

Effect of *Nigella sativa* seeds on the concentration of some plasma proteins

Dr.NAJDAT ALI AL –KADHI

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Dr.NAJDAT ALI AL –KADHI

Department of Med.Lab.Tech.

Technical college – Kirkuk

Foundation of Technical institute

Iraqi – Kirkuk

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Abstract

The study was designed to investigate the effect of *Nigella sativa* seeds as crude and oil form on the concentration of some plasma proteins. forty eight male female volunteers (20-35) years old in Kirkuk city were included in the experiment .Twenty four of them received orally crude *Nigella Sativa* seeds 2gm/ day for six weeks. The other twenty four volunteers were received orally *Nigella Sativa* oil (NSO) 10ml/ day for six weeks. Blood were collected by vein puncture at the beginning of the treatment and considered as control and then at the end of second , fourth and sixth weeks of treatment and considered as treated groups . The serum were separated by centrifugation .Tests were carried out for measuring some serum proteins. Statistically significant differences between group means for control and treated values were identified with the use of analysis of variance (F-test). Results revealed that treatment of female volunteers by NSO increased the concentration of serum globulin and total protein at the end of fourth and sixth weeks of treatment significantly($p \leq 0.05$) .The treatment of male volunteers by NSO increased the concentration of serum globulin and total protein at the end of sixth week of treatment significantly($p \leq 0.05$). Treatment of volunteers with crude *Nigella Sativa* seeds increased the concentration of serum albumin in females at the end of the second and fourth weeks significantly($p < 0.05$) . Concentration of serum globulin and total protein in both sexes significantly increased($p < 0.05$) at the end of second week and continued to the end of the experiment. The globulin to albumin decrease significantly ($p \leq 0.05$) compared to control in both sexes with both types of treatment during different periods of treatment resulting from the increment of globulin levels. It is concluded that the treatment of volunteers with *Nigella Sativa* seeds as crude and oil fraction revealed to have a significant effect on serum proteins specially globulin and total protein during different periods . The crude form possesses more potent effect than oil fraction to increase serum protein levels .

Key words : *Nigella sativa* , plasma protein, cumin black seed ,*Nigella sativa* oil

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تأثير بذور الحبة السوداء على تركيز بعض بروتينات بلازما الدم

د. نجدت علي شفيق

قسم تقنيات التحليلات المرضية

الكلية التقنية / كركوك

هيئة التعليم التقني - العراق / كركوك

الخلاصة

اجريت هذه الدراسة لمعرفة تأثير الحبة السوداء بشكلها الزيتي وال خام على تراكيز بعض بروتينات بلازما الدم وتحديد ايهما اكثر تأثيرا مع تحديد الفترة الزمنية لأظهار هذه التأثيرات. إذ خضع ٤٨ من المتطوعين الاصحاء من الذكور والاناث في مدينة كركوك تتراوح اعمارهم بين (٢٠ – ٣٥) سنة. تم معالجة اربعة وعشرون منهم ببذور الحبة السوداء الخام بجرعة (٢ غم) يوميا عن طريق الفم لمدة ستة اسابيع، ومعالجة اربعة وعشرون اخرون بزيت بذور الحبة السوداء بجرعة (١٠ مل) يوميا عن طريق الفم ولمدة ستة اسابيع ايضا.

