Potential Protective Effects of *Nigella Sativa* and *Allium Sativum* Against Fructose-Induced Metabolic Syndrome in Rats

Nawal Al-Rasheed¹, Nouf Al-Rasheed¹, Yieldez Bassiouni¹, Laila Faddah¹ and Azza M Mohamad²*

¹ Department of Pharmacology, College of Pharmacy, King Saud University, Saudi Arabia P. O. Box 22452, Riyadh 11495, KSA
² Department of Biochemistry, Faculty of Science for Girls, King Abdulaziz University, P. O. Box 51459, Jeddah 21453, KSA

Abstract: Among famous medicinal plants with known antioxidant activity; black seed (*Nigella sativa*, *NS*) and garlic (*Allium sativum*) which have been used in traditional medicine. In recent years, rates of metabolic syndrome (MS) have been increasing globally. The present work was designed to study the potential protective effects of black seed and raw garlic homogenate against fructose-induced MS in rats and to assess the benefits gained from their combination. Fifty male albino Wistar rats were divided into 5 groups. A control group was allowed to feed on normal chow and drink tap water. MS group was fed the same diet plus 10% fructose in drinking water. Treated groups received NS or garlic either alone or in combination as oral supplements along with high fructose diet for 8 weeks. Results revealed that body weight, liver weight, fasting blood glucose, serum triglycerides (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels were significantly increased while high density lipoprotein cholesterol (HDL-C) and the activities of Lactate dehydrogenase (LDH), glucose -6-phosphate dehydrogenase (G-6-PHD) and catalase in liver tissues were significantly decreased in MS group compared to the control group. Administration of NS or garlic either alone or in combination significantly ameliorated all the above-mentioned altered parameters. Treatment with both NS and garlic showed the utmost reduction in serum LDL-C and TG levels and could restore the activities of the studied enzymes in liver nearly to normal values. It was concluded that both NS and garlic were effective in attenuating multiple abnormalities of MS. Combination of these medicinal plants may have additional effectiveness in reducing serum TC, LDL-C and increasing HDL-C levels which could be a step in the prevention and management of MS.

Key words: metabolic syndrome, dyslipidemia, *Nigella sativa*, garlic, rats.

1 INTRODUCTION

Metabolic syndrome (MS) is a combination of medical disorders linked to overweight, central obesity, insulin resistance, dyslipidemia and hypertension¹,². Patients suffering from MS have increased chance of having heart diseases, stroke and type 2 diabetes³,⁴. MS is diagnosed if a person have at least three of the following metabolic risk factors; large waistline, high blood pressure, high fasting blood sugar, high TG and low level of HDL-C⁵,⁶.

It was estimated that prevalence of MS in the USA to be up to 25% of all US adults⁷,⁸. In Saudi Arabia, a study showed that the prevalence of MS is high accounting to 39.3% and that Saudi subjects from urban areas have significantly higher prevalence compared to those living in rural areas. The authors concluded that low HDL-C plays a major role in the contribution to MS in Saudi Arabia⁹. The upsurge in the incidence of MS over the past two decades has coincided with the marked increase in the consumption of calories of refined carbohydrates specially fructose¹⁰,¹¹. Because it is so sweet and inexpensive, intake of fructose in the form of table sugar and high-fructose syrups in soft drinks, fruit drinks, syrups and candies has been increasing globally, in parallel with changes in human environment; increased restaurant and fast-food meals¹².

In recent years, increasing scientific evidence has emerged on the role of dietary antioxidants as promising therapeutic agents and their impact on human health as they can delay the onset of and prevent many human dis-
Among famous medicinal plants with known antioxidant activity; the black seed and garlic which have been used for thousands of years in traditional medicine. *Nigella sativa* (NS) seeds have been used to promote health and fight disease for centuries especially in the Middle East and Southeast Asia. Muslims have been using and promoting the use of the black seed for hundreds of years. NS has been in the treatment and prevention of some common illnesses as it possesses antioxidant, anti-inflammatory, antifungal, antibacterial, anticonvulsant and anticancer properties. On the other hand, the medicinal value of garlic has been understood by herbalists for at least 2000 years. Garlic has antineoplastic, antiviral and antimicrobial properties. Also, garlic has cardio-protective effects as it may help decrease TC, LDL-C and blood pressure while raising high HDL-C. The potential protective effects of NS and garlic if given together against fructose-induced metabolic syndrome. The present study was conducted to find out if the co-administration of NS and garlic with 10% fructose in drinking water could prevent or ameliorate criteria of MS in male albino rats. The study also aimed to assess the benefits gained from the combination of these medicinal plants.

