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## Effect of commercial oil of *Nigella sativa* L. seeds on lipids parameters and weight in sheep

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### Abstract

**Objective:** This study investigated the effects of feeding commercial oil of *Nigella sativa* (*N. sativa*) on the levels of serum various lipid fractions in sheep. Serum samples were collected every two weeks for the determination of total cholesterol, low density lipoprotein (LDL-c), high density lipoprotein (HDL-c), triglycerides concentrations, and the body weights.

**Material & Methods:** Twelve male cross breed sheep were divided into two groups on the basis of their weights. Each cage was supplied with fattening ration prepared as pellets at the rate of one kilogram per day. Barseem and water were supplied ad libitum throughout the day. The sheep were fed the commercial ration for two weeks as an adaptation period and then they were subjected to a feeding programme for 6 weeks as follows: In group B (control group) sheep's received pellets. In group A (treated group) received pellets mixed with 4.7% (47 gram) of *Nigella sativa* oil seeds.

**Results:** *N. sativa* significantly raised cholesterol, LDL-C, HD, and the body weights after 8 weeks. The treated group showed significant with reference to FBG and LDL-cholesterol.

**Conclusions:** Feeding of *Nigella sativa* oil to the diets of sheep, resulted in significant elevation in Serum total cholesterol, LDL-C, HDL-C, triglycerides concentrations, and the body weights.

**Keywords:** *Nigella sativa*, Cholesterol, Lipoprotein, Triglycerides.

### 1. Introduction

*Nigella sativa* (*N. sativa*) is an annual erect herb belongs to the family *Ranunculaceae* buttercup family. It is commonly known as: black seed, Habat Elbaraka and in Sudan it is known as (Black cumin). *N. sativa* is one of the very important medicinal plants, which used for centuries in Middle East as a healer of many complains and disease [1]. In many Arabian, Asian and African countries, its oil used as a natural remedy for a wide range of diseases, including various allergies. The plant's mechanism of action is still largely unknown, due to the lack of study data on its efficiency in allergies [2]. Also, seeds are used for edible and medicinal purposes, in many countries. The average proximate analysis of *N. sativa* seeds on dry matter basis are 216 g/kg crude protein, 406 g/kg fat, 45 g/kg ash, 84 g/kg crude fiber and 249 g/kg nitrogen free extract [3]. The oil content of *N. sativa* seeds from three different regions is 29.4, 29.5 and 29.7. The major fatty acids in *N. sativa* seed oil are linoleic 60%, oleic 22% and palmitic 12% [4].

Previous studies reported the lowering effect of *N. sativa* on the serum and egg lipids and cholesterol in various animals including rabbits, rats and poultry [5-8]. Also the effect of *N. sativa* on body weight was reported by [9]. However, no sufficient information was found available in literature about the effect of *N. sativa* oil on serum lipids in sheep or other ruminants. The objectives of this study were to determine the effect of feeding *N. sativa* oilseeds on the following parameters: 1- Total cholesterol. 2- Low density lipoprotein cholesterol. 3- High density lipoprotein cholesterol. 4- Triglycerides. 5- Body weight.

### 2. Material and Methods

#### 2.1 Animals

The present study was conducted on Twelve males crossbreed sheep, (Hamary and desert sheep) from University Farm and Abu Zeid market during summer from April to May. The age groups of these sheep were varied from 3 to 6 months of age. The animals were individually housed in 1 x 1.5 m separate cages in the same environmental conditions. The cage sides were of a wire mesh set over a meter brick side wall. Before the insertion of the animals, they got a bath with cypermethrin as antiticks and antimanges in a dilution of 1 ml per liter of water (produced by Veterinary and Agricultural products Mfg. Co. Ltd, Jordan), and the cages were cleaned manually, disinfected with cypermethrin in dilution of 2 ml per

