Effect of prolonged drenching of aqueous extract of *Nigella sativa* seeds on body weight, some blood parameters and serum enzymatic activities of female rabbits *(Lepus cuniculus)*

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Abstract

This work had been done on thirty six immature female rabbits of mean body weight of 485 gms and average age of 1.5 months randomly allocated into three groups of twelve animal each. First and second groups were drenched 200 and 500 mg/kg/day aqueous extract of defatted *Nigella sativa* seeds extract for sixteen weeks. The third group was drenched the same volume of physiological saline only for the same period and regarded as controls .All the animals were weight at the beginning of the experiment then every week till the end of the experiment. Also blood samples were taken from each animal at the end of the experiment to estimate level of haemoglobin (Hb gm/dl), total protein level; blood glucose level; clotting time and also serum enzymatic activity of SGOT and SGPT which were determined photometrically.Results obtained were as follow:

1- Group drenched 500 mg/kg/day for 16 weeks showed significant increase in body weight gain during first 5 weeks but then showed significant decrease in body weight gain till the sixteen weeks of the treatment.
2- Females treated with 200 mg/kg/day for 16 weeks showed significant decrease in average body weight gain.
3- Also results showed significant decrease in Hb% in both treated groups of 200 and 500 mg/kg/day as ($P<0.05$)and ($P<0.01$) respectively.
4- Highly significant decrease ($P<0.01$) in blood sugar level was found in both treated groups.
5- Highly significant ($P<0.01$) prolong in blood clotting time in both treated groups were found.
6- Highly significant increase($P<0.01$)in SGOT and SGPT in group drenched 500 mg/kg/day while no significant effect of these two enzymes in group drenched 200 mg/kg/day.

Introduction

Nigella sativa L. belongs to the family Ranunculaceae. The herb is widely grown in different parts of the world and its seed are used as a condiment. In South Asia it is known “Kalonji” and its Arabic name is “Habat-al Souda” (Nadkarni, 1979). In the western world, it is known as: Black Cumin”(Ghazanfer,1994).In the traditional system of medicine , it is recommended in a wide range of ailments including, asthma, chronic headache, migraine, chest congestion, dysmeno-rrhoea, infections (both fungal and bacterial), obesity, paralysis, rheumatism, hypertension and gastro- intestinal problems like dyspepsia and flatulence (Nadkarni,1979 and Usman-ghani *et al.*, 1997). It has also been used as stimulant: diuretic and anthelmentic. Chemical components studies on seeds revealed the presence of volatile oil (1.5%);fixed oil (37.5%); nigellin; melanthin and arabic acide (Nadkarni, 1976), and thimoquinone (Houghton *et al.*,1995).The volatile oil consists mainly of carvone (45-60%); carvene and cymene (Nadkarni, 1976) and thymoquinone (El-Tahir and Ashour, 1993).Pharmacological effects of the isolated alkaloids from *N. sativa* seeds such as nigellidine, nigellimine and nigellicine have not been reported (Rehman *et al.* ,1995), (Rehman *et al.*,1992) and (Rehman and Malik,1985). Consequently, Kalonji has been extensively studied particularly in the Islamic world, which justifies its broad traditional therapeutic value. It was found to have wound healing effect (Ahmed *et al.*, 1995). Antioxytocic effect (Aqel and

Shaheen, 1996), antilipamic effect (Bashandy, 1996), anti-plague (Nambal *et al.*, 1985), CNS depressant and analgesic (Khanna and Zaidi, 1991), anticancer (Salomi *et al.*, 1992), histamine release inhibitor (Chakaravarty, 1993), anti-hypertensive (El-Tahir and Ashour, 1993), and anti inflammatory (Mutabagani and El-Mahdy,1997).This work aimed to study the effect of prolonged administration of aqueous extract of defatted *N. sativa* seeds on body weight, some blood parameters and serum enzymatic activities of local female rabbits (*Lepus cuniculus*).

