Asian Pacific Journal of Tropical Biomedicine

Original Article

doi: 10.4103/2221-1691.260396 www.apjtb.org

Trigonella foenum-graecum seed extract modulates expression of lipid metabolism-related genes in HepG2 cells

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ARTICLE INFO

Article history:
Received 14 December 2018
Revision 9 January 2019
Accepted 30 May 2019
Available online 17 June 2019

Keywords:
Fenugreek seed extract
Diosgenin
4-Hydroxy-*L*-isoleucine
Dyslipidemia
Obesity

ABSTRACT

Objective: To investigate anti-dyslipidemic effects of hydroalcoholic fenugreek seed extracts, diosgenin, and 4-OH-Ile on HepG2 cell line.

Methods: HepG2 cells were treated with hydroalcoholic fenugreek seed extracts, diosgenin, 4-OH-Ile, and orlistat. IC_{20} was calculated using the MTT method. The cells were then pre-treated with IC_{20} concentrations for 24 and 48 h. Real time PCR was employed to measure expression of liver X receptor alpha (*LXR α*), sterol regulatory element-binding protein-1C (*SREBP-1C*), acetyl-CoA carboxylase (*ACC*), fatty acid synthase (*FAS*), fibroblast growth factor 21 (*FGF21*), peroxisome proliferator-activated receptor gamma (*PPAR γ*), and low-density lipoprotein receptor (*LDLR*).

Results: The results showed that *LXR α* (P=0.003, P<0.001), *SREBP-1C* (P<0.001, P<0.001), *ACC* (P=0.002, P=0.006), and *FAS* (P<0.001, P<0.001) were downregulated significantly, while *FGF21* (P<0.001, P<0.001), *PPAR γ* (P=0.004, P<0.001), and *LDLR* (P<0.001, P<0.001) were upregulated significantly in HepG2 cells treated with the IC_{20} of hydroalcoholic fenugreek seed extracts, diosgenin, 4-OH-Ile, and orlistat in 24 and 48 h, respectively.

Conclusions: Hydroalcoholic fenugreek seed extracts, diosgenin, and 4-OH-Ile significantly modulate the expression of some important lipid metabolism related genes, which is similar to orlistat. *Trigonella foenum-graecum* seed extract or its derivatives should be further investigated for their dyslipidemia effects and its complications.

1. Introduction

Dyslipidemia is an abnormal lipid and lipoprotein metabolism, which leads to an increase in the total cholesterol, low density lipoprotein, triglycerides, and a decrease in the high-density lipoprotein cholesterol[1], being associated with diabetes, cardiovascular diseases, inflammation, obesity, and some related

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Funding: This work was supported by the RUMS by the grant number 20.1094

How to cite this article: Mohammad-Sadeghipour M, Mahmoodi M, Falahati-pour SK, Khoshdel A, Fahmidehkar MA, Mirzaei MR, et al. *Trigonella foenum-graecum* seed extract modulates expression of lipid metabolism-related genes in HepG2 cells. Asian Pac J Trop Biomed 2019; 9(6): 240-248.

disorders[2]. The prevalence of obesity and dyslipidemia is on the rise throughout the world every year[3]. Obesity is a chronic disease[4] that occurs due to the imbalance between energy intake and energy consumption[5]. Over the years, many treatments have been introduced to manage dyslipidemia and obesity, including statins, HMG-CoA reductase inhibitors, as well as niacin as a lipase inhibitor in the adipose tissue, omega-3 fatty acid, and fibrates[6–8]. Orlistat is currently one of the medicines approved by the Food and Drug Administration for the treatment of obesity, with the commercial brand of Xenical, which acts as a potent, specific, and irreversible inhibitor of pancreatic and gastrointestinal carboxyl ester lipase[9–11]. However, it has an unpleasant effect on the gastrointestinal tract (steatorrhea)[10]. Evidence has revealed that medicinal plants, such as fenugreek can decrease the incidence rate of various diseases and play an important role in the management of dyslipidemia and obesity as well as associated complications through exerting protective effects on the oxidative damage and reducing inflammation; besides, it can be a suitable alternative for chemical drugs[12,13].

