

Effects of *Nigella sativa* L. Seed Extract on Fatigue, Blood Biochemical Parameters and Thyroid Function in Male Mice

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Received 3 January 2014; revised 8 February 2014; accepted 24 February 2014

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Abstract

Nigella sativa L. (Black seed), is a traditional herbal medicine that has been used for many purposes. The present study was designed to investigate the effects of hydro-alcoholic extract of *nigella sativa* L. (NS) on performance of Forced Swimming Test (FST), blood biochemical parameters related to fatigue and thyroid functions. Therefore, Blood Urea Nitrogen (BUN), Creatine Kinase (CK), Lactic Dehydrogenase (LDH), and Total Protein (TP), triiodothyronine T3, thyroxin T4 and TSH tests were investigated. Thirty five male adult mice were randomly divided into five groups: three NS-fed groups, one fluoxetine treated group and one control group. Three NS experimental groups received hydro-alcoholic extract of NS at doses of 50, 100 and 200 mg/kg orally for two weeks. Immobility time decreased in all NS groups compared with control group. Administration of NS significantly increased the concentration of T3 and T4 of the treatment groups. On the contrary, the amount of BUN, CK, LDH, TP and TSH decreased. In conclusion, black seed extract at the experimented doses showed anti-depressant, anti-fatigue and hyperthyroid effects.

Keywords

Nigella sativa Hydro-Alcoholic Extract; Black Seed; Thyroid Functions; Forced Swimming Test; Blood Biochemical Parameters Related to Fatigue

1. Introduction

The genus *Nigella* is an annual plant that belongs to the *Renunculaceae* family and comprises about eight species [1].

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Nigella sativa L. is one of the spices that commonly known as black seed. It is native to many areas such as southern Europe and western Asia [2] which has been used as an herbal medicine for more than 2000 years.

Compound of black seed contains fixed and essential oils alkaloid, steroids, proteins, carbohydrates, fatty acids, and flavonoids. Also, it contains many bioactive constituents such as thymoquinone, thymohydroquinone, pinene, p-cymene, dithymoquinone [3] [4] that have been shown to possess biological, pharmacological and biochemical actions, including antibacterial [5], bronchodilator [6]. Besides it has activity against diabetes [7] and prevents lipid peroxidation [8]. Moreover, in some studies, NO exhibits anti-oxidant and immune-potentiating effects [9] [10].

Forced Swimming Test (FST) is a behavioral test for rodents, which evaluates the efficacy of antidepressant treatment and physical stamina [10]-[13]. Blood urea nitrogen (BUN), lactic dehydrogenase (LDH), creatine kinase (CK), and total protein (TP) are blood biochemical parameters related to fatigue. The BUN test is a routine test used primarily for evaluating renal functions. Serum CK and LDH in the body normally exist in muscle, and an increase in the amount of serum CK and LDH in the blood indicates that muscle damage has occurred [14]. TP is a rough measure of serum protein. Protein measurements can reflect the nutritional state, liver disease, kidney disease, and many other conditions [15]. Serum levels of Triiodothyronine (T3), thyroxin (T4) and thyroid stimulating hormones (TSH) are usually used to assess thyroid functions. Regarding NS, different kinds of extraction have been used on different animals and effects on blood biochemical parameters and thyroid function have been reported. So far however, the results are controversial [16]-[18].

The present study was undertaken to determine the effects of *Nigella sativa* L. hydro-alcoholic extract on FST performance, plasma concentration of BUN, CK, LDH, TP and thyroid functions.

2. Materials and Methods

2.1. Animals

35 Male adult mice Balb/c weighing 23 ± 5 g (Pasteur Institute, Karaj Production and Research Center, Iran) were used in this study. The animals were randomly divided into 5 groups of 7 each and treated according to the experimental protocol for 2 weeks. Animals housed under the following laboratory conditions: temperature $22^{\circ}\text{C} \pm 1^{\circ}\text{C}$, humidity 40% - 60%, 12 h Light/Dark cycle, lights on at 07:00 h. Mice were maintained in polyethylene cages with enough food and water available *ad libitum*. All measurements were performed between 10:00 and 14:00 h in the animal testing room. Mice were treated by the current law of Medical Sciences Research Center, Tehran Medical Branch, Islamic Azad University, Tehran, Iran, in accordance with the National Institutes of Health (NIH) Guide for Care and Use of Laboratory Animals. Control Group administrated distilled water orally. One group administered Fluoxetine, 10 mg/kg/day and three other groups administered NS at doses 50, 100 and 200 mg/kg/day.

