EFFECT OF BLACK CUMIN (NIGELLA SATIVA) POWDER ON SERUM LIPID PROFILE, MALONDIALDEHYDES, NITRITES, sICAM-1 AND sVCAM-1 IN EXPERIMENTALLY INDUCED Atherosclerosis

ABSTRACT

Objective: Black cumin (Nigella Sativa), have been used for nutritional and medicinal purposes in many countries. This work was done to study the effect of black cumin powder during and after induction of atherosclerosis in cholesterol-enriched diet fed rabbits.

Methods: Thirty male New Zealand White rabbits, (4-5 months old, average body weight \( \approx 1.5 \) kg) were used. Rabbits were divided into five groups: negative control group, positive control group (fed atherogenic diet for 20 weeks); prophylactically treated group (each rabbit in this group fed atherogenic diet and given black cumin powder 150 mg/kg body weight /day by gavage for 20 weeks to show the possible protective effect of black cumin on atherosclerotic process); positive control group for development of atherosclerosis (fed atherogenic diet for 12 weeks); and Atherosclerotic treated group (each rabbit in this group fed atherogenic diet for 12 weeks and after the development of atherosclerosis received black cumin powder 150 mg/kg body weight /day by gavage to show the effect of black cumin on the progression of atherosclerosis).

Results: Administration of black cumin during the development of atherosclerosis produced significant inhibition of aortic atherosclerotic changes. Also, administration of black cumin during or after the development of atherosclerosis produced significant decrease (\( P < 0.05 \)) in total-cholesterol (TC), LDL-cholesterol, Triglycerides (TG), Malondialdehydes (MDA), sICAM-1 and sVCAM-1 while HDL-cholesterol and nitrite was significantly increased.

Conclusion: Black cumin has anti-atherosclerotic effects which may be exerted by inhibiting the release of free radicals, hypocholesterolemic effect and through their effect on adhesion molecules.

Keywords: Atherosclerosis, Black cumin, Rabbits, Cholesterol, sICAM, sVCAM, MDA, Nitrate.

INTRODUCTION

Atherosclerosis is a chronic inflammatory process that is characterized by the formation of plaques consisting of foam cells, immune cells, vascular endothelial cells (ECs), smooth muscle cells (SMCs), platelets, extracellular matrix, and a lipid-rich core with extensive necrosis and fibrosis of surrounding tissues [1, 2]. Hyperlipidemia is an important risk factor for cardiovascular disease. Its major role in the pathogenesis of atherosclerosis has been implicated by several clinical and epidemiological studies [3]. In addition, hyperlipidemia has an indirect role by stimulating the production of oxygen free radicals (OFRs) from polymorphonuclear leukocytes (PMNLs) and monocytes [4].

Atherosclerosis is a focal disease affecting discrete regions of the vasculature, such as vessel curvatures and bifurcations [5]. These regions are characterized by disturbed oscillatory flow that induces upregulation of proinflammatory adhesion molecules such as soluble Inter Cellular Adhesion Molecule-1 (sICAM-1), soluble Vascular Cellular Adhesion Molecule-1 (sVCAM-1) [6, 7].

It is evident that most of the hypocholesterolemic drugs must be used for several weeks to be effective. This may expose patients to several side effects, especially liver injury [8]. Black cumin (Nigella Sativa), commonly known as black seeds has been used for nutritional and medicinal purposes in many Middle Eastern countries and other parts of the world [9].

Evidence concerning the antiatherosclerotic effect of N. sativa seeds in animals and human is inconclusive [10]. Therefore, The aim of the present work was to study the potential effect of Black cumin on the progress of atherosclerosis events in diet induced atherosclerosis in rabbits, and in-vivo estimation of nitrite, MDA, sICAM-1 and sVCAM-1 in a trial to determine the possible mechanisms of action of Black cumin in this respect.

MATERIAL AND METHODS

Drugs and chemicals used

The black cumin seeds of nigella sativa (NS) were purchased from the local market in Mansoura, Egypt. The seeds were grinded with a grinder into powder and dissolved in freshly prepared carboxymethyl cellulose (CMC) and each animal received black cumin at a dose of 150 mg/kg body weight everyday by intragastric intubation [11].

