 6.50	12.10 ± 2.40	7.15 ± 0.30 (−63.33%)

Values are expressed as mean ± SD ($n = 6$). [a]: $P < 0.001$, [b]: $P < 0.01$ and [c]: $P < 0.05$ between diabetic treated groups and negative control as healthy control groups (analyzed by One-way ANOVA Dunnett's HSD test). − and + before the number of percentage, it means decrease and increase of the level of weight and blood glucose compared to the baseline.

extent of reduction showed by methanol, ethyl acetate and chloroform (64.20%, 54.32% and 45.51%, respectively) (Table 6). Hence, the methanol extract was also considered the most effective in anti-hyperglycemic effect.

3.4. Total phenolics in *O. tenuiflorum* extracts

Figure 1 illustrates the total phenolic contents of the different extracts obtained through maceration. The hexane extract contained less phenolic compounds, with (72.47 ± 0.30) μg/mL gallic acid equivalent. The aqueous extract contained the highest total phenolic compounds [(144.13 ± 0.15) μg/mL], followed by the phenolic contents of methanol, ethyl acetate, and chloroform extracts.

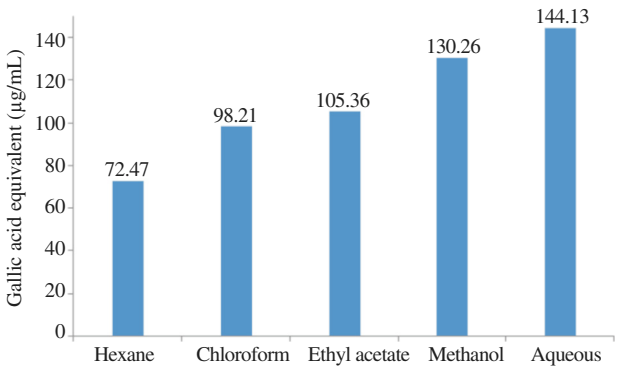

Figure 1. Total phenolic content of *O. tenuiflorum* leaves extract ($n = 3$).

3.5. Total flavonoids in *O. tenuiflorum* extracts

The total flavonoid contents of the different extracts of *O. tenuiflorum* leaves are shown as follows. The ethyl acetate extract yielded the highest total flavonoid content, with (38.56 ± 0.41) μg/mL quercetin equivalent. The aqueous extract [(27.74 ± 0.02) μg/mL] and the methanol extract [(30.26 ±0.01) μg/mL] contained lower total flavonoid contents. Hexane and chloroform extracts also yielded similar amounts of total flavonoid contents [(31.76 ± 0.01) μg/mL].

4. Discussion

Various *in vivo* models, such as diazoxide, alloxan, or STZ-induced diabetic rats, have been traditionally used to evaluate medicinal plants with suspected hypoglycemic potentials.

In this study, diabetes mellitus was induced by intraperitoneal injection of STZ at single dose of 55 mg/kg body weight in rats. This dose reliably induced diabetes mellitus in the treated rats 7 days post incubation. This result is consistent with that described in previous studies on the treated rats with STZ-induced hyperglycemia 5–7 days after intraperitoneal injection [20]. Our study demonstrated that the minimum hyperglycemic dose was 1 mg/kg body weight of the rats in all of the extracts. This finding is consistent with that observed in a previous study [21]. Using this diabetic model, we screened serial non-polar to polar *O. tenuiflorum* extracts to determine their hypoglycemic and anti-hyperglycemic effects. The glucose-lowering test in normal rats aimed to evaluate the tendency of an extract/drug to produce hypoglycemia or side effects of some anti-diabetic drugs. The glucose-lowering test in diabetic rats aimed to examine the anti-diabetic property of an extract/drug. In this study, *O. tenuiflorum* extracts did not significantly affect FBG at an acute dose of 1 g/kg until 2 h; after 7 h, these extracts significantly decreased FBG ($P < 0.000\,1$). This feature is desirable because hypoglycemia, a side effect of most oral hypoglycemic agents, can lead to seizures and even death; in other cases, hypoglycemia induces permanent brain damage. The response to acute dose of glucose of the diabetic rats indicated that all of the five extracts of *O. tenuiflorum* decreased FBG at some points within 7 h. The methanol extract induced reduction of FBG until 7 h to a greater extent than metformin did. This finding suggested that the methanol extract combination may be used to isolate the active anti-diabetic compound in *O. tenuiflorum*. Therefore, the methanol extract could be considered as a good candidate to develop novel anti-diabetic natural products or standardized herbal formulation. *O. tenuiflorum* extracts were also evaluated to determine the influence on the tolerance level of parenteral glucose administration in non-diabetic and diabetic rats. In this model, glucose loading was administered subcutaneously rather than orally to prevent the possibility of a false positive response that could result from delayed glucose absorption because of its interaction with sticky or waxy components and various extracts, especially hexane and chloroform extracts. However, the extracts except hexane significantly retained the same blood glucose level in the normal rats within 15–90 min of glucose loading. This effect in SGTT test was also replicated in the STZ-induced diabetic rats.

