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THE EFFECT OF ORAL ADMINISTRATION OF *NIGELLA SATIVA* L. SEEDS AND OIL ON SOME BLOOD PARAMETERS

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تمت إجراء دراسة مقارنة لمعرفة تأثير بذور نبات حبة البركة (الحبة السوداء) المنزرع فى الأردن والزيت المستخلص منها والمصاغ بطريقة الانتشار السطحى الصلب على بعض القياسات الحيوية لدى عدد من الطلاب المتطوعين ومتوسط أعمارهم ٢٢ سنة مثل التأثير على نسبة سكر الدم والكوليسترول والليبيدات عالية الكثافة والكرياتنين والبروتاكتين والكرياتين كاينار وكذا دراسة التأثير على عدد كريات الدم الحمراء والبيضاء والصفائح الدموية ومستوى الهيموجلوبين فى الدم وقد تناول كل متطوع جرعة من بذور حبة البركة (٢ جم كل يوم) موزعة كل جرعتين وكذا كمية مماثلة من الزيت (١٢٥ مجم مرتين يوميا) مجهزة فى كبسولة. وقد أخذت عينات الدم من المتطوعين قبل أخذ المستحضر بأسبوع ومرة أخرى بعد أسبوع من التعاطى والعينة الثالثة بعد أسبوعين.

وقد اثبتت الدراسة أن كلا من مسحوق البذور والزيت المحضر أدى إلى انخفاض ذو دلالة احصائية على نسب السكر والكوليسترول والكرياتنين وزيادة نسبة الليبيدات عالية الكثافة. بينما أظهر الزيت نقص كبير على نسبة هرمون البروتاكتين مقارنة مع المتطوعين الذين أخذوا البذور وعلى العكس أظهرت البذور تأثير ملموس على زيادة زمن التجلط. وأتضح من الدراسة أيضا أنه ليس هناك تأثير ملموس على كرات الدم الحمراء والبيضاء والصفائح الدموية ونسبة الهيموجلوبين وكذا أنزيم الكرياتين كاينار. وقد تم عمل مسح كيميائى أولى للبذور الجافة لمعرفة مكوناتها الاساسية وكذلك قياس بعض الثوابت الفيزيوكيميائية للزيت. وباستخدام كروماتوجرافيا العمود للجزء الغير متصبن نتج فصل مركبين والتعرف عليهما وهما بيتاسيتوستيرول وحمض الاوليانوليك كمكونين أساسين.

A comparative study was carried about the effects of two weeks daily ingestion of 2 g of Nigella sativa L. seeds cultivated in Jordan and the equivalent amount of the formulated oil prepared by surface solid dispersion method on the blood levels of glucose, cholesterol, high density lipoprotein (HDL), and other parameters such as: creatinine, prolactin, creatine kinase (CK) activities and RBCs, WBCs, platelets, hemoglobin, and clotting time. The study was carried on male pharmacy students volunteers of an average age of 22 years. Ten students each administered two capsules containing 500 mg Nigella sativa crushed seeds twice daily (group I) and another group of ten students (group II) received one capsule containing an equivalent amount of the oil twice daily for two weeks. Blood samples were taken from each student one week before administration, one week and two weeks after administration. Both crushed seeds and the oil produced a significant decrease in blood glucose, cholesterol, and creatinine level, meanwhile there was an increase in high density lipoprotein. The oil produced a significant lowering effect on prolactin level ($P < 0.05$) while the crushed seeds exhibited a significant increase in clotting time ($P < 0.01$) after one week and continued for two weeks of administration. Insignificant effects were observed on RBCs, WBCs, platelets, hemoglobin and creatine kinase activity.

The phytochemical screening of the dried powdered seeds was done and the physico-chemical constants of the oil were determined. Column chromatography of unsaponifiable matter results in the isolation of β-sitosterol and oleanolic acid as the main constituents.

