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Comparison between the Efficacy of *Nigella Sativa* Aqueous Extract and Its Oil on Methimazole-induced Hypothyroidism in Albino Mice

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ABSTRACT

Objective: This study was conducted to evaluate the impact of both aqueous extract of *Nigella sativa* and its oil in treating the hypothyroid induced by methimazole in female of white mice in order to determine which one of them is more effective in treating of hypothyroidism, aiming to use it as a natural treatment instead of the chemical treatments which have dangerous side effects.

Materials and Methods: The study included 40 female white mice dived into 4 groups 10 mice each one. Group 1 (control): orally administered and treated with distilled water. Group 2 (HYP): orally treated with methimazole at a dose 0.05-mg/kg/ body weight/day for 3 weeks. Group 3 (NSE): the hypothyroidism was induced as in group 2, then treated orally with aqueous *Nigella sativa* extract at a dose of 400mg/kg/body weight/ day for 4 weeks. Group 4 (NSO): the hypothyroidism was induced as group 2, then treated orally with *Nigella sativa* oil at a dose of 1 ml/kg/body weight/ day for 4 weeks.

Results: This study showed that both NSE and NSO caused a significant decreasing $p<0.05$ of TSH level and a significant increase of FT3 level. Also, NSO caused increasing of FT4 level, while the increase of FT4 was un-significant under NSE treatment.

Conclusions: The efficacy of NSO is higher than the NSE in treating hypothyroidism.

Keywords: Aqueous extract, *Nigella sativa*, FT4, FT3, TSH, Hypothyroidism.

INTRODUCTION

Many medicinal plants have been used in curing illnesses for many years. The whole plant or some parts were used after soaking or boiling without determining the effective material. Due to the development of chemical and pharmaceutical sciences, the chemical effective materials of plants were isolated in order to determine the perfect form to use it (aqueous extract, alcoholic extracts by its various types or oils). Some of these plants are used in curing many diseases instead of chemical medications that have dangerous side effects. The World Health Organization (WHO) has designated medical plants within pharmaceutical safety standards in many countries.

Nigella sativa is seed of herbal plants belonging to Ranunculaceae family known as "Black cumin", its original habitat in the Mediterranean countries, Pakistan, and India [1]. It's rich in nutrients, 1000 g of *N. sativa* contains 210g of protein, 350g of carbohydrate, 350-380g of oils. Many amino acids were isolated and the most important amino acid are tyrosine and lysine [2]. Minerals (Cu, Zn, Se, Fe), vitamins (A, B1, B2, B3, C). It also contains many special secondary metabolites such as alkaloids (Nigellicimine, Nigellicimine-N-oxide, Nigellidine) and saponies [3]. it has also some special oil which contains saturated fatty acids (Linoleic acid, oleic acid) and many unsaturated fatty acids, it's mainly

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concentrated in fixed oil of Nigella Sativa, and also it contains essential oil and violate oil, that has the most effective components of Nigella Sativa ,which are phenols and terpenoid as thymoquinon, thymol, limonene, and p-cymen [4,5], and polyphenols compound as α,β,γ tocopherol (pro-vitamin E)[6] .

Nigella sativa was used in traditional medicine to treat headache, toothache, intestinal warms, nasal congestion, increase milk production, back pain, hypertension and gastrointestinal [7].

Recent studies have indicated to the efficiency *Nigella sativa* aqueous extract analgesic and anti-inflammatory [8], increasing the red blood cells and decreasing the white blood cells with modification in formula of white blood cells [9], Treating diabetes[10] . As well as *Nigella Sativa* oil increases the levels of (LH, FSH) [11], and treats hepatitis-c and non-alcoholic fatty liver disease (NADFL)[12,13], and it has the ability to treat most allergic and respiratory diseases because it inhibits the release of histamine and decreases the production of eosinophil and IL-10 [14].

The thyroid gland is the largest endocrine in the human body, it's controls metabolism [15], and sensitivity to other hormones. As well as regulate the growth and functioning of many other systems in the body[16] . It's secreting three hormones (Thyroxin T4, Triiodothyronine T3, and calcitonin). Thyroid stimulating Hormone produced by anterior lobe of the pituitary gland, regulate the production of hormones (T3, T4), also its under control of (TRH) [17]

Many Studies refereed to the existence of relationship between the use of *N. sativa* and increasing of thyroid gland hormones in blood serum. Where the oral delivery of *N. sativa* ethanol extract to thyroid healthy rats increases the levels of (T3, T4) hormones in blood serum[18]. As well as hydro-alcoholic extract increased the level of thyroid hormones thyroid of healthy white mice female too[19]. Another study clarified that using powdered *N. sativa* increased the level of T3 hormone in which had Hashimoto's thyroiditis [20].

