Effect of *Nigella sativa* L. on heart rate and some haematological values of alloxan-induced diabetic rabbits

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Summary

This study was designed to investigate the effect of an extract of *Nigella sativa* L. on the heart rate and some haematological values in alloxan-induced diabetic rabbits. Fifteen New Zealand male rabbits were divided into three experimental groups: control, diabetic and *N. sativa* L.-treated diabetic. At the end of the experimental period (2 months), animals in all three groups were fasted for 12 hours and blood samples were taken for the determination of glucose levels, RBC and WBC (red and white blood cell) counts, packed cell volume (PCV), and haemoglobin (Hb) concentration. Heart rates were also measured by a direct-writing electrocardiograph before the blood withdrawals. It was found that *N. sativa* L. treatment increased the lowered RBC and WBC counts, PCV and neutrophil percentage in diabetic rabbits. However, the WBC count of the *N. sativa* L. treated diabetic group was still lower than the control. *N. sativa* L. treatment also decreased the elevated heart rate and glucose concentration of diabetic rabbits. It is concluded that oral *N. sativa* L. treatment might decrease the diabetes-induced disturbances of heart rate and some haematological parameters of alloxan-induced diabetic rabbits.

Introduction

This study was part of our investigations of the responses of alloxan-induced diabetic rabbits to a water extract of *Nigella sativa* L. *(Meral et al., 2001; Meral et al., 2003).* Diabetes Mellitus (DM) is one of the most common metabolic disorders, with a worldwide prevalence estimated to be between 1 % and 5 %. The increasing prevalence of DM in the world is a cause for concern. DM leads to abnormalities in carbohydrate, protein and lipid metabolism and increases the risk of developing atherosclerotic arterial disease by two- to six fold *(Sacks, 1997)*. It has been suggested that the heart rate is higher, and the red and white blood cell (RBC and WBC) counts lower, in type diabetes than in non-diabetic individuals (*Palmieri et al., 2001; Yenigun, 1997*). In long-term diabetes, cardiomyopathy and congestive heart failure may also develop as a result of impaired left ventricular function (*Ozturk et al., 1998*).

N. sativa L. is a spice plant belonging to the family Ranunculaceae *(Aqel & Shaheen, 1996)*. It is a medicinal plant that contains black seeds and has been used as a natural remedy for a variety of illnesses. It has been shown that *N. sativa* L. seeds contain > 30 % of a fixed oil and 0.40-0.45 % w/w of a volatile oil (VO). The VO has been shown to contain 18.4-24 % thymoquinone and a total of 46 % of many monoterpenes such as p-cymene and α-pinene *(El-Tahir et al., 1993a)*, and to have bronchodilator *(El-Tahir et al., 1993b)*, antibacterial *(Hanafy & Hatem, 1991)*, diuretic and hypotensive *(Zaoui et al., 2000)*, and immunopotentiating *(El-Kadi & Kandil, 1987)* activities.

Because there is evidence that the function of the cardiovascular system may be altered in DM *(Palmieri et al., 2001; Yenigun, 1997; Ozturk et al., 1998),* this study was undertaken to determine whether or not N. sativa L. could restore the altered cardiovascular system function to normal.

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Materials and Methods

Preparation of Extract

An extract of *N. sativa* L. seeds was prepared using the method described by Farida et al. (1987). Briefly, *N. sativa* L. seeds were purchased from a local herb store, authenticated by Dr. Fevzi Ozgokce, Department of Botany, University of Yuzuncu Yil, washed and air-dried. A voucher specimen (F-5427b) has been kept in the VANF herbarium for future reference. An extract of *N. sativa* L. seeds in drinking water (5 %) was prepared fresh daily by boiling the seeds (50 g) in drinking water (1000 ml) for 10 min and then filtering through 4 layers of surgical gauze to obtain the water extract used for the experiment.

