The Effects of 8-week *Nigella sativa* Supplementation and Aerobic Training on Lipid Profile and VO$_2$ Max in Sedentary Overweight Females

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ABSTRACT

Background: Regular moderate intensity physical activity and lipid lowering effects of *Nigella sativa* (*N. sativa*) supplementation may be appropriate management for sedentary overweight females. Therefore, the aim of the present study was to examine the effects of long-term *N. sativa* supplementation and aerobic training on lipid profile and maximal oxygen consumption (VO$_2$ max) in sedentary overweight females.

Methods: In this randomized, double-blind, controlled trial, which was conducted in Kerman city (Iran), 20 sedentary overweight females were divided into two groups and assigned to *N. sativa* supplementation (*N. sativa* capsules) or a placebo for the 8 weeks, both groups participated in an aerobic training program (3 times/week). Each *N. sativa* capsule contained 500 ± 10 mg *N. sativa* crushed seeds and subjects had to take 2 g *N. sativa* per day for 8 weeks. Blood lipids and VO$_2$ max were determined at baseline and at the end of 8 weeks.

Results: *N. sativa* supplementation lowered total cholesterol (TC) ($P < 0.01$), triglyceride ($P < 0.001$), low-density lipoprotein (LDL) ($P < 0.001$) and body mass index ($P < 0.01$) and increased high density lipoprotein (HDL) and VO$_2$ max ($P < 0.01$). Aerobic training program lowered TC ($P < 0.001$) and LDL ($P < 0.01$) and increased VO$_2$ max ($P < 0.01$). Furthermore, we observed a significant effect of aerobic training program and *N. sativa* supplementation lowered LDL and HDL ($P < 0.05$).

Conclusions: The present study demonstrates that 8-week aerobic training plus *N. sativa* supplementation have a synergistic effect in improve profile lipid parameters.

Keywords: Aerobic training, lipid profile, *Nigella sativa*, overweight

INTRODUCTION

World-wide, at least 2.8 million people die each year as a result of being overweight or obese and an estimated 35.8 million (2.3%) of global DALYs are caused by overweight or obesity. Overweight and obesity lead to adverse metabolic effects on blood pressure, cholesterol, triglycerides (TG) and insulin resistance.[1]

Some natural products have been considered to play a good role as an alternative to synthetic chemicals in this clinical

condition. A wide variety of plants has been reported to have lipid lowering activity.[2] Among such plants *Nigella sativa* (*N. sativa*) (black seed) in several studies has been investigated for its lipid lowering effects on animals.[3,4] Nigella seeds have many pharmaceutical uses. The seeds of the plant have been used in the Southeast Asia, Middle and Far East as a natural remedy to treat many diseases, including anti-microbial, anti-oxidant, anti-inflammatory, antitumor, hematologica, anti-hypertensive, anti-hypertensive, anti-diabetic, antilipidemic, hypophagic and anti-obesity effects and respiratory health.[5,6]

In fact, this plant has occupied a special place for its wide range of medicinal value in the Islamic civilizations. Due to the sayings of the Holy prophet, Mohammad (peace be upon him) that "in the black seed there is healing for every illness except death."[7] Anwar and Tayyab[8] reported that *N. sativa* in the diet has a favorable effect on the lipid profile by lowering the TG, total cholesterol (TC) and low-density lipoprotein (LDL)-cholesterol and increasing the high density lipoprotein (HDL)-cholesterol in rats.

Exercise is most important for every living being; in other words, we can also say that physical inactivity results in several types of diseases in the body.[9] A sedentary life-style is associated with an increased risk for an acute myocardial infarction and death from coronary heart disease (CHD). The findings are consistent and show that sedentary people have about twice the risk of developing or dying from CHD, compared to active people.[10] Physical inactivity is now recognized by the American Heart Association as an independent risk factor, comparable to the other established risk factors for CHD. Increasing physical activity is strongly recommended to improve the coronary risk profile.[11] It is well-established that habitual physical activity improves physical fitness in middle-aged men and women.[12]

Yar *et al.*[13] had monitored the effect of *N. sativa* supplementation on cardiac reserve in rats. Their results suggest that *N. sativa* generated homogenous cardiac hypertrophy similar to that provoked by exercise training. Despite the aforementioned beneficial effects of exercise and *N. sativa*, no study exist that investigated the effects of *N. sativa* combined with exercise. Thus, the purpose of this randomized, double blind, controlled trial was to investigate whether 8 weeks of aerobic training combined with

N. sativa supplementation could improve lipid profile and maximal oxygen consumption (VO$_2$ max) in sedentary overweight females.

