The effect of dietary supplementation of *Nigella sativa L.* on serum lipid profile in rats

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ABSTRACT

الأهداف: فحص أثر المعالجة الفموية للجرذان من نوع ألبينو ويستار بمختلف الجرعات من نيجيلا ساتيفا إل (NS) بذور البودرة على مستويات مصل الدهون.

الطريقة: أجريت هذه الدراسة بمركز البحوث وتطبيقات العلوم الطبية بجامعة دايكل (DUSAM) بتركيا، خلال الفترة ما بين فبراير 2003م وحتى ديسمبر 2008م. شملت الدراسة إجمالي عدد 75 ذكر جرذ من نوع ألبينو ويستارن تم تزويد 60 منهم بـ(NS) إضافي، 15 من الحيوانات مثلوا مجموعة التحكم تم إدراجهم في الدراسة. قُسمت المجموعة التي تلقت (NS) إلى أربع مجموعات رئيسية بعدد 15 لكل مجموعة. تم استخدام أربعة جرعات من (100،200،400،600mg/kg/day) (NS) في اليوم. قُسمت كل مجموعة إلي المزيد من ثلاث مجموعات فرعية بعدد 5 جرذان لكل مجموعة، تم إكمال التغذية بـ(NS) لمدة أسبوع، أسبوعين، وأربعة أسابيع. كما تم تقسيم مجموعة التحكم إلى ثلاثة مجموعات رئيسية بعدد 5 من الجرذان لكل مجموعة. تمت التضحية بالجرذان عند الأسبوع الأول، الثاني والرابع بعد كل تغذية. قيست نسبة الدهون.

النتائج: تمت معالجة الجرذان بجرعة مقدارها (400mg) لمدة أسبوع وأظهرت النتيجة زيادة ملحوظة في مستويات الكولسترول عالي الكثافة (HDL–C). كان هنالك انخفاض ملحوظ في مستويات الكولسترول منخفض الكثافة (LDL–C) لمدة أسبوع واحد لجرعة (400mg) و (600mg) وجميع الجرعات لمدة أسبوعين، وبعد أربعة أسابيع لجرعة (200mg) و(600mg) عند المقارنة مع مجموعة التحكم. كان هنالك انخفاض ملحوظ في مستويات الكولسترول المنخفض الكثافة للغاية (VLDL–C) لمدة أسبوع لـ(600mg, 400mg, 200mg) وجميع الجرعات لمدة أسبوعين وأربعة أسابيع. أدت الجرعة التي كان مقدارها (400mg) لمدة أسبوعين وجميع الجرعات لمدة أربع أسابيع إلى انخفاض ملحوظ في مستويات الدهون الثلاثية (TG). كان هنالك انخفاض ملحوظ في مستويات الكولسترول الكامل (TC) لجميع الجرعات خلال 4 أسابيع من التغذية.

خاتمة: تشير هذه النتائج إلى أن (NC) قد يحسن من التبديل في مستويات الدهون الناتجة عن الأمراض أو العوامل السامة.

Objectives: To investigate the effect of oral treatment of Wistar albino rats with different doses of Nigella sativa L. (NS) powdered seeds on the levels of serum lipids.

Methods: This study was performed in the Medical Science Application and Research Center of Dicle University, Diyarbakir, Turkey, from February 2003 to December 2008. A total of 75 Wistar albino male rats, 60 of them with NS supplementation and 15 animals acting as controls, were included in the study. The NS groups were divided into 4 main groups of 15 each. Four doses of NS were used (100, 200, 400, and 600mg/kg/day). Each dose group was further divided into 3 duration subgroups of 5 rats each, the feeding of NS seeds continued for one, 2, and 4 weeks. Control animals were divided into 3 main groups of 5 rats each. The rats were sacrificed at one, 2, and 4 weeks after feeding. Lipid parameters were measured.

