

THE MENOPAUSE: BIOCHEMICAL CONSEQUENCES OF *NIGELLA SATIVA* AND HORMONAL REPLACEMENT THERAPY

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ABSTRACT

The study aims to determine in obese postmenopausal women, the influence of *Nigella sativa* administration with and without hormone replacement therapy (HRT) on pro-inflammatory cytokines and total antioxidant capacity. Their inter-relationship with indices of platelet activation, lipid peroxidation and lipoprotein status would be evaluated.

The study included sixty selected obese postmenopausal women (mean age 62.7 ± 5.3) were equally classified into four groups: Group I (GI, n=15) involved postmenopausal cases not receiving any therapeutic medication. Therapeutic management of cases for 6 months involved *Nigella sativa* for group II (GII, n=15); combined HRT for group III (GIII, n=15); and both *Nigella sativa* and HRT for group IV (GIV, n= 15).

The results showed increased total antioxidant capacity (AOC%), high-density lipoprotein-cholesterol (HDL-c) and its subunits in response to therapeutic management. In parallel, a reduction in indices of pro-inflammatory cytokines, platelet activation, lipid peroxidation product, lipoprotein (a), and low-density lipoprotein-cholesterol (LDL-c) was evident.

In conclusion, supplementation of both *Nigella sativa* and HRT proved a more effective regimen for minimizing atherogenic and

thrombogenic potentials in obese postmenopausal women having insulin resistance and elaborating a sedentary life style.

Key words: Menopause, Nigella sativa, hormone replacement therapy, proinflammatory cytokines, platelet activation and lipid peroxidation.

INTRODUCTION

Menopause, although not a disease yet, it is associated with "dis-ease", annoying physiologic changes and symptoms such as hot flashes and vaginal atrophy. With the onset of menopause, the need for health maintenance measures may include hormone replacement therapy (HRT) (Gelfand, 1995; Thacker, 1996). Hence, the five target areas affected by menopause involve the cardiovascular system, the skeleton, the genitourinary system, the neuro-endocrine system, and the integument (Wenger, 1996). The association between medical, gynecologic, as well as dietary history and exercise habits, provide the basis for health assessment of the menopausal woman (Kushi et al., 1997). Thus, excess weight gain, central obesity, diet changes, and lack of exercise, more frequently with advancing age, all concur to alter glucose tolerance and increase insulin resistance during the post-menopause (Gaspard et al., 1995).

The nuisance of adverse effects of HRT such as continued bleeding, premenstrual syndrome-like effects, increased susceptibility of gall bladder disease and fear of breast and endometrial cancer prevent many women from receiving HRT (Kenemans, 1995; Colditz et al., 1995). However, reported HRT benefits including relief of vasomotor symptoms, prevention of postmenopausal osteoporosis and fractures as well as reduction of cardiovascular risk, reversal of genitourinary atrophy, possible improvement in mood, energy and recent memory may outweigh the risk of HRT (Coli et al., 1997).

The pre-existing psychological characteristics of women who subsequently used HRT may aid in identifying possible patient factors that determine hormone use. However, as obese women tend to have higher levels of circulating estrogens (due to conversion of androstenedione to estrone in fat tissues) they may have fewer symptoms and seek treatment with HRT less often. Although they may have a worse cardiovascular risk factor profile yet, they would predominantly be categorized as non-HRT users (Herzog et al., 1995; Matthews et al., 1996). Such cases are usually associated with dyslipidemia, insulin resistance, and their sequelae of events (Kamel & Lotfy, 1998).

On the other hand, in HRT users, the cardio-protective effects of estrogens influencing development of arteriosclerosis and foam cell formation

which is associated with atherosclerosis must be mediated through a direct influence on cellular or biochemical events and immuno-inflammatory response occurring in the arterial intima (Cheang et al., 1994). This includes protection from the reactive oxygen species (ROS)-induced vascular endothelial damage, via reduction of the lipoprotein-induced arterial smooth muscle cell proliferation, inhibition of platelet aggregation and decrease of collagen and elastin production by arterial smooth cells. Besides, the beneficial estrogen-mediated endothelium-dependent vasodilatation provides a favorable impact on clotting mechanisms for HRT users (Herzog et al., 1995).