Animals used

Thirty male New Zealand White rabbits, (4-5 months old, average body weight \( \approx 1.5 \) kg) were used throughout the study. Animal care and experiments were performed in accordance with NIH guide to the care and use of laboratory animals. The local ethical committee approved the study. Rabbits were housed under similar standard laboratory conditions. Male rabbits were used to avoid the variability secondary to sex differences in this experimental model. Rabbits were housed individually with free access to water. Each rabbit was fed 100 gm per day of a standard commercial rabbit chow and had free access to water. The animals received a standard diet without cholesterol during the 2-week "pretreatment period" for adaptation to the environment. Atherosclerosis was induced experimentally by feeding rabbits on cholesterol-enriched diet for 12 weeks. To prepare the cholesterol containing chow, cholesterol was dissolved in coconut oil and mixed manually with the standard chow (1% cholesterol plus 4% coconut oil mixed with standard commercial rabbit chow). This mixture was chosen because Sun et
Burstein et al. (1970) have demonstrated that this mixture when given for a period of 10 to 12 weeks, it results in marked elevation in serum cholesterol and induction of diffuse, aortic atherosclerosis. A similar procedure was used for the placebo chow (negative control group), except that no cholesterol was added.

### Animal grouping

Rabbits were classified into two major groups.

- **Negative control Group** = Group A (n = 6): Each rabbit in this group received standard rabbit chow and 1 mL of freshly prepared carboxymethyl cellulose (CMC) by gavage for 20 weeks.
- **Cholesterol-enriched diet Group** (n = 24), rabbits in this group received cholesterol containing diet “cholesterol enriched diet”. They were classified into 4 equal groups (B, C, D & E) as follow:
  - **Group B** (n = 6): Each rabbit in this group fed atherogenic diet and given 1 mL of CMC by gavage for 20 weeks. (They served as positive control group).
  - **Group C** (n = 6): Each rabbit in this group fed atherogenic diet for 12 weeks and given 1 mL of CMC by gavage for 12 weeks. They served as positive control group for development of atherosclerosis.
  - **Group D (n = 6)**: Each rabbit in this group fed atherogenic diet and given black cumin powder (150 mg/kg body weight /day) dissolved in 1 mL of CMC by gavage for 20 weeks to show the possible protective effect of black cumin on atherosclerotic process.
  - **Group E (n = 6)**: Each rabbit in this group fed atherogenic diet for 12 weeks and after the development of atherosclerosis (as confirmed by pathological examination & lipogram assay in group C), received black cumin powder (150 mg/kg body weight /day) dissolved in 1 mL of CMC by gavage [11] with atherogenic diet for another 8 weeks to show the effect of black cumin on the progression of atherosclerosis.

At the end of the experiment, all rabbits were sacrificed with the knife after overnight (14 hours) fasting. Trunk blood of each rabbit was collected and centrifuged at 1000 rpm. for 15 minutes. The unhaemolysed serum samples were separated from the clot carefully and were stored at -30°C until used for the assay of:

1) Triglycerides according to the method of Fassati, (1982) [13], while enzymatic determination of serum total cholesterol was determined according to the method described by Richmond, (1973) [14]. HDL-cholesterol was determined according to the method of Burgzin et al (1978) [15]. LDL-cholesterol was determined according to Friedewald equation.

2) sICAM-1 & sVCAM-1 was determined using a commercially available solid phase sandwich ELISA kit, according to Casensky, (1996) [16]. The kit was provided from Immunotech, Marseille, Cedex, France.

3) Nitrite (Nitric oxide): Nitrite levels were estimated by colorimetric enzymatic determination of serum total-cholesterol was significant decrease of serum concentration of triglycerides, total-cholesterol, LDL-cholesterol, sICAM-1, sVCAM-1 and MDA while fasting serum triglyceride, total-cholesterol, LDL-cholesterol, MDA, sICAM-1 & sVCAM-1 while fasting serum HDL-cholesterol and Nitrite significantly increase compared with cholesterol-enriched diet group.

B) Immunohistochemistry

The animals in this group exhibited histopathological alterations related to atherogenesis in the endothelium, subendothelium, membrane elastica interna and tunica intima, also smooth muscle actin immunohistochemical staining revealed normal smooth muscle cells as showed in fig. 1, 2.

### Pathology Results

Group A: Negative control: (Type 0 lesions)

None of the animals in this group exhibited histopathological alterations related to atherogenesis in the endothelium, subendothelium, membrane elastica interna and tunica intima, also smooth muscle actin immunohistochemical staining revealed normal smooth muscle cells as showed in fig. 1, 2.
Group B: Cholesterol rich diet for 20 week: (type 2, 3, 4 lesions)

Typical atheromatous plaque formation with marked endothelial disarrangement & numerous foam cells in most sub endothelial areas. The luminal lipid-endothelial interactions show marked irregularities. Higher magnification (x400) revealed excess lipid laden macrophages, soft lipid-rich core with abundant smooth muscle cells within the interstitium underneath the injured endothelium as evident by actin immunostaining. These features are shown in fig. 3 and 4.

