Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Study of Glucose Uptake Enhancing Potential of Fenugreek *(Trigonella foenum graecum)* Leaves Extract on 3T3 L1 Cells Line and Evaluation of its Antioxidant Potential

Debarupa Hajra, Santanu Paul

Department of Botany, Laboratory of Cell and Molecular Biology, University of Calcutta, Kolkata, West Bengal, India

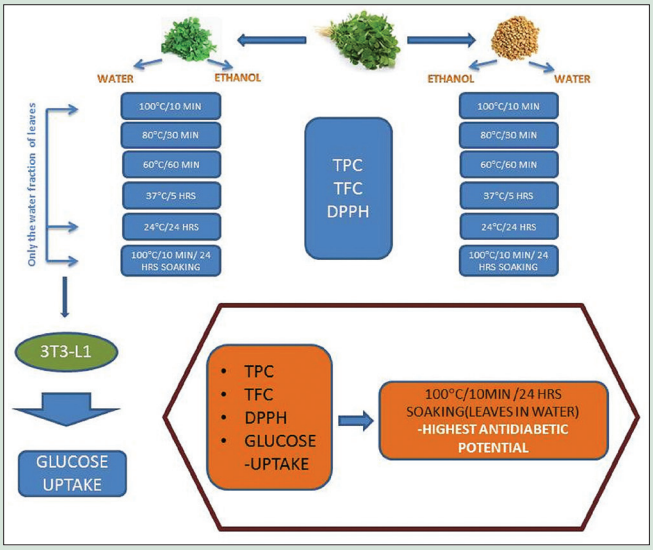
ABSTRACT

Background: The incidence of diabetes mellitus (DM) is increasing at an alarming rate globally. There is a need for suitable natural alternatives for its treatment, as the regular use of drugs causes several secondary health issues. **Aim:** This study aimed to quantitatively evaluate the phenol and flavonoid content; antioxidant and anti-diabetic activity of fenugreek seeds and leaves in water and ethanolic extracts. **Materials and Methods:** Dried and powdered seeds and leaves of fenugreek were treated at different time and temperature combinations (100°C for 10 min; 80°C for 30 min; 60°C for 1 h; 37°C for 6 h; 24°C [room temperature] for 24 h; 100°C for 10 min followed by overnight soaking). Total phenolic content, total flavonoid content, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and glucose uptake assays on 3T3-L1 cell lines were performed on the extracts. **Results:** The results show that fenugreek leaves treated at 100°C for 10 min and overnight soaking yield extracts with the highest concentrations of desired phenols (46.08 ± 0.15 mg GAE/g sample) and flavonoid content (13.02 ± 0.44 mg/g sample) and antioxidant activity (DPPH) (45.41 ± 2.1%) with enhanced glucose uptake activity in 3T3-L1 cell lines. **Conclusion:** The phenolic and flavonoid content, DPPH radical scavenging activity and antidiabetic activity was highest in the water extract of fenugreek leaves treated at 100°C for 10 min and subsequent soaking for 24 h before filtration as compared to the other protocols tested. Furthermore, water extracts showed enhanced activity as compared to the ethanol extracts in case of both seeds and leaves and in all the treatment combinations.

Key words: 3T3-L1 cell lines, antioxidant, diabetes, fenugreek, glucose uptake

SUMMARY

- Fenugreek leaves have higher phenolic and flavonoid content and antioxidant activity as compared to fenugreek seeds
- The aqueous extract of both fenugreek leaves and seeds yield higher quantity than their ethanolic counterparts
- The extract which was obtained by boiling the leaves at 100°C for 10 min and letting it soak for 24 h at room temperature showed enhanced phenolic and flavonoid content, antioxidant activity and glucose uptake activity on 3T3-L1 cell lines as compared to the other protocols
- Hence, it can be concluded that this extract has enhanced antidiabetic potential.



Abbreviations Used: 2NBDG: (2-(N-(7-Nitrobenz-2-oxa-1, 3-diazol-4-yl) Amino)-2-Deoxyglucose), DM: Diabetes mellitus, DMEM: Dulbecco's Modified Eagle's Medium, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, GA: Gallic Acid, GAE: Gallic Acid Equivalents, PBS: Phosphate Buffered Saline, TFC: Total Flavonoid Content, TPC: Total Phenolic Content

Correspondence:
Dr. Santanu Paul,
Department of Botany, Laboratory of Cell and Molecular Biology, University of Calcutta, 35 Ballygunge Circular Road, Kolkata - 700 019, West Bengal, India.
E-mail: spaul_1971@yahoo.com

DOI: 10.4103/pr.pr_50_18

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

In the developed world, diabetes mellitus (DM) is among the top 5 most significant diseases that are severely affecting human life. Currently, there are 171 million people who are suffering from diabetes.[1]

Diabetes or DM is a metabolic disorder[2-5] wherein the blood sugar level is higher than normal. This is due to the disturbed secretion or improper functioning of insulin.[6-9]

Insulin is a peptide hormone. It is secreted by the beta-cells of the pancreatic islets of Langerhans. Its main function is to maintain normal blood glucose levels. It facilitates cellular glucose uptake and regulates the metabolism of carbohydrate, protein, and lipid.[10] For treating diabetic patient, anti-diabetic drugs such as metformin, rosiglitazone, gliclazide, and such other or synthetic human insulin are prescribed.

Cite this article as: Hajra D, Paul S. Study of glucose uptake enhancing potential of fenugreek (*Trigonella foenum graecum*) leaves extract on 3T3 L1 cells line and evaluation of its antioxidant potential. Phcog Res 2018;10:347-53.

Insulin and hypoglycemic drugs are mostly used to treat DM. Although effective they cause several other health problems. Hence, there is an inclination toward naturally derived medicines or medicines made from various phytochemicals.

DM is affected by the oxidative stresses that develop in one's body[6] due to impaired insulin secretion and increased insulin resistance.[11,12] Antioxidants are substances that protect the cells of our body from oxidation by itself getting oxidized in the process. Hence, antioxidants help to protect the body and can be used to treat this disorder.[13] Antioxidants derived from plant sources are harmless and hence reduce the chance of associated or secondary health problems. Antioxidants are generally found in food such as Vitamin C and Vitamin E. Foods such as spinach, fenugreek, other green leafy vegetables, beetroot, and citrus fruits are also rich in antioxidants.