This study aims to determine in obese postmenopausal women, the influence of *Nigella sativa* with and without hormone replacement therapy (HRT) on pro-inflammatory cytokines and total antioxidant capacity. Their inter-relationship with indices of platelet activation, lipid peroxidation and lipoprotein status would be evaluated.

MATERIALS AND METHODS

Sixty selected postmenopausal women (mean age 62.7 ± 5.3 years) were equally classified into four groups: Group I (GI, n=15) involved postmenopausal cases not receiving any therapeutic medication representing the control group. Therapeutic management of cases for 6 months involved *Nigella sativa* for group II (GII, n=15) receiving 450 mg *N. sativa* oil/day (Baraka Capsules provided by Pharco Pharmaceuticals, Egypt); combined HRT for group III (GIII, n=15) taking combined estrogen and progestin (Prempack Tablets, one tablet administered cyclicly); and both *Nigella sativa* and HRT for group IV (GIV, n= 15).

Postmenopausal cases were selected from those attending the Outpatient Clinic of the University Hospitals of Tanta (Egypt) and King Abdulaziz (Jeddah, SA). All cases were submitted to thorough clinical examination. They were non-diabetic and free from any neuro-endocrine disorders. They all showed no history of hypertension, liver, kidney or heart disease.

Women included in the four studied groups underwent investigations to fulfill the following parameters:

- Obesity involving body mass index (BMI) $> 27 \text{ Kg/m}^2$ (Keys et al., 1972); waist to hip ratio (WHR) > 0.8 (Garrow & Webster, 1985) and impaired glucose tolerance test (GTT) (Yudkin et al., 1990).
- Indices of oxidative stress including percent of total antioxidant capacity (AOC%) estimated by the method of Bonnetont et al., 1989; lipid peroxidation product (malondialdehyde, MDA) according to the method of

Satoh, 1978; and vitamin E content of LDL by HPLC (Milne & Botnen, 1986).

- Indices of immuno-inflammatory response involving the pro-inflammatory cytokines (interleukin-1 β "IL-1 β " and tumor necrosis factor alpha "TNF- α ") assessed by enzyme-linked immunosorbent assay (ELISA) by the method of Ledur et al., 1995.
- Indices of platelet activation including platelet endothelial cell adhesion molecule-1 (PECAM-1), platelet-derived endothelial cell growth factor (PD-ECGF), β -thromboglobulin (β TG), and platelet factor 4 (PF-4). All these parameters were determined by ELISA using commercial kits provided by Abbott Co., USA.
- Lipoprotein profile involving lipoprotein (a) [Lp (a)] determined by immunoprecipitin method of Walton et al., 1974; low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) were assessed by the method of Wilson & Spiger, 1974. HDL subfractions (HDL₂ and HDL₃) were measured according to the method of Warnick et al., 1982.

RESULTS

Table (1) represents the values of indices of oxidative stress and immune response in the studied groups. The data showed a statistically significant increase in total antioxidant capacity (AOC%), vitamin E content of LDL and a statistically significant reduction in values of lipid peroxidation product (MDA) and pro-inflammatory cytokines (IL-1 β and TNF- α). These changes indicate an improvement in the antioxidant status and immune inflammatory response after administration of *Nigella sativa* or HRT. The maximum effect was shown in cases of GIV who received both regimens for 6 months. The data in table (2) indicated a statistically significant decrease in indices of platelet activation (PECAM-1, PD-ECGF, β -thromboglobulin, and platelet factor-4). This reduction was more prominent in cases of GIV indicating a greater decline in thrombogenic potential in this group. The lipoprotein profile shown in table (3) demonstrated a statistically significant decrease in lipoprotein (a), LDL-c, and a statistically significant increase in HDL-c and its subunits (HDL₂, HDL₃). These results followed the same pattern of change showed in table (1) and (2) indicating a significant reduction in atherogenic potential specially in cases of GIV.

Table 1: Indices of oxidative stress and immune response in groups under study
Data are Mean ± SD.