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INTRODUCTION

Nigella sativa L (F. *Ranunculaceae*) is an annual herbaceous plant growing in countries bordering the Mediterranean sea. The seeds of the plant are known in Arabic under the names "Al-Habah Al-Sawda" (black corn)[1] and Habbet Al-Barakah (corn of blessing).[2] It exhibit special importance for Muslems because the authentic saying (hadeeth) by prophet Mohammad (peace be upon him) stating "Al-Habbah Al-Sawda is a cure for every thing except death".[3] The Arabian authors[4-6] reported that the seeds are useful mainly in headache, respiratory appression, asthma and expelling urinary calculus, as well as lactogogue, emmenagogue[7] and diuretic. In Egyptian folk medicine, the seeds are used as carminative, and as a flavouring agent to breads while the expressed oil is used in asthma, respiratory appression and cough.[8] Reviewing the available literature,[9-17] several authors had investigated the fixed and volatile oils to isolate and identify the active constituents. The isolation of thymoquinone from the volatile oil was ascribed to El-Dakhakhny[10] and the thymohydroquinone to El-Alfy *et al.*[11]

Many studies have been conducted referring to the pharmacological and biological effects of *Nigella sativa* seeds, volatile oil, fixed oil and thymoquinone[19,20] as anti-inflammatory effect[21,22] hypoglycemic effect[23-27] and antihistaminic.[28-30] Several studies documented the ability of *N. sativa* to modulate the immune system.[19,31-34] Antibacterial[35-41] and antiparasitic[40] activities were studied and the antitumor activity has also been reported.[42-44] In rabbit, *N. sativa* produced shortening in bleeding time, inhibition of fibrinolytic activity and shortening of clotting time.[45] The different pharmacological activities and wide medicinal uses of *N. sativa* seeds and oil encourage us to carry out this study.

The aim of this study is to investigate the differences in effects between the use of *N. sativa* crushed seeds and oil prepared by surface solid dispersion method on the blood levels of glucose, cholesterol, HDL, creatinine, CK, prolactin, RBCs, WBCs, hemoglobin (HB) and clotting time of male healthy volunteers. Since the oil will contain the lipophylic substances while the seeds contain both lipophylic substances and polar compounds as saponins and tannins[46-50] which will not dissolve in the oil.

EXPEREMENTAL

Materials, equipment and methods

Plant material

N. sativa seeds were obtained from the plants cultivated in the experimental station of medicinal plants, Faculty of Pharmacy, Al-Isra University, Jordan, in 1997. Preparation of fixed oil was done by two methods:

a- By expression methods:
Two kg of *N. sativa* seeds were wormed at a temperature not exceeding 40°, expressed by hydrolic pressure, oil was filtered, 250 g of oil were obtained (yield 12.5% w/w).

b- By extraction method:
100 g of the crushed seeds were subjected to extraction with n-hexane in Soxhelt till exhaustion, the extract was concentrated under reduced pressure till free from solvent and weighed, 30.4 g of extract were obtained (Yield ≈30%).

Materials and equipment

- Manual capsules filling, 60 capsule capacity (Germany).
- Centrifuge: Herareas Sepatch, Labofuge 200 Max. Dehzahl, 5300 r.p.m. (Germany).
- Hematology instrument: Coulter counter; Coulter-T-860 (Germany).
- Spectrophotometer (single beam) Cecil, CE 1020 (Germany).
- Electrothermal model used for determination of melting points.
- EDTA tubes {EDTAK3 (15%)}. Nipro, Japan, plain tubes (Silicone coated, Nipro, Japan) and Glass tubes (Japan).
- Glucose kit TECO Diagnostic (USA).
- Cholesterol kit Arcomex (Jordan).
- Triglyceride kit Biosystem (Spain).
- HDL kit Arcomex (Jordan).
- Creatine kinase kit Human (Germany).

- Creatinine kit BioMerieux (France).
- Prolactin kit Quram (Spain).
- Oleanolic acid and ß-sisterol (Sigma, USA).