Fenugreek is a herb, belonging to the Fabaceae family.[14,15] Its scientific name is *Trigonella folium*. Fenugreek is known to possess antidiabetic properties.[16] It possesses a number of properties that are beneficial for human health such as anti-inflammatory,[17,18] antioxidant, antifungal, antibacterial, antilipidemia, hypocholesterolemic, anticarcinogenic, and also antidiabetic activities.[19-21] The soluble dietary fiber fraction of fenugreek enhances glycemic control by inhibiting lipid hydrolyzing and carbohydrate-hydrolyzing enzymes in the digestive system.[22] It has high antioxidant activity that is beneficial for human health. It is reported that germinating seeds show better results as compared to ungerminated seeds.[23,24]

Flavonoids present in plants contribute to the color of fruits and flowers. They are polyphenolic compounds. These are known to possess antioxidant, antitumor, and antibacterial activities.[25] They are known to be present in fenugreek as well.

In traditional or ethnomedicine the extracts of both the seeds and leaves of this fenugreek are recommended by doctors as a measure to control diabetes. In addition, it is an age-old practice in different parts of the world and in ethnomedicine or traditional medicine to soak the fenugreek seeds in water and then consume the water after filtration. However, till now, there is comparatively less literature available on the water extracts of the leaves and the seeds. The research done till date mostly deals with the ethanolic or methanolic and acidified water extracts of the herb and its parts.[26,27] The current study deals with studying the effects of different time and temperature treatments on both water and ethanol extracts of the seeds and leaves of the fenugreek herb. The study also aims to compare the water extract with those obtained by applying the same time and temperature combinations to ethanolic extracts of fenugreek seeds and leaves. The whole research deals with the collection and proper treatment of the seeds and leaves of the herb. The antioxidant and antidiabetic properties are due to the phenolic and flavonoid compounds that are present in the different parts of the plant. The estimation of the total phenolic content, the total flavonoid content, and the free radical scavenging activity was carried out. The free radical scavenging activity was estimated by the help of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay.

MATERIALS AND METHODS

Chemicals

All the reagents used were of analytical grade. Quercetin, Gallic acid (GA) (Sigma-Aldrich), Methanol, Sodium Carbonate, Folin-ciocalteu reagent, Aluminum Chloride, Sodium Nitrate, Sodium Hydroxide (all from SRL, India), and DPPH (Rankem).

Collection of samples and preparation of extracts

Fenugreek seeds were collected from the local market of Kolkata, India, and leaves Gurgaon, India. The samples were washed, dried using a tray drier at 45°C and powdered. Each of these, seeds and leaves were extracted in water and ethanol simultaneously [Table 1]. The extractions were made in different time and temperature combinations

- SET 1–100°C for 10 min, (100FLW/100FSW/100FLE/100FSE)
- SET 2–80°C for ½ h, (80FLW/80FSW/80FLE/80FSE)
- SET 3–60°C for 1 h, (60FLW/60FSW/60FLE/60FSE)
- SET 4–37°C for 6 h, (37FLW/37FSW/37FLE/37FSE)
- SET 5–24°C (room temperature) for 24 h, (24FLW/24FSW/24FLE/24FSE).
- SET 6–100°C for 10 min followed by overnight cooling (FLW/FSW/FLE/FSE).
- FLW: Fenugreek Leaves in Water
- FSW: Fenugreek Seeds in Water
- FLE: Fenugreek Leaves in Ethanol
- FSE: Fenugreek Seeds in Ethanol.

The samples 2 g each were dissolved in 20 ml water and ethanol separately in conical flasks and shaken well and were covered with aluminum foil.

Water bath was used to achieve 37°C. Hot air oven was used to achieve 80°C and 60°C. 100°C was achieved by using a gas oven. After extraction processes, the solutions were filtered separately. The time and temperature combinations are such that they would be easily available at home.

After the extraction was over, the extracts were tested for antioxidant activity, total phenolics, and total flavonoids. To determine the above total phenolic content (TPC), total flavonoid content (TFC), and DPPH assay were carried out. These were observed for all the water extracts of the leaves and seeds. These values were then compared with those obtained from the ethanolic extracts.

Also, for those aqueous extracts with the highest TPC, TFC, and antioxidant values, glucose uptake measurement was performed.

Glucose uptake is performed to study the uptake of glucose by cells both when stimulated by insulin and without insulin treatment. In this study,

Table 1: The time-temperature treatment of the fenugreek seeds and leaves

Code name	Fenugreek sample	Solvent	Temperature (°C)	Time
T1 (100 FSW)	Seeds	Water	100	10 min
T2 (100 FLW)	Leaves	Water	100	10 min
T3 (100 FSE)	Seeds	Ethanol	100	10 min
T4 (100 FLE)	Leaves	Ethanol	100	10 min
T5 (80 FSW)	Seeds	Water	80	30 min
T6 (80 FLW)	Leaves	Water	80	30 min
T7 (80 FSE)	Seeds	Ethanol	80	30 min
T8 (80 FLE)	Leaves	Ethanol	80	30 min
T9 (60 FSW)	Seeds	Water	60	60 min
T10 (60 FLW)	Leaves	Water	60	60 min
T11 (60 FSE)	Seeds	Ethanol	60	60 min
T12 (60 FLE)	Leaves	Ethanol	60	60 min
T13 (37 FSW)	Seeds	Water	37	5 h
T14 (37 FLW)	Leaves	Water	37	5 h
T15 (37 FSE)	Seeds	Ethanol	37	5 h
T16 (37 FLE)	Leaves	Ethanol	37	5 h
T17 (24 FSW)	Seeds	Water	24	24 h
T18 (24 FLW)	Leaves	Water	24	24 h
T19 (24 FSE)	Seeds	Ethanol	24	24 h
T20 (24 FLE)	Leaves	Ethanol	24	24 h
T21 (FSW)	Seeds	Water	100°C 10 min/cooling	24 h
T22 (FLW)	Leaves	Water	100°C 10 min/cooling	24 h
T23 (FSE)	Seeds	Ethanol	100°C 10 min/cooling	24 h
T24 (FLE)	Leaves	Ethanol	100°C 10 min/cooling	24 h

FSW: Fenugreek seeds in water; FLW: Fenugreek leaves in water; FSE: Fenugreek seeds in ethanol; FLE: Fenugreek leaves in ethanol

3T3-L1 cell lines were treated with different fenugreek extracts and their respective glucose uptake potential was measured.

