

Antibacterial Activity of Total Extracts and Essential oil of *Nigella Sativa* L. Seeds in Mice

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ABSTRACT

The purpose of this study was to evaluate the antibacterial activity of total crude extracts and essential oil (EO) of *Nigella sativa* L. seeds in male mice infected intraperitoneally with *Staphylococcus aureus* or *Escherichia coli* (0.1mL from 10⁶ colony forming units/ml suspension). After 24 hours, the infected mice were subjected to different doses of TE or EO or received 33 mg/kg of gentamicin (a positive control) or 0.4 mL of normal saline (a negative control). After 24 hours, aspirated specimens from intraperitoneal fluids were cultured on a soybean casein digest agar plate surface. The inhibitory effect of the methanol extract at a dose of 2.14 g/kg in mice infected with *S. aureus* was 87.5%. The doses of 1.2 and 2.14 g/kg in mice infected with *E. coli* were 100% compared with mice who received saline (the negative control). While the aqueous extract did not show any inhibitory effect on either micro-organism, the effect of the chloroform extract at dose of 2.6 g/kg and 33 mg/kg gentamicin (the positive control) was 100 %. The EO at dose of 0.3 g/kg in mice infected with *S. aureus* and *E. coli* showed 100% inhibitory effect compared with mice who received saline. *N. sativa* methanol and chloroform seed extracts as well as its essential oil have dose dependent antibacterial activities on the Gram-positive and Gram-negative organisms.

KEYWORDS: *Nigella sativa*, Black cumin, antibacterial activity, *Staphylococcus aureus*, *Escherichia coli*.

INTRODUCTION

Nigella sativa Linn. is indigenous to the Mediterranean region but has been cultivated into other parts of the world including Saudi Arabia, northern Africa and parts of Asia. The plant is known by names, such as black cumin (English), black-caraway seeds (USA) and shonaiz (Persian) (Khan, 1999). Different pharmacological effects such as isulintropic (Fararh et al., 2002), hypoglycemic (El-

Dakhakhny et al., 2002), anticancer (Mabrouk et al., 2002; Salomi et al., 1992), antinociceptive, anti-inflammatory (Abdel-Fattah et al., 2000; El-Dakhakhny et al., 2002; Ghannadi et al., 2005), hepatoprotective (Mahmoud et al., 2002), neuroprotective (Kanter et al., 2006a), antihistamine, antiulcer (Kanter et al 2006 b) and bronchodilator (Gilani et al., 2001) activities have been reported for this plant. Black cumin has been

traditionally used in the Indian continent, Arabian countries and Europe for culinary and medicinal purposes as a natural remedy for a number of illnesses and conditions that include asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza. The seeds or its oil are used as a carminative, diuretic, lactagogue and vermifuge (Ali and Blunden, 2003).

N. sativa seeds contain 36%–38% fixed oils, proteins, alkaloids, saponin and 0.4%–2.5% essential oil. The fixed oil is composed mainly of unsaturated fatty acids. The essential oil was analyzed using GC-MS. Many components were characterized, but the major ones were thymoquinone (27.8%–57.0%), ρ -cymene (7.1%–15.5%), carvacrol (5.8%–11.6%), t-anethole (0.25%–2.3%), 4-terpineol (2.0%–6.6%) and longifoline (1.0%–8.0%). Thymoquinone readily dimerizes to form dithymoquinone. Four alkaloids have been reported as constituents of *N. sativa* seeds. Two, nigellicine and nigellidine have an indazole nucleus, whereas nigellimine and its N-oxide are isoquinolines (Ali and Blunden, 2003).

As the public becomes more interested in herbal medicine and bacterial pathogens become more resistant to commercial antibiotics, scientists are increasingly investigating the antibacterial properties of plant extracts and fractions (Fazly Bazzaz et al., 1997; Hassanzadeh et al., 2001; Taskova et al., 2002; Kariba, 2002). Antimicrobial

activities have been reported for the extracts of *N. sativa* seed *in vitro* (Hanafy and Hatem, 1991; Morsi, 2000). To extend this activity and show this effect in a body, the antibacterial activity of crude extracts and essential oil of *N. sativa* seed were studied on infected mice with *Staphylococcus aureus* and *Escherichia coli*.

METHODOLOGY

Animals

Male BALB/c mice (25 ± 3 g) were obtained from a random bred colony of maintained with laboratory pellet chow (Khorassan Javane Co, Mashhad, I.R. Iran) in animal house of Mashhad University of Medical Sciences. Animals were housed in a colony room with a 12/12 hour light/dark cycle at 24 ± 1 °C. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee acts.

Plant materials

N. sativa seeds were purchased from Gonabad (Khorasan Province). The methanol extract (ME) of seeds was prepared using Soxhlet apparatus. The aqueous extract (AE) was decocted with hot water for 15 min. The chloroform extract (CE) was macerated with chloroform for 72 hour. The extracts were then concentrated under reduced pressure to volume desired. The residual water was evaporated to dryness at 30 °C on a water bath. In this study ME, CE and AE were called total extract (TE). Essential oil (EO)

was prepared by hydrodistillation (Samsam-Shariat and Moatar, 1996).

Test microorganisms

The strains of bacteria used in this study were *Staphylococcus aureus* (ATCC 29737) and *Escherichia coli* (ATCC 8739).

