Efficiency of black cumin seeds on hematological factors in normal and hypercholesterolemic rabbits

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Abstract

BACKGROUND: Hypercholesterolemia is among the most common health problems treated with traditional remedies. Nigella sativa (NS) is an effective plant for treating hypercholesterolemia. However, the effects of this herb on hematologic factors and hemostasis system have not been elucidated. This study was designed to investigate the effects of NS on these factors in both normal and hypercholesterolemic rabbits.

METHODS: In this research, twenty rabbits were randomly distributed into four groups of five. The groups received four different diets, namely normal, normal + NS (5%), hypercholesterolemic (1% cholesterol), and hypercholesterolemic (1% cholesterol) + NS (5%), for 8 weeks. After this period, WBC (white blood cell), RBC (red blood cell), HTC (hematocrit), HGB (hemoglobin content), PLT (platelet), fibrinogen (FIB) and factors VII (F VII) were measured.

RESULTS: Using NS significantly increased PLT count in the normal group. In addition, it significantly decreased WBC counts in the hypercholesterolemic group (P < 0.05). However, dietary use of NS did not have any effects on other hematologic factors including RBC, HTC, HGB, FIB, and F VII (P < 0.05).

CONCLUSION: Increased PLT numbers might cause enhanced coagulation. The achieved results call for more research on the effects of various diets (hypercholesterolemic and normal diet) supplemented with NS on different coagulation factors and hemostasis system.

Keywords: Hematological Factors, Hypercholesterolemia, Nigella Sativa L., Rabbit.

Introduction

Plants have been an important source of medicine for thousands of years. The World Health Organization (WHO) estimated that up to 80% of people still rely on herbal remedies for their health care. Nigella sativa (NS) (Ranunculaceae), commonly known as "black cumin", is an erect, herbaceous annual plant. Several beneficial pharmacological effects, including antihistaminergic, antihypertensive, hypoglycemic, antimicrobial, mast cell stabilizing and anti-inflammatory activities, have been attributed to various crude and purified components of black seeds. Researches support the traditional use of NS and its derivatives as a treatment for dyslipidemia and hyperglycemia. NS oil has hypocholesterolemic effects on patients with hyperlipidemia. Hyperlipidemic states, especially hypercholesterolemia, have been largely under consideration. Hypercholesterolemia is one of the crucial risk factors for the development of atherosclerosis and subsequent cardiovascular diseases. Cholesterol-rich diets are associated with free radicals production, followed by oxidative stress and hypercholesterolemia. Although the effects of NS on homeostasis is known in traditional medicine, the pharmacological investigations on the effects of NS on homeostasis are few. Al-Jishi did not find any changes in blood cells when NS oil was given to normal rats. Previous studies clearly showed that chronic treatment with NS fixed oil effectively influenced blood homeostasis in rats and seemed to induce transient changes in coagulation activity. In a study by Al-Hader et al., while NS fixed oil significantly...
decreased serum lipids and glucose levels, and leukocytes and platelets counts in rats, it significantly increased hematocrit and hemoglobin concentrations. However, treatment with NS extract has been reported to significantly protect mice and rats from cisplatin-induced falls in leukocytes counts, and to influence leukocytes activities.

Anomalous results have been reported about the effects of NS on hematological factors and homeostasis system. Thus, we investigated the effects of NS on hematological factors and homeostasis.

Materials and Methods

Grouping and treatment
This experimental study and was carried out in 2006. It included 20 male 10-week old rabbits with a mean weight of 2-2.5 kg. The animals were provided by the Pasteur Institute and acclimatized under the standard rabbit chow basic diet for a period of 4 weeks before starting the experiment. Then, they were randomly divided into 4 groups of five, namely normal diet, normal diet + NS (5%), hypercholesterolemic diet (1% cholesterol) and hypercholesterolemic (1% cholesterol) + NS (5%). They were all treated under the special diets for 8 weeks.

Identifying and preparing the NS powder
NS seeds were obtained from Roshan Dasht in east of Isfahan. Genus and species of this plant were identified and authenticated by Iraj Mehregen, a botanist in the herbarium of Shiraz School of Pharmacology. NS was powdered using an electrical mill. It was then screened by a mesh device (number 20) to deliver a homogenous powder. Afterwards, 5 g of this was powder mixed with 95 g powdered basic diet. To prepare hypercholesterolemic diet, 1 g pure cholesterol powder was added to 99 g powdered basic diet.