تم الحصول على الدم من المتطوعين عن طريق وخز الوريد اولا قبل بدأ المعالجة مباشرة واعتبرت مجموعة السيطرة ومن ثم في نهاية الاسبوع الثاني والرابع والسادس من المعالجة واعتبرت مجاميع المعالجة. وفصل مصل الدم من النماذج بواسطة المنبذة وحفظت تحت التجميد لحين قياس تراكيز بعض بروتينات مصل الدم. استخدم تحليل التباين (F-test) لايجاد الفروقات المعنوية بين مجاميع المعالجة والسيطرة. وبينت النتائج أن معالجة الاناث بزيت بذور الحبة السوداء ادى الى زيادة معنوية ($p < 0.05$) بتركيز كلوبولين والبروتين الكلي لمصل الدم في نهاية الاسبوع الرابع والسادس من المعالجة بينما في الذكور سجلت زيادة معنوية ($p < 0.05$) في تراكيزهما في نهاية الاسبوع السادس من المعالجة. في حين ان المعالجة ببذور الحبة السوداء الخام قد سجلت زيادة معنوية ($p < 0.05$) في تركيز البومين مصل الدم في نهاية الاسبوع الثاني والرابع من المعالجة للاناث فقط، وسجل كل من كلوبولين والبروتين الكلي لمصل الدم زيادة معنوية ($p < 0.05$) في كلا الجنسين عند نهاية الاسبوع الثاني من المعالجة واستمرت لغاية نهاية البحث. بينما سجلت نسبة البومين الى كلوبولين انخفاضاً معنوياً بالمقارنة مع السيطرة في كلا الجنسين وفي كلا النوعين من المعالجة مصاحبا للزيادة الحاصلة في تركيز كلوبولين مصل الدم بالمقارنة مع تركيز البومين مصل الدم ولغاية نهاية البحث.

نستنتج من هذه الدراسة بأن معالجة المتطوعين ببذور الحبة السوداء بشكلها الزيتي وال خام لها تأثير معنوي على زيادة تركيز كلوبولين والبروتين الكلي في مصل الدم الا ان شكلها الخام اظهر تأثيرا اقوى من شكلها الزيتي من خلال احداثها تأثيرا معنوياً مبكراً.

الكلمات الدالة: بروتينات بلازما الدم، بذور الحبة السوداء، زيت الحبة السوداء، البومين، كلوبولين.

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Introduction

Nigella Sativa (NS) belongs to the botanical family of ranunculaceae and commonly grows in Europe, Middle East and Western Asia. *Nigella Sativa* has been used as flavoring additive to bread and prickles (1). The seeds of *Nigella Sativa*, commonly known as black cumin, contains both fixed and essential oils (32-40%), alkaloids, saponins, coumarins, minerals, vitamins, proteins, carbohydrates, carotinoids, flavonoids(2).

Nigella Sativa seeds or their oil fraction has been reported in many studies from potential prophylactic, antitumor effects (3). *Nigella Sativa* seeds also been reported to have a protective effect against diabetes, nephrotoxicity, hepatotoxicity and hemotoxicity (4). Many research's have conducted with laboratory animals to reveal the mechanism action of black cumin seeds and to be identified their effects on plasma proteins and other functions of body organs (5). A study were carried out on rats, revealed that the administration of black cumin seeds or oil in their diets led to an increase in serum albumin (6,7). El-Daly (1998) and Mohammad et al(2008) showed a decrease in serum albumin and total plasma protein concentration or had no influence(11). Other studies on rats showed inconsistency with regard to hematological values as the administration of black cumin seeds or oil, and found to decrease the white blood cells (12) or to increase their count (9) or to have no effect (11,13).

Other studies about the influences of black cumin seed or oil on red blood cells count showed an increases of count (9) or to have no effect (7).

Studies on rabbits showed that the inclusion of either 10 or 15 % black cumin seed in the feed did not effect serum concentration of total protein, albumin and globulin, but the level of 20% caused a depressed effect (14).

The ameliorated effects of *Nigella Sativa* seeds were carried out by Meral I et al(2004) and they concluded that ameliorated effects of *Nigella Sativa* seeds effect on some hematological values of alloxan-induced diabetic rabbits. While Amina et al, (2010) concluded that the morphological, biochemical and alteration in blood cells were ameliorated by the administration of an aqueous suspension of *Nigella sativa* orally 50 mg /kg of body weight for swiss albino mice.

The literature data mentioned above represents conflicting results on the effect of black cumin seeds on plasma proteins and blood cells count in laboratory animals.

This prompted us to undertaken the present study with healthy male and female human volunteers for describing the influences of crude and the oil fraction of the *Nigella Sativa* seeds on the concentration of plasma proteins and to investigation the more potent one.

Materials & Methods

The cumin black seeds *Nigella Sativa*(N.S), were obtained from local market. The crude form and its oil were used as treatment of volunteers in Kirkuk city. The oil form of N.S. obtained by the extraction process starts with the selection of black seeds which are then sorted and cleaned. Then, the oil were extracted using the cold pressing method –a mechanical process without using any chemical additives or heat.