METHODS

Subjects

A total of 20 adult females with a mean age of 34.31 ± 7.9 years, body mass index (BMI) ≥25 kg/m^2 and sedentary life-style for at least 2 years participated in this study voluntarily. The main criterion for entry of adult females was a TC concentration more than 200 mg/dl. Subjects taking lipid-altering medication within 8 weeks prior to the study were excluded. Other exclusion criteria were pregnant, current smoking, lactation, having diabetes mellitus, cardiovascular disease (CVD), renal or liver disease, thyroid dysfunction and myopathy. Descriptive characteristic are listed in Table 1.

Supplementation

According to the subject's profiles number and the random figures tables, they were randomized to receive either *N. sativa* capsules (NS group) or placebo (PLA group). Each black seed capsule contained 500 ± 10 mg *N. sativa* crushed seeds and subjects had to take 2 g of *N. sativa* per day for 8 weeks. They were instructed to take two capsules before breakfast and two capsule in the afternoon prior their food.[14] Subjects were also asked not to change their usual daily diet and physical activity during the study.

Training program

Subjects in two groups performed slow running (10 min), stretching muscles and loosening joints (10 min), aerobic training program (30 min) and cool down (10 min). The intensity of training was 60-70% of target pulse rate calculated by Karvonen *et al.* protocol.[15] These training were completed 3 days in a week during 8 weeks.

Table 1: Physical characteristics of the study participants*

Variables	PLA group (*n*=10)	NS group (*n*=10)
Age (year)	33±4.34	34.14±10.54
Height (cm)	170±0.09	174±0.06
Weight (kg)	72.5±7.98	76.85±3.71
BMI (kg/m^2)	25.85±1.45	25.4±0.81

*Data presented as mean±standard deviation. BMI=Body mass index; PLA=Placebo group; NS=*N. sativa* group

Target heart rate (HR) zone = ([HR maximum− HR rest] × [60-70%] + HR rest) HR maximum = 220 − age.

Resting HR

Resting HR was measured in the morning immediately after the participants were awake. If the subjects are not able to take a measurement first thing in the morning, make sure subjects lie down for at least 10 min before taking a measurement. Measurement was taken from the radial artery with forefinger and the middle finger of the right hand placed horizontally across the subject's wrist while sitting on the chair. After that, the number of pulse beats multiplied by two to give the 1 min HR.[16]

Measurements

Cardio-respiratory assessment

Cardio-respiratory fitness was determined by 1609 m (1 mi) walk test using Rockport fitness test.[17] After a brief warm up, the subject walked as briskly as possible for 1609 m (1 mi) with a HR monitor. Tester recorded HR (beats/min) and time to completion. The formula used to calculate VO_2 max was:

132.853− (0.0349 × mass [kg]) − (0.3877 × age [year]) + (6.315 × gender) − (3.2649 × time [min]) − (0.1565 × HR [bpm]).

The validity of the test was nearly high $R = 0.88$ and standard error of the test was 5 ml/kg/min.[18] Cardio-respiratory measurement was taken before and after 8 weeks of aerobic training program and supplementation.

Blood samples

A total volume of 20 ml blood was drawn from the subjects in their forearm vein after a 12 h overnight fast immediately before and after 8 weeks of aerobic training program and supplementation in the sitting position after a 20-min rest between 8:00 and 9:00 a.m. Plasma was separated by centrifugation and samples were stored at −10°C until assays were determined (within 48 h) in all samples.

Statistical analysis

The statistical analysis was initially performed using the changes in the one-sample Kolmogorov-Smirnov test. All variables presented normal distribution. Statistical analysis was performed using the Statistical Package for the Social Sciences statistical software. Data was analyzed using paired *t*-test for within group's comparison and unpaired *t*-test for between-groups comparison. Statistical significance was accepted as $P \leq 0.05$.

RESULTS

Numbers of four subjects were excluded during the study, because of altering their usual aerobic training program and being irregular in taking capsules, so we did our statistical analysis on 16 subjects (8 subjects in NS group and 8 subjects in PLA group) who fully completed the study [Figure 1]. There were no differences among groups for age, bodyweight, height and BMI [Table 1]. Changes of lipid profiles after 8 weeks for two groups are shown in Table 2. TC ($P < 0.001$) and LDL-cholesterol ($P < 0.01$) were reduced in PLA group and VO_2 max ($P < 0.01$) increased. However, these changes were not statistically significant for TG, BMI and HDL-cholesterol ($P > 0.05$).

In the NS group TC ($P < 0.01$), TG ($P < 0.001$) and LDL-cholesterol ($P < 0.001$) levels were decreased by 4.79%, 7.97% and 5.02% respectively and BMI ($P < 0.01$) was significantly reduced by 2.76% and HDL-cholesterol ($P < 0.01$) and VO_2 max ($P < 0.01$) significantly increased by 5.76% and 2.53% respectively. Furthermore, there were significant differences between the NS and PLA groups for LDL-cholesterol ($P < 0.05$) and HDL-cholesterol ($P < 0.05$) values [Table 2].