Biochemical factors measurement
After the 8-week period, blood was obtained from central sinus of the rabbits. Blood samples were used for biochemical factors analysis including WBC (white blood cell), RBC (red blood cell), HTC (hematocrit), HGB (hemoglobin content) and PLT (platelet) measurement. Plasma FIB (fibrinogen) and factor VII (F VII) were measured in Isfahan Cardiovascular Research Center and Blood Transfusion Center, respectively. The number of blood cells was determined by a cell counter. FIB was calculated according to clot formation time using Mahsa Yaran kit. In addition, F VII was estimated based on its percentage of activity in plasma by comparing clot formation time with a standard sample.

Statistical analysis
Statistical evaluations were conducted by SPSS13 and the results were expressed as mean ± SD. Kruskal-Wallis test was performed to compare the means of measured biochemical factors between the groups. Mann-Whitney test was applied for two by two comparisons.

Results
Based on the results, using NS in the normal group significantly increased platelet count (P < 0.05) but did not have any effects on other blood factors (WBC, RBC, HGB, HTC, FIB and F VII). Significant differences between normal and normal + NS (5%) groups confirmed these results (Figures 1, 2 and 3). As shown in the Figure 1 and 3, non-significant increases in RBC, HGB, HCT, FIB and PLT and a non-significant decrease F VII were observed in hypercholesterolemic (1% cholesterol) + NS (5%) group compared with the hypercholesterolemic group (1% cholesterol). Using NS in hypercholesterolemic diet (1% cholesterol) caused a significant decrease in WBC (P < 0.05) (Figure 2).

Discussion
Previous research showed that use of NS enhanced decreased levels of HGB, WBC and RBC in rabbits treated with carbon tetrachloride. Aflatoxin can be causes hematological changes whereby using NS makes resistance in rabbits under the aflatoxin diet. This plant has been shown to modify established chaos in hematological parameters of diabetic rabbits and increase defensive mechanisms of body versus infection in these rabbits. NS improved anemia resulted from increased non-enzymatic glycosylation in membrane proteins of RBC in diabetics. This plant also decreases anemia via lipid peroxidation reduction in RBC hemolysis and does not affect RBC levels directly. Results of this research confirmed that NS does not have an effect on blood cells directly.

Decreased coagulation factor in hypercholesterolemic (1% cholesterol) + NS (5%) rabbits may be due to the effects this plant on cholesterol and triglycerides (TG) levels. The positive relation between F VII and cholesterol levels, especially TG, along with the non-significant difference in F VII between normal and normal + NS (5%) groups, suggest that NS reduces F VII through an effect on cholesterol and TG levels and does not directly affect this factor. Since PLT counts increased in this research, NS might have increased coagulation activity.
Figure 1. The effects of using NS on red blood cell counts (A), hemoglobin (B) and hematocrit (C) in rabbits under the normal and hypercholesterolemic diets (1% cholesterol) (1- normal group; 2- normal + NS (5%) group; 3- hypercholesterolemic group (1% cholesterol); 4- hypercholesterolemic (1% cholesterol) + NS (5%) group)

* Significant difference means between hypercholesterolemic group (1% cholesterol) and normal group (P < 0.05)

Figure 2. The effects of using NS on white blood cell counts in rabbits under the normal and hypercholesterolemic diets (1% cholesterol) (1- normal group; 2- normal + NS (5%) group; 3- hypercholesterolemic group (1% cholesterol); 4- hypercholesterolemic [(1% cholesterol) + NS (5%) group]
While Ghoneim et al. reported an enhancement of coagulation after parenteral administration of NS extract,27 El-Naggar and El-Deib reported an anticoagulant effect after oral administration of powdered NS seeds.28 Enomoto et al. found NS oil to have repressive effect on PLT accumulation and suggested it to have strong anticoagulation effects.29 On the other hand, AL-Jishi and Abuo used 360 mg/KgRat/day of NS for a period of 2 weeks and observed increased FIB levels and prolonged prothrombin time. Thus, coagulation and anticoagulation effects of the extract were simultaneously detected.18 It can be concluded that these effects existence jointly show different components effect of NS on various coagulation factors. Therefore, further research is needed to recognize the effects of different NS components on various factors of homeostasis system.

**Conflict of Interests**

Authors have no conflict of interests.

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