Results: Rats treated with the 400mg dose for one week's duration showed a significant increase in high-density lipoprotein-cholesterol levels. There was a significant decrease in low density lipoprotein-cholesterol levels after one week for 400 and 600mg doses, and all doses after 2 weeks and 4 weeks for 200 and 600 mg doses when compared to control groups. There was a significant decrease in very low-density lipoprotein-cholesterol levels after one week for 200, 400, and 600 mg doses, and all doses for 2 and 4 weeks. A 400 mg dose for 2 weeks, and all doses for 4 weeks caused a significant decrease in triglyceride levels. There was a significant decrease of total cholesterol levels in all doses after 4 weeks of NS feeding.

Conclusion: These results indicate that NS may ameliorate the alteration in the lipid levels caused by diseases or toxic agents.

Saudi Med J 2009; Vol. 30 (7): 893-896

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Received 14th April 2009. Accepted 8th June 2009.

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Nigella sativa L. (NS) is an annual Ranunculaceae herbaceous plant and its seeds contain alkaloids, fixed and volatile oils, and a variety of pharmacologically active substances.[1] The NS seeds and its crude or essential oils have been widely used in traditional nutritional and medicinal applications.[2] The seeds of NS are known as black seed or black cumin. Its Arabic name is Habat-ul-Sauda.[2] The NS seeds have long been used in the Middle East and Far East as a traditional medicine to promote health and fight disease. It is known for its hypotensive,[3,4] immunomodulatory,[5-7] hepatoprotective,[8-10] antihelmintic, and antibacterial agents.[11,12] The risk of death from coronary heart disease (CHD) is determined by a number of factors including high blood pressure, dyslipidemia, diabetes and most importantly high total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) concentrations, and decreased high-density lipoprotein cholesterol (HDL-C).[13] Saturated fats and cholesterol in the diet play a major role in the causation of hypercholesterolemia.[14,15] The NS seeds have a low atherogenic index (TC/HDL), and may be recommended in dislipidemic patients or normal individuals.[16] The addition of NS seeds to diet is a remedy that may prove to be useful in the prevention and treatment of hyperlipidemia and hypercholesterolemia. Therefore, the aim of the present study was to evaluate the effect of varying doses and duration of NS powdered seeds on lipid parameters in rats.

Methods. This study was performed in the Medical Science Application and Research Center of Dicle University, Diyarbakir, Turkey, from February 2003 to December 2008. Approval from the University Ethical Committee was obtained. A total of 75 Wistar albino male rats, weighing approximately 180-220 g were included; 60 were administered NS powdered seeds, and 15 animals acted as controls. The NS groups were divided into 4 main groups of 15 each. The rats in all treatment groups were fed with different amounts of NS seeds of 100, 200, 400, and 600 mg/kg/day. The NS seeds were purchased from a local herb store, in Diyarbakir, Turkey. The seeds of NS were powdered in a mixer and mixed with flour, making a small amount of dough approximately 2.5 g weight before feeding. Each main group was further divided into 3 duration subgroups of 5 rats each, the feeding of NS continued for one, 2, and 4 weeks. Group Ia - rats received 100 mg/kg/day NS for only one week. Group Ib - rats received 100 mg/kg/day NS for 2 weeks. Group Ic - rats received 100 mg/kg/day NS for 4 weeks. Group IIa - rats received 200 mg/kg/day NS for only one week. Group IIb - rats received 200 mg/kg/day NS for 2 weeks. Group IIc - rats received 200 mg/kg/day NS for 4 weeks. Group IIIa - rats received 400 mg/kg/day NS for only one week. Group IIIb - rats received 400 mg/kg/day NS for 2 weeks. Group IIIc - rats received 400 mg/kg/day NS for 4 weeks. Group IVa - rats received 600 mg/kg/day NS for only one week. Group IVb - rats received 600 mg/kg/day NS for 2 weeks. Group IVc - rats received 600 mg/kg/day NS for 4 weeks. Control animals were divided into 3 main groups of 5 rats each. Each group was only given plain flour dough of approximately 2.5 g. All animals were subsequently allowed free access to normal food and water. After 12 hours of overnight fasting, the blood samples were obtained from each rat at the end of each duration for both treated and control rats. All animals underwent sodium pentobarbitone (40 mg/kg) anesthesia and a mid sternotomy was performed in all animals. The blood samples were withdrawn from the ascending aorta for the measurement of lipid parameters. These parameters were analyzed in the same samples. The levels of TC, TG, LDL-C, VLDL-C, and HDL-C were determined using a C-16000 biochemical Aeroset autoanalyzer (Abbott Laboratories Inc., Abbott Park, North Chicago, IL, USA).

