Full Length Research Paper

Preventive effect of *Nigella sativa* on metabolic syndrome in menopause induced rats

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In women facing menopause, end of menstrual activity is accompanied by lower levels of estrogen and gradual weight gain. Postmenopausal weight gain sounds an alarm for women's health and may lead to hyperlipidemia, a lipid increase and glucose intolerance. These phenomena are connected to lifestyle-related diseases such as hypertension, type II diabetes mellitus, arteriosclerosis and metabolic syndrome, making it essential to prevent weight gain in women. This study was conducted using an ovariectomized rat model to determine the metabolic impact of *Nigella sativa* in experimental menopause induced rats*.* Forty ovariectomized Sprague Dawley rats, weighting 250 to 350 g were used in the study and randomly allotted into one of five experimental groups. Animals were given either different doses of *N. sativa* (300, 600, 1200 mg/kg/day) as treatment groups or distilled water (1 ml) and conjugated equine estrogen (CEE) (200 µg/kg/day) by intra-gastric gavage as negative and positive control group respectively for 21 days. Food and water intake were measured daily and body weight and biochemical parameters were measured at baseline, 11th day and at the end of experiment. The treatment groups showed significant ($P < 0.05$) improvement with reference to daily body weight gain, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and blood glucose ($P < 0.05$). There were no significant differences between groups in serum triglyceride concentration. These results suggested that treatment with *N. sativa* exert a therapeutic and protective effect by modifying weight gain, improving lipid profile and blood glucose as well as hormonal level which is believed to play an important role in the pathogenesis of metabolic syndrome during menopause.

Key words: Menopause, Metabolic Syndrome, Nigella sativa, Ovariectomized Rats.

INTRODUCTION

For menopausal women, end of menstrual activity is accompanied by lower levels of estrogen and gradual weight gain. This postmenopausal weight gain sounds an alarm for women's health (Schneider et al., 2006) since it can lead to hyperlipidemia, a lipid increase and abnormalities in blood sugar concentrations. These phenomena are all connected to lifestyle-related diseases such as hypertension, type II diabetes mellitus, arteriosclerosis and metabolic syndrome (Kopelman, 2000; Spiegelman and Flier, 2001) making it essential to prevent weight gain that occurs as the hormone levels are reduced. Ovariectomized rats (OVR) are well known as a model for obesity from limited estrogen function (Hioki et al., 2004; Lee et al., 2006).

Obesity is reaching epidemic proportions worldwide; it is correlated with various comorbidities, among which the most relevant are dyslipidemia (Fried et al., 2008), diabetes mellitus (Pagotto et al., 2008), cardiovascular (CV) diseases such as heart failure (HF) and coronary heart

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disease (CHD) (Lavie et al., 2008). It is generally recognized that natural products with a long history of safety can modulate obesity. Nowadays there is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs which may have adverse effects. Traditional medicinal plants are often cheaper, locally available, and easily consumable (raw or as simple medicinal preparations) (Amin and Nagy, 2009).

The seeds of *Nigella sativa* plant have been used to promote health and fight disease for centuries especially in the Middle East and Southeast Asia. In South Asia, it is called Kalonji, its Arabic name is Habat-ul-Sauda and its English name is Black cumin. This plant has been a great focus of research and has several traditional uses and consequently has been extensively studied for its chemical constituents and biological activities (Najmi et al., 2008). A lot of animal studies have already been done to determine the various activities of *N. sativa* on different components of the metabolic syndrome for example blood sugar (Bamosa et al., 1997) and blood pressure (Aqel, 1992).

In spite of large number of pharmacological studies carried out world wide on *N. sativa* seed, no experimental studies have been done in OVX rats as an animal model of menopause. This experimental study was undertaken to know the adjuvant effect of *Nigella sativa* on various clinical and biochemical parameters of the metabolic syndrome in menopause-induced rat model. Thus the aim of current study was to determine the metabolic impact of *N. sativa* in ovarictomizeded rats.

MATERIALS AND METHODS

Plant materials

N. sativa seeds (imported from India) were purchased from a local shop in Serdang, Malaysia. The seed was identified and authenticated by Professor Dr. Nordin Hj Lajis, Head of the Laboratory of Natural Products, Institute of Bioscience, University Putra Malaysia. Voucher specimens of seeds were kept at the Cancer Research Laboratory, Institute of Bioscience, University Putra Malaysia. Seeds were cleaned under running tap water for 10 min, rinsed twice with distilled water and air-dried in an oven at 40°C overnight.

