Effect of nigella (*Nigella sativa L.*) seeds on hematological parameters in rats

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Nigella (*Nigella sativa L.* [NS]) is an important medicinal herb distributed and cultivated in various parts of the world. The effect of NS on hemostasis is known in traditional medicine. However, few studies have been conducted on its effect on hemostasis and biochemical function. El-Naggar and El-Deib[1] reported an anticoagulant effect after oral administration of NS powdered seeds. Al-Jishi and Abuo Hozaifa[2] and Zaoui et al[3] investigated the effect of NS on different blood coagulation parameters and the effect of varying dose and duration of NS administration on these parameters. Kökdil et al[4] reported that treatment with NS fixed oil have effect on blood biochemical and total antioxidant status in rat. Therefore, the aim of the present study was to evaluate the effect of NS powdered seeds on blood hematological parameters in rats, and the effect of varying doses, and duration of NS administration on these parameters.

This study was performed in the Medical Science Application and Research Center of Dicle University (DUSAM), Diyarbakir, Turkey from February 2003 to December 2007. Ethical approval was obtained, and the study performed according to Health Guidelines on the Care and Use of Laboratory Animals. A total of 75 Wistar albino male rats, 60 of them with NS supplementation, and 15 animals acting as controls, were included in the study. Four doses of NS were used (100, 200, 400, and 600 mg/kg). Each dose was given for 3 durations: one, 2, and 4 weeks. The NS group received locally cultivated NS powdered seeds mixed with flour dough, and the control group was given plain flour dough. Each dose was mixed with flour dough that was prepared for the animals before feeding. The 60 rats in the test group were divided into 4 main groups, each including 15 rats, and fed as follows: Group I was fed 100 mg/kg rat/day of NS. Group II was fed 200 mg/kg rat/day of NS. Group III was fed 400 mg/kg rat/day of NS. Group IV was fed 600 mg/kg rat/day of NS. Each main group including the controls and test groups were subdivided into 3 subgroups, each involving 5 rats each, according to the duration of feeding of NS, which continued for one, 2, and 4 weeks. Biochemical and hematological measurements were performed at weeks one, 2, and 4 following NS seeds powder administration. At the end of the experimental period, animals underwent sodium pentobarbitone (40 mg/kg) anesthesia and a midsternotomy was performed in all animals. A 5 ml blood sample was withdrawn from the ascending aorta for the measurement of biochemical and hematological parameters. Serum albumin, alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), bilirubin, and uric acid levels were measured on a C 16000 biochemical analyzer (Abbott Diagnostics kit, North Chicago, IL, USA) using the enzymatic colorimetric method. Hematological parameters were measured using a Cell-Dyn 3700 analyzer (Abbott Diagnostics kit, Abbott Park, IL, 60064, USA). Activated partial thromboplastin time (APTT), antithrombin III (ATIII), and fibrinogen levels were determined by using ACL TOP blood count machine (Beckman Diagnostics kit, Lexington, MA, 02421-3125, USA).

Significant differences between the treated, and control group means were determined by the paired-t test. One way ANOVA test followed by Tukey HSD, and Dunnet's test were used to determine significant differences between different test subgroups. Normal distributions were evaluated using the Kolmogorov-Smirnow test, and homogenity was evaluated using Levene test. All data were analyzed by the Statistical Package SPSS version 11.0 for Windows. The significance of test results was ascertained at $p<0.05$.

The effects of NS on erythrocytes, leukocytes, and platelets counts, levels of hematocrit (Htc), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular content of hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) are shown in **Table 1**. There was no significant effect of NS on erythrocytes, and leukocyte counts, MCHC, and ATIII level at any dose for any duration. The NS induced a statistically significant increase in platelet counts, only when given in high dose after one week of NS feeding compared to the control values ($p<0.05$), and significantly decreased to the group II dose after 4 weeks of feeding compared to control values ($p<0.05$). The administration of the group IV dose for one, and 2 weeks caused a significant increase in platelet count when compared to the groups receiving other doses ($p<0.01$). There was a significantly increase in Htc level of group IV dose for one, and 2 weeks and group II dose for 4 weeks when compared to the group I dose and controls ($p<0.001$). There was a significant increase in hemoglobin level of group IV dose for one week when compared to the control, and paralleling that of the groups taking other doses for 4 weeks ($p<0.05$). There was a significant increase in MCV level of group IV dose for one, and 2 weeks, and group I dose for 4 weeks when compared to that of the control values ($p<0.05$). There was a significant MCH level of group I dose for 4 weeks when compared to the controls ($p<0.05$). There was a significant increase in key hepatic enzyme AST

Table 1 - Effect of feeding with different doses of NS for one, 2, and 4 weeks on hematological parameters.