3T3-L1 cell line has been derived from the mouse 3T3 cells. These differentiate into adipocyte-like phenotype. These have lipid storage and glucose homeostasis properties. These increase the synthesis and accumulation of triglycerides. 3T3-L1 is a most frequently employed adipocyte cell lines. This has been isolated from Swiss 3T3 cells. Swiss 3T3 cells are in turn derived from disaggregated 17–19-day mouse embryos.

Determination of total phenolic content of the extracts

The total phenol content was quantified using the previously used colorimetric assay by Mashkor Idries,[28] Chakraborty *et al.*,[29] Yogesh *et al.*[30] with some modification. Approximately 4.5 ml of distilled water and 100 µl of Folin-Ciocalteu reagent are added to 100 µl fenugreek extracts each and covered and mixed well. To this, 0.3 ml of 2% sodium carbonate is added and incubated for 2 h at room temperature with periodic shaking every 40 min. The absorbance was measured at 760 nm. For estimation of the sample activity calibration curve of GA is used and the result is expressed as GA equivalents (GAEs), mg GAEs per ml extract or per mg sample.

Determination of total flavonoid content of the prepared extracts

The total flavonoid content was determined by the method based on that reported by SB.[31] To 125 µl of sample 1.025 ml of distilled water and 37.5 µl of 5% sodium nitrite are added and allowed incubation time of 5–6 min at room temperature. To this 75 µl of 10% aluminum chloride and incubated for 5 min at room temperature followed by addition of 0.25 ml of 1 N sodium hydroxide. The absorbance is measured at 510 nm. Quercetin was used as the standard for the calibration curve. The result is expressed as mg flavonoid per ml of extract or per mg of sample.

Determination of DPPH radical scavenging activity of the extracts

Quantitative estimation of the DPPH radical scavenging activity of the extracts was based on the previously reported work of Mridha *et al.*[32] and Al-Tamimi *et al.*[33] with a few modifications. DPPH solution was prepared by adding 6 mg of DPPH in 100 ml methanol. To 30 µl of sample 3 ml of DPPH is added. The absorbance was measured at 517 nm using 3 ml DPPH solution in 30 µl of sample as control and methanol as the reference for the spectrophotometer. The DPPH scavenging activity was calculated using the equation: Percentage of antioxidant activity = $([A_c - A_s]/A_c) \times 100$; where A_c represents the absorbance of the control and A_s is the absorbance of the sample.

Determination of glucose uptake

The preliminary results showed that the phenol and flavonoid content and the antioxidant activity of three aqueous extracts of leaves are the highest. These are FL001 (extract prepared by soaking the leaves at room temperature for 1 day), FL002 (extract prepared by heating the leaves at 100°C for 10 min), and FL003 (extract prepared by heating at 100°C for 10 min and soaking for 24 h before filtering). Hence, the glucose uptake enhancement potential of the above three samples was observed.

Glucose uptake was performed using the method described by Chowdhury *et al.*[34] and Gulati *et al.*[35] with minor modification.

At day 9 of differentiation, the adipocytes were incubated for 24 h with the respective test solutions (10 µg/mL).[36] The medium or 1X phosphate buffered saline (PBS) was used as negative control and metformin as a positive control. Its concentration was 10 µM; the second positive control used was insulin (10 µM). Twenty-four hours later, the cells were rinsed with 1X PBS and incubated for 60 min at 37°C in DMEM containing 80 µM of the fluorescent glucose analog, 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino)-2-Deoxyglucose (2-NBDG) and in the presence of the extracts for basal glucose uptake measurement but in the absence of light. The cells were then treated with insulin - the second positive control, during the 2-NBDG incubation to measure the insulin-stimulated glucose uptake. The reaction of 2-NBDG uptake was terminated by washing the cells with precooled 1X PBS. The remaining fluorescence activity in the cells was measured by using fluorescence microplate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Fluorescence activity in the absence of 2-NBDG was subtracted from all values.

RESULTS

Total phenol content

Phenolic is partly responsible for the antioxidant activity of plants.[30] Hence, the determination of the phenol content is an essential step in understanding the antioxidant properties of the compounds. As shown in Figure 1, it has been observed that the highest amount of phenol was observed in the extract, FLW (0.4608 ± 0.015 GA eqs mg/ml). The descending order of phenol content of rest of the fenugreek leaves samples treated at different time-temperature combinations are 60FLW >100FLW>80FLW>37FLW>24FLW. In case of fenugreek seeds the phenol, content was highest in FSW (0.43 ± 0.021). The phenol concentrations of the rest of the fenugreek seed extracts in descending order are 100°C – 10 min >60°C – 60 min >24°C – 1 day >80°C – 30 min >37°C – 5 h [Figure 1].

Total flavonoid content

The TPC assay revealed that the highest content of flavonoid has been observed in the extract FLW, 1.30 ± 0.044 Quercetin Equivalents mg/ml. The flavonoid content of the rest of the aqueous extract of the fenugreek leaves are 100FLW > 37FLW > 80FLW > 60FLW > 24FLW. Among the fenugreek seeds aqueous extract, the highest flavonoid content has been observed in the FLW, 1.023 ± 0.0056 Quercetin Equivalents mg/ml. The flavonoid content of the rest of the aqueous fenugreek seeds extract can be arranged in decreasing order as 24FSW > 100FSW > 80FSW > 37FSW > 60FSW [Figure 2].

