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Impact of alcoholic extract of *Nigella sativa* on serum levels of thyroid hormones and transforming growth factor β (TGF-β) in male rats with induced hypothyroidism

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ABSTRACT

Background: Hypothyroidism is also associated with metabolic derangements, oxidative stress and enhanced inflammatory mediators that could result in tissue damage. As a way, natural products like Nigella sativa have received more attention because of their antioxidant, anti-inflammatory and immunomodulatory effects. Objectives: This work was conducted to investigate the effect of alcoholic extract of Nigella sativa on transforming growth factor-β (TGF-β) in hypothyroid male rats.

Methods: A total 60 male rats were randomly assigned into three experimental groups. The G1 was not treated and fed a normal diet. Group II - received alcoholic extract of Nigella sativa. Another group was the experimentally induced hypothyroid rats. The treatments were continued for 60 days. Blood samples were taken at the end of test period, and TGF-β levels in serum were determined by using established biochemical assays. Differences between groups were assessed by statistical analysis at a significance level of $P < 0.05$.

Results: TGF-β levels differed statistically in the experimental groups. TGF-β concentration in the hypothyroid group was significantly higher than that in the G1. Conversely, rat treatment with Nigella sativa extract resulted in decreased TGF-β levels compared to the hypothyroid group. The present findings indicate the potential to mitigate inflammatory response related to thyroid dysfunction by the plant extract.

Conclusions: Nigella sativa alcoholic extract also might have protective effects against hypothyroidism-related inflammation by modulating TGF-β levels. Conclusively, these results suggest potential therapeutic significance for Nigella sativa as a natural agent in the management of endocrine-related inflammatory disorders. More studies are needed to clarify its mechanisms.

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INTRODUCTION

Hypothyroidism, which is one of the most common endocrine disorders seen in clinical practice, results from inadequate production of thyroid hormones mainly triiodothyronine (T3) and thyroxine (T4) that have a role in metabolic processes, growth regulation and maintenance of physiological homeostasis. The disorder can be caused by autoimmune diseases, iodine deficiency, drug-induced thyroid dysfunction or damage to thyroid tissue. Reduced levels of thyroid hormones cause many physiological disturbances like metabolic inhibition, poor energy homeostasis and disruption in multiple organ systems (Farhangi et al.,2016).

Animal models are widely used to investigate hypothyroidism and test new therapeutic drugs. Hypothyroidism in laboratory animals is commonly induced using antithyroid drugs (i.e. propylthiouracil, PTU; carbimazole) that block thyroid hormone synthesis and

constitute the most reproducible endocrine setting to study changes and therapeutic intervention. Such models are useful for elucidating the biochemical and molecular alterations induced by a deficiency of thyroid hormone and for testing natural or pharmacological agents that can restore hormonal homeostasis (Asiaei et al., 2017). Hypothyroidism is not only limited to metabolic derangement, but also associated with oxidative stress and inflammation and dysregulation of intracellular signaling pathways leading to tissue injury and systemic complications (Mancini et al., 2016).

Transforming growth factor beta (TGF-β) is one of the signaling molecules involved in inflammatory and fibrotic processes which has garnered much interest. TGF-β is a pleiotropic cytokine that inhibits cell proliferation, induces differentiation and apoptosis, as well as production of extracellular matrix components. It is involved in tissue remodeling and immunoregulation, and abnormal expression of this mediator has been associated with a variety of pathological processes including autoimmune disease, fibrosis, and endocrine diseases (Sanjabi et al., 2017). There is evidence that changes in thyroid hormone levels can alter (activate) cytokines expression, including fibrogenics such as TGF-β, modulating inflammatory responses and signaling pathways in endocrine tissues. The relationship between thyroid dysfunction and TGF-β signaling should shed light on the molecular mechanisms of hypothyroidism-associated tissue damage (Lasa & Contreras-Jurado, 2022).

In recent decades, there has been increasing interest in using medicinal plants as potential therapeutic agents for endocrine disorders. Herbal medicine use are mostly popular because of availability, inexpensive and possible pharmacological properties. Among these, Nigella sativa L., which is known as black seed or black cumin, has been widely studied for its activity in various biological aspects. The seeds of Nigella sativa were traditionally, for centuries have been used in many countries (Middle Eastern, Asian & North African) to treat diseases such as inflammation, metabolic disorders and immune dysfunction (Farhangi et al., 2016).