The seeds and rat chow pellet were ground to a powder using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 10 min. Grounded seeds and chow pellet mixed with water into three doses of 300, 600 and 1200 mg/kg to yeild a dough shape. Afterward the dough was rolled in a tray to reach 1 cm thick. Then the dough were cut into small pieces to facilitate balking process and feeding. The pellet supplements were baked in an oven, at 40°C overnight until receiving to the initial weight. The baking pellet supplements were packed in a sealed plastic container to prevent humidity and to maintain its quality.

Chemicals and reagents

Conjugated equine estrogen (CEE 0.625 mg) was purchased from Wyeth, Montreal, Canada. CEE (Wyeth Montreal, Canada), prepared in a dosage of 0.2 mg/kg (Genazzani et al., 2004;

Oropeza et al., 2005; Araujo et al., 2006) by dissolving it in distilled water (Hajdu et al., 1965; Genazzani et al., 2004; Parhizkar et al., 2011) and was used as a positive control for the purpose of comparison with the treated groups. All other reagents and chemicals were of analytical grade.

Animals

The protocol of the study was approved by Animal Care and Use Committee(ACUC) with reference number of UPM/FPSK/PADS/BR/UUH/F01-00220 in accordance to "Guide for care and use of laboratory animals" set by the ACUC of Faculty of Medicine and Health Sciences, University Putra Malaysia. The experiment was carried out using 16 week-old female albino Sprague-Dawley rats, weighing 250 to 350 g. They were housed in cages under standard laboratory conditions with a period of 12 h light/dark at 29 to 32°C and 50 to 60% relative humidity in the Animal House, Faculty of Medicine and Health Sciences, University Putra Malaysia. The animals were allowed to acclimatize for at least 10 days before the start of the experiments. The rats had access to a standard rat chow pellet and drinking water *ad libitum*. Hygienic condition was maintained by changing the bedding weekly. All animal handling were conducted between 08.00 and 10.00 am to minimize the effects of environmental changes. Lipid profile, blood glucose and body weight were measured at baseline (day 0), 11th, and at the end of experiment (21st days).

Experimental design

Forty rats were ovariectomized in order to induce menopause and to investigate biochemical changes following *N. sativa* supple-mentation. Their ovariectomy was performed during a distrous cycle to keep the consistent lowest levels of sex hormones in rats. Surgery of the animals was conducted under a combination of xylazine and ketamine (10 + 75 mg/kg, i.p. respectively) anesthesia. Bilateral ovariectomy was performed through a dorso-lateral approach with a small lateral vertical skin incision. The ovariec-tomized animals were acclimatized at the Animal House of Faculty of Medicine and Health Sciences for one month prior to supple-mentation. The ovariectomized rats were divided equally into five groups (8 animals in each group). The grouping of rats include of negative control (1 ml distilled water by intra-gastric gavage), positive control (0.2 mg/kg/day CEE diluted in distilled water by intra-gastric gavage) and the rest groups receiving different doses of *N. sativa.* The test group consist of low dose *N. sativa*-LNS (300 mg/kg NS), moderate dose *N. sativa*-MNS (600 mg/kg NS), and high dose *N. sativa*-HNS (1200 mg/kg NS). Supplementations with *N. sativa,* CEE and distilled water were continued for 3 weeks.

Statistical analysis

Data were expressed as means ± standard deviation. The data were analyzed using SPSS windows program version 15 (SPSS Institute, Inc., Chicago, IL, USA). The one-way analysis of variance (ANOVA) and general linear model (GLM) followed by Duncan multiple range test (DMRT) were used to determine which *N. sativa* concentration shows the most significant effect. A p-value less than 0.05 ($P < 0.05$) was considered to be significant.