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liter of water. After that the animals were treated with ivermectin as antiparasitic (intermectin 1%, Holland) at a dose of 0.5 ml s/c, oxytetracyclin as antibacterial (Rasomycin-5, star laboratories (PVT) Ltd, Pakistan) at a dose rate of 1 ml per 10 kg 1/M for 3 days and multivitamins (Ultravit M, Avico, Jordan) at gram per liter of drinking water for 5 days. Then the animals were divided into two groups on the basis of their weights with  $\pm 113$  kg for each group. The sheep were fed the commercial ration for two weeks as an adaptation period and then they were subjected to a feeding programme for 6 weeks as follows: group (B) received Kenana feed as control group, and group (A) received Kenana feed mixed with 4.7% (47 gram) of *N. sativa* oil seeds as treated group. The percentage of *N. sativa* oil is determined by the difference between the highest levels of fats in ruminants' diet (7%)<sup>[10]</sup>; and the crude fat percentage in Kenana feed (2.3%).

## 2.2. *N. sativa* seeds

*Nigella sativa* seed oil was obtained from commercial sources in the local market. Before production of the oil, the viability of seeds was confirmed by germination test according to International seed testing agency<sup>[11]</sup>. The seeds were tested for germination rate and purity. The germination rate must be at least 85% in order for the seed to be certified by the agency. Hundred seeds selected randomly and distributed into 4 Petri-dishes (25 seeds in each), containing filter papers moisten with distilled water. The dishes were incubated at 20 °C for 7 days. On the 4<sup>th</sup> day, the normal seedling that developed was counted. At the end of the 7<sup>th</sup> day, the final count was done as follows: normal seedling, abnormal seedling and dead seedling. All the four sets of germinated seeds should have a germination rate of at least 85%. A germination rate of 85% is 85 normal seedlings for all sets of 100 seeds.

### 2.2.1 Feeding programme

Each cage was supplied with fattening ration prepared as pellets (Product of Kenana Sugar Company Ltd) at the rate of one kilogram per day. Barseem and water were supplied *ad libitum* throughout the day. The components of Kenana ratio are: sorghum, molasses, wheat bran, groundnut cake, bagasse, calcium salts, urea and sodium chloride. The proximate analysis of feed pellets is including 87.6 dry matters (on dry matter basis), 17.5 Crude proteins, 11.9 Crude fibers, 2.3 Crude fats, 11.2 Ash and 10.5 metabolizable energy (Metabolizable energy per mega joule per kg dry mater MJ/Kg DM).

### 2.3 Blood sample collection

The first blood samples were collected as zero levels before the application of *N. sativa* oil, and then the other blood samples were taken every two weeks for the rest of the experimental period. Fasting blood samples were taken in the morning before feeding as (5 ml of blood) from the jugular vein of each sheep into clean glass tubes. The blood was allowed to clot at 4°C for overnight, and then centrifuged at 4000 rpm for 5 minutes; clear serum was then separated and stored at -20 °C till used for biochemical analysis.

### 2.4 Statistical Analysis

Statistical software known as SPSS has been used to get the means and standard errors by utilizing the one sample t-test. The experimental design analysis and the mean separation were obtained using another computer package named SAS, (1982), software utilizing completely randomized design analysis and Duncan's multiple range lists for mean separation.

Pre and post intervention mean  $\pm$  standard deviation of each parameter was calculated for both groups. Paired T-test was applied to know the intergroup difference of each variable before and after intervention. Then unpaired t test was applied to know about intergroup difference between both groups. P-values < 0.05 were considered statistically significant.

## 3. Results

### 3.1 Serum Total cholesterol

The effect of feeding the commercial oil of *N. sativa* seed on serum cholesterol concentration is represented in Table (1). At time zero, there was no significant difference between the two groups, though group B showed apparently higher value compared to group A. After feeding *N. sativa* oil to the treated group (A), also no significant differences were observed between the two groups until the end of the experiment. At week 2, group A showed a significant (P<0.05) elevation in the cholesterol level compared to time zero, while group B showed slightly lower value in the cholesterol level compared to the level of time zero. A none significant reduction was also observed at week 4 in group B compared to week 2 while in group A the reduction was significant (P<0.05) compared to week 2. At the end of week 6, group A showed a significant (P<0.05) higher total cholesterol level compared to time zero, and group B showed a non-significantly lower value compared to time zero. There was no significant difference between the total means, but the treated group showed slightly higher total mean compared to the control group.

