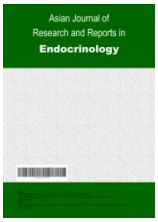

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Fenugreek Oil and Metformin Improve Insulin Resistance via Increase of GLUT4 and PPARγ in Metabolic Syndrome-induced Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EAZ designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study, managed the literature searches and wrote the first draft of the manuscript. Authors HAAEL and AAAS designed the study, wrote the protocol and reviewed the first draft of the manuscript and author AEMKEM managed the analyses of the study. Author AAZ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Fructose consumption and desserts sweetened with fructose increase risk of metabolic syndrome (MS). Features of MS include insulin resistance, dyslipidemia, visceral obesity, and hypertension. The aim of this study was to evaluate the role of fenugreek oil and metformin in ameliorating features of MS.

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Methods: MS was induced in rats by high-fructose high-fat fed diet for 8 weeks. They were randomly divided into five groups: normal control, MS control group treated with saline, MS groups given fenugreek oil (4 ml/kg), or metformin (100 mg/kg) daily for 4 weeks. Body weight, relative organ weight, glucose, insulin, adiponectin, and lipid profiles were estimated. Also glucose transporter 4 (GLUT-4) content and peroxisome proliferator-activated receptor-gamma (PPAR-γ) protein expressions were determined.
Results: fenugreek oil and metformin caused decrease in both MS-induced increase in body weight and glucose. They reduced insulin level and resistance with increased adiponectin, and correction of MS-induced hyperlipidemia. Treatment also increased GLUT-4 and PPAR-γ protein expression compared with MS control group.
Conclusion: Fenugreek oil and metformin improve features of MS via increased GLUT4 and PPARγ expression.

Keywords: Insulin resistance; fenugreek oil; lipid; metabolic syndrome; metformin.

ABBREVIATIONS

GLUT-4 : Glucose transporter 4
HDL-C : High-density lipoprotein-cholesterol level
HFHFD : High-fructose high-fat diet
HOMA-IR : Homeostasis model assessment of insulin resistance (HOMA-IR)
MS : Metabolic syndrome
PPAR-γ : Peroxisome proliferator-activated receptor-gamma
QUICKI : Quantitative insulin sensitivity check index
TC : Total cholesterol
TG : Triglyceride
VLDL : Very-low-density lipoprotein

1. INTRODUCTION

Metabolic syndrome (MS) is a growing health problem that has reached pandemic proportions [1]. Features of MS include insulin resistance, dyslipidemia, visceral obesity, and hypertension [2]. The term MS has been developed with the purpose to assist in identification of individuals at high risk of type 2 diabetes and cardiovascular disease (CVD) to put in place preventative measures that can decrease their risks [3]. Two features: the visceral obesity and impaired insulin in particular stand out as potential etiologies underlying the associated abnormalities of MS [2].

Aging and hormonal changes as well as potential multiple gene combinations have also been implicated in the development of MS [4]. Impaired insulin action in the liver, muscle, and adipose tissues have been considered as the core disorders in the MS and at the origin of risk factors that tend to cluster together as well as to occur commonly in insulin resistant individuals [5].

Diets containing high amount of fat and simple carbohydrate, especially fructose, are strongly associated with MS [6]. Insulin resistance represents the earliest signs in the development of type 2 diabetes [7]. In addition, dyslipidemia plays a major role in coronary artery disease, stroke, and peripheral vascular disease [8]. Insulin-induced glucose uptake into skeletal muscle is governed by a variety of factors such as glucose transporter 4 (GLUT-4). Previous study documented that decreased glucose GLUT4 plays an important role in insulin resistance [9]. In addition, improved insulin sensitivity was shown to be increased by activating peroxisome proliferator-activated receptor-gamma (PPAR-γ) through regulation of different metabolic pathways [10].

Fenugreek (*Trigonella foenum-graecum* L. Leguminosae) is one of the oldest medicinal plants with medicinal properties such as hypocholesterolemic, antibacterial, gastric stimulant, anorexigenic, antidiabetic, galactogogue, hepatoprotective and anticancer. These beneficial physiological effects including the antidiabetic and hypocholesterolemic effects of fenugreek are mainly attributable to the intrinsic dietary fiber constituent which have promising nutraceutical value [11,12].

