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The effect of dietary *Nigella sativa* seeds on the blood cholesterol and lipoprotein levels of rabbits

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Key words
Cholesterol, diet, lipid, *Nigella sativa*, rabbits

1 SUMMARY

The present study investigated the effects of dietary *Nigella sativa* seeds on levels of cholesterol and lipoproteins in the blood of rabbits. Eighteen healthy female New Zealand white rabbits, 5-6 months of age were randomly allotted into two treatments of nine animals each, with three animals per replicate. The rabbits were fed on diets supplemented with *N. sativa* seeds at one of two levels (0.0 and 2.5%), or 15 gram of egg yolk. The experiment lasted for four weeks, and the blood samples were collected pre-treatment and at the end of the experimental period for the determination of serum total cholesterol, triacylglycerol, low density lipoprotein (LDL), and high density lipoprotein (HDL). The results showed that feeding rabbits on egg yolk increased serum levels of total cholesterol, triacylglycerol and LDL ($P < 0.01$), while HDL was not affected. Diet with *N. sativa* seeds showed insignificant effects on serum total cholesterol, triacylglycerol and LDL. However, HDL significantly increased ($P < 0.05$) after feeding on Nigella's seeds. This trial provides strong evidence supporting the use of *N. sativa* seeds in the amelioration of abnormal lipid metabolism.

2 INTRODUCTION

Black seed or black cumin, *Nigella sativa* is a spicy plant that is widely used in North Africa and the Middle East Its seeds are used mostly for edible purposes such as for seasoning of many kinds of bread, yoghurt and cookies. Nigella seed is a good source of oil and protein, containing significant amounts of sterols. The oil is rich in β-sitosterol that inhibits the absorption of dietary cholesterol (Atta, 2003). It is known for its hypotensive (Zaoui *et al.*, 2000), immunomodulatory (Swamy & Tan, 2000), and hepatoprotective (Mahmoud *et al.*, 2002) effects. It is also used for medical purposes for treatment of some diseases (Utun *et al.*, 1990, Houghton *et al.*, 1995). Chakravarty (1993) reported that low concentration of Nigellone (carbonyl polymer of thymoquinone extracted from *N. sativa* has antihistamine effect. *N sativa* seeds have also been found to be very effective against intestinal cestodes (Akhtar *et al.*, 1991). Moreover, Hanafy and Hatem (1993) reported the antimicrobial activity of *N. sativa* against pathogenic bacteria and yeasts.

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Inclusion of *Nigella sativa* seeds in diets have been shown to reduce levels of lipids and cholesterol in blood in rabbit and man (Bamosa *et al*., 1997; Abdel-Majeed, 1999; Omer, 2001). Defects in cholesterol metabolism are major causes of cardiovascular diseases. It is for this reason that cholesterol becomes of paramount importance in clinical biochemistry (Zubay *et al*., 1995). At the end of 4-weeks feeding on *N. sativa*, rats had lower fasting plasma levels of triglycerides, and higher HDL-cholesterol as compared to pair-fed controls. The petroleum ether extract of *N. sativa* has a slight anorexic effect, and it contains hypolipidemic activity previously associated with the plant (Le *et al*., 2004).

The petroleum ether extract of *N. sativa* significantly reduced plasma triglycerides while increasing HDL-cholesterol, while the volatile oil is as efficient as the cholesterol-reducing drug simvastatin (Settaf *et al*., 2000). *N. sativa* oil (NSO) may have protective effects on lipid peroxidation process during IRI in rat hippocampus (Hosseinzadeh *et al*., 2007). With the increasing incidence of heart disease and abnormal lipid metabolism, provision for alternative cholesterol-lowering agents is crucial.

Therefore, this study was designed to investigate the effect of feeding *Nigella sativa* seeds at 2.5% on blood cholesterol level of rabbits, aiming to determine how people can achieve normal cholesterol level by using *Nigella sativa* seeds as protective agent from hypercholesterolemia.

3 MATERIALS AND METHODS

Experimental animals: Eighteen healthy female New Zealand white rabbits, of 5-6 months old and weighing 2.4-2.7 kg were obtained from Balsam Medicine Company, Khartoum, Sudan. The rabbits were allotted randomly into two treatments, 9 animals per treatment with 3 replicates of 3 animals each. The rabbits were kept for four weeks adaptation period on basal diet and with free access to water and feed.

Experimental diets: Two kilograms of *Nigella sativa* seeds (Black cumin) were purchased from the local market, in addition to the other ingredients (Table 1). To animals in each replicate 300 grams basal diet were provided daily, supplemented with 15 grams boiled egg yolk as a source of cholesterol (1 gram of egg yolk approximately equivalent to 14 mg of cholesterol). The animals were further fed on *N. sativa* seeds at 0 and 2.5% daily throughout the experimental period.

