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Original Research Article

Anti-Atherosclerotic Effect of *Nigella sativa L.* in High-Fat diet Fed Wistar Rats

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ABSTRACT

Black cumin (*Nigella sativa* L.) is thought to have an anti-atherosclerotic effect, including the potential to prevent foam cell formation, a key factor in atherosclerosis and cardiovascular disease. This study evaluated the impact of black cumin extract on body weight and foam cell formation in Wistar rats fed a high-fat diet. A post-test-only control group design was used, with five groups: a high-fat diet group with 500 mg/day of black cumin extract (P1), a high-fat diet group with 40 mg/day of atorvastatin (P2), a high-fat diet group with both atorvastatin (40 mg/day) and black cumin (500 mg/day) (P3), a normal diet group (N), and a high-fat diet group (P). Rats' body weights were measured weekly, and at the study's end, euthanasia was performed using xylazine and ketamine injection, followed by cervical dislocation, to collect aortic samples, which were stained with Mason Trichrome and examined for foam cells. The results showed average body weights of 286.6 ± 6.54 g (P), 264.8 ± 7.57 g (N), 233.6 ± 11.05 g (P2), 229.6 ± 7.50 g (P1), and 188.8 ± 11.00 g (P3). The average number of foam cells per fields was 6.6 ± 1.03 (N), 19.4 ± 1.34 (P), 7.08 ± 0.98 (P1), 10.2 ± 2.32 (P2), and 11.28 ± 3.57 (P3). The highest number of foam cells was found in the high-fat diet group (P), significantly higher than in other groups. In conclusion, black cumin reduced both body weight and foam cell formation, showing effects similar to atorvastatin.

Keywords: Atherosclerosis, *Nigella sativa*; Atorvastatin; Body Weight; Foam Cells; High-Fat Diet; Rat

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality globally, claiming 17.9 million lives annually and accounting for 32% of all deaths. Among the various types of CVD, coronary artery disease (CAD) has been reported to have significant rates of morbidity and mortality.[1] CAD is primarily caused by atherosclerosis, a chronic, progressive artery condition marked by plaque rupture, calcification, fat accumulation, inflammatory cell infiltration, and intima damage.[2] In addition, atherosclerosis causes changes in blood vessel morphology, leading to the development of atherosclerotic lesions. These lesions are characterized by smooth muscle proliferation, the formation of foam cells, the presence of fat and macrophage infiltration, thickening of the intima and media, and infiltration of inflammatory cells.[3] Several studies have shown that foam cells play an essential role in the pathophysiology of atherosclerosis. In addition, the formation and accumulation of cells in the subendothelial region of a damaged artery are key drivers in the progression of atherosclerosis.[4]

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Histological assay have been reported to be the major method for detecting structural alterations at this stage because it might occur without presenting any symptoms.[5] One promising strategy for managing atherosclerosis is to target the production of foam cells in the atherosclerotic plaques. Lipid uptake, cholesterol esterification, and cholesterol export are the three main interconnected biological processes that cause the production of foam cells. This indicates that by inhibiting lipid uptake, cholesterol esterification, and increasing cholesterol ester hydrolysis and cholesterol efflux, a variety of natural substances and pharmaceutical medicines can prevent the development of foam cells and exhibit antiatherosclerotic properties.[6] One of the substances known to have the potential to improve metabolic conditions and inhibit foam cells formation is black cumin.[7]
Black cumin is a popular therapeutic herb worldwide and is one of 14 species in the genus *Nigella* belonging to the Ranunculaceae family. From the Mediterranean valley to Iran, Pakistan, India, North Africa, and South Asia, it flourished in both southern and eastern nations. Several countries had different names for this plant, including siyah daneh (Persian), kezah (Hebrew), chamushka (Russian), kalonji (Indian), black cumin (England), and black seed (English and Arabic).[8] The plant is used extensively as an analgesic, diuretic, antimicrobial, antihypertensive, and anti-diarrhea. Thymoquinone (30 to 48%), thymohydroquinone, dithymoquinone, p-cymene (7 to 15%), carvacrol (6 to 12%), 4-terpineol (2 to 7%), t-anethol (1 to 4%), sesquiterpene longifolene (1 to 8%), α-pinene, and thymol were among the several active components of black cumin that were present. In addition, this plant had saponins, pentacyclic triterpenes, and α-hederin. Calcium, potassium, phosphorus, magnesium, sodium, copper, zinc, and iron were abundant in the seeds.[9–12] According to previous studies, black cumin offered pharmacological advantages that needed more study. These advantages included hepatoprotective, nephroprotective,