2 EXPERIMENTAL

2.1 Chemicals

A commercial formulation of fructose powder (Vivis, Fructose sucrant poudre, France) was used. NS seeds were cleaned, dried, powdered in an electrical grinder before use. Kits for determination of serum TG, TC, LDL-C and HDL-C were supplied by Randox Laboratories Ltd., UK. All chemicals used in the study were of high analytical grade obtained from Sigma and Merck companies.

2.2 Animals and treatments

Fifty male Wistar albino rats weighing 180-220 grams were obtained from the Animal Care Center at College of Pharmacy, King Saud University. Animals were housed in wire cages (5 per cage) and maintained under a daily controlled lighting cycle (12 h-light and 12 h-dark) at 22 ± 2°C and 60% humidity with free access to rat diet and tap water for one week to adapt to the laboratory environment prior to the experiments.

Animal experimental protocol was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of King Saud University, College of Pharmacy.

Animals were randomly divided into the following 5 groups (10 rats per group) and weighed at the start of the study. Control group: rats were fed a modified diet containing 65% cornstarch (Cat no. d11708b, Research diet, USA), and allowed to drink tap water *ad libitum*. MS group: rats were fed the same diet plus free access to 10% fructose (w/v) in drinking water for induction of MS for 8 weeks.

MS + NS group: rats fed the same diet and allowed to drink 10% fructose in drinking water. NS was co-administered orally in a dose of 200 mg/day. MS + Garlic group was fed the same diet, allowed to drink 10% fructose solution, and received raw garlic homogenate as an oral dose of 250 mg/kg/day. Whole garlic cloves were homogenized with water to make a fresh paste every day. MS + NS + Garlic group: rats were fed the same diet, allowed to drink 10% fructose solution and received both NS and garlic in the same doses as mentioned before. NS powder and raw garlic homogenate were suspended separately in 2% gum acacia and co-administered to MS rats by oral gavage for 8 weeks.

The body weight and food intake (per cage) were measured once a week. The food intake was calculated per cage, using the formula:

\[
\text{Food intake} = \frac{\sum \text{Body weight of the rats in each cage} \times \text{grams}}{\text{Daily food intake (grams)}}
\]

The calculated daily food intake was approximately equivalent to 10 g/100 g.

At the end of the experiment, rats were weighed, sacrificed by cervical decapitation after overnight fast. Blood was collected and allowed to stand. Serum was separated by centrifugation. Liver was dissected out, weighed and put in ice cold saline and then thoroughly rinsed. The serum and isolated liver of each rat were stored at −80°C until use.

2.2.1 Biochemical serum analysis

Blood glucose was measured using glucometer (Accu-Chek Go). Serum was used for the determination of TG, TC, LDL-C and HDL-C levels. TG was determined using Hi-tech diagnostic kit. Both TC and HDL-C were estimated in serum using Randox laboratories (England) kits. LDL-C was calculated according to Friedewal et al. 

\[
\text{LDL-C} = \text{Total Cholesterol} - \text{HDL-C} - \frac{\text{Triglycerides}}{5}
\]

2.2.2 Biochemical analysis of liver tissues

Liver tissue was homogenized in a known volume of cold distilled water and used for the estimation of the following enzymatic activity. LDH activity was evaluated in a reaction mixture containing tris buffer (50 mM, pH 7.5), sodium pyruvate (0.6 mM), NADH (0.18 mM). The rate of NADH consumption is determined at 340 nm and is directly proportional to LDH activity in the sample. G-6-PDH activity was assayed in a reaction mixture containing triethanolamine buffer (86 mM, pH 7.6), MgCl2 (6.9 mM), glucose-6-phosphate (1 mM), NADP (0.39 mM). The reduction of NADP was followed at 340 nm. Catalase activity was determined by monitoring the decomposition of hydrogen peroxide as described by Aebi.

840
2.3 Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used, followed by Bonferroni test for multiple comparisons. Differences were considered significant at $p<0.05$.

3 RESULTS

Effect of administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination for 8 weeks on body weight (BW), liver weight g/100 g BW and blood glucose in male albino Wistar rats (Table 1).

