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RESEARCH ARTICLE

Natural approach for Calcium regulating hormone maintenance: Beneficial effects of Black seed

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ABSTRACT

Introduction: Menopause is a permanent cessation of menstruation with the decreasing of ovarian follicular functions. Hormone Replacement Therapy (HRT) is the most effective method in reducing the rate of bone loss in post-menopausal women with increasing the risks of breast cancer and uterine hyperplasia. So it is necessary to find a suitable treatment which is mimic to the estrogen role in maintaining normal calcium balance without its side effects.

Objectives: objective of the study was to determine the effect of Black seed on calcium homeostasis by measuring the level of calcium regulating hormones, i.e. calcitonin, PTH; and serum calcium concentration in menopause induced animal model.

Methods and Materials: Thirty six ovariectomized (OVX) Sprague-Dawley female rats with 250 – 350 g of weight were supplemented with Black seed for 28 days. The rats were divided into 6 group as followed; Non-OVX + 0 mg/day Black seed, OVX + 0 mg/day Black seed, OVX + 300 mg/day Black seed, OVX + 600 mg/day Black seed, OVX + 1200 mg/day Black seed and OVX + 0.05 mg/day Estradiol valerate. Statistical analyses have done through 2-way ANOVA using SPSS.

Results: A significant PTH reduction was revealed with 1200 mg/day Black seed treatment ($p<0.05$). A significant increase of calcitonin level on day 28 of 600 and 1200 mg/day of Black seed treatment groups was noted in comparison with control group ($p<0.05$). Meanwhile serum calcium significantly increased ($p<0.05$) with supplementation duration on day 20 and 28 compared to day 0 of supplementation.

Conclusion: In conclusion, although the study does not completely describing the whole spectrum of osteoporotic pathogenesis prevalence, the study has reflected Black seed as a probable candidate possessing estrogenic-activity in regulating calcium-regulating hormones, responsible for calcium homeostasis for menopausal women.

Key words: Black seed, Calcitonin, Calcium, Menopause.

1. INTRODUCTION

Menopause usually occurs at the age between 45 and 54 years when the ovarian function begins to decrease[(1)]. In women facing menopause, end of menstrual activity is accompanied by lower levels of estrogen and some psychological and physical symptoms ranging from short to long term complication. Post-menopausal osteoporosis is the most common cause of age-related bone loss[(2)]. It is characterized by reduced amount of bone leading to diminished physical strength of the skeleton and increased susceptibility to fracture[(3)]. Menopause results in elevated bone turnover, an imbalance between bone formation and bone resorption and net bone loss[(4)]. In osteoporosis, studies have shown a significant reduction in bone mass and the change in its microarchitecture,

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leading to an increased susceptibility to fractures[5]. Parathyroid hormones, a central regulator of calcium homeostasis, functions as a protein that remodels the bone, reclaim filtered calcium in the kidney and facilitate absorption of calcium from the gastrointestinal tract via its action to stimulate the renal production of 1, 22-dihydroxy-vitamin D[6]. A key hypothesis implicating parathyroid hormone in the development of osteoporosis relates either to the increase in parathyroid hormone concentrations with age or to enhance sensitivity at target organs[7,8]. However, Bilezikian et al. (2002) proposed that the estrogen deficiency actions are not primarily at skeletal sites but rather at the gastrointestinal and renal systems [9]. The extra skeletal actions of estrogen deficiency lead to increased urinary calcium excretion and reduced calcium absorption. The parathyroid hormone increases secondarily and responsible in ensuing bone loss[10]. The increase in parathyroid hormone has also been associated with a similar rise in bone turnover as assessed by bone markers[11]. Hormone replacement therapy (HRT) is the most popular treatment that has been proven to reduce menopausal symptoms worldwide. As HRT prevent long-term development of post-menopausal osteoporosis [12], Estrogen therapy is used to alleviate the problem of accelerating bone loss rate by reducing the rate of post-menopausal bone loss. The effect persists for as long as therapy is continued. However, bone density reaches a plateau after the initial 1 or 2 years of therapy. Bone mass remains stable thereafter throughout the treatment period. When estrogen replacement is stopped, bone loss resumes[13]. It is shown that estrogen treatment increases calcium absorption with a concomitant increase in parathyroid hormone levels[9]. Apart from the beneficial effect gained from HRT, it has been controversially associated with an increased risk of breast and endometrial cancers[14]. Therefore, it would be a need-based study to find a naturally occurring substance that minimizes bone loss in postmenopausal women but decreasing the necessity for drug therapy.

