Indian J Physiol Pharmacol 2002; 46 (2) : 195–201

THE EFFECT OF THYMOQUINONE ON BLOOD LIPIDS IN RATS

ABDULLAH O. BAMOSA*, BASIL A. ALI AND
ZUBAIDA A. AL-HAWSAWI

Department of Physiology,
College of Medicine,
King Faisal University,
PO Box 2114, Dammam, 31451
Saudi Arabia

(Received on April 16, 2001)

Abstract : We have studied the effect of thymoquinone on the blood levels of cholesterol, triglycerides, HDL and LDL in albino rats. A total of 200 rats, 150 test group and 50 as controls, were included in the study. Six doses of thymoquinone (0.5, 1, 2, 4, 6, and 8 mg/kg/day) were given through intraperitonial injections at 8 Am. The drug was administered for 5 durations 1, 4, 7, 10, and 14 days.

Thymoquinone produced significant reduction in the blood level of all parameters studied. There was no linear dose or time dependent effect on these parameters. The effect of thymoquinone started after 4 days with all doses and continued, with some swings, in the rest of the duration. The dose of 8 mg/kg was found to be toxic. It is concluded that thymoquinone has a hypocholestrolemic as well as a reducing effect on triglycrides, HDL and LDL. Therefore, we recommend further research on the therapeutic effect of thymoquinone in related diseases in humans and animals.

Key words : blood lipids cholesterol triglycerides HDL LDL *Nigella sativa* thymoquinone

INTRODUCTION

Thymoquinone is an important ingredient of the volatile oil of *Nigella sativa* (1). *Nigella sativa* is a type of plant that belongs to the Ranunculaceae family (2). It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavour in many countries. *Nigella sativa* volatile oil has been recently shown to possess 67 constituents many of which are capable of inducing therapeutic effects in humans (3).

Nigella sativa has been reported to possess many pharmacological effects (4–11). The carbonyl fraction of *Nigella sativa* (polythymoquinone) was found to protect guinea-pigs against histamine induced bronchospasm and increase bile excretion

*Corresponding Author

in dogs (4). In rabbits, the fat and petroleum ether extracts of *Nigella sativa* produced shortening in bleeding time and inhibition of fibrinolytic activity, while the petroleum ether extract induced shortening of clotting time (5). Experimental observation in rats also indicated a hypotensive effect of *Nigella sativa* (6). In humans, *Nigella sativa* was found to enhance immunity by increasing T4:T8 ratio as well as NK cell (natural killer cell) activity (7). *Nigella sativa* possesses antibacterial (8), anti-tumour activity (9), as well as an enhancing effect on the phagocytic activity of macrophages (10, 11).

Research on the effect of *Nigella sativa* on blood lipids is limited. A significant decrease in blood cholesterol was observed by the end of the first week of daily treatment with 2 g *Nigella sativa* in humans (2). El-Zawahry (13) reported an increase in serum total lipids and triglycerides and a decrease in total LDL cholesterol and LDL/HDLC ratio in rats receiving daily dose of 36 mg of *Nigella sativa* seeds for 6 weeks. However, results of large dose of *Nigella sativa* (10% of diet) on rats were mostly opposite to those of the low dose.

The aim of this research was to study the effect of thymoquinone on the blood lipids and to determine effective dose range and the best interval of treatment.

METHODS

A total of 150 as test and 50 controls female albino rats weighing 180–220 g, were included in the study. Animals were obtained from our animal house and were fed on standard diet ad libitum and normal drinking water. This study was approved by research and ethic committee of King Abdulaziz city for science and technology, the body which supported this research. Test animals were divided into 6 groups of 25 rats each. Animals in each group were given intraperitoneal injections of different concentrations of thymoquinone (0.5, 1, 2, 4, 6 and 8 mg/kg body weight respectively) (Sigma, UK), dissolved in ethanol and then diluted with normal saline. Thymoquinone injections were all given at 8 AM daily. All test animals were subsequently allowed free access to normal food and water.

