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

Nehal M. Bahgat [1*] and Ghada Z. A. Soliman[2]

[1]Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
[2]National Nutrition Institute (NNI), Cairo, Egypt
[*]nehalgamil@ yahoo.com

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 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 carried out on 52 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 in the form of pure fructose) and metabolic syndrome/*Nigella sativa* group M/NS (n=18) fed high fructose diet as M group but mixed with ground seeds of *Nigella sativa* (1.7 g/ Kg diet) 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 weights, 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.
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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 (ATPIII) 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 morbididty and mortality *(Sanisoglu 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 (**N**ational **N**utrition **I**nstitute) & GOTHI (**G**eneral **O**rganization for **T**eaching **H**ospitals and **I**nstitutes).

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 (m^2) *(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 – 80^{o}C 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),* insuln 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 Fruchart, 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 zylol 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 differences. 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/m^2), 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).

Groups	IBW (g)	FBW (g)	WG (g)	Food intake (g)	BMI (Kg/m^2)	VF (g)	ADPN (ng/ml)
C*(n=20)*	295.6±3.7	356.8±5	61.2±2.3	30.7±0.6	8.8±0.1	9.6±0.2	1.06 ±0.04
M*(n=14)*	296.2±6.7	364.2±7.6	68.1±4	31.1±0.5	8.5±0.3	25.6±2.1[a]	0.4±0.02[a]
M/NS*(n=18)*	305.1±3.5	367.5±1.8	62.3±2.7	31.7±0.6	8.4±0.1	11.8±0.3[b]	0.5±0.01[ab]
P	*NS*	*NS*	*NS*	*NS*	*NS*	*<0.001*	*<0.001*

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).

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

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).

Groups		Glucose (mg/dl)	Insulin (µU/ml)	HOMA-R	TC (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)	ADPN (ng/ml)
M *(n=14)*	*r*	0.79	0.87	0.85	0.87	0.82	0.95	-0.77
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
M/NS*(n=18)*	*r*	0.71	0.88	0.83	0.7	0.68	0.47	-0.77
	P	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.005*	*<0.001*

r: correlation coefficient *P is significance at 0.05 level*

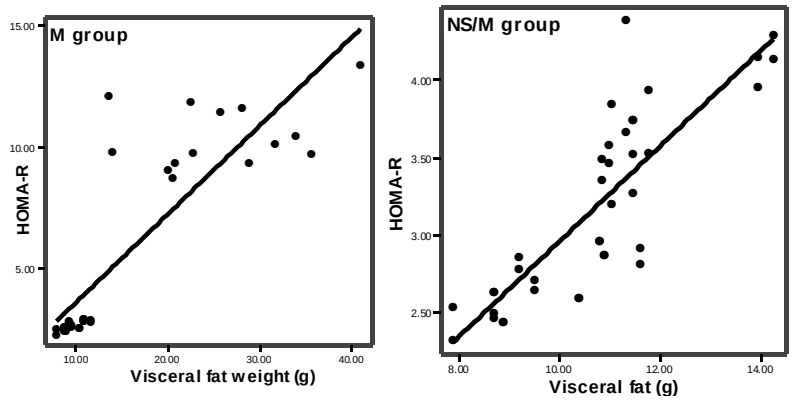


Fig. (1);Correlation of visceral fat weight (g) versus The homeostasis model assessment of insulin resistance (HOMA-R) in metabolic syndrome group (M) and metabolic syndrome/*Nigella Sativa* group (M/NS).