Treatment of Rabbits

Fifteen New Zealand male rabbits, weighing 1.5 kg and averaging 12 months old, were obtained from the Department of Animal Science, Faculty of Agriculture, Yuzuncu Yil University, Van, Turkey. The rabbits were divided into three experimental groups (control, diabetic, and diabetic with *N. sativa* L. treatment), each containing 5 rabbits. At the start of the experiment the animals in the latter two groups were injected intravenously with 150 mg/kg of 10 % alloxan (Sigma Chemical Co., St Louis, MO, USA) dissolved in isotonic NaCl to induce diabetes. The control group was injected only with the same volume of isotonic NaCl as the diabetic groups received. Three days after alloxan injection DM was confirmed by the demonstration of hyperglycaemia (blood glucose ≥ 300 mg/dl). The rabbits were not treated with insulin at any time during the experiment. The diabetic-*N.sativa* L.-treated group was given the aqueous extract of *N. sativa* L. seeds orally at 20 ml/kg (substituted for drinking water) everyday for two months after the induction of DM was confirmed. All animals were housed in stainless cages under standard laboratory conditions (light period 7.00 a.m. to 8.00 p.m., 21±2 ^{o}C, relative humidity 55 %, ground wheat-soybean meal based-diet and water freely available), and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Haematological Analysis

At the end of the experimental period (2 months), the animals in all three groups were fasted for 12 hours and blood samples were taken for the determination of glucose levels, RBC and WBC counts, packed cell volume (PCV), and haemoglobin (Hb) concentration. Serum glucose concentration was measured immediately by the glucose oxidise method *(Yenson, 1986)*. The PCV was measured by the microhaematocrit centrifuge *(Mitruka & Rawnsley, 1977)*. Hb concentration was determined by the cyanmethemoglobin technique *(Mitruka & Rawnsley, 1977)*. The RBC and WBC counting methods were based on the dilution of obtained blood with diluting fluids (*Hayem & Turk*) in RBC and WBC counting pipettes *(Mitruka & Rawnsley, 1977)*. Individual cells were then counted in the counting chamber (haemocytometer). Giemsa's staining method was used for the differential count of RBC. The heart rates of the rabbits were measured by a direct-writing electrocardiograph (Cardiofax 02611; Nihon Kohden, Tokyo) before the blood withdrawals.

Statistical Analysis

The data were expressed as mean±standard deviation (SD) and analysed using analysis of variance (ANOVA). Tukey's test was used to test for differences among means for which ANOVA indicated a significant ($P \leq 0.05$) F ratio.

Results

Comparative haematological values, serum glucose concentration and heart rates of control and treatment groups are shown in Table 1.

Table 1. Comparative haematological values, serum glucose concentration and heart rates of control and the treatment groups.

Parameters	*Control*	*Diabetic*	*Diabetic with N. sativa L. treatement*
RBC (x 10^6/µl)	5.7 ± 0.3[a]	4.1 ± 0.1b	5.2 ± 0.7 [a]
Hb (g/dl)	12 ± 0.9	12 ± 1.2	12 ± 1.1
WBC (x 10^3/µl)	5.4 ± 0.4[a]	3.1 ± 0.1[b]	4.4 ± 0.3[c]
Neutrophils (%)	30 ± 3.7[a]	25 ± 3.2[b]	33 ± 3.9[a]
Basophils (%)	2 ± 0.2	3 ± 0.6	3 ± 0.3
Eosinophils (%)	3 ± 0.2	4 ± 0.6	4 ± 0.5
Lymphocytes (%)	61 ± 6.7	63 ± 5.8	57 ± 5.4
Monocytes (%)	4 ± 0.3	5 ± 0.8	3 ± 0.3
PCV (%)	37 ± 2.9[a]	29 ± 3.3[b]	35 ± 2.8[a]
Heart Rate (beats/min)	183.7 ± 32.6[a]	268.1 ± 37.7[b]	205.4 ± 29.3[a]
Glucose (mg/dl)	96.32 ± 8.37[a]	340.43 ± 45.76[b]	194.41 ± 27.33[c]

[abc]: Means in the same row with different superscripts significantly differ (P<0.05)
Values are expressed as mean ± SD
Number of rabbits in each group = 5

Serum glucose concentration increased significantly (P≤0.05) in diabetic rabbits. *N. sativa* L. treatment decreased the elevated glucose concentration significantly (P≤0.05) in treated diabetic rabbits; however, their glucose concentrations were still significantly higher (P≤0.05) than those of the control group.