Each dose group was further divided into 5 subgroups of 5 rats each. Each subgroup received the same dose of thymoquinone through intraperitoneal injections but for different duration (1, 4, 7, 10 and 14 days respectively).

The rest of animals were divided into 5 duration groups of 10 rats each and served as controls for the different durations to which the tested groups were subjected to (1, 4, 7, 10 and 14 days respectively) regardless of the dose of thymoquinone. Animals in the control groups received intraperitoneal injections of the same volume and concentration of ethanol (1%) as experimental groups and at the same time (8:00 AM) daily. All control animals were subsequently allowed free access to normal food and water.

At the end of each duration a sample of blood was obtained after 2 hours of fasting (i.e. at 10.00 AM) from each rat for

both test and control animals. The blood was withdrawn from abdominal aorta, following abdominal incision, after anesthetizing the animal with 1.25 g/kg. phenobarbitone.

From each blood sample, plasma was obtained and the blood levels of cholesterol, triglyceride, HDL and LDL parameters were measured spectrophotometrically (Spectronic instruments, USA) utilizing standard kits (bioMerieux, France).

Mean of each blood parameter from each test group was compared to its corresponding parameter in the control groups using unpaired Student's t-test. The level of statistical significance was set at P-values below 0.05.

RESULTS

All doses (0.5 to 6 mg/kg) of thymoquinone, except the dose of 8 mg, were tolerable and animals showed no signs of discomfort or toxicity. Most of the animals injected with the highest dose of thymoquinone (8 mg/kg) died by the end of first week of treatment. Animals, which survived and could tolerate the 8 mg/kg dose of thymoquinone showed signs of peritonitis on opening the abdomen for blood withdrawal. Their abdomens were full of fluid, pus, and adhesions and had a greenish color all over. Therefore, this dose was discontinued and no data could be reported for it.

Effect of thymoquinone injections on total blood cholesterol level

All five thymoquinone doses failed to produce any significant change in blood cholesterol level when administered for 1 day (Table I). Thereafter all doses, except the 6 mg/kg in 14 days group, produced significant reduction in blood cholesterol level over the rest of the duration. The highest effect for most doses seemed to have occurred following 10 days. The level of cholesterol started to swing after 4 days, the direction of swing with the smaller dose (1 mg/kg) was opposite to that with the higher doses (4 and 6 mg/kg).

TABLE I: Changes in cholesterol level (mg/dl) in normal rats treated with different doses of thymoquinone injected for different duration compared with control.

Animal groups	*Dose (mg/kg)*	*Duration*				
		1 day Mean±SD	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
Control♣	0	68±14.0	72±10.0	83±13.5	63.6±11.3	70.6±15.3
Test◆	0.5	74±6.0	57±10.4*	43.2±5.0***	51.6±6.9	44.2±6.6**
	1.0	79±18.0	54±6.0**	38±13.5***	47.6±4.2*	40.8±3.5**
	2.0	69±9.4	48.2±5.0***	46.4±5.0***	40.8±2.8**	45.8±3.8**
	4.0	64.4±10.7	43.8±5.0***	49.6±8.2***	37.2±2.3***	42.2±4.7**
	6.0	64±6.2	43±5.3***	57.6±8.6**	31.6±4.3***	72.0±4.0

♣10 Animal for each of the five duration regardless of the dose (a total of 50 control animals).
◆5 Animals for each of the five duration of each dose (a total of 150 tested animals).
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

Effect of thymoquinone injection on blood triglycerides level

All injected doses of thymoquinone produced significant reduction in triglyceride level within one day of treatment except the 1 mg/kg dose (Table II). Significant reduction in triglyceride level caused by 1 mg/kg dose was only obtained in the 10 days group. The 0.5 mg/kg dose lost its effect later on, while the 2 mg/kg maintained its effect almost in all duration. The 4 and 6 mg/kg doses caused an upward swing (at the 7th day) between two significant reductions in the 4 and 10 days groups. These higher doses tended to lose their effect by the end of the 14th day.