In our study, 1 g/kg of *O. tenuiflorum* extracts was administered daily to STZ-induced diabetic rats for 14 days. Body weight and blood glucose were monitored within 14 days of treatment. The body weight of the diabetic control rats was lower than that of the normal control rats. Body weight gain is an indicator of efficient glucose homeostasis. Other alternative fats and tissue proteins are broken down to produce energy accounting for the loss in body weight [22]. Of the five treatments in our study, the methanol extract induced a significant recovery in body weight after 7 and 14 days of treatment compared with that observed in the diabetic control rats. This result indicated improvement in insulin secretion, and control of glycemic levels [14,15,23].

All of the extracts except the aqueous extract significantly reduced the blood glucose levels of the diabetic rats 14 days after the treatment. All of the diabetic rats treated with the hexane extract died 1 day before sacrificed date on Day 13. By contrast, the normal rats treated with the same dose of the hexane extract survived until the last day of treatment. In additional result, the body weight of rats showed reduction between 7 and 14 days after treatment.

However, among the extracts, the methanol extract induced the highest decrease on blood glucose level at Days 7 (53.14%) and 14 (64.20%). This decrease was almost greater than that induced by metformin. The polarity of methanol is 5.1 compared with that of other solvents characterized by moderate polarity. Therefore, regarding this result it is expected that the extracts with a moderate concentration of active compounds elicit anti-hyperglycemic effects compared with high and low polarity concentration of active compound that extracted by other solvents. The present study also supports most of previous reports that polar leaves extract can decrease blood glucose level [22,24].

Phenolic compounds, such as flavonoids, phenolic acids, and tannins are considered as major contributors to the antioxidant capacity of plant foods. These antioxidants also exhibit diverse biological activities. It seems that these activities are related to their antioxidant activity and α-amylase and α-glucosidase inhibitory activity. *Solanum xanthocarpum*, *O. sanctum*, and *Acacia pennata* can be used in the dietary management of type 2 diabetes mellitus because of high contents of polyphenolics like, caffeic acid and *p*-coumaric acid [25,26]. Polyphenolic compounds, especially flavonoids, are widely investigated because of their anti-diabetic properties [27]. Flavonoids are natural polyphenolic molecules from plants known for their antioxidant, anti-inflammatory, and anti-carcinogenic properties [28]. Flavonoids may be included in diets as an effective alternative treatment for diabetes; these substances may also be administered to reduce the risk of diabetes [29].

Thus, the total phenolic and total flavonoid contents of different *O. tenuiflorum* leaf extracts were also evaluated [29]. Polyphenolic compounds likely stabilize lipid oxidation and participate in antioxidant activity [30]. Polyphenolic compounds also elicit inhibitory effects on mutagenesis and carcinogenesis in humans; at 1.0 g, these compounds are ingested daily from a diet rich in fruits and vegetables [27]. Owing to previous result, the flavonoids have been shown to have an important antidiabetic activity and are able to affect the regeneration of pancreatic beta cells in additional phenolic compounds of plants extracts which have been reported to reduce blood glucose level [31]. Our results also revealed that the phenolic and flavonoid contents were respectively the highest in aqueous and ethyl acetate extracts but were approximately similar to those of the methanol extract. The phenolic and flavonoid contents also increased the polarity of solvents, and this finding is similar to the observed anti-hyperglycemic activity of the extracts. The aqueous extract with a high phenolic content and the ethyl acetate extract with high flavonoid content elicited an anti-hyperglycemic effect to a less extent than the methanol extract did. The fluctuation enhanced interaction of hydroxyl and/or carboxylic groups in polar solvent to promote the dissolution of phenolics in the solvent [32]. However, the interaction is decreased by the benzene ring present in the structure, thus restricting the solubility of phenolic compounds. Plant extracts contain numerous compounds, whose net observed pharmacological

actions or interaction largely depend on synergistic or antagonistic interactions among these compounds [33]. The selective and non-predictive interaction of compounds in the methanol extract of *O. tenuiflorum* leaves may account for its potent anti-hyperglycemic effect rather than the actual phenolic and flavonoid contents.