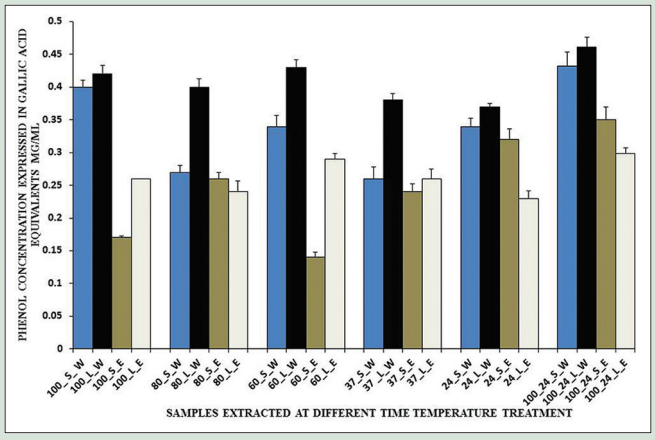


Figure 1: The total phenol concentration of fenugreek seeds and leaves in water and ethanol extracts at different time-temperature treatment expressed as gallic acid equivalents. The results represent mean ± standard division of three independent experiments

Total antioxidant content

Recent studies show that oxidative stress can be a cause for increasing insulin resistance.[12] Hence, antioxidants can be used as a treatment of diabetes. DPPH radical scavenging activity was performed to determine the antioxidant activity of the extracts. The highest antioxidant activity has been observed in the sample FLW, 45.41% ±2.10%. The antioxidant activity of the rest of the aqueous fenugreek leaves extract in descending order is 80 FLW > 37 FLW > 100 FLW > 60 FLW > 24 FLW. The highest antioxidant activity in case of fenugreek seeds has been observed in the FSW extract, 30.33% ±0.83%. The antioxidant activity of the rest of the aqueous fenugreek seeds extract in descending order is as follows: 100FSW > 37FSW > 80FSW > 24FSW > 60FSW [Figure 3].

Glucose uptake

The effects of the extracts on 3T3-L1 cell lines for glucose uptake activity both in the presence and in the absence of insulin stimulation expressed as relative fluorescence intensity, in percentage was measured [Figure 4]. The M− and M+ bar graph represents the effect of the control on the 3T3-L1 Cell lines in the absence and presence of insulin stimulation, respectively. The graph also depicts the effect of Metformin as represented as Mt− and Mt+ with or without insulin stimulation on the cell lines. The rest of the bar graphs show the effects of the extracts FL001, FL002, and FL003 at two different concentrations 10 μg/ml and 100 μg/ml and in the absence and presence of insulin stimulation each. The extract FL001 was prepared by soaking the fenugreek leaves in water at room temperature for 24 h. The extract FL002 was prepared by heating the leaves in water for 10 min and filtering, while the extract FL003 was prepared by heating the leaving for 10 min and letting it soak for 24 h before filtering.

On comparative analysis with the blank without insulin stimulation, it can be observed that the extracts FL001, FL002, and FL003 show enhanced glucose uptake activity without insulin stimulation. The highest activity is observed in case of FL003 in case of both the concentrations, i.e., 10 and 100 μg/ml. The extract FL003 at 100 μg/ml shows the greatest glucose uptake activity among all, 215% ±5.87 in the absence of insulin. The activity of the extracts without insulin stimulation in descending order is as follows: FL003 (100) >FL002 (100) >FL001 (100) > Fl003 (10) >FL002 (10) >FL001 (10) >M−. This shows that the glucose uptake activity of all the extracts at 100 μg/ml concentration in the absence of insulin is greater than those at the 10 μg/ml concentration in each case, FL001, FL002, and FL003.

Second, on comparing the glucose uptake activity of the control, in the presence of insulin, M+, with the rest of the extracts, it can be observed that a similar trend is followed with a few notable exceptions. The highest glucose uptake activity is found for the extract FL003 at 100 μg/ml in the presence of insulin stimulation, 254% ± 4.88%. The activity of all the extracts in comparison with the blank in the presence of insulin can be arranged in descending order as follows: FL003 (100) >FL002 (100) >FL003 (10) >FL001 (100) > FL002 (10) >M+>FL001 (10). It can be concluded that the glucose uptake activity of FL003 at 10 μg/ml concentrations is more than those of FL002 at 10 μg/ml and FL001 at both 100 and 10 μg/ml. Furthermore, the glucose uptake activity of Fl001 at 100 μg/ml is more than that of Fl002 at 10 μg/ml. Another exception is that the glucose uptake activity of the extract FL001 at 10 μg/ml is less than that of the control with insulin stimulation.

On comparison with Metformin Mt−, in the absence of insulin stimulation, it is found that the highest glucose activity is found in case of Fl003 at 100 μg/ml concentrations. In this comparative analysis, the glucose uptake activities of all the extracts at 100 μg/ml concentrations are higher than those at 10 μg/ml. In addition, the glucose uptake activity of the extracts FL001 and FL002 at 10 μg/ml do not show much variation. The glucose uptake activity of the extracts on the 3T3-L1 cell lines can be arranged in the following manner FL003 (100) >FL002 (100) >FL001 (100) >FL003 (10) >FL002 (10) > FL001 (10) >Mt− in descending order.

On analyzing the values of Metformin with insulin stimulation, Mt + and comparing it with those of the extracts, the best result is obtained in case of FL003 at 100 μg/ml. The glucose uptake activity of all the extracts can be arranged as FL003 (100) > FL002 (100) >FL003 (10) >FL001 (100) >FL002 (10) >Mt+>FL001 (10). It can be observed that the glucose uptake activity of FL001 at 10 μg/ml concentrations is less than that of the Metformin in the presence of insulin. Furthermore, the extract FL003 at 10 μg/ml shows enhanced glucose uptake activity than FL002 at 10 μg/ml and FL001 at both 10 and 100 μg/ml concentrations. Hence, in this case, it cannot be concluded that all the extracts at 100 μg/ml concentrations showed enhanced glucose uptake activity than all the extracts at 10 μg/ml.