All data were analyzed using the statistical package SPSS version 11.0 for Windows. All values were expressed as the mean ± SD. Significant differences between the treated and control group means were determined by the paired-t test. Normal distributions were evaluated using the Kolmogorov-Smirnov test, and homogeneity was evaluated using Levene's test. The significance of test results was ascertained at $p<0.05$.

Results. The effect of NS seeds on HDL-C, LDL-C, VLDL-C, TC, and TG levels are shown in **Table 1**. Rats treated with 400 mg/kg/day dose for one week duration showed a significant increase in HDL-C levels when compared to control groups ($p<0.05$). There was a significant decrease in LDL-C levels of after one week for 400, and 600 mg/kg/day doses ($p<0.05$, $p<0.001$) and all doses for 2 weeks ($p<0.001$), and after 4 weeks for 200 and 600 mg/kg/day doses when compared to control groups ($p<0.05$). There was a significant decrease in VLDL-C levels after one week for 200, 400, and 600 mg/kg/day doses ($p<0.001$), and for all doses after 2, and 4 weeks ($p<0.001$). A 400 mg/kg/day dose for 2 weeks ($p<0.05$), and all doses for 4 weeks were caused a significant decrease in TG levels ($p<0.001$). As compared to the control, there was a significant decrease of TC levels of all doses after 4 weeks of NS feeding ($p<0.05$). Treatment with all doses for one had no effect on TC levels.

Discussion. In our study, we showed that NS seeds favorably affect serum lipid profile in albino rats with significantly decreased TC, LDL-C, VLDL-C,

TG, while increasing HDL-C. This result agrees with those obtained by El-Dakhakhany,[17] and Kokdila et al.[18] The findings in the present study were also in agreement with the results obtained by Buriro and Tayyab,[19] and Hostmark et al,[20] who observed the hypertriglyceridemic effect of polyunsaturated fat. Therefore, they reported that the hypertriglyceridemic effect of NS seeds could be the result of their choleretic activity. This choleretic function of NS is either in reducing the synthesis of cholesterol in the hepatocytes, or decreasing its fractional reabsorption from the small intestine.[17] Zaoui et al[1] reported that serum TC and TG levels were significantly decreased in NS seeds (1 ml/kg for 12 weeks) treated rats. Mai Le et al[21] studied the effect of the petroleum ether extract of NS seeds for 4 weeks on blood glucose, insulin, and lipids in rats. At the end of the 4-week treatment, the NS-treated rats had lower fasting plasma levels of insulin and TG, and higher HDL-C as compared to the controls. Their results suggested that the petroleum ether extract of NS has a slight anorexic effect, and that it ?contains the hypolipidemic activity. Also, in our study, we noticed a significant decrease of TC levels in all doses after 4 weeks when compared to control groups. Similar to our results, Bamosa et al[22] and Najmi et al[16] reported that NS had a hypocholesterolemic effect. Researchers suggested various mechanisms by which NS may be effective in lowering the cholesterol level. It was supposed that the seeds may either inhibit de novo cholesterol synthesis, or stimulate bile acid excretion.[22,23] Another mechanism that was proposed is that NS increases the production of LDL receptors.[24] The various unsaturated fatty acids, arachidonic, linoleic, linolenic, oleic, palmitic, myristic, stearic, and palmitoleic acid, may be responsible for the improvement in the lipid profile.[25] Nigella sativa when given in a higher concentration induced a significant decrease in TC, LDL-C, and TG levels, while induced a significant increase in HDL-C levels. All these effects are beneficial for patients with CHD, and those individuals who are predisposed to this problem.[19] In our study, we noticed a significant increase in HDL-C levels after one week for the 400 mg/kg/day dose when compared to

Table 1 - Changes in lipoprotein levels (mg/dl) in normal rats treated with different doses of Nigella sativa (NS) seeds given for different durations compared with control.