The hypoglycemic and anti-hyperglycemic activities of the methanol extract and metformin were similar. This finding may provide insights into the anti-diabetic mechanism of the methanol extract. Metformin elicits its anti-hyperglycemic effect primarily by inhibiting the increase in the rates of hepatic gluconeogenesis and by improving insulin sensitivity through the stimulation of peripheral glucose uptake in skeletal muscles and adipose tissues [34]. The methanol extract may induce its effect via similar mechanisms. However, the rapid normalization of blood glucose level in the rats treated with the methanol extract relative to the control rats suggested the existence of residual β-cells, which must have been sensitized by compounds, such as flavonoids and phenolics in the extract [35].

Thus, *O. tenuiflorum* must be considered as an excellent candidate for future studies on diabetes mellitus. Further comprehensive pharmacological investigations, including active crude extract fractionation and bioactive compound studies, should be performed.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was funded by a grant of USM (101/PTEKIND/812176). The financial support of PhD fellowship from USM for author Leila Mousavi was gratefully acknowledged.

References

[1] Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: a review. *Nutr J* 2002; **1**: 4.
[2] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012; **35**: S64-71.
[3] Man YBC, Sin KK. Processing and consumer acceptance of fruit leather from the unfertilised floral parts of jackfruit. *J Sci Food Agric* 1997; **75**(1): 102-8.
[4] Guillén A, Granados S, Rivas KE, Estrada O, Echeverri LF, Balcázar N. Antihyperglycemic activity of *Eucalyptus tereticornis* in insulin-resistant cells and a nutritional model of diabetic mice. *Adv Pharmacol Sci* 2015; **2015**: 418673.
[5] Yajnik CS. The insulin resistance epidemic in India: fetal origins, later lifestyle, or both. *Nut Rev* 2001; **59**: 1-9.
[6] Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. *J Ethnopharmacol* 2005; **99**(1): 75-81.
[7] Balamurugan K, Nishanthini A, Mohan VR. Antidiabetic and antihyperlipidaemic activity of ethanol extract of *Melastoma malabathricum* Linn. leaf in alloxan induced diabetic rats. *Asian Pac J Trop Biomed* 2014; **4**: S442-8.
[8] Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: an overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac J Trop Biomed* 2012; **2**(5): 411-20.
[9] Gupta S, Mediratta PK, Singh S, Sharma KK, Shukla R. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimum sanctum* (Linn) seed oil. *Indian J Exp Biol* 2006; **44**: 300-4.
[10] Patil R, Patil R, Ahirwar B, Ahirwar D. Isolation and characterization of anti-diabetic component (bioactivity-guided fractionation) from *Ocimum sanctum* L. (Lamiaceae) aerial part. *Asian Pac J Trop Med* 2011; **4**(4): 278-82.
[11] Patil RN, Patil R, Ahirwar D. Study of some medicinal plants for antidiabetic activity in alloxan induced diabetes. *Pharmacologyonline* 2010; **1**: 53-60.
[12] Kothari SK, Bhattacharya K, Ramesh S. Essential oil yield and quality of methyl eugenol rich *Ocimum tenuiflorum* L.f. (syn. *O. sanctum* L.) grown in south India as influenced by method of harvest. *J Chromatogr A* 2004; **1054**(1–2): 67-72.
[13] Rai V, Vajpayee P, Singh SN, Mehrotra S. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Sci* 2004; **167**(5): 1159-69.
[14] Nabi SA, Kasetti RB, Sirasanagandla S, Tilak TK, Kumar MVJ, Rao CA. Antidiabetic and antihyperlipidemic activity of *Piper longum* root aqueous extract in STZ induced diabetic rats. *BMC Complement Altern Med* 2013; **13**: 37.