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The oil packaged in clean small bottles and stored at 5-10 C° until use. The study carried out on forty eight adult male and female volunteers with age 20-35 years old were continued until the end of the experiment . The volunteers were divided and treated as follows:

- Twelve male received orally crude N.S. seeds 2gm daily for six weeks.
- Twelve female received orally crude N.S. seeds 2gm daily for six weeks.
- Twelve male received orally N.S. oil 10 ml daily for six weeks.
- Twelve female received orally N.S. oil 10 ml daily for six weeks.

Blood samples were taken from volunteers by vein puncture using disposable syringe at beginning of the experiment (considered control) after end of second , fourth and sixth weeks of treatment (considered treated groups).The blood samples were collected in tubes and the serum was obtained by centrifugation and kept frozen until protein analysis.

Serum total protein was analyzed colorimetrically using copper sulphate as reagent. Serum albumin was determined by use of bromocrysol green. Total globulins were calculated as difference between total protein and albumin, while A/G ratio calculated by division of albumin over globulin.

Statistical analysis:-

The data are represented as group mean and S.E. at the average for the twelve blood samples taken during the experiment. Statistically significant differences between group means for control and treated values were identified with the use of analysis of variance (F-test). Duncun's multiple range used for comparison of means and the level of statistical significance were pre-set at $P \leq 0.05$.

Results

Results of this study shown in table (1&2) the effects of orally administration of *Nigella Sativa* oil (NSO) 10ml daily in male and female volunteers for six weeks of treatment, no significant effects were recorded on the concentration of serum albumin in both sexes when compared within treated groups and with the control. While the concentration of serum globulin and total protein in male recorded significant increase ($P < 0.05$) at the end of the sixth week of treatment when compared with the other treated and control groups. The results in the female recorded a significant increase in their concentration ($P < 0.05$) at the end of the fourth and sixth weeks of treatment when compared with the control. Table (3&4)revealed that the orally administration of volunteers with the crude N.S. seeds 2gm daily for six weeks of treatment recorded a significant increase ($p < 0.05$) in serum albumin in females at the end of the second and fourth week of treatment when compared with the control .While the concentration of serum globulin recorded a significant increase ($p < 0.05$) in male and female at the end of the second week. This increment was more remarkable in female at the end of the fourth and sixth weeks of treatment when compared with control . The concentration of serum total protein in male and female volunteers recorded a significant increase ($p < 0.05$) at the end of the second week of treatment when compared with control. This increment was more remarkable in both sexes at the end of the fourth and sixth weeks of treatment when compared with the control .On the other hand the ratio of serum A/G of volunteers treated with the NSO recorded a significant decrease ($p < 0.05$) in males at the end of second and fourth weeks of treatment. In females a significant decrease ($p < 0.05$) of serum A/G were recorded at the end of fourth and sixth weeks of treatment when

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compared with control . when the volunteers of both sexes treated with the crude form of the N.S. seeds, a significant decrease ($p < 0.05$) in the ratio of serum A/G were recorded at the end of second week of treatment. This decrement was more remarkable at the end of sixth week of treatment when compared with control

Tab.1 Effect of *Nigella sativa* oil(10 ml daily) on concentration of plasma proteins(mg/dl) in male

Treatment	No.of sample	Albumin $\bar{X} \pm S.E$	Globulin $\bar{X} \pm S.E$	Total protein $\bar{X} \pm S.E$	A/G $\bar{X} \pm S.E$
Control	12	4.98 $\pm$ 0.08 A	2.73 $\pm$ 0.09 B	7.71 $\pm$ 0.09 b	1.82 $\pm$ 0.08 b
After 2 weeks	12	4.94 $\pm$ 0.07 A	2.65 $\pm$ 0.8 b	7.63 $\pm$ 0.09 b	1.91 $\pm$ 0.07 a
After 4 weeks	12	5.08 $\pm$ 0.11 A	2.67 $\pm$ 0.26 b	7.76 $\pm$ 0.24 b	1.90 $\pm$ 0.57 a
After 6 weeks	12	5.3 $\pm$ 0.23 A	2.95 $\pm$ 0.33 a	8.26 $\pm$ 0.24 a	1.79 $\pm$ 0.2 b

