Effect of *Nigella Sativa* Supplementation in Diet on Metabolic Syndrome in Aged Rats

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Abstract: Metabolic syndrome is a serious health problem that is increasing worldwide particularly in aged people due to increased fructose intake in processed foods as well as physical inactivity. The present study was conducted on aged rats to investigate the effect of dietary supplementation with ground seeds of *Nigella sativa* on the criteria of metabolic syndrome in aged rats. The present study was conducted on aged Wistar male albino rats (18-20 months). Rats were allocated into the following 3 groups: Control rats C (n=20) fed standard rat diet; metabolic syndrome group M (n=14) fed high fructose diet (60% of diet as high fructose as M group but mixed with ground seeds of *Nigella sativa* (180 mg/Kg b.w)) to achieve daily intake of *Nigella sativa* (180 mg/Kg b.w). Throughout the study, rats were examined for daily food intake and weekly body weight. After 4 weeks, rats were subjected to estimation of the following parameters: final body weight, body mass index (BMI) and serum levels of glucose, insulin, total cholesterol, HDL-c, LDL-c, VLDL-c and adiponectin. Insulin resistance was estimated by calculating HOMA-R. Histopathological examination of rat livers, kidneys and brains was also done. Obtained results revealed that visceral fat weight increased significantly in M group compared to C group and decreased significantly in M/NS group compared to M group. Both M and M/NS groups had significant increase in serum levels of fasting glucose, insulin, total cholesterol, LDL-c, VLDL-c and HOMA-R as well as significant decrease in serum adiponectin compared to C group. However M/NS group showed significant decrease of serum levels of fasting glucose, insulin, total cholesterol, LDL-c and HOMA-R as well as significant increase of serum adiponectin compared to M group. Histopathological examination revealed vascular congestion in the liver and kidneys, necrosis of hepatocytes and renal tubular cells as well as focal cerebral hemorrhage in M group and almost normal histological picture in M/NS group. In conclusion: *Nigella sativa* seeds co-feeding with high fructose diet improved some criteria of metabolic syndrome in aged rats.


Key words: HOMA-R, metabolic syndrome, dyslipidemia, fatty liver, adiponectin, visceral adiposity.

Introduction:

Third Report of the National Cholesterol Education Program -Adult Treatment Panel III (ATP III) report (2002) identified the metabolic syndrome as a clustering of metabolic complications of obesity and that it constitutes a multiple of risk factors that deserve more clinical attention. Individuals with metabolic syndrome are susceptible to cardiovascular disease (CVD), type II diabetes, polycystic ovary syndrome, fatty liver, cholesterol gallstones, asthma, sleep disturbances, and some forms of cancer (Lorenzo et al., 2007). ATP III report (2002) identified 6 components of the metabolic syndrome that relate to cardiovascular risk which are abdominal obesity, atherogenic dyslipidemia, raised blood pressure, insulin resistance ± glucose intolerance, proinflammatory state, prothrombotic state. An individual that meets three or more of these criteria yields a clinical diagnosis of metabolic syndrome (Kraja et al., 2006).

The number of individuals with metabolic syndrome is increasing worldwide, constituting a major social problem in many countries (Lim et al., 2010). Several population studies have reported an increase in the prevalence of the metabolic syndrome with age with more susceptibility to morbidity and mortality (Sanisoglou et al., 2006 and Hildrum et al., 2007).

The black seed, *Nigella sativa* (NS), a member of the family of ranunculaceae, contains more than 30% of fixed oil and 0.4-0.45 % wt/wt of volatile oil which contains 18.4-24% thymoquinone (TQ) and 46% many monoterpenes such as p-cymene and α-piene (El-Kadi and Kandil, 1987). Clinical and animal studies have shown that extract of the black seeds has immunomodilative (Hanafy and Hatem, 1991), antibacterial (Zaoui et al., 2000), hypotensive (Turkdogan et al., 2001), hepatoprotective (Kanter et al., 2003) and antidiabetic effects (Bamosa et al., 2002).

The present study was conducted on aged rats fed high fructose diet to find out if *Nigella sativa* ground seeds co-feeding with high fructose diet could prevent or ameliorate criteria of metabolic syndrome.
Material and Methods

Experimental animals:
This study was approved by the high society of scientific ethic committee of NNI (National Nutrition Institute) & GOTHI (General Organization for Teaching Hospitals and Institutes).