immunomodulatory, antidiabetic, anticancer, analgesic, antibacterial, anti-inflammatory, spasmolytic, bronchodilator, and antioxidant qualities. In addition, black cumin was one of the natural remedies with scientific backing because of its therapeutic properties.[7,13–15] This study aims to investigate anti-atherosclerotic effect of black cumin, by measuring body weight and the number of foam cells after the administration of black cumin in Wistar rats.

Material and Methods

Animals

This study was conducted for 8 months at the Experimental Animal Laboratory of the Faculty of Veterinary Medicine at Syiah Kuala University. This study was ethically approved by the Medical Research Ethics Commission of the Faculty of Veterinary Medicine, Syiah Kuala University, and registered with number 243/KEPH/VII/2023 on July 31, 2023. The ARRIVE guidelines were utilised in the reporting of this animal study. The Federer formula was used to determine the treatment sample size.

Male Wistar rats (n=30), weighing between 50 and 100 grams at 4 weeks of age and in good health, was used. The rats were purchased from the University, cared for appropriately, maintained at a steady temperature of $\pm$24°C, and allowed unrestricted access to food and beverages. A high-fat diet included 92.8% ordinary food, 0.2% cholic acid, 2% egg yolk, 5% goat fat, and vitamin D_3. This feed was created by combining 1000 g rat food in the form of maize rice with 100 g goat fat and 50 g egg yolk. After melting goat fat and extracting the yolk from boiled eggs, 1000 g corn rice was combined with the goat fat and egg yolk. Rats were fed 20 mg each day.[16] All rats were fed a portion of regular food and given a week to get used to their new environment. A total of 6 rats were given a regular diet for the first stage (weeks 2 through 5), while 24 rats were randomly assigned to a high-fat diet. In this study, every group was placed in a different cage. At the second stage (6th to 9th week), 24 rats that were fed a high-fat diet were split into 4 groups, while 6 rats remained on the standard diet as a negative control (N). The study groups were as follows: the Negative control (N) group consisted of normal rats fed a regular diet (n=6); the Positive control (P) group was composed of rats on a high-fat diet (n=6); the P1 group included rats on a high-fat diet and administered 500 mg/day of black cumin extract (n=6); the P2 group consisted of rats on a high-fat diet and given 40 mg/day of atorvastatin (n=6); and the P3 group was composed of rats on a high-fat diet and administered both 40 mg/day of atorvastatin and 500 mg/day of black cumin extract (n=6).

Rats were sacrificed at the end of the ninth week by and intraperitoneal injections of 5-10 mg/kg xylazine (Xyla; Interchemie, Netherlands) and 15-20 mg/kg ketamine (Ilium ketamil; Troy Laboratories, Australia), followed by cervical dislocation. Aortic preparations were obtained for Masson Trichrome staining, and then the number of foam cells was counted. The Institutional Animal Care and Use Committee's Animal Research on *in vivo* Experiments (ARRIVE) Guidelines were cited in the preclinical study protocol.

Black Cumin Collection, Identification, and Extraction

Nigella sativa seed was obtained from Surakarta, Jawa Tengah, Indonesia, and produced by Javaplant Factory (-7.628995, 111.091874) in 2023. Ulfah, Maria and Kartini (PT. Tri Rahardja, Indonesia) identified the plant. To obtain 1 kg of *Nigella sativa* extract, 4 kg of *Nigella sativa* seeds were used which were macerated using ethanol solvent for 3 different 24-hour extractions and a water solvent for a 24-hour extraction. After that, the filtrate was evaporated using a rotary evaporator to obtain viscous extract (ethanol extract). The end extract form was crude oil with residual ethanol solvent NMT 5000 ppm. This product is registered with the number 2065J91N, production number: JP 701.02.00, and batch number: 03231501A/Lot A.