### 3.2 Serum low density lipoprotein cholesterol (LDL-c)

The effect of feeding *N. sativa* on serum LDL-c is represented in Table (1). There was no significant difference between two groups at time zero, though group B showed higher value compared to the group A. At week 2, group A showed a significantly (P<0.05) higher value compared to time zero, while group B showed a no significant decrease value. At week 4 the serum LDL-c level in group A was none significantly decrease to near the level of time zero, while group B showed a significant (P<0.05) lower levels it week 4 and week 6 compared to the level of time zero. At the end of experiment, group A showed none-significantly higher LDL-c level compared to time zero, but significantly (P<0.05) higher value compared to group B. No significant difference between the total means, but the treated group showed higher total mean compared to the control group.

### 3.3 Serum high density lipoprotein-cholesterol (HDL-c)

The result of feeding *N. sativa* seeds oil on serum HDL-c is represented in Table (1). At time zero there was no significant difference between the treated group and the control group, but the control group showed slightly higher value compared to the treated group. Then the two groups showed nearly the same gradual increase in the HDL-c level until the end of the experiment. In group A there were significant (P<0.05) differences in the rest of weeks when compared to time zero, but it week 2 group A showed a significant (P<0.05) increase compared to group B. Group B showed significant (P<0.05) differences it week4 and 6 compared to time zero. The total mean values showed no significant difference, but the total mean in group A was very slightly higher compared to group B.

### 3.4 Serum Triglycerides

The result of feeding *N. sativa* seed oil on serum triglycerides

level is represented in Table (1). At time zero there was no significant difference between the two groups, but group B showed apparently higher value in TG level compared to group A. After feeding *N. sativa* oil for two weeks to group A, no significant difference was observed compared to time zero, while group B showed a significant decrease in T.G level. At week 4, there was a non-significant increase in the two groups, but at week 6 the T.G level in group A was significantly ( $P < 0.05$ ) higher compared to time zero, while in group B the T.G level increased to the same first level but this was not significantly. There was no significant difference between the

total means, but group (A) showed slightly higher total mean compared to group (B).

### 3.5 Body weights

The effect of feeding *N. sativa* seed oil on the body weights is represented in Table (2). There was no significant difference between the two groups before and after the feeding of cumin seed oil. This was also seen in the control group, but the treated group showed a significantly ( $P < 0.05$ ) higher weights at the end of the experiment compared to time zero. The total means showed nearly the same value, but was not significantly.

**Table 1:** The effect of feeding commercial oil of *N. sativa* on lipids parameters in sheep. Means  $\pm$  SE (N = 6):