Materials and Methods

The dried seeds of *Nigella sativa* were purchased form local market in Basrah, authenticated with the help of a botanist at Basrah University; The sample identified at the herbarium of Basrah University at the college of science.Seventy five gms of *N. sativa* seeds were powdered and defatted with (n-hexan).The combined n-hexan extract was concentrated below 50c under reduced pressure in a rotary evaporator to get 25 gms of dark brown oily mass. This mass was dried at room temperature and then further extracted with distal water (500 ml) and the combined extract with distal water was concentrated under reduced pressure below 50c to get the aqueous extract (Harborn, 1986).

Animals: Thirty-six local immature female rabbits weighing between (380-530) gms and aging between (1-1.5) mouths were divided into three equal groups randomly.First and second groups were drenched 200 and 500mg/kg/day of aqueous extract of *N. Sativa* respectively for 16 weeks. The third group was regarded as the control group and drenched the same volume of the daily dose of normal saline for the same period. These animals maintained in air conditioned quarters (24C$^{\circ}$) under standard husbandry condition with alternate 12 hours light/dark period. Animals were given a prepared ration composed of wheat flour, bran mash powdered milk and 1% NaCl in addition to green alfalfa and tab water *ad labium*. The following criteria were studied:

Effect on body weight: Rabbit in each group were weighed at the beginning of the experiment then every week till the end of the experiment which lasted 16 weeks. The body weight gain of each group was calculated and recorded.

Effect on some blood parameters: For revealing the different hematological changes that may occur following oral administration of aqueous extract of *N. sativa* seeds at the end of the experiment blood sample (2ml) were taken from each female rabbit from the heart in a clean dry test tube containing EDTA as anticoagulant, and the following criteria were measured: Hemoglobin %, total protein level, glucose level, and clotting time were calculated immediately after blood sampling.

A-Determination of Hemoglobin level: Determination of Hb level (mg/dl) was carried out as explained by (Coles, 1986) as hemoglobin reaction with hydrochloric acid to form acid hematin which could be measured by Sahli haemometer (Hawksly Comp. England).

B-Determination of total protein level: Total protein level was determined in plasma of blood samples by the Buiret method (Henry *et al*., 1974) using total protein kits.

C-Determination of glucose level: Glucose was determined in plasma by the glucose oxidase method (Teuscher and Richterich, 1971) and (Barham and Trinder, 1972) using liquid Glucose GOD-PAP kits.

D-Determination of clotting time: Determination of clotting time of the blood samples by two methods: Capillary tube method and Lee and White method (Coles, 1986) and (Shalm *et al*., 1981)

E-Effect on serum enzymatic activity: For revealing the changes in the activity of some enzymes after prolonged oral administration of the studied plant, rabbits were bled to obtain 1 ml of blood from each rabbit, samples were left to clot, then centrifuged at 1500 r.p.m. for 10 minutes and the obtained sera were subjected for the following studies:The activities of serum glutamic oxaloacetic transaminase

(SGOT) and serum glutamic pyruvic tranaminase (SGPT) were determined photometrically as described by (Ritman and Frankel, 1957).

Statistical analysis: The data obtained were computed using (SPSS) software and later analyzed using one way analysis of variance (ANOVA) test.

Results

A-General body weight: The mean difference of increase in general body weight of female rabbits previously administrated aqueous extract of *Nigella sativa* Seeds (200 and 500 mg/kg/day) for 16 successive weeks are recorded in table (1).The obtained data revealed that the aqueous extract of the studied plant significantly increase the general body weight of treated females, especially (500 mg/kg/day)which occurs during the first 6 weeks of treatment but after that period there was no change in general body weight till the end of the treatment, when body weight become near the body weigh of the control group while at the sixteenth week the body weight showed a significant decrease ($P<0.05$) in the average body weight compared with controls. Female rabbits treated with (200mg/kg/day)did not show any difference in body weight till 14 weeks of treatment but after that at 15-16 weeks of treatment they showed a significant increase ($p<0.05$) in average body weight.

B- weekly body weight gain: The mean difference in the increase of weekly body weight gain previously administrated aqueous extract of defatted *N. sativa* seeds (200 and 500 mg/kg/day) for 16 successive weeks are recorded in table(2).The obtained data revealed that aqueous extract of the tested plant show increase in the average body weight gain between significant ($P<0.05$)and highly significant ($P<0.01$) for females treated with 500 mg/kg/day during the first five weeks of treatment while there was decrease in average weekly gain between significant ($P<0.05$)and highly significant ($P<0.01$) for females treated by 200mg/kg/day especially during the weeks(6; 13-15)of treatment period.