Effect of thymoquinone injections on HDL level

All doses, except 4.0 mg, showed no significant effect in the one-day groups. Both 0.5 and 1 mg/kg doses had non-significant reducing effect on HDL in almost all remaining duration. The other three doses (2, 4 and 6 mg) of thymoquinone produced significant reduction in the 4 and 7 days groups. Thereafter, the 6 mg dose tended to lose its significant effect, and the 2.0 mg dose lost its effects in the 14 days group (Table III).

TABLE II: Changes in triglycerides level (mg/dl) in normal rats treated with different doses of thymoquinone injected for different duration compared with control.

Animal groups	*Dose (mg/kg)*	*Duration*				
		1 day Mean±SD	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
Control♣	0	79±13.0	61.6±11.7	75.6±22	66.2±7.1	68.2±13.0
Test◆	0.5	58±20.0*	64.4±11.7	57.8±19	57.6±10.2	57±23.0
	1.0	71±13.0	56.8±9.0	53.2±13	49±10.0**	56±11.0
	2.0	51±17.2*	48.4±10.0	50±11.7*	44.4±4.3***	49.4±12.6*
	4.0	60±11.0*	48.4±8.0*	58±19.4	40.2±3.3***	55.4±19.3
	6.0	58±13.0*	46.4±5.0*	74±11.6	34.8±4.9***	63±10.3

♣10 Animal for each of the five duration regardless of the dose (a total of 50 control animals).
◆5 Animals for each of the five duration of each dose (a total of 150 tested animals).
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

TABLE III: Changes in HDL level (mg/dl) in normal rats treated with different doses of thymoquinone injected for different duration compared with control.

Animal groups	*Dose (mg/kg)*	*Duration*				
		1 day Mean±SD	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
Control♣	0	46.6±9.7	39.2±3.5	45±5.7	41.1±4.5	41.7±8.7
Test◆	0.5	53±7.4	36±3.0	39±1.0*	38.2±3.0	44±3.4
	1.0	48.4±7.7	35±4.0	40.4±8.5	28.8±2.6	40.8±8.3
	2.0	48.4±6.5	33.8±3.3*	37.4±5.2*	32.6±4.0**	40.2±6.7
	4.0	60.4±11.0*	34.6±4.7*	30.6±4.0***	30.6±4.0**	29.4±2.7*
	6.0	35±12.0	29.2±2.6***	31.8±4.0***	35.6±5.6	32.4±6.0

♣10 Animal for each of the five duration regardless of the dose (a total of 50 control animals).
◆5 Animals for each of the five duration of each dose (a total of 150 tested animals).
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

Effect of thymoquinone injections on LDL level

Thymoquinone injections failed to produce any significant effect on LDL level in the one day group. In general, all doses produced significant reduction in LDL level after the 4th day and continued in the rest of the duration. However, the significant LDL lowering effect of the 0.5 mg was evident from day 7 thereafter. Swinging in the level of LDL was seen after 7 days in rats treated with 4 mg and 6 mg. For all doses, except that of the 6 mg, the longer the duration of treatment the greater was the effect (Table IV).

Effect of thymoquinone injections on HDL/LDL and HDL/total cholesterol ratios

The one day group showed no changes in both ratios. Thymoquinone produced significant elevation in HDL/LDL ratio in most doses and duration studied (Table V). However, its effect was less consistent for HDL/total cholesterol ratio (Table VI). While the doses of thymoquinone between 0.5–2 mg elevated the ratio in the 7 and 14 days groups, it almost failed to do so in the 4 and 10 days groups. The 4 and 6 mg doses, behaved differently and produced a significant elevation in the 4 and 10 days groups only.

TABLE IV: Changes in :LDL level (mg/dl) in normal rats treated with different doses of thymoquinone injected for different duration compared with control.