Methods

Phytochemical screening of the total powder

The dried powdered seeds of *N. sativa* L. cultivated in Jordan was screened for its constituents of volatile oil, saponins, carbohydrates and/or glycosides,[51,52] unsaturated sterols and/or triterpenes,[53,54] tannins,[55] flavonoids,[56] anthraquinones,[57] cyanogenic glycosides,[58] coumarins[58] and alkaloids and/or nitrogenous bases.[59]

Investigation of the unsaponifiable matter

Ten grams of the lipid portion obtained by extraction was saponified using N/2 alcoholic KOH and the unsaponified matter was spotted over silica gel TLC where 4 major spots were detected, then fractionated on column of neutral alumina. The effluents was monitored on precoated silica gel plates using hexane-ethyl acetate (8:2) as mobile phase and 10% methanolic H_2SO_4 as spraying reagent. The column was developed with benzene-ethyl acetate by gradient elution technique where two compounds were isolated and purified by re-crystallization.

Determination of the physico-chemical characteristics

The prepared oil was used for the determination of ester value by the normal method[60] and to determine the iodine number by Wij's solution.[60] The physico-chemical characteristics of the prepared oil were determined and compared with those obtained from other studies.

Preparation of oil formula

Preparation of the loaded mixture (Surface solid dispersion)

To prepare a loaded mixture containing 50 % of Nigella oil, 18.75 g of aerosil (porous silica) was added gradually to 18.75 g of oil in a glass mortar with continuous trituration. Further homogenization was achieved by mixing in a mechanical mixer (Moulinex, France). The prepared loaded mixture was stored in a desiccator over $CaCl_2$ under vacuum until further use. The prepared loaded mixture was filled in capsules No: 0 after determining the tapped bulk density of the mixture. The amount of oil equivalent to 2 g of the crushed seeds was 250 mg daily, so divided to 125 mg twice daily and formulated with aerosil and lactose in dried form and dispensed in capsule as mentioned before.

Volunteer groups

Group I

Ten subjects (volunteers) aged between 21 and 23 years, each (individual) received a daily dose of 2 g of crushed seeds dispensed in 500 mg capsules twice daily for (14) days.

Group II

Ten subjects received the prepared oil formula (oil adsorbed on aerosil and dispensed in capsule). The amount of oil is equivalent to the amount of oil present in 2 g of crushed seeds (i.e. 125 mg of oil twice daily).

Sampling

Blood samples: 10 ml were taken from each volunteer as control in day zero (before administration of drug) and repeated each week interval for two weeks. The blood samples were divided into 3 fractions:

Fraction I

Three glass tubes were used for determining the clotting time for each sample using Lee and White method.[61]

Fraction II

EDTA tubes were used for hematological tests (WBCs, RBCs, HB and platelets)[61] each test was carried out using hematology instruments (Coulter-T-860) which was calibrated using Uni-T-PAK Kit.

Fraction III

Plain tubes, The blood samples were centrifuged to separate the sera to be used for the determination of blood glucose level,[62] cholesterol,[62] creatinine,[63,64] creatine kinase,[65-67] HDL-cholesterol[68,69] and prolactin.[70,71]

RESULTS AND DISCUSSION

I- Results of biological study

The results of two subjects in the group taking *N. sativa* oil were excluded due to their noncompliance in taking the oil capsules.

The results of this study showed a significant reduction in blood glucose level (BGL) after ingestion of *N. sativa* seeds ($P < 0.05$) and equivalent amount of oil ($P < 0.01$) after one week of treatment. However the blood glucose level showed slight increase in the second week in case of *N. sativa* seeds but yet still insignificantly lower than before treatment, while in case of oil, the BGL remain significantly reduced. The blood cholesterol levels (BCL) was also significantly reduced after one week of administration of the seeds, ($P < 0.01$) (Tables 1 and 3) and the equivalent amount of the oil ($P < 0.05$) (Tables 2 and 5). While the high density lipoprotein (HDL) level was significantly increased after one week of administration of both seeds ($P < 0.01$) and oil ($P < 0.05$) and this was insignificantly increased after the two weeks but still higher than the first reading. Both the crushed seeds and prepared oil showed non significant effect on WBCs, RBCs, platelets, hemoglobin and creatine kinase (CK) activity. These parameters was elevated after two weeks of administration of seeds and the oil, but still lower than before treatment (Tables 3, 4 and 6).