RESULTS

Body weight

Over the period of treatment, the body weight of control group increased 8% compare to baseline. The body
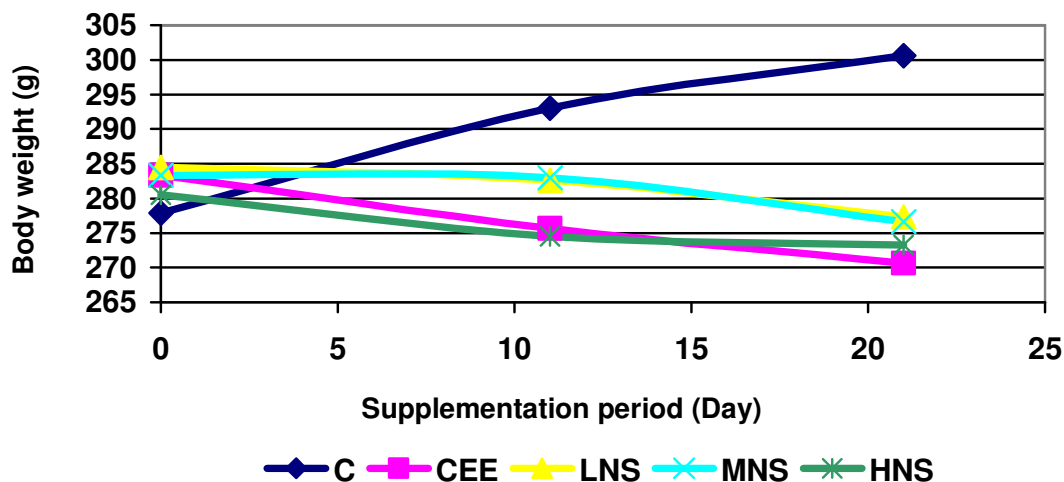
Figure 1. Body weight changes among rats supplemented with various doses of *N. sativa* or CEE for 21 days.Treatment: C= control (1 ml distilled water), CEE= conjugated equine estrogen (0.2mg/kg), LNS= low dose of *N. sativa* (300 mg/kg), MNS= medium dose of *N. sativa* (600 mg/kg), HNS= high dose of *N. sativa* (1200 mg/kg) groups. Data expressed as mean.

Table 1. GLM of average daily gain (gram/day) of OVX rats supplemented with various doses of *N. sativa* or CEE.

Source	DF	Sum of squares	Mean square	F value
ADG11	4	21.7078	5.4269	16.71***
Model	35	11.3646	0.3247	
Total	39	33.0725		
ADG21	4	14.5897	3.6474	18.39***
Model	35	6.9407	0.1983	
Total	39	21.5305		

*** **-** Significantly different at $P < 0.001$.

weight of CEE group had 5% reduction. Supplementation with *N. sativa* for three weeks tended to reduce the body weights of all *N. sativa* groups as compared to control group (Figure 1). Although, body weight decline was not significant, but average daily gain (ADG) in 11th and end of study (21st day) showed a significant differences between treatment groups and control group ($P < 0.001$) (Table 1).

Lipid profile

The sequential changes in serum TC, TG, LDL.C and HDL.C are summarized in Table 2. *N. sativa* supplementations for 21 days in OVX rats significantly improved triglyceride and HDL levels ($P < 0.05$) while no effects were observed on the total and LDL cholesterol. The OVX rats in group fed on low dose *N. sativa* supplementation showed significant rise in HDL cholesterol level as compared to negative and positive control groups.

Blood glucose

There was significant differences ($P < 0.05$) in blood glucose level in treatment groups (LNS and HNS). *N. sativa* supplementation can decline blood glucose levels especially in low and high doses of NS (16.28 and 23.12% respectively). In contrast positive and negative control groups showed an elevation in blood glucose that is CEE group, where it increased significantly ($P < 0.05$) over the period of treatment. Surprisingly, the level of blood glucose remained stable through second and third sampling in all groups (Figure 2).

DISCUSSION

The phenomenon of increased body weight gain in ovariectomized rats is well-documented (Turner et al., 1987; Arjmandi et al., 1998; Ishida et al., 1998; Tansey et al., 1998; Tollba et al., 2010). In addition, previous

Table 2. Means of Lipid profile level (mmol/L) of OVX rats supplemented with various doses of *N. sativa* or CEE.