DISCUSSION

Elevated LDL-cholesterol is a major cause of CHD. The relationship between LDL-cholesterol and CHD risk is continuous over a broad range of LDL-cholesterol levels: The higher the LDL-cholesterol level, the greater the CHD risk.[19] The increased permeability of the endothelium in the presence of elevated concentration of LDL promotes accumulation of LDL particles in the intima. These particles undergo oxidative modification, which may lead to inflammatory reactions and initiate atherosclerosis process. Therefore, lowering LDL-concentration and improving antioxidant defense of the body are two important ways to prevent atheriovascular disease.[20]

The relationship between the dietary fats and CVD, especially CHD, has been extensively investigated, with strong and consistent associations emerging from a wide body of evidence accrued from animal experiments, as well as observational studies, clinical trials and metabolic studies

conducted in diverse human populations.[21] The evidence shows that intake of saturated fatty acids is directly related to cardiovascular risk and raise total and LDL-cholesterol.[22,23] When substituted for saturated fatty acids in metabolic studies with unsaturated fatty acids lower plasma total and

LDL-cholesterol concentrations; polyunsaturated fatty acids are somewhat more effective in this respect, especially linoleic acid.[24]

This double-blind, randomized placebo-control study examined the long-term *N. sativa* supplementation and aerobic training on lipid
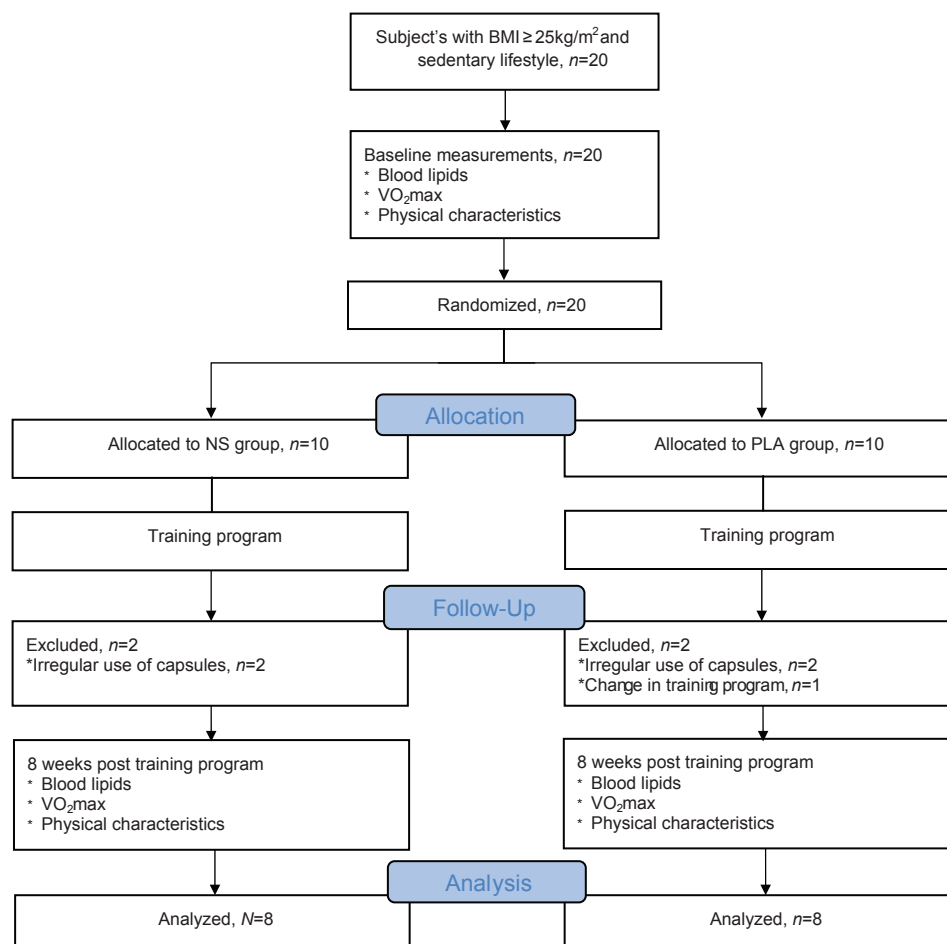

Figure 1: The schematic diagram of the study

Table 2: Changes in the health related physical fitness parameters and blood lipids in PLA and NS group