Fenugreek (*Trigonella foenum-graecum*) is an annual plant in the family of Leguminosae[14]. It is native to the eastern shores of the Mediterranean and North Africa. According to some researchers, fenugreek was initially native to Iran and then distributed to other regions[15]. The chemical composition of this plant consists of diosgenin, 4-OH-Ile, fiber[16], galactomannan[17], terpenes[18], trigonellin, sapogenins, and mucilage[19]. Based on past research, the long-term use of fenugreek controls appetite and helps in the treatment of obesity[20–23]. Other studies have shown that the use of the fenugreek seed extract leads to a decrease in the blood glucose, total cholesterol, triglycerides, and low density lipoprotein in types Ⅰ and Ⅱ diabetes mellitus[24–26]. Studies have shown that diosgenin [(25R)-5-Spirosten-3 β -ol, 3 β -Hydroxy-5-spirostene], (2S, 3R)-2-Amino-4-hydroxy-3-methylpentanoic acid, and 4-OH-Ile are the bioactive compounds present in the fenugreek plant. Diosgenin is a bioactive steroidal saponin in fenugreek[27], and 4-OH-Ile is a branched-chain amino acid found in fenugreek seeds[28]. Diosgenin is known as a lipid-lowering, anti-diabetes, anti-cancer, and anti-oxidative stress agent[16]. In addition, 4-OH-Ile plays a significant role in the improvement of dyslipidemia, diabetes, obesity, and metabolic syndrome[16,29].

The present study explored the alteration of vital genes, including low-density lipoprotein receptor (*LDLR*), liver X receptor alpha (*LXR α*), sterol regulatory element-binding protein-1C (*SREBP-1C*), acetyl-CoA carboxylase (*ACC*), fatty acid synthase (*FAS*), peroxisome proliferator-activated receptor gamma (*PPAR γ*), and fibroblast growth factor 21 (*FGF21*) in the HepG2 cell line, involved in lipid metabolism. In this study, apart from the hydroalcoholic fenugreek seed extract (HFSE), the effects of diosgenin and 4-OH-Ile, as the two major components of fenugreek seeds, were compared with orlistat as a most common hypolipidemic drug.

2. Materials and methods

2.1. Materials

The HepG2 cell line was purchased from the Pasteur Institute (Iran, Tehran). The materials required for cell culture included RPMI-1640, fetal bovine serum, penicillin-streptomycin and trypsin enzymes from Gibco-BRL (Grand Island, NY, USA), dimethyl sulfoxide (DMSO) from Merck (USA), MTT powder, diosgenin with the purity of 93%, 4-OH-Ile with the purity of ≥ 98% (TLC), orlistat with the purity of ≥ 98% from Sigma (Sigma-Aldrich, USA), the RNA extraction and cDNA synthesis kit purchased from PARS Tous (Iran), and the SYBR Green Premix Ex Taq Ⅱ Kit (Takara, Japan), which were all prepared.

2.2. Preparation of HFSE

Fenugreek seeds were taken from the Davaran region located in the Rafsanjan County in Iran and were identified and confirmed by the Botany Research Division of the Vali Asr University of Rafsanjan, Iran. The seeds were dried at room temperature and powdered by a laboratory blender (the Waring Product Division, USA). The extraction process was performed using a Soxhlet extractor (BAKHSHI Laboratory Industrial Co., Iran). Next, 5 grams of the powder were weighed, poured in a paper filter, and placed in the specific part of the device embedded inside the beakers. Two thirds of the beakers were then filled with 70% ethanol and placed in a specific part of the device. The solution started boiling at 80 ℃ for 100 min to deposit a concentrated solution at the bottom of the container. In the next step, the resulting solution was poured in sterilized petri dishes that were placed in a freeze dryer (model: VaCo5-D, Zirbus Technology Co., Germany) at -70 ℃ for 72 h until it turned into a dry yellow and crystalline powder. The extract powder was kept in a freezer at -20 ℃. Other concentrations of the extract were obtained by RPMI 1640.

2.3. Preparation of diosgenin, 4-OH-Ile, and orlistat

Stock solutions were prepared from diosgenin in ethanol[30], 4-OH-Ile in phosphate buffered saline (PBS)[31], and orlistat dissolved in the sterile DMSO[32].

2.4. Cell culture

The liver and hepatocytes play an important role in fat metabolism, since the production of lipids and oxidation of fatty acids occur in them. The HepG2 cell line is suitable for the study of fat metabolism in the liver. Thus, in this study, HepG2 cells were cultured in the RPMI 1640 culture medium containing 10% FBS, 100 IU/mL of penicillin, and 100 g/mL of streptomycin, and then incubated at

37 ℃ at the presence of 5% CO_2 and 95% humidity. When the cell density reached 70%-80%, the treatment would proceed at the different concentrations of HFSE, diosgenin, 4-OH-Ile, and orlistat for 24 and 48 h.