2.2. Drugs

Black seeds were purchased from a local herb market, cleaned, dried, mechanically powdered, extracted with 70% ethanol and dried, with rotary evaporator to render the extract alcohol free and kept in refrigerator at 4 degree centigrade until used. The NS seeds extract was provided at doses of 50, 100, 200 mg/kg.

2.3. Forced Swimming Test

FST is generally used for evaluating anti depressive effects. Recently, the FST has also been used as an anti-fatigue test. During the FST, the animals were placed in a Plexiglas cylinder with 25 cm height and 10 cm width, which was filled with water ($25^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$).

The total duration of immobility was measured during a period of 4 min, after a delay of 2 min [19]. Each mouse was consider to be immobile when it ceased struggling and remained floating in the water, making only these movements necessary to keep its head above water.

2.4. Sample Collection

At the end of the experimental period (2 weeks) animals in all 5 groups were killed with chloroform and the blood samples were taken from the heart. Plasma was separated by centrifugation at speed 3000 rpm for 5 mi-

nutes and placed in plain Containers and stored at -10°C until analysis.

2.5. Hormones Estimation

T3, T4 and TSH levels were measured by electrochemiluminescence method (Elecsys2010). In order to clarify its anti-fatigue mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST. Contents of BUN, LDH, CK and TP were determined by an auto analyzer (Hitachi 747, Hitachi Japan).

2.6. Statistical Analysis

To evaluate significance differences among treatment groups with control groups, one-way analysis of variance (ANOVA) was applied. The data were expressed as mean \pm standard error of the mean (SEM). A value of $p < 0.05$ or less was considered to show statistical significance.

3. Results

3.1. Forced Swimming Test

Immobility time or exhaustion of the control and Fluoxetine group was measured 95.28 ± 19.81 s, 31.66 ± 2.94 , respectively. Data of the FST test (**Figure 1**) demonstrated that administration of NS were significantly decrease the immobility time of the treatment groups which were 49.00 ± 6.03 s, 40.83 ± 6.17 s, 50.66 ± 3.88 s respectively ($p < 0.01$, $F = 36.89$).

3.2. Effect of NS on Biochemical Parameters

In order to clarify its anti-fatigue mechanisms, we assessed the levels of several blood biochemical parameters in mice after FST (**Table 1**). All the biochemical parameter such as BUN, LDH, CK and TP levels significantly tended to decrease in compared with control group ($p < 0.001$).

3.3. Thyroid Function Tests

These results of T3, T4 and TSH serum level of control and treatment groups are shown in **Table 2**. The plasma concentration of T3 and T4 are significantly ($P \leq 0.001$) increased and TSH serum level significantly ($p < 0.001$) decreased in treatment groups compared with control groups.

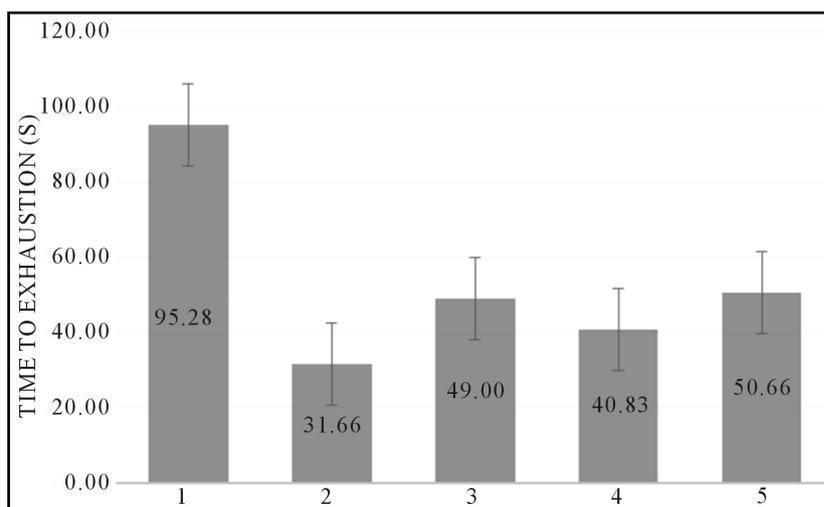


Figure 1. Time to exhaustion in swimming test ($n = 7$). Group 1: control mice administered distilled water, Group 2: administered Fluoxetine 10 mg/kg/day, Group 3: administered NS 50 mg/kg/day, Group 4: administered NS100 mg/kg/day, Group 5: administered NS 200 mg/kg/day. Values are means \pm S.D.