Parameter	Day	*N. sativa* suplementation (mg/kg/day)				CEE (mg/kg/day)	Total
		0	300	600	1200		
T.C	0	1.56± 0.34 [bc]	1.74±0.20 [abc]	1.56± 0.28 [bc]	1.53± .32 [bc]	1.38± 0.33 [c]	1.55± 0.30 [X]
	11	1.49± 0.31 [bc]	1.65± 0.42 [a]	1.87± 0.30 [ab]	1.66± .30 [abc]	1.54± 0.32 [bc]	1.70± 0.36 [X]
	21	1.53± 0.32 [bc]	2.02± 0.58 [a]	1.71±0.28 [abc]	1.57± .21 [bc]	1.48± 0.23 [bc]	1.66± 0.39 [X]
Total		1.53±0.31 [BC]	1.90± 0.43 [A]	1.71± 0.30 [B]	1.58± 0.27 [BC]	1.46± 0.29 [C]	
TG	0	0.67± 0.28 [bc]	0.79± 0.33 [abc]	0.84± 0.17 [abc]	0.61± 0.16 [bc]	0.45± 0.12 [c]	0.67± 0.26 [X]
	11	0.82± 0.24 [abc]	0.64± 0.12 [bc]	0.63± 0.34 [bc]	0.66± 0.49 [bc]	0.97± 0.66 [ab]	0.74± 0.41 [X]
	21	1.03± 0.51 [ab]	1.18± 0.64 [a]	0.72± 0.21 [bc]	0.78± 0.40 [abc]	1.06± 0.52 [ab]	0.95± 0.49 [X]
Total		0.84± 0.38 [A]	0.87± 0.47 [A]	0.73± 0.26 [A]	0.69± 0.36 [A]	0.83± 0.54 [A]	
HDL	0	1.17± 0.22 [bc]	1.20± 0.12 [bc]	1.17± 0.17 [bc]	1.15± 0.31 [bc]	1.07± 0.30 [c]	1.15± 0.23 [X]
	11	1.11± 0.21 [bc]	1.50± 0.30 [a]	1.49± 0.25 [a]	1.13± 0.29 [bc]	1.15± 0.26 [bc]	1.27± 0.31 [Y]
	21	1.12± 0.22 [bc]	1.38± 0.34 [ab]	1.33± 0.23 [abc]	1.10± 0.18 [bc]	1.11± 0.16 [bc]	1.21± 0.25 [XY]
Total		1.13± 0.21 [B]	1.36± 0.29 [A]	1.33± 0.24 [A]	1.13± 0.25 [B]	1.11± 0.24 [B]	
LDL	0	0.25± 0.07 [bc]	0.25± 0.05 [bc]	0.23± 0.07 [bcd]	0.32± 0.08 [ab]	0.24± 0.04 [bcd]	0.26± 0.07 [X]
	11	0.22± 0.10 [bcd]	0.31± 0.10 [ab]	0.28± 0.08 [abc]	0.37± 0.12 [a]	0.19± 0.07 [cd]	0.27± 0.31 [X]
	21	0.21± 0.07 [cd]	0.25± 0.11 [bcd]	0.27± 0.08 [bc]	0.32± 0.10 [ab]	0.15± 0.05 [d]	0.24± 0.10 [X]
Total		0.23± 0.08 [BC]	0.27± 0.09 [B]	0.26± 0.07 [B]	0.34± 0.10 [A]	0.19± 0.06 [C]	

Data are expressed as Mean ± SD, ABCD: Comparison of the means between columns of the same parameter significant at $P < 0.05$, XYZ: Comparison of the means between rows of the same parameter significant at $P < 0.05$, abcdef: Comparison of the means between column and row significant at $P < 0.05$.
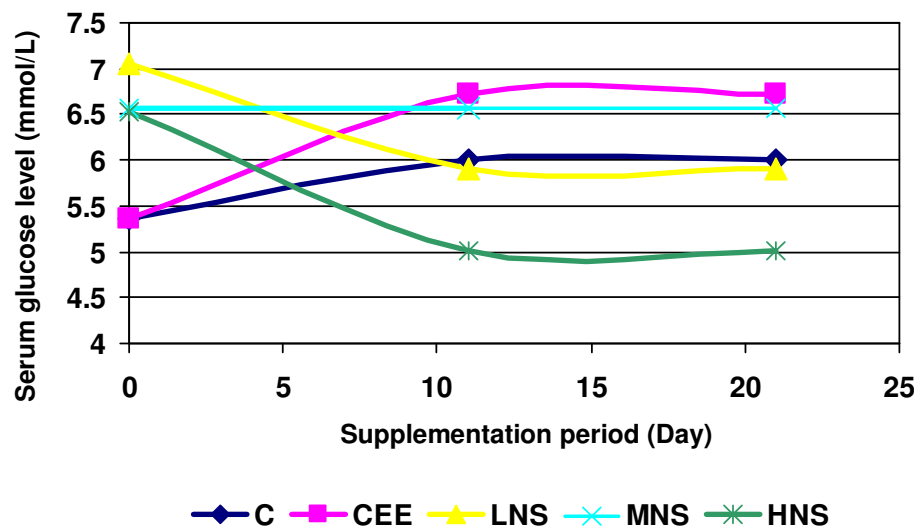

Figure 2. Changes of serum glucose level (mmol/L) of OVX rats supplemented with various doses of *N. sativa* or CEE. Treatment: C= control (1 ml distilled water), CEE= conjugated equine estrogen (0.2 mg/kg), LNS= low dose of *N. sativa* (300 mg/kg), MNS= medium dose of *N. sativa* (600 mg/kg), HNS= high dose of *N. sativa* (1200 mg/kg) groups. Data expressed as mean.