Different clinical and experimental studies were conducted to approve the therapeutic potential of Nigella sativa. Extracts or oils from Nigella sativa are known to positively impact metabolic parameters, oxidative stress, and hormonal activity according to multiple reports. For instance, its administration has an effect on thyroid stimulation by changing levels of hormones effective on thyroid and lowering indices of thyroid function. A randomized controlled trial showed a significant reduction in serum thyroid-stimulating hormone (TSH) and an increase in triiodothyronine (T3), indicating improved status of the thyroid, as a result of providing these patients with Hashimoto's Thyroiditis Nigella sativa supplementation (Overduin et al. 2016). Likewise, experimental studies in animals showed that the oil of Nigella sativa is capable to affect thyroid hormone levels and antioxidant capacity positively in models for thyroid dysfunction (Avci et al., 2022).

Most of the benefits of Nigella sativa is mediated by its antioxidant and anti-inflammatory activities. Oxidative stress is another factor playing a significant role in the pathogenesis of hypothyroidism and other endocrine disorders, resulting in cellular injury as well as disruption of hormonal signaling pathways. Thymoquinone, an antioxidant component of Nigella sativa, has been found to scavenge free radicals and increase the activity of antioxidant enzymes while decreasing inflammatory mediators. These features might protect the thyroid against oxidative damage and promote restoration of normal endocrine function (Elghareeb et al., 2024).

There has been growing evidence on potential pharmacological efficacy of Nigella sativa, but very few studies have explored therapeutic effects of Nigella sativa on cytokine signaling pathways, particularly TGF-β in cases of dysfunctional thyroid. The alcoholic extract of Nigella sativa can modulate TGF-β levels in hypothyroidism, which is important to help elucidate the mechanisms in the protective or therapeutic effects on the medicinal plant; More studies will be needed to carefully characterize this extract. These kinds of investigations are crucial because cytokine-induced inflammation and tissue remodeling play key roles especially during the progression of endocrine disorders (Ahmad et al., 2013).

Thus, the aim of the current study was to assess serum thyroid hormones and TGF-β levels following administration of alcoholic extract of Nigella sativa in male rats with induced experimental hypothyroidism. Analysis of hormonal and cytokine changes can offer insight into the therapeutic potential of Nigella sativa in mediating endocrine and inflammatory pathways implicated in thyroid dysfunction.

METHODS

Experimental Animals

The study is experimental, and it was conducted on 36 healthy adult male albino rats with weights of (220–260 g). The animals were obtained from the animal house of College of Veterinary Medicine, University of Karbala — Iraq at which this experiment was executed. Rats were kept in well-ventilated, polypropylene cages under standard laboratory conditions using a 12-h light/12-h dark cycle with controlled temperature (22–25°C) and relative humidity of approximately 50–60%. Animals were given free access to standard pellet diet and drinking water ad libitum during the experiments. Prior to the experiment, animals under laboratory conditions were acclimatized for two weeks to minimize environmental stress. All the experimental protocols were implemented according to the guidelines established for the care and handling of laboratory animals, approved by the Animal Ethical Clearance Committee, University of Karbala.

Preparation of Alcoholic Extract of Nigella sativa.

Nigella sativa seeds were obtained from local markets of Karbala, Iraq and the botanical identity was verified by one expert in Department of biology, College of Science, University of Karbala. The seeds were washed, air dried and subsequently milled into

a fine powder by means of an electric grinder. Powdered seeds were macerated with 95% ethanol for extraction. Four hundred mL of ethanol was added to approximately 100 g powdered seeds and incubated at room temperature for 72 h with intermittent shaking. The final mixture was filtered through Whatman No.1 filter paper to remove solid residues. The obtained filtrate was thoroughly concentrated at reduced pressure at 40 °C by rotary evaporator to obtain a viscous semi-solid extract. The extract was kept in the dark glass bottles at 4 °C until use. The extract was freshly prepared in the study and were supplied freshly during test to maintain its active constituents stability.

Experimental Design

Following the acclimatization period, rats were randomly assigned to four experimental groups of twelve animals each (n = 12):
Group 1 (G1): (Hypothyroid group + distilled water): rats were administered distilled water orally over the experimental period.
Group 2 (Hypothyroid group + Nigella sativa group 100 mg/kg): rats with induced hypothyroidism were treated by alcoholic extract of Nigella sativa with dose 100 mg/kg body weight/day orally.
Group 3 (Hypothyroid + Nigella sativa group 200 mg/kg): rats with induced hypothyroidism were treated by alcoholic extract of Nigella sativa with dose 200 mg/kg body weight/day orally.
Six rats from each group were scarified after 30 days; while the other 6 rats were scarified after 60 days of treatment; body weights were recorded every week and the dosage was adjusted to account for fluctuations in body weight. General health status, activity levels, feeding behavior and signs of toxicity were observed daily.

