ASIAN JOURNAL OF BIOMEDICAL & PHARMACEUTICAL SCIENCES

e-ISSN: 2249-622X

RESEARCH ARTICLE

Modulatory effects of *unsaturated fatty acids* on metabolic syndrome in menopause induced rats

Saadat Parhizkar[1,2*], Gholamhossein Shahraki[3], Behrooz Hassanpour[4], Parvin Abedi[5]

[1] Medicinal Plants Research Center, Yasuj University of Medical Sciences (YUMS), Iran

[2]Faculty of Medicine and Health Sciences, University Putra Malaysia (UPM), Malaysia

[3] Faculties of Health, Yasuj University of Medical Sciences (YUMS), Iran

[4] Dept. of Rural Development and Agricultural Economics Agricultural and Natural Resources Research Centre, Kohgiluyeh-va-Boyerahmad Province, Yasuj, Iran

[5] Reproductive Health Research Center, Ahvaz Jundishapur University of Medical Sciences, Iran

Received:
16th Nov 2012
Received in revised form:
4th Dec 2012
Accepted:
10th Dec 2012
Available online:
15th Dec 2012

OPEN ACCESS

Online ISSN 2249–622X
http://www.jbiopharm.com

ABSTRACT

Aims: This study was aimed to compare the metabolic impact of unsaturated fatty acids in experimental menopause induced rats

Study design: Animal model experimental design

Methodology: Forty female Sprague Dawley rats, aged 16 weeks were ovariectomized in order to induce menopause pattern and were randomly allotted into five groups: negative control (taking 1 ml olive oil/ day); positive control (taking 0.2 mg/kg/day Conjucated Equine Estrogen: CEE); experimental groups (taking 50 mg/kg/day *Linoleic acid* or 10 mg/kg/day Gamma *Linolenic acid* or 15mg/kg/day *Thymoquinone*). All of supplements administered via intragastric gavage for 21 consecutive 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.

Results: All treatment groups showed significant (P<.05) improvement with reference to blood glucose and serum HDL level in the first 10 days while the serum glucose level increased significantly (P<.05) until the end of experiment in all groups. Similarly, all treatment groups showed a decline in serum triglyceride 10 days, but the effect was much higher in LA and GLA groups respectively. Significant weight reducing effect (P<.05) existed in gamma linolenic group.

Conclusion: These results suggested that supplementing with unsaturated fatty acids exert a therapeutic and protective effect by modifying weight gain, improving lipid profile and blood glucose which can reduce menopause burden in this context.

Keywords: *Gamma-Linolenic acid*, Linoleic acid, Menopause, Metabolic Syndrome.

1. INTRODUCTION

The risk of metabolic syndrome and cardiovascular disease increases in women after menopause due to changes in the body (increase accumulation of abdominal fat) that occur in response to a decrease in estrogen levels [1-3]. Therefore, postmenopausal status might be a predictor of metabolic syndrome in this area [4,5]. Studies have shown that approximately half of the coronary events in women can be attributed to metabolic syndrome [6]. *N*igella *sativa* seeds have traditionally been used in Middle Eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years. The seeds of *N*igella *sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years [7-9]. It has been shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid [10]. *Nigella sativa* oil is a rich source of linoleic acid (LA) an omega-6 fatty acid. Although there have been no studies to determine the specific impact of LA and other principles of *Nigella sativa* on metabolic syndrome, previous studies have shown LA impact on some of the syndrome's components. Several studies have shown decreased blood pressure with higher LA intake [11,12].

*Corresponding author: Saadat Parhizkar, | Medicinal Plants Research Center, Yasuj University of Medical Sciences (YUMS), Iran|
Email: parhizkarsa@gmail.com

Diets rich in omega-6 polyunsaturated fatty acids (PUFA) have been shown to decrease total cholesterol levels, low density lipoprotein (LDL) levels and the ratio of total cholesterol to HDL [12]. Since *Nigella sativa* has shown beneficial effect on metabolic syndrome, we hypothesized that its active ingredients would be more effective than negative control in reducing plasma lipids concentration among ovariectomized (OVX) rats. This study sought to examine the impact of some ingredients of *Nigella sativa* including linoleic acid, gamma linolenic acid and thymoquinone on the attenuation of the risk factors of metabolic syndrome in ovariectomized rats as menopause induced animal model. Therefore the present study aimed to evaluate modulatory effects of some active ingredients of *Nigella sativa* on metabolic syndrome components among OVX rats.

