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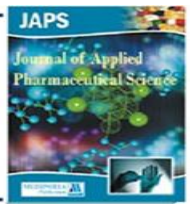

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Metabolic Impact of *Nigella sativa* extracts on Experimental Menopause Induced Rats

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ABSTRACT

The aim of current study was to investigate the effects of different extracts of Nigella sativa on metabolic profile of ovariectomized rats. Forty ovariectomized Sprague Dawley rats were used in the study and randomly allotted into one of five experimental groups: treated with Supercritical Fluid Extraction (SFE), Methanol Extract and Hexane Extract of *N.S* (300mg/kg/day) compared to vehicle control (Olive oil 1ml) and estrogen group (0.2mg/kg Conjugated Equine Estrogen) 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 low density lipoprotein cholesterol (LDL-C) and blood glucose ($P < 0.05$). There were no significant differences between groups in total cholesterol, high density lipoprotein and serum triglyceride concentration. These results suggested that treatment with *Nigella sativa* extracts 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.

Keywords: Menopause, Metabolic Syndrome, Nigella sativa, Ovariectomy.

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization and for a long time, mineral, plant and animal products were the main sources of drugs used for therapeutic purpose (Hernandez-Ceruelos *et al.*, 2002). Plants have always been a major source of nutrition and health care for both humans and animals. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Schwartsmann *et al.*, 2002). The *Nigella sativa* (black seed*)* is a type of plant that belongs to the *Ranunculaceae* family (El-Dakhakhny *et al.*, 2000). It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavouring agent in many countries. The black seed oil is reported to be beneficial due to its content of over a hundred components such as aromatic oils, trace elements, and vitamins (Ali and Blunden, 2003). Recently, clinical and animal studies have shown that extract of the black seeds have many therapeutic effects such as immunomodulative (Hanafy and Hatem, 1991), antibacterial (Zaoui *et al.*, 2000), anti-tumor (Turkdogan *et al.*, 2001), diuretic and hypotensive (Kanter *et al.*, 2003), hepatoprotective and antidiabetic (Houghton *et al.*, 1995; Kanter *et al.*, 2003). *Nigella sativa* is also accepted in folk medicine as estrogenic activity which can help regulate menstrual cycles in women. Since estrogen plays an important role in regulating various body systems in the female, drastic change or reduction in estrogen levels can result in elevated blood pressure, increased

glucose intolerance and dyslipidemia which may lead to development of metabolic syndrome.

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), blood pressure (Aqel, 1992), but there are limited number of studies which focused on metabolic effects caused by menopause. Therefore current study was undertaken to know the adjuvant effect of different extracts of *Nigella sativa* on various clinical and biochemical parameters of the metabolic syndrome in ovarictomizeded rats as an animal model of menopause.

MATERIALS AND METHODS

Plant Materials and extractions

Nigella sativa seeds (imported from India) were purchased from a local herb store in Serdang, Malaysia. Voucher specimens of seeds were kept at the Cancer Research Laboratory of Institute of Biosciences and the seed was identified and authenticated by Professor Dr. Nordin Hj Lajis, Head of the Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia. After cleaning the seeds under running tap water for 10 min, they were rinsed twice with distilled water and air-dried in an oven at 40 °C overnight until a constant weight was attained. The seeds were grounded to a powder shape using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 6 minutes. Homogenized and grounded samples (100g) were soaked overnight with solvents at a ratio of 1:5 (w/v ratio). Two different solvents were used: n-hexane (Pu: 99%, Merk, Darmstadt, Germany) and methanol. The mixture of sample and solvent were covered with aluminum foil, and were shacked using a Shaking Incubator (Heidolph Unimax 1010, Germany) at 5-7 rpm for 90 minutes. Then solvents were filtered using Whatman paper number 1. The residues were resoaked with fresh solvent two times to ensure the complete extraction of the oil. Solvents were completely evaporated using a rotatory evaporator (Heidolph Laborata, Germany) at 50°C and 90 rpm that yielded a blackish-brown and yellowish concentrates for methanol and hexane extract respectively which kept at -20° C prior to use. The extractive values (w/w %) of the methanol and hexane extract were 29 % and 33 %, respectively.

Extraction of essential oil from the seed of Nigella sativa also was done using the speed supercritical fluid extraction (SFE) instrument. The seed powder of Nigella Sativa was measured to 150gr with digital scale (Shimadzu Model, Japan) balance before placing in the extraction vessel. The oil extract was obtained at 60 MPa and 40°C by means of Supercritical Fluid Extraction set (SFE-1000F Thar US Technology, USA). SFE flow-rates were maintained at 20.00 ml/min using a variable flow restrictor. The yellowish-brown color yield was collected within 3 hours and its value (w/w %) was 26% which stored at -20° C prior to use. The collected pressure and temperature were 0.1 MPa and 25°C, respectively. The extraction was carried out with pure CO2.

