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Nigella Sativa and Ginger Increase GLUT4 and PPARγ in Metabolic Syndrome-Induced Rats

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Abstract: Increased fructose intake has been linked to the epidemiology of insulin resistance, type 2 diabetes mellitus, renal damage, and metabolic syndrome (MS). As oxidative stress plays a pivotal role in the pathology of insulin resistance, the present study was conducted to investigate the effects of Nigella Sativa (NS) and ginger as potent antioxidants on fructose-induced MS in rats. Male rats were fed with a high-fructose high-fat-fed diet for 8 weeks. By the end of the 8th week, rats were divided into four groups; one was left untreated (normal control) and MS control group was treated with saline. MS groups were given Nigella sativa (4 ml/kg) and ginger (500 mg/kg) daily for 4 weeks. Markers chosen for assessment included the effect on body weight gain, glucose, insulin, adiponectin levels, and lipid profile. Also, protein expressions were estimated by glucose transporter 4 (GLUT4) content and peroxisome proliferator-activated receptor-gamma (PPARγ). Nigella sativa and ginger ameliorated some manifestations of MS, including an increase in body weight, glucose, insulin level, and resistance. Besides, both drugs lowered insulin resistance, induced hyperlipidemia and increased adiponectin level. Drugs also increased GLUT4 and PPARγ protein expression compared with MS control group. Nigella sativa and ginger ameliorated parameters of MS via increased GLUT4 and PPARγ expression.

Keywords: Metabolic syndrome; *Nigella sativa*; ginger; insulin resistance; lipid.

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1. Introduction

Metabolic syndrome (MS) is characterized by insulin resistance, hyperlipidemia, obesity, and increased risk for developing non-alcoholic fatty liver disease, type 2 diabetes mellitus, cardiovascular and renal diseases [1]. Consumption of large amounts of dietary fructose is one of the major factors contributing to obesity and MS [2].

Underlying factors for fructose-induced insulin resistance are varied. Fructose is more lipogenic than glucose, leading to more significant elevations of triglycerides (TG) in the skeletal muscle and, consequently, insulin resistance [3].

Glucose uptake into skeletal muscle is primarily through glucose transporter 4 (GLUT-4), which is modulated by insulin signaling or the alternative pathway via activation of AMP-activated protein kinase (AMPK) [4].

Activation of AMPK leads to increased glucose uptake and the fatty acid influx into cells and is accompanied by up-regulation of peroxisome proliferator-activated receptor-gamma co-activator 1-alpha (PGC-1α), a potent transcriptional cofactor in regulating mitochondrial biogenesis and function [5].

There has been much interest in potential strategies to treat and prevent metabolic syndrome, including non-pharmacological interventions [6]. *Nigella sativa* and ginger were chosen in this study. *Nigella sativa* (NS) seeds possess antioxidant and hypotensive activity [7]. Moreover, NS is known for its hepatoprotective [8], immunomodulatory effects [9], and anti-diabetic activity [10]. Similarly, ginger can treat hyperlipidemia [11], platelet aggregation [12], and hypertension [13]. Also, ginger is reported to possess anti-inflammatory, hypoglycemic activity [14], renoprotective [15], and immunomodulatory effects [16]. Furthermore, they exert antithrombotic activity [17].

The study aimed to investigate the role of *Nigella sativa* and ginger compared with metformin on MS-induced insulin resistance.

2. Materials and Methods

2.1. Animals.

Adult male Sprague Dawley rats, weighing 200 to 230 g, were used. They were purchased from the animal house of the National Research Center Institute (Cairo, Egypt). The animals were housed under appropriate laboratory conditions of controlled humidity, temperature, and light during the study. The study was carried out according to the regulation of the ethics committee of the faculty of Pharmacy Cairo University.

