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Effects of *Nigella sativa* fixed oil on blood homeostasis in rat

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Abstract

We investigated the effects of the fixed oil of *Nigella sativa* seeds in rats by monitoring blood homeostasis and body weight as well as toxicity. Animals were treated daily with an oral dose of 1 ml/kg body weight of the *N. sativa* seed fixed oil for 12 weeks. Changes in key hepatic enzymes levels were not observed in *N. sativa* treated rats after 12 weeks of treatment. The serum cholesterol, triglycerides and glucose levels and the count of leukocytes and platelets decreased significantly by 15.5, 22, 16.5, 35 and 32%, compared to control values, respectively; while haematocrit and haemoglobin levels increased significantly by 6.4 and 17.4%, respectively. In parallel, significant slowdown of the body weight evolution was observed in *N. sativa* treated animals comparatively to the animal control group. On the other hand, no mortality was noted for ten times the therapeutic dose in mice, during 15 days period after the oil administration (10 ml/kg p.o.). These results support the traditional use of *N. sativa* seeds as a treatment of the dyslipidemia and the hyperglycaemia, and related abnormalities; however, indicate a relative toxicity of this plant. Acute and chronic toxicity, and the mode of the action of the *N. sativa* fixed oil must be studied. © 2002 Published by Elsevier Science Ireland Ltd.

Keywords: *Nigella sativa*; Fixed oil; Biochemical parameters; Haematological parameters; Toxicity

1. Introduction

Nigella sativa L. (Ranunculaceae), commonly known as 'black cumin', is an erect herbaceous annual plant. It grows in Mediterranean countries and is also cultivated in the north of Morocco. *N. sativa* seeds have traditionally been used in Middle Eastern folk medicine as a natural remedy for various diseases as well as a spice for over 2000 years. The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years. It has been shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid (Houghton et al., 1994). *N. sativa* seeds decrease the serum total lipids and body weight in *Psammomys obesus* sand rat (Labhal et al., 1997), decrease the fasting plasma glucose in rabbit (Al-Hader et al., 1993), increase serum total protein (Haq et al., 1995), and shows diuretic and hypotensive effects in spontaneously hypertensive rat (Zaoui et al., 2000). Studies in mice and rats have shown that treatment with *N. sativa* extract significantly protects from cisplatin-induced falls in leukocytes counts, haemoglobin level, mean osmotic fragility and haematocrit increase (Nair et al., 1991; El-Daly, 1998), influences leukocytes activities (Haq et al., 1995; Houghton et al., 1994) and causes the death of mice lymphocytes in vitro (Salomi et al., 1992). In Morocco *N. sativa* and its derived products are consummated abusively for traditional treatment of blood homeostasis abnormalities. This study was therefore undertaken to determinate the effects of a chronic treatment with *N. sativa* fixed oil on blood biochemical and haematological parameters in rat.

2. Materials and methods

2.1. Preparation of the *N. sativa* seed extract

The plant seeds were harvested in the North of Morocco. The plant material was identified and authen-

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ticated as *N. sativa* (L.) (Ranunculaceae), by Professor A. Ouyahya, a plant taxonomist from the Scientific Institute of Rabat. A voucher specimen has been deposited at the repository in our institute in Rabat. The *N. sativa* seeds were powdered mechanically. The extract was obtained by cold shocking of the powdered seeds in 3×1.5 l of hexane during 3×24 h. The solvent was removed from the extract under reduced atmospheric pressure. The obtained oily extract from *N. sativa* seeds has a chestnut colour and agreeable perfume.

2.2. Experimental animals

Wistar kyoto rats were purchased from IFFA (Credo, France) and studied at 8 weeks of age. Animals were maintained on a 12 h light cycle and fed standard lab chow ad libitum. Rats were randomly assigned to two experimental groups of 12 animals each. The *N. sativa* treated rat group (Ns-rats) received daily administrations of 1 ml/kg body weight of *N. sativa* fixed oil by oral gavage (force-feeding) for 12 weeks period. Control rat animals (C-rats) were treated in an identical fashion with 1 ml/kg body weight of water.

Body weight was measured at J0 and 2, 4, 6, 8, 10 and 12 weeks.

2.3. Metabolic and haematological measurements

Metabolic and haematological measurements were realised at J0 and, 4, 8 and 12 weeks following oil administration. Animals were studied after an (15 h) overnight fast. Afterwards, blood was obtained from the retro-orbital sinus (2 ml). Metabolic measurements were realised spectrophotometrically, according to standardised procedures, using commercially available kits, purchased from BOEHRINGER Mannheim (Meylan, France). Haematological parameters were determined automatically by ABX COBAS LO.