The antioxidant potential of *Nigella sativa* oil, as measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)) methods, is indicated by the colour change of DPPH free radicals from purple to yellow and the colour change of ABTS free radicals from greenish-blue to colorless. The antioxidant strength was determined by the IC_{50} value using a UV-Vis spectrophotometer. An IC_{50} value of less than 50 ppm is classified as a very strong antioxidant, an IC_{50} value of 50–100 ppm is classified as a strong antioxidant, an IC_{50} value of 100–150 ppm is classified as a moderate antioxidant, and an IC_{50} value of 151–200 ppm is classified as a weak antioxidant.[17–19]

Body Weight and Number of Foam Cells Measurement

To monitor weight increase, body weight was measured every week. Preparations from aorta were taken and cut into tissue pieces with the following stages (a) Fixation using neutral buffer formalin solution, (b) Dehydration using graded percentage of alcohol, (c) Clarification using xylol solution, (d) Embedding using paraffin, (e) Blocking using an iron mold, and (f) Cutting using a microtome. After the preparations were processed, Masson Trichrome was used as a strainer, and histopathological examination was carried out using a Meiji brand microscope. Foam cell calculations were conducted using the Image J application.[20]

Statistical Analysis

The Shapiro-Wilk test was used to determine the normality of the data, and one-way ANOVA test (α=0.05) was used to evaluate the differences in body weight and foam cell counts across groups. SPSS Statistics version 26 for Windows was applied to conduct this statistical test. To identify significant changes across groups, Duncan Test was used to do post hoc testing (α=0.05).

Results and Discussion

Phytochemical Analysis

The results of the phytochemical study indicated that the extract from black cumin contained flavonoids, tannins, saponins, triterpenoids, and alkaloid, but no steroids, quinones, or polyphenols were detected. The IC_{50} value of *Nigella sativa* oil, tested using the DPPH method, was 3.76 ppm, classifying it as a very strong antioxidant.

Body Weight

Body weight was measured at the beginning of the trial (week 0), at the beginning of the treatment (week 5), and at weekly intervals during the course of the treatment (weeks 6, 7, 8, and 9). The groups that had regular feed (N) and a high-fat diet (P, P1, P2, P3) exhibited significantly different body weights at the end of the fifth week. At the end of the study, the average body weights in order from the highest was the P (286.6 ± 6.54 g), N (264.8 ± 7.57 g), P2 (233.6 ± 11.05 g), P1 (229.6 ± 7.50 g), and P3 (88.8 ± 11.00 g), as shown in Table 1 and 2.

Number of Foam Cells

The number of foam cells was counted using a microscope at 400x magnification and 5 fields of view for each sample. In addition, average number of foam cells after treatment was 6.6 ± 1.03 foam cells per field (N), 19.4 ± 1.34 foam cells per field (P), 7.08 ± 0.98 foam cells per field (P1), 10.2 ± 2.32 foam cells per field (P2), and 11.28 ± 3.57 foam cells per field (P3). The number of foam cells varied significantly between groups, as indicated by the P value of 0.002. The results of post hoc with Duncan Test showed that the number of foam cells was highest in the positive control group (P) and was significantly different from the other 4 groups. The negative control group was the group with the lowest number of foam cells (Figure 1, Table 3).

Administration of Black Cumin Extract and Body Weight

The results of this study were consistent with several previous studies which stated that giving black cumin could prevent weight gain. According to previous studies on animals, black cumin could reduce body weight by enhancing energy expenditure and decreasing appetite.[21] However, the mechanisms underlying the effect on anthropometric parameters and weight control are conflicting. While some studies found that black cumin reduced body weight,[22,23] others did not find that it had any beneficial benefits on body weight.[24] In

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comparison to a placebo, Al Asoom found that a daily intervention of 2 g of black cumin powder for 6 to 12 weeks could significantly reduce body weight and other measures of anthropometry.[25] Badar and associates, however, discovered that supplementing with black cumin at a dose of 2 g/day did not considerably raise body mass index.[26]