Metformin is a biguanide agent that is currently used in treatment of diabetes mellitus. Metformin improves insulin sensitivity and decreases insulin resistance, targeting a primary defect in type 2 diabetes [13]. Metformin suppresses hepatic glucose production and increases glucose utilization, which only occurs in the presence of insulin as metformin enhances insulin action at

the post receptor level in peripheral tissues. The principal site of action of metformin is the liver where it inhibits hepatic glucose production [14.15].

The aim of the study was to investigate the role of fenugreek oil and metformin on MS-induced insulin resistance.

2. MATERIALS AND METHODS

2.1 Animals

Thirty male Sprague Dawley rats weighting 200 to 230 g were used in the current study. They were purchased from the animal house of the National Research Center Institute (Cairo, Egypt). During the study, the animals were housed under conventional laboratory conditions on a 12 hours light/dark cycle and constant temperature (22 ± 1°C).

Fenugreek oil was purchased from local herbal store, Metformin was. Fructose was purchased from El Nasr Pharmaceutical, (Cairo, Egypt). Insulin, adiponectin, GLUT4, and PPARγ enzyme-linked immunosorbent assay (ELISA) Kits were purchased from Bioassay Technology Laboratory Company, (Shanghai, China). Total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol level (HDL-C), and glucose kits were purchased from Spectrum Diagnostics, (Obour, Egypt).

2.2 Experimental Design

MS was induced by feeding rats a high-fat diet consisting of standard rodent chow in addition to 10% fat, 3% NaCl, and fructose 20%solution in drinking water for 8 weeks according to modified method described by Calvo-Ochoa et al. 2014. [16].

Diet and fructose solution were freshly prepared every day. Rats were provided with a high-fructose high-fat diet (HFHFD) for 8 weeks. Rats were randomly allocated into five groups (six rats each) as follows:

Group 1 : this group received normal laboratory diet, tap water ad libitum and given saline daily during the time of experiment.

Group 2 : this group fed HFHFD for 12 weeks and given saline daily during the time of experiment.

Group 3 : this group fed HFHFD for 12 weeks and fenugreek oil (4ml/kg) for the last 4 weeks.

Group 4 : this group fed HFHFD for 12 weeks and metformin (100 mg/kg) for the last 4 weeks [17].

At the end of treatment, the animals were fasted for 12 hour weighed and blood samples were withdrawn from the retro-orbital plexus under light anesthesia [18]. Serum was separated by centrifugation at (1,509g, 15 min, 4°C) and divided into small aliquots that were stored for the estimation of the levels of fasting glucose, insulin, TC, TG, HDL-C, and adiponectin.

In addition, homeostasis model assessment of insulin resistance (HOMA-IR) score as an indicator of insulin resistance and quantitative insulin sensitivity check index (QUICKI) were calculated.

Furthermore serum low-density lipoprotein-cholesterol level (LDL-C) and very-low-density lipoprotein (VLDL) were calculated.

Animals were killed by cervical dislocation, heart, liver, and visceral adipose tissue were rapidly excised, washed with saline, weighed and homogenized in ice-cold phosphate buffer saline to prepare 10% homogenates mixture of the three tissues that divided into several aliquots and stored at −80°C to determine GLUT-4, and PPAR-γ protein expression. Mixed homogenate was used for quantitative estimation of total GLUT-4 and PPAR-γ as a measure for the overall improvement in MS. Analysis of GLUT-4 in tissues mixture can give identification of distinct storage compartments that are recruited by treatment induced increase in insulin.

Table 1. Nutritional composition of diets

Nutrient composition	Normal control	HFHFD
Fat (%)	4	14
Carbohydrates (total) (%)	50	50
Fructose (%)	0	20
Maltodextrin 10 (%)	15	15
Protein (%)	22	22

Abbreviation: HFHFD, high-fructose high-fat diet.