On comparative analysis of the concentrations of the extracts in the absence of insulin, all the extracts at 100 μg/ml show higher glucose

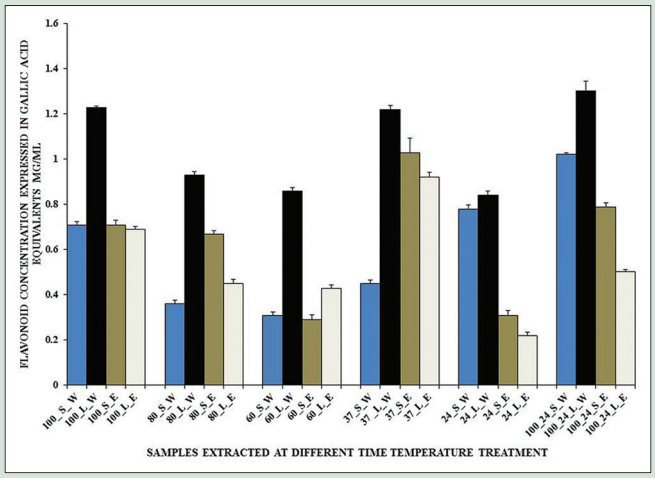


Figure 2: The total flavonoid concentration of fenugreek seeds and leaves in water and ethanol extracts, at different time-temperature treatment. The results represent mean ± standard division of three independent experiments

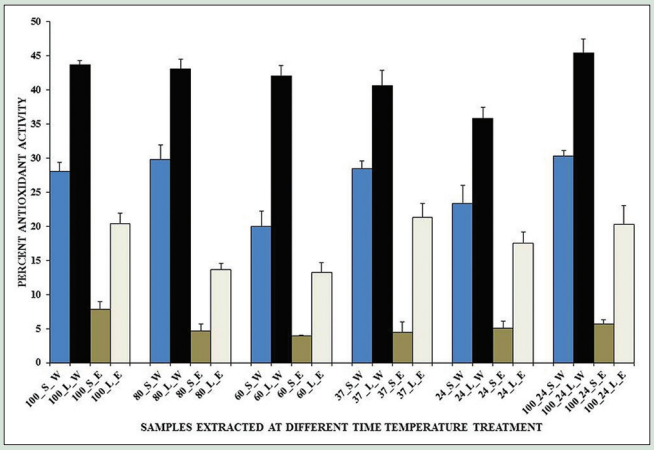


Figure 3: The 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity of fenugreek seeds and leaves in water and ethanol extracts at different time-temperature treatment. The result represents mean ± standard division of three independent experiments

uptake activity than those at 10 μg/ml. FL003 (100) >FL002 (100) >FL001 (100) >FL003 (10) >FL002 (10) >FL001 (10).

However, when the cells are stimulated with insulin, we find that FL003 (100) >Fl002 (100) >FL003 (10) >FL001 (100) >FL002 (10) >FL001 (10). In this case, the FL003 at 10 μg/ml has higher concentration than FL002 at 10 μg/ml and the FL001 at both 10 and 100 μg/ml concentrations. Similarly, the extract FL001 at 100 μg/ml concentration shows enhanced glucose uptake activity as compared to FL002 at 10 μg/ml.

The data taken together show that in case of both with and without insulin stimulation and at both the concentrations, i.e., 10 μg/ml and 100 μg/ml the extract, which was boiled in water for 10 min and soaked for 24 h before filtering, FL003 provides the best results [Figure 4].

Relative percentage increase in glucose uptake by the 3T3-L1 cell lines in response to the extracts FL001, FL002, and FL003 at two different concentrations each has been observed [Figure 5]. The percentage increase in glucose uptake, in this case, has been compared with the control without insulin stimulation. The extract FL003 at 100 μg/ml concentration shows the best result with 115% increase in glucose uptake. The lowest has been observed for the FL001 at 10 μg/ml extract.

Next, we evaluated the relative percentage increase in glucose uptake by 3T3-L1 cell lines in response to extracts in comparison with control with insulin stimulation. The highest percentage of relative glucose uptake has been observed in case of FL003 at 100 μg/ml, 84.06%. The extract FL001 at 10 μg/ml shows negative relative percentage uptake when compared to the control when the cells are stimulated with insulin [Figure 6].

The highest value of relative percentage of glucose uptake with respect to metformin treatment in the absence of insulin stimulation was observed for the extract FL003 at 100 μg/ml, 106.73%. The FL001 at 10 μg/ml show the lowest relative percentage glucose uptake, 7.69% [Figure 7].

Similarly, the relative percentage increase in glucose uptake by insulin-stimulated 3T3-L1 cell lines on treatment with the extracts, with respect to metformin has been observed [Figure 8]. The highest relative activity has been observed in case of the FL003 extract at 100 μg/ml, 68.21%. The extract FL001 at 10 μg/ml showed negative relative percentage glucose uptake by insulin-stimulated cell lines with respect to metformin.

All the above results show that the extract FL003 provide the highest relative glucose uptake both in comparison with control and metformin and in absence or presence of insulin. Hence, a comparative analysis of the relative percentage of glucose uptake by the cells on addition of FL003 extract with respect to the control, both in the absence and presence of insulin, and metformin also in the absence and presence of insulin [Figure 9]. The extract shows more than 60% increase in glucose uptake by 3T3-L1 cell lines on comparison with metformin.

DISCUSSION

DM is an emerging health disorder worldwide. Every year there are more and more reports of people suffering from this deadly disease. Many drugs are available in the market, which is used to treat diabetes. However, over the years, it has been realized that these drugs come with a huge toll on health. They cause many secondary health problems one of them being obesity and slowly damage the liver. Hence, with time, there has been a shift to traditional medicines. These have been known since time immemorial but were never fully explored. Traditional medicines from plant sources have tremendous potential to cure diabetes. Among them, fenugreek is known to possess antidiabetic potential.

The current study contributes to the scientific knowledge regarding the phenol and flavonoid content and the antioxidant activity of fenugreek seeds and leaves. The study also helps to conclusively proof that the fenugreek leaves can also enhance glucose uptake in 3T3-L1 cell lines.

The temperatures that have been used are such that they can be achieved by a person at home. 100° is the temperature that will be attained when the water will boil at 80°C when the stove will be in medium flame and 60°C when the flame will be at its minimum power. The 37°C will be attained when the seeds and leaves will be dissolved in lukewarm water or when it has been kept under sun for 6 h.