Black cumin could reduce body weight and this was linked to several mechanisms such as its effect on appetite suppression,[27] metabolic effects,[28] anti-inflammatory properties,[29] improving insulin sensitivity,[30] and enhanced fat oxidation.[31] Treatment with black cumin led to reduced body weight and food intake, while not affecting water intake. This weight loss could be linked to appetite suppression, potentially involving neuronal circuits that regulated catecholaminergic, serotonergic, and peptidergic systems.[27] The effect was also mediated by leptin, signaling in the brain's satiety center, contributing to reduced food consumption.[32] In addition, previous studies indicated that black cumin consumption could increase ghrelin levels, a peptide involved in appetite regulation.[33] Black cumin also enhanced lipid metabolism, leading to reduced fat accumulation. This was shown to lower levels of low-density lipoprotein (LDL) cholesterol and triglycerides, which could contribute to weight loss.[28] The anti-inflammatory effects of black cumin mitigated chronic inflammation, promoting healthier metabolism and weight management.[29] Previous studies also suggested that black cumin could improve insulin sensitivity, which aids in better blood sugar control and weight management.[30] In addition, it could also promote fat oxidation, leading to greater energy expenditure and weight loss.[31] Overall, these results suggested that black cumin reduced body weight and prevented high-fat diet induced increase in body weight or dyslipidemia, indicating its potential use in managing dyslipidemia.

Table 1: Body weight measurement at the end of stage I (g)

Groups	Mean ± SEM	SD	95% CI	P value
Negative control [a]	198.8 ± 7.57	16.93	177.7 – 219.8	0.013
Positive control [b]	245.4 ± 6.54	14.19	227.8 – 263.0	
P1 (*Nigella sativa*) [b]	231.8 ± 7.50	16.78	210.9 – 252.6	
P2 (Atorvastatin) [b]	236.2 ± 11.05	24.73	205.5 – 266.9	
P3 (*Nigella sativa* and Atorvastatin) [b]	238.4 ± 11.00	24.58	207.9 – 268.9	

Different superscripts indicated significant differences.

Table 2: Body weight measurement during the study (g)

	Weeks					
Groups	0	5	6	7	8	9
Negative control	65.0 ± 1.64	198.8 ± 7.57	214.6 ± 8.09	226.2 ± 12.52	248.6 ± 14.04	264.8 ± 13.93
Positive control	63.6 ± 1.03	245.4 ± 6.35	262.6 ± 8.68	261.0 ± 10.34	276.4 ± 14.16	286.6 ± 12.31
P1 (*Nigella sativa*)	64.6 ± 1.5	231.8 ± 7.51	219.2 ± 9.44	216.0 ± 10.67	218.2 ± 8.42	229.6 ± 10.53
P2 (Atorvastatin)	66.2 ± 1.02	236.2 ± 11.06	233.6 ± 18.77	224.4 ± 14.68	229.8 ± 18.95	233.6 ± 14.47
P3 (*Nigella sativa* and Atorvastatin)	64.2 ± 1.02	238.4 ± 10.99	203.8 ± 13.63	196.2 ± 12.4	195.2 ± 11.28	188.8 ± 10.45

Different superscripts indicated significant differences.