Table (1)Effect of oral administration of aqueous extract of *Nigella sativa* seeds on general body weight of female rabbits

Control group 3 ml of normal saline (n=12)		200 mg / day of aqueous extract (n=12)		500 mg / kg /day of aqueous extract (n=12)	
0 wk	403.80±3.13	405.06±3.63	a	425.0±7.49	a
1 wk	414.75±4.57	430.83±13.38	ab	481.66±6.31	b*
2 wk	465.0±5.04	480.0±13.38	a	543.41±6.23	b*
3 wk	520.5±5.00	526.41±12.96	a	611.0±7.93	b**
4 wk	563.5±6.56	571.66±4.23	a	670.16±7.27	b*
5 wk	600.74±8.13	619.16±3.75	a	745.83±6.31	a
6 wk	669.74±6.41	709.66±5.77	ab	778.83±7.98	a
7 wk	738.5±6.59	743.91±6.49	a	8.7.58±6.7	a
8 wk	776.5±8.86	780.16±5.95	a	828.76±8.42	a
9 wk	830.58±4.81	821.08±4.44	a	869.16±8.92	a
10 wk	898.66±6.84	893.75±3.97	a	910.66±10.56	a
11 wk	940.33±7.45	937.16±4.64	a	975.5±10.9	a
12 wk	1007.25±4.86	985.25±6.41	a	1007.0±10.5	a
13 wk	1064.91±7.09	1024.25±8.02	a	1054.50±11.95	a
14 wk	1121.75±8.09	1054.83±3.39	a	1086.58±10.26	a
15 wk	1187.25±6.31	1086.75±5.46	b*	1121.83±12.29	ab
16 wk	1258.0±6.76	1119.75±6.89	b**	1156.83±10.61	b*

(P<0.05)*
(P<0.01)**

Table (2) Effect of administration of aqueous extract of *Nigella sativa* seeds on weekly body weight gain of female rabbits.

Control group of 3 ml of normal saline (n=12)		200 mg / day of aqueous extract (n=12)		500 mg / kg /day of aqueous extract (n=12)	
1 wk	10.99±1.44	25.77±9.75	b	56.66±1.18	a*
2 wk	50.29±0.47	49.17±0.0	a	61.75±0.08	b*
3 wk	55.5±0.04	46.41±0.42	a	67.59±1.7	b**
4 wk	43.0±1.56	45.25±8.73	a	59.16±0.66	b*
5 wk	43.24±1.57	47.5±0.48	a	75.67±0.96	b**
6 wk	63.0±1.72	90.5±2.02	a	33.0±1.67	a
7 wk	68.78±0.18	34.25±0.72	b	28.75±1.28	a
8 wk	38.0±2.27	36.25±0.54	b	21.18±1.72	a
9 wk	54.08±4.05	40.92±1.54	a	40.4±0.5	a
10 wk	68.08±2.03	72.67±0.44	c	41.5±1.64	a
11 wk	41.67±0.61	43.41±0.67	a	64.84±0.34	b*
12 wk	66.92±0.61	48.09±1.77	a	31.5±0.39	a
13 wk	57.66±2.23	39.0±1.61	b	47.5±1.44	a
14 wk	56.84±1.0	30.58±4.63	b	32.08±1.69	a
15 wk	65.5±1.78	31.92±2.07	b	35.35±2.03	a
16 wk	70.75±0.45	33.0±1.52	b	35.0±1.68	a

(P<0.05)*
(P<0.01)**

C-Hemoglobin concentration: There is a significant decrease (P<0.05) in hemoglobin concentration in females rabbits treated by 200 mg/kg/day and highly significant decrease (P<0.01) in hemoglobin concentration in females treated with 200 mg/kg/day.

Table (3) Effect of prolonged oral administration of aqueous extract of *Nigella sativa* seeds on hemoglobin concentration in blood of female rabbits.