Table 1. Effect of NS on blood biomedical parameters in mice treated with NS after FST.

	BUN (mg/dl)	TP (mg/dl)	CK (U/l)	LDH (U/l)
Group I	42.44 ± 3.18	11.61 ± 1.99	1058.50 ± 427.72	5288.50 ± 2288.27
Group II	29.93 ± 7.96**	8.04 ± 1.97**	481.42 ± 334.58*	2561.28 ± 392.66*
Group III	23.42 ± 2.36**	6.75 ± 0.49**	55.16 ± 12.38**	1659.20 ± 346.36**
Group IV	16.26 ± 3.80**	6.05 ± 1.25**	90.14 ± 87.02**	1198.66 ± 370.65**
F	31.33	13.97	18.10	13.97
P	<0.001	<0.001	<0.001	<0.001

Values are the means ± S.D. (n = 7). * p < 0.05 indicates significant difference from the control group and ** p < 0.001 indicates significant difference from the control group. Group I: control mice administered distilled water, Group II: administered NS 50 mg/kg/day, Group III: administered NS 100 mg/kg/day, Group IV: administered NS 200 mg/kg/day.

Table 2. Thyroid function tests before and after supplementation of NS.

	TSH	T3	T4
Group I	0.30 ± 0.10	1.24 ± 0.20	67.66 ± 1.52
Group II	0.08 ± 0.02	1.75 ± 0.19*	74.33 ± 5.13
Group III	0.005 ± 0.01**	1.63 ± 0.12*	76.80 ± 8.07
Group IV	0.005 ± 0.01*	1.95 ± 0.05*	102.33 ± 7.50*
F	29.21	11.96	16.24
P	<0.001	<0.001	<0.001

Values are the means ± S.D. (n = 7). * p < 0.05 indicates significant difference from the control group and ** p < 0.001 indicates significant difference from the control group. Group I: control mice administered distilled water, Group II: administered NS 50 mg/kg/day, Group III: administered NS 100 mg/kg/day, Group IV: administered NS 200 mg/kg/day.

4. Discussion

First, we investigated the effect of NS on FST, which is commonly accepted an experimental exercise model used for assessing anti-depressive and anti-fatigue effects. Increases in concentrations of NS treated mice showed significant improvement in the swimming test. It suggests that the decreased duration of immobility in mice is related to change of certain metabolites in the system. These results agree with Roshan, who reported feeding NS could protect neural cell against oxidative stress [20]. In addition, there is evidence suggesting that the anti-depressant effect of NS was most probably related to its anti-oxidant effects [8] [17].

In order to investigate its mechanisms, we assessed biochemical serum markers after FST. BUN, CK, LDH and TP levels are related to fatigue. In the present study, CK level which is known to be an accurate indicator of muscle damage tended to decrease and less leakage of CK occurring during the swimming exercise. These results indicated that muscle damage in the treated groups, as reflected by the serum CK levels, was minimized by NS. Moreover, LDH is known to be an accurate indicator of muscle damage [21]. Therefore, the LDH serum level increases after exercise. In this study, however, the LDH level tended to decrease; thus, our results indicate that NS could influence the fatigue metabolism. Also, NS could significantly reduce BUN level which may be due to inhibition of stimulation of sympathetic nervous system. In fact, further studies are needed to clarify the detailed mechanisms involved in the anti-fatigue-like properties of NS in order to support present results.

The main findings of our study were that NS significantly increases the concentration of T3 and T4 and decreases the TSH in experimental groups compared to untreated mice. These results are in agreement with previous studies by Sharif *et al.* who indicated that treatment with oral administration of NS increased T4 levels in rabbits [18]. These results indicated that oral administration of NS lead to hyperthyroidism in mice; likewise, other study reports that the oral administration of NS not only increased serum T3 and decreased TSH but also has an anti-oxidant effect [22]. Yet, Marel reported that treatment oral administration with NS could raise T3 level without changing T4 and TSH serum concentration levels [23]. Further work is suggested for evaluating the effect of NS on the serum concentration of thyroid hormones to clarify the possible mechanism of action.

5. Conclusion

In brief, in the present study, swimming time in NS-fed groups increased during FST. NS also increased the blood concentration of T3 and T4, leading to the decrease of TSH serum level. Taken together, it is suggested that NS not only has anti-depressive effect and anti-fatigue like effects that might be useful in the development of physical strength, but also leads to hyperthyroidism in mice, so it can be tested in thyroid function disorders.

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