2. MATERIAL AND METHODS

2.1. Experimental Design

In order to induce menopause and to investigate metabolic changes following supplementation with different ingredients 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 [13]. All surgical procedures were performed by a veterinary surgeon. High degree of aseptic procedure was maintained throughout the operation. 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/day), group 2, positive control (0.2mg/kg/day CEE diluted in distilled water), group 3 Linoleic acid (daily 50 mg/kg LA which calculated based on yielding *Nigella sativa* fixed oil (29%) and concentration of LA (57%) in fixed oil), group 4, Thymoquinone (daily 15mg/kg TQ which calculated based on yielding *Nigella sativa* fixed oil (29%) and concentration of TQ (16.1%) in fixed oil which analyzed and reported by Latiffah et al. [14] on the same plant source) and group 5, Gamma Linolenic acid (GLA-daily 10 mg/kg considering presence of GLA in methanolic extract and production of it through conversion from LA with taking in account recommended dosage for maintaining health). Dosage of the ingredients were selected based on the optimum desired effect of *Nigella sativa and* its extracts in the previous experiments[15,16], which was at low dose (300mg/kg BW/day) and were administered by intra-gastric gavage for 3 weeks. All ingredients were diluted in olive oil as vehicle to reach its total volume equal to 1ml. Lipid profile, blood glucose and body weight were measured at baseline (day 0), 11th days, and at the end of experiment (21st day).

2.2. 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*.

2.3. Chemicals

Linoleic Acid (95%), Gamma-Linolenic Acid and thymoquinone (99%) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Conjugated Equine Estrogen (CEE 0.625mg) was purchased from Wyeth, Montreal, Canada and prepared in a dosage of 0.2mg/kg [17-19] by dissolving it in distilled water [16, 17, 20] 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.

2.4. 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. Triglycerides (TG), LDL, high density lipoprotein (HDL), total cholesterol (TC) and glucose level in the serum were determined using kits (Roche System) in the Hitachi Chemical Analyzer (Model 902, Japan). All tests were performed according to the manufacturers' instructions.

2.5. 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) statistical packages. Variables exhibited a normal distribution, so 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 was considered to be significant.

3. RESULTS

3.1. Body weight

Over the period of treatment, the body weight of OVX rats tended to increase in Control, TQ and LA, while there was a tendency to reduce mean of body weight among CEE and specially GLA groups (Figure 1).

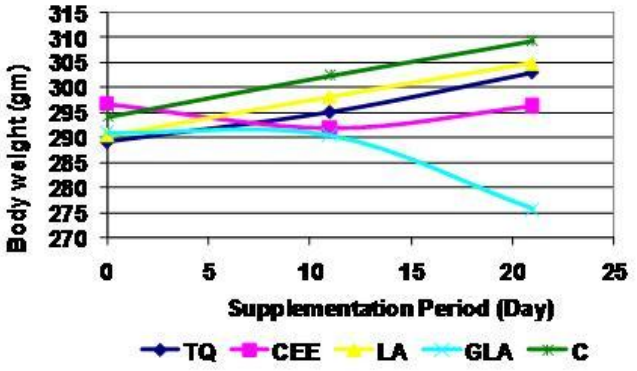

Figure1: Changes of body weight (gm) of OVX rats supplemented with various ingredients of Nigella sativa or Conjugated Equine Estrogen for 21 Days.

Data expressed as mean, TQ=Thymoquinone (15mg/kg/day); LA=linoleic Acid (50mg/kg/day); GLA= Gamma Linolenic Acid (10mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day)groups.