Groups		Hemoglobin concentration g/dl
Control (3 ml of normal saline)		11.51±0.53 a
aqueous extract of defatted *Nigella sativa* seeds	200mg/kg/day	11.07±0.53 b*
	500mg/kg/day	10.92±0.34 c**

Number of animal in each group=12
Mean±S.D, *=(P<0.05) ,**=(P<0.01)

D-Effect on sugar level: Highly significant decrease (P<0.01) in blood sugar (89.41 mg/100ml and 99.41 mg/100) blood in groups drenched (200 and 500 mg/kg/day) of aqueous extract respectively compared with normal value (128 mg/100ml) blood, while there was no significant effect of treatment on plasma total protein.

Table(4) Effect of prolonged oral administration of aqueous extract of *Nigella sativa* seeds on blood sugar and plasma total protein of female rabbits (n=12).

Groups		Sugar level in plasma mg/100ml	Level of total protein g/dl
Control (3 ml of normal saline)		128±6.16 a	7.32±0.89
aqueous extract of defatted *Nigella sativa* seeds	200mg/kg/day	89.41±12.95 b**	7.066±0.63 a
	500mg/kg/day	79.41±7.05 c**	6.94±0.71 a

Mean ±S.D
*=(P<0.05)
**=(P<0.01)

E-Effect on clotting time: The aqueous extract of *N. sativa* seeds had significantly prolonged clotting time (P<0.01) of blood of treated females with doses of (200 and 500mg/kg/day).

Table (5). Effect of prolonged oral administration of aqueous extract of *Nigella sativa* seeds on clotting time of female rabbits (n=12) (X±SD)

Groups		Lee-white method	Capillary tube method
Control (3 ml of normal saline)		166.166±44.74 a	174.83±41.46 a
aqueous extract of *N. sativa* seeds	200mg/kg/day	261.25±50.99 b*	233.75±38.32 b*
	500mg/kg/day	269.58±90.01 b*	255±92.68 b*

*=(P<0.01)

F-Effect on serum enzymatic activity: The influence of prolonged oral administration of aqueous extract on the enzymatic activity are demonstrated in table(6). Results obtained showed that aqueous extract of the studied plant significantly increased serum glutamic oxaloacetic transaminase (SGOT) and also significantly increased the activity of serum glutamic pyruvic tranaminase (SGPT)of female treated with 500mg/kg/day for 16 weeks whereas this aqueous extract of the tested plant did not alter the activity of the two enzymes when given at the dose of 200mg/kg/day for 16 weeks.

Table (6)Effect of prolonged oral administration (16 weeks) of aqueous extract of *N. sativa* on some enzymatic activity of female rabbits (N=12).

Groups		SGOT unit/L	SGPT unit/L
Control (3 ml of normal saline)		6.16±2.28 a	6.41±0.51 a
aqueous extract of *defatted Nigella sativa* seeds	200mg/kg/day	7.33±1.87 a	7.25±2.0 a
	500mg/kg/day	9.58±2.31 b*	10.0±1.8 b*

Mean±S.D
*=(P<0.01)

Discussion

The obtained results demonstrated that aqueous extract of defatted *Nigella sativa* seeds cause significantly decreased (P<0.05) body weight gain of females rabbits drenched this extract for 16 successive weeks. These effects may be due to anemia induced by this extract recorded by this investigation, as well as our findings showed that extract significantly (P<0.05) decreased hemo-globin content of the treated groups compared with the control one. This effect may be attributed to the effect of the tested extract on the haemopoetic tissue. This effect is agreement with that recorded by Kandil *et al*. (1993) that found the aqueous extract of *N. sativa* seeds caused highly significant decrease in body weight gain of rats drenched this extract for 12 weeks and this effect agreed with results of Al-Okbi *et al*. (1988) when recorded that the aqueous and alcoholic extracts caused decrease in body weight gain of rats. This study showed highly significant decrease (P<0.01) in fasting blood glucose level in all treated groups. This finding suggested that the hypoglycemic effect of *N. sativa* increased glycolysis in peripheral tissues, decreases glyconeogenesis and inhibits release of counter-regulatory hormones such as glucagons, cortisol and growth hormone which are the possibilities that may be considered. This effect resembles that of Akhtar and Ali (1985) and Hawsawi *et al*. (2001).Statistically the effect of aqueous extract of *N. sativa* seeds showed non significant effect on total protein level which is agreed with that of Al-Okbi *et al*. (1998). This study also showed significant prolong in clotting time of treated animals compared with controls which may be due to the cause of the used extract to inhibit the formation of arachidonic acid and adenosine diphosphate which were the inducer of platelets aggregation. This effect is in agreement with that of Enomoto *et al.* (2001). The obtained results denoted that aqueous extract of the tested plant significantly increased the activity of serum glutamic-oxaloacetic trnsaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT). These effects may be attributed to the influence of this extract on liver and heart. This effect is in agreement with results of Ibraheim *et al*. (2001) and Tennkoon *et al*. (1991) when reported that aqueous extract of *N. sativa* lead to increase (GPT) in serum of blood of male rats and this result is also agreed with results of Kandil *et al*. (1993).Conclusively although the studied extract altered some blood criteria and enzymatic activities at the tested dosage, the plant was used effectively in therapy. In this respect the authors recorded that aqueous extract of *N. sativa* seeds in concentration above 50 mg/ml can be used effectively in treatment of various skin diseases caused by pathogenic bacteria and fungi. In addition extract of tested plant showed a potent antispasmodic activity in concentration above 10mmg/ml and significantly decrease blood glucose level and prolongs clotting time so it can be used in treatment of hyperglycemic and hypertension.