Parameters	Groups									
	Group A (Treated group fed <i>N. sativa</i> )					Group B (Control group)				
	Time 0	2Wks	4 Wks	6Wks	T. mean	Time 0	2Wks	4Wk	6wks	T. mean
<b>T.C (mg/dl)</b>	61.81 <sup>Ca</sup> $\pm$ (5.01)	99.99 <sup>Aa</sup> $\pm$ (5.26)	77.68 <sup>Ca</sup> $\pm$ (10.19)	93.9 <sup>Aa</sup> $\pm$ (3.64)	83.35 <sup>a</sup> (8.58)	90.8 <sup>Aa</sup> $\pm$ (12.48)	82.29 <sup>Aa</sup> $\pm$ (11.48)	65.39 <sup>Aa</sup> $\pm$ (8.65)	87.69 <sup>Aa</sup> $\pm$ (6.36)	81.54 <sup>a</sup> (9.74)
<b>LDL-c</b>	43.61 <sup>Ba</sup> $\pm$ (5.58)	69.13 <sup>Aa</sup> $\pm$ (6.43)	45.82 <sup>Ba</sup> $\pm$ (7.06)	54.72 <sup>Ba</sup> $\pm$ (4.21)	53.32 <sup>a</sup> (5.79)	72.87 <sup>Aa</sup> $\pm$ (12.08)	54.66 <sup>Aa</sup> $\pm$ (10.45)	40.4 <sup>Ba</sup> $\pm$ (7.91)	33.79 <sup>Bb</sup> $\pm$ (7.98)	50.43 <sup>a</sup> (8.66)
<b>HDL-c</b>	14.76 <sup>Ba</sup> $\pm$ (1.56)	27.61 <sup>Aa</sup> $\pm$ (1.32)	27.9 <sup>Aa</sup> $\pm$ (1.70)	30.08 <sup>Aa</sup> $\pm$ (2.05)	25.10 <sup>a</sup> (3.49)	18.17 <sup>Aa</sup> $\pm$ (0.76)	22.31 <sup>Aa</sup> $\pm$ (1.70)	25.25 <sup>Ba</sup> $\pm$ (1.05)	31.14 <sup>Ba</sup> $\pm$ (2.65)	24.22 <sup>a</sup> (2.73)
<b>TG</b>	14.05 <sup>Ba</sup> $\pm$ (1.85)	12.34 <sup>Ba</sup> $\pm$ (4.59)	22.73 <sup>Ba</sup> $\pm$ (3.33)	41.58 <sup>Aa</sup> $\pm$ (14.66)	22.67 <sup>a</sup> (6.70)	27.48 <sup>Aa</sup> $\pm$ (6.97)	9.37 <sup>Ba</sup> $\pm$ (1.54)	16.76 <sup>Aa</sup> $\pm$ (2.33)	27.04 <sup>Aa</sup> $\pm$ (2.89)	20.16 <sup>a</sup> (4.37)

Serum total cholesterol (T.C), Serum low density lipoprotein cholesterol (LDL-c), Serum high density lipoprotein-cholesterol (HDL-c) and Serum Triglycerides (TG). Means in the same row having different capital letters are significantly different at ( $P < 0.05$ ). Means in the same column followed by different small letters are significant different at ( $P < 0.05$ ). A= control group. B= treated group fed *N. sativa* seeds oil. Means  $\pm$  SE. (N = 6).

**Table 2:** The effect of feeding commercial oil of *N. sativa* on body weights (kg) of sheep. Means  $\pm$  SE (N = 6):

Group	Time zero	Week 6	Total mean
<b>A</b>	19.00 <sup>Ba</sup> $\pm$ (1.77)	24.75 <sup>Aa</sup> $\pm$ (0.89)	21.88 <sup>a</sup> (2.88)
<b>B</b>	18.83 <sup>Aa</sup> $\pm$ (2.06)	23.67 <sup>Aa</sup> $\pm$ (1.83)	21.25 <sup>a</sup> (2.42)

Means in the same row having different capital letters are significantly different at ( $P < 0.05$ ).

Means in the same column followed by different small letters are significant different at ( $P < 0.05$ ).

A = control group. B = treated group fed *N. sativa* seeds oil.

## 4. Discussion

### 4.1 Serum total cholesterol

Cholesterol is an amphipathic lipid present in tissues and plasma. Cholesterol levels play a central role in the genesis of atherosclerosis and coronary heart disease [12, 13]. Previous findings showed that *N. sativa* has promising effect resembling to those drugs that reduce serum cholesterol and decrease its atherogenic pathological effect [5, 14]. In the current study the level of serum cholesterol showed fluctuating manner. Group A showed significant ( $P < 0.05$ ) increase compared to time zero after two weeks, and then significantly ( $P < 0.05$ ) reduced it week 4 and significant ( $P < 0.05$ ) increased at week 6. Control group B showed slight decline it week 2 and week 4, then slight increase at week 6. This finding was in line with another report which suggested that, feeding 2.5% *N. sativa* with 15 g