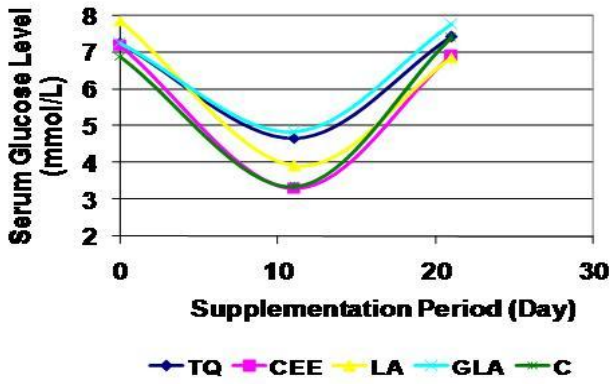

Figure 2: Changes of serum glucose level (mmol/L) of OVX rats supplemented with various ingredients of *Nigella sativa* or Conjugated Equine Estrogen for 21 Days.

Data expressed as mean. TQ=Thymoquinone (15mg/kg/day); LA=linoleic Acid (50mg/kg/day); GLA= Gamma Linolenic Acid (10mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day) groups.

3.2. Blood glucose

All experimental groups showed decline in the concentration of blood glucose in the first 10 days which were significant in all groups (P<.05) compared to baseline, but surprisingly the blood glucose return to the baseline at the end of experiment (Figure 2).

3.3. Lipid Profile

The sequential changes in serum TC, TG, LDL and HDL are summarized in Table 1. Supplementations with different ingredients of N. sativa for 21 days in OVX rats significantly improved HDL (P<.05), while no effects were observed on the total cholesterol, low density lipoprotein and serum triglyceride concentration. The higher tendency of ingredients to exert effect on lipid profile was belong to thymoquinone.

4. DISCUSSION

It is well known that estrogen deficiency significantly increased the weight gain in ovariectomized rats which can relief by estrogen therapy [21]. As expected, over the period of the experiment, the body weight of OVX rats increased significantly (P<.05), while body weight of estrogen treated group remained unchanged. In contrast, gamma linoleic acid in OVX rats treated group showed significant reduction in body weight which is consistent with Kaku study's in both human and animals [22-24].

The present study clearly showed that all active ingredients of *Nigella sativa* and both negative and positive control groups possessed a significant glucose lowering effect on OVX rats in the first 10 days of intervention. The effect of CEE and LA groups were more than other groups. After continuing of supplementation, the effect was disappeared and serum glucose level increased significantly until the end of experiment and the increment was more pronounced in the TQ, GLA and negative control groups. Results indicated that active ingredients of NS have a promising reducing effect on the blood glucose levels in OVX rats. Similarly, Hawsawi [25] also showed desirable effect of TQ on blood glucose of normal rats. Some studies [21] showed that ovariectomy increased the level of plasma total cholesterol and LDL cholesterol causing the development of atherosclerosis and CHD. The result of the present study illustrated the beneficial effect of active ingredients of *Nigella sativa* on lipid profile which caused a lowering effect on total cholesterol, triglyceride and LDL. The PUFAs of the n-6 and n-3 series have beneficial effects on plasma lipids. Those of the n-6 series are more effective in reducing cholesterol [26] whereas those of the n-3 series exert their main effect on triglycerides and their effect on lipoproteins is still unknown [27].

Some studies [21] showed that ovariectomy increased the level of plasma total cholesterol and LDL cholesterol causing the development of atherosclerosis and CHD. The result of the present study illustrated the beneficial effect of active ingredients of *Nigella sativa* on lipid profile which caused a lowering effect on total cholesterol, triglyceride and LDL. The PUFAs of the n-6 and n-3 series have beneficial effects on plasma lipids. Those of the n-6 series are more effective in reducing cholesterol [26] whereas those of the n-3 series exert their main effect on triglycerides and their effect on lipoproteins is still unknown [27].