Sample collection and analysis: Blood samples were collected before feeding the rabbits on the experimental diet and at the end of the experimental period. After overnight fasting, blood samples were collected from each rabbit (5 ml) from the ear vein into centrifuge tubes. The blood was allowed to clot at room temperature for one hour, and then the serum was separated by centrifugation at 3000 rpm for 15 minutes, clear serum was stored at 20°C until analyzed. Commercial kits from Biosystem Company were used for the estimation of serum levels of total cholesterol, LDL and HDL using spectrophotometer.

4 RESULTS AND DISCUSSION

In the current study, feeding egg yolk to rabbits confirmed the well-known association between hypercholesteramia and animal products in diets. The mean serum levels of total cholesterol increased more than 150% (from 113 to 296 mg/dl), while triacylglycerol increased almost by 38%. Moreover, LDL increased approximately six times compared to the initial value, while HDL showed insignificant change. From a clinical point of view, the reduction in cholesterol status would reduce the incidence of coronary heart disease, and this can be achieved if people eat less eggs. The composition of the ration used in this experiment is presented in Table 1.

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Table 1: The composition of the experimental ration fed to rabbits.

Ingredient	%
Sorghum grains	39
Wheat bran	39
Groundnut cake	20
Molasses	1
Oyster shell	0.25
Salt	0.5
Minerals and multivitamins	0.25
Total	100

Table 2 summarizes the effect of feeding egg yolk on serum lipid levels of rabbit. The result showed that dietary egg yolk significantly ($P < 0.01$) increased serum total cholesterol, triacyglycerol, and LDL. However, HDL was not affected by this treatment. In this study, there was an insignificant effect of supplementation of rabbits with egg yolk on lipid profile. This finding disagreed with that of Omer (2001) showing that dietary *N. sativa* seeds reduce serum total cholesterol. The inconsistency between our finding and the previous ones may be due to the amount of cholesterol consumed, the type of diet and genetic variation between the animals used. In addition to that the duration of the experimental period of this study may have an effect. There is a large body of evidence indicating a direct relationship between high cholesterol and coronary heart disease (Murray *et al*., 1999).

Table 2: The effects of feeding egg yolk on serum lipids of rabbits.

Parameter (mg / dl)	Initial	Post feeding egg yolk
Total Cholesterol	$113.67^{a} \pm 4.92$	$296.13^{b} \pm 21.17$
Triacyglycerol	$94.67^{a} \pm 5.69$	$131.17^{b} \pm 6.35$
Low density lipoprotein (LDL)	$36.98^{a} \pm 5.68$	$213.27^{b} \pm 21.95$
High Density lipoprotein (HDL)	$57.75^{a} \pm 4.49$	$56.75^{a} \pm 3.09$

$^{a, b}$Means on the same raw with different superscript are significantly different at $P < 0.05$.

Table 3 summarizes the effect of dietary *N. sativa* seeds on serum lipid level of rabbit. The dietary *N sativa* seed had no significant effect ($P < 0.05$) on serum total cholesterol, triacylglycerol or LDL. However, there was a numerical increase of LDL, and total cholesterol of those fed on 2.5% *N sativa* seed. On the other hand, serum HDL increased significantly ($P < 0.05$) after feeding on *Na sativa* seeds.

Table 3: The effects of feeding *Nigella sativa* on serum lipid profile of rabbits.

Parameter (mg / dl)	0.00	2.5%
Total Cholesterol	$296.13^{a} \pm 21.2$	$323.25^{a} \pm 27.88$
Triacyglycerol	$131.7^{a} \pm 6.25$	$119.25^{aa} \pm 4.27$
Low density lipoprotein (LDL)	$213.28^{a} \pm 21.95$	$233.68^{a} \pm 29.17$
High Density lipoprotein (HDL)	$56.75^{a} \pm 3.09$	$65.75^{b} \pm 3.47$

^{a,b}Means on the same raw with different superscripts are significantly different at $P < 0.05$.

Coupling the beneficial effects with its use in folk medicine, *N. sativa* seed is a promising source of active ingredients with potential therapeutic application in different clinical settings (Salem, 2005). Thus, our study indicates that petroleum ether extracts of *N. sativa* seeds contain cholesterol-lowering components as previously reported (Le *et al*., 2004).

Dietary *N sativa* seeds appeared to increase high-density lipoprotein (HDL), which is clinically important. Thus, encouraging human consumption of *N sativa* seed as food additives can reduce a major risk factor for heart disease. The present study clearly shows that *N. sativa*

seeds have cholesterol- lowering potential. Our work thus provides strong evidence to support the ethnopharmacological use of *N sativa* seeds to improve lipid metabolism.

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