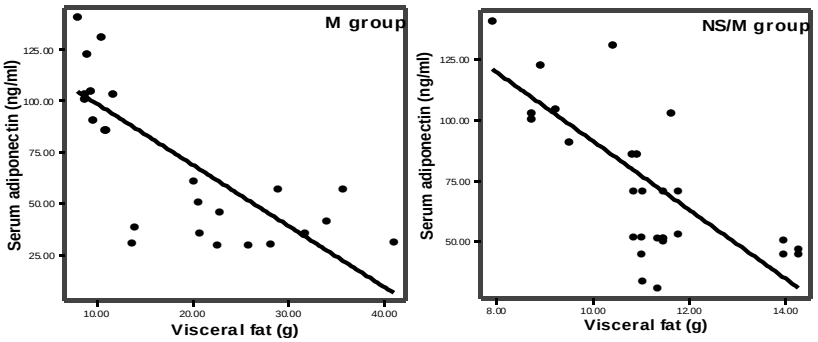
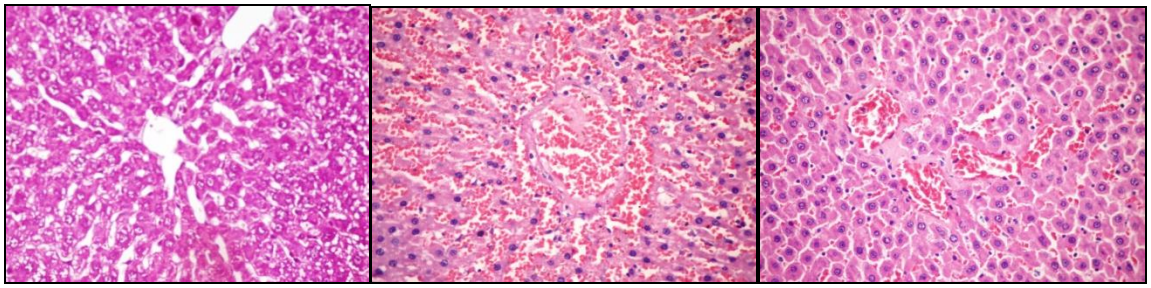
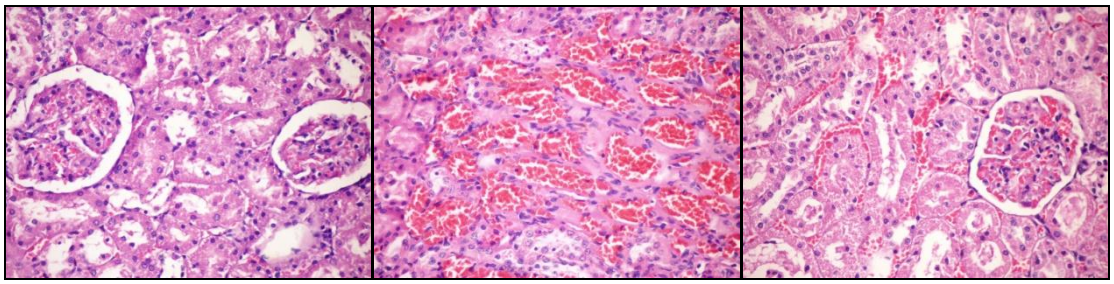


Fig. (2); Correlation of visceral fat weight (g) versus The homeostasis model assessment of insulin resistance (HOMA-R) in metabolic syndrome group (M) and metabolic group /*Nigella Sativa* (M/NS).



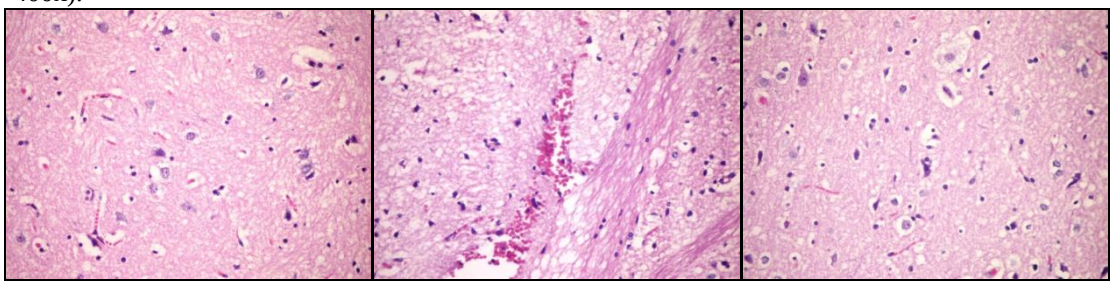
A **B** **C**

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).



A **B** **C**

Fig. (4): (A) Microscopic examination of kidney of C rat showing normal histological picture of renal corpuscles and tubules. (B) Kidneys of M rats showing congestion of renal blood vessels and necrosis of tubular cells. (C) Kidneys of M/NS group showing less vascular congestion and less necrosis of tubular cells (Hx & E 400x).



A **B** **C**

Fig. (5): (A) Microscopic examination of brain of C rat showing normal histological appearance of brain tissue. (B) Brain of M rats showing focal gliosis, pyknosis of neurons and focal cerebral hemorrhage. (C) Brain of M/NS rats showing pyknosis of some neurons (Hx & E 400x).