Phytoestrogen and herbal preparations are often used by women as they are perceived to be safe alternatives rather than traditional hormone therapies[15]. *Nigella sativa* L., commonly known as black cumin seed, belongs to the botanical family of Ranunculaceae[16]. It has been used in many Middle Eastern countries as a natural remedy for 2000 years. *N.sativa* (NS) is an amazing herb with a rich historical and religious background[16,17]. The desirable effects of *N.sativa* has been tested widely on cardiovascular system[18-21] immune system[22-24] reproductive system[25] and digestive system[26,27] as well as homeostasis[19,21,27-29]. As a traditional medicine, *N. sativa* (Black seed) increases milk production and promotes menstruation in the female. As no specific study have been conducted to investigate the effect of *Nigella sativa* on maintaining calcium balance in post-menopausal women, this study is designed to discover the potential of *Nigella sativa* in the role of regulating normal plasma calcium concentration and the calcium-related hormones.

2. MATERIAL AND METHODS

Plant Material

Nigella sativa in a full-grind form was obtained from Tsabit Banani Sdn. Bhd.; a herbal product pharmaceutical company located in Kota Bharu, Kelantan, 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 physiology laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia. The rat chow pellet were grounded to a powder form using an electric grinder (National, Model MX-915, Kadoma, Osaka, Japan) for 10 min. Grounded chow pellet and *Nigella sativa* powder mixed with water into three doses of 300, 600 and 1200 mg/kg and backed in an oven at 80°C until receiving instant weight. The pellets were made exclusively for each rat and each preparation contained 10% of body weight of basal diet- composed commercial pellets.

Chemicals and reagents

PTH and calcitonin EIA (Enzyme Immunoassay kit) purchased from Phoenix Pharmaceutical, Inc. U.S.A; and Roche Calcium reagent for Hitachi 912. All other reagents and chemicals were of analytical grade.

Experimental animals

The protocol of the study was approved by animal care and use committee (ACUC) with reference number of UPM/FPSK/PADS/BRUUH/00236 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 12 week-old female albino Sprague-Dawley rats, weighing 250 to 350 g. They were housed in cages under standard laboratory conditions within 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.

Experimental design

Thirty six rats were used for study. 30 rats were subjected to ovariectomy in order to induce menopause, while the

remaining rats were left unovariectomized. Their ovariectomy was conducted 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 (30). The ovariectomized animals were acclimatized at the Animal House of Faculty of Medicine and Health Sciences for one month prior to supplementation. The rats were divided into 6 group (6 animals in each group)as followed; Non-OVX + 0 mg/day Black seed, OVX + 0 mg/day Black seed, OVX + 300 mg/day Black seed, OVX + 600 mg/day Black seed, OVX + 1200 mg/day Black seed and OVX + 0.05 mg/day Estradiol valerate. Supplementations with *N. sativa* were continued for 4 weeks. Serum PTH, calcium, calcitonin and body weight were measured at baseline (day 0), 14th, and at the end of experiment (28th days).

Statistical analysis

Data were expressed as means ± standard error. 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.