Biochemical Parameters	Control Group (GI, n=15)	PM Women Receiving NG (GII, n=15)	PM Women Receiving HRT (GIII, n=15)	PM Women Receiving Both NG and HRT (GIV, n=15)
AOC %	71.3±19.2	95.2±23.4**	86.7±20.6*	104±28.5***
Lipid Peroxidation Product (nmol MDA/ml)	8.9±2.8	6.5±2.2*	6.9±2.4*	5.6±2.0***
Vitamin E content of LDL (µmol/g LDL)	2.18±0.62	3.21±1.12**	2.81±0.83*	4.05±1.51***
IL-1β (pg/ml)	10.8±3.6	7.7±2.6*	8.3±2.9*	6.8±2.1***
TNF-α (pg/ml)	7.9±2.6	5.6±1.9*	6.1±2.0*	5.1±1.3***

Statistically significant values at (P<0.05)* (P<0.01)** and (P<0.001)***

PM=Postmenopausal

NG=Nigella sativa

HRT=Hormone replacement therapy

AOC%=Percent of total antioxidant capacity

MDA=Malondialdehyde

LDL=Low-density lipoprotein

IL-1β=Interleukin-1 beta

TNF-α=Tumor necrosis factor alpha

Table 2: Indices of Platelet activation in groups under study
Data are Mean ± SD.

Biochemical Parameters	Control Group (GI, n=15)	PM Women Receiving NS (GII, n=15)	PM Women Receiving HRT (GIII, n=15)	PM Women Receiving Both NG and HRT (GIV, n=15)
PECAM-1 (ng/ml)	51.2±14.7	41.6±10.3*	37.3±12.4**	31.6±9.6***
PD-ECGF (U/ml)	7.75±2.62	5.76±2.11*	5.01±1.95**	4.52±1.34***
β-thromboglobulin (IU/ml)	23.2±6.2	18.9±5.1*	16.8±4.5**	14.7±3.8***
Platelet Factor-4 (IU/ml)	7.4±2.6	5.6±2.0*	4.9±1.6**	4.1±1.3***

Statistically significant values at (P<0.05)* (P<0.01)** and (P<0.001)***

PM=Postmenopausal

NS=Nigella sativa

HRT=Hormone replacement therapy

PECAM-1=Platelet endothelial cell adhesion molecule-1

PD-ECGF= Platelet endothelial cell growth factor

Table 3: Lipoprotein Profile in groups under study
Data are Mean \pm SD.

Biochemical Parameters	Control Group (GI, n=15)	PM Women Receiving NG (GII, n=15)	PM Women Receiving HRT (GIII, n=15)	PM Women Receiving Both NG and HRT (GIV, n=15)
Lp (a) (μ g/ml)	126 \pm 33.0	99.5 \pm 27.3*	88.4 \pm 23.8**	67.9 \pm 17.5***
LDL (mg/dl)	147 \pm 44.0	118 \pm 31.8*	98.5 \pm 27.4**	87.9 \pm 23.6***
HDL (mg/dl)	43.5 \pm 15.7	58.9 \pm 18.0*	67.4 \pm 20.1**	78.2 \pm 23.5***
HDL ₂ (mg/dl)	38.4 \pm 9.3	46.5 \pm 11.2*	52.1 \pm 12.2**	59.6 \pm 14.7***
HDL ₃ (mg/dl)	14.2 \pm 4.5	18.0 \pm 5.1*	21.3 \pm 6.1**	24.7 \pm 7.3***

Statistically significant values at (P<0.05)* (P<0.01)** and (P<0.001)***

PM=Postmenopausal

NG=Nigella sativa

HRT=Hormone replacement therapy

Lp(a)=Lipoprotein (a)

LDL= Low -density lipoprotein

HDL= High-density lipoprotein

DISCUSSION

In postmenopausal women, partly in relation to advancing age, and partly due to estrogen deficiency, there is a frequent increase in body weight, and more specifically, in android fat distribution as evidenced herein. References indicate that impaired glucose tolerance and diabetes mellitus may be found in nearly 20% of women aged 55-65 years (Gaspard et al., 1995). It is evident from the present data in obese non-HRT users that the reduced antioxidant potential which develops with aging becomes potentiated furthermore with sedentary life style and obesity. It is relevant to declining estrogen levels during menopause, insulin resistance, and dyslipidemia expressing thrombogenic liability. In parallel, there appeared increased levels of indices of platelet activation (PECAM-1, PD-ECGF, β -thromboglobulin " β TG", and platelet factor 4 "PF-4") indicative of endothelial damage, platelet aggregation and sequelae of events. Evidently, assessed decrements in AOC% and vitamin E content of LDL versus increments in IL-1 β , TNF- α and insulin verifies the interactions between reactive oxygen species (ROS), immune mechanisms and insulin resistance. These are associated with increased lipid peroxidation product (MDA), Lp (a) and LDL-c versus decreased HDL-c, HDL₂, and HDL₃ representing cardiovascular risk factors (Berg et al., 1997).