Induction of Hypothyroidism

Rats of Groups 3 and 4 were experimented to induce hypothyroidism with propylthiouracil (PTU), which is an antithyroid drug that prevent thyroid hormones synthesis by inhibiting the activity of thyroid peroxidase. PTU (10 mg/kg body weight/day) dissolved in drinking water was given orally for 4 weeks to induce hypothyroidism. Such a treatment regimen is commonly applied to generate a reproducible hypothyroidism model in laboratory animals. Induction of hypothyroidism was successful as evidenced by assessment of serum thyroid hormone (T3 and T4) and TSH (thyroid-stimulating hormone) concentrations, because lower levels of the thyroid hormones with stimulation of TSH reflect a state associated with hypothyroidism.

Blood Sampling and Biochemical Analysis

At the end of experimental period rats were fasted overnight and then anesthetized by light ether anesthesia. Blood samples were obtained from cardiac puncture and under sterile conditions. Blood was then transferred into plain tubes and clotted at room temperature. Serum was obtained from this sample by centrifuging samples for 10 min at 3000 rpm. Serum samples were kept at −20°C before being assayed biochemically.

Biomarker serum levels were assessed as follows:

1. Thyroid-stimulating hormone (TSH)
2. Triiodothyronine (T3)
3. Thyroxine (T4)
4. Transforming growth factor-β (TGF-β)

Commercial enzyme-linked immunosorbent assay (ELISA) kits specific for rat hormones were used to determine TSH, T3 and T4 concentrations according to the manufacturer's protocols. Serum TGF-β levels were also detected by using a rat-specific ELISA kit according to the sandwich immunoassay technique. Each was performed in duplicate, all measured to ensure accuracy and reproducibility.

Statistical Analysis

Statistical analysis was carried out through Statistical Package for the Social Sciences (SPSS) software version 25. Data are presented as mean ± SD. Differences between experimental groups were assessed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test to identify pairwise differences between the groups. Statistical significance was defined as p-value < 0.05.

RESULTS

The serum levels of thyroid hormones in male rats after the 30 day treatment period with alcoholic extract of Nigella sativa is shown in Table 1. The results also note small variations in TSH, T3 and T4 levels in the groups studied. However, statistical analysis indicated that none of these differences were significant (P > 0.05). Rats that treated with nigella sativa extract (G2) showed an increasing level of T3 and T4 slightly with low TSH as compared to the G1, which may reflect an improvement of the activity of the thyroid gland. On the other hand, the hypothyroid group (G3) showed increased TSH levels and decreased levels of T3 and T4 which corroborates with the hormonal response usually attributed to hypothyroidism. Despite these tendencies, none of the differences were statistically significant, indicating that a 30-day treatment may not be necessary for significant alterations to occur in circulating thyroid hormone concentrations under the experimental conditions utilized in the present study. This pattern may indicate an early stage of endocrine compensation, or it may be that longer duration or a higher dose is necessary for physiological effects to achieve significance.

Table 1. Thyroid hormones levels in after 30 days administration of Nigella sativa alcoholic extract in male rats

Groups	TSH (µIU/mL) Mean±SD	T3 (ng/dL) Mean±SD	T4 (µg/dL) Mean±SD
G1	1.82 ± 0.35	98.40 ± 8.12	5.61 ± 0.74
G2	1.76 ± 0.31	101.25 ± 9.05	5.74 ± 0.69
G3	1.90 ± 0.40	95.60 ± 7.85	5.48 ± 0.71
P value	0.34 NS	0. 29 NS	0.42 NS

NS: Non-significant at P >0.05

Table 2 also shows the effect of alcoholic extract of Nigella sativa on serum Transforming growth factor beta (TGF-β) levels in hypothyroid male rats after treatment for 30 days. The hypothyroid control group (G1) treated with distilled water had average TGF-β level of 26.80 ± 3.45 Rats treated with Nigella sativa extract, however, exhibited a gradual decrease of TGF-β levels, being 24.60 ± 3.12 in the (G2) group and 22.95 ± 2.84 for the (G3) group. The data showed a dose-dependent reduction of levels of TGF-β following treatment with increasing doses of nigella sativa; yet statistical analysis confirmed that these differences were not significant ($P = 0.08$; $P > 0.05$). This means that in the period applied for the experiment and within the doses, Nigella sativa alcoholic extract does not significantly modulate TGF-β levels in hypothyroid rats.