2.5. Cell viability assay

The MTT assay, as a direct colorimetric reaction, was used to study cytotoxic effects and determine the IC_{50} of HFSE, its active components, and orlistat. Furthermore, HepG2 cells (5×10^3 cells per well) were cultured in a 96-well plate with the culture medium containing FBS to let the cells grow and get attached. The plates were incubated at 37 ℃ overnight. On the next day, the wells were washed once with PBS, followed by adding HFSE at various concentrations (0-2 000 μg/mL), as well as diosgenin, 4-OH-Ile, and orlistat at the concentrations of 0-32 μg/mL, 0-16 μg/mL, and 0-48 μg/mL, respectively, on the HepG2 cells. Three replicates were assigned to each dilution. Three wells containing cells and complementary culture medium were considered as controls. The cells were then incubated at 37 ℃ with 5% CO_2 and under the 95% atmospheric pressure. After 24 and 48 hours of incubation, the supernatant was discarded and 10 μL of the MTT solution (5 mg/mL in PBS) was added to each well and incubated at 37 ℃ for 4 h with 5% CO_2 in the dark. Next, the medium containing the MTT was removed carefully, and 100 μL of DMSO was added to each well to dissolve the insoluble purple formazan. The resulting mixture was blended thoroughly by a shaker for 5 min. Next, optical density (OD) was read by the ELISA reader, based on the color intensity of the blue formazan at 570 nm, with the relative growth rate (cell viability) calculated using the following equation[33]:

Relative growth rate (%) = $(OD_{treatment}/OD_{control}) \times 100$

An average inhibitory concentration of 20% (IC_{20}) will result in 80% cell survival. The IC_{20} value was partly non-toxic where the HepG2 cells exhibited an approximate viability of 80%. This concentration was selected for the subsequent treatment in HepG2 cells. To continue the study, HepG2 cells were treated with 300 μg/mL of HFSE, 6.21 μg/mL of diosgenin, 1 μg/mL of 4-OH-Ile, and 6.19 μg/mL of orlistat for 24 and 48 h.

2.6. RNA extraction and cDNA synthesis

After the treatment of HepG2 cells with the IC_{20} concentration of HFSE, its active components, and orlistat, the total mRNA was extracted using the RNA extraction kit (Pars Tous, Iran)[34], according to the manufacturer's instructions. The mRNA quantity was obtained by the NanoDrop device (model: DS-11 FX+ Spectrophotometer/Fluorometer, Avayeh Tejarateh Atiyeh Co., USA), at the wavelength of 260 nm. For this purpose, 1 λ of the RNA was delivered to the device to read OD and the concentration. Diethyl pyrocarbonate solution was used as the blank. The RNA purity was assessed by measuring the absorbance ratio of 260/280 nm, with the OD ratio being between 1.8 and 2 for all samples. The intergrity of RNA was also determined by 1.2% agarose gel electrophoresis. cDNA was synthesized using the Pars Tous Kit, according to the manufacturer's protocol. A total of 3-5 μg of pure mRNA, oligo dT, random hexamer, and reverse transcriptase enzymes were used to synthesize cDNA[35].

2.7. Real time PCR

To determine the expression alteration of the genes *LDLR*, *LXR α*, *SREBP-1C*, *ACC*, *FAS*, *FGF21*, and *PPAR γ*, the real time PCR technique was used. The real time PCR process was followed using specific primers in triplicate. The specificity of the primers designed for each gene was controlled on the basis of BLAST on the NCBI site (Table 1). The *β-actin* gene (housekeeping gene) was used for the normalization purpose as the internal control. The real time PCR process was followed using specific primers and the SyberGreen method by means of the Takara Bio SYBR Green Master Mix Kit (TaKara, Japan) in the final volume of 20 μL, by the ABI Step One Plus™ Real-Time PCR System (Applied Biosystems, USA), according to the following protocol:

1) The initial denaturation of the templates at 95 ℃ for 30 s in one cycle

2) Denaturation at 95 ℃ for 5 s in 40 cycles

3) Annealing/extension for 30-60 s in 40 cycles. At this stage, the temperature of each gene was different as shown below.

LXR α: 57 ℃, *PPAR γ* and *FGF21*: 58 ℃, *SREBP-1C*, *ACC*, and *β-actin*: 60 ℃, *LDLR*: 61 ℃, and *FAS*: 62 ℃.

To estimate the fold change of the genes studied, the threshold cycle (CT) data were analyzed by the software Step One ver.2.3 and the formula $2^{-\Delta\Delta Ct}$. Additionally, the relative levels of target genes were calculated by $2^{-\Delta\Delta Ct}$ where ΔCt= Ct (target genes) – Ct (reference gene) and ΔΔCt= ΔCt (treated groups) - ΔCt [untreated group (Control)][36,37].