MATERIALS AND METHODS

Experimental animals

In this study 40 adult Albino female mice/ Balb-c were used, with weights between 25-30g, obtained from the Scientific Research center, Damascus at the age 5-6 weeks, were placed in the physiology laboratory at Tishreen University for 4 weeks in order to adapt them to the condition of experiment (were placed in special cages furnished with sawdust, good ventilation, food made of wheat and dried bread and source of water), in addition to the light system(12 hours of lighting and 12 hours of darkness), and temperature of 28-30 c^{o}.

Plant Material

Taxonomic Classification of *Nigella Sativa*

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family : Ranunculaceae

Genus: *Nigella*

Species: *N. Sativa*

Plant material of Nigella Sativa Targeted to the aqueous extract was collected from experimental fields of the General Commission for Scientific Agricultural Research, Hama center.

Material was preserved in paper bags for further Extraction process .

Preparation of extract and oil *Nigella sativa*

-The aqueous extract of *Nigella sativa* was prepared according to the Herndez method [21]:

Seeds were crushed by the grinder, then 20g of seeds powder was added to 400ml of distilled water, it was mixed with a magnetic mixer for an hour, the mixture was left for 24 hours at laboratory room temperature, the mixture was then filtered using several layers of medical gauze to get rid of residue, the filtrate was distributed in plastic tubes, it was centrifuged at 300 rpm for 10 minutes,

the filtrate was taken and filtered again using filter papers which permeability is 0.01, the product was dried in the oven and then kept in the refrigerator until use.

-The *Nigella Sativa* oil:

The used oil was extracted using pressure and cooling method of seeds collected from local market of Hama city.

Experimentally induced hypothyroidism

Animals were received methimazole (TABAZOL) anti thyroid drug at dose of 0,05 mg/kg body weight / day for successive 3 week [22].

Experimental design

40 female mice were divided into 4 groups each group consisted 10 mice.

Group 1 (cont.): control group was treated orally with distilled water at a dose of 0.05 mg/kg/body weight /day for 3 week.

Group 2 (HYP): Hypothyroidism group, hypothyroidism was induced by oral delivery of methimazole at dose of 0.05 mg/kg/body weight/day for 3 weeks

Group 3 (NSA): hypothyroidism was induced as in group 2, and then it was treated orally with aqueous extract at dose of 400 mg/kg/ body weight/day for 4 weeks.

Group 4 (NSO): hypothyroidism was induced as in group 2, and then it was treated orally with oil at dose of 1ml /kg/ body weight/day for 4 weeks.

Sample collection

Animals in 4th group were killed with chloroform and blood sample were taken from heart by cardiac puncture. Plasma was separated by centrifuge at speed 3000 rpm for 30 minutes then placed in plain containers and stored at 4^{o}c until analysis.

Hormone estimation

The levels of TSH, FT4 and FT3 were determined using the Siemens® Kit, Siemens healthcare diagnostics products Ltd, UK by IMMULITE- 1000/ Siemens® , the device uses the immunoassay analyzer system, which based on competitive binding of hormones.

Results:

Table (1): Thyroid function tests before and after supplementation of *Nigella Sativa*

Group	TSH ulU/ml	FT3 pg/dl	FT4 g/dl
Group 1 (cont)	0.12±0.03 A	C 412.20±75.80	C 1.93±0.56
Group 2 (HYT)	B 2.13±1.03	A 191.90±49	A 0.82±0.14
Group 3 (NSA)	A 0.42±0.01	B 339.80±34.43	AB 1.02±0.35
Group 4 (NSO)	A 0.05±0.02	B 341.20±54.51	B 1.20±0.23
LSD 5%	0.48	50.89	0.33

Values are the means ± S.D. (n = 10). Group 1: control mice administered distilled water, Group 2: administered Methimazole 0.05 mg/kg/day, Group 3: administered *Nigella Sativa* aqueous Extract 400mg/kg/day, Group 4: administered *Nigella Sativa* oil 1ml/kg/day.

Comparison of the means value of TSH in studied group:

Table (1) and figure (1) show a significant increase occurring ($p<0.05$) means value of TSH after induced hypothyroidism and significantly decrease after the treatment by *Nigella Sativa* aqueous extract and *Nigella Sativa* oil. There were no significant differences compared to the control group and group 3 and group 4, this means that aqueous extract and oil of *Nigella sativa* restored to semi normal value to control.