A= Albumin

G= Globulin

a•b•c letters:- (similar letters indicate to non significant differences between groups vertically and visa versa)

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Tab.2 Effect of *Nigella sativa* oil(10 ml daily) on concentration of plasma proteins(mg/dl) in female

Treatment	No.of sample	Albumin $\bar{X} \pm S.E$	Globulin $\bar{X} \pm S.E$	Total protein $\bar{X} \pm S.E$	A/G $\bar{X} \pm S.E$
Control	12	5.06±0.11 a	2.40±0.16 b	7.38±0.11 b	2.21±0.17 a
After 2 weeks	12	4.90±0.1 a	2.26±0.14 b	7.16±0.11 b	2.37±0.21 a
After 4 weeks	12	4.98 ±0.13 a	2.92 ±0.12 a	7.9 ±0.41 a	2.01 ±0.3 b
After 6 weeks	12	4.93 ±0.21 a	2.91 ±0.33 a	7.84 ±0.16 a	1.70 ±0.3 c

A= Albumin

G= Globulin

a·b·c letters:- (similar letters indicate to non significant differences between groups vertically and visa versa)

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Tab.3 Effect of crude *Nigella sativa* seeds(2 gm daily) on concentration of plasma proteins(mg/dl) in male

Treatment	No.of sample	Albumin $\bar{X} \pm S.E$	Globulin $\bar{X} \pm S.E$	Total protein $\bar{X} \pm S.E$	A/G $\bar{X} \pm S.E$
control	12	5.01 $\pm$ 0.16 a	2.38 $\pm$ 0.15 c	7.4 $\pm$ 0.1 c	2.15 $\pm$ 0.2 a
After 2 weeks	12	4.9 $\pm$ 0.09 a	2.99 $\pm$ 0.2 a	7.89 $\pm$ 0.23 b	1.61 $\pm$ 0.09 b
After 4 weeks	12	4.85 $\pm$ 0.19 a	2.95 $\pm$ 0.25 a	7.8 $\pm$ 0.2 b	1.76 $\pm$ 0.1 b
After 6 weeks	12	5.2 $\pm$ 0.17 a	3.06 $\pm$ 0.15 a	8.26 $\pm$ 0.29 a	1.38 $\pm$ 0.3 c

A= Albumin

G= Globulin

a•b•c letters:- (similar letters indicate to non significant differences between groups vertically and visa versa)

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Tab.4 Effect of crude *Nigella sativa* seeds(2 gm daily) on concentration of plasma proteins(mg/dl) in female

Treatment	No.of sample	Albumin $\bar{X} \pm S.E$	Globulin $\bar{X} \pm S.E$	Total protein $\bar{X} \pm S.E$	A/G $\bar{X} \pm S.E$
control	12	4.8±0.19 b	2.17±0.21 c	7.0±0.22 d	2.21±0.14 a
After 2 weeks	12	5.04±0.13 a	2.44±0.14 b	7.48±0.23 c	2.06±0.15 a
After 4 weeks	12	5.11 ±0.3 a	3.21 ±0.23 a	8.32 ±0.4 a	1.6 ±0.42 b
After 6 weeks	12	4.88 ±0.22 b	2.96 ±0.57 a	7.85 ±0.56 b	1.65 ±0.54 b

A= Albumin

G= Globulin

a·b·c letters:- (similar letters indicate to non significant differences between groups vertically and visa versa)

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Discussion

Several studies described the traditional therapeutics and ameliorating effects of *Nigella Sativa* seeds on some disorders of the human body. Improvement of the beneficial physiological efficacy of the body organs was concluded (17). This study focused on observation and improvement of some possible effects of crude *Nigella Sativa* seeds and oil form on the concentrations of some serum proteins in apparently healthy male and female volunteers (20-35) years old.