Table 1. Nucleotide sequence of primers used in this study.

Gene	Sequence (5′→3′)
LXR α	Forward: TCCCCATGACCGACTGATGT
	Reverse: TCCGGAGGCTCACCAGTTT
SREBP-1C	Forward: TGCAGACAGGGCCTTTGC
	Reverse: CAGTGGGACTGTTGCCAAGA
ACC	Forward: GGATCCGGCGCCTTACTT
	Reverse: CTCCGATCCACCTCATAGTTGAC
FAS	Forward: TTGGAAGGCCTGCATCATG
	Reverse: CACCTGGAGGACAGGGCTTA
FGF21	Forward: GACCAGAGCCCCGAAAGTCT
	Reverse: TTTGAATAACTCCCGGCTTCA
PPAR γ	Forward: TCAGGGCTGCCAGTTTCG
	Reverse: GCTTTTGGCATACTCTGTGATCTC
LDLR	Forward: ACTGGGTTGACTCCAAACTTCAC
	Reverse: GGTTGCCCCCGTTGACA
β-actin	Forward: GATCAGCAAGCAGGAGTATG
	Reverse: GTGTAACGCAACTAAGTCATAG

2.8. Statistical analysis

All data were reported as the mean ± SD of at least three independent experiments. The data were analyzed by the one-way ANOVA (SPSS, version 18.0 for Windows) for cell viability. In addition, for evaluation of gene expression, treated groups were compared with the untreated control group, using a one-way ANOVA accompanied by the Dunnett's *post hoc* test. Also, the *post hoc* Tukey test was used to evaluate different groups to determine significant differences. The independent *t*-test was used to compare schedules in each group. All tests were performed with the significance level of $P<0.05$.

3. Results

3.1. Effects of HFSE, its active components, and orlistat on cell viability

The cell viability percentage decreased in a dose-dependent manner 24 h and 48 h after treatment with HFSE, diosgenin, 4-OH-Ile, and orlistat (Figure 1 A-D). The HFSE, diosgenin, 4-OH-Ile, and orlistat at their respective hightest concentration decreased the cell viability most significantly ($P<0.001$).

3.2. Effects of HFSE, diosgenin, 4-OH-Ile and orlistat on the gene expressions of HepG2 cells

HFSE, diosgenin, 4-OH-Ile, and orlistat groups significantly downregulated the gene expressions of *LXR α* , *SREBP-1C*, *ACC*, and *FAS* for 24 and 48 h, respectively. Meanwhile, these treatment groups significantly upregulated the gene expression of *FGF21*. *PPAR γ* was also found to increase in the groups treated with HFSE, diosgenin, 4-OH-Ile, and orlistat, but there was no significant difference between HFSE and control group for both 24 and 48 h, as well as between 4-OH-Ile and control group for 48 h. Moreover, all treatment groups significantly increased the gene expression of *LDLR*, except HFSE which increased the expression without statistical significance for both 24 and 48 h. Comparing the results of 24 and 48 h in each group, statistically signficant difference was found in the gene expression of *SREBP-1C* in HFSE and 4-OH-Ile groups, *FAS* in diosgenin group, *FGF21* in orlistat and 4-OH-Ile groups, *PPAR γ* in orlistat group as well as *LDLR* in HFSE and 4-OH-Ile groups. On the other hand, the gene expressions of *PPAR γ* ($P=0.043$) and *SREBP-1C* ($P=0.020$) were better in diosgenin group than 4-OH-Ile group in 24 h, respectively. The gene expression of *LDLR* in 4-OH-Ile group increased more significantly than other groups in 24 h and 48 h ($P=0.001$). Moreover, the 48-hour results showed a significant difference in *FGF21* gene between HFSE and diosgenin groups ($P=0.010$) (Table 2).