3. RESULTS

Body weight

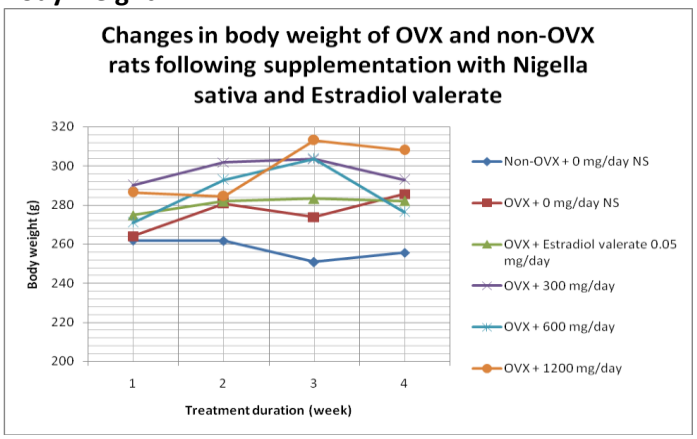

Figure 1: Body weight changes among OVX and non-OVX rats following supplementation of *Nigella sativa* and Estradiol valerate and in changes in means of non-ovariectomized rats

Figure 1 illustrated the mean body weight of all group of rats (OVX and Non-OVX) following supplementation of *Nigella sativa* and Estradiol valerate. Over the period of treatment, there was a significant difference in body weight in rats upon treatment ($P < 0.05$). Duncan Multiple Comparison test showed that the body weight was statistically higher in OVX group without *Nigella sativa* supplementation compared to non-OVX normal rats group. However, finding revealed that there was significant weight increment ($P < 0.05$) in the body weight of groups supplemented with *Nigella sativa* of 300 mg/day and 600 mg/day, while there was no significant difference between 600 mg and estradiol valerate group compared to OVX group without supplementation in body weight. In addition, there was no significant difference in body weight between all *Nigella sativa* treatment groups.

Serum PTH Level

There was a reduction in PTH level of all NS supplemented groups on day-28 compared to baseline, which was statistically significant only among 1200 mg/day treatment group ($p<0.05$). While no significant difference of PTH level observed between non-OVX and OVX group without supplementation and estradiol valerate supplementation.

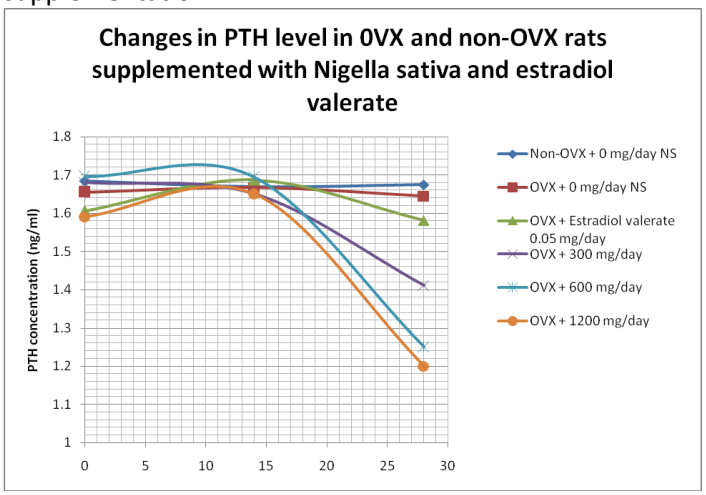

Figure 2: PTH level changes in OVX and non-OVX rats following supplementation of *Nigella sativa* and Estradiol valerate and changes in means of non-ovariectomized groups.