On the other hand, the beneficial cardioprotective effect of HRT in postmenopausal women herein coincided with increasing HDL, HDL₂, HDL₃ and decreasing LDL that could favorably influence the prostacyclin/thromboxane balance noted elsewhere (Fiodart et al., 1991). Hence, the estrogen component of

HRT was reported to prevent cholesterol and oxidized LDL particles from accumulation on the arterial wall and suppresses hepatic lipase activity subsequently elevating levels of HDL, HDL₂, and HDL₃ were observed (Tikkanen et al., 1982; Sacks & Walsh, 1990; Walsh et al., 1991). Moreover, LDL-c was lowered possibly by increasing its rate of clearance from plasma (Sacks & Walsh, 1990; Walsh et al., 1991; Fiodart, 1996). Evidently, the use of certain progestin (as Medroxyprogesterone acetate) with low levels of androgenic activity showed slight or no effects on lipoprotein levels when combined with estrogen (Nabulsi et al., 1993).

Furthermore, the effect of combined HRT on reduction of Lp (a) as evidenced herein is interesting, because Lp (a) appears to be genetically determined for the most part and resistant to most environmental influences or life style factors (Hegele, 1989; Soma et al., 1991). However, loss of ovarian function is associated with the development of a more atherogenic profile with potentially an irregular increase in Lp (a) with increased triglycerides, LDL-c and its smaller dense subfractions and decreased HDL and HDL₂. HRT is implicated in counteracting all these changes towards a less atherogenic profile which was verified herein (GIII) (Gaspard et al., 1995). The Lp (a) presented a novel mechanism for its atherogenicity influencing the increments of PECAM-1 verified herein and elsewhere (Allen et al., 1998).

Conceivably, the beneficial outcome of HRT supplementation with *Nigella sativa* herein coincides with the antioxidant (Badr El-Din & Iskander, 2000) and immuno-modulatory potency of *N. sativa* (Haq et al., 1999) besides its ability to reduce insulin resistance as reported elsewhere in diabetic subjects (Al-Hader et al., 1993). Possibly, aligning with oxidative stress, the pathogenic mechanisms involved herewith in obese postmenopausal women constitute immuno-inflammatory response to reactive oxygen species (ROS) generated with insulin resistance. This was confirmed herein by increments of pro-inflammatory cytokines IL-1 β and TNF- α which were confronted and managed by the immuno-modulatory response to *N. sativa* supplementation alongside HRT. Hence, a marked anti-inflammatory activity of whole powdered seeds of *N. sativa* L. family *Rununculaceae* was reported alongside its antioxidant and hepato-protective functions (Rahman et al., 1997; Schwenke, 1998).

In fact, insulin resistance appears as a complex phenomenon that is not exclusively restricted to the diabetes syndrome. Thus, in obesity, insulin sensitivity of target tissues decreases (even in the absence of diabetes), and serum levels of insulin may be elevated to compensate for insulin resistance. The cellular and molecular basis of insulin resistance is not entirely clear. There is a decrease in the number of insulin receptors, and, more important, the post-

receptor signaling by insulin is impaired (Kahn, 1994). Estrogen deficiency may be further responsible for decreased pancreatic insulin secretion and alteration of its metabolic clearance rate-changes that can be reversed towards improved insulin secretion and sensitivity by estrogen treatment in small doses (Gaspard et al., 1995).

Also, the high antioxidant potential of thymoquinone (TQ), which is the main constituent of the volatile oil of *N. sativa* seeds, was identified in relation to its marked protective effect from hyperlipidemia associated with doxorubicin-induced nephropathy (Badary et al., 2000). It aligns with the impact of *N. sativa* with HRT on assessed lipoprotein profile herein. Moreover, the inhibition of eicosanoid generation and lipid peroxidation was noted to occur by the fixed oil of *N. sativa* and by its content of thymoquinone (TQ) and C_{20:2} unsaturated fatty acids (Houghton et al., 1995).