Group C: Cholesterol rich diet for 12 week: (Type 1 lesions)

Endothelial disarrangement and many foam cells were seen in some sub endothelial areas. Higher magnification of these areas revealed lipid laden macrophages, smooth muscle cells and connective tissue fibers. Protrusion and mild irregularities in the endothelial cells and luminal lipid-endothelial interactions were observed in some areas. Immunohistochemistry revealed mild smooth muscle cell proliferation. These features are shown in fig. 5 and 6.

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Table 2: Serum Lipid profile, Nitrite, MDA, sICAM-1 and sVCAM-1 of Atherosclerotic rabbits after administration of black cumin (150 mg/Kg/day) for 20 weeks (n = 6. t-test, M ± SEM)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Standard diet</th>
<th>Cholesterol-enriched diet (20 weeks)</th>
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<td>Triglyceride (mg/dl)</td>
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<td>Total-cholesterol (mg/dl)</td>
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<td>HDL-cholesterol (mg/dl)</td>
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<td>Nitrite (µ mol/µl)</td>
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<td>MDA (µmol/ml)</td>
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* Significant difference between Standard diet (negative control) group & other groups (P < 0.05), ^ Significant difference between Cholesterol-enriched diet fed group and black cumin treated groups (P < 0.05).

Table 1: Serum Lipid profile, Nitrite, MDA, sICAM-1 and sVCAM-1 of Rabbits treated with atherogenic diet alone or concomitantly with black cumin (150 mg/Kg/day), for 20 weeks (n = 6. t-test, M ± SEM)

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**Fig. 6**: Smooth muscle immunohistochemical staining show mild smooth muscle proliferation (Immunoperoxidase staining, hematoxylin counter-stain X 100)

**Fig. 7**: Few scattered foam cells on the intimal surface (H&E X 100)

**Fig. 8**: Smooth muscle immunohistochemical staining show near normal aortic thickness (Immunoperoxidase staining, hematoxylin counter-stain X 100)

**Fig. 9**: Irregular endothelial alignments with foam cells (H&E X 100)

**Fig. 10**: Smooth muscle immunohistochemical staining show hypertrophied smooth muscle cells (Immunoperoxidase staining, hematoxylin counter-stain X 100)

**DISCUSSION**

In the present study, feeding rabbits with Cholesterol-enriched diet for 12 or 20 weeks led to well-developed atherosclerosis and strongly increased foamy macrophages in the aortic tissues of rabbits. Also, feeding rabbits with cholesterol enriched diet induced a significant increase of serum triglycerides, total-cholesterol and LDL-cholesterol while HDL-cholesterol was significantly decreased compared to control group. These results are similar to that obtained by Heinleh et al., [22] and Ghanya et al., [10]. The addition of cholesterol to the diet is partly responsible for the increase in the plasma concentration of total-cholesterol and LDL-cholesterol [23]. The major bulk of LDL-cholesterol is removed by hepatic LDL receptor, so suppression of hepatic LDL receptors can explain the elevated level of LDL-cholesterol that occurs with cholesterol-enriched diet [24]. The increased level of plasma triglycerides observed in this study could be explained by the study of Fungwe et al., [23] who proved that, addition of cholesterol to diet leads to stimulation of triglycerides synthesis, reduced fatty acid oxidation or both. Also, dietary cholesterol accelerates VLDL secretion from the liver [25].

Feeding rabbits on the cholesterol-enriched diet for 12 or 20 weeks led to the significant decrease in fasting serum nitrite and significant increase in fasting serum MDA compared to control group. These results are similar to that obtained by Seinosuke K and Mitsuhiro Y., [26], since they reported that atherosclerosis in both human and animal studies is associated with an impairment of endothelium-dependent relaxations, which represents the reduced bioavailability of nitric oxide (NO), produced from endothelial NO synthase (eNOS). Also, Philip w., [27], reported that early hypercholesterolaemia-induced vascular disease is characterized by an attenuated capacity for endothelial production of the antiatherogenic molecule nitric oxide (NO), which is generated by eNOS. Similar to our results, In a cholesterol-induced atherosclerotic rabbit model; plasma MDA level was significantly higher and plasma superoxide dismutase (SOD) activities were lower in rabbits fed regular chow (control group) and there was a positive correlation between the extent of aortic atherosclerosis and plasma MDA values [28]. Hypercholesterolemic atherosclerosis was associated with an increase in aortic tissue MDA and a decrease in the antioxidant reserve that are known to produce endothelial cell injury that represents a critical initiating event in the development of atherosclerosis [29].