investigations have indicated that estrogen deficiency significantly increased the weight gain in ovariectomized rats, an effect that was attenuated by estrogen treatment (Liu et al., 2004; Sarkaki et al., 2008). The results of the present study showed that treatment of OVX rats with *N. sativa* for 3 weeks induced a transient initial weight loss

and a sustained reduction in food but not water intake. This action was not correlated to the toxicity of *N. sativa*, since no physical or behavioral signs of toxicity like lethargy, hyperactivity, restlessness, respiratory distress or convulsions could be revealed. The same observations have been made by Liu et al. (2004) in normal rats treated with the petroleum ether extract of *N. sativa* for 4 weeks. Recent studies using *N. sativa* also failed to show any in vivo toxicity of *N. sativa* in rats (Izwan et al., 2008; Hafanizam et al., 2008; Meddah et al., 2009). None-theless, our results are in line with previous studies showing that *N. sativa* aqueous extract normalized body weight in the obese diabetic models of *Psammomys obesus* (Labhal et al., 1997; Le et al., 2004) or *Meriones shawi* (Labhal et al., 1999) sand rats. Similarly, *N. sativa* supplemented pellet and the fixed oil of *N. sativa* was found to slow the growth rate of normal rats (Hawsawi et al., 2001; Zaoui et al., 2002). Recent study revealed that methanolic extract and the commercial oil of *N. sativa* displayed appetite-reducing components inducing the loss of weight (Houcher et al., 2007). These effects may be related to the action of *N. sativa* on lipid metabolism. Labhal and colleagues stated that this effect was accompanied by concomitant alterations in plasma insulin levels, which might suggest an insulin-mediated mecha-nism of action (Labhal et al., 1997). It also might be related to the serum lipids and glucose levels decrease as a consequence of a possible reduction in food intake by the drug administration.

Estimations of lipid profile included TG, TC, HDL and LDL cholesterol. Supplementation of *N. sativa* seeds to the diet had a favorable effect on the lipid profile. It decreased TG in the first 10 days and kept TC and LDL cholesterol stable without any undesirable changes which expected for OVX rats and also increased HDL cholesterol as compared to controls. Although, estrogen has effectiveness to ameliorate lipid profile, particularly in post menopause, but in some parameters such as HDL, the effect of *N. sativa* was more favorable rather than CEE.

Serum cholesterol is a major risk factor for the develop-ment of CVD. This study investigated whether *N. sativa* was effective at preventing an increase in serum lipid profile in an OVX rat model of menopause. Undesirable changes in the lipoprotein spectrum in ovariectimized rats are well known (Fait et al., 2002; Liu et al., 2004). Estrogen indirectly influences serum lipoprotein and total cholesterol profiles (Babiker and Leon, 2002). The findings on the effect of *N. sativa* on lipid profile in the present study are consistent with those reported by other scholars (Buriro and Tayyab, 2007; Pourghassem-Gargari et al., 2009).The hypo-triglyceridaemic effect of *N. sativa* is possibly due to its cholerectic activity as reported by El-Dakhakhany (2000). The cholerectic function of *N. sativa* is either by reducing the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine (Qidwai et al.,

2009). The finding that *N. sativa* caused significant increased in HDL cholesterol level was in conformity with the results of Najmi and colleagues (2008). Liu and colleagues (2004) reported that ovariectomy caused elevation of plasma total cholesterol and LDL cholesterol leading to the development of atherosclerosis and CHD. Plasma total cholesterol has received too much impor-tance because of its strong and consistent association with CHD. Arsenault and colleagues (2007) reported that elevation of LDL cholesterol is positively associated while elevation of HDL cholesterol is negatively associated with the development of CHD. *N. sativa* is also a source of soluble fiber which is associated with lower cholesterol and improved blood glucose and insulin levels (Hawsawi et al., 2001; Farah et al., 2002; Mai Le et al., 2004) and has also been found to improve other metabolic syndrome abnormalities including hypertension (Zaoui et al., 2002; Roghani and Kamkhah, 2008), weight control (Labhal et al., 1999; Hawsawi et al., 2001; Zaoui et al., 2002; Houcher et al., 2007; Meddah et al., 2009) and improved insulin sensitivity (Le et al., 2004; Meddah et al., 2009).