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تأثير التجريع طويل الأمد للمستخلص المائي لبذور نبات الحبة السوداء على وزن الجسم وبعض معايير الدم ومستوى نشاط بعض الأنزيمات في إناث الأرانب

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الخلاصة

أجريت هذه الدراسة على ستة وثلاثون من إناث الأرانب المنزلية الغير بالغة والتي كانت معدل أوزانها 485 غم ومعدل أعمارها شهر ونصف. قسمت عشوائيا الى ثلاث مجموعات متساوية كل مجموعه احتوت على 12 أنثى أرنب .جرعت المجموعتان الأولى و الثانية 200 و 500 ملغم/كغم /يوم من المستخلص المائي لبذور نبات ألحبه السوداء المنزوعة الدهن لمدة ستة عشر أسبوعا .إما المجموعة ألثالثه فجرعت نفس الحجم من المحلول الملحي الطبيعي لنفس المدة و اعتبرت مجموعة سيطرة وزنت جميع الحيوانات في بداية التجربة وفي نهاية كل اسبوع بعد ذلك لغاية نهاية التجربة . كذلك أخذت نماذج دم من كل حيوان عند نهاية التجربة وقيست نسبة خضاب الدم و مستوى البروتين الكلي ومستوى السكر في الدم وزمن تخثر الدم و كذلك تم قياس مستوى أنزيمات SGOT وSGPT والتي قيست باستعمال المطياف الضوئي . و قد تم الحصول على النتائج التالية :-

1- المجموعة التي جرعت 500 ملغم/كغم/اليوم لمدة 16 أسبوعا اظهرت زيادة معنوية في وزن الجسم الخمسة أسابيع الأولى و لكنها أظهرت انخفاض معنوي في الزيادة الوزنية لغاية الاسبوع السادس عشر من التجربة .
2- المجموعة التي جرعت 200 ملغم /كغم /اليوم لمدة 16 أسبوع أظهرت انخفاض معنوي في معدل الزيادة الوزنية .
3- كذلك بينت النتائج ان هناك انخفاض معنوي في نسبة خضاب الدم في كلتا المجموعتين المعاملتين ب 200 و 500 ملغم /كغم /اليوم بمستوى($P<0.05$) و($P<0.01$) على التوالي.
4- وجد إن هناك انخفاض عالي المعنوية ($P<0.01$) في مستوى السكر في الدم في كلتا المجموعتين المعاملتين.

5- ظهر إن هناك زيادة عالية المعنوية ($P<0.01$) في زمن تخثر الدم في كلتي المجموعتين المعاملتين .
6- وجد ان هناك ارتفاع عالي المعنوية ($P<0.01$) من إنزيمي SGOT و SGPT في المجموعة المعاملة ب 500 ملغم /كغم /اليوم بينما لم يكن هناك أي تأثير معنوي للإنزيمين في المجموعة المعاملة ب200ملغم /كغم / اليوم.