Some studies [21] showed that ovariectomy increased the level of plasma total cholesterol and LDL cholesterol causing the development of atherosclerosis and CHD. The result of the present study illustrated the beneficial effect of active ingredients of *Nigella sativa* on lipid profile which caused a lowering effect on total cholesterol, triglyceride and LDL. The PUFAs of the n-6 and n-3 series have beneficial effects on plasma lipids. Those of the n-6 series are more effective in reducing cholesterol [26] whereas those of the n-3 series exert their main effect on triglycerides and their effect on lipoproteins is still unknown [27]

Parameters	Day	NS ingredients			Control Groups		Total
		TQ	LA	GLA	-ve (C)	+ve(CEE)	
TC	0	1.62± 0.27^{abc}	1.49± 0.26^{bc}	1.49± 0.26^{bc}	1.42± 0.25^{bc}	1.74± 0.54^{ab}	1.55± 0.34^{XY}
	11	1.92± 0.22^{a}	1.65± 0.22^{ab}	1.52± 0.24^{bc}	1.47± 0.27^{bc}	1.51± 0.37^{bc}	1.62± 0.30^{X}
	21	1.56± 0.28^{bc}	1.53± 0.21^{bc}	1.56± 0.14^{bc}	1.41± 0.17^{bc}	1.32± 0.29^{c}	1.47± 0.23^{Y}
	Total	1.70± 0.29^{A}	1.56± 0.23^{AB}	1.52 ± 0.21^{B}	1.44 ± 0.23^{B}	1.52± 0.44^{B}	
TG	0	0.91± 0.39^{def}	1.37± 0.42^{def}	1.13± 0.45^{def}	0.89± 0.36^{def}	0.96± 0.37^{def}	1.05± 0.42^{X}
	11	0.82± 0.25^{ef}	0.75± 0.20^{ef}	0.65± 0.09^{f}	0.73± 0.30^{ef}	0.78± 0.50^{ef}	0.75± 0.29^{Y}
	21	2.23± 0.65^{a}	1.53± 0.65^{bcd}	2.00± 0.89^{ab}	1.89±1.00^{abc}	1.10± 0.99^{def}	1.75± 0.90^{Z}
	Total	1.32± 0.79^{A}	1.22± 0.56^{AB}	1.26± 0.79^{AB}	1.17± 0.80^{AB}	0.95 ± 0.66^{B}	
HDL	0	1.36± 0.28^{ab}	1.20± 0.19^{bc}	1.26± 0.21^{bc}	1.20± 0.27^{bc}	1.38± 0.39^{ab}	1.28± 0.27^{X}
	11	1.59± 0.24^{a}	1.38± 0.19^{ab}	1.30± 0.18^{bc}	1.22± 0.20^{bc}	1.28± 0.32^{bc}	1.35± 0.26^{X}
	21	1.17± 0.23^{bc}	1.17± 0.17^{bc}	1.17± 0.11^{bc}	1.06± 0.14^{c}	1.06± 0.28^{c}	1.12± 0.19^{Y}
	Total	1.37 ± 0.29^{A}	1.25± 0.20^{AB}	1.24± 0.17^{AB}	1.16± 0.21^{B}	1.24± 0.35^{AB}	
LDL	0	0.20± 0.06^{bcd}	0.16± 0.09^{cd}	0.15± 0.07^{cd}	0.20± 0.06^{bcd}	0.24± 0.17^{bc}	0.19± 0.10^{X}
	11	0.38± 0.07^{a}	0.29± 0.09^{b}	0.24± 0.07^{bc}	0.18± 0.07^{cd}	0.21± 0.09^{bcd}	0.26± 0.10^{Y}
	21	0.18± 0.04^{cd}	0.17± 0.08^{cd}	0.17± 0.08^{cd}	0.13± 0.05^{d}	0.17± 0.07^{cd}	0.17± 0.07^{X}
	Total	0.25± 0.11^{A}	0.21± 0.10^{AB}	0.19± 0.08^{B}	0.17± 0.06^{B}	0.21± 0.12^{AB}	

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

Data are expressed as Mean ± SD.