The results of the study revealed that the treatment of male and female volunteers with *Nigella sativa* seeds by crude and oil form recorded a significant increase ($p < 0.05$) in the concentration of serum globulin and total protein accompanied with the significant decrease ($p < 0.05$) in ratio of serum A/G. These results may reflect the important components of the *Nigella sativa* seeds and oil form which have an anabolic and stimulatory effects. They alter the functions of various body organs specially liver and enhances their enzyme activities in human and rats. This plays an important role for synthesis of plasma proteins in human and rats (18). The above results with their explanation agree with the results of the other studies. Some of these studies observed that the *Nigella sativa* seeds contain an important active ingredients specially essential and saturated fatty acids such as linoleic acid (omega – 6) and linolenic acid (omega – 3). These substances can not be manufactured in the body (8), and are considered as building blocks of cells (19). In addition to their important role in the regulation of metabolic process and promote liver function (20). It has been suggested in previous studies that the increased globulin and protein levels might be due to an increase in certain carrier proteins such as transferrin, ceruloplasmin, hormone binding globulin and thyroxine binding prealbumin (21). *Nigella sativa* seeds contain a large number of flavonoid compounds relative to other plant resources, so possesses slightly estrogenic effects (22&23). This estrogenic effect seems to be responsible for the increased total plasma proteins (24). It has been also established that the serum albumin, globulin and total protein levels tend to be more potent and exert their effects early in females than males (table 2&4). This result may have been established by the synergism effects exerted by the original estrogen in the females with the estrogenic effects of the flavonoid compounds in the *Nigella sativa* (25). On the other hand the crude form of *Nigella sativa* recorded a significant effect than the oil form. Crude form recorded a significant effect at the end of second week of treatment in the concentration of serum globulins and total proteins to the end of experiment. These results indicate the richness

of the crude form to have many different types of active ingredients with high quantities and qualities than their oil form. Results also suggested the inclusion of the crude form for different types of antioxidant such as flavonoids, carotinoids...etc which are known as an important substances for scavenging of free radicals and protect the plasma proteins and vital organic materials from peroxidation process (11). This enhances the enzymes that promote the synthesis of plasma proteins specially globulins which play an important role in immune system (26). The above results about the influences of crude *nigella sativa* seeds or oil were also improved by Nagi et al (20). They concluded that the pretreatment of mice with *nigella sativa* seeds against Carbon tetrachloride (CCl₄) induced liver damage, restored Lactate dehydrogenase (LDH) enzyme to normal level with an increase in L-alanine aminotransferase (ALT) and Aspartate

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serum transaminase (AST) enzymes level via an antioxidant mechanism . Also Ekanem and Yusuf(2008) concluded that the treatment of rats infected by *T.brucei* with the NSO enhances the activities of some hepatic enzymes such as alkaline phosphatase and Glutamate oxaloacetate transaminase (GOT) enzymes with significant increase in the concentration of liver proteins. This study shows that albumin / globulin ratio in all treated volunteers on oral crude and oil forms of N.S. seeds were significantly decreased ($p \leq 0.05$) compared with control . This disparity in ratio is apparently due to the significantly increase in serum globulin levels compared with control in both sexes . Also the result was compatible with the changes and fluctuations occurred in the levels of plasma proteins during six weeks of treatment. Finally , we concluded the crude *nigella sativa* seeds and oils have a significant effects on the level of serum globulins and total proteins during different periods of treatment. The crude form possesses a potent effects on the studied parameters than their oil form.

Conclusion

1. Both forms of N.S. seeds have a stimulatory effects on synthesis of plasma proteins.
2. The oil form of N.S. seeds was more potent and exerted early influence on females than males.
3. The crude N.S. seeds have more potent influence on the concentration of plasma proteins than oil form in male and female.
4. Both forms of N.S. seeds have an enhancement effects for the globulins which may promote the function of immune system.

Abbreviations

N.S.	<i>Nigella Sativa</i>
N.S.O	<i>Nigella Sativa</i> oil
A/G	Albumin over globulin ratio
GOT	Glutamate oxaloacetate transaminase
LDH	Lactate dehydrogenase
ALT	L-alanine aminotransferase.
AST	Aspartate serum transaminase
CCL4	Carbon tetrachloride
<i>T . brucei</i>	<i>Trypanosoma brucei</i>

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