It is well known that vitamin E in LDL represents an index of LDL susceptibility to oxidative modification which is an essential step prior to LDL uptake by macrophages which are then transformed into foam cells (Napoli et al., 1995). Moreover, it has been suggested that vitamin E has an inhibitory effect on LDL-induced production of adhesion molecules and adhesion of monocytes to endothelial cells via its antioxidant function (Martin et al., 1997). Concomitantly, together with increased lipid peroxidation verified by increased MDA herein, such an aspect provides the link between atherogenic potential and reduced vitamin E in LDL with increased oxidative stress and increased Lp (a) in cases of GI. Hence, increased Lp (a) levels were noted to be involved in the recruitment of monocytes to the vessel wall and stimulates expression of adhesion molecules on coronary artery endothelial cells participating in the atherogenic process (Poon et al., 1997; Allen et al., 1998). This was more effectively counteracted in GIV cases by *N. sativa* with HRT. Hence, *N. sativa* exhibited multidisciplinary events associated with endothelial damage in cell layers of vessels and spares vitamin E from oxidation via its antioxidant potential.

Moreover, extracts of *N. sativa* seeds were noted to have a potentiating effect on the cellular immune response via its cytotoxic effect to cancer that may influence and protect against the carcinogenic potential of HRT (Swamy & Tan, 2000). This coincides with reported evidence that purified components of *N. sativa* seeds, thymoquinone (TQ) and dithymoquinone (DTQ) were found to be cytotoxic for multidrug resistant human tumor cell lines (Worthern et al., 1998). As well, *N. sativa* counteracts the toxic side effects of cisplatin (a chemotherapeutic drug) that induce falls in leukocyte count, hemoglobin levels, and mean osmotic fragility of erythrocytes and also prevents the increase of hematocrit (El-Daly, 1998). Besides, *N. sativa* in itself has a significant anti-

fertility activity which is comparable to 17-ethinylestradiol (Keshri et al., 1995), therefore adding to the estrogenic impact.

Conceivably, the assessed altered glycemic status and insulin resistance is known to be associated with changes in the hemostatic mechanisms (Schneider et al., 1993; Kwaan, 1992). These include abnormal platelet function leading to platelet activation which is expressed herein by the increase in levels of β -thromboglobulin (β TG), platelet factor 4 (PF-4) and an increase in PECAM-1 and PD-ECGF in non-HRT cases. It has been reported that indices of platelet activation (β TG and PF-4) may antedate parameters of coagulation (Ballageer et al., 1992). Referencewise, platelet activation factor (PAF) which is a phospholipid-derived mediator with a broad spectrum of inflammatory effects. PAF causes enhanced leukocyte adhesion, chemotaxis, leukocyte degranulation, and the oxidative burst. Also, PAF acts directly on target cells via specific receptors, and it stimulates the synthesis of other mediators, particularly eicosanoids (Goetzl, 1995).

On the other hand, the platelet endothelial cell adhesion molecule-1 (PECAM-1) is expressed on the surface of circulating platelets, monocytes, neutrophils, and selectively T cell subsets being the major component of the endothelial cell intercellular junction (Varon et al., 1998). Thus, leukocyte-endothelial cell interactions, which are mediated by various adhesion molecules are crucial events in inflammatory reactions potentiating atherosclerosis (Martin et al., 1997). It appears to be related to previous reports indicating that human fibroblasts release ROS in response to IL-1 or TNF- α (Meier et al., 1989) as reflected herein by assessed parameters in non-HRT users. This would denote PECAM-1 link with the immuno-inflammatory response associated with insulin resistance observed in non HRT users herein.