TQ=Thymoquinone (15mg/kg/day); LA=linoleic Acid (50mg/kg/day); GLA= Gamma Linolenic Acid (10mg/kg/day); CEE= conjugated equine estrogen (0.2mg/kg/day); and C= control (1 ml Olive Oil/day)groups. AB: Comparison of the means between columns within row of the same parameter significant at p<.05. XYZ: Comparison of the means between rows within column of the same parameter significant at p<.05. abcdef: Comparison of the means between column and between row of the same parameter significant at p<.05

Some studies [21] showed that ovariectomy increased the level of plasma total cholesterol and LDL cholesterol causing the development of atherosclerosis and CHD. The result of the present study illustrated the beneficial effect of active ingredients of *Nigella sativa* on lipid profile which caused a lowering effect on total cholesterol, triglyceride and LDL. The PUFAs of the n-6 and n-3 series have beneficial effects on plasma lipids. Those of the n-6 series are more effective in reducing cholesterol [26] whereas those of the n-3 series exert their main effect on triglycerides and their effect on lipoproteins is still unknown [27] .

Similarly, Bregman [28] reported that animals fed a normal linoleic acid diet showed a worse lipid profile (higher concentrations of LDL and lower levels of HDL) when compared to those fed with high linoleic acid diet. Conjugated linoleic acids (CLAs) is a group of naturally occurring isomers of linoleic acid that differ in the position or geometry of their double bonds [29, 30], in a hypercholesterolemic diet in rabbits led to a significant reduction in serum triglyceride (TG), LDL and cholesterol (CHO) levels [31]. Similar results also have been reported for hamster hypercholesterolemia models [32, 33].

In this study, there was no significant decrease in total cholesterol, LDL and increase in HDL level in LA group. Purred linoleic acid was used via intera gastric gavage. Recent in vitro and in vivo studies suggested that the individual CLAs isomers may have different effects on lipid metabolism. In human studies regarding lipid profile parameters were inconsistent with the animal ones. The inconsistency of results may arise from the differences between LA and CLA and also differences of spices as mentioned by Braun et al.,[34] that rodents are especially inadequate models to study some aspects of lipid

metabolism in reference to humans. Moreover, the rout of administration was different, gavage vs supplementation to food. Aryaeian and colleagues [35] stated that the effects of the CLAs-enriched dairy products on blood lipids were more complex. In our study similar to Desroches and colleagues' [36] study after oral administration of LA supplements, LDL, HDL and total cholesterol concentrations were not significantly changed. Other studies have also shown that CLAs supplementation had no effect on HDL lipid composition [32, 37, 38].

In current study, LDL increased in the first 10 days in all groups except controls and afterward reduced at the baseline levels and the difference between LA and TQ groups was significant. In this study, TQ was more effective than LA in reducing LDL and total cholesterol. On the other hand, it was showed that all active ingredients of *Nigella sativa* reduced plasma triglycerides in the first 10 days while further supplementation until the end of study increased it. This result was consistent with our first and second experiments using either different dosages or different extracts of *Nigella sativa*, where it showed an increase in the TG level following the initial reduction. As Cascio and his colleagues [39] mentioned free fatty acids (FFA) are not indeed only essential fuels for the organism. They also act as legends for both membrane and nuclear receptors involved in different signalling pathways.

5. CONCLUSION

In conclusion our study provided convincing evidence that ingredients of *Nigella sativa* can reduce metabolic syndrome burden in the context of menopause which provides novel evidence in support of the traditional use of *Nigella sativa* as an anti aging remedy. Moreover, further studies are required to establish the beneficial effect of active principles of *Nigella sativa* seeds in human beings as well as its molecular sites of action responsible for desirable effects of those ingredients.

6. ACKNOWLEDGEMENT

The authors would like to thank University Putra Malaysia for its financial support of this research project.

7. COMPETING INTERESTS

Authors have declared that no competing interests exist.