It was also found that the RBC and WBC counts, PCV and neutrophil percentage decreased (P≤0.05), and the heart rate increased (P≤0.05) in diabetic rabbits. *N. sativa* L. treatment increased (P≤0.05) the lowered RBC and WBC counts, PCV and neutrophil percentage in diabetic rabbits. However, the WBC count of the *N. sativa* L – treated diabetic group was still lower than those of control values. *N. sativa* L. treatment also decreased the elevated heart rate of diabetic rabbits to normal level.

Discussion

The present study indicated that N. sativa L. treatment might ameliorate some disturbed haematological parameters of diabetic rabbits. It has been suggested that anaemia occurrence in DM is due to the increased non-enzymatic glycosylation of RBC membrane proteins, which correlates with hyperglycaemia *(Kennedy & Baynes, 1984)*. Oxidation of these glycosylated membrane proteins and hyperglycaemia in DM cause an increase in the production of lipid peroxides causing a hemolysis of RBC. In this experiment, we did not measure the RBC membrane lipid peroxide levels in diabetic rabbits. However, Meral et al. (2001) demonstrated that serum lipid peroxide level increased in diabetic rabbits. They also demonstrated that *N. sativa* L. treatment for two months after inducing DM decreased the elevated lipid peroxide level to normal level. Thus, increased RBC count of *N. sativa* L. treatment rabbits could be due to the lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBC to hemolysis. Since non-enzymatic glycosylations of membrane proteins correlate with hyperglycaemia *(Kennedy & Baynes, 1984)*, it might be said that *N. sativa* L. produced its effect by decreasing the elevated glucose

concentration in *N. sativa* L. treatment rabbits. However, more studies by measuring the RBC fragility, and serum folic acid, iron, cobalt, vitamin B_{12} and calcium levels are needed to demonstrate the exact mechanism of action of *N. sativa* L. on increased RBC count of diabetic rabbits.

Neutrophils ingest and kill bacteria and have been called the body's first line of defence against bacterial infections *(Ganong, 1991)*. It has been suggested that the body's defence mechanism against infections was disturbed due to the disturbed neutrophile function in diabetes *(Yenigun, 1997)*. In this experiment, we demonstrated that *N. sativa* L. treatment increased the lowered neutrophil percentage of WBC to control level. This result indicated that *N. sativa* L. treatment might also increase the defence mechanism of the body against infections in diabetic rabbits.

Alloxan-induced diabetes increased the heart rate while *N. sativa* L.-treatment decreased it to control level. The increased heart rate in diabetic rabbits was probably due to the increased sympathetic output produced by diabetes-induced anaemia. El-Tahir et al. (1993a) studied the cardiovascular actions of the volatile oil of *N. sativa* L. seeds in non-diabetic rats, and similar to our result they found a decreased heart rate. The effect of the volatile oil of the *N. sativa* L. seeds was antagonised by the ganglionic blocker hexamethonium and the non-selective muscarinic receptor blocker atropine indicating that its heart rate-reducing effect occurred by activating cholinergic mechanisms *(El-Tahir et al., 1993a)*. In the present study, it was found that the heart rate decreased and also RBC count increased to control level in *N. sativa* L. treated rabbits. Therefore, decreased heart rate could also be due to a normalised RBC count in these rabbits.

It is concluded that oral *N. sativa* L. treatment might decrease the diabetes-induced disturbances of heart rate and some haematological parameters of alloxan-induced diabetic rabbits.

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