Consistently, the increase in levels of PECAM-1 herein aligns with the assessed increments in platelet-derived endothelial cell growth factor (PD-ECGF) (GI>GII>GIII>GIV). Platelet-derived growth factor which induces fibroblasts, smooth muscle cells, and monocytes migration and proliferation and other pro-inflammatory properties as well (Birchmeier, 1995). Platelet aggregation at the subendothelial space results in the release of products such as PD-ECGF. These are thought to be involved in the development of atherosclerotic lesions by stimulating migration and proliferation of smooth muscle cells and fibroblasts in the arterial media. It is possible that PD-ECGF counteracts the atherogenic effect of other platelets by promoting repair of endothelial cell layers (Ishikawa et al., 1989).

In conclusion, reduced antioxidant potential with aging, lack of exercise with obesity and inappropriate life style specially dietary measures would induce insulin resistance. This participates in mild subclinical immuno-inflammatory manifestations observed herein (GI). This appears to influence the vascular endothelium with disbalanced lipid profile (HDL, LDL) inducing endothelial cell damage and thrombogenic potential viz platelet aggregation and activation (involving β TG, PF-4, AOC%, MDA, IL-1 β , TNF- α and vitamin E content of LDL). *Nigella sativa*, owing to its favorable effect on insulin resistance and its immuno-modulatory, antioxidant and hepato-protective functions, would coordinate a hypothetical equilibration of such alterations (GII). A more favorable outcome occurs when supplemented with HRT (GIV) which exerts a cardioprotective effect attributable to lipid-related estrogen effect, improved insulin secretion, and insulin sensitivity, all of which influence platelet activation and thrombogenic potential.

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التلخيص

التأثيرات الكيميوحيوية لتناول حبة البركة والهرمونات التعويضية في السيدات بعد انقطاع الطمث

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يهدف هذا البحث إلى تقييم تأثير تناول حبة البركة "تيجيلا ساتيفا" مع أو بدون تناول علاج هرمونى تعويضى في السيدات البدينات بعد انقطاع الطمث على بعض القياسات الكيميوحيوية مثل السيوكينز والقدرة الكلية لمضادات الأكسدة وعلاقة هذه القياسات بالتغير فى مؤشرات تنشيط الصفائح الدموية وفوق أكسدة الدهون ونمط البروتينات الدهنية.

أجريت هذه الدراسة على ٦٠ سيدة بدنية فى سن ما بعد انقطاع الطمث (يبلغ متوسط أعمارهن 62.7 ± 5.3 عاماً). تم تقسيم هؤلاء السيدات إلى أربع مجموعات متساوية: مجموعة (١) ضمت ١٥ سيدة لا يتناولن أى علاج ويمثلن المجموعة الضابطة، مجموعة (٢) شملت ١٥ سيدة تتناولن حبة البركة "تيجيلا ساتيفا" لمدة ٦ أشهر (كمسحوق "بركة" تحتوى على ٤٥٠ مجم زيت حبة البركة يوميا) ، مجموعة (٣) ضمت ١٥ سيدة تتناولن علاج الهرمونات التعويضية لمدة ٦ أشهر (أقرص "بريمالك" تحتوى على الإستروجين والبروجسترون بطريقة دورية). أما المجموعة (٤) فشملت ١٥ سيدة تتناولن كل من حبة البركة والهرمونات التعويضية بالطريقة المذكورة لمدة ٦ أشهر.

أوضحت نتائج هذه الدراسة أن هناك تحسناً ملحوظاً فى القياسات البيوكيميائية بعد تناول حبة البركة أو الهرمونات التعويضية وظهر هذا التحسن بصورة أوضح فى المجموعة التى تناولت العلاجين سوياً (مجموعة ٤). فقد حدثت زيادة ذات دلالة إحصائية فى مستوى كل من القدرة الكلية لمضادات الأكسدة والبروتينات الدهنية عالية الكثافة ومشتقاتها. كما حدث نقص ذو دلالة إحصائية فى مستوى كل من السيوكينز ، مؤشرات تنشيط الصفائح الدموية ، فوق أكسدة الدهون ، السيروتين الدهنى (أ) والبروتينات الدهنية منخفضة الكثافة.

تستنتج من هذه الدراسة أن تناول حبة البركة والهرمونات التعويضية سوياً له التأثير الأكبر فى زيادة كفاءة الجسم لمقاومة العوامل المسببة لتصلب الشرايين وحدوث الجلطات خاصة فى السيدات البدينات اللاتى يعشن حياة كمسولة غير نشطة.