8. REFERENCES

1. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA. 2002;287(3):356-359.
2. Carr M C. The emergence of the metabolic syndrome with menopause. J. Clin. Endocrinol. Metab. 2003; 88(6):2404-2411.
3. Paul S, Smith L. The metabolic syndrome in women: a growing problem for cardiac risk. J Cardiovasc Nurs. 2005;20(6):427-432.
4. Marjani M and Moghasemi S. The Metabolic Syndrome among Postmenopausal Women in Gorgan. Int J Endocrinol. 2012.doi:10.1155/2012/953627
5. Heidari R, Sadeghi M, Talaei M, Rabiei K, Mohammadifard N, Sarrafzadegan N. Metabolic syndrome in menopausal transition: Isfahan Healthy Heart Program, a population based study. Diabetol Metab Syndr. 2010;2:59.
6. Wilson PWF, Kannel WB, Silbershatz H, D'Agostino RB. Clustering of Metabolic Factors and Coronary Heart Disease. Arch Intern Med.1999. 159(10):1104-1109.
7. Coban S, Yildiz F. "The effects of *Nigella sativa* on bile duct ligation induced-liver injury in rats." Cell Biochem Funct. 2010;28(1): 83-88.
8. Al-Nazawi MH, and El-Bahr SM. Hypolipidemic and Hypocholestrolemic Effect of Medicinal Plant Combination in the Diet of Rats: Black Cumin Seed (*Nigella sativa*) and Turmeric (*Curcumin*). J Anim Vet Adv. 2012;11: 2013-2019.
9. Akhtar M, Maikiyo AM. "Ameliorating effects of two extracts of *Nigella sativa* in middle cerebral artery occluded rat." J Pharm Bioallied Sci. 2012;4(1): 70-75.
10. Houghton PJ, Zarka R, Heras BDL, Hoult JRS. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica. 1994;61, 33–36.
11. Harris WS, Mozaffarian D, Rimm E. Omega-6 fatty acids and risk for cardiovascular disease: a scienceadvisory from the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. Circulation. 2009;119(6):902-907.
12. Czernichow S, Thomas D, Bruckert E. n-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations. Br J Nutr.2010 1-9.
13. Parhizkar S, Rashid I, and Latiffah AL. Incision Choice in Laparatomy: a Comparison of Two Incision Techniques in Ovariectomy of Rats. WASJ. 2008;4 (4): 537-540.
14. Latiffah AL, Hassanzadeh Ghahramanloo K, Hanachi P. Comparative analysis of essential oil composision of Iranian and Indian *Nigella sativa* extracted by using Supercritical Fluid Extraction (SFE) and solvent extraction. Clin Biochem. 2011; 44(13):S20.
15. Parhizkar S, Latiffah AL, Sabariah AR, Dollah MA. Preventive effect of *Nigella sativa* on metabolic syndrome in Menopause Induced Rats. J Med Plants Res. 2011; 5(8);1478-1484.
16. Parhizkar S, Latiffah AL, Sabariah AR, Dollah MA, Hanachi P. Assessing estrogenic activity of Nigella sativa in ovariectomized rats using vaginal cornification assay. Afr J Pharm Pharmacol. 2011; 5(2):137 – 142.
17. Genazzani, A. R., Stomati, M., Bernardi, F.,Luisi S., Casarosa, E., Puccetti, S., Genazzani, A.D., Palumbo, M. and Luisi, M. (2004). Conjugated equine estrogens reverse the effects of aging on central and peripheral allopregnanolone and beta-endorphin levels in female rats. *Fertility and Sterility,* 81(1): 757-766.
18. Oropeza, M.V., Orozco, S., Ponce, H. and Campos, M.G. (2005). Tofupill lacks peripheral estrogen-like actions in the rat reproductive tract. *Reproductive Toxicology,*20: 261–266.
19. Araujo, L.B.F., Soares, J.M., Simoes, R.S. Ricardo Santos, S., Calió, P.L., Ricardo Oliveira-Filho, M., Simões, M.J., Haidar, M.A. and Baracat, E.C. (2006). Effect of conjugated equine estrogens and tamoxifen administration on thyroid gland histomorphology of the rat. *Clinics*,61: 321-326.
20. Hajdu, A., Chappel, C. I. and Rona, G. (1965). The influence of estrogens on scorbutic bone lesions in guinea-pigs. *CMLS.,* 21(8): 466-467.
21. Liu M, Xu X, Rang W, Li Y, and Song Y. Influence of ovariectomy and 17β-estradiol treatment on insulin