2.2. Drugs and chemicals.

Nigella sativa oil was purchased from Pharco Pharmaceuticals (Alexandria, Egypt). Ginger was purchased from an Arab company for pharmaceuticals & Medicinal plants (MEPACO-MEDIFOOD) (Sharkeya, Egypt). Metformin was purchased from Minapharm Pharmaceutical (Cairo, Egypt). 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, high-density lipoprotein-cholesterol level (HDL-C), and glucose kits were purchased from Spectrum Diagnostics (Obour, Egypt).

2.3. Experimental design.

Rats were divided into five groups, each consisting of 6 rats. Rats in the first group were fed with normal laboratory chow, whereas rats in the remaining groups were fed with a high-fructose high-fat diet (HFHFD) composed of standard rodent chow in addition to 10% fat, 3% NaCl, and fructose 20% solution in drinking water for 8 weeks according to a modified method described by Calvo-Ochoa *et al.* [18]. After 8 weeks of diet initiation, animals in the four groups were treated as follows:

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

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

Group 3: this group was fed with HFHFD for 12 weeks and *Nigella sativa* oil (4ml/kg) for the last 4 weeks [19].
Group 4: this group was fed with HFHFD for 12 weeks and metformin (100 mg/kg) for the last 4 weeks [20].
Group 5: this group was fed with HFHFD for 12 weeks and ginger (500 mg/kg) for the last 4 weeks [21].

At the end of the study, blood samples were withdrawn from the retro-orbital plexus of all rats.[22] Serum was separated by centrifugation at (3000 rpm, 15 min, 4°C) and divided into small aliquots that were stored for the estimation.

2.4. Biochemical assays.

Percentage of body weight gain and organ weights were calculated. Serum samples were used to estimate the levels of fasting glucose, insulin, TC, TG, HDL-C, and adiponectin.

Besides, the homeostasis model assessment of insulin resistance (HOMA-IR) score as an indicator of insulin resistance was calculated according to the equation provided by Matthews *et al.* [23]. The quantitative insulin sensitivity check index (QUICKI) was calculated according to the equation provided by McAuley *et al*. [24].

LDL-C and VLDL were calculated from the formula described by Friedewald *et al.* [25], where LDL-C=TC−(HDL + TG/5)

VLDL = TG/5.

PPARγ and GLUT4 were assayed in tissue homogenate using ELISA kits.

2.5. Statistical analysis

Data were expressed as mean ± standard deviation (SD). Results were analyzes using one-way analysis of variance followed by Tukey's post hoc test using SPSS software. For all statistical tests, the level of significance was set at $P < 0.05$.

3. Results and Discussion

At the end of 8 weeks of the feeding with HFHFD, the body weight gain and relative organ weight were significantly higher in non-treated MS-induced rats than normal control rats (Table 1).

Table 1. Effect of Nigella sativa and ginger on body weight gain and relative organs weight in MS-induced rats.

Parameters/ Treatment	Body eight 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#
Nigella sativa group (4ml/kg)	-43±15.05*@#	0.024±0.24*#	0.002±0.02@#	0.02±0.11*@#
Ginger group (500 mg/kg)	-35±10.84*#	0.032±0.18*#	0.003±0.03	0.021±0.18*@#

Abbreviations: ANOVA, analysis of variance; MS, metabolic syndrome. Results are expressed as mean ± SD (n = 6). The statistical comparison of the 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 the control values at $P < 0.05$.

After 4 weeks of oral treatment of MS-induced rats with NS (4ml/kg), ginger (500 mg/kg) suppressed body weight gain by 67% and 65%, respectively, compared with the non-treated MS-induced group. The non-treated MS-induced rats had significantly higher relative liver, heart, and visceral fat weight than the normal control group (Table 1). Meanwhile, MS-induced rats treated with the NS and ginger exhibited decreased relative liver, heart, and visceral adipose tissue weight.

The non-treated MS-induced group showed a higher serum glucose level than the normal control (Table 2). A significant reduction in glucose level was seen in MS-induced rats treated with NS and ginger by 64% and 57 %, respectively, compared to the MS-induced group (Table 2).