egg yolk (as a source of cholesterol) to rabbits showed no significant effect on serum total cholesterol and LDL-C [15]. But it in contrast to another study, when the authors investigated the effect of fixed oil of *N. sativa* seeds in rats, on serum cholesterol, triglycerides, glucose, and the body weight and reported that serum cholesterol decreased significantly in rats [9], and these results was also agreed and supported with the same results found in other report [16]. Also, an earlier study was suggested that, the *N. sativa* seed oil might improve hyperlipidemic nephropathy in rats [17]. From this study, one can conclude that, feeding 4.7% of *N. sativa* seed oil with sheep, result in a significant ( $P < 0.05$ ) elevation in the serum cholesterol level compared to time zero, and non-significant elevation compared to control group in contrast to findings in monogastric animals.

#### 4.2 Serum LDL-c cholesterol

LDL-c is one of the four major groups of lipoproteins which are important physiologically and in clinical diagnosis. The LDL-c transports cholesterol from liver to the peripheral tissues, it is called (Bad cholesterol) and its concentration in blood has positive correlation with incidence of cardiovascular diseases [18]. In this study the levels of LDL-c followed the same fluctuating manner of the serum total cholesterol levels in the treated group, which lead to a significant ( $P < 0.05$ ) difference at week 2 from time zero, and at week 6 from the control group. While in the control group B there were significant decreases at week 4 and week 6. This result showed that, the elevation of serum LDL cholesterol concentration has a positive correlation with the elevation of serum total cholesterol concentration this suggest that, the increase of serum total cholesterol, is due to increase of the serum LDL cholesterol fraction. LDL-C levels decreased gradually in the control group and reached significantly ( $P < 0.05$ ) lower levels at week 4 and 6 compared to time zero. This finding showed that feeding *N. sativa* oil influenced the lowering effect of the ration to the LDL-C in the control group. This result does not agree with [15] who reported that there was no significant effect on serum LDL-C when 2.5% of *N. sativa* and 15g boiled egg yolk were fed to rabbits. And it is in contrast to the finding reported by [6] who found that, the administration of seed oil to rats orally, caused significant lowering in serum LDL cholesterol.

#### 4.3 Serum HDL-c cholesterol

HDL-c is the main transport form of cholesterol from peripheral tissue to liver which is later excreted through bile. The level of HDL in serum is inversely related to the incidence of myocardial infarction. As it is (anti atherogenic), HDL-c is known as (good cholesterol) [18]. In the present work, the effect of *N. sativa* oil caused a significant increase in HDL-c cholesterol from time zero until the end of experiment in group A, and there was a significant ( $P < 0.05$ ) difference between the two groups at week 2. In the last week group A and B showed similar levels of HDL cholesterol. Diets containing monounsaturated fatty acids (like olive oil) have been known to increase serum HDL-c and decrease LDL-c levels [19], this effect was observed by many authors previously, in monogastric experimental animals fed *N. sativa*. [6] cited that oral administration of seed oil to rats orally resulted in significant elevation in serum HDL cholesterol; cited the same result [16]. In the present work *N. sativa* oil, when fed to sheep, increased significantly ( $P < 0.05$ ) both the LDL-c and the HDL-c this could be due to the biohydrogenation of the unsaturated fatty acids in the rumen together with a possible effect of the rumen atmosphere to the thymoquinone, the active ingredient of the *N. sativa*.

