EFFECT OF *NIGELLA SATIVA* (BLACK SEED) AND THYMOQUINONE ON BLOOD GLUCOSE IN ALBINO RATS

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The black seed *Nigella sativa* (*N. sativa*) is a type of plant that belongs to the *Ranunculaceae* family.[1] It has been used as a herbal medicine for more than 2000 years. It is also used as a food additive and flavor in many countries. *N. sativa* volatile oil has recently been shown to possess 67 constituents, many of which are capable of inducing beneficial pharmacological effects in humans.[2]

Studies on the effect of *N. sativa* on blood glucose levels in normal and diabetic animals seem to be conflicting. In 1985, Al-Awadi et al.[3] reported a significant decrease in blood glucose produced by a plant mixture containing *N. sativa* in normal and streptozotocin-induced diabetic rats, however, when *N. sativa* was used alone it produced no effect on the blood glucose level of both normal and diabetic rats.[4] Also, El-Naggar and El-Deib[5] reported that oral administration of powdered *N. sativa* seeds for three weeks produced minimal insignificant reduction in blood glucose in normal and alloxan-induced diabetic rats. On the other hand, the intraperitoneal administration of volatile oil of *N. sativa* to fasting normal and alloxon-diabetic rabbits produced significant hypoglycemic effects.[6] A plant mixture containing *N. sativa* administered once daily at doses of 0.5-1.5 mL/kg body weight for one month to normal and diabetic rats produced significant reductions in serum glucose level only in diabetic rats.[7] Another plant mixture containing *N. sativa* was also reported to produce a significant hypoglycemic effect in alloxan-induced diabetic rats.[8] The only study which has been done on humans reported a significant decrease in blood glucose level after one week of oral ingestion of *N. sativa* powder at a dose of 2 g/day.[9] The glucose level went up but remained insignificantly below baseline by the end of two weeks. We conducted the current study in an attempt to shed some light on the effect of *N. sativa* on blood glucose.

Materials and Methods

A total of 300 tested and 100 control white female albino rats were included in the study. The rats, which weighed 180-220 g, were fed on standard *ad labitum* and normal drinking water. The tested animals were divided into two main groups of 150 each. One group received *N. sativa* seeds and the other was given intraperitoneal injections of thymoquinone (Sigma Chemicals, USA). Each tested group was then subdivided into six groups of 25 rats each, with each group receiving different drug doses. *N. sativa* groups were fed different amounts of black seed of 50, 100, 200, 300, 400 and 500 mg/day, respectively. Each dose was mixed with flour, making a small amount of dough of around 2.5 g weight, before feeding. Thymoquinone groups were given intraperitoneal injections of different thymoquinone concentrations dissolved in ethanol and diluted with normal saline, and were 0.5, 1, 2, 4, 6 and 8 mg/kg body weight/day, respectively. All tested animals were subsequently allowed free access to normal food and water. Each dose group was further divided into five duration subgroups of five rats each, in which the feeding of *N. sativa* or intraperitoneal injections of thymoquinone continued for 1, 4, 7, 10 and 14 days, respectively.

Control animals were divided into two main groups of 50 each. One group was only given dough of around 2.5 g and served as controls for rats fed with *N. sativa* seeds. The other group received intraperitoneal injections of ethanol and served as controls for the thymoquinone-injected rats. All control animals were subsequently allowed free access to normal food and water. Each main control group was further divided into five duration groups of 10 rats each and served as controls for one of the different durations to which the tested groups were subjected (1, 4, 7, 10 and 14 days, respectively), regardless of the dose of either *N. sativa* seeds or thymoquinone. Feedings of *N. sativa* seeds or injections of thymoquinone were given around 8 a.m. in order to avoid the effect of diurnal variation. Controls had the same timing for feedings and injections. After two hours of fasting, a blood sample was obtained at 10 a.m. from each rat at the end of each duration for both tested and control animals. The blood was extracted from the abdominal aorta following abdominal incision, after anesthetizing the animal with 1.25 g/kg phenobarbitone. From each blood sample, plasma was obtained and the following blood parameters were measured spectrophotometrically (Spectronic Instruments, USA)

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TABLE 1. *Changes in blood glucose levels (mmol/L) in normal rats treated with different doses of* Nigella sativa *seeds given for different durations compared with control.*

		Duration (Mean±SD)				
Animal groups	Dose mg/day	1 day	4 days	7 days	10 days	14 days
Control†	0	5.5±0.57	6.1±0.4	5.8±0.44	6.1±0.36	5.95±0.52
Tested††	50	5.1±0.33	5.93±0.36	3.6±0.44***	6.17±0.42	5.73±0.91
	100	4.97±0.33	5.34±0.41**	4.0±0.22***	5.36±0.61	4.89±1.06*
	200	5.56±0.67	5.07±0.35***	3.8±0.48***	4.88±0.52***	4.15±1.07**
	300	5.08±0.52	4.34±0.24***	4.34±0.41***	4.73±0.48***	4.31±0.78***
	400	5.48±0.67	4.31±0.31***	4.99±0.72*	4.65±0.65***	5.36±0.81
	500	5.3±0.56	5.0±0.42***	5.53±0.26	5.24±0.36**	5.73±0.33

†Ten animals for each of the five durations regardless of the dose (a total of 50 control animals); ††Five animals for each of the five durations of each dose (a total of 125 tested animals); **P*<0.05; ***P*<0.01; ****P*<0.001.