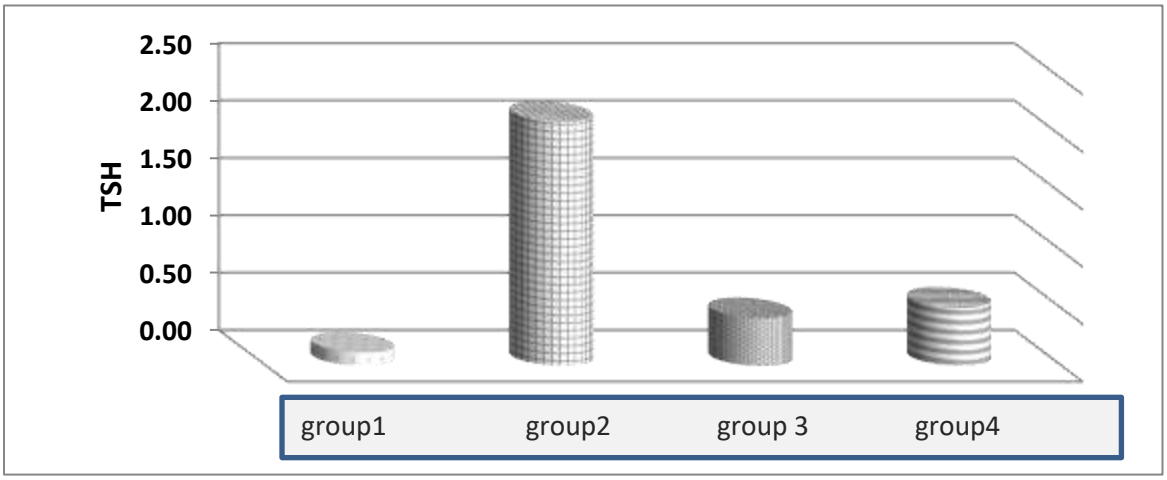

Figure 1: the effect of aqueous extract (group 3) and oil (group 4) on TSH compared to control (group 1) and Hypothyroidism group 2.

Comparison of the means value of FT3 in the studied group:

Table (1) and scheme (2) show that the means value of FT3 has significantly decreased $p<0.05$ after inducing the hypothyroidism to increase significantly $p<0.05$ after treatment with extract and oil.

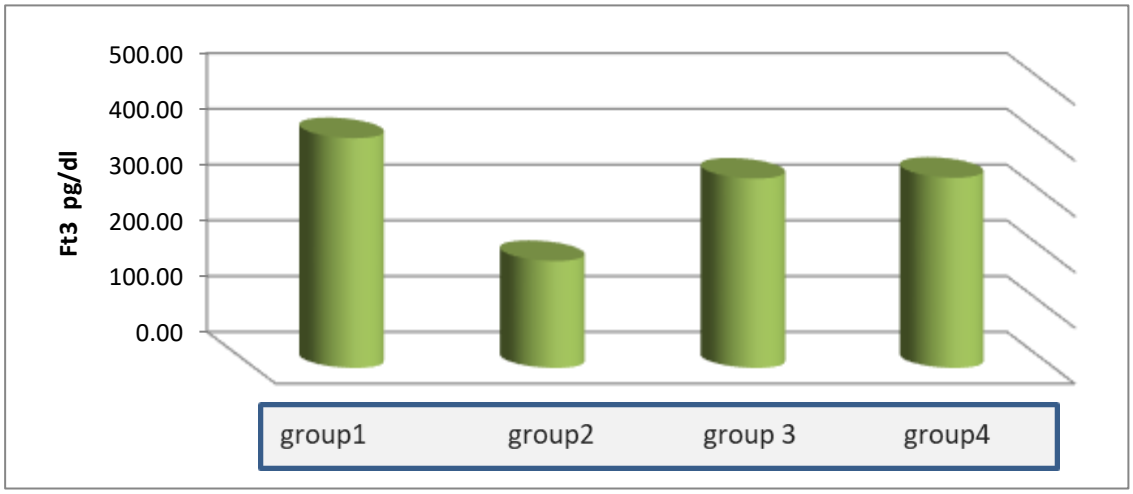

Figure 2: the effect of aqueous extract (group 3) and oil (group 4) on FT3 compared to control (group 1) and Hypothyroidism group 2.

Comparison of the means value of FT4 in the studied group:

Table (1) and scheme (3) show that the means value of FT4 has significantly decrease $p<0.05$ after induced the hypothyroidism to insignificantly increased $p>0.05$ after treating with extract, while it was significantly increased $p<0.05$ after treating with oil.