On analyzing the results, it is found that enhanced phenolic content, flavonoid content and DPPH radical scavenging activity has been found in the leaves extract as compared to the seeds. This can be conclusively stated for all the six treatments. In each of these cases, quantitatively the

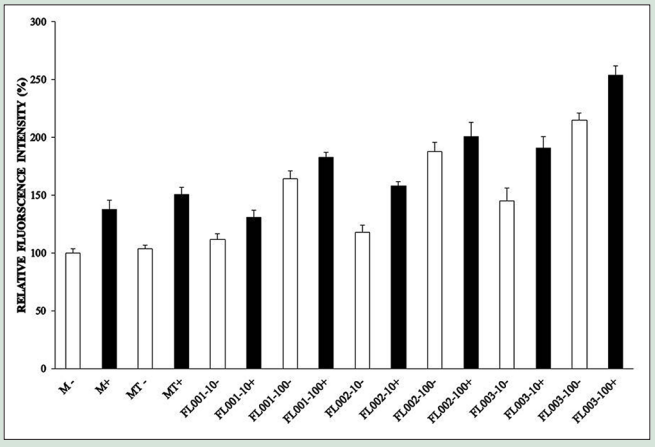


Figure 4: Effect of fenugreek leaves extract at different concentrations on basal and insulin-stimulated glucose uptake in 3T3-L1 adipocyte cells. The results represent mean ± standard division of three independent experiments. M+-only medium, MT-Metformin, FL001-the fenugreek leaves extracted by soaking for 24 h at room temperature; FL002-fenugreek leaves treated at 100°C for 10 min; FL003-the fenugreek leaves treated at 100°C for 10 min and soaked for 1 day before filtering; (+) sign-insulin stimulated treatment; (–) sign-without insulin; (10) and (100) denote the concentrations of the samples

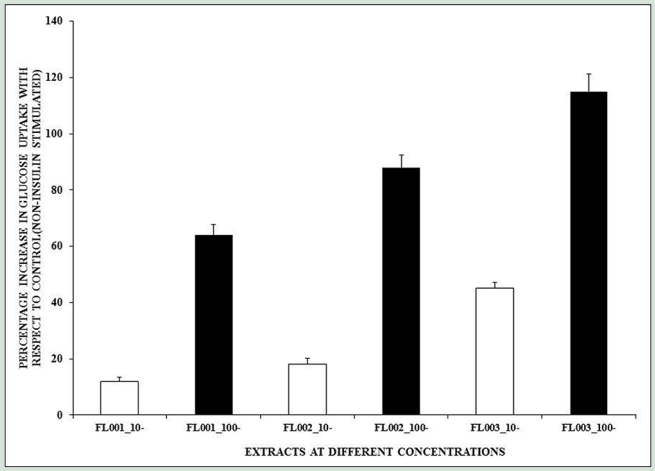


Figure 5: Relative percentage increase in glucose uptake by 3T3-L1 cell lines in response to extracts in comparison with control (noninsulin stimulated [–]). FL001_10-represents the extract FL001 at 10 μg/m;, FL001_100-, extract FL001 at 100 μg/ml; FL002_10-, extract FL002 at 10 μg/ml; FL002_100-extract FL002 at 100 μg/ml; FL003_10-, extract FL003 at 10 μg/ml and FL003_100-, extract FL003 at 100 μg/ml. The results represent mean ± standard division of three independent experiments

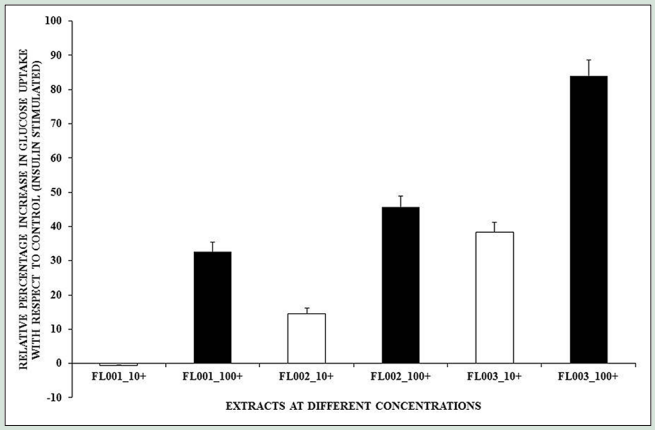


Figure 6: Relative percentage increase in glucose uptake by 3T3-L1 cell lines in response to extracts in comparison with control (insulin-stimulated [+]). The FL001_10+ and FL001_100+ denotes the extract FL001 at 10 μg/ml and 100 μg/ml; FL002_10+ and FL002_100+ denotes the extract FL002 at 10 and 100 μg/ml; FL003_10+ and FL003_100+ denotes the extract at concentration 10 and 100 μg/ml. The results represent mean ± standard division of three independent experiments

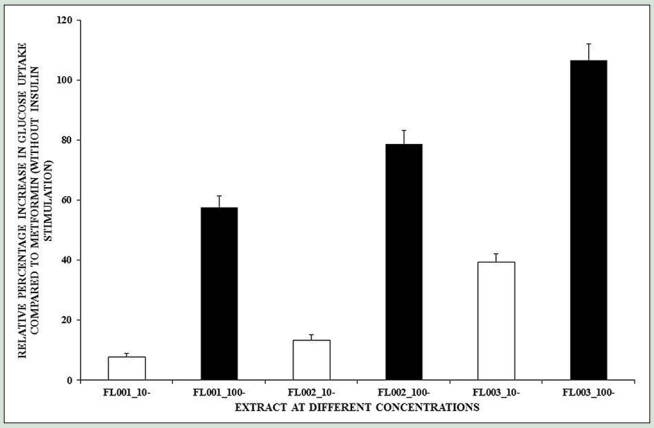


Figure 7: Relative percentage increase in glucose uptake by 3T3-L1 cell lines in response to extracts in comparison with metformin (without insulin stimulation [-]). FL001_10-and FL001_100-represent the extract FL001 at 10 and 100 μg/ml; FL002_10-and FL002_100-represent the extract FL002 at 10 and 100 μg/ml; FL003_10-and FL003_100-represent the extract FL003 at 10 and 100 μg/ml. The results represent mean ± standard division of three independent experiments