Chemicals

Conjugated Equine Estrogen (CEE 0.625mg) was purchased from Wyeth, Montreal, Canada and prepared in a dosage of 0.2mg/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., 2011a, b) 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

Forty female Sprague–Dawley rats weighing between 250 and 350g aged 4 months were used in this study. They were supplied by animal house of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (Serdang, Selangor, Malaysia). Rats were individually housed in stainless steel cages in a well-ventilated room with a 12/12h light/dark cycle at an ambient temperature of 29–32 °C and 50- 60 % relative humidity. Experiments were carried out according to the guidelines for the use of animals and approved by the Animal Care and Use Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia with UPM/FPSK/PADS/BR/UUH/F01-00220 reference number for notice of approval. They were fed standard rat chow pellets purchased from As-Sapphire (Selangor, Malaysia) and allowed to drink water *ad libitum*.

Experimental Design

In order to induce menopause and to investigate reproductive changes following supplementation with different extracts of *Nigella sativa*, the rats were ovariectomized under a combination of xylazine and ketamine (10 mg/kg + 75 mg/kg, i.p. respectively) anesthesia. Bilateral ovariectomy was performed via a dorso-lateral approach with a small lateral vertical skin incision (Parhizkar et al., 2008). The ovariectomized animals were acclimatized at the Animal House of Faculty of Medicine and Health Sciences for one month prior to supplementation. Five experimental rat groups were established with 8 rats per group. The groups were as follows: group 1, negative control (1 ml Olive Oil), group 2, positive control (0.2mg/kg/day CEE diluted in distilled water), group 3 Supercritical Fluid Extract-SFE extract treated group (300 mg/kg in Olive Oil), group 4, methanol extract treated group (300 mg/kg in Olive Oil) and group 5, hexane extract treated group (300 mg/kg in Olive Oil). Dosage of the extracts were selected based on the optimum desired effect of *Nigella sativa* in the previous experiment (Parhizkar et al., 2011a), which was at low dose (300mg/kg BW/day) and were administered by intra-gastric gavage for 3 weeks. Lipid profile, blood glucose and body weight were measured at baseline (day 0), 11th days, and at the end of experiment (21st day).

Blood collection

Fasting blood samples were collected under the deep ether anaesthesia by cardiac puncture using sterile disposable syringes at baseline (pre-treatment), day 11 (during treatment) and day 21 (after treatment). The blood samples were then centrifuged at 3000

rpm for 10 minutes to separate the serum. The serum was stored at -80°C until assays were carried out. All tests were performed according to the manufacturer's instructions.

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) or SAS (SAS Institute Inc., Cary, NC) statistical packages. The One-Way Analysis of Variance (ANOVA) and General linear Model (GLM) followed by Duncan Multiple Range Test (DMRT) were used to determine which extract of *Nigella sativa* showed optimum effects. 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 5% compare to baseline. The body weight of CEE group had no changes compare to baseline. Supplementation with different extract of *N. sativa* for three weeks slightly tended to increase the body weights but less than control group (Figure 1).

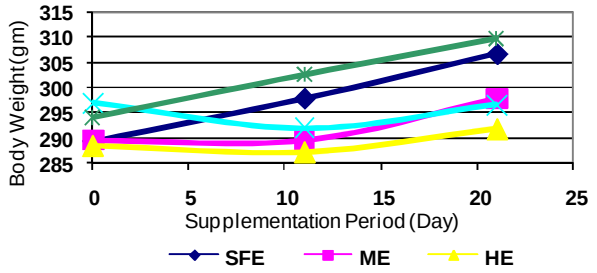

Fig 1: Changes of body weight (gm) of OVX rats supplemented with various extracts of *Nigella sativa* or Conjugated Equine Estrogen.

Treatment: Data expressed as mean SFE= Supercritical Fluid Extract of *Nigella sativa* (300mg/kg/day); ME= Methanol Extract of *Nigella sativa* (300mg/kg/day); HE= Hexane Extract of *Nigella sativa* (300mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day) groups.

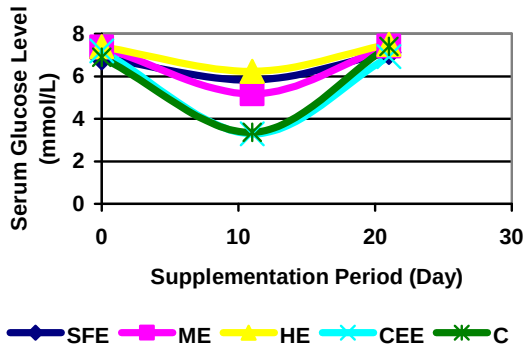

Fig 2: Changes of serum glucose level (mmol/L) of OVX rats supplemented with various extracts of *Nigella sativa* or Conjugated Equine Estrogen for 21 Days.