[15] Kumar R, Pate DK, Prasad SK, Sairam K, Hemalatha S. Antidiabetic activity of alcoholic leaves extract of *Alangium lamarckii* Thwaites on streptozotocin-nicotinamide induced type 2 diabetic rats. *Asian Pac J Trop Med* 2011; **4**(11): 904-9.
[16] Shirwaikar A, Rajendran K, Barik R. Effect of aqueous bark extract of *Garuga pinnata* Roxb. in streptozotocin-nicotinamide induced type-II diabetes mellitus. *J Ethnopharmacol* 2006; **107**(2): 285-90.
[17] Eknayake S, Jansz ER, Nair BM. Proximate composition, mineral and amino acid content of mature *Canavalia gladiata* seeds. *Food Chem* 1999; **66**(1): 115-9.
[18] Moreno DA, Carvajal M, López-Berenguer C, García-Viguera C. Chemical and biological characterisation of nutraceutical compounds of broccoli. *J Pharm Biomed Anal* 2006; **41**: 1508-22.
[19] Gursoy N, Sarikurkcu C, Cengiz M, Solak MH. Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food Chem Toxicol* 2009; **47**(9): 2381-8.
[20] Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. *J Clin Invest* 1969; **48**(11): 2129-39.
[21] Khan MRI, Islam MA, Hossain MS, Asadujjaman M, Wahed MII, Rahman BM, et al. Antidiabetic effects of the different fractions of ethanolic extracts of *Ocimum sanctum* in normal and alloxan induced diabetic rats. *J Sci Res* 2010; **2**(1): 158-68.
[22] Atangwho IJ, Ebong PE, Eyong EU, Asmawi MZ, Ahmad M. Synergistic antidiabetic activity of *Vernonia amygdalina* and *Azadirachta indica*: biochemical effects and possible mechanism. *J Ethnopharmacol* 2012; **141**(3): 878-87.
[23] Pandhare RB, Sangameswaran B, Mohite PB, Khanage SG. Antidiabetic activity of aqueous leaves extract of *Sesbania sesban* (L) Merr. in streptozotocin induced diabetic rats. *Avicenna J Med Biotechnol* 2011; **3**(1): 37-43.
[24] Algariri K, Atangwho IJ, Meng KY, Asmawi MZ, Sadikun A, Murugaiyah V. Antihyperglycaemic and toxicological evaluations of extract and fractions of *Gynura procumbens* leaves. *Trop Life Sci Res* 2014; **25**: 75-93.
[25] Orhan N, Aslan M, Süküroğlu M, Deliorman Orhan D. *In vivo* and *in vitro* antidiabetic effect of *Cistus laurifolius* L. and detection of major phenolic compounds by UPLC-TOF-MS analysis. *J Ethnopharmacol* 2013; **146**: 859-65.
[26] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. *Crit Rev Food Sci Nutr* 1998; **38**(6): 421-64.
[27] Coman C, Rugina OD, Socaciu C. Plants and natural compounds with antidiabetic action. *Not Bot Horti Agrobot* 2012; **40**(1): 314-25.
[28] Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of *Sonchus asper* (L.) Hill. *Chem Cent J* 2012; **6**(1): 12.
[29] Pinent M, Castell A, Baiges I, Montagut G, Arola L, Ardévol A. Bioactivity of flavonoids on insulin-secreting cells. *Compr Rev Food Sci Food Saf* 2008; **7**: 299-308.

[30] Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. *Phytomedicine* 2000; **7**(1): 7-13.

[31] Shewamene Z, Abdelwuhab M, Birhanu Z. Methanolic leaf exctract of *Otostegia integrifolia* Benth reduces blood glucose levels in diabetic, glucose loaded and normal rodents. *BMC Complement Altern Med* 2015; **15**: 19.

[32] Mota FL, Queimada AJ, Pinho SP, Macedo EA. Aqueous solubility of some natural phenolic compounds. *Ind Eng Chem Res* 2008; **47**(15): 5182-9.

[33] Rasoanaivo P, Wright CW, Willcox ML, Gilbert B. Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malar J* 2011; **10**: S4.

[34] Hui H, Tang G, Go VL. Hypoglycemic herbs and their action mechanisms. *Chin Med* 2009; **4**: 11.

[35] Islam MA, Akhtar MA, Islam MR, Hossain MS, Alam MK, Wahed MI, et al. Antidiabetic and hypolipidemic effects of different fractions of *Catharanthus roseus* (Linn.) on normal and streptozotocin-induced diabetic rats. *J Sci Res* 2009; **1**(2): 334-44.