Animal groups	*Dose (mg/kg)*	*Duration*				
		1 day Mean±SD	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
Control♣	0	42.4±7.5	30±3.6	40±7.0	23.6±4.7	34±6.6
Test◆	0.5	40±10.	27±4.0	17.4±9.5***	15.8±4.3**	13.6±1.7***
	1.0	40±7.2	21.8±2.7**	18±7.0***	16.6±4.2*	15.6±2.9***
	2.0	41.6±5.0	21.4±3.0***	20.8±3.1***	14.8±3.7**	15±1.9***
	4.0	40.4±9.0	18.6±2.3***	15.4±4.7***	16.8±4.8*	10.2±3.0***
	6.0	43±9.0	17±4.2***	19.6±4.4***	20.2±3.5	18.4±14.4*

♣10 Animal for each of the five duration regardless of the dose (a total of 50 control animals).
◆5 Animals for each of the five duration of each dose (a total of 150 tested animals).
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

TABLE V: Effect of different doses of thymoquinone on HDL/LDL ratio in normal rats injected for different durations compared with control.

Dose (mg/kg)	*1 day Mean±SD*	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
0♠	1.1±0.5	1.2±0.2	1.2±0.3	1.8±0.4	1.2 ±0.4
0.5	1.3±0.5	1.3±0.3	3±1.8**	1.2±0.1*	3.5 ±0.7**
1	1±0.1	1.5±0.3	2.9±1.3**	1.7±0.4	2.7 ±1**
2	1±0.3	1.5±0.3*	1.8±0.4**	1.9±0.3	2.7 ±0.5**
4	1±0.4	1.7±0.3**	1.8±1	2.4±1	3 ±0.8**
6	1.4±0.6	1.6±0.4**	1.7±0.5*	2.2±1	2.7 ±1.6*

♠Control Group
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

TABLE VI: Effect of different doses of thymoquinone on HDL/cholesterol ratio in normal rats injected for different durations compared with control.

Dose (mg/kg)	*1 day Mean±SD*	*4 days Mean±SD*	*7 days Mean±SD*	*10 days Mean±SD*	*14 days Mean±SD*
0♠	0.6±0.2	0.6±0.2	0.6±0.1	0.7±0.1	0.6±0.1
0.5	0.8±0.2	0.5±0.1	0.9±0.1***	0.6±0.1*	1±0.2**
1	0.7±0.1	0.5±0.1	1±0.3***	0.7±0.1	1±0.3**
2	0.6±0.2	0.7±0.1	0.8±0.2**	0.9±0.3	0.9±0.2**
4	0.8±0.2	0.8±0.1*	0.6±0.1	1±0.2**	0.7±0.1
6	0.7±0.2	1±0.1***	0.6±0.1	1±0.2**	0.5±0.1*

♠Control Group
(Asterisks indicate level of significance: No asterisk = Not significant; *=P<0.05; **=P<0.01; ***=P<0.001)

DISCUSSION

Our results indicate that thymoquinone has a reducing effect on the blood levels of: cholesterol, HDL and LDL in normal rats, if administered for at least 4 days. The result, however, fail to show a linear consistent dose or time dependent effect of thymoquinone on the various parameters studied.

Thymoquinone failed to show an effect on cholesterol, HDL and LDL when administered for one day only. This may indicate that the therapeutic use of thymoquinone for lipids disorders for short duration is not effective. Exception to that, the significant reducing effect on blood triglycerides observed with most doses when given for one day. The significant reducing effect on triglycerides observed after one day raises the possibility that the mode of action of thymoquinone on triglycerides is different from that on other parameters. The swings observed in certain parameters (cholesterol, triglycerides and LDL) during the 2 weeks of treatment with some doses are hard to explain. One plausible explanation for these swings could be attributed to the heterogenicity of animals, as different animals were used for different duration groups. If this is true, then either self-control or increasing the sample size of each duration group might minimize such swinging. However, such swings warn researchers to exercise caution in interpreting an effect of a single dose of thymoquinone used for one duration.