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 hypertriglyceridemia 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 antinflammatory *(Alkharfy et al., 2011)* ,antioxidant *(Kruk et al., 2000),* cardioprotective *(Kondo et al.,2010)*and hepatoprotrctive *(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

old age. The contribution of visceral adiposity to insulin resistance was demonstrated in our study by the significant positive correlation between visceral fat and HOMA-R and was in accordance with previous reports in human and animal models of metabolic syndrome *(Barzilai et al., 1999; Frayn, 2000 and Yatagai et al., 2003).* However, the mechanism underlying this correlation has not been well elucidated. Proposed mechanisms included visceral fat lipolysis and enhanced non-esterified free fatty acid flux into the portal vein blood to the liver *(Frayn, 2000)* as well as hypo-adiponectemia *(Yatagai et al .,2003).* Hypercholesterolemia observed in M rats in the current study was due to increased LDL and VLDL fractions rather than HDL which was consistent with the ATPIII (2000) criteria of metabolic syndrome. Insulin resistance might have contributed to this unfavorable lipid profile due to increased LDL and VLDL synthesis by the liver as well as decreased removal from the circulation *(Ganong , 2001).*

Nigella Sativa seeds supplementation with the high fructose diet in M/NS rats decreased visceral adiposity, insulin resistance, hyperlipidemia hypoadiponectinemia and alleviated histopathological abnormalities seen in M group. This beneficial outcome of *Nigella Sativa* came in accordance with the results of *Hildrum et al. (2007); Najmi et al. (2008) and Parhizkar et al. (2011)***.** Amelioration of all criteria of metabolic syndrome by *Nigella sativa* despite continued intake of the high fructose diet indicates that it targeted the mechanism responsible for eliciting this syndrome which might be visceral adiposity or insulin resistance. Nigella sativa seeds was reported to possess hypoglycemic *(Benhaddou–Andaloussi et al., 2010),* hypolipidemic, antinflammatory and antioxidant properties *(Abd–El–Fattah et al., 2000 ; Kruk et al., 2000 and Alkharfy et al., 2011).* The cholesterol- lowering effect of *Nigella Sativa* was reported to be due to either inhibition of *de novo* cholesterol synthesis by downregulating 3 -hydroxy -3-methylglutaryl-coenzyme A reductase (HMGCR) genes in HepG2 cells as reported by *Al-Naqeep et al. (2009)* or stimulation of bile acid excretion *(Bamosa, 1997)***.**The significant decrease in LDL-c was reported to be due to upregulation of LDL –receptor gene *(Al-Naqeep et al., 2009).*

Previous interventions to metabolic syndrome aimed at modification of the life style of patients by restricting caloric intake, promoting physical activity and encouraging intake of polyunsaturated fatty acids. However, elderly people may not always yield to these approaches partially due to low compliance or the presence of associated health problems that limit physical activity.

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 degenerat 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.

Corresponding author
Nehal M. B. Gamil; Physiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
*nehalgamil@ yahoo.com

References:

Abd–El–Fattah A, Matsumoto K, and Watanabe H., (2000) . Antihypertensive effects of Nigella sativa oil and its major component, thymoquinone, in mice. Eur. J. Pharmacol., 14: 89–97.

Alkharfy KM, Al-Daghri NM, Al-Attas OS, and Alokail MS., 2011.The protective effect of thymoquinone against sepsis syndrome morbidity and mortality in mice. Int Immunopharmacol., 11:250-4.

Allain CC, Poor LS, Chan CSG, Richmond W, and Fu PC. , 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20: 470-475.

Al-Naqeep G, Ismail M , and Allaudin Z. 2009; Regulation of low-density lipoprotein receptor and 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression by thymoquinone-rich fraction and thymoquinone in HepG2 cells. J Nutrigenet Nutrigenomics. 2(4-5):163-72.

Armitage P, and Berry G., 1987.Statistical Methods in Medical Reserve in left ventricular hypertrophy. : Hypertension, 5: 192-197.

Barham D and Trinder P., 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst, 97: 142-145.

Bamosa AO., 1997. Effect of oral ingestion of Nigella sativa seeds in some blood parameters. Saudi Pharm J., 5:126-9.

Bamosa AO, Ali BA, al-Hawsawi ZA., 2002. The effect of thymoquinone on blood lipids in rats . Indian J physiol Pharmacol., 195-201.