Parameter (unit)	Animal group Dose mg/ kg/day	NS-rats treatment time (weeks)		
		1 week	2 weeks	4 weeks
Platelets (x10^3/µl)	Control	628 ± 42	630 ± 38	637 ± 28
	I	618 ± 43	642 ± 58	666 ± 21[b]
	II	618 ± 43	580 ± 57	560 ± 65[a]
	III	598 ± 48	590 ± 54	590 ± 42
	IV	726 ± 90[a,b]	696 ± 45[b]	590 ± 42
Hematocrite (%)	Control	43 ± 6.45	43 ± 6.38	43 ± 7.2
	I	42 ± 6.70	44 ± 4.18	46 ± 4.1
	II	47 ± 6.70	48 ± 4.18	53 ± 5.7[a,b,c]
	III	48 ± 8.36	50 ± 6.12	52 ± 5.7[a,c]
	IV	60 ± 7.90[a,b,c]	57 ± 9.81[a,b,c]	52 ± 5.7[a,c]
Hemoglobin (g/dl)	Control	12.36 ± 1.80	12.0 ± 1.87	12.40 ± 1.51
	I	12.40 ± 1.80	14.4 ± 1.44	15.60 ± 0.86[a]
	II	13.04 ± 1.00	14.1 ± 1.22	14.64 ± 0.80[a]
	III	13.64 ± 1.63	13.9 ± 1.53	14.64 ± 1.36[a]
	IV	15.20 ± 1.30[a]	14.0 ± 1.58	14.64 ± 1.36[a]
MCV (fl)	Control	56.90 ± 9.87	56.26 ± 8.93	56.40 ± 11.05
	I	57.80 ± 10.73	63.74 ± 4.42	64.80 ± 7.03[a]
	II	69.20 ± 12.5	66.08 ± 9.06	69.26 ± 9.37
	III	59.22 ± 12.3	66.26 ± 9.96	66.26 ± 9.37
	IV	77.46 ± 13.1[a]	76.12 ± 9.81[a]	66.26 ± 9.37
MCH (pg)	Control	16.34 ± 2.92	15.86 ± 2.78	16.16 ± 2.05
	I	17.12 ± 2.57	20.42 ± 3.88	22.12 ± 3.35[a]
	II	19.22 ± 3.35	19.28 ± 1.28	19.08 ± 1.31
	III	16.76 ± 2.28	17.20 ± 2.58	18.66 ± 2.60
	IV	19.40 ± 2.03	20.54 ± 2.82	18.66 ± 2.60
MCHC (gHb/dl0)	Control	28.88 ± 4.54	28.34 ± 3.66	29.38 ± 5.26
	I	29.89 ± 5.12	33.19 ± 5.57[a]	28.08 ± 4.20
	II	28.26 ± 5.79	29.54 ± 3.79	27.82 ± 3.15
	III	28.68 ± 3.01	27.94 ± 4.03	28.36 ± 3.79
	IV	25.62 ± 3.51	23.58 ± 2.63	28.36 ± 3.79

Data are mean ± SD. Group I fed 100 mg/kg/day, Group II fed 200 mg/kg/day, Group III fed 400 mg/kg/day, Group IV fed 600 mg/kg/day, NS-rats - *Nigella sativa* treated rats group, [a]Significantly different from corresponding control at $p<0.05$, [b]Significantly different from corresponding between the groups at $p<0.01$ and, [c]Significantly different from corresponding the groups I and controls at at $p<0.001$, MCV - mean corpuscular volume, MCH - mean corpuscular content of hemoglobin, MCHC - mean corpuscular hemoglobin concentration

level of group II dose for 2 and 4 weeks when compared to control values ($p<0.05$). The administration of the group I dose for 4 weeks caused a significant increase in AST level when compared to the group receiving a similar dose for 2 weeks ($p<0.01$). There was a significant increase of ALT level group II dose for one, and 4 weeks when compared to the control values ($p<0.05$). The administration of the group II dose for 4 weeks, and group IV dose for 2 weeks caused a significant increase of ALT level when compared to the other doses ($p<0.01$). There was a significant increase of bilirubin level of group II for one, and 4 weeks when compared to the control values ($p<0.05$). There was a significant increase of uric acid level of group IV for one week, and group III and group IV dose for 4 weeks when compared to the control values ($p<0.05$). There was no significant effect of NS on ATIII level at any dose for any duration when compared to the control values ($p<0.05$). As compared to the control, there was a significant shortening of APTT of group IV dose for one week of NS feeding ($p<0.05$). The group IV dose administration for one week caused a significant shortening of APTT when compared to the groups receiving a similar dose for 2, and 4 weeks ($p<0.01$). A significant increase of

fibrinogen level of group I dose for one week was noted parallel to a decrease of similar dose for 4 weeks when compared to the control ($p<0.05$). The administration of the group I dose for one week caused an increase of fibrinogen level when compared to groups II and III. The group I dose administration for one and 2 weeks caused a significant increase in fibrinogen level when compared to the groups receiving a similar dose for 4 weeks ($p<0.01$). The group II dose administration yielded a significant increase of fibrinogen level after 4 weeks when compared to the groups receiving a similar dose for one, and 2 weeks ($p<0.05$). There was no significant effect of NS on fibrinogen level in the groups III and IV dose for any duration. There was a significant increase of albumin level of group IV dose for one week, and group II dose for 2, and 4 weeks when compared to the control values ($p<0.05$). The administration of the group II dose for 4 weeks caused a significant increase of albumin level when compared to the groups receiving a similar dose for one and 2 weeks ($p<0.01$). The group IV dose for one week caused a significant increase in albumin level when compared to the groups for 2 and 4 weeks ($p<0.01$).

The study show that NS feeding within the doses used seems to induce changes in the hematological parameters of rats. On the other hand, there was no linear dose or time dependent effect of NS on these parameters. Similar to our results, Al-Jishi and Abuo Hozaifa[2] demonstrated that there was no significant effect of NS on ATIII level at any dose for any duration when compared to control values. As compared to the control, there was a significant shortening of APTT of group IV dose for one week of NS feeding. This result agrees with that obtained by Al-Jishi and Hozaifa.[2] They reported that APTT reduction is most likely due to the effect of NS on the intrinsic pathway. Similar to our results, Al-Jishi and Abuo Hozaifa[2] and Kökdil et al[4] demonstrated that albumin and fibrinogen levels were increased in rats treated with NS powdered seeds.

In our study, we noticed a significant increase of fibrinogen level group I dose for one week, parallel to the decrease of similar dose for 4 weeks when compared to the control. This discrepancy may be explained by the difference in the dosage administered, and the difference in species response.

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