Thymoquinone produced a highly significant reduction in blood cholesterol. The hypocholesterolemic effect observed in studies using *Nigella sativa* both in rats (13) and humans (12) could therefore be, at least in part, attributed to thymoquinone. The reducing effect of thymoquinone on triglycerides was far less significant than on cholesterol. Another ingredient of *Nigella sativa* namely, poly-unsaturated fatty acid has been reported to have a reducing effect on triglycerides (14, 15). Our results also showed a significant elevation in both HDL/LDL and HDL/cholesterol ratio which persisted up to 2 weeks. This indicates a promising role for thymoquinone

in long term treatment of lipids disorders.

In conclusion, the reducing effect of thymoquinone on cholesterol and triglycerides and its tendency to elevate both HDL/LDL and HDL/total cholesterol, make it a potential drug for preventing and treating atherosclerosis.

ACKNOWLEDGEMENTS

Thanks are due to King Abdulaziz City for Science and Technology for their kind support of the project. We are also thankful for Mr. Kaisar, for technical assistance, and Mr. Hasany and S. Fakir for secretarial assistance.

REFERENCES

1. El-Dakhakhny M. Studies on the Egyptian *Nigella sativa* L. Some pharmacological properties of the seeds active principle in comparison to its dihydrocompound and its polymer. *Arzneim Forsch (Drug Res)* 1965; 15: 1227–1229.
2. Saad S. Classification of flowering plants, The General Egyptian Book Co., Alexandria, 2nd edition, 1975; 412–413.
3. Aboutabl EA, El-Azzouny AA, Hammerschmidt FJ. Aroma Volatiles of *Nigella sativa* L. seeds. *Prog Essent Oil Res, Proc Int Symp Essent Oils* (edt Brunke EJ) 1986; 44–55, De Gruyter, Berlin, Germany.
4. El-Dakhakhny M. Some pharmacological properties of some constituents of *Nigella sativa* L. seeds: The carbonyl fraction of the essential oil. Studies in Islamic Medicine and Advantages of Herbal treatment, Kuwait 1982; 426–431.
5. Ghoneim M, El-Gindy A, El-Alami E, Shoukry R, Yaseen, S. Possible effect of some extracts of *Nigella sativa* L. seeds on blood coagulation system and fibrinolytic activity. *Proceeding of 2nd International Conference on Islamic Medicine, Kuwait* 1982; 528–535.
6. El-Tahir K, Ashour M, Al-Harbi M. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: Elucidation of the Mechanism of action. *Gen Pharmacol* 1993; 24: 1123–1131.
7. El-Kadi A, Kandil O. Effect of *Nigella sativa* (the black seed) on immunity. Proceeding of the fourth International conference on Islamic medicine. *Bull of Islam Med* 1986; 4: 344–348.
8. Topozada H, Mazolum H, El-Dakhakhny M. The antibacterial properties of *Nigella sativa* seeds, active principle with some clinical applications. *J Egypt Med Assoc* 1965; 48: 187–202.
9. Salomi N, Nair S, Jayawardhanan K, Varghese C, Panikkar K. Antitumor Principles from *Nigella sativa* seeds. *Cancer Letters* 1992; 63(1): 41–46.
10. Ali BA, Erwa H. Effect of *Nigella sativa* on ingestion ability of mice peritoneal macrophages. *Saudi Pharmac J* 1993; 1(1): 18–21.
11. Haq A, Abdullatif M, Lobo PI, et al. *Nigella sativa*: Effect on human lymphocytes and polymorphonuclear leukocyte Phagocytic activity. *Immunopharmacology* 1995; 30: 147–155.
12. Bamosa A, Ali BA, Sowayan S. Effect of oral ingestion of *Nigella sativa* seeds on some blood parameters. *Saudi Pharmac J* 1997; 5(2–3): 126–129.
13. El-Zawahry B. Effect of *Nigella sativa* on certain aspects of metabolism after feeding normal and hyperlipidemic diet in adult and old animals. MD Thesis, Faculty of Medicine for Girls, Al-Azhar University 1997.
14. Mattson F, Grundy S. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J Lipid Res* 1965; 26: 194–202.
15. Ventura M, Woollett L, Spady D. Fish oil stimulates hepatic LDL transport in the rat. *J Clin Invest* 1989; 84: 528–537.