Barzilai N, She L, Liu BQ, P, P, Wang J and Rossetti L., 1999. Surgical removal of visceral fat reverses hepatic insulin resistance. Diabetes, 48: 94-98.

Benhaddou–Andaloussi A, Martineau LC, Vallerand D, Haddad Y, Afshar A, and Settaf A, *et al.* ,2010. Multiple molecular targets underlie the antidiabetic effect of *N. sativa* seed extract in skeletal muscle, adipocyte and liver cells. Diabetes Obes. Metab.,12 : 148–57.

Buriro MA and Tayyab M. 2007.Effect Of Nigella Sativa On Lipid Profile In Albino Rats. Gomal Journal of Medical Sciences Jan–June, Vol. 5, No. 1

Burstein M, Scholnick HR, and Monfin R., 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J. Lipid Res., 11: 585-595.

Cartier A, Lemieux I, Alméras N, Tremblay A, Bergeron J, and Després JP. , 2008. Visceral obesity and plasma glucose-insulin homeostasis: contributions of interleukin-6 and tumor necrosis factor-alpha in men. J Clin Endocrinol Metab., 93:1931-8.

Drury RA, and Wallington EA.,1980. Carleton's Histological Techniques.Fifth Edition Oxford University, p 139.

El-Kadi A, and Kandil O. ,1987. The black seed (Nigella sativa) and immunity: its effect on human T cell subset. Fed Proc., 46: 1222.

Frayn KN., 2000. Visceral fat and insulin resistance — causative or correlative? British Journal of Nutrition, 83: S71-S77.

Friedewald WT, Levy RI, and Fredrickson DS. , 1972. Estimation of the concentration of low-Density lipoprotein cholesterol in plasma without use of the preparative ultra centrifuge." Clin. Chem., 18: 499-502.

Fruchart GC. ,1982. LDL-Cholesterol determination after separation of low density lipoprotein. Rev. Fr. Des. Lab., 7: 103-117.

Ganong WF.,2001. *Review of* Medical Physiology; 20th edition (Middle Eeast Edition). Chapter 19, page 332. Mc Graw Hill; LIBRAIRIE DU LIBANE.

Guyton and Hall. (2006): Textbook of medical physiology; Eleventh edition. Unit XIII ; page: 872. Elesevier Saunders.

Hamed GM, Bahgat NM, Abdel Mottaleb FI and Emara MM., 2011. Effect of Flavonoid Quercetin Supplement on the Progress of Liver Cirrhosis in Rats. Life Science Journal, 8:641-651.

Hanafy MS, and Hatem ME. , 1991. Studies on the antimicrobial activity of Nigella sativa seed (black cumin). J Ethnopharmacol., 34: 275-278.

Hildrum B, Mykletun A, Hole T , Midthjell K and Dahl AA. , 2007. Age-specific prevalence of the metabolic syndrome defined by the International Diabetes Federation and the National Cholesterol Education Program: the Norwegian HUNT 2 study. BMC Public Health, 7:220

Kanter M, Meral I, Yener Z, Ozbek H, and Demir H. ,2003. Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by Nigella sativa L. in streptozotocin-induced diabetic rats. Tohoku J Exp Med., 201: 213-219.

Kasim-Karakas,SE, Vriend,H, Almario,R, Chow,LC, and Goodman,MN. 1996. Effects of dietary carbohydrates on glucose and lipid metabolism in golden Syrian hamsters. J Lab Clin Med 128:208-213.

Kondo K, Shibata R, Unno K, Shimano M, Ishii M, Kito T, Shintani S, Walsh K, Ouchi N, and Murohara T. ,2010. Impact of a single intracoronary administration of adiponectin on myocardial ischemia/reperfusion injury in a pig model. Circ Cardiovasc Interv., 3:166-73.

Korner J, Savontaus E, Chua SC Jr, Leibel RL. and Wardlaw SL. ,2001. Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J. Neuroendocrinol. 13:959-966.

Kraja AT, Borecki IB,North K, Tang W, Myers RH, Hopkins PN, Arnett D, Corbett J, Adelman A and Province MA. ,2006. Longitudinal and age trends of metabolic syndrome and its risk factors: The Family Heart Study. Nutrition & Metabolism, 3:41.

Kruk I, Michalska T, Lichszteld K, Kladna A, and Aboul–Enein H., 2000. The effect of thymol and its derivatives on reactions generating reactive oxygen species. Chemosphere, 411059–1064.