Serum Calcitonin level

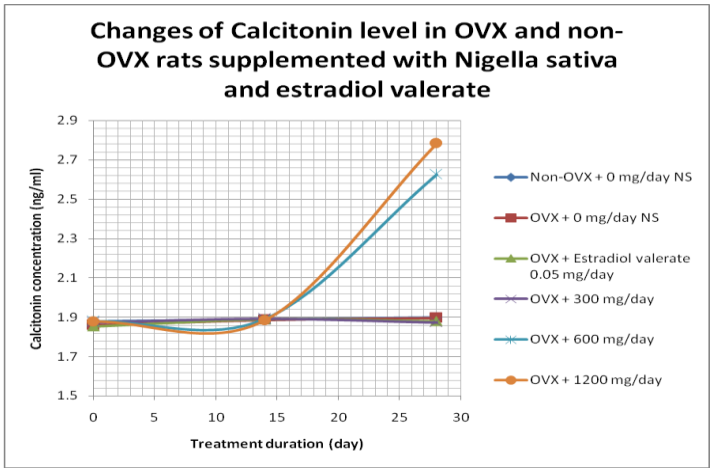

Figure 3: Changes in calcitonin level of non-OVX and OVX rats supplemented with *Nigella sativa* and Estradiol valerate

Over the period of treatment, the supplemented groups with 600 and 1200 mg/day *Nigella sativa* revealed a significant increment of calcitonin level ($p<0.05$) compared with control groups as well as baseline. Meanwhile, there was no significant difference of calcitonin level in OVX group without any

supplementation compared to non-ovariectomized group. The finding showed in Figure 3.

Serum calcium level

Supplementation with *N. sativa* for 4 weeks tended to increase the serum calcium of all *N. sativa* groups and stradiol valerate as compared to control group (Figure 4). The finding indicated that increment of serum calcium level following supplementation were statistically significant for 600 and 1200 mg/day *Nigella sativa* supplementation groups.

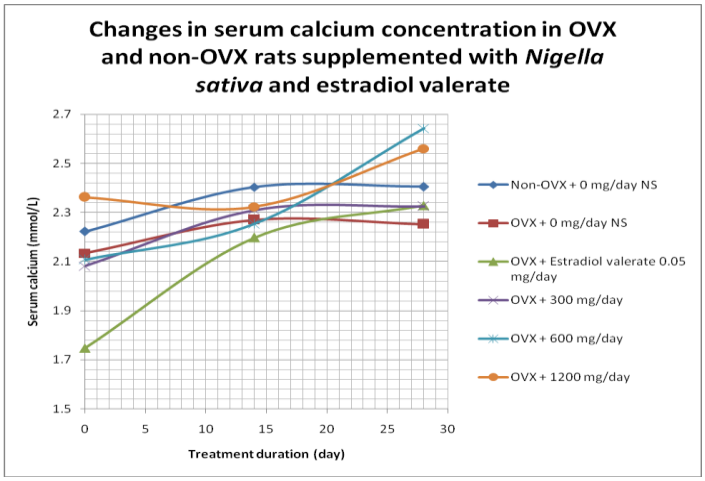

Figure 4: Changes in serum calcium level of non-OVX and OVX rats following supplementation of *Nigella sativa* and Estradiol valerat

4. DISCUSSION

According to the findings of the study, it was shown that ovariectomy resulted in elevated body weight. A significant weight increment was observed in OVX rats without treatment given, compared to that of normal rats. The elevation of body weight was known to be associated with reduced level of estrogen in OVX rats[31]. The increment of the body weight of OVX rats was mainly due to an increase in the percentage of total body fat[32,33]. Thus, the increment of body weight in non-supplemented OVX rats compared to supplemented non-OVX rats as observed in this study was desired to serve as a tool of estrogen deficiency model.

There was a significant body weight elevation in 300 mg/day and 600 mg/day *Nigella sativa* supplementation group compared to untreated OVX group. The significant increment might be due to the increased appetite following supplementation. Apart from that, normal growing phase of the rats and healthy status with no immunologically-suppressed condition were reflected by the body weight increment of the rats. The body weight increment also indicated that the rats did consume the supplement pellets containing *Nigella sativa* as desired. However, study conducted by Labhal et al (1997), showed that the serum total lipid and body weight in Psammomys obesus sand rat reduced upon supplementation of aqueous suspension of *Nigella sativa*[34]. Difference in result obtained in this finding might be due to different species of rats used in the experiment conduction and estrogen deficient state following ovariectomy of rats in this study was used.