The present study was carried out on 52 aged male Wistar albino rats (18-20 months) purchased from Helwan Animal Farm and were housed in National Nutrition Institute (NNI). All rats were housed individually in wire meshed cages. Animals were fed ad libitum on water and the standard rat diet (AIN-93 M diet formulated for adult rodents) prepared according to the National Research Council (NRC), 1978 and Reeves et al. (1993). Rats were randomly allocated in three groups:

Control group C (n=20): comprised of rats fed standard rat diet.
Metabolic syndrome group M (n=14): comprised of rats fed high fructose diet (60 % of diet in the form of pure fructose). Fructose was added as 100% pure powder (SAFI) as described by Kasim-Karakas et al. (1996).
Metabolic syndrome and Nigella sativa group M/NS (n= 18): comprised of rats fed high fructose diet as M group and supplemented with ground seeds of Nigella sativa (1.7 g/ Kg diet) to achieve a daily dietary intake of (180 mg/kg b.w) modified from Buriro and Tayyab (2007). The mean daily intake of Nigella sativa per rat was calculated to be 54±1.5 mg.

Throughout the study period, rats were examined for daily food intake and weekly body weight. After 4 weeks, rats were fasted overnight, weighed and anesthetized by thiopental sodium (40mg/kg b.wt.: i.p). The animal was placed on its back, fixed on the dissecting table, and the length of the rat was measured from the tip of the nose (while the neck is extended) to the anus to calculate body mass index (BMI) according to the following equation BMI= Body weight (Kg)/ length (m²) (Guyton & Hall 2006). A midline abdominal incision was made, the abdominal aorta was exposed and blood samples were collected in plastic tubes, centrifuged at 4000 r.p.m. for 15 minutes to separate serum which was stored at – 80ºC for later biochemical study. Visceral fat was excised and weighed with 5 Digit-Melter balance (AK 163).

Biochemical assay of serum levels of:
Glucose using (Randox kit) according to Barham and Trender (1972), insulin using rat insulin ELISA kit EIA 2018 (DRG international inc, USA) according to Korner et al. (2001). Total cholesterol (TC), using Bio Mérieux kit according to Richmond (1973 ) and Allain et al. (1974). HDL-c using Bio Mérieux kit (Burstein et al., 1970 and Lopes Virella et al., 1977), LDL-C was determined using Bio Mérieux kit (Friedewald et al., 1972, Levy et al., 1981 and Fruchtart, 1982), serum adiponectin using Alpco ELISA kit for rat adiponectin (ALPCO Diagnostics) according to the method described by Shimada et al., (2004).

VLDL-c was determined by using the following equation: VLDL-c=total cholesterol-(HDL-c +LDL-c).

The homeostasis model assessment of insulin resistance (HOMA-R), an index of insulin resistance was calculated from the product of the fasting concentrations of plasma insulin (microunits per milliliter) and plasma glucose (millimoles per liter) divided by 22.5 according to Matthews et al., (1985).

Histopathological study
Livers, kidneys and brains of rats were excised and kept in 10% formaline for histopathological examination, dehydrated, cleared in yzoil and embedded in parablast. Paraffin sections were cut serially at 6 µm thickness and stained by Hematoxylin and Eosin (Hx & E) as described by Drury and Wallington (1980).

Statistical Analysis:
All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1 according to Armitage and Berry (1987). Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between means of different groups; further analysis was made by LSD (least significance difference) to find intergroupal difference. A probability of P< 0.05 was considered statistically significant. Correlations and Lines of Regression were calculated by linear regression analysis using the Least Square Method. A probability of (P<0.05; 2tailed) was considered statistically significant .All data were expressed as mean ±SEM.

Results
In the fourth week of the study M rats exhibited 30% death rate compared to 10 % death rate in M/NS rats.

Food intake, initial and final body weight, weight gain and BMI were not significantly different among the three studied groups. However, visceral fat weight increased significantly (P<0.05) in M group compared to C group and decreased significantly (P<0.05) in M/NS group compared to M group approaching normal control values. Serum adiponectin level decreased significantly (P<0.05) in both M and M/NS groups compared to C group but
increased significantly (P<0.05) in M/NS group compared to M group (Table 1).

Serum levels of glucose, insulin as well as HOMA-R increased significantly (P<0.05) in M and M/NS groups compared to C group and decreased significantly (P<0.05) in M/NS group compared to M group (Table 2).

Serum levels of total cholesterol, LDL-c and VLDL increased significantly (P<0.05) in M and M/NS groups compared to C group. Total cholesterol and LDL-c showed significant (P<0.05) decrease in M/NS group compared to M group, while HDL-c was not significantly different among the three studied groups (Table 2).