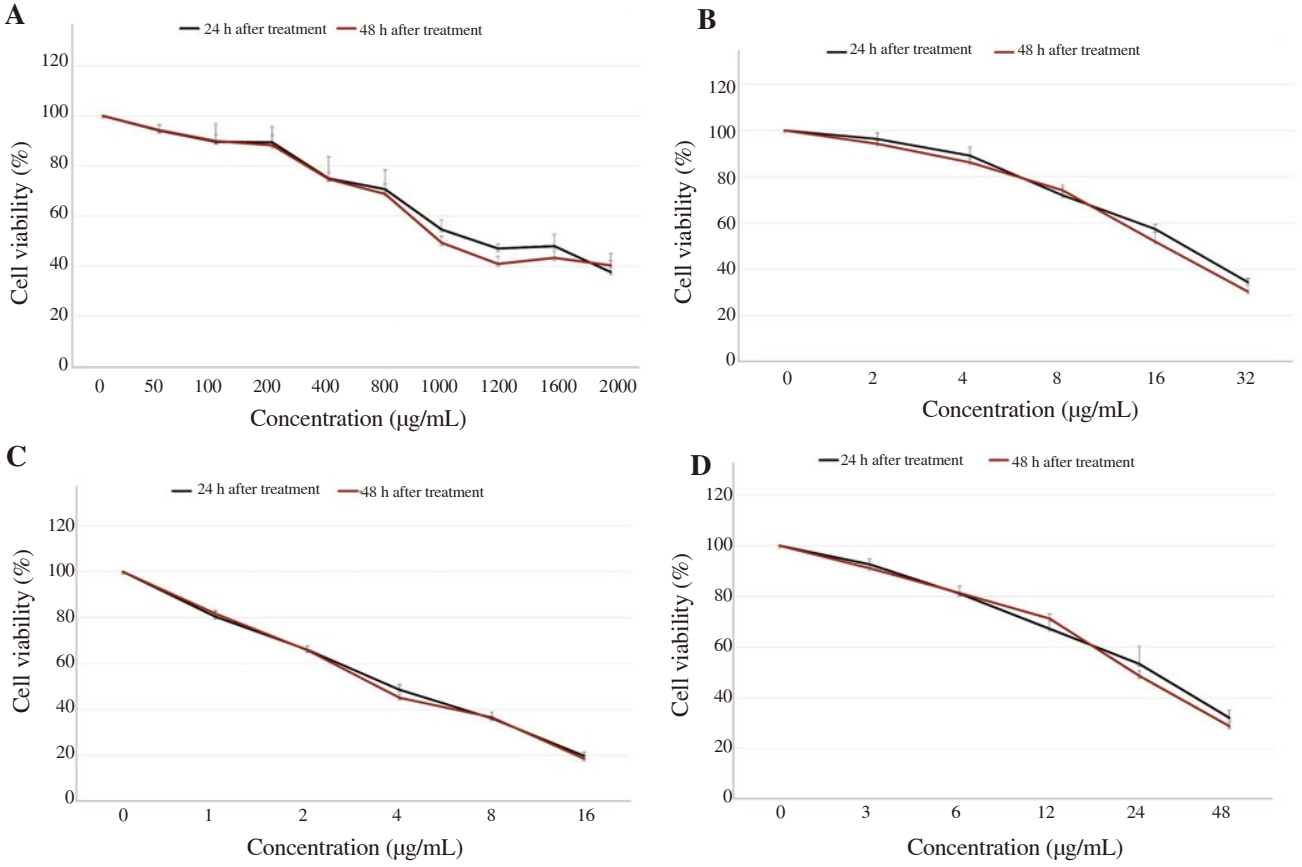

Figure 1. Percentage of viable HepG2 cells following 24 and 48 hours of treatment with hydroalcoholic fenugreek seed extract (A), diosgenin (B), 4-OH-Ile (C), and orlistat (D) in terms of different concentrations measured by MTT assay. Results are obtained from three independent experiments and data are presented as mean ± SD ($P<0.001$).

Table 2. Effects of hydroalcoholic fenugreek seed extract, diosgenin, 4-OH-Ile, and orlistat on lipid metabolism related genes in HepG2 cells (mean ± SD).