PTH level of non-supplemented OVX rats was observed not to differ significantly from that of normal rats. This was due to the ability of the body to regulate to normal homeostasis of calcium, even though in the condition of estrogen deprivation. Although this contradicted with the theory describing OVX will immediately cause bone resorption, and will result high PTH level, maintenance of normal feedback loop of regulation of calcium in non-supplemented group OVX rats might be explanatory for the PTH level in OVX rats not to alter significantly.

There was a significant decrement of PTH level observed in 1200 mg/day *Nigella sativa* supplementation group. This result was desirable as an agent intended to replace conventional estrogen must mimic the role of estrogen in preventing excessive bone resorption, which include the mechanism of lowering the PTH level. On the other hand *Nigella sativa* at 600 mg/day supplementation showed significant decrement of PTH level only at day 28 of supplementation which has failed to affect the total mean. This might be an indication that *Nigella sativa* exhibit effect in a time-dependent manner. As a significant decrement of PTH level observed in 1200 mg/day *Nigella sativa* supplementation group, it was shown that *Nigella sativa* involved in bone resorption reduction by lowering the PTH level. Thus, insignificant decrement of 300 and 600 mg/day *Nigella sativa* supplementation might indicate that both of above doses were not the optimal and effective dose in preventing bone resorption on menopause-induced rats. As higher dose of *Nigella sativa* successfully exerted desired effect of PTH decrement, thus *Nigella sativa* supplementation was shown to be dose-dependent.

There appeared to be a significant increment of calcitonin level in two groups of *Nigella sativa* supplementation (600 and 1200 mg/day). In addition, there was a significant increment of calcitonin level on day 28 of 600 and 1200 mg/day of *Nigella sativa* supplementation. As the effect was observable after day 28, *Nigella sativa*, can be predicted exhibiting time-dependent effect. It was as well noted that *Nigella sativa* supplementation group exhibit elevated pattern of calcitonin level depending on different doses of *Nigella sativa*. Thus, it was shown that *Nigella sativa* acted in a dose-dependent manner. As lower calcitonin levels are seen in women after the menopause and their response to calcium load is diminished[35], increment in calcitonin level was an indicator of inhibition of bone resorption. This result could be indicating increasing bone formation, as well as

osteoclast apoptosis, which will lead to decreased bone resorption.

In summary, *Nigella sativa* supplementation was shown to mimic estrogenic role in regulating calcium homeostasis, by increasing PTH and serum calcium level and decreasing calcitonin level. 300, 600, and 1200 mg/day *Nigella sativa* was proven to be effective as it reduced PTH concentration. PTH reduction would indicate a reduction of bone resorption and osteoclastic activities. 600 and 1200 mg/day of *Nigella sativa* in the other hand was proven to be the most effective dose in increasing calcitonin level. Increased calcitonin level was an indication of increased osteoclastic inhibition, which will result in less bone resorption. The increment of serum calcium was significant on day 28 of 600 mg/day Nigella sativa supplementation. As the increased *Nigella sativa* dose would result further decrement of PTH level and further increment of calcitonin level, it was shown that *Nigella sativa* acted in a dose-dependent manner. As the effect of *Nigella sativa* on PTH, calcitonin and serum calcium was observed only after day 28 of supplementation, thus it was found that *Nigella sativa* acted in a time-dependent manner as well.