Correlation study in M and M/NS groups revealed that visceral fat weight correlated significantly and positively with serum levels of glucose, insulin, HOMA-R, total cholesterol, LDL-c, VLDL-c and negatively with serum levels of adiponectin (Table 3; Figs. 1, 2).

Histopathological examination of livers of M rats showed congestion of central vein and blood sinusoids, necrosis of hepatocytes in the form of pyknosis of their nuclei and hepatocellular vacuolations. Livers of M/NS group exhibited less extensive changes in the form of congestion of central veins (Figs. 3; a.b.c).

Histopathological examination of the kidneys from M rats showed necrobiotic changes of epithelial lining of renal tubules and congestion of renal blood vessels. These changes were less extensive in the kidneys of M/NS rats (Figs. 4; a.b.c).

Histopathological examination of brains from M rats showed focal gliosis, pyknosis of neurons and focal cerebral hemorrhage. Brains from M/NS rats showed pyknosis of some neurons and neurophagia of pyknotic neurons (Figs. 5; a.b.c).

Table (1): Changes in initial body weight (IBW, g), final body weight (FBW, g), weight gain (WG, g), food intake (g), Body mass index (BMI, Kg/m²), visceral fat (VF, g) and serum adiponectin (ADPN, ng/ml) in control group (C), metabolic syndrome group (M) and metabolic syndrome/Nigella sativa group (M/NS).

<table>
<thead>
<tr>
<th>Groups</th>
<th>IBW (g)</th>
<th>FBW (g)</th>
<th>WG (g)</th>
<th>Food intake (g)</th>
<th>BMI (Kg/m²)</th>
<th>VF (g)</th>
<th>ADPN (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C(n=20)</td>
<td>295.6±3.7</td>
<td>356.8±5</td>
<td>61.2±2.3</td>
<td>30.7±0.6</td>
<td>8.8±0.1</td>
<td>9.6±0.2</td>
<td>1.06±0.04</td>
</tr>
<tr>
<td>M(n=14)</td>
<td>296.2±6.7</td>
<td>364.2±7.6</td>
<td>68.1±4</td>
<td>31.1±0.5</td>
<td>8.5±0.3</td>
<td>25.6±2.1</td>
<td>0.4±0.02</td>
</tr>
<tr>
<td>M/NS(n=18)</td>
<td>305.1±3.5</td>
<td>367.5±1.8</td>
<td>62.3±2.7</td>
<td>31.7±0.6</td>
<td>8.4±0.1</td>
<td>11.8±0.3</td>
<td>0.5±0.01</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

a: significance by LSD at significance level P< 0.05 from C group.
b: significance by LSD at significance level P< 0.05 from M group.
P: significance by one way ANOVA among the three studied groups.
NS: not significant
In parenthesis is the number of rats.

Table (2): Changes in serum levels of glucose (S.glucose, mg/dl), insulin (S.insulin, µU/ml), total cholesterol (TC, mg/dl), high density lipoprotein cholesterol (HDL-c, mg/dl), low density lipoprotein cholesterol (LDL-c, mg/dl), very low density lipoprotein cholesterol (VLDL-c, mg/dl) and HOMA-R in control group (C), metabolic syndrome group (M) and metabolic syndrome/Nigella sativa group (M/NS).

<table>
<thead>
<tr>
<th>Groups</th>
<th>S. glucose (mg/dl)</th>
<th>S. insulin (µU/ml)</th>
<th>HOMA-R</th>
<th>TC (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
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<tr>
<td>C(n=20)</td>
<td>87.9±0.8</td>
<td>12.2±0.1</td>
<td>2.6±0.04</td>
<td>84.6±0.8</td>
<td>34.7±0.3</td>
<td>34.1±0.4</td>
<td>15.8±0.7</td>
</tr>
<tr>
<td>M(n=14)</td>
<td>138.1±4.4</td>
<td>30.8±0.3</td>
<td>10.5±0.3</td>
<td>219.4±2.1</td>
<td>35.2±0.5</td>
<td>154.7±1.4</td>
<td>29.4±2.4</td>
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<tr>
<td>M/NS(n=18)</td>
<td>106.7±1.6</td>
<td>14.2±0.2</td>
<td>3.7±0.08</td>
<td>120.5±1.8</td>
<td>35.1±0.7</td>
<td>58.8±1.2</td>
<td>26.6±1.2</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
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</table>