2.3 Biochemical Assays

The animals were weighed three times at the baseline, once before commencement of dosing and once on the day of sacrificed killed. Changes in body weight of rats were noted individually, to note the differences in between the control and experimental samples. On the day of killed, body weight was reported as a percent change relative to baseline.

Percentage body-weight gain was calculated as:

(Body weight on sacrifice day (g) - initial body weight)/initial body weight × 100.

Relative weight of heart, liver, and visceral adipose tissue of each animal was then calculated as follows:

Relative organ weight = absolute organ weight (g) × 100/body weight of rat on sacrifice day (g).

Serum sample were used for estimation of the level of fasting glucose, insulin, adiponectin, TC, TG, and HDL-C. In addition, the prepared 10% homogenates mixture of the three tissues (heart, liver, and adipose tissue) was used for quantitative estimation of total GLUT-4 and PPAR-γ [19].

LDL-C and VLDL were calculated using the formula:

LDL-C=TC− (HDL + TG/5) [20].
VLDL = TG/5.

HOMA-IR was calculated according to the equations provided by Matthews et al. 1985 [21].

HOMA-IR = serum glucose (mmol/L) × serum insulin (μLU/mL)/22.5.
QUICKI was calculated according to using the following equation:

QUICKI = 1/(log insulin + log glucose in mg/dL).

Rats are considered as insulin resistant, when QUICKI ≤0.33 [22].

GLUT-4 and PPAR-γ were assayed in tissues homogenate using ELISA kits.

Formula used to calculate the percentage of change (%) = (treated value−control value)/control value × 100.

2.4 Statistical Analysis

Statistical analysis was performed using one-way analysis of variance followed by Tukey's post hoc test using SPSS software v21 (SPSS Inc, Chicago, IL). Data were expressed as mean ± standard deviation (SD) and P values of less than 0.05 were considered as statistically different.

3. RESULTS

During the 8 weeks feeding of HFHFD. An increase in body weight was observed by initiation of the diet. The body weight gain and relative organ weight were significantly higher in nontreated MS-induced rats when compared with normal-control rats (Table 2). After 4 weeks of oral treatment of MS-induced rats with fenugreek oil (4 ml/kg), and metformin (100 mg/kg) suppression of body weight gain by 92%, and 85%, respectively, when compared with MS-induced group was observed. MS-induced rats had significantly higher relative liver, heart, and visceral fat weight than normal control group (Table 2). Meanwhile MS-induced rats treated with the fenugreek oil, and metformin exhibited decrease in relative liver, heart, and in visceral adipose tissue weight.

At the same time MS-induced group showed higher serum glucose level than control (Table 3). Significant reduction in glucose level was seen in MS-induced rats treated with fenugreek oil, and metformin by 59%, and 56%, respectively, when compared with MS-induced group (Table 3).

Similarly, serum insulin level of MS-induced group was significantly increased compared with those in control group. In comparison to the MS-induced group, fenugreek oil, and metformin administration to MS-induced rats significantly reduced serum insulin level by 67%, and 44%, respectively.

HOMA-IR in MS-induced rats was significantly higher than the control group (Table 3). MS-induced rats given fenugreek oil, and metformin nearly normalized HOMA-IR index. A statistically significant decrease in QUICKI index was observed in MS-induced rats than those in control group. MS-induced group treated with fenugreek oil, and metformin give significantly ($P < 0.05$) elevated level of QUICKI index compared with MS-induced rats.