Parameter/groups	Dose mg/day	NS-rats treatment time (Mean ± SD)		
		1 week (n=5)	2 weeks (n=5)	4 weeks (n=5)
HDL-C (mg/dl)				
Control group	0	39 ± 4.18	46 ± 5.47	47 ± 5.7
Treated group	100	41 ± 4.18	42 ± 2.73	46 ± 4.18
	200	42 ± 2.73	45 ± 3.53	50 ± 3.53
	400	47 ± 5.70*	41 ± 4.18	45 ± 3.27
	600	42 ± 2.73	44 ± 6.65	49 ± 6.51
LDL-C (mg/dl)				
Control group	0	44 ± 5.47	42 ± 4.95	38 ± 13.03
Treated group	100	36 ± 15.16	21 ± 5.47†	23 ± 12.04
	200	31 ± 11.4	21 ± 7.41†	16 ± 5.47*
	400	22 ± 8.36*	18 ± 4.47†	22 ± 13.03
	600	16 ± 8.94†	16 ± 8.94†	14 ± 5.47*
VLDL-C (mg/dl)				
Control group	0	14.24 ± 2.70	14.18 ± 1.02	14.26 ± 0.96
Treated group	100	12.82 ± 1.49	10.70 ± 0.94†	8.62 ± 1.65†
	200	0.68 ± 0.90†	8.56 ± 1.65†	7.56 ± 1.65†
	400	8.30 ± 1.32†	7.02 ± 0.80†	6.16 ± 0.93†
	600	7.02 ± 0.80†	6.12 ± 0.98†	4.94 ± 1.19†
Triglyceride (mg/dl)				
Control group	0	60 ± 50	59 ± 4.18	60 ± 50
Treated group	100	54 ± 4.18	51.6 ± 3.20	47 ± 2.12†
	200	57 ± 5.70	51 ± 4.18	44 ± 4.18†
	400	56 ± 2.94	50 ± 6.12*	44 ± 3.56†
	600	53 ± 8.31	51 ± 7.41	45 ± 50†
Total cholesterol (mg/dl)				
Control group	0	82 ± 2.73	83 ± 4.47	83 ± 4.47
Treated group	100	81 ± 4.18	78 ± 2.73	74 ± 4.18*
	200	82 ± 2.73	78 ± 2.73*	74 ± 4.18*
	400	80 ± 6.12	79 ± 6.51	74 ± 4.18*
	600	78 ± 5.70	80 ± 7.90	74 ± 4.18*

All values are mean ± SD.
*Significantly different from corresponding control at $p<0.05$, †significantly different from corresponding control at $p<0.001$

control groups. Previous studies had reported varying results with regard to the effect of NS on HDL-C levels. Some studies reported[16,25] that NS had no effect on HDL-C level, while others reported that NS had an increasing effect on HDL-C level.[19,26] Kokdila et al[18] reported that NS oil (1 ml/kg orally for 4 weeks) had an increasing effect on HDL-C level. This discrepancy may be explained by administering different dosages and because of difference in response of various species. Nigella sativa powdered seed feeding has a beneficial effect on plasma lipids. Our findings showed that NS feeding in the various doses and duration decreased TG, TC, VLDL, and LDL-C level and increased HDL-C levels when compared to controls. Generally, all doses of NS seeds caused a significant decrease in the serum lipids. However, there was no linear dose or time dependent effect of NS on lipid parameters. The effective dose of NS seemed to be between 400-600 mg/kg.

We conclude that dietary supplementation of NS seed is a remedy that may prove to be useful in the prevention and treatment of the hyperlipidemia and hypercholesterolemia. However, further investigations will be required to clarify the beneficial effect of NS powdered seeds in rats and human beings.

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