Also the creatinine blood level was reduced in case of seed after one week ($P < 0.01$) and in case of oil ($P < 0.05$) and this continued for the second week (Tables 1 and 3). After ingestion of *N. sativa* seeds there is a non significant effect on blood prolactin level after one and two weeks while in case of oil, there is a non significant effect after one week and a highly significant decrease after two weeks of administration (Table 2). Both of the crushed seeds and oil produced a non significant effect on creatine kinase activity (Tables 1, 2 and 5), and the number of WBCs, RBCs, platelets and hemoglobin after one week of administration (Tables 3, 4 and 6). The effect of *N. sativa* seeds and oil on clotting time was controversial, the seeds showed non significant increase after one week and highly significant increase after two weeks ($P < 0.01$) while the oil showed a significant decrease after one week ($P < 0.01$) and non significant increase after two weeks.

II- Results of phytochemical screening, standard values and chromatography

Preliminary phytochemical screening of the dried powdered seeds of *N. sativa* revealed the presence of volatile oil, carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, saponins, tannins, alkaloids and/or basic nitrogenous compounds and trace amount of flavonoids.The percentage of fixed oil obtained from *N. sativa* seeds cultivated in Jordan varied according to the method used. The oil prepared by compression was found to be 12.5% w/w and this ratio was similar to those published for *N. sativa* cultivated in Egypt[12,39] and India.[15] The study of the unsaponifiable content in *N. sativa* seeds revealed that the percentage of the unsaponificable matter in the oil prepared by compression was 0.65% w/w, TLC of the unsaponificable matter showed the presence of 4 major spots, Column chromatography of the unsaponificable matter resulted in isolation and identification of ß-sitosterol and oleanolic acid (m.p, m.m.p, IR and co-chromatography with authentic samples). The oil is yellow in colour, refractive index 1.4720, density 0.91, iodine value 99.0/100 g, saponification value 199.2 mg/g and unsaponifiable matter 0.65%.

The prepared oil showed the same physico-chemical constants nearly similar to those reported for *N. sativa* oil cultivated in Egypt[10,22,39] and India[15] except the iodine value and this may be due to the lower amount of unsaturated fatty acids in the oil. The major substances of the unsaponificable matter were ß-sitosterol and oleanolic acid. The oil was prepared in capsule dosage form, because it is more convenient to use it in a such simple dosage form.

Table 1: Effect of *N. sativa* seeds on different parameters in 10 test subjects.

Item	Reading No.	Mean ± S.D.	t-calculated	p-value
Glucose (mg/dl)	0	99.10 ± 15.20		
	Wk1	62.60 ± 20.71	3.100	**0.05**
	Wk2	86.80 ± 28.90	1.241	0.10
Cholesterol (mg/dl)	0	175.70 ± 47.62		
	Wk1	151.82 ± 43.00	4.801	**0.01**
	Wk2	172.21 ± 48.91	0.56	0.10
HDL (mg/dl)	0	46.91 ± 3.10		
	Wk1	56.42 ± 7.00	4.430	**0.01**
	Wk2	48.11 ± 4.20	1.000	0.10
Creatinine (mg/dl)	0	1.00 ± 0.10		
	Wk1	0.95 ± 0.08	5.001	**0.01**
	Wk2	0.94 ± 0.10	2.660	**0.05**
Creatine kinase (U/L)	0	56.92 ± 7.61		
	Wk1	55.41 ± 6.50	1.320	0.10
	Wk2	55.80 ± 5.76	0.800	0.10
Prolactin (ng/ml)	0	14.40 ± 4.60		
	Wk1	15.41 ± 7.52	0.436	0.10
	Wk2	9.02 ± 4.93	2.069	0.10

Readings,

0: Pre-treatment
Wk1: after one week of treatment
Wk2: after two weeks of treatment
S.D.: Standard deviation

Table 2: Effect of *N. sativa* seed oil on different parameters in 8 test subjects.