Gene		hydroalcoholic fenugreek seed extract	Diosgenin	4-OH-Ile	Orlistat	Control	*P* value
LXR α	24 h	0.371±0.085**	0.250±0.082**	0.318±0.025**	0.234±0.128**	1±0.167	0.003
	48 h	0.314±0.071***	0.186±0.033***	0.228±0.094***	0.279±0.018***	1±0.074	< 0.001
SREBP-1C	24 h	0.401±0.032$^{***\#}$	0.216±0.063$^{***\,\S}$	0.467±0.051$^{***\,\#\#}$	0.276±0.001***	1±0.152	< 0.001
	48 h	0.289±0.036***	0.152±0.020***	0.260±0.029***	0.239±0.067***	1±0.149	< 0.001
ACC	24 h	0.448±0.061**	0.366±0.146**	0.410±0.152**	0.297±0.249**	1±0.149	0.002
	48 h	0.343±0.101*	0.293±0.180**	0.331±0.288**	0.210±0.069**	1±0.304	0.006
FAS	24 h	0.401±0.097***	0.579±0.094$^{**\#}$	0.224±0.103***	0.181±0.029***	1±0.152	< 0.001
	48 h	0.371±0.139***	0.267±0.089***	0.292±0.056***	0.151±0.060***	1±0.212	< 0.001
FGF21	24 h	2.419±0.378**	2.655±0.410***	2.041±0.008$^{**\,\#\#}$	2.901±0.265$^{***\,\#\#}$	1±0.239	< 0.001
	48 h	2.465±0.163***†	3.475±0.028***	2.931±0.101***	4.675±0.493***	1±0.345	< 0.001
PPAR γ	24 h	1.571±0.417	2.805±0.549$^{**\,\S}$	2.283±0.155*	2.494±0.698$^{**\#}$	1±0.094	0.004
	48 h	1.729±0.264	2.704±0.765**	2.185±0.332	4.329±0.764***	1±0.065	< 0.001
LDLR	24 h	1.154±0.217$^{\#\omega}$	2.242±0.279$^{**\,\omega}$	2.470±0.419$^{***\,\#\#}$	2.130±0.282$^{**\omega}$	1±0.145	< 0.001
	48 h	1.828±0.316$^{\omega}$	2.631±0.702$^{**\,\omega}$	3.822±0.278***	2.137±0.477$^{**\omega}$	1±0.025	< 0.001

$^{*}P$<0.05, $^{**}P$<0.01, $^{***}P$<0.001 compared with the untreated control. $^{\#}P$<0.05, $^{\#\#}P$<0.01 compared between 24 and 48 h in the indicated groups. $^{\S}P$<0.05 compared between diosgenin and 4-OH-Ile in 24 h. $^{\dagger}P$=0.010, compared between hydroalcoholic fenugreek seed extract and diosgenin in 48 h. $^{\omega}$4-OH-Ile (P=0.001) compared with other treatment groups in 24 h and 48 h.

4. Discussion

Dyslipidemias are commonly known as multifactorial diseases[38]. In fact, disorder of triglyceride and the cholesterol metabolism results in the abnormal levels of plasma lipids that are the causes of a pathologic state called dyslipidemia. Recent studies have shown a significant relationship between dyslipidemia and obesity[39]. The prevalence of overweight and obesity is increasing at an alarming rate in all societies and age groups around the world. Overweight and obesity are the two major causes of diseases and mortality, which can be prevented[40].

They lead to weight-related diseases and a decrease in the quality of life[41]. The risk of developing diseases such as arthritis, lung diseases, type 2 diabetes, insulin resistance, metabolic syndrome, hypertension, hyperlipidemia, thromboembolism, infertility, coronary heart disease, and the heart failure increases due to overweight and obesity[41,42].

Among synthetic drugs, only orlistat and sibutramine have been approved for the long-term use in the treatment of obesity and overweight, which have very limited efficacy in the treatment of obesity apart from their high cost and significant side effects. Orlistat, Xenical®, and Alli® inhibit triacylglycerol lipases in the gastrointestinal tract. The commercial type of this drug, called Xenical®, is prescribed at the dose of 120 mg, three times a day, and Alli®'s commercial type is prescribed at the dose of 60 mg, three times a day. They have their own side effects, such as soft stool, anal leakage, the potential association with the breast cancer, and the potential deficiencies of lipid soluble vitamins. Therefore, other drugs and methods with fewer complications and more efficacies are required for the treatment of obesity and overweight. Besides, plants are an important source of new drugs for preventing or treating obesity and overweight. The findings of complementary and alternative therapies have revealed that medicinal plants and their active ingredients are safe and effective in achieving weight loss goals and treating obesity[43].

Fenugreek seeds have been used for long for the herbal treatment of metabolic and nutritional disorders. Research has demonstrated that fenugreek seeds exert effects on the eating behavior of animals, and that the fenugreek hydroalcoholic extract at the daily dose of 1 176 mg reduces the daily fat intake in healthy volunteers with a normal weight[44].

Cholesterol homeostasis is regulated at the transcriptional level by *LXR α* [45]. *LXR α* is a nuclear transcription factor that regulates lipid expression by regulating a lipogenic transcription factor (*SREBP-1C*)[46]. It plays a significant role in inducing lipogenic genes in the liver, both through a direct activity to increase the expression of the genes *ACC* and *FAS*, or indirectly by increasing the expression of *SREBP-1*[47]. In the present study, it was observed that HFSE, diosgenin, and 4-OH-Ile decreased the expression of the genes *ACC*, *FAS*, and *SREBP-1C* through the modulation of *LXR α* . The highest reduction rate in the gene expression of *LXR α* was found in orlistat in 24 h (a 4.26 fold) and diosgenin in 48 h (a 5.35 fold), respectively.