Results revealed that the supplementation to OVX rats with 300 and 600 mg/kg/day of *N. sativa* only provoked a significant reduction of blood glucose during the first ten days and then blood sugar remained stable until the end of experiment. The results, however, fail to show a linear consistent dose or time dependent effect of *N. sativa*. Houcher et al. (2007) reported reduction of glucose following treatment of diabetic rats with *N. sativa* oil in the first 10 days and after that this antihyperglicaemic effect was disappeared. Some similar results have been shown by other teams of research which data indicated that volatile oil of *N. sativa* could lead a normoglycaemia in either rats and diabetic rabbits (Al-Awadi et al., 1991; Al-Hader et al., 1993; Hedaya, 1995; Farah et al., 2002; Haddad et al., 2006). These results indicate that the anti hyperglycaemic effect of *N. sativa* is independent of its insulinotropic action (Meral et al., 2001). The cytotoxicity of alloxan or streptozotocin provoked a severe damage on the β- cells of the pancreas and therefore the insulino-secretion is absent in this model of diabetes (Farah et al., 2004). These results were consistent with the hypogly-cemia obtained with a *N. sativa*-containing plant mixture (Al-Awadi et al., 1985) or with the fixed oil of *N. sativa* (Zaoui et al., 2002) in normal rats. Despite of current study, Le and collaborators (2004) reported that *Nigella sativa*-treated animals displayed stable and normal fasting glucose levels throughout the 4-week treatment period with the petroleum ether extract. Hawsawi et al. (2001) observed that the higher doses of *N. sativa*, particularly 500 mg, tended to lose their effect after two weeks of daily treatment. A similar finding was observed in previous study in humans (Bamosa et al., 1997), which may support the possibility that the dose used in the human study (2 g/day) was also high. The hypoglycemic effect of *N. sativa* found in this study is in agreement with

previous reports in normal and alloxan induced diabetic rabbits (Al-Hader *et al.,* 1993), alloxan-induced diabetic rats (El-Shabrawy and Nada, 1996; Eskander et al., 1995), and in human subjects (Bamosa et al., 1997). On the other hand, our results seem to be in contradicting with other studies (Al-Awadi and Gumaa, 1987; El-Naggar and El-Deib, 1992; El-Shabrawy and Nada, 1996). Al-Awadi and Gumaa (1987) reported that there was no significant change in fasting blood glucose level when *N. sativa* (40 mg/day) was administered to normal and streptozotocin-induced diabetic rats. El- Naggar and El-Deib (1992) also found that there was no significant reducing effect of *N. sativa* (36 mg/day) on blood glucose level in normal rats. However, it seems that the doses of *N. sativa* used by both groups were subtherapeutic. The third group (El-Shabrawy and Nada, 1996) who reported a negative effect of *N. sativa* on blood glucose of normal rats was actually because the plant mixture was used rather than the pure *N. sativa* and their dose could not be calculated. Despite of current study, Le and collaborators (2004) reported that *N. sativa*-treated animals displayed stable and normal fasting glucose levels throughout the 4-week treatment period with the petroleum ether extract.

Conclusion

In summary, the present study clearly shows that *N. sativa* seeds possess significant anti-metabolic syndrome potential that is unrelated to any toxic effect of the plant. Indeed, *N. sativa* seeds had a slight anorexic effect, exerted a beneficial action on serum lipids and weight gain. More research is required to find out the various mechanisms by which *N. sativa* acts on the various components of the metabolic syndrome.

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REFERENCES

Al AFM, Fatania H, Shamte U (1991). The effect of a plant mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. Diabetes Res., 18: 163-168.

Al HA, Aqel M, Hasan Z (1993). Hypoglycemiceffect of volatile oil of *Nigella Sativa* seeds. Int. J. Pharmacog., 31:96-100.

Amin KA, Nagy MA (2009). Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. Diabetol. Metab. Syndr.*,* 16(1): 1-17.

Aqel M (1992). The relaxing effect of the volatile oil of *Nigella sativa* seeds on vascular smooth muscle. Dirasat Series B, Pure Appl. Sci.,19: 91-100.

Araujo LBF, Soares JM, Simoes RS, Ricardo SS, Calió PL, Ricardo OFM, Simões MJ, Haidar MA, Baracat EC (2006). Effect of conjugated equine estrogens and tamoxifen administration on thyroid gland histomorphology of the rat, Clin., 61: 321-326.

Arjmandi BH, Birnbaum R, Goyal NV, Getlinger MJ, Juma S, Alekel L, Hasler CM, Drum ML, Hollis BW, Kukreja SC (1998). Bone-sparing

effect of soy protein in ovarian hormone-deficient rats is related to its isoflavone content. Am. J. Clin. Nutr., 68: 1364S-1368S.

Arsenault BJ, Lemieux I, Després JP, Wareham NJ, Luben R, Kastelein JJP, Khaw KT, Boekholdt SM (2007).Cholesterol levels in small LDL particles predict the risk of coronary heart disease in the EPIC-Norfolk prospective population study. Eur. Heart J., 28(22): 2770-2777.

Babiker F, Leon J (2002). Estrogenic hormone action in the heart: regulatory network and function. Cardiovasc. Res., 53: 709-719.

Bamosa AO, Ali BA, Sawayan SA (1997). Effect of oral ingestion of *Nigella Sativa* seed on some blood parameters. J. Saudi Pharm., 5: 126-129.