Table 2. TGF-β levels in study groups after 30 days administration of Nigella sativa alcoholic extract in male rats

Groups	TGF-β Mean±SD
G1	26.80 ± 3.45
G2	24.60 ± 3.12
G3	22.95 ± 2.84
P value	0.08 NS

NS: Non-significant at P >0.05

The Serum concentrations of thyroid hormones of male rats after 60 days of treatment with the alcoholic extract of Nigella sativa are shown in Table-3 Salivary thyroid hormone changes demonstrated significant differences between groups over the longer treatment period, consistent with an experimental effect. TSH, T3 and T4 remained within the physiological range for healthy rats over time in the G1. An increase in the T3 and T4 level with a slight decrease in TSH in the second group treated with Nigella sativa extract demonstrated that the plant extract probably enhances gland function or stimulate hormone synthesis and/or secretion by the gland itself. The effects could be explained through the bioactive components of Nigella sativa, especially thymoquinone which has an antioxidant and endocrine modulatory activity. In contrast, the hypothyroid group (G3) showed a significant increase in TSH level along with a significant decrease in T3 and T4 levels, a hallmark of a hypothyroid state. Elevated serum TSH represents a compensatory response by the pituitary gland to lower circulating thyroid hormone levels. Analysis using statistical methods showed a highly significant difference ($P = 0.003$) for TSH level and significant difference ($P < 0.05$) for T3 and T4 levels between the study groups. An extended treatment duration, as seen in 60-day treatments, may cause more significant hormonal changes than is seen with more standard shorter treatment durations, emphasizing that treatment duration is an important factor in determining the physiological effects of herbal extracts.

Table 3. Thyroid hormones levels in after 60 days administration of Nigella sativa alcoholic extract in male rats

Groups	TSH (µIU/mL) Mean±SD	T3 (ng/dL) Mean±SD	T4 (µg/dL) Mean±SD
G1	1.80 ± 0.32 A	100.45 ± 7.60 A	5.70 ± 0.68 A
G2	1.62 ± 0.28 A	108.30 ± 8.12 B	6.15 ± 0.72 B
G3	3.42 ± 0.55 B	70.85 ± 6.95 C	3.60 ± 0.58 C
P value	0.003 HS	0.03 S	0.02 S

S: Significant at P <0.05: HS; High Significant at P <0.01
A,B,C different letters refer to significant difference at P <0.05

The results in table 4 show the effect of differences in using alcoholic extract of Nigella sativa on serum levels of Transforming growth factor beta (TGF-β) after 60 days treatment in male rats with hypothyroidism. Group (G1), which received distilled water

as the hypothyroid control, showed the highest mean level of TGF-β 30.25 ± 3.88. On the other hand, once rats were treated with the alcoholic extract of Nigella sativa, there was a significant decrease in TGF-β. The mean value of group administered with 100 mg/kg (G2) was 24.60 ±3.05, whereas that of the other group fed 200 mg/kg (G3), showed a low reading (21.80 ± 2.74). Statistical analysis showed significant difference between the study groups (P = 0.04). Different letters superimposed (A, B, C) indicate significant differences between groups at P < 0.05 where the administration of Nigella sativa extract resulted in a dose-dependent decrease in TGF-β contents compared with the hypothyroid control group after such an extended use period.

Table 4. TGF-β levels in study groups after 60 days administration of Nigella sativa alcoholic extract in male rats

Groups	TGF-β Mean±SD
G1	30.25 ± 3.88 A
G2	24.60 ± 3.05 B
G3	21.80 ± 2.74 C
P value	0.04 S

S; Significant at P <0.01

A,B,C different letters refer to significant difference at P <0.05

DISCUSSION

This study aimed to assess the impact of 30 days of oral Nigella sativa alcoholic extract administration on the concentration of the transforming growth factor-beta (TGF-β) in male rats. Statistical analysis of results found that there is no statistically significant difference among groups (G1 vs treated group G2 vs G3). P value (P = 0.08 values) is greater than 0.05. These results indicate that Nigella sativa extract for the experimental time course produced no significant changes in TGF-β among the experimental animals.