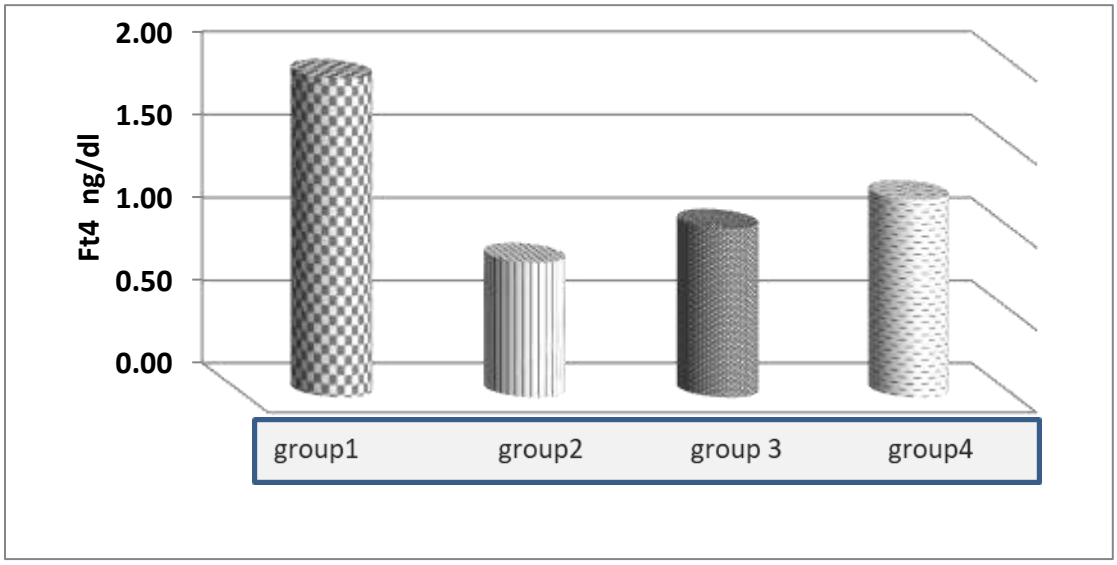

Figure 3: clarifies the effect of aqueous extract (group 3) and oil (group 4) on FT4 compared to control (group 1) and Hypothyroidism group 2.

DISCUSSION

The orally administered white mice a dose of 0.05 mg/kg/body weight /day for 3 weeks caused significant deceasing (P<0.05)in hormone level (FT4,FT3), it also caused significant increasing (P<0.05) in TSH level hormone, that is a result of induced the hypothyroidism in these mice, where methimazole acts as a false Iodide for thyroid peroxidase, thus blocking the iodination of thyrosine residues within thyroglobulin, subsequently occurring decreasing its hormones levels in blood serum[23], this stimulates the anterior lobe of the pituitary gland to increase TSH secretion to retrieve this decreasing, in sense that the thyroid gland is under control of the regulation of the hypothalamic-pituitary-thyroid axis (Feedback Negative), and this explains the level height of TSH after orally methimazole[24].

As well as after orally administered of aqueous extract of *N. sativa* and its oil, significant increasing p<0.05 happened in FT3 levels, and significant decrease p<0.05 in TSH levels, also significant increasing<0.05 happened in FT4 levels when Nigella Sativa oil was dosed, but this increasing was un significant p>0.05 in the level of FT4 when Aqueous extract of Nigella Sativa was dosed.

The results of this study had agreed with Ebrahim *et al* it had shown that oral administration of thyroid healthy male rats with *N. sativa* alcoholic extract at doses (25,50,100)mg/kg/weight body/day for a month causes a significant increasing p<0.05 in T3 level, and insignificant in T4 level, that because the *N. sativa* contents of amino acids specially tyrosine which consider the essential substrate to make the thyroid hormone, besides antioxidant that improve the functioning of thyroid gland [25]. It also agreed with Abou- Zena et al were adding *N. sativa* powder 2% to fodder which feed goats' kids to significantly increasing in T3 and insignificantly increasing in T4, also the researcher found semi-results when adding supplement which contents (Vit E, Zn) [26]. And Sharif et al. 2012 who pointed to Orally treatment by *N. sativa* ethanol extract with a daily 1g/kg caused a significant increasing in thyroid hormones levels and the cause of this large increasing in T3 level is activity increasing enzyme deodenase-5 which works on

transforming T4 to T3. As well as agreed with Khalawi et al (2013) which clarified that 400mg/kg dose of Nigella Sativa oil for a month had changed PTU induced hypothyroidism statue to hyperthyroidism [27].