Item	Reading No.	Mean ± S.D.	t-calculated	p-value
Glucose (mg/dl)	0	90.70 ± 14.51		
	Wk1	63.60 ± 17.72	4.901	**0.01**
	Wk2	79.60 ± 10.41	5.000	**0.01**
Cholesterol (mg/dl)	0	206.51 ± 62.40		
	Wk1	153.02 ± 40.83	2.551	**0.05**
	Wk2	177.00 ± 49.83	1.002	0.10
HDL (mg/dl)	0	50.11 ± 3.61		
	Wk1	59.51 ± 5.91	3.421	**0.05**
	Wk2	52.32 ± 7.12	0.820	0.10
Creatinine (mg/dl)	0	0.97 ± 0.08		
	Wk1	0.93 ± 0.07	2.850	**0.05**
	Wk2	0.91 ± 0.08	3.112	**0.01**
Creatine kinase (U/L)	0	54.60 ± 10.01		
	Wk1	58.82 ± 11.02	1.061	0.10
	Wk2	58.82 ± 11.02	1.061	0.10
Prolactin (ng/ml)	0	12.37 ± 5.21		
	Wk1	15.78 ± 5.21	1.070	0.10
	Wk2	6.22 ± 1.60	3.462	**0.05**

Readings,

0: Pre-treatment
Wk1: after one week of treatment
Wk2: after two weeks of treatment
S.D.: Standard deviation

Table 3: Effect of *N. sativa* seeds on some blood counts in 10 test subjects.

Item	Reading No.	Mean ± S.D.	t-calculated	p-value
WBCs ($x\ 10^9$/L)	0	7.16 ± 1.40		
	Wk1	7.19 ± 1.87	0.100	0.10
RBCs ($x\ 10^{12}$/L)	0	5.33 ± 0.26		
	Wk1	5.27 ± 0.25	1.520	0.10
Platelet ($x\ 10^9$/L)	0	255.00 ± 63.91		
	Wk1	241.36 ± 82.62	1.260	0.10
Hemoglobin (g/dl)	0	14.67 ± 1.04		
	Wk1	14.42 ± 0.83	2.000	0.10
Clotting time (minutes)	0	5:23 ± 0.76		
	Wk1	5:43 ± 1.24	1.000	0.10
	Wk2	8:06 ± 0.99	6.860	**0.01**

Table 4: Effect of *N. sativa* seed oil on some blood counts in 8 test subjects.

Item	Reading No.	Mean ± S.D.	t-calculated	p-value
WBCs ($x\ 10^9$/L)	0	7.53 ± 1.53		
	Wk1	7.80 ± 1.30	0.580	0.10
RBCs ($x\ 10^{12}$/L)	0	5.49 ± 0.26		
	Wk1	5.45 ± 0.35	0.310	0.10
Platelet ($x\ 10^9$/L)	0	270.00 ± 44.61		
	Wk1	264.00 ± 47.82	0.690	0.10
Hemoglobin (g/dl)	0	15.41 ± 1.43		
	Wk1	15.03 ± 1.35	2.000	0.10
Clotting time (minutes)	0	6:15 ± 1.10		
	Wk1	5:24 ± 0.77	3.580	**0.01**
	Wk2	7:12 ± 1.43	2.020	0.10

Readings,

0: Pre-treatment
Wk1: after one week of treatment
Wk2: after two weeks of treatment
S.D.: Standard deviation

Table 5: Comparison between the effects of *N. sativa* seeds and its oil on some blood parameters.

Item	Week 1				Week 2			
	Seed	p-value	Oil	p-value	Seeds	p-value	Oil	p-value
Glucose (mg/dl)	62.60	**0.05**	63.6	**0.01**	86.80	0.10	79.6	**0.01**
Cholesterol (mg/dl)	151.82	**0.01**	153.0	**0.05**	172.21	0.10	177.0	0.10
HDL (mg/dl)	56.42	**0.01**	59.5	**0.05**	48.11	0.10	52.3	**0.01**
Creatinine (mg/dl)	0.95	**0.01**	0.93	**0.05**	0.94	**0.05**	0.91	**0.05**
Creatine kinase (U/L)	55.41	0.10	58.8	0.10	55.80	0.10	58.8	0.10
prolactin (ng/ml)	15.41	0.10	15.78	**0.05**	9.02	0.10	6.22	**0.05**

Table 6: Comparison between the effects of *N. sativa* seeds and its oil on some blood counts.