a: significance by LSD at significance level P< 0.05 from C group.
b: significance by LSD at significance level P< 0.05 from M group.
P: significance by one way ANOVA among the three studied groups.
NS: not significant
In parenthesis is the number of rats.
Table (3): Correlations of visceral fat weight versus serum levels of total cholesterol, LDL-c, VLDL-c, glucose, insulin, adiponectin (ADPN) and HOMA-R in metabolic syndrome group (M) and metabolic syndrome/Nigella Sativa group (M/NS).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (µU/ml)</th>
<th>HOMA-R</th>
<th>TC (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
<th>VLDL-c (mg/dl)</th>
<th>ADPN (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (n=14)</td>
<td>0.79</td>
<td>0.87</td>
<td>0.85</td>
<td>0.87</td>
<td>0.82</td>
<td>0.95</td>
<td>-0.77</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
</tr>
<tr>
<td>M/NS (n=18)</td>
<td>0.71</td>
<td>0.88</td>
<td>0.83</td>
<td>0.7</td>
<td>0.68</td>
<td>0.47</td>
<td>-0.77</td>
</tr>
<tr>
<td></td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.001</td>
<td>P &lt;0.005</td>
<td>P &lt;0.001</td>
</tr>
</tbody>
</table>

r: correlation coefficient

Fig. (1): Correlation of visceral fat weight (g) versus The homeostasis model assessment of insulin resistance (HOMA-R) in metabolic syndrome group (M) and metabolic syndrome/Nigella Sativa group (M/NS).

Fig. (2): Correlation of visceral fat weight (g) versus The homeostasis model assessment of insulin resistance (HOMA-R) in metabolic syndrome group (M) and metabolic group/Nigella Sativa (M/NS).

Fig. (3): (A) Microscopic examination of liver of C rat showing normal histological picture. (B) Liver of M rat showed congestion of central vein and blood sinusoids, necrosis of hepatocytes in the form of pyknosis of their nuclei and vacuolations. (C) Livers of M/NS group with almost normal picture (Hx & E 400 x).
Discussion

Metabolic syndrome is increasing worldwide due to increased fructose intake and sedentary life style. The current investigation revealed that rats fed high fructose diet (M group) for 4 weeks developed three criteria of the metabolic syndrome namely visceral adiposity, insulin resistance and atherogenic dyslipidemia in the form of increased total cholesterol, LDL-c and VLDL-c. The high death rate in M compared to M/NS rats suggests development of fatal complications by the end of the study. Histopathological examination rat livers, kidneys and brains revealed vascular congestion, cellular degeneration, necrosis and cerebral hemorrhage in M group which might explain the higher death rate in this group. The observation that M rats developed significant visceral adiposity as early as 4 weeks of high fructose feeding without significant change in final body weight indicates that visceral adiposity and not obesity that contributed to the development of metabolic syndrome and its complications. The observation that food intake was not significantly changed among the three studied groups suggests that metabolic syndrome might evolve with normal food and energy intake if fructose comprised an increasing proportion of the ingested food. Fructose-induced visceral adiposity might be due to hypertriglyceridermia and development of hepatic insulin resistance (Tappy et al., 2010). Excess visceral fat was found to increase level of inflammatory mediators like IL-6 and TNF-α which were reported to be implicated in insulin resistance (Cartier et al., 2008). Our study demonstrated that visceral fat weight correlated significantly and positively with insulin resistance and dyslipidemia which agree with Yatagai et al. (2003) and negatively with serum adiponectin, a correlation previously validated in human patients with increased waist –hip ratio (Nasseri et al., 2008) as well as normal men (Nakamura et al., 2009). The absence of any significant difference in BMI between M and C group excludes the contribution of total body fat to hypoadiponectinemia as recently reported by Nasseri et al. (2008). Decreased seum adiponectin with increased visceral adiposity would be expected to deprive the animal from a natural anti-inflammatory (Alkharfy et al., 2011), antioxidant (Kruk et al., 2000), cardioprotective (Kondo et al., 2010) and hepatoprotective (Hamed et al., 2011 and Latif et al., 2011) molecule which might explain the vascular and cellular microscopic changes observed in M rat livers, kidneys and brains. Finding the causative link between visceral adiposity and hypoadiponectinemia in metabolic syndrome might be helpful in establishing a prophylactic approach to the complications of metabolic syndrome particularly in
The present study showed that increased fructose content in diet for 4 weeks resulted in development of metabolic syndrome in aged rats with appearance of vascular and cellular degenerative changes. *Nigella Sativa* co-feeding with high fructose diet conferred protection against the development of three criteria of metabolic syndrome which could be of value for aged people particularly those who cannot engage in other therapeutic or prophylactic regimens.

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**References:**