Transforming growth factor-beta (TGF-β) is a multi-functional cytokine that regulates diverse biological processes, including cell proliferation, immune regulation, tissue repair, and extracellular matrix production. In addition, it is also known to be involved in the regulation of inflammatory response or/and in fibrotic processes in different tissues. Involved in pathogenic processes like chronic inflammation, fibrosis, and tissue injury, alterations in TGF-β expression are common. Consequently, measuring its degree in preclinical investigations is crucial for assessing promising therapeutic treatments for any anti-inflammatory and/or antifibrotic potential (Tie et al., 2022).

The absence of statistical significance for changes in TGF-β levels following treatment with Nigella sativa alcoholic extract in the present study may instead reflect the dosage and treatment duration did not allow for quantifiable differences in this cytokine to be achieved in healthy tissue. An alternative explanation is that the basal TGF-β levels are stable in healthy animals under normal physiologic conditions and the effects of Nigella sativa on TGF-β may only manifest under pathological conditions such as oxidative stress, inflammation, and metabolic disorder (Darand et al., 2019). Various other studies previously published have indicated that Nigella sativa has strong anti-inflammatory, antioxidative and immunomodulatory properties possibly influencing cytokine production and signaling pathways. The biological activities of Nigella sativa mainly ascribe to its bioactive compounds, of which thymoquinone has been proven to modulate inflammatory mediators and perturb oxidant/antioxidant balance (Arshad et al., 2025).

An experimental study evaluating the protective role of Nigella sativa oil in irradiated rats found that irradiation increased levels of inflammatory mediators and fibrogenic markers such as TGF-β, and Nigella sativa treatment significantly improved tissue repair and regulated these inflammatory parameters. This indicates that Nigella sativa seems to modulate TGF-β expression state-dependent, especially in regards to tissue injury or stress (Radwan & Mohamed, 2018).

In a similar line of studies on the model of diabetic nephropathy, it was found that the administration of Nigella sativa oil or its active compound thymoquinone resulted in a significant down-regulation of expression of TGF-β1 and other extracellular matrix genes in diabetic kidneys (Al-Trad et al., 2016). The authors indicated that the protective effects of Nigella sativa were linked to inhibition of fibrotic pathways and maintenance of renal tissue structure. These results additionally converge that Nigella sativa dubiously influence TGF-β signaling in diseased conditions where inflammation and fibrosis are predominant in nature.

In another experimental study, they found that Nigella sativa decreased expression of TGF-β1 in experimental autoimmune encephalomyelitis rats, implicating it as a modulator of inflammatory and immune response. These findings demonstrate the plant's immunomodulatory activities and indicate that, at least for the TGF-β pathway, the effects of the plant may be contingent on (disease) state or presence of immune activation (Mohamed et al., 2015).

These findings had also been confirmed through more recent histological and immunohistochemical studies. Similarly, irradiation prompted evident fibrosis and higher expression of TGF-β in a rat parotid gland model of radiation-induced damage. Although the TGF-β expression was significantly higher in Ni–Ni than in Ni rats, Nigella sativa oil treatment greatly downregulated the TGF-β and fibrotic effect of the Ni, and successfully reduced the excess tissue fibrosis (Ahmed & Bakr, 2024).

The non-significant difference as shown in the present findings contrasts with these studies and could be attributed to differences in experimental designs including health of the experimental animals, extract preparation, dosage, treatment duration and targeted tissues. The majority of the studies reporting a significant modulation of TGF-β were conducted during complex disease models of e.g. diabetes, radiation injury or autoimmune inflammation. On the other hand, under the physiological condition, the effect of herbal extracts on cytokines is possibly more subtle (Ali et al., 2024).

TGF-β is a regulatory cytokine that maintains immune homeostasis, which could also contribute to the results observed. Under physiological environment, its generation is strictly regulated, and maximal oscillations may not be seen unless stimulated by tissue damage or inflammation. Thus, this lack of important modifications in TGF-β levels in the present study might imply that the administration of Nigella sativa did not disturb the appropriate immune equilibrium, a favorable sign of its safety in healthy beings (Sanjabi et al., 2017).

CONCLUSION

The results of this research demonstrate that oral administration of Nigella sativa alcohol extract for male rats does not alter the serum levels of TGF-β after 30 days. Nigella sativa for 60 days might have protective effects against hypothyroidism-related inflammation by modulating TGF-β levels. Conclusively, these results suggest potential therapeutic significance for Nigella sativa as a natural agent in the management of endocrine-related inflammatory disorders. Additional studies are needed to clarify its mechanisms.

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