SREBP-1C increased the expression of the genes involved in cholesterol synthesis that were downregulated in HepG2 cells treated with HFSE, diosgenin, and 4-OH-Ile. It was shown that the reduction in the expression of *SREBP-1C* and its target genes, such as *ACC* and *FAS*, inhibited lipogenesis[48,49]. The highest reduction rate in the gene expression of *SREBP-1C* was observed in diosgenin for both 24 and 48 h.

These results are partly confirmed by Khound and colleagues[50]. They reported that dietary fenugreek seed inhibits the liver SREBP-1C activity and lipogenesis by an increase in the expression of insulin-inducible gene-1 (Insig-1) and gene-2 (Insig-2) (two proteins regulating the SREBP activity in the liver). Besides, the accumulation of the liver fat and very-low-density lipoprotein secretion decreased significantly. These functions led to an increase in the insulin sensitivity and improved hyperlipidemia. SREBP1-C is the main regulator of FAS and ACC. These enzymes play a major role in fatty

acid biosynthesis[50]. Therefore, in the present study, HFSE and its active ingredients affected the mRNA expression of the genes *FAS* and *ACC* directly, and indirectly, by reducing the expression of the *SREBP-1C* gene leading to a decrease in the expression of the genes *FAS* and *ACC*, thereby improving lipid abnormalities.

According to the currect research, among SREBPs isoforms, including SREBP-1a, SREBP-1c, and SREBP-2, the expression of the genes involved in the synthesis of fatty acids was regulated in particular by SREBP-1c, while SREBP-2 regulated the expression of the genes involved in cholesterol synthesis[51]. In nutritional status, the expression of SREBP-1c is induced by insulin or glucose in the liver and fat tissues. Insulin is the key anabolic hormone that stimulates the expression and activity of SREBP-1c *via* PI3K. The activity of SREBP-1c leads to an increase in the expression of many adipogenic genes, including *FAS*, *ACC*, *SCD1*, and *LPL*, and induces adipogenesis. Therefore, another hypothesis about the mechanism of HFSE and its effective ingredients implies that they inhibit the expression of the gene *SREBP-1c* through the inhibition of PI3K, thereby leading to the improvement of dyslipidemia, which requires further studies.

Previous research showed that diosgenin enhanced the phosphorylation of AMPK and ACC in HepG2 cells and inhibited the accumulation of fats due to the high amount of glucose; it also decreased the expression of *SREBP-1C* and *LXR α* mRNA in the liver cells. Therefore, it can be anticipated that HFSE and its active ingredients cause AMPK phosphorylation, resulting in the phosphorylation of ACC and HMG-CoA reductase. ACC inhibition by AMPK reduces the content of Malonyl-CoA and the synthesis of fatty acids. Besides, the allosteric regulator, carnitine palmitoyl transferase type 1, is activated in response to the reduction in Malonyl-CoA and the increase in the beta-oxidation of fatty acids. Moreover, following the inactivation of HMG-CoA reductase, the synthesis of cholesterol decreases[52].

FGF21 is one of the important targets of PPAR γ [53]. FGF21 is expressed in the liver and regulates lipid metabolism in response to nutritional conditions[54]. Besides, FGF21 regulates liver lipolysis and the use of fatty acids[45]. It has also been shown that FGF21 has the ability to reduce body weight and low-density lipoprotein (LDL) cholesterol, and increase high-density lipoprotein levels in obese humans[55], yet the mechanism is not fully known. A recent study revealed that the gene *FGF21* had at least one LXR response element, and that there was a negative correlation between the level of expression of the gene *LXR α* and the gene *FGF21* in the liver[45]. In the present study, the treatment of HepG2 cells with HFSE, diosgenin, and 4-OH-Ile upregulated the expression of the gene *FGF21*. Diosgenin and 4-OH-Ile exerted a better effect than HFSE in 48 h.

In addition, the findings of this study showed that HFSE, diosgenin, and 4-OH-Ile increased the expression of the genes involved in fatty acid catabolism by the modulation of PPAR γ and the expression of its target genes. Besides, diosgenin and 4-OH-Ile showed a better effect than HFSE in 24 and 48 h.