Buriro MA and Tayyab M (2007). Effect of *Nigella sativa* on Lipid Profile in Albino Rats. Gomal J. Med. Sci. (GJMS), 5(1): 28-31.

El DM, Mady NL, Halim MA (2000). *Nigella sativa* L. oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneim. Forsch., 50(9): 832-836.

El NARM, El DAEM (1992). A study of some biological activities of *Nigella sativa* (black seeds) "Habbat El-Barka". J. Egypt Soc. Pharmacol. Exp. Ther., 11(2): 781-800.

El SOA, Nada SA (1996). Biological evaluation of Multi component tea used as hypoglycemic in rats. Fitoterapia, 15(2):99-102.

Enazzani AR, Stomati M, Bernardi F, Luisi S, Casarosa E, Puccetti S, Genazzani AD, Palumbo M, Luisi M (2004). Conjugated equine estrogens reverse the effects of aging on central and peripheral allopregnanolone and beta-endorphin levels in female rats. Fertil Steril.*,* 81(1): 757-766.

Eskander H, Emad F, Won JA, Ibrahim K, Abelal WE (1995). Hypoglycemic effect of a herbal formulation in alloxan-induced diabetic rats. Egypt. J. Pharm. Sci., 36: 253-270.

Fait T, Malkova J, Zivny J (2002). Effect of hormone replacement therapy on the cardiovascular system. Ceska. Gynekol., 67(5): 285–293.

Fararh KM, Atoji Y, Shimizu Y, Takewaki T (2002). Isulinotropic properties of *Nigella sativa* oil in Streptozotocin plus Nicotinamide diabetic hamster . Res. Vet. Sci.*,* 73(3): 279-282.

Fried M, Hainer V, Basdevant A, Buchwald H, Dietel M, Finer N, Greve JW, Horber F, Mathus VE, Scopinaro N, Steffen R, Tsigos C, Weiner R, Widhalm K (2008). Interdisciplinary European guidelines on surgery for severe obesity. Rozhl Chir., 87:468-476.

Haddad PS, Martineau L, Lyoussi B, Le PM (2006) Middle Eastern and African medicine. In: A. Soumyanath, Editor, *In* Traditional Herbal Medicines for Modern Times: Antidiabetic Plants, CRC Press, Boca Raton, FL, pp. 221–241.

Hafanizam MBH, Dollah MA, Latiff AL, Rahman SA (2008). Toxicity effects of *Nigella sativa* supplementation on the liver function of rats. Universiti Putra Malaysia, Unpublished Thesis.

Hajdu A, Chappel CI, Rona G (1965). The influence of estrogens on scorbutic bone lesions in guinea-pigs. *CMLS.,* 21(8): 466-467.

Hawsawi ZA, Ali BA, Bamosa AO (2001). Effect of *Nigella sativa* (Black seed) and thymoquinone on blood glucose in Albino rats. Ann. Saudi Med., 21(3-4): 242-244.

Hedaya SA (1995). Effect of *Nigella sativa* seeds (black seeds) extract on some heamatologica and biochemical parameters in rats. Alexandria J. Vet. Sci., 11(2): 95-99.

Hioki C, Yoshimoto K, Yoshida T (2004). Efficacy of Bofu-tsusho-san, an oriental herbal medicine, in obese Japanese women with impaired glucose tolerance. Clin. Exp. Pharmacol. Physiol., 31: 614–619.

Houcher Z, Boudiaf Kh, Benboubetra M, Houcher B (2007). Effects of Methanolic Extract and Commercial Oil of *Nigella sativa* L. on Blood Glucose and Antioxidant Capacity in Alloxan-Induced Diabetic Rats. Pteridines*,* 18: 8-18.

Izwan MEM, Dollah MA, Latiff AL, Rahman SA (2008). Toxicity effects of *Nigella sativa* supplementation on the kidney function of rats. Universiti Putra Malaysia, Unpublished Thesis.

Kopelman PG (2000). Obesity as a medical problem. Natare, 404: 635–643.

Labhal A, Settaf A, Bennani KN, Cherrah Y, Slaoui A, Hassar M (1997). Action anti-obésité, hypocholestérolémiante et hypotriglycéridémiante de *Nigella sativa* chez le Psammomys obesus. Caducée, 27: 26-28.

Labhal A, Settaf A, Zalagh F, Cherrah Y, Hassar M, Slaoui A (1999). Propriétés anti-diabétiques des graines de *Nigella sativa* chez le

Meriones shawi obese et diabétique. Espérance Médicale, 47: 72-74.

Lavie CJ, Artham SM, Milani RV, Ventura HO (2008). The obesity paradox: impact of obesity on the prevalence prognosis of cardiovascular diseases. Postgrad.Med., 120: 34-41.