Table 2. Effect of fenugreek oil, and metformin on body weight gain and relative organs weight in MS-induced rats

Parameters/ Treatment	Bodyweight gain (g)	Relative Liver weight (g)	Relative Heart weight (g)	Relative Visceral fat tissue weight (g)
Normal control	52±5.94*†	0.022±0.32†	0.002±0.02*	0.018±0.26*†
MS-induced group	100±13.57†#	0.043±0.35†#	0.06±0.04†#	0.34±0.19†#
Fenugreek oil group (4ml/kg)	8±14.07*@#	0.026±1.66*	0.002±0.02*	0.029±0.23*@#
Metformin group (100 mg/kg)	-15±10.49*#	0.029±0.12*#	0.002±0.02*	0.028±0.14*#

Abbreviations: ANOVA, analysis of variance; MS, metabolic syndrome. Results are expressed as mean ± SD (n = 6). The statistical comparison of difference between the control and the treated groups were carried out using one-way ANOVA. Relative organ weight = (organ weight/body weight) × 100

**Statistically significant from the MS-induced rats treated with HFHFD only at P < 0.05.*

†Statistically significant from metformin treated group at P < 0.05.

#Statistically significant from the control values at P < 0.05.

Table 3. Effect of fenugreek oil, and metformin on blood glucose homeostasis in non MS-induced rats

Parameters/ Treatment	Blood glucose (mg/dl)	Insulin (mU/l)	HOMA-IR	QUICKI
control	74.33±2.48*	7.02±0.39*†	1.28±0.00*†	0.37±0.00*†
MS-induced group	164.27±3.56†#	26.42±2.2†#	10.61±0.02†#	0.27±0.00†#
Fenugreek oil group (4ml/kg)	67.67±2.73*#	8.62±0.4*@#	1.42±0.08*	0.36±0.00*@
Metformin group (100 mg/kg)	71.73±1.33*	14.8±0.77*#	2.59±0.01*#	0.33±0.00*#

Abbreviations: ANOVA, analysis of variance; HOMA-IR, homeostasis model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index. Results are expressed as mean ± SD (n = 6). The statistical comparison of difference between the control and the treated groups were carried out using one-way ANOVA.

**Statistically significant from the MS-induced rats treated with HFHFD only at P < 0.05.*

†Statistically significant from metformin treated group at P < 0.05.

#Statistically significant from the control values at P < 0.05.

There was significant reduction of PPARγ expression in MS-induced rats as compared with normal control group (Table 4). Meanwhile, significant increase of PPARγ expression was observed in MS-induced group treated with fenugreek oil, and metformin by 314%, and 182%, respectively, when compared with MS-induced group.

The amount of tissue GLUT4 in MS-induced group was significantly reduced compared with control group. In addition, significant increase in tissue GLUT4 was observed in MS-induced group treated with fenugreek oil, and metformin 281%, and 512%, respectively when compared with MS-induced group.

Serum adiponectin level of MS-induced group it was not associated with any significant change when compared with those observed in control group as it show intial increase in the induction of Ms and then return to normal level by the end of 8 weeks. MS-induced group treated with fenugreek oil, and metformin showed significant increased serum adiponectin level by 718%, and 1194%, respectively when compared with MS-induced group.

Moreover, MS was accompanied by marked dyslipidemia as evidenced by the observed increase. In Table 5, MS-induced rats showed a significant increase in levels of cholesterol, TG, LDL-C, VLDL-C and decrease in HDL-C in comparison with control group. In comparison to nontreated MS-induced group, oral administration of fenugreek oil, and metformin showed a significant decrease in the levels of cholesterol by 57%, and 56%, respectively.

Table 4. Effect of fenugreek oil, and metformin on biomarkers affecting insulin resistance MS-induced rats

Parameters / Treatment	Adiponectin (mg/l)	GLUT-4 (ng/ml)	PPAR γ (ng/ml)
control	6.35±0.18†	7.53±0.3*†	8.58±0.65*
MS-induced group	4.68±0.17†	4.33±0.22†#	3.06±0.18†
Fenugreek oil group (4ml/kg)	21.92±1.42*@	16.5±1.87*@#	13.5±1.26*@#
Metformin group (100 mg/kg)	34.67±2.99*#	26.5±1.8*#	8.615±0.4*

Abbreviations: ANOVA, analysis of variance; GLUT-4, glucose transporter 4; MS, metabolic syndrome; PPARγ, peroxisome proliferator-activated receptorγ. Results are expressed as mean ± SD (n = 6). The statistical comparison of difference between the control and the treated groups were carried out using one-way ANOVA.