Item	Week 1				Week 2			
	Seed	p-value	Oil	p-value	Seeds	p-value	Oil	p-value
WBCs (x 10^9/L)	7.19	0.10	7.80	0.10	---	---	---	---
RBCs (x 10^{12}/L)	5.27	0.10	5.45	0.10	---	---	---	---
Platelet (x 10^9/L)	241.36	0.10	264.00	0.10	---	---	---	---
Hemoglobin (g/dl)	14.42	0.10	15.03	0.10	---	---	---	---
Clottnig time (minutes)	5:43	0.10	5:24	**0.01**	8:06	**0.01**	7:12	0.10

---: not measured.

Both of the crushed seeds and seed oil showed a significant lowering effects on blood glucose level after one week, this effect was continued with oil group but elevated after two weeks in case of seeds, this might be due to a down regulation or a possible desensitization related to the relatively high dose of *N. sativa* seeds[28] and adequate amount of oil used, the possible mechanism of hypoglycemic effect of *N. sativa* was suggested to enhance peripheral utilization of glucose by the tissue and this may call for a further investigation.[28] The authors suggested that this may be due to stimulate the glycogenesis which is an active process utilizing energy through the breakdown of phospho-creatine to creatinine and they provide this by increase creatinine level in their study.[28] But in our study creatinine level was significantly decreased and this suggests another mechanism of action.

Concerning the effect of *N. sativa* on blood glucose level of animals, different reports have been in literature; some authers[72,73] reported that, there is no effect on blood glucose of either normal or diabetic rats, others[74,22] proved that the volatile oil or the extracts produced a significant hypoglycemic effect on blood sugar level. The administration of the volatile oil did not alter basal insulin level which might suggest a non-insulin mediated mechanism of action for the hypoglycemic activity. The hypoglycemic study on volunteers showed a significant decrease on blood glucose level.[28]

The level of blood cholesterol was significantly decreased in case of both seeds and oil after one week of administration. The reduction of the cholesterol level is more significant in case of the crushed seeds ($P < 0.01$) than the oil ($P < 0.05$), this may be due to the presence of ß-sitosterol which is the dominant sterol in the *N. sativa* seeds and its oil as confirmed from the phytochemical results as well as the reported literature.[75,76] Also, it was suggested that the lowering of cholesterol level may be attributed to the enhancing effect of *N. sativa* on cholesterol excretion in the bile,[28] or due to initial increase in HDL level as clear from this study (Tables 1, 2 and 5), since the HDL is significantly elevated after one week of receiving of both seed and oil.

Both the seeds and the seed oil produced a non significant decrease in haemoglobin. Studies on animals showed a significant increase of blood haemoglobin when the alcoholic extract of the seeds was administered to rats.[22]

Surprisingly the oil showed a significant decrease in prolactin level after two weeks of administration (Tables 2 and 5) meanwhile the seeds showed a non significant decrease in this parameter (Tables 1 and 5). Prolactin usually increases after delivery because of the significant drop of progesterone levels.[77] By other hand, a fall of estradiol and progesterone levels combined with stress can lead to an increased pituitary secretion of prolactin in women.[78] So we propose that it may be of much concern if the progesterone levels are proved to be increased simultaneously with the decrease of prolactin levels in females treated with *N. sativa* seed oil. This proposal may elucidate a dopaminergic like effect of *N. sativa* by inhibiting prolactin secretion which may lead to one other use of *N. sativa* oil to decrease prolactin levels in some cases of hyperprolactinemia. The study of the effect of *N. sativa* seeds and formulated oil on female volunteers is in progress now.

So, this study reports that the effects of oral administration of *Nigella sativa* seeds and seed oil on some blood parameters are consistent with the use of the seeds in folk medicine. Further studies, however, are required before these seeds and oil can be stated as useful for the treatment of hyperglycemia, hypercholesteremia, and hyperprolactinemia.

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