sensitivity, lipid metabolism and post-ischemic cardiac function. Int J cardiol. 2004;97(3): 485-493.
22. Vaddadi KS, and Horrobin D F. Weight loss produced by evening primrose oil administration in normal and schizophrenic individuals. IRCS Med. Sci. 1999;1979;7: 52.
23. Takada R, Saitoh M, and Mori T. Dietary gamma-linolenic acid-enriched oil reduces body fat content and induces liver enzyme activities relating to fatty acid beta- oxidation in rats. J. Nutr. 1994;124(4):496-74.
24. Kaku S, Ohkura K, Yunoki S, Nonaka M, Tachibana H, Sugano M, and Yamada K. Dietary gamma-linolenic acid dose-dependently modifies fatty acid composition and immune parameters in rats. Prostag Leukotr Ess. 2001;65(4); 205-210.
25. Hawsawi ZA, Ali BA, Bamosa AO. Effect of Nigella sativa (Black seed) and thymoquinone on blood glucose in Albino rats. Ann Saudi Med. 2001;21(3-4), 242-244.
26. Katan MB. Omega-6 polyunsaturated fatty acids and coronary heart disease. Am J Clin Nutr. 2009;89:1283–4
27. Skulas-Ray AC, Kris-Etherton PM, Harris WS, Heuvel JPV, Wagner PR, and West ShG. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and endothelial function in healthy persons with moderate hypertriglyceridemia. Am J Clin Nutr. 2010; doi: 10.3945/ajcn.110.003871.
28. Bregman R, Lemos CCS, Caldas ML, and Gregório SMP. Effect of dietary linoleic acid on the progression of chronic renal failure in rats. Braz J Med Biol Res. 2002;35(5); 573-579.
29. Kelly GS. Conjugated linoleic acid: A review. Altern Med Rev. 2001;,6:367–82.
30. Bhattacharya A, Banu J, Rahman M. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem. 2006;17:789–810.
31. Lee KN, Kritchevsky D, and Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis. 1994;108:19–25.
32. Nicolosi RJ, Rogers EJ, and Kritchevsky D. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery. 1997; 22:266–77.
33. Wilson TA, Nicolosi RJ, and Chrysam M. Conjugated linoleic acid reduces early aortic atherosclerosis greater than linoleic acid in hypercholesterolemic hamsters. Nutr Res. 2000;20:1795–805.
34. Braun A, Rigotti A, Trigatti BL. Myocardial infarction following atherosclerosis in murine models. Curr Drug Targets. 2008;Mar;9(3):217-23.
35. Aryaeian N, Shahram F, Djalali M, Eshragian MR, Djazayeri A, Sarrafnejad A, Naderi N,Chamari M, Fatehi F, and Zarei M. Effect of conjugated linoleic acid, vitamin E and their combination on lipid profi les and blood pressure of Iranian adults with active rheumatoid arthritis. Vasc Health Risk Manag. 2008;4(6);1423–1432.
36. Desroches, S., Chouinard, P.Y., Galibois, I. (2005). Lack of effect of dietary conjugated linoleic acids naturally incorporated into butter on the blood lipid profi le and body composition of overweight and obese men. *Am J Clin Nutr*, 82:309–19.
37. Gavino VC, Gavino, G, Leblanc MJ. An isomeric mixture of conjugated linoleic acids but not pure cis-9, trans-11-octadecadienoic acid affects body weight gain and plasma lipids in hamsters. J Nutr. 2000;130:27–9.
38. Stangl GI. High dietary levels of conjugated linoleic acid mixture alter hepatic glycerophospholipid CLAss profi le and cholesterol-carrying serum lipoproteins of rats. J Nutr Biochem. 2000;11:184–91.
39. Cascio G, Schiera G, Di Liegro I. Dietary fatty acids in metabolic syndrome, diabetes and cardiovascular diseases. Curr Diabetes Rev. 2012;8(1):2-17.

Conflict of Interest: None Declared