2.4. Toxicity

A group of ten mice were given *N. sativa* fixed oil at the dose of 10 ml/kg p.o. ($10 \times$ therapeutic dose). The animals were observed for gross effects and mortality during 15 days.

2.5. Statistical analysis

All data are expressed as mean $\pm$ SD. Student's and Snedecor's tests were applied.

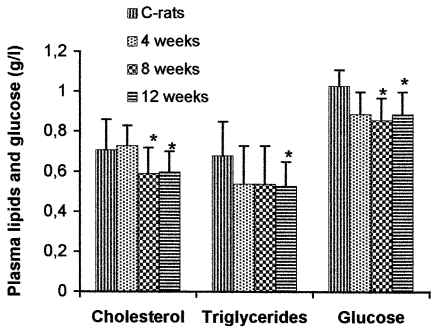

Fig. 1. Effects of *N. sativa* fixed oils (1 ml/kg/day) on plasma lipids and glucose in rat. Plasma cholesterol, triglycerides and glucose were measured as described in Section 2. Rats were treated with *N. sativa* seed extract (Ns-rats; $n = 12$) for 12 weeks. Plasma lipid and glucose values are given for the 4, 8 and 12 weeks treatment points. Values are expressed as mean $\pm$ SD. Values in control rats (C-rats) were similar at all points and are grouped for the sake of simplicity. *Significantly different from C-rats group by Student's test, $P < 0.05$.

3. Results

3.1. Biochemical parameters

Fig. 1 shows the effects of *N. sativa* fixed oil on the metabolism of plasma lipids and glucose. After 12 weeks of daily treatment (1 ml/kg), serum cholesterol, triglycerides and glucose levels were decreased significantly by 15.5, 22, 16.5%, respectively when compared to the control values observed in placebo animals ($P < 0.05$).

Table 1
Effects of *N. sativa* fixed oils (1 ml/kg/day) on plasma key hepatic enzymes, bilirubin, uric acid and creatinin in rat as a function of treatment time

Parameter (unit)	C-rats	Ns-rats treatment time (weeks)		
		4	8	12
ASAT (U/l)	128 ± 59	120 ± 40	156 ± 76	171 ± 72
ALAT (U/l)	47.5 ± 9.2	41.1 ± 12.1	38.4 ± 14.6	43.4 ± 15.7
ALP (U/l)	213.2 ± 68.4	187.8 ± 61.4	149.0 ± 57.4*	139.4 ± 59.3*
GGT (U/l)	4.40 ± 2.77	5.08 ± 2.15	3.85 ± 1.95	3.18 ± 1.83
T-bilirubin (mg/l)	1.34 ± 1.0	1.10 ± 0.94	1.50 ± 1.2	1.10 ± 0.7
Uric acid (mg/l)	15.8 ± 5.8	15.5 ± 5.13	17.1 ± 7.3	14.2 ± 4.2
Creatinin (mg/l)	5.92 ± 1.32	6.36 ± 0.87	5.21 ± 0.85	6.76 ± 0.77

C-rats, control rats group; Ns-rats, *N. sativa* treated rats group; ASAT, aspartate-aminotransferase; ALAT, alanine-aminotranferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase. Values are expressed as mean $\pm$ SD ($n = 12$).

* Significantly different from C-rats by Student's test, $P < 0.05$.

Table 2
Effects of *N. sativa* fixed oils (1 ml/kg/day) on haematological parameters

Parameter (unit)	C-rats	Ns-rats treatment time (weeks)		
		4	8	12
Erythrocytes (10^6/mm^3)	7.23 ± 1.03	7.05 ± 0.57	6.74 ± 0.44	6.91 ± 0.68
Leukocytes (10^3/mm^3)	7.25 ± 1.71	8.23 ± 1.6	7.6 ± 1.3	4.7 ± 1.5**
Platelets (10^3/mm^3)	743 ± 121	680 ± 82	570 ± 109**	504 ± 110**
Haematocrit (%)	40.99 ± 4.32	39.26 ± 2.49	40.73 ± 3.15	43.60 ± 3.11*
HGB (g/dl)	13.12 ± 1.45	13.53 ± 0.62	14.3 ± 0.94*	15.4 ± 0.64**
MGV (μm^3)	53.35 ± 0.14	52.75 ± 2.96	55.56 ± 2.01	56.3 ± 3.53**
$MCCH_1$ (pg)	17.97 ± 0.95	18.77 ± 1.41	19.13 ± 1.48*	19.26 ± 1.22**
$MCCH_2$ (g/dl)	32.94 ± 1.81	34.82 ± 2.12	34.70 ± 1.84*	34.80 ± 1.32**

C-rats, control rats group; Ns-rats, *N. sativa* treated rats group; HGB, haemoglobin; MGV, mean globular volume; $MCCH_1$, mean corpuscular content of haemoglobin; $MCCH_2$, mean corpuscular concentration of haemoglobin. Values are expressed as mean ± SD ($n = 12$).