Le PM, Benhaddou AA, Settaf A, Cherrah Y, Haddad PS (2004). The petroleum ether extract of *Nigella sativa* exerts lipid lowering action in the rats. J. Ethanopharmacol., 94(2-3): 251-259.

Lee YS, Kim WS, Kim KH, Yoon MJ, Cho HJ, Shen Y (2006). Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin resistant states. Diabetes, 55: 2256–2264.

Liu M, Xu X, Rang W, Li Y, Song Y (2004). Influence of ovariectomy and 17β-estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function. Int. J. Cardiol.,97(3): 485-493.

Mai LP, Benhaddou AA, Elimadi A, Settaf A, Cherrah Y, Haddad PS (2004). The petroleum ether extract of *Nigella sativa* exerts lipid-lowering and insulin-sensitizing actions in the rat. J. Ethnopharmacol., 94(2-3): 251-259.

Meddah B, Ducroc R, El Abbes Faouzi M (2009). *Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J. Ethnopharmacol., 121: 419–424.

Meral I, Yener Z, Kahraman T, Mert N (2001).Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defense system and liver damage in experimentally-induced diabetic rabbits. J. Vet. Med. A., 48: 593-599.

Najmi A, Haque ShF, Khan RA, Nasiruddin M (2008). Therapeutic Effect of *Nigella Sativa* Oil on Different Clinical and Biochemical Parameters in Metabolic Syndrome. *Int* J. Diabetes Metabol.,16: 85-87.

Oropeza MV, Orozco S, Ponce H, Campos MG (2005). Tofupill lacks peripheral estrogen-like actions in the rat reproductive tract. Reprod. Toxicol.,20: 261–266.

Pagotto U, Vanuzzo D, Vicennati V, Pasquali RG (2008). Pharmacological therapy of obesity. G. Ital. Cardiol., (Rome), 9(4 Suppl 1):83S-93S.

Parhizkar S, Latiff L, Rahman S, Dollah MA (2011). Assessing estrogenic activity of *Nigella sativa* in ovariectomized rats using vaginal cornification assay. Afr. J. Pharm. Pharmacol., (AJPP). [In Press].

Pourghassem GB, Ebrahimzadeh AV, Rafraf M, Gorbani A (2009). Effect of dietary supplementation with *Nigella sativa* L. on serum lipid profile, lipid peroxidation and antioxidant defense system in hyperlipidemic rabbits. J. Med. Plant. Res., 3(10): 815-821.

Qidwai W, Hamza HB, Qureshi R, Gilani A (2009). Effectiveness, Safety, and Tolerability of Powdered *Nigella sativa* (*Kalonji*) Seed in Capsules on Serum Lipid Levels, Blood Sugar, Blood Pressure, and Body Weight in Adults: Results of a Randomized, Double-Blind Controlled Trial. J. Altern. Complement. Med., 15(6): 639-644.

Roghani DF, Kamkhah AF (2008). Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension. Fundam. Clin. Pharm., 22(4): 447-453.

Sarkaki A, Amani R, Badavi M, Safahani M, Aligholi H (2008). Effect of Ovariectomy on Reference Memory Version of Morris Water Maze in Young Adult Rats. Iranian Biomed. J. (IBJ)., 12 (2): 123-128.

Schneider JG, Tompkins C, Blumenthal RS, Mora S (2006). The metabolic syndrome in women. Cardiol. Rev., 14: 286–291.

shida H, Uesugi T, Hirai K, Toda T, Nukaya H, Yokotsuka K, Tsuji K (1998). Preventive effects of the plant isoflavones, daidzin and genistin, on bone loss in ovariectomized rats fed a calcium-deficient diet. Biol. Pharm. Bull., 21: 62-66.

Spiegelman BM, Flier JS (2001). Obesity and the regulation of energy balance. Cell. 104: 531–543.

Tansey G, Hughes CLJ, Cline JM, Krummer A, Walmer DK, Schmoltzer S (1998). Effects of dietary soybean estrogens on the reproductive tract in female rats. Proc. Soc. Exp. Biol. Med., 217: 340-344.

Tollba AAH, Shabaan SAM, Abdel MMAA (2010). Effects of using aromatic herbal extract and blended with organic acids on productive and physiological performance of poultry 2 – the growth during cold winter stress. Egypt. Poult. Sci., 30(I): 229-248.

Turner RT, Vandersteenhoven JJ, Bell NH (1987). The effects of ovariectomy and 12-beta-estradiol on cortical bone histomorphometry in growing rats. J. Bone Miner. Res., 2: 115–122.

Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M (2002). Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomed., 9: 69–74.