ORIGINAL ARTICLE

EFFECT OF NIGELLA SATIVA ON SERUM CONCENTRATIONS OF THYROID HORMONES AND THYROID STIMULATING HORMONE IN ALLOXAN-INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Background: Thyroid hormones and thyroid stimulating hormone levels are altered in diabetes mellitus. This study aims to assess the effect of Nigella Sativa (NS) seeds on serum concentrations of these hormones in type 1 diabetes.

Material & Methods: It was a Randomized Control Trials study on 30 albino rats. They were divided into Controls, Diabetics, and NS-treated Diabetics groups. Alloxan was used to produce type 1 diabetes. Control group animals were neither given NS seeds nor alloxan. Diabetic animals were given only alloxan. NS-treated diabetic animals were given both alloxan and NS seeds. After 12 weeks, blood was collected for estimation of these hormones. Statistical analysis was done using SPSS version 16.0..

Results: The mean concentrations of serum T3 in control, diabetic, and NS-treated groups were 0.78 ±0.16 ng/ml, 0.45 ±0.07 ng/ml and 0.55 ±0.07 ng/ml respectively, mean concentration of T4 were 3.65 ±0.52 μg/dl, 2.25 ±0.32 μg/dl and 2.82 ±0.42 μg/dl respectively, while that of TSH were 0.75 ±0.13 μU/ml, 1.42 ±0.15 μU/ml, and1.03±0.22 μU/ml respectively. The levels of T3 and T4 were significantly ($p<0.001$) decreased in diabetic group as compared to controls. However, in NS-treated diabetic group, serum T3 and T4 were significantly ($p<0.001$) increased as compared to diabetics. The mean concentration of TSH was significantly ($p<0.001$) increased in diabetic group as compared to controls. However, in NS-treated diabetic group, the mean value of serum TSH was significantly ($p< 0.001$) decreased when compared with diabetics.

Conclusion: This study concludes that T3, T4 levels were increased whereas that of TSH decreased significantly in diabetic animals after treatment with Nigella sativa.

Key words: Nigella Sativa; Type 1 Diabetes; Thyroid hormones; Thyroid stimulating hormone.

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INTRODUCTION

Nigella sativa (NS) is a member of botanical family Ranunculaceae and is commonly found in Western Asia, Middle East, and Europe. In English language, NS is known as black seed; while in Pakistan and India it is called as Kalvanji. In Arab countries, the names for NS seed are Khodria, Schuniz, and Al-Habbah Al-Sawda.[1] NS seed is used as a natural remedy for many diseases like asthma, rheumatism, diarrhea, skin disorders worldwide.[2] Nigella sativa has been widely investigated for its biological activities and therapeutic potential and shown to possess wide spectrum of activities i.e. antidiabetic, antihypertensive, anticancer and immunomodulatory, spasmolytic, renal protective, analgesic and anti-inflammatory, diuretic, and anti-oxidant properties. It is also used as appetite stimulant, digestive agent, hepatotonic, and to support body immune system.[3-8] Thymoquinone is the major chemical agent of the NS oil used for treatment of different diseases. NS seeds are used in foods as a flavoring agent in pickles and

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breads due to least toxicity.[9]

Various studies show that thyroid hormones (T_3 & T_4) and thyroid stimulating hormone (TSH) concentrations change in DM. The plasma concentrations of T_3 and T_4 are lowered, whereas that of TSH is raised in type 1 diabetes mellitus. Association between diabetes and thyroid disorders and their mutual influence have been reported by different studies.[10] It has been reported that both subclinical and overt hypothyroidism were more frequent among diabetic patients, having significantly lowered concentrations of both T_3 and T_4 and increased concentration of TSH as compared to normal subjects.[11-13] Thyroid dysfunction and thyroid autoimmunity are widely prevalent in young population with type 1 DM. The close relationship between type 1 DM and hypothyroidism suggests that all type 1 diabetic patients should undergo yearly screening by measurement of serum TSH to detect thyroid dysfunction.[14]

Therefore, this study was done to assess the effects of NS seeds (in powdered form) on the altered levels of serum thyroid hormones and thyroid stimulating hormone in type 1 DM.