The results of the present work revealed that MS rats showed highly significant increase ($p<0.001$) in body weight (BW), blood glucose as compared with those of the control group. Co-administration of either NS or garlic alone or in combination as oral supplements to MS rats for 8 weeks significantly reduced BW, blood glucose. The greatest reduction in blood glucose level was achieved by co-administration of both NS and garlic. There were insignificant changes in liver weight/100 g BW between all groups.

Effect of administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination for 8 weeks on lipid profile in male albino Wistar rats (Fig. 1).

MS rats showed highly significant ($p<0.001$) increase in serum TG, TC and LDL-C levels while HDL-C was insigni-

Table 1 Effect of daily administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination on body weight (BW), liver weight / 100 g BW and blood glucose for 8 weeks in male albino Wistar rats.

<table>
<thead>
<tr>
<th>Groups Parameter</th>
<th>Control group</th>
<th>MS group</th>
<th>MS+NS group</th>
<th>MS+Garlic group</th>
<th>MS+NS+Garlic group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW) (g)</td>
<td>208.38 ± 6.31</td>
<td>282.00 ± 7.15a</td>
<td>201.75 ± 7.88*</td>
<td>230.13 ± 8.18*</td>
<td>197.13 ± 11.14*</td>
</tr>
<tr>
<td>Liver weight g/ 100g BW</td>
<td>3.89 ± 0.23</td>
<td>3.92 ± 0.20</td>
<td>3.50 ± 0.22</td>
<td>3.53 ± 0.30</td>
<td>4.01 ± 0.43</td>
</tr>
<tr>
<td>Blood Glucose (mg/dl)</td>
<td>100.00 ± 2.36</td>
<td>132.10 ± 2.50a</td>
<td>107.60 ± 2.29*#</td>
<td>104.00 ± 2.48*#</td>
<td>92.50 ± 2.91*</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM of 10 rats. a Significant as compared to control group.
* Significant as compared to MS group. # Significant as compared to MS + NS + garlic.
- Control group was fed normal diet and allowed to drink tap water.
- MS groups were fed normal diet plus free access to 10% fructose (w/v) in drinking water.

Fig.1 Effect of administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination for 8 weeks on lipid profile in male albino Wistar rats. Data are presented as mean ± SEM of 10 rats. a Significant as compared to control group. * Significant as compared to MS group. # Significant as compared to MS + NS + Garlic.
Effect of administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination for 8 weeks on the activity of LDH, G-6-PDH and catalase in liver tissues of male albino Wistar rats. Enzymes are expressed as \( \mu \text{mole/min/mg proteins} \). Data are presented as mean ± SEM of 10 rats. a Significant as compared to control group. * Significant as compared to MS group. $ Significant as compared to MS+NS group. # Significant as compared to MS + NS+G group.

4 DISCUSSION

The general increase in the consumption of calories over long periods, and specifically of fructose, is clear and correlates positively with the alarming increases in MS\(^{34,35}\). Because of its lipogenic properties, excess fructose in the diet can cause glucose & fructose malabsorption and greater elevations in TG and cholesterol compared to other carbohydrates\(^{36}\). Rayssiguier et al.\(^{37}\) have shown that fructose-fed rat model has been used as a model for investigating the development of MS. Metabolic syndrome can be induced experimentally either by feeding rats with a high-fructose diet (60%)\(^{38,39}\) or by adding fructose to drinking water (10–20%)\(^{25,26,39}\). Diets specifically high in fructose cause metabolic disturbances in animal models mainly weight gain, hyperlipidemia\(^{40}\) and hypertension\(^{41}\).

The results of the present study revealed that rats maintained on fructose 10% in drinking water for 8 weeks (MS group) showed significant increases in BW, fasting blood glucose, serum TG, TC, and LDL-C levels, while HDL-C level was significantly decreased as compared with control rats. These results are in accordance with many studies which have shown that fructose feeding in rats contributes to hyperglycemia and dyslipidemia\(^{42-45}\).

Fig. 2 Effect of administration of 10% fructose in drinking water and co-administration of NS or garlic either alone or in combination for 8 weeks on the activity of LDH, G-6-PDH and catalase in liver tissues of male albino Wistar rats. Enzymes are expressed as \( \mu \text{mole/min/mg proteins} \). Data are presented as mean ± SEM of 10 rats. a Significant as compared to control group. * Significant as compared to MS group. $ Significant as compared to MS+NS group. # Significant as compared to MS + NS+G group.
will enhance the expression of hepatic sterol response element binding protein with subsequent activation of hydroxyl methylgluteryl Co-enzyme A (HMG-CoA) reductase and fatty acid synthase involved in cholesterol biosynthesis.