Variables	PLA group		P^1	NS group		P^1	P^2
	Pre-test	**Post-test**		**Pre-test**	**Post-test**		
TC (mg/dl)	232.75±12.85	220±15.92	0.000*	221.50±31.34	210.88±31.50	0.002*	0.43
TG (mg/dl)	173.80±50.43	144.95±68.30	0.116	167.88±18.61	154.50±16.18	0.000*	0.42
LDL (mg/dl)	120.5±10.39	109.88±12.01	0.001*	119.10±11.96	113.12±11.78	0.000*	0.01†
HDL (mg/dl)	48.37±8.61	49.62±7.90	0.305	45.62±10.02	48.25±10.40	0.004*	0.01†
VO$_2$ max (kg/ml/min)	34.19±2.47	34.81±2.66	0.002*	34.78±4.81	35.66±5.03	0.008*	0.35
BMI (kg/m^2)	25.85±1.45	25.6±1.71	0.099	25.39±0.75	24.69±0.75	0.001*	0.24

P^1=Significantly difference between pre- and post-test, P^2=Significantly difference compared with PLA group, TC=Total cholesterol, TG=Triglyceride, LDL=Low density lipoprotein, HDL=High density lipoprotein, BMI=Body mass index, NS=*N. sativa* group,PLA=Placebo group

profile and VO₂ max in sedentary overweight females. The main finding of this study was that 8 weeks of aerobic training with *N. sativa* supplementation improved HDL-cholesterol and impaired LDL-cholesterol.

Results of the present study are in consistent with other previous studies that found improvements in health related parameters of overweight and obesity females participants as a result of aerobic exercise program also *N. sativa* for the treatment of hyperlipidemia.[14,18,25,26] Since, we asked all subjects not to change their usual daily diet, it seems that this changes may be due to the result of consuming black seeds and regular aerobic training. Furthermore, these results are also in agreement with the results obtained by Anwar and Tayyab who observed that polyunsaturated fat have hypotriglyceridemic effect.[8] The hypotriglyceridemic effect of *N. sativa* is possibly due to its choleretic activity. The choleretic function of *N. sativa* is either by reducing the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine. Furthermore, it has been proved that volatile oil of *N. sativa* has two main constituents, i.e. nigellone and thymoquinone which play a key role in heart disease prevention.[27]

N. sativa is a rich source of unsaturated fatty acids, mainly linoleic acid (50-60%), oleic acid (20%), eicosadienoic acid (3%) and dihomo linolenic acid (10%)[6] that cholesterol lowering effect of *N. sativa* may be attributed to the presence of phytosterols like beta-sitosterol, polyunsaturated fatty acids and its antioxidant activity. *N. sativa* may be able to reduce synthesis of cholesterol by hepatocytes and lower its absorption from the small intestine.[28] It may also activate LDL-receptor by decreasing intracellular cholesterol, which leads to rapid clearance of LDL-cholesterol from blood circulation.[7] The effect of *N. sativa* on increasing cholesterol secretion in the bile is another probable mechanisms, which can enhance its cholesterol lowering properties.[29]

Furthermore, physical exercises performed regularly have effects on obesity, cardiovascular system, physical fitness and healthy life of the middle-aged individuals.[25] Exercise may promote reverse cholesterol transport by increasing pre-β HDL-cholesterol recycling from α HDL-cholesterol substrates that have been induced by exercise

itself. This mechanism can explain the greater cholesterol efflux from cultured human fibroblasts when exposed to serum of activity people's versus sedentary controls. Several studies have confirmed this belief[10,25] and the results of the present study are in agreement with the above statement. The concentration of HDL is inversely correlated with the risk of CHD. Results of the present study is consistent with Arazi *et al*.[10] in PLA group that applied at the end of 8-week of regular aerobic exercises on middle-aged sedentary women, found that decreased in LDL-cholesterol and increased in the HDL-cholesterol levels. In addition, Bemelmans *et al*.[30] applied at the end of 12-day of walking exercises on middle-aged sedentary men and women, found in their study that decreased in the LDL-cholesterol and TC levels and increased in the HDL-cholesterol levels.

In addition, aerobic exercise training increases the percentage of skeletal slow-twitch ☐bers, which have a higher capacity to metabolize fatty acids liberated by lipoprotein lipase from TG-rich lipoproteins.[31] Also, exercise decreases the presence of small, dense LDL-cholesterol and increases that of larger, more buoyant LDL-cholesterol particles, in a relationship that appears dose-dependent with the amount and intensity of exercise.[32] Because over the last 10 years, there have been a growing number of studies that show the benefits of moderate intensity physical activity for cardiovascular health so in our study intensity of training was 60-70% of target pulse rate.[33] Despite the aforementioned results, *N. sativa* Supplementation may facilitate additional lipid profile improvements in exercise by hypotriglyceridaemic effect and increasing cholesterol secretion in the bile.

CONCLUSIONS

Our results suggest that 2 g/day of *N. sativa* combined with an aerobic exercise program provides significant improvements in LDL-cholesterol and HDL-cholesterol that are known to influence CVD risk in sedentary overweight females. However, further studies with a larger sample size and diet assessment are still needed.

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