5. CONCLUSION

Nigella sativa of 300 mg/day, 600 mg/day and 1200 mg/day decreases PTH level while *Nigella sativa* of 600 mg/day and 1200 mg/day are capable of increasing calcitonin level. The pattern of serum calcium increment, although the changes were not significant compared to non-supplemented ovariectomized group, was a normal physiological response to high PTH, forming a simple calcium negative feedback loop. The effect of *Nigella sativa* was observed only after day 28 of supplementation. In conclusion, *Nigella sativa* possesses anti-resorptive and bone-forming properties, and the mechanism might mimic the estrogenic activity exhibited by estrogen. *Nigella sativa* acted on a time-dependent and dose-dependent manner in the regulation of calcium regulating hormones. Although the study does not completely describing the whole spectrum of osteoporotic pathogenesis prevalence, the study has reflected *Nigella sativa* as a prospective candidate possessing estrogenic-activity in regulating calcium-regulating hormones, responsible for calcium homeostasis.

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Competing interests:

Authors have declared that no competing interests exist.

7. REFERENCES

1. Kaplan JR, Manuck SB. Ovarian dysfunction and the premenopausal origins of coronary heart disease. Menopause. 2008;15:768-776.
2. North American Menopause Society, Clinical challenges of perimenopause: Consensus opinion of the North American Menopause Society. Menopause. 2000;*7*:5-13.
3. Kanis JA, Diagnosis of osteoporosis and assessment of fracture risk. Lancet. 2002; 359:1929–1936.
4. Feng X, McDonald JM. Disorders of bone remodeling. Annu Rev Pathol. 2010;28;6:121-45.
5. Marie P J, Osteoporosis: a disease of bone formation. Medicographia. 2010; 32:10-17.
6. Webb GP, An Overview of Dietary Supplements and Functional Food. In Dietary Supplements and Functional Foods, 1st ed.; Blackwell Publishing: Oxford, UK. 2006.
7. Kotowicz MA. Klee GG, Kao PC, O'Fallon WM, Hodgson SF, Cedel SL, Eriksen EF, Gonchoroff GD, Judd HL, and Riggs BL. Relationship between serum intact parathyroid hormone concentrations and bone remodeling in type I osteoporosis: Evidence that skeletal sensitivity is increased. Osteoporosis Int. 1990; 1:14-22.
8. Riggs BL, Khosla S. and Melton LJ. A Unitary Model for Involutional Osteoporosis: Estrogen Deficiency Causes Both Type I and Type II Osteoporosis in Postmenopausal Women and Contributes to Bone Loss in Aging Men. J Bone Miner Res. 1998;13:763-773.
9. Bilezikian JP, Raisz LG, Rodan GA. Principles of Bone Biology. Academic Press. Columbia University, New York. 2002.
10. Hough S, Ascott-Evans B, Brown S. for the National Osteoporosis Foundation of South Africa (NOFSA). NOFSA Guideline for the Diagnosis and Management of Osteoporosis. JEMDSA. 2010;15(3, suppl 1):107-108.
11. Singer FR, and Eyre DR. (2008). Using biochemical markers of bone turnover in clinical practice. Cleve Clin J Med. 2008; 75:739–750.
12. Srivastava M, and Deal M. Osteoporosis in elderly: prevention and treatment. Clin Geriatr Med. 2002;18:529–555.
13. Lindsay R, Hart DM, and Kraszewski A. Prospective double-blind trial of synthetic steroid (Org OD 14) for preventing postmenopausal osteoporosis. Br Med J. 1980; 280: 1207–1209.
14. Hickey M, Davi SR, and Sturdee D. Treatment of menopausal symptoms: what shall we do now? Lancet. 2005; 366: 409–21.
15. Panay N, and Rees M. Alternatives to hormone replacement therapy for management of menopause symptoms. Curr Obst Gynaecol. 2005;15:259–266.