#### 4.4 Serum triglycerides (TG)

Triglyceride are the major form of lipid in the animal body, and has a role in lipid transport and storage body fat, and in many diseases such as obesity and diabetes mellitus, but it is lesser correlated with coronary heart disease than cholesterol [13]. In the present study, feeding of *N. sativa* oil resulted in obvious increase in serum TG level in the treated group, which was significantly ( $P < 0.05$ ) higher compared to time zero at the end of experiment, While in control group there were significant decreases at week 2, then increase again to near the first level of TG. Mustafa AE [15] reported that there was no significant effect on serum T.G. when 2.5% of *N. sativa* and 15

g boiled egg yolk were fed to rabbits. Furthermore, another study found that, the addition of *N. sativa* seeds with 0.25 and 0.75% resulted in significant decrease in the concentration of triglycerols [20]. Also, there was report cited that feeding 1 and 3 % of *N. sativa* seeds to laying hens resulted in significant reduction in serum triglycerols [8]. Whoever, another report suggested that, the oral administration of seeds oil to rats caused significant reduction in serum TG [6]. From this result, it is clear that the dietary *N. sativa* oil does not reduce serum TG level in sheep as in monogastric animals, but has an effect of increasing it significantly. Indeed, it was found that the fractionate the neutral lipid (NL) classes of *N. sativa* seed oil, TG were the major NL class (80.8 – 83.1) of the total NLs. This could be the reason of this elevation of serum T.G level in sheep, which passed as saturated lipids from the rumen to the lower intestine [21].

#### 4.5 Body weight (BW)

There was no significant difference in the body weight between the two groups at the beginning of the experiment, but group A the treated group showed significant ( $P < 0.05$ ) increase in body weight at the end of experimental period compared to time zero. This could be explained as a result of the increase in all serum lipids studied total cholesterol, TG, LDL-c, and HDL-c. These results disagree with previous study, when authors reported that the effect of fixed oil of *N. sativa* in rats resulted in a significant decrease in the body weight [9]. In the present work it was observed that, feed intake was increased in the treated group, this suggest that the commercial oil of *N. sativa* has a slight appetizer effect. In addition to that, the effect of dietary *N. sativa* oil, lead to increase the levels of serum cholesterol, LDL-c, HDL-c, triglycerides, and the body weights. This can be explained as that, the rumen atmosphere may destroy the active constituents of *N. sativa* oil, specially thymoquinone which is considered as active ingredient responsible for the pharmaceutical interest of the plant [22]. Also, previous study had been reported that, the high ratio of polyunsaturated fatty acid in the diet is a major lowering plasma cholesterol concentration by dietary means and hence *N. sativa* seeds contain unsaturated fatty acid it may be the cause of reduction of serum cholesterol concentration in monogastric animals [13]. But in the ruminant animals although they consume a diet that predominantly contains polyunsaturated fatty acids (PUFA) as part of plant triglycerols and glycolipids, bacteria in the rumen split off the FA (and sugar) from the glycerol backbone (Hydrolysis process), and the resulting free fatty acids are acted upon by microbial enzymes which convert them ultimately into saturated FA (stearic acid) (Biohydrogenation Process) [23]. This suggested that, supplementation of *N. sativa* oil, to ruminants' diet, act as additional source of PUFA which later will be converted to saturated FA, passes to the small intestine and absorbed.

#### 5. Conclusion

Feeding of 4.7% of *N. sativa* oil to the diets of sheep, resulted in significant ( $P < 0.05$ ) elevation in Serum total cholesterol, LDL-C, HDL-C, triglycerides concentrations, and the body weights. These results in contrast to previous findings in the monogastric animals, which reported a lowering effect of *N. sativa* on the serum total cholesterol, low density lipoprotein cholesterol, triglycerides concentration with an increase in the high density lipoprotein cholesterol levels. Also, different effects were observed for the body weights.

## 6. Recommendations

Future studies are recommended to: investigate about the fate of thymoquinone in the rumen. To confirm and identify the presence of appetizer component in the commercial oil of *N. sativa*; Although, to confirm the presence of the intermediate compounds (conjugated linoleic acid) (CLA) in the body fat which having potent anti-cancer effect specially after the elevation in the serum lipids observed after feeding *N. sativa* oil of sheep in this work, since *N. sativa* oil is known to contain their origins (linoleic and linoleinic acid).

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