Latif HA, Assal HS, Mahmoud M, and Rasheed WI., 2011. Role of serum adiponectin level in the development of liver cirrhosis in patients with hepatitis C virus. Clin Exp Med. Jun; 11:123-9.

Levy RI., 1981 .Cholesterol lipoprotein, apolipoproteins, and heart disease: Present status and future properties. Clin. Chem., 27: 653-662.

Lim S, Cho YM, Park KS, Lee HK, and Ann N Y., 2010. Persistent organic pollutants, mitochondrial dysfunction, and metabolic syndrome. Acad Sci., 1201:166-76.

Lopes-Virella MF, Stone PG, Ellis S, and Coldwell JA. , 1977. Cholesterol determination in high density lipoprotein separated by three different method. Clin. Chem., 23: 882-884.

Lorenzo C, Williams K, Hunt KJ and Haffner SM., 2007.The National Cholesterol Education Program–Adult Treatment Panel III, International Diabetes Federation, and World Health Organization Definitions of the Metabolic Syndrome as Predictors of Incident Cardiovascular Disease and Diabetes. Diabetes Care, 30 : 8-13

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, and Turner RC**.,** 1985. Homeostasis model assessment. Diabetologia, 28:412–419.

Najmi A, Nasiruddin M, Khan RA, and Haque SF., 2008. Effect of Nigella Sativa oil on various clinical and biochemical parameters of insulin resistance syndrome. Int J Diabetes Dev Ctries.,28 : 11-14.

Nakamura Y, Sekikawa A, KadowakI T, Kadota A, Kadowaki S, Maegawa H, Kita Y, Evans RW, Edmundowicz D, Curb JD and Ueshima H. ,2009.Visceral and Subcutaneous Adiposity and Adiponectin in Middle-aged Japanese Men: The ERA JUMP Study.Obesity,**17** : 1269–1273.

Nasseri E, Hosseini M, Dorosti AR and Chamari M., 2008.Relationship of visceral adiposity with plasma adiponectin concentration: effect of weight loss. *Endocrine* Abstracts 16:560.

National Research Council (NRC) Committee on Animal Nutrition. (1978): Nutrient requirement of laboratory animals. No. 10 3rd revised edition. National academy of science, National Research Council, Washington, DC.

Parhizkar S, Latiff LA1, .Rahman SA and Dollah MA. , 2011. Preventive effect of Nigella sativa on metabolic syndrome in menopause induced rats. Journal of Medicinal Plants Research, 5: 1478-1484.

Reeves PG, Nielson FH, and Fahey GC Jr. (1993): Ain 93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition and HOC Writing Committee on the Reformation of the Ain 76 A rodent diet. J Nutr 123: 1939-1952.

Richmond W., 1973. Preparation and properties of cholesterol oxidase from Nocardia Sp. And its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.

Sanisoglu SY, Oktenli C, Hasimi A, Yokusoglu M, and Ugurlu M. ,2006.Prevalence of metabolic syndrome-related disorders in a large adult population in Turkey. BMC Public Health, 6:92.

Shimada, K., T. Miyazaki, and Daida H., 2004. Adiponectin and atherosclerotic disease. Clin Chim Acta., 344 : 1-12.

Tappy L, Lê KA, Tran C, and Paquot N. (2010); Fructose and metabolic diseases: new findings, new questions. Nutrition, 26:1044-9.

Third Report of the The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. National Heart, Lung, and Blood Institute, National Institutes of Health , NIH Publication No. 02-5215, 2002; Page II-26.

Turkdogan MK, Agaoglu Z, Yener Z, Sekeroglu R, Akkan HA, and Avci ME., 2001. The role of antioxidant vitamins (C and E), selenium and *Nigella sativa* in the prevention of liver fi brosis and cirrhosis in rabbits: new hopes. Dtsch Tierarztl Wochensch, 108: 71-73..

Yatagai T, Nagasaka S, Taniguchi A , Fukushima M, Nakamura T, Kuroe A, Nakai Y, and Ishibashi S. ,2003. Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism - Clinical and Experimental**,** 52 ; 1274-1278.

Zaoui A, Cherrah Y, Lacaille-Dubois MA, Settaf A, Amarouch H, and Hassar M. ,2000. Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat. Therapie, 55: 379-382.

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