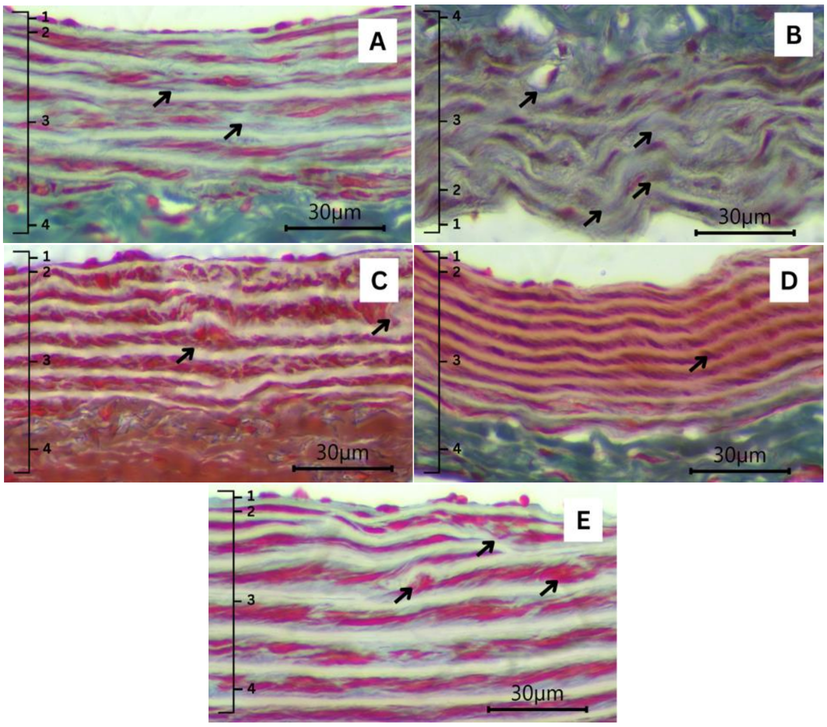

Figure 1: Foam cell images in the 5 study groups (black arrows). (A) Normal regular diet control group. (B) High-fat diet control group. (C) High-fat diet group with 500 mg/day of black cumin extract. (D) High-fat diet group with 40 mg/day of atorvastatin. (E) High-fat diet group with 40 mg/day of atorvastatin and 500 mg/day of black cumin. The images of bleeding in the tunica intima of each group are visible. Foam cells were more common in group B than in the other groups. 1: Aortic lumen, 2: Tunica intima, 3: Tunica media, 4: Tunica adventitia (Masson Trichrome; 40x10).

Administration of Black Cumin Extract and Number of Foam Cells

The results indicated that the highest number of foam cells was in control group on a high-fat diet (P) and significantly different from the other 4 groups: control group with a regular diet (N); high-fat diet group administered 500 mg/day of black cumin extract (P1); high-fat diet group administered 40 mg/day of atorvastatin (P2), and high-fat diet group administered 40 mg/day of atorvastatin and 500 mg/day of black cumin extract (P3). Control group with a regular diet (N) was the group with the lowest number of foam cells. This study provided additional evidence that black cumin had a role in slowing the development of atherosclerosis, by inhibiting the number of foam cells and reducing the integrity of the plaques. Foam cell formation is one of the important processes in the development and origin of atherosclerosic plaques. The formation of fatty streaks detected on the walls of blood vessels is due to the formation of foam cells and the development of atherosclerosis. In addition, foam cells are involved in the formation of primary atherosclerotic plaques which could be disrupted resulting in ischemic conditions.[4]

Targeting the formation of foam cell in atherosclerotic lesions is a potential approach to treating and prevent atherosclerosis. This process is determined by the balanced effects of interrelated biological processes, namely lipid uptake, cholesterol esterification, macrophage proliferation, and cholesterol efflux. Natural products, such as black cumin, were one of the potential sources to be the main agents in inhibiting foam cell formation and therefore exhibiting antiatherosclerotic effect by suppressing lipid uptake, cholesterol esterification, or enhancing cholesterol ester hydrolysis as well as cholesterol efflux.[6] Black cumin could lower levels of low density lipoproteins (LDL) cholesterol and triglycerides, potentially reducing the substrate available for foam cell formation.[34,35] Previous studies suggested that black cumin could improve the ability of macrophages to process lipids without becoming foam cells.[36] Incorporating black cumin into the diet may offer protective effects against cardiovascular diseases by influencing the mechanisms that lead to foam cell formation. However, more study was needed to fully understand its mechanisms and therapeutic potential.

Table 3: Number of Foam Cells (per fields)

Groups	Mean ± SEM	SD	95% CI	P value
Negative control [a]	6.6 ± 1.03	2.3	3.64 – 9.56	0.002
Positive control [b]	19.4 ± 1.34	3.0	15.67 – 23.13	
P1 (*Nigella sativa*) [a]	7.08 ± 0.98	2.2	4.33 – 9.83	
P2 (Atorvastatin) [a]	10.2 ± 2.32	5.2	3.71 – 16.69	
P3 (*Nigella sativa* and Atorvastatin) [a]	11.28 ± 3.57	8.0	1.35 – 21.21	

Different superscripts indicated significant differences

Conclusion

In conclusion, black cumin indicated potential to reduce both body weight and the foam cells formation in high-fat diet fed rats, thereby reducing the risk of atherosclerosis and associated cardiovascular diseases. These effects emphasized the potential of black cumin as a beneficial agent in managing dyslipidemias. Further studies are needed to fully elucidate its mechanisms and optimize its therapeutic applications.

Conflict of Interest

The authors declare that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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