TABLE 2. *Changes in blood glucose levels (mmol/L) in normal rats treated with different doses of thymoquinone injected for different durations compared with controls.*

		Duration (Mean±SD)				
Animal groups	Dose mg/day	1 day	4 days	7 days	10 days	14 days
Control†	0	6.23±0.5	6.22±0.37	6.0±0.65	5.81±0.34	6.12±0.39
Tested††	0.5	6.39±0.14	5.77±0.35*	6.0±0.4	5.6±0.26	5.62±0.62
	1.0	5.89±0.51	5.64±0.37*	3.89±0.51***	5.35±0.61	3.91±0.5***
	2.0	6.26±0.43	5.44±0.55**	3.76±0.42	5.33±0.4*	3.9±0.96***
	4.0	6.28±0.15	5.1±0.36***	3.63±0.33***	4.94±0.64**	3.65±0.37***
	6.0	6.23±0.47	5.18±0.46***	4.0±0.5***	5.33±0.54	3.76±0.5***

†Ten animals for each of the five durations regardless of the dose (a total of 50 control animals); ††Five animals for each of the five durations of each dose (a total of 125 tested animals); **P*<0.05; ***P*<0.01; ****P*<0.001.

utilizing standard kits (Bio-Merieux, France). The mean of each blood parameter from both tested groups was compared to its corresponding parameter in the control groups using unpaired Student's *t*-test.

Results

All animals fed with the six doses of *N. sativa* tolerated the drug over the period of treatment and showed no sign of toxicity or discomfort. Similarly, all doses (0.5 to 6 mg/kg) of thymoquinone, except the dose of 8 mg, were tolerable and animals showed no sign 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 extraction. Their abdomen was 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 N. sativa *Feeding on Blood Glucose Levels*

Treatment with all doses for one day produced no effect on blood glucose. Rats treated with 50 mg dose showed a significant reduction in their blood glucose only in the seven days' duration. Treatment with 100, 200 and 300 mg produced a significant reduction in blood glucose levels in all durations except that of one day. However, the reduction induced by 200 and 300 mg doses seemed to be greater by the end of 14 days' treatment. The 400 and 500 mg doses produced a significant decrease in the groups treated for 4 and 10 days, however, the effect was lost after 14 days.

Effect of Thymoquinone Injections on Blood Glucose Levels

Thymoquinone intraperitoneal injections with the 5 doses (0.5, 1, 2, 4 and 6 mg/kg) failed to produce a change of significance in blood glucose level after one day of treatment. Injections with 0.5 mg/kg gave a significant reduction in blood glucose after four days of treatment but were lost thereafter. The rest of the doses used produced a significant reduction in blood glucose in almost all the four duration periods, but the blood glucose level was swinging up and down over these durations. However, the effect was well maintained even in the animals treated with the highest dose given for the longest duration.

Discussion

Our results indicate that *N. sativa* seeds and their active ingredient, thymoquinone, have a promising reducing effect on the blood glucose levels in normal rats. The results, however, fail to show a linear consistent dose or time-dependent effect of both drugs on the parameter studied. The effective dose range of *N. sativa* seemed to lie between 100 and 300 mg/200 g rat/day for most of the duration studied. The higher doses of *N. sativa*, particularly 500 mg, tended to lose their effect after two weeks of daily treatment. A similar finding was observed in our previous study in humans,[9] which may support the possibility that the dose used in the human study (2 g/day) was also high.

The hypoglycemic effect of *N. sativa* reported here is in agreement with previous reports in normal and alloxan-induced diabetic rabbits,[6] alloxan-induced diabetic rats,[7,8] and in human subjects.[9] On the other hand, our results seem to be in conflict with other studies.[4,5,7] Al-Awadi and Gumaa[4] reported no significant change in fasting blood glucose level when *N. sativa* (40 mg/day) was administered to normal and streptozotocin-induced diabetic rats. El-Naggar and El-Deib[5] had also found no significant reducing effect of *N. sativa* (36 mg/day) on blood glucose level in normal rats. However, it seems that the doses of *N. sativa* used by both groups were subtherapeutic. The third group,[7] who reported a negative effect of *N. sativa* on blood glucose of normal rats, were actually using a plant mixture rather than pure *N. sativa* and their dose could not be calculated.

The hypoglycemic effect of *N. sativa* in our study took four days to show, which is close to the duration (6 days) reported by Al-Hader et al.[6] The similarity between the results of thymoquinone and those produced by *N. sativa* seeds indicates the role of thymoquinone in the hypoglycemic effect of *N. sativa*. Furthermore, thymoquinone results support and validate the findings with *N. sativa*. Interestingly, the higher doses of thymoquinone did not lose their effect in the 14 days' group, as happened in the *N. sativa* feeding. This indicates that perhaps another ingredient of *N. sativa* reduces its effect at higher doses. However, it is no doubt better to use thymoquinone rather than raw seeds in order to induce a hypoglycemic effect.

In conclusion, both *N. sativa* and thymoquinone produced significant hypoglycemic effects in normal rats. We recommend further studies on the effect of these drugs in diabetic animals and humans.

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