16. Kamal A, Arif JM, Ahmad IZ. Potential of *Nigella sativa* L. seed during different phases of germination on inhibition of bacterial growth. EJBPR. 2010; 1: 009-013.
17. Swamy SMK, and Tan BKH. Extraction, Isolation and Characterization of Antitumor Principle, ephedrin from the seeds of *Nigella sativa*. Planta Med. 2001;67: 29–32.
18. Ali BH, and Blunden G. Pharmacological and toxicological properties of *Nigella sativa.* Phytother. Res. 2003;17: 299-305.
19. Najmi A, Haque SF, Khan RA, Nasiruddin M. Therapeutic Effect of *Nigella Sativa* Oil On Different Clinical And Biochemical Parameters In Metabolic Syndrome. Int. J. Diabetes Metabol. 2008;16: 85-87.
20. Qidwai W, Hamza HB, Qureshi R, Gilani A. 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. Alternative. Compl. Med. 2009; 15: 639-644.
21. Tasawar Z, Siraj Z, Ahmad N, Lashari MH. The Effects of *Nigella sativa* (Kalonji) on Lipid Profile in Patients with Stable Coronary Artery Disease in Multan, Pakistan. PJN. 2011;10:162-167.
22. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J. Ethnopharmacol. 2001; 76: 45-48.
23. Al-Naggar TB, Gomez-Serranillos MP, Carreto ME, Villar AM. Neuropharmacological activity of *Nigella sativa L.* extracts. J. Ethnopharmacol. 2003;88: 63-68.
24. Alsaif MA. Effect of *Nigella sativa* Oil on Metabolic Responses to Prolonged Systemic Injury in Rats. J. Biol. Sci. 2008; 8: 974-983.
25. Parhizkar S, Latiff L, Rahman S, Dollah MA. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. Afr. J. Pharm. Pharmacol. 2011; 5: 137-142.
26. Mahmoud MR, El-Abhar HS, Salch S. The effect of *Nigella sativa* oil against the liver damage induced by Schistosoma mansoni infection in mice. J. Ethnopharmacol. 2002;79: 1-11.
27. Abdel-Sater KA. Gastroprotective effects of *Nigella Sativa* oil on the formation of stress gastritis in hypothyroidal rats. Int. J. Physiol. Pathophysiol. Pharmacol. 2009;1: 143-149.
28. Houcher Z, Boudiaf Kh, Benboubetra M, Houcher B. Effects of Methanolic Extract and Commercial Oil of *Nigella sativa* L. on Blood Glucose and Antioxidant Capacity in Alloxan-Induced Diabetic Rats. Pteridines. 2007;18: 8-18.
29. El-Bagir NM, Farah ITO, Alhaidary A, Mohamed HE, Beynen AC. Clinical laboratory serum Values in Rabbits Fed Diets containing Black Cumin Seed. J. Anim. Vet. Adv. 2010; 9: 2532-2536.
30. Parhizkar S, Rashid I, Latiffah AL. Incision Choice in Laparatomy: a Comparison of Two Incision Techniques in Ovariectomy of Rats. WJAS. 2008;4: 537-540.
31. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB. and Cooke PS. Increased adipose tissue in male and female estrogen receptor alpha knockout mice. Proc Natl Acad Sci USA.2000;97:12729-12734.
32. Boulbaroud S, A El-Hessni A, Azzaoui FZ, Mesfioui A. Sesame seed oil and flaxseed oil affect plasma lipid levels and biomarkers of bone metabolism in ovariectomized Wistar rats. Biol Med. 2012; 4 :102-110.
33. Lucas EA, Mahajan SS, Soung Do Y, Lightfoot SA, Smith BJ, Arjmandi BH. Flaxseed but not flaxseed oil prevented the rise in serum cholesterol due to ovariectomy in the Golden Syrian Hamsters. J Med Food. 2011;14: 261–267.
34. Labhal A, Settaf A, Bennani KN, Cherrah Y, Slaoui A, Hassar M. Action anti-obésité, hypocholestérolémiante et hypotriglycéridémiante de *Nigella sativa* chez le Psammomys obesus. Caducée. 1997; 27: 26-28.
35. Deftos LJ, Burton DW, Watkins WB, Catherwood BD. Immunohistological studies of artiodactyl and teleost pituitaries with antisera to calcitonin. Gen Comp Endocrinol. 1980;42:9–18

Conflict of Interest: None Declared