MATERIAL AND METHODS

This study was conducted in Animal House of Postgraduate Medical Institute (PGMI), Lahore, Pakistan, from 1st December, 2010 to 1st March, 2011. It was an experimental animal study (Randomized Control Trials), on 30 albino rats, equal in number of either sex, weighing 180-230 gm (average weight about 200 gm), extending over a period of twelve weeks. This study was approved by ethical committee of PGMI, Lahore. Average age of the animals was 10 weeks. The animals were obtained from Animal House of National Institute of Health (NIH), Islamabad, Pakistan. Before starting the experiment, they were acclimatized for 2 weeks. During this period, they were given daily food and water at libitum and kept in iron cages under hygienic conditions and optimum temperature (24±2°C). The animals were divided into three groups i.e. Control, Diabetic, and NS-treated diabetic. Each group comprised of ten animals of either sex, i.e. 5 males and 5 females rats. After acclimatization for two weeks, procedure was started. Alloxan, a diabetogenic agent, was used to produce animal models of type 1 diabetes. Intraperitoneal injection of 150mg/kg bwt of 10% alloxan dissolved in 0.9% NaCl was given to animals in the latter two groups. Animals in control group were injected only with the same volume of 0.9% NaCl. After 72 hours, DM was confirmed by determining the glucose concentration with glucometer. Animals in diabetic and NS-treated groups demonstrated hyperglycemia (blood glucose>350mg/dl).

Nigella sativa seeds were made free of particulate impurities manually and washed with tap water thrice to remove all the dust. Then the seeds were placed on a steel tray, in an incubator. They were dried at 40°C for one hour and powdered. Until preparation of the diets, the prepared powder was kept in clean, air-tight glass bottles in a refrigerator at 4°C. The diets were prepared according to the table-1. Percentage composition of each ingredient is given. Diet (D1) was given to animals in control and diabetic groups. Diet (D2) was fed to animals in NS-treated diabetic group.

Prepared diets were dispensed in special containers purchased from local market, placed in the cages. Before starting to feed the animals, each rat was weighed. Then weight measurements were made on weekly basis. To ensure that rats in NS-treated diabetic group consume the required dose of NS seed powder (30mg/kg bwt), each rat was kept in separate cage. The required dose of NS was mixed with 10 gms of experimental diet and was served to each rat twice a day. Rats in control and diabetic groups were fed on control diet at a rate of 10 gms of diet per rat. Food was changed twice daily at interval of 12 hours i.e. at 08.00 AM and 08.00 PM. Water was made available ad libitum.

Blood sampling was done at the end of 12th week. After an overnight fasting of animals, samples were collected in the morning by cardiac puncture with 5 ml sterile disposable syringes after giving deep ether anesthesia. About 4 ml blood was collected from each rat and directly transferred to a micro-test tube for T3, T4, and TSH estimation. Blood was centrifuged and serum was separated. Serum for hormones estimation was stored at -20°C in freezer till the time of measurement. Concentrations of serum T3, T4, and TSH were estimated by using ELISA kits.

The data were entered into computer using SPSS-16 version for analysis. The data were described in terms of mean ±standard deviation (SD). Statistical significance was calculated using one-way ANOVA (analysis of variance) by computing F-ratio among the three groups. A 5% significance level ($p<0.05$) was used as per convention. In all the significance testing p-values were also calculated. Tukey's HSD (honestly significant difference) was used to know the differences observed within any two groups.

RESULTS

Serum T3 concentration is shown in table-2. Mean concentration of T3 was 0.78 ±0.16 ng/ml for animals in control group. For animals in diabetic group and NS-treated diabetic group, the mean concentration of serum T3 was 0.45 ±0.07 ng/ml and 0.55 ±0.07 ng/ml respectively. The mean concentration of serum T3 was significantly ($p<0.001$) decreased in animals of diabetic group as compared to that of animals in control group. However, in animals of NS-treated group, mean serum T3 concentration was significantly ($p<0.001$) increased compared to

Table-1: Percentage (%) composition of control and experimental diet

Ingredients	D1(gm/100gm)	D2(gm/100gm)
Wheat starch	62.1	62.1
Casein	20.0	20.0
Glucose	10.0	10.0
Choline & Methionine	0.5	0.5
Mineral mixture	3.5	3.5
Vitamin mixture	1.0	1.0
Fat	2.9	2.9
Nigella sativa (30mg/kg bwt)	–	+

(Adapted from Dahri AH et al, 2000)

Key: D1:Control diet.(−):Nigella sativa was missed.
D2: Experimental diet.(+): Nigella sativa was added.

Table-2: Mean serum concentrations of T3, T4, and TSH

Parameters	Control	Diabetic	NS-treated diabetic	P-value
T3 (ng/ml)	0.78 ±0.16	0.45 ±0.07	0.55 ±0.07	0.001
T4 (μg/dl)	3.65 ±0.52	2.25 ±0.32	2.82 ±0.42	0.001
TSH (μU/ml)	0.75 ±0.13	1.42 ±0.15	1.03 ±0.22	0.001

Results are expressed as mean ±SD.

diabetic group.