Another study reported that the aqueous extract of fenugreek seeds improved some parameters, including insulin, glucose, adiponectin, and triglyceride significantly. This study showed the positive effects of fenugreek seeds through three mechanisms, *i.e.* the direct insulin-like effect, the increase in the adiponectin level, and the increase in the expression of the PPAR γ protein[26]. Similar to previous research, the possible cause of the increase in the *PPAR γ* gene in the present study was to justify that HFSE and its active ingredients could reduce the synthesis of fatty acids, increase beta-oxidation, as well as improve dyslipidemia and obesity through two pathways. The first pathway functioned by activating the *PPAR γ* gene, binding it to retinoid X receptor (RXR), attaching this complex to the PPAR response element called PPRE, and transcribing the adiponectin gene, which further reduced the expression of the genes *SREBP-1C*, *ACC*, and *FAS* and increased the expression of the genes *PPAR α* and *ACO* (acyl-CoA oxidase). As a result, the oxidation of lipids increased, and the synthesis of lipids decreased. The second pathway had a direct effect on the expression of the reduction in the genes *ACC*, *FAS*, and *SREBP-1C*, which in turn reduced the synthesis of fatty acids and triglyceride[56].

Research has shown that LXR α forms a heterodimer complex by binding to RXR (a nuclear factor), and then the complex attaches to cysteine elements found in the promoter of the *SREBP-1C* gene and activates the transcription of this gene, thereby regulating lipogenesis. Besides, PPARs, like LXR α , undergoes heterodimerization with the RXR nuclear factor and alters the transcription of the target genes[57]. Thus, by increasing the expression of the *PPAR γ* gene under the influence of HFSE and its active ingredients, it competes in the heterodimerization of the LXR α /RXR complex, thereby resulting in a reduction in the transcription of the *SREBP-1C* gene and its target genes. In general, liver lipid hemostasis is the result of a complex process among a number of transcription factors, including LXR, PPARs, and SREBP.

It was also found that HFSE, diosgenin, and 4-OH-Ile treatments increased significantly the expression of genes coding for the LDL receptor (LDLR) in HepG2 cells. This receptor increased the uptake of LDL and removed cholesterol from it[58]. Among the four substances used, 4-OH-Ile showed a significant effect at 24 and 48 h. These results are consistent with the study by Vijayakumar *et al*, which showed that at the molecular level, the thermostable extract of fenugreek seeds inhibited fat accumulation by modifying the expression of adipogenic factors, such as *aP2*, *GAPDH*, *PPAR γ* , *SREBP-1C*, and *c/EBP α* . It was also demonstrated that the thermostable extract of fenugreek seeds reduced triglyceride and cholesterol levels in HepG2 cells by reducing the expression of the SREBP-1C protein and mRNA. In the sterol-enriched status, this extract adjusted the LDLR expression and increased LDL absorption[2].

Finally, it can be stated that HFSE and its active ingredients

resulted in an increase in the *PPAR γ* gene expression. This increase can be compared with the *LXR α* gene in binding to RXR that leads to a reduction in the expression of the *LXR α* gene. This decrease led to a reduction in the expression of the *SREBP-1C* gene and its target genes, including *ACC* and *FAS*, and it also had a negative correlation with the expression of the *FGF21* gene.

In conclusion, the results of this study showed that HFSE and its two active compounds, *i.e.* diosgenin, and 4-OH-Ile, upregulated and downregulated significantly the expression of some important genes involved in lipid metabolism, similar to orlistat. Such effects are presumably effective in the treatment of dyslipidemia and its complications, including obesity. However, HFSE or its derivatives should be further investigated for these effects. Additional studies are required on treatments in this field to determine the effects of various concentrations of the mentioned substances used on the expression of other genes involved in fat metabolism. In addition, the study of the synergistic effects of HFSE, diosignin, 4-OH-Ile, and orlistat on lipid metabolism is recommended. More laboratory studies on other cell lines, on humans and animals, the evaluation of the results at serum and tissue levels with their comparisons, the use of other techniques, such as determining the level of protein expression, immunohistochemistry, and the use of the Western blot are also recommended in the future by other researchers.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

The authors thank the Molecular Medicine Research Center (MMRC) from Rafsanjan University of Medical Sciences (RUMS) of Iran for providing the necessary equipment of this work. The support of vice chancellor to the research of RUMS is acknowledged. This work was supported by the RUMS by the grant number 20.1094 and approved by the RUMS ethical committee by the number of IR.RUMS.REC.1395.109.

Funding

This work was supported by the RUMS by the grant number 20.1094.

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