Treatment: Data expressed as mean. SFE= Supercritical Fluid Extract of *Nigella sativa* (300mg/kg/day); ME= Methanol Extract of *Nigella sativa* (300mg/kg/day); HE= Hexane Extract of *Nigella sativa* (300mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day) groups.

The sequential changes in serum TC, TG, LDL-C and HDL-C are summarized in Table 1. Supplementations with different extracts of *N. sativa* for 21 days in OVX rats significantly improved low density lipoprotein cholesterol (LDL-C) ($P < 0.05$), while no effects were observed on the total cholesterol, high density lipoprotein and serum triglyceride concentration. The higher tendency of extracts to exert effect on lipid profile was belong to methanol extract.

Blood glucose

All experimental groups showed decline in the concentration of blood glucose in the first 10 days which were significant in methanol and hexane extracts as well as positive control groups ($p<0.05$), but surprisingly the blood glucose return to the baseline at the end of experiment (Figure 2).

DISCUSSION

Over the period of treatment, the body weight of rats treated with vehicle (Control) and SFE groups was initially increased then maintained. The body weight of CEE group reduced during first 10 day of the experiments, but it then increased towards the end of experiment. However the body weights of the M.E and H.E groups showed slight increased until the end of the experimental period.

A slowing of body weight gain was observed in methanol and hexane extract treated rats, as compared to control animals. These effects may be related to the action of *Nigella sativa* on lipid metabolism. The same results have been obtained through oral and intaperitoneal administration of *Nigella sativa* fixed oil which provoked a slowing of the growth rate of normal rats (Zaoui *et al.*, 2002). Similarly, another survey revealed that *Nigella sativa* oil diet exerted a slowing of the weight gain in normal rats (Alsaif, 2008).

These results contrast with the previous reports about body weight reduction in normal and obese diabetic rats fed *Nigella sativa* extracts (Labhal *et al.*, 1997; Labhal *et al.*, 1999; Le *et al.*, 2004; Meddah *et al.*, 2009). Since OVX rats were used as a menopause model in our study, so animal model differences and duration of trial may be implicated in this discrepancy because in those studies, researchers used normal or diabetic animals in a long term treatment rather than OVX rats for 3 weeks.

The results obtained in the present study clearly showed that different *N*igella *sativa* extracts were effective in influencing lipid profile in OVX rats which caused a lowering effect on total cholesterol, triglyceride and LDL. This study confirm the previous studies with the aqueous extract of *Nigella sativa* seeds (Labhal *et al.*, 1997, 1999), petroleum ether extract (Le *et al.*, 2004) or with their fixed oil (Zaoui *et al.*, 2002). Another study has also previously reported that the volatile oil of *Nigella sativa* was as efficient as the cholesterol-reducing drug simvastatin (Settaf *et al.*, 2000). Thus, this study suggests that all extracts examined contain the cholesterol-lowering components of *Nigella sativa* seeds. On the other hand, this study also revealed that the potential of methanol extract was higher than hexane and SFE extracts.

Table 1: Lipid Profile level (mmol/L) of OVX rats at different days of supplementation with extract from various methods of extraction of *Nigella sativa* or Conjugated Equine Estrogen