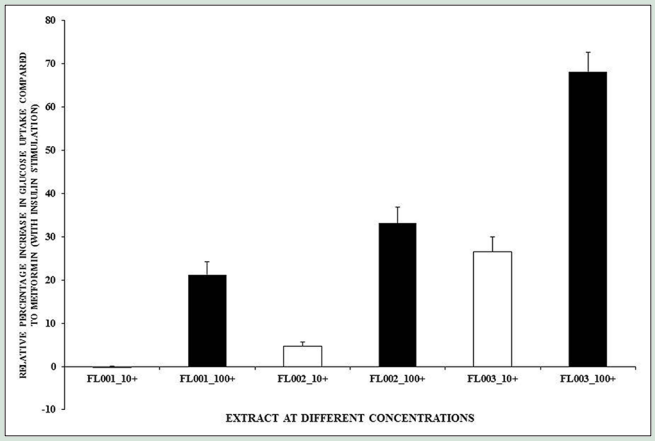


Figure 8: Relative percentage increase in glucose uptake by 3T3-L1 cell lines in response to extracts in comparison with metformin (with insulin stimulation [+]). FL001_10+ and FL001_100+ represent the extract FL001 at 10 and 100 μg/ml; FL002_10+ and FL002_100+ represent the extract FL002 at 10 and 100 μg/ml; FL003_10+ and FL003_100+ represents the extract FL003 at 10 and 100 μg/ml. The results represent mean ± standard division of three independent experiments

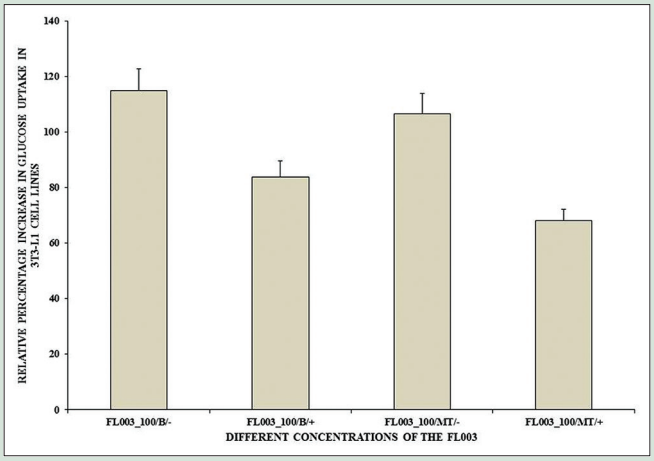


Figure 9: Comparative analysis of the relative percentage increase in glucose uptake by 3T3-L1 cells on addition of FL003 at 100 μg/ml w. r. t. control without insulin, FL003/C/-; w. r. t. control with insulin FL003/C/+; w. r. t metformin without insulin FL003/MT/-; and w. r. t metformin with insulin FL003/MT/+. The results represent mean ± standard division of three independent experiments

leaves yield much more than the seeds samples subjected to the same treatments. The highest concentration of phenols, flavonoids, and the percentage antioxidant activity are found in the extract that is obtained by the Set 6 treatment or FSW.

The water extract contains quantitatively higher concentrations of phenolics, flavonoid, and DPPH radical scavenging activity value than the ethanolic extracts. All the data are taken together proof that water extract of fenugreek leaves show enhanced concentrations of phenols, flavonoids, and antioxidants activity.

Conclusively, it can be recommended that the Set 6 treatment or FSW is the best method for extraction of the above-mentioned phytochemicals, yielding aqueous extracts with the highest concentrations of the desired phytochemicals. The phenol and flavonoid concentration of the aforementioned extract is 46.08 ± 0.15 mg of GAEs per gram of the sample, and 13.02 ± 0.44 mg of flavonoid concentrations per g of sample, respectively. The antioxidant activity, measured in terms of DPPH radical scavenging activity, of this extract is 45.41% ± 2.1%.

As mentioned earlier three types of fenugreek leaves extracts, namely FL001, FL002, and FL003 have been tested on the 3T3-L1 cell lines for measuring the glucose uptake activity. This helped to realize the effect of the extract on the cells for glucose uptake on insulin stimulation and without insulin. The result shows that the extract FL003 shows 115% increase in glucose uptake at 100 μg/ml when compared with the blank in the absence of insulin stimulation. On comparison with the blank when the cells are stimulated with insulin, the extract FL003 at 100 μg/ml shows 84.06% glucose uptake activity. This extract showed 106.73% increase in glucose uptake without insulin stimulation as against

metformin and 68.21% increase with insulin stimulation compared to metformin.

From the above discussions, it can be conclusively stated that fenugreek leaves on boiling in water for 10 min and letting it soak for 24 h before filtering shows enhanced glucose uptake activity when compared to the rest. It also has the highest phenol and flavonoid content and antioxidant activity.