Serum insulin level of the non-treated MS-induced group was significantly increased compared to those in the normal control group. Compared to the MS-induced group, NS and ginger administration to MS-induced rats significantly reduced serum insulin levels by 75% and 40 %, respectively.

HOMA-IR in the non-treated MS-induced rats was significantly higher than the normal control group (Table 2). MS-induced rats given NS and ginger nearly normalized the HOMA-IR index.

A statistically significant decrease in QUICKI index was observed in non-treated MS-induced rats than those in the normal control group. MS-induced group treated with NS and ginger give significantly ($P<0.05$) elevated QUICKI index levels compared to non-treated MS-induced rats.

Table 2. Effect of Nigella sativa and ginger on blood glucose homeostasis in MS-induced rats.

Parameters/ Treatment	Blood glucose (mg/dl)	Insulin (mU/l)	HOMA-IR	QUICKI
Normal 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#
Nigella sativa group (4ml/kg)	57.7±1.56*@#	6.55±0.42*@	0.92±0.00*@	0.39±0.00*@#
Ginger group (500 mg/kg)	69.85±5.67*#	15.8±0.85*#	2.69±0.00*#	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 the difference between the control and the treated groups was carried out using one-way ANOVA.
*Statistically significant from the MS-induced rats treated with HFHFD only at $P < 0.05$.
#Statistically significant from the control values at $P < 0.05$.

There was a significant reduction of PPARγ expression in non-treated MS-induced rats compared to the normal control group (Table 3). Meanwhile, a significant increase of PPARγ expression was observed in the MS-induced group treated with NS and ginger by 353% and 420%, respectively, compared to non-treated MS-induced groups.

The amount of tissue GLUT4 in the non-treated MS-induced group was significantly reduced compared to the normal control group. Meanwhile, a significant increase in tissue GLUT4 was observed in the MS-induced group treated with NS and ginger 814% and 512%, respectively, compared to non-treated MS-induced groups.

Serum adiponectin level of the non-treated MS-induced group did not significantly reduce compared to those observed in the normal control group. The MS-induced group treated with NS and ginger showed significantly increased serum adiponectin level by 1212%, and 1256%, respectively, compared to the non-treated MS-induced group.

Table 3. Effect of Nigella sativa and ginger on biomarkers affecting insulin resistance MS-induced rats.

Parameters / Treatment	Adiponectin (mg/l)	GLUT-4 (ng/ml)	PPAR γ (ng/ml)
Normal 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
Nigella sativa group (4ml/kg)	35.17±1.47*#	39.6±1.6*@#	13.85±0.76*@#
Ginger group (500 mg/kg)	36.33±2.15*#	26.5±1.8*@#	15.92±1.42*@#

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 the difference between the control and the treated groups was carried out using one-way ANOVA.
*Statistically significant from the MS-induced rats treated with HFHFD only at $P < 0.05$.
#Statistically significant from the control values at $P < 0.05$.

MS-induced rats showed a significant increase in cholesterol, triglyceride LDL-C, VLDL-C, and a decrease in HDL-C than the normal control group (Table 4). Compared to the non-treated MS-induced group, oral administration of NS and ginger showed a significant decrease in cholesterol levels by 64 % and 55 %, respectively. Triglyceride levels also decreased by 58 % and 51 %, respectively, compared to the non-treated MS-induced group. Furthermore, the LDL-C level was suppressed by 95 % and 87 %, respectively, compared to the non-treated MS-induced group. VLDL-C level was decreased ($P<0.05$) by 59 % and 31 %, respectively, compared to the non-treated MS-induced group. On the other hand, an increase in HDL-C by 288% and 244%, respectively, was observed compared to the non-treated MS-induced group.