**Statistically significant from the MS-induced rats treated with HFHFD only at P < 0.05.*

†Statistically significant from metformin treated group at P < 0.05.

#Statistically significant from the control values at P < 0.05.

Table 5. Effect of fenugreek oil, and metformin on lipid profile in MS-induced rats

Parameters / Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL (mg/dl)
control	82±1.43*	79.85±1.6*†	30.05±2.2*	36.06±2.71*	15.97±0.32*
MS-induced group	199.33±6.74†#	156±1.41†#	13.5±2.7†#	154.63±8.6†#	31.2±0.28†#
Fenugreek oil group (4ml/kg)	85.33±2.58*	78.17±3.18*	49.5±2.16*@#	20.2±2.4*@#	15.63±0.63*
Metformin group (100 mg/kg)	88.67±2.94*#	80.17±2.92*	43.55±3.28*#	31.97±6.9*	31.97±6.9*

Abbreviations: ANOVA, analysis of variance; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; MS, metabolic syndrome; VLDL, very low density lipoprotein. Results are expressed as mean ± SD (n = 6). The statistical comparison of difference between the control and the treated groups were carried out using one-way ANOVA.

**Statistically significant from the MS-induced rats treated with HFHFD only at P < 0.05.*

†Statistically significant from metformin treated group at P < 0.05.

#Statistically significant from the control values at P < 0.05.

TG level also decreased by 50%, and 49%, respectively when compared with MS-induced group. Furthermore, LDL-C level was suppressed by 87%, and 79%, respectively when compared with MS-induced group. VLDL-C level was decreased (P < 0.05) by 50%, and 49%, respectively when compared with MS-induced group. On the other hand an increase in HDL-C by 267%, and 201%, respectively when compared with MS-induced group was observed.

4. DISCUSSION

HFHFD resulted in increased glucose level, insulin resistance, and hyperlipidemia associated with decreased adiponectin, GLUT4, and PPARγ protein expression. Adiponectin is a hormone playing a vital role in lipid and glucose metabolism [23].

Dyslipidemia might be attributed to increased flux of free fatty acids into the liver in accordance to insulin deficiency or insulin resistance, [24] which leads to excessive accumulation of fatty acids in the liver and converted to TG [25]. The elevation of VLDL-C and TG levels causes a decreased level of HDL-C and an increased accumulation of small LDL-C particles by activated lipoprotein lipase and lecithin acyl-cholesterol transferase [26].

The impaired ability of the fat cell to store excess TG adequately is a major step in the underlying hypertriglyceridemia of insulin resistance [27].

High fructose consumption disturbs normal hepatic carbohydrate metabolism leading to disturbance in glycolytic pathway which enhance rate of de novo TG synthesis and decrease in expression of hepatic PPARγ. PPARγ plays an important role in differentiation of fat cell, storage of lipid, and insulin sensitivity [28]. Furthermore PPARγ influence insulin sensitivity positively by control of the expression of numerous factors secreted from adipose tissue (eg leptin) and up regulates GLUT4 expression [29].

GLUT4 represents glucose transporter in skeletal muscle and adipose tissue, which is controlled by insulin. Insulin promotes the translocation of GLUT4 from the intracellular compartment to the cell membrane [30].

The administration of fenugreek seed extracts have the potential to slow enzymatic digestion of carbohydrates, reduce gastrointestinal absorption of glucose, and thus reduce post-prandial glucose levels and HbA1c [31]. In diabetic rats, trigonelline ingestion increased insulin sensitivity and reduced blood glucose levels [32].

In the present study, the administration of fenugreek oil provoked a significant increase in GLUT-4 expression which may lead to decreased IR and increased glucose uptake. Kouzi et al. [33] showed that 4-hydroxyisoleucine contained in fenugreek stimulated glucose uptake by enhancing the translocation of GLUT-4 to the cell surface via a PI3-K/Akt-dependent mechanism.