Serum T4 concentration is shown in table-2. The mean serum T4 concentration was 3.65 ±0.52 μg/dl for animals in control group. For animals in diabetic group and NS-treated diabetic group, the mean serum T4 concentration was 2.25 ±0.32 μg/dl and 2.82 ±0.42 μg/dl respectively. The mean concentration of serum T4 was significantly ($p<0.001$) decreased in animals of diabetic group as compared to that of animals in control group. However, in animals of NS-treated diabetic group, the mean serum concentration of T4 was significantly ($p<0.001$) increased compared to diabetic group.

Serum TSH concentration is shown in table-2. The mean serum concentration of TSH was 0.75 ±0.13 μU/ml for animals in control group. For animals in diabetic group and NS-treated diabetic group, the mean serum concentration of TSH was 1.42 ±0.15 μU/ml and 1.03±0.22 μU/ml respectively. The mean concentration of serum TSH was increased significantly ($p<0.001$) in animals of diabetic group as compared to that of animals in control group. However, in animals of NS-treated diabetic group, the mean value of serum TSH was significantly ($p<0.001$) decreased compared to diabetic group.

DISCUSSION

Changes in level of thyroid hormones are a common feature in diabetic patients.[15] There is interaction between thyroid hormones and insulin for cellular metabolism so that DM and thyroid diseases can mutually affect each other. When DM occurs in individuals with normal thyroid functions, it results in abnormal thyroid function tests with no clinical manifestation.[16] Although, so far, the causes of thyroid dysfunction in DM are unknown, it has been observed by different researchers that metabolic changes caused by DM, or lack of insulin itself, can affect some aspects of thyroid function directly.[17] Thyroid disease frequency increases with age and hypothyroidism is the most common thyroid disorder in the normal and diabetic populations.[12] The available literature regarding the use of NS seeds for alleviation of deranged serum levels of TSH and T_3, T_4 in diabetics is very meager and, at best, inconclusive. Therefore, this study was done to investigate whether NS seeds could normalize the altered levels of TSH and thyroid hormones in experimentally-induced diabetes in albino rats.

At the end of 12 week, significantly decreased concentrations of serum T_3 and T_4 were observed in diabetic animals as compared to control group animals whereas serum concentration of TSH significantly increased in diabetic animals compared to control group animals. Other studies have also shown that in type 1 diabetes, patients had lower serum T_3 and T_4 levels and comparatively increased TSH level than the non-diabetic subjects.[18-20] The most prevalent immunological diseases in type 1 diabetics are autoimmune thyroid disorders. The prevalence of positive thyroid peroxidase (TPO) an-

tibodies has been reported in about 80% of patients with type 1 DM and increased TSH and decreased T_4 levels in these patients as well. These studies have also suggested that serum TSH screening is more sensitive for detecting thyroid abnormalities in children and adolescents with type 1 diabetes, the presence of positive serum anti-TPO antibodies may be an earlier marker for thyroid disease, therefore, serum TSH estimation should be done in patients with positive antibodies at yearly intervals.[14]

In our study, upon administration of NS seed powder in a prescribed dose to diabetic animals in NS-treated diabetic group for 12 weeks, the serum concentrations of the respective hormones reversed significantly i.e. the serum concentrations of both T3 and T4 increased whereas that of TSH decreased significantly. Only one study is available conducted by Meral et al, showing the effect of an extract of NS on serum concentrations of thyroid hormones in alloxan-induced diabetic rabbits.[21] Their study has shown significant increase in the level of serum T_3 which is in accordance with the results of our study. The same study showed no improvement in the levels of both T_4 and TSH which is in contrast with the results shown in our study. Our study indicated significant increase in serum T4 and decrease in serum TSH levels at the end of 12 weeks. They used a small sample size and also the duration of their study was 8 weeks, in contrast, we used an almost double sample size and extended the experiment for 12 weeks. So further longitudinal studies using larger study sample and employing varying doses of NS must be carried out to elaborate and ascertain the complete efficacy of this treatment in terms of normalization of all these study parameters.

CONCLUSION

The present study concludes that levels of serum T3 and T4 increased whereas that of TSH decreased significantly in diabetic animals after treatment with Nigella seed powder for 12 weeks. These altered levels of thyroid hormones improved significantly but not completely. So the administration of powdered NS seeds could partially normalize the altered serum levels of these hormones in type 1 DM. Therefore, it is advisable that Nigella sativa may be beneficial as an adjunct to other existing diabetic therapies.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
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None declared.

AUTHORS' CONTRIBUTION

Conception and Design:	SZ, AI
Data collection, analysis & interpretation:	SZ, AI, AR
Manuscript writing:	SZ, AI, AR, RJ