Exposure of liver to large quantities of fructose leads to rapid stimulation of de novo lipogenesis which plays an important role in fructose-induced fatty liver and hypertriglycerideremia. It was reported that rats fed high fructose diet had sustained elevations in triacylglycerol during the entire time fructose was fed and fell promptly when a standard chow diet was instituted. Simultaneously, fructose inhibits hepatic lipid oxidation, thus favoring fatty acid re-esterification and very low density lipoprotein (VLDL)–TG synthesis. While, fructose stimulates TG production, it impairs TG removal; creating the known dyslipidemic profile. High blood TG concentration in the form of VLDL induces cholesterol ester transfer protein activity, resulting in an increased transfer of TG from VLDL to HDL and a subsequent increase in HDL clearance and decreased HDL concentration.

Supplementation of NS in MS rats reduced BW, blood glucose, serum TC, TG & LDL and increased HDL. The beneficial effects of NS as evidenced by the amelioration of all criteria of MS despite continued intake of high fructose diet came in accordance with the results of Bahgat and Soliman and others who investigated the effect of NS supplementation against high fructose feeding in different animal models as well as in diabetic and dyslipidemic patients.

The cholesterol-lowering effect of NS was explained to be due to either stimulation of bile acid excretion or inhibition of de novo cholesterol synthesis by down-regulating HMG-CoA genes while the significant decrease in LDL-C was reported to be due to upregulation of LDL–receptor gene.

On the other hand, garlic is one of the best-researched herbal remedies. Traditionally, it has been used in treatment of infections, rheumatism, heart diseases, diabetes, and many other disorders. Experimentally, it has been shown to exert antihypertensive, antibacterial, and hypoglycemic actions.

The obtained results showed that garlic supplementation along with high fructose in drinking water reduced BW, blood glucose, serum TC, TG & LDL and increased HDL. The antioxidant effects of garlic were demonstrated by Vazquez-Prieto et al. who reported that aqueous garlic extracts prevented oxidative stress and vascular remodeling in Wistar rats maintained on 10% fructose in drinking water. Similarly, Padiya et al. reported that administration of raw garlic to fructose fed rats significantly reduced serum glucose, TG and normalized serum levels of nitric oxide and hydrogen sulphide as well as hepatic glutathione levels.

In hypertensive patients, Durak et al. showed that garlic extract supplementation improves blood lipid profile, strengthens blood antioxidant potential and decreases the level of malondialdehyde in blood samples. In patients suffering from coronary artery disease, administration of garlic significantly reduced serum TC and TG, and increased HDL-C. Also, combination of garlic with metformin improved the glycemic control and showed additional antihyperlipidemic activity. The authors concluded that garlic may be a good addition in the management of patients with diabetes and hyperlipidemia.

The data obtained from the present study showed that administration of 10% fructose in drinking water for 8 weeks resulted in significant decreases in the activities of LDH, G-6-PDH and catalase in livers of MS rats as compared to the control group. LDH and G-6-PDH have important role in maintaining the homeostasis of blood glucose which stated that the induced hyperglycemia was due to obstruction of glucose utilization by the tissues through glycolysis. On the other hand, G-6-PDH is considered as one of the antioxidant enzymes. It is required for NADPH generation which is needed for the maintenance of GSH in its reduced form. Inhibition of G-6-PDH activity may reduce the capacity of the tissue to protect itself from the oxidative stress because less amount of NADPH is produced. Also, the decline in the activity of the free-radical scavenging enzymes; catalase, may be due to inactivation by excess reactive oxygen species (ROS) production. This will damage the first line of enzymatic defense against hydrogen peroxide. This finding is supported by Rajasekar et al. who showed that high fructose-feeding in rats, resulted in an increase in the oxidative stress in rats as evidenced by the significant increase in plasma levels of thio-barbituric acid reactive substances, lipid hydroperoxides and conjugated diens. In addition, Polizio et al. found that addition of fructose to diet, diminished the activity of the antioxidant enzymes in the liver and kidney of rats. Besides, some authors stated that a defect in the antioxidant defense mechanisms and increased free radical production caused the oxidative damage brought about by dietary fructose. In addition, it was reported that hyperglycemia was associated with decreased plasma insulin, glucose-6-PDH activity in plasma and liver, NADPH/NADP+ ratio and glutathione levels in the liver and pancreas. Also Jana et al. reported that hyperglycemia has an inhibitory effect on activities of key carbohydrate metabolic enzymes like hexokinase, glucose-6-phosphatase, and glucose-6-PDH, as well as the antioxidant enzymes like catalase and superoxide dismutase along with the effect on the lipid peroxidation level in hepatic tissues.
The present study showed that administration of NS and/or garlic to high fructose fed rats markedly ameliorated the decrease in the activities of LDH, G-6- PDH and catalase in livers of treated rats compared to MS rats. A previous study revealed that there were significant decreases in LDH and G-6- PDH activity in hyperglycemic rats and that oral administration of thymoquinone (TQ); the main active principle in the volatile oil of NS seeds significantly reversed the activities of the above-mentioned enzymes to near normal. TQ upregulates the activities of these enzymes in hepatic tissues through insulin release and thereby it enhances the utilization of glucose for cellular biosynthesis, which is marked by the significant decrease in plasma glucose levels. Similarly, Heinle and Betz reported that hypercholesterolemia induced alterations in G-6-PDH activity in rat liver and that intake of garlic significantly protected this enzyme. In another study, it was found that aqueous garlic extract could restore the decreased activity of G-6-PDH in liver of arsenic-intoxicated mice.