Feeding rabbits on cholesterol-enriched diet for 12 or 20 weeks led to the significant increase in both serum sICAM-1 and sVCAM-1 compared to control group. Similar to our results, Becker et al., [30], reported that increased plasma levels of sICAM-1 and sVCAM-1 are associated with an increased risk of atherothrombosis. Also, Sakai A et al., [31] reported that sVCAM-1 and sICAM-1 are expressed by activated endothelial cells and play an important role in very early stages of atherogenesis as well as in more advanced atherosclerotic lesions, where intimal cells and endothelial cells of intimal neovessels in atherosclerotic plaques express them.

In the present study administration of black cumin (N. sativa) during or after the development of atherosclerosis produced significant inhibition of aortic atherosclerotic changes when compared with the
aorta of the positive control groups. Also, administration of black cumin during or after the development of atherosclerosis produced significant decrease in total-cholesterol (TC), LDL-cholesterol, Triglycerides and MDA while HDL-cholesterol and nitrite significantly increased. These findings were similar to those results reported by Prasad [32] and Ghanya et al. [10]. The beneficial effect of black cumin on lipid profile observed in the present study can be explained by its high content of vitamin E and total antioxidant activity as it was reported that, vitamin E administered to hypercholesterolemic rabbits significantly reduced the plasma LDL-cholesterol and TC [10, 33]. It was also found that oil extracted from N. sativa seeds is rich in unsaturated fatty acids, which could be responsible for the decrease of TC and LDL cholesterol levels as reported by other researchers [9, 32]. The hypocholesterolemic effect of black cumin could also be attributed to the seeds contents of total dietary fiber, insoluble dietary fiber and soluble dietary fiber as it was found that several dietary fibers significantly decrease plasma cholesterol levels in human subjects and thereby may reduce the risk of coronary heart diseases [34-35].

Regarding the effect of black cumin on serum MDA & serum nitrite, our results are in agreement with previous studies [36-38]. In contrast to our results, oral administration of N. sativa oil to rats had no significant effect on the levels of heart tissue MDA [39]. Kanter et al. [36-37] found that treatment with the volatile oil of N. sativa decreased blood MDA levels, increased the antioxidant defense system activity in carbon tetrachloride treated rats and reduced the spinal cord tissue MDA following the experimental spinal cord injury in rats [36-37]. Moreover, in an experimental study in diabetic rabbits, N. sativa extract decreased the elevated blood MDA concentration and increased the lowered glutathione and ceruloplasmin concentrations [38].

The antioxidant effect of black seed seems to be due to its oil, thymoquinone (TQ), flavonoids and antioxidant vitamins like ascorbic acid. The N. sativa oil and TQ inhibit non-enzymatic lipid peroxidation in liposomes and both of them, especially TQ, work as a scavenger of various reactive oxygen species, including superoxide anion and hydroxyl radicals [40]. In addition flavonoids are a class of polyphenolic compounds that seem to have antioxidant properties by suppressing reactive oxygen and nitrogen species formation, scavenging reactive oxygen and nitrogen species and protecting the antioxidant defense system [41].

In the present study administration of black cumin (N. sativa) during or after the development of atherosclerosis produced the significant decrease of serum sVCAM-1 & sICAM. Previous studies with experimental animals have revealed increased expression of VCAM-1 and ICAM-1 in atherosclerosis plaques [42]. To the best of our knowledge, Literature survey showed no work has been carried out on the effect of black cumin on sICAM & sVCAM and the precise molecular mechanism(s) of its effects on sICAM & sVCAM discovered in the present study need further investigations to be explained. In the present work, the antioxidant activity, the decreased serum total-cholesterol, LDL-cholesterol and Triglycerides by black cumin may in part explain their effect on sICAM-1 and sVCAM-1.

CONCLUSION

Black cumin has anti-atherosclerotic effects; these anti-atherosclerotic effects may be exerted by inhibiting the release of free radicals, hypocholesterolemic effect and through their effect on adhesion molecules leading to inhibition of the inflammatory atherosclerotic process.

CONFLICT OF INTERESTS

Declared None

REFERENCES