Nigella Sativa contains thymoquinone which plays an important role in thyroid hormones biosynthesis, because of this compound ability to increase thyroid hormone production and deletion oxidative stress accompanying to hypothyroidism [28] ,this compound is soluble in oils (Fat) [29]which makes NSO superior to aqueous extract in improving FT4 level, as well as Nigella Sativa oils contain triterpene saponin and many other terpenes and phenolic compounds, which play roles as antioxidants to[30,31].And polyphenols compounds as α,β,γ Tocopherol (Pro-Vit E) that many functions in increasing thyroid hormones levels [32]. In addition to fatty acids function especially linoleic acid (Omega 3) that regulate thyroid hormones secretion and antioxidant function[33, 34] .

In this context NSE and its oil contain many important minerals to thyroid gland function besides the antioxidant role such as Zn and Cu[35] . selenium which is considered accompanying to deodenas-5 enzyme and additionally, it's a part of glutathione peroxides(one of the most important antioxidants in thyroid gland)[36].

The result of this study had also agreed with many studied of medical plants to treat hypothyroidism. Where *Ginge*r extract has a role in the treatment of hypothyroidism albino mice, it caused significant increasing $p<0.05$ in T3, T4 levels, and significant decreasing $p<0.05$ in TSH level [37] . And phenolic extract of convolvulus arvensis had related effect to the levothyroxine efficiency in treating the hypothyroidism [38] .And agreed with Abu-Fotouh et al that shows the effect of curcumin extract with 100mg/kg dose in treating hypothyroidism induced by potassium dichromate in white mice , because curcumin contains polyphenol yellow dye which function as a free radicals remover [39] . It also agreed with Osman et al which referred to the aqueous extract ability with 10% concentration in treating the induced hypothyroidism in white mice, this goes back to content of the extract of antioxidants for example thymol and other phenols [40, 41] .

Statistical analysis

The results were statistically analyzed using the social package for social sciences program (SPSS). A on way test of variance (ANOVA) was conducted to determine if there were significant differences among the studied groups, then the LSD5% test was used to located these differences, and the result of LSD test are shown in a letter method, where the means are arranged in ascending order, then every two groups have a common letter, the difference between them is not significant (the difference between them is less than the LSD value)

CONCLUSION

We studied the efficacy of aqueous *Nigella sativa* extract and its oil in treating hypothyroidism induced methimazole in white mice and both of them had effectiveness in increasing FT3 level and decreasing TSH level, but *Nigella Sativa* oil had larger effect in increasing FT4 level (in sense that increasing was significant $p<0.05$) and it was not significant in FT4 level when using aqueous extract.

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مقارنة بين تأثير المستخلص المائي للحبة السوداء وزيتها على قصور الدرق المستحدث بالميثمازول لدى الفئران البيضاء

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ملخص

الهدف: أجريت هذه الدراسة بهدف تقييم تأثير كل من المستخلص المائي للحبة السوداء وزيتها في علاج قصور الدرق المستحدث بالميثمازول لدى الفئران البيضاء بهدف الوصول إلى علاج طبيعي لقصور الدرق بدلاً من العلاجات الكيميائية ذات التأثيرات الجانبية الخطيرة.

المواد المستخدمة والطرق: شملت الدراسة 40 من إناث الفئران البيضاء البالغة، وزعت على أربع مجموعات، المجموعة 1 (شاهدة فيزيولوجية): جرعت بمحلول فيزيولوجي 0.09 طيلة فترة التجربة. المجموعة2 (شاهدة مرضية) جرعت بعقار الميثمازول بتركيز 0.05 ملغ/كغ يومياً لمدة 3 أسابيع لاستحداث القصور الدرقي. المجموعة 3: استحدث فها قصور الدرق ثم عولجت بمستخلص الحبة السوداء المائي بجرعة 400 ملغ/كغ لمدة شهر. المجموعة 4: استحدث فيهل قصور الدرق ثم عولجت بزيت الحبة السوداء بجرعة 1كل/كغ.

النتائج: بينت النتائج أن كلا المستخلص والزيت سبب انخفاضاً معنوياً$P<0.05$ في مستو هرمون TSH، وارتفاعاً معنوياً $P<0,05$ في مستو هرمون FT3 ، وسبب زيت الحبة السوداء ارتفاعاً معنوياً في مستوى هرمون FT4 بينما لم يكن الارتفاع معنوياً عند استخدام المستخلص المائي للحبة السوداء.

الكلمات الدالة: زيت الحبة السوداء، المستخلص المائي للحبة السوداء، FT3، FT4، TSH.

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