* Significantly different from C-group by Student's test, $P < 0.05$.

** Significantly different from C-group, $P < 0.01$.

Table 1 illustrates the effects of *N. sativa* fixed oil on plasma key hepatic enzymes, bilirubin, uric acid and creatinin in rats as a function of treatment time. After 12 weeks of daily treatment (1 ml/kg), plasma key hepatic enzymes, bilirubin, uric acid and creatinin did not increase significantly compared to the control values observed in placebo animals.

3.2. Haematological parameters

Table 2 illustrates the haematological parameters in *N. sativa* treated rats as a function of treatment time. After 12 weeks of treatment, the leukocytes and platelets counts decreased significantly when compared to the control values ($P < 0.01$), whereas heamatocrit and haemoglobin level (HGB) increased significantly ($P < 0.05$). Consequently, mean globular volume (MGV), mean corpuscular content of haemoglobin ($MCCH_1$), and mean corpuscular concentration of haemoglobin ($MCCH_2$) were increased significantly ($P < 0.01$).

3.3. Effect on body weight

Fig. 2 shows the evolution of the mean body weight in the *N. sativa* treated and control rat groups. The progression of body weight was not similar in both groups. Indeed, the *N. sativa* treated rats had significantly lower body weights than their control rat counterparts; this effect is statistically significant from the 6 weeks treatment point onward ($P < 0.01$).

3.4. Toxicity

N. sativa fixed oil, when given at 10 ml/kg p.o., showed no adverse effects or mortality during the observation period of 15 days following the oil administration in mice.

4. Discussion

The results obtained in the present study clearly show that *N. sativa* fixed oil chronic treatment was effective in influencing blood homeostasis in rat. Serum lipids and glucose levels, and leukocytes and platelets counts was decreased significantly, whereas the haematocrit and haemoglobin concentration was increased significantly. The serum key hepatic enzyme concentrations did not change significantly. In parallel, a slight slowdown of body weight was observed.

The effect of *N. sativa* seeds on blood homeostasis is not without precedent. Previous studies in normal and alloxan-induced diabetic rabbits have shown that treatment with the volatile oil of *N. sativa* seeds significantly diminishes plasma glucose levels (Al-Hader et al., 1993). Analogous results, accompanied with decreases in serum lipids level and body weight have also been

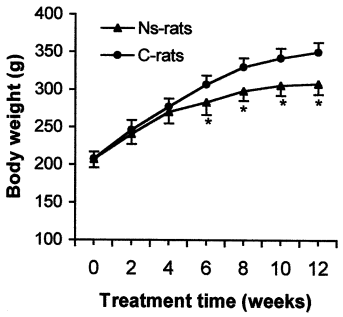

Fig. 2. Effects of *N. sativa* fixed oils (1 ml/kg/day) on body weight evolution. C-rats, control-rats group ($n = 12$); Ns-rats, *N. sativa* treated rats group ($n = 12$). Values are expressed as mean ± SD. *Significantly different from C-rats group by Student's test, $P < 0.05$.

observed in sand rats treated with an aqueous suspension of *N. sativa* seeds (Labhal et al., 1997). In rat, *N. sativa* seeds increase serum total protein (Al-Gaby, 1998).

Studies in mice and rats have shown that treatment with *N. sativa* extract significantly protects from cisplatin-induced falls in leukocytes counts, haemoglobin levels, mean osmotic fragility and haematocrit increase (Nair et al., 1991; El-Daly, 1998), influences leukocytes activities (Haq et al., 1995; Houghton et al., 1994) and causes the death of mice lymphocytes in vitro (Salomi et al., 1992).

While the underlining effects were observed at 1 ml/kg body weight of *N. sativa* extract in rats, no evidence of toxicity was noted in ten times this dose in mice, suggesting, only a seeming margin of safety for the used therapeutic doses of *N. sativa*. The changes in haemoglobin metabolism and the fall in leukocytes and platelets counts must be taken into consideration.

The slowdown of body weight evolution in *N. sativa* treated rats might be related to the serum lipids and glucose levels decrease as a consequence of a possible reduction in food intake by the drug administration. Other explanations are also possible, like a toxic effect.

In conclusion, these results support the traditional use of *N. sativa* and its derived products as a treatment for the dyslipidemia and the hyperglycaemia, and related abnormalities; however, indicate a relative toxicity of this plant extract. Acute and chronic toxicity, and the mode of the action of the *Nigella sativa* fixed oil must be studied.

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