Table 4. Effect of Nigella sativa and ginger 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)
Normal 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#
Nigella sativa group (4ml/kg)	71.67±3.77*@#	63.67±2.16*@#	52.48±2.0*@#	6.45±0.43@#	12.73±0.43*@#
Ginger group (500 mg/kg)	81.67±6.73*@	76.5±3.08*@#	46.52±3.65@#	19.85±6.1*@#	15.3±0.61*@#

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 the 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 the control values at $P < 0.05$.

Feeding rats with HFHFD resulted in hyperglycemia, hyperinsulinemia, and hyperlipidemia associated with decreased adiponectin, GLUT4, and PPARγ protein expression.

Fructose does not stimulate insulin secretion in the short term; insulin resistance and obesity induced by fructose feeding resulted in compensatory hyperinsulinemia [26].

Reduction of GLUT-4 and PPAR-γ expression in MS-induced rats could lead to decreased insulin sensitivity and glucose uptake. It was reported that GLUT-4 translocation does not occur efficiently, and GLUT-4 transporters remain inside, where they are not functioning [27]. This results in decreased glucose uptake by muscle cells, contributing significantly to elevated blood glucose levels [28].

Similarly, the significant decrease in expression of PPAR-γ in MS-induced rats leads to decreased insulin sensitivity and decreased glucose uptake. Previous data reported that high fructose consumption disturbs normal hepatic carbohydrate metabolism leading to disturbance in the glycolytic pathway, which may enhance the rate of de novo TG synthesis and decrease the expression of PPAR-γ. PPAR-γ plays an essential role in the differentiation of fat cells, lipid storage, and insulin sensitivity [29].

The anti-adipose activity of NS Weight loss is related to decreasing serum lipids and glucose levels [43]. A previous study revealed that methanolic extract and NS's commercial oil displayed appetite-reducing components inducing weight loss [30].

A previous study reported that NS reduced plasma lipids concentrations [31]. The mechanisms of NS favorable effects may be due to its choleretic activity, as reported by Kaatabi *et al.* [32].

Administration of NS to MS-induced rats increased PPARγ protein expression. Previous studies reported that NS to rats fed a high-fat diet improved insulin resistance by thymoquinone, a bioactive constituent of NS that interacts with the ligand-binding pocket of PPAR γ reported to be critical for its activity [33]. In addition, Benhaddou-Andaloussi reported that NS stimulated PPARγ in cultured adipocytes and increased the total amount of GLUT-4 glucose transporters in skeletal muscle [34].

Also, NS in the MS-induced group increased adiponectin [35]. Ginger decreased the glucose level in MS-induced rats with reduced insulin level and resistance. Increased insulin sensitivity was also seen in this study and as reported before [36]. Improvement of insulin resistance by ginger could be related to the observed increase in adiponectin, GLUT4, and PPARγ expression.

Ginger significantly decreased MS-induced hyperlipidemia, which was also reported before.[37] The hypocholesterolemic effects of ginger stem from the inhibition of cellular cholesterol synthesis. The attenuation of cholesterol synthesis results in augmenting the LDL receptor activity, leading to the elimination of LDL from plasma.

The mechanism of the hypolipidemic action of ginger may be due to inhibition of dietary lipid absorption in the intestine or stimulation of biliary secretion of cholesterol and excretion of cholesterol in feces [38].

Furthermore, ginger stimulates glucose uptake and increases translocation of GLUT-4 in the membrane surface of the cells together with small increases in total GLUT-4 protein expression.[39] Activation PPAR γ expression by ginger may be due to the presence of 6-shogaol, which was identified as PPARγ activator, which was founded to be a novel effect [40].

4. Conclusions

In conclusion, NS and ginger for 4 weeks decreased insulin resistance and reduce glucose levels compared with metformin. They improve the lipid profile and insulin sensitivity. These effects could be related to increased adiponectin, GLUT4, and PPAR protein expression.

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Conflicts of Interest

The authors have no conflicts of interest.

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