In a recent study, Dollah et al. reported that supplementation of NS powder in different doses up to one gram/kg for a period of 28 days in male Sprague Dawley rats did not exert any toxic effects on liver function evaluated by measuring liver enzymes and through histopathological examination of liver tissue. There were insignificant changes in serum alanineaminotransferase (ALAT) and aspartateaminotransferase (ASAT) between treatment groups. Histopathological study showed very minimal and mild changes in normal and high doses of NS-treated groups. Also, the hepatoprotective effect of NS alcoholic extract (NSE) was investigated on D-Galactosamine/Lipopolysaccharide-induced hepatotoxicity in Wistar rats and it was found that NSE restored the levels of ALAT, ASAT and alkaline phosphatase (ALP) nearer to control level.

On the other hand, it was reported that low dose of garlic (250 mg/kg/day) has the potential to enhance the endogenous antioxidant status by reducing thiobarbituric acid reactive substances and enhancing catalase and superoxide dismutase while high doses (500 and 1000 mg/kg/day doses) reduced endogenous antioxidants and caused marked histopathological and ultrastructural changes in both rat liver and kidneys. Also, Samson et al. reported that oral administration of garlic extracts at different concentrations (200, 400, 600 mg/kg/d) in male albino rats significantly decreased serum levels of ALAT and ASAT in a dose-dependent manner indicating that these doses have no potential risk of hepatotoxicity.

5 CONCLUSION

The results of the present study clearly indicate that Nigella sativa and garlic are capable of attenuating multiple abnormalities of MS in Wistar rats like hyperglycemia and dyslipidemia. Also, they could restore the activities of lactate dehydrogenase, glucose 6-phosphate dehydrogenase and catalase in liver tissues nearly to normal values. Combination of NS and garlic as supplements to food may have additional effectiveness in reducing serum TC, LDL-C and increasing HDL-C levels thus protecting against coronary artery disease and could be a step in the prevention and management of MS. An increase in dietary intake of NS and garlic could represent a potential strategy to reduce the risk of MS, diabetes and cardiovascular diseases especially in elderly patients who cannot engage in other therapeutic or prophylactic regimens.

ACKNOWLEDGMENTS

This research project was supported by a grant from “Research Center of Center for Female Scientific and Medical Colleges” Deanship of Scientific Research, King Saud University.

REFERENCES

6. Grundy, S. M.; Cleeman, J. I.; Daniels, S. R.; Donato, K. A.; Eckel, R. H.; Franklin, B. A.; Gordon, D. J.; Krauss, R. M.; Savage, P. J.; Smith, S. C. Jr; Spertus, J. A.; Costa, F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific State-
Nigella Sativa and Allium Sativum against metabolic syndrome


21) Mohamed, E. F. Antiviral properties of garlic cloves juice compared with onion bulbs juice against potato virus Y (PVY). J. Am. Sci. 6(8), 302-310 (2010).


34) Bray, G. A.; Nielsen, S. J.; Popkin, B. M. Consumption


61. Kaafari, H.; Babamsa, A. O.; Lebda, F. M.; Al Elq, A. H.;
Nigella Sativa and Allium Sativum against metabolic syndrome