Acknowledgment

The authors are indebted to UGC, UGC-UPE, and UGC-CAS program at the Department of Botany, University of Calcutta for their support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hasan M, Rahman M. Effect of fenugreek on type 2 diabetic patients. Int J Sci Res Publ 2016;6:2250-3153.
2. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2010;33 (Suppl 1):S62-S69.
3. Lindberg G, Lindblad U, Melander A. Sulfonylureas for treating type II diabetes mellitus. Cochrane Database Syst Rev 2002;Issue 4.
4. Mane K, Chaluvaraju K, Niranjan M, Zaranappa T, Manjuthej T. Review of insulin and its analogues in diabetes mellitus. J Basic Clin Pharm 2012;3:283-93.
5. Jangir RN, Jain GC. Evaluation of antidiabetic activity of hydroalcoholic extract of *Cassia fistula* Linn. pod in streptozotocin-induced diabetic rats. Pharmacogn J 2017;9:599-606.
6. Asadi-Samani M, Moradi MT, Mahmoodnia L, Alaei S, Asadi-Samani F, Luther T, *et al*. Traditional uses of medicinal plants to prevent and treat diabetes; an updated review of ethnobotanical studies in Iran. J Nephropathol 2017;6:118-25.
7. Mirhoseini M, Baradaran A, Rafieian-Kopaei M. Medicinal plants, diabetes mellitus and urgent needs. J Herbmed Pharmacol 2013;2:53-4.
8. Suji G, Sivakami S. Approaches to the treatement of diabetes mellitus: An overview. Cell Mol Biol (Noisy-le-grand) 2003;49:635-9.
9. Achari AE, Jain SK. L-cysteine supplementation increases insulin sensitivity mediated by upregulation of GSH and adiponectin in high glucose treated 3T3-L1 adipocytes. Arch Biochem Biophys 2017;630:54-65.
10. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev 2005;26:19-39.
11. Montonen J, Knekt P, Järvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 2004;27:362-6.
12. Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab 2012;16:S267-71.
13. Manna P, Achari AE, Jain SK. Vitamin D supplementation inhibits oxidative stress and upregulate SIRT1/AMPK/GLUT4 cascade in high glucose-treated 3T3L1 adipocytes and in adipose tissue of high fat diet-fed diabetic mice. Arch Biochem Biophys 2017;615:22-34.
14. Prabhakar, P. K. Doble M. Mechanism of action of natural products used in the treatment of diabetes mellitus. Chin J Integr Med 2011;17:563–574.
15. Ota A, Ulrih NP. An overview of herbal products and secondary metabolites used for management of type two diabetes. Front Pharmacol 2017;8:436.
16. Pradeep SR, Srinivasan K. Amelioration of hyperglycemia and associated metabolic abnormalities by a combination of fenugreek (*Trigonella foenum-graecum*) seeds and onion (*Allium cepa*) in experimental diabetes. J Basic Clin Physiol Pharmacol 2017;28:493-505.
17. Mandegary A, Pournamdari M, Sharififar F, Pournourmohammadi S, Fardiar R, Shooli S, *et al*. Alkaloid and flavonoid rich fractions of fenugreek seeds (*Trigonella foenum-graecum* L.) with antinociceptive and anti-inflammatory effects. Food Chem Toxicol 2012;50:2503-7.
18. Pournamdari M, Mandegary A, Sharififar F, Zarei G, Zareshahi R, Asadi A, *et al*. Anti-inflammatory subfractions separated from acidified chloroform fraction of fenugreek seeds (*Trigonella foenum-graecum* L.). J Diet Suppl 2018;15:98-107.
19. Yadav UC, Baquer NZ. Pharmacological effects of *Trigonella foenum-graecum* L. In health and disease. Pharm Biol 2014;52:243-54.
20. Olaiya CO, Soetan KO. A Review of the Health Benefits of Fenugreek (*Trigonella foenum-graecum* L.): Nutritional, Biochemical and Pharmaceutical Perspectives. AJSIH 2014;3-12.
21. Nathiya S, Durga M, Devasena T. Therapeutic role of *Trigonella foenum graecum* [fenugreek] a review. Int J Pharm Sci Rev Res 2014;12:74-80.
22. Hannan JM, Ali L, Rokeya B, Khaleque J, Akhter M, Flatt PR, *et al*. Soluble dietary fibre fraction of *Trigonellafoenum-graecum* (fenugreek) seed improves glucose homeostasis in animal models of type 1 and type 2 diabetes by delaying carbohydrate digestion and absorption, and enhancing insulin action. Br J Nutr 2017;97:514-21.
23. Murlidhar M, Goswami TK. A review on the functional properties, nutritional content, medicinal utilization and potential application of fenugreek. J Food Process Technol 2012;3:9.
24. Ahmad A, Alghamdi SS, Mahmood K, Afzal M. Fenugreek a multipurpose crop: Potentialities and improvements. Saudi J Biol Sci 2016;23:300-10.
25. Kwon Y, Kim H, Park S, Jung S. Enhancement of solubility and antioxidant activity of some flavonoids based on the inclusion complexation with sulfobutylether β-cyclodextrin. Bull Korean Chem Soc 2010;31:3035-7.
26. Eidi A, Eidi M, Sokhteh M. Effect of fenugreek (*Trigonellafoenum-graecum* L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats. Nutr Res 2007;27:728-33.
27. Yadav M, Tomar R, Prasad GB, Jain S, Yadav H. Complementary hypoglycemic and anti-hyperglycemic activity of various extracts of fenugreek seeds in rats. Asian J Biochem 2008;3:182-7.
28. Mashkor Idries MA. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res 2014-15;6:841-4.
29. Chakraborty T, Pal Basak A, Mridha A, Gopal PK, Paul S. Anti-cancer and anti-oxidant potential of Indian carpet weed *Glinus oppositifolius* (L.) Aug. DC. J Pharmacogn Phytochem 2017;6:464-8.
30. Yogesh K, Jha SN, Ahmad T. Antioxidant potential of aqueous extract of some food grain powder in meat model system. J Food Sci Technol 2014;51:3446-51.
31. Bukhari SB, Bhanger MI, Memon S. Antioxidative activity of extracts from fenugreek seeds (*Trigonellafoenum-graecum*). Pak J Anal Environ Chem 2008;9:78-83.
32. Mridha A, Nandi C, Pal R, Paul S. Studies on few fresh water green algal species reveals *Spirogyra triplicata* as the repository of high phenolic and flavonoid content exhibiting enhanced anti-oxidant property. J Pharmacogn Phytochem 2017;6:1291-7.
33. Al-Tamimi MA, Rastall B, Abu-Reidah IM. Chemical composition, cytotoxic, apoptotic and antioxidant activities of main commercial essential oils in palestine: A comparative study. Medicines (Basel) 2016;3. pii: E27.
34. Chowdhury A, Kunjiappan S, Bhattacharjee C, Somasundaram B, Panneerselvam T. Biogenic synthesis of *Marsilea quadrifolia* gold nanoparticles: A study of improved glucose utilization efficiency on 3T3-L1 adipocytes. *In Vitro* Cell Dev Biol Anim 2017;53:483-93.
35. Gulati V, Gulati P, Harding IH, Palombo EA. Exploring the anti-diabetic potential of Australian aboriginal and Indian ayurvedic plant extracts using cell-based assays. BMC Complement Altern Med 2015;15:8.
36. Shim EH, Lee MS, Lee JA, Lee H. Do in seung GiTang extract suppresses adipocyte differentiation in 3T3L1 cells. Mol Med Rep 2017;15:3549-54.