Additionally, enhanced PPAR-γ expression by fenugreek was observed in this study may play a key role in maintaining glycemic control. Diosgenin raised PPAR-γ level in white adipose tissue [34]. Tharaheswari et al. [35] found that methanolic extract of fenugreek increased PPAR-γ mRNA levels in high fat fed rats.

In the present study, a highly significant increase in adiponectin level was observed in animals supplied with fenugreek oil. Arshadi et al. [36] and Knott et al. [37] reported that fenugreek supplementation increased adiponectin expression in subcutaneous adipose tissue.

The antiadipose activity of fenugreek fiber significantly increased satiety and decreased hunger leading to reduction in body weight as observed. Mathern et al. [38] postulated that galactomannan present in seeds flushes out the sugars from the body before it enters the blood stream, thus resulting in weight loss. Furthermore it has been suggested that weight loss induced by fenugreek may be due to reduction of intestinal fat absorption or inhibition of pancreatic lipase activity [39] or reducing appetite by decreasing the levels of leptin in the adipose tissue [40]. Also, it was reported that saponins present in fenugreek suppressed the appetite signals in the hypothalamus leading to reduced food intake and body weight gain [41,42].

In addition fenugreek significantly improved lipid profile. Kumar et al. [43] reported that galactomannan, and polyphenols contained in fenugreek seeds regulated dyslipidemia in obese and diabetic rodents. The hypolipidemic effect of fenugreek on adipocytes and liver cells may be due to enhanced LDLR-mediated LDL uptake which is responsible for improvement in serum lipid profile and body weight. Fenugreek lowered the hepatic TGs and cholesterol levels by increasing the excretion of bile acids and cholesterol in the feces of rats in a dose-dependent manner [9,34].

Another postulated mechanism of fenugreek hypolipidemic effect might be due to the activation of several enzymes, such as lecithin-cholesterol acyltransferase, TG lipase, and lipoprotein lipase [44,45]. Also, saponin present in fenugreek reduces cellular triglyceride accumulation by stimulating AMPK phosphorylation [46].

Metformin improved body weight gain through activation of AMPK which reduces the activity of acetyl CoA carboxylase enzyme leading to decrease in the biosynthesis of fatty acids, increase β-oxidation, and decrease the adipogenesis which promotes weight loss [47]. Duan et al. [48] reported that metformin reduced food intake through induction of AMPK in the hypothalamus.

Furthermore, metformin reduced serum total cholesterol, triglycerides, low density lipoprotein and fatty infiltration of hepatic parenchyma and increased high density lipoprotein level [49]. Kashi et al. [50] reported that metformin in addition, to lowering BMI and glucose levels, influenced the therapeutic outcomes of the lipid-lowering drug atorvastatin which was evident by the decrease in TG, TC, LDL-C, and the increase in HDL-C levels.

Metformin decreases the production of glucose in liver [51], reduces the absorption of glucose from intestine, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization [52]. The improvement in insulin sensitivity by metformin could be ascribed to its positive effects on insulin receptor expression and tyrosine kinase activity [14]. Metformin reported to increase plasma levels of glucagon like peptide 1 (GLP-1) and induces islet incretin receptor gene expression [53].

Increase of insulin sensitivity caused by metformin could also related to increased PPAR-γ protein expression PPAR-γ protein expression further support the data of Elia et al. [54] and Aminuddin et al. [55].

In addition metformin provoked a significant increase in GLUT-4 expression. Grisouard et al. [56] and Lee et al. [57] have proven that metformin increases the expression of GLUT-4 mRNA via AMPK activation, favoring glucose uptake in insulin-dependent tissues such as adipose and skeletal muscle tissues.

5. CONCLUSIONS

In conclusion, administration of fenugreek oil and metformin for 4 weeks decreased insulin resistance and reduce glucose level. They improve the lipid profile and insulin sensitivity. These effects could be related to increased adiponectin, GLUT-4, and PPAR-γ protein expression.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It's not applicable.

ETHICAL APPROVAL

The experimental design was carried out according to the regulation of ethic committee of faculty of Pharmacy Cairo University (Approval number: PT 1305).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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