Parameters	Day	NS Extracts (300mg/kg/day)			Control Groups		Total
		SFE	ME	HE	-ve (C)	+ve(CEE)	
T.C	0	1.58± 0.21^{cd}	1.91±0.26^{ab}	1.46± 0.29^{cd}	1.42± 0.25^{cd}	1.74± 0.54^{abc}	1.62± 0.37^{X}
	11	1.61± 0.19^{bcd}	1.96± 0.31^{a}	1.51± 0.32^{cd}	1.47± 0.27^{cd}	1.51± 0.37^{cd}	1.61± 0.33^{X}
	21	1.46± 0.11^{cd}	1.71±0.31^{abc}	1.40± 0.26^{cd}	1.41±0.17^{cd}	1.32± 0.29^{d}	1.46± 0.26^{X}
	Total	1.55± 0.18^{B}	1.86± 0.30^{A}	1.46± 0.28^{B}	1.44± 0.23^{B}	1.52± 0.44^{B}	
TG	0	1.15± 0.58^{bcde}	1.26± 0.14^{bcde}	1.49± 1.75^{bcde}	0.89± 0.36^{de}	0.96± 0.37^{cde}	1.15± 0.84^{X}
	11	1.03± .35^{cde}	1.09± .46^{cde}	0.67± 0.18^{e}	0.73± 0.30^{e}	0.78± .50de	0.86± 0.39^{X}
	21	2.09±1.05^{ab}	2.77± 1.32^{a}	1.74± 1.26^{bcd}	1.89±1.00^{abc}	1.10± 0.99^{cde}	1.92±1.20^{Y}
	Total	1.42± 0.84^{AB}	1.71±1.09^{A}	1.30±1.28^{AB}	1.17± 0.80^{B}	0.95± 0.66^{B}	
HDL	0	1.33± 0.18^{abcd}	1.55± 0.20^{a}	1.08± 0.34^{d}	1.20± 0.27^{cd}	1.38± 0.39^{abc}	1.31± 0.31^{X}
	11	1.33± 0.17^{abcd}	1.52± 0.30^{ab}	1.24± 0.24^{bcd}	1.22± 0.20^{cd}	1.28± 0.32^{abcd}	1.32± 0.26^{X}
	21	1.15± 0.09^{cd}	1.27± 0.22^{abcd}	1.10± 0.18^{cd}	1.06± 0.14^{d}	1.06± 0.28^{d}	1.13± 0.20^{Y}
	Total	1.27± 0.17^{B}	1.45 ±. 026^{A}	1.14± 0.26^{B}	1.24± 0.35^{B}	1.24± 0.35^{B}	
LDL	0	0.17± 0.10^{def}	0.22± 0.07^{bcde}	0.18± 0.050^{def}	0.20± 0.06^{cdef}	0.24± 0.17^{bcd}	0.20± 0.10^{X}
	11	0.29± 0.08^{abc}	0.35± 0.12^{a}	0.30± 0.09^{ab}	0.18± 0.07^{def}	0.21± 0.09^{bcdef}	0.27± 0.11^{Y}
	21	0.11± 0.06^{f}	0.13± 0.06^{ef}	0.16± 0.07^{def}	0.13± 0.05^{ef}	0.17± 0.07^{def}	0.14± 0.06^{Z}
	Total	0.19± 0.11^{AB}	0.23± 0.12^{A}	0.22± 0.09^{AB}	0.17± 0.06^{B}	0.21± 0.12^{AB}	

Data are expressed as Mean ± SD. Treatment: SFE= Supercritical Fluid Extract of *Nigella sativa* (300mg/kg/day); ME= Methanol Extract of *Nigella sativa* (300mg/kg/day); HE= Hexane Extract of *Nigella sativa* (300mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day) groups. ABCD: Comparison of the means between columns within row of the same parameter significant at $p<0.05$. XYZ: Comparison of the means between rows within column of the same parameter significant at $p<0.05$. abcdef: Comparison of the means between column and between row of the same parameter significant at $p<0.05$.

Furthermore, total cholesterol and LDL slightly increased in the first 10 days and then reduced considerably until the end of experiment which this favorable effect was higher than estrogen treated group.

On the other hand, this study showed that all type of extracts of *Nigella sativa* reduced plasma triglycerides in the first 10 days while increasing afterward until the end of study. This result was consistent with our previous experiment using different dosages of *Nigella sativa* seed in powder form which showed an increase in the TG level following the initial reduction (Parhizkar et al., 2011).

In addition, hexane extract of *Nigella sativa* exert rise in the level of HDL, but SFE and methanol extracts failed to exert an improvement in the level of HDL in OVX rats. The positive and negative group also showed decline in HDL level.

At the first 10 days of treatment, serum glucose level reduced in all groups compare to baseline while in M.E, H.E and CEE groups showed significant reduction ($P<0.05$) and the level of glucose in all extracts differed significantly ($P<0.05$) from CEE and control groups. In contrast all groups showed significant ($P<0.05$) increase in glucose level until end of the treatment.

The results indicated that *Nigella sativa* extracts have a promising reducing effect on the blood glucose levels in OVX rats but the effect was disappeared after 10 days. The results, however, failed to show a linear consistent dose or time dependent effect of the herb on the blood glucose. These results were consistent with the previous studies by Bamosa *et al.*, (1997), Hawsawi *et al.*, (2001) and Houcher *et al.*, (2007) who reported that *Nigella sativa* tended to lose their anti hyperglycemic effect after 10-14 days. The finding also confirmed our previous experiment which exerted glucose lowering effect in the first 10 days of experiment (Parhizkar et al., 2011b).

CONCLUSION

In conclusion our study provides novel evidence in support of the traditional use of *Nigella sativa* as an anti aging remedy. Moreover, our results provide convincing evidence that *Nigella sativa* can reduce metabolic syndrome burden in the context of menopause. Further studies are required to establish the beneficial effect of *Nigella sativa* seeds in human beings.

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