All bacteria were cultured from soybean casein digest agar (Merck) stock cultures already at 4°C into the fresh prepared agar and cultured overnight at 35° C. The bacterial suspensions were prepared (10⁶ cfu/ml) using normal saline from these cultures.

Assay procedure

240 mice were used in this study. 30 groups of animals were used, each of which contained 8 mice. Each group of eight mice was infected with 0.1 ml (10⁶ cfu/ml) of *S. aureus* or *E. coli* suspension intraperitoneally. Twenty four hour later, each group received different treatments. Groups were designed as following:

Negative control: Group 1 received 0.4 ml normal saline against *S. aureus* and group 2 received 0.4 ml normal saline against *E. coli*.

Positive control: Group 3 received 33 mg/kg gentamicin against *S. aureus* and group 4 received 33 mg/kg gentamicin against *E. coli*.

ME: Groups 5-7 received 0.3, 1.2 and 2.14 g/kg the methanol extract against *S. aureus*, respectively and groups 8-10 received 0.3, 1.2 and 2.14 g/kg the methanol extract, respectively against *E. coli* **CE:** Groups 11-13 received 0.38, 1.5 and 2.6 g/kg the chloroform

extract, respectively against *S. aureus* and groups 14-16 received 0.38, 1.5 and 2.6 g/kg the chloroform extract, respectively against *E. coli*

AC: Groups 17-19 received 0.24, 0.96 and 1.68 mg/kg the aqueous extract, respectively against *S. aureus* and groups 20-22 received 0.24, 0.96 and 1.68 mg/kg the aqueous extract, respectively against *E. coli*

EO: Groups 23-26 received 0.03, 0.12, 0.21 and 0.3 g/kg the essential oil, respectively against *S. aureus* and groups 27-30 received 0.03, 0.12, 0.21 and 0.3 g/kg the essential oil, respectively against *E. coli*

After 24 hours, 0.1 ml aspirated specimens from intraperitoneal fluid were cultured on soybean casein digest agar (Merck) plate surface. Plates were incubated overnight at 35° C and the number of colonies formed on each plate was counted.

Statistical analysis

The resulting colonies were counted and expressed in terms of percent colonies formed from each aspiration sample. This number was compared with the negative control group and tested with Fisher's exact test. The p-values less than 0.05 were considered to be statistically significant.

RESULTS

Gentamicin, as positive control, completely inhibited the appearance of colonies of both

micro-organisms (Table 1-4). The methanol extract showed significant antibacterial activity against both micro-organisms. The

effect of this extract was more significant on *E. coli* (Table 1). The chloroform extract also showed significant antibacterial activity

Table 1. Effects of intraperitoneal injection of methanol extract (ME) of *N. sativa* seed on infected mice with micro-organisms.

Treatment	Dose	%Activity	
		<i>E.coli</i>	<i>S. aureus</i>
Normal saline	0.1 ml	0	0
Gentamicin	33 mg/kg	100***	100***
ME	0.3 g/kg	87.5**	50
ME	1.2 g/kg	100***	75**
ME	2.14 g/kg	100***	87.5**

** P< 0.01, *** P< 0.001, compared with normal saline, Fisher's exact test, N=8

against both micro-organisms. The effect of this extract on *S. aureus* and *E. coli* was almost equivalent. Even with high doses, the aqueous extract (e.g. 1.68 g/kg: P=0.2281 against *S. aureus* and P=0.1126 against *E. coli*) did not show significant antibacterial

activity against both micro-organisms (Table 3). The essential oil with lower doses inhibited the growth of both micro-organisms (Table 4). This effect of oil was more considerable against *S. aureus*.

Table 2. Effects of intraperitoneal injection of chloroform extract (CE) of *N. sativa* seed on infected mice with micro-organisms.

Treatment	Dose	%Activity	
		<i>E.coli</i>	<i>S. aureus</i>
Normal saline	0.4 ml	0	0
Gentamicin	33 mg/kg	100***	100***
CE	0.38 mg/kg	75**	75**
CE	1.5 mg/kg	75**	87.5**
CE	2.6 mg/kg	100***	100***

** P< 0.01, *** P< 0.001, compared to normal saline, Fisher's exact test, N=8

DISCUSSION

In this study, the *N. sativa* seed as an essential oil (Table 4), as well as methanol (Table 1) and chloroform (Table 2) extracts showed significant antimicrobial activities *in vivo*.

However, the effect of the aqueous extract (Table 3) was not significant (e.g. 1.68 g/kg: P=0.2281 against *S. aureus* and P=0.1126 against *E. coli*).

Table 3. Effects of intraperitoneal injection of aqueous extract (AC) of *N. sativa* seed on infected mice with micro-organisms.

Treatment	Dose	%Activity	
		<i>E.coli</i>	<i>S. aureus</i>
Normal saline	0.1 ml	0	0
Gentamicin	33 mg/kg	100***	100 ***
AC	0.24 g/kg	2.5	14.2
AC	0.96 g/kg	25	14.2
AC	1.68 g/kg	33.3	37.5

*** P< 0.001, compared with normal saline, Fisher's exact test, N=8

There are *in vitro* studies documenting that *N. sativa* seed have *in vitro* antibacterial activity against pathogens such as *S. aureus*, *E. coli*, *Shigella spp.* and *Vibrio cholerae* (Hanafy and Hatem, 1991; Morsi, 2000; Ferdous et al., 1992; Rathee et al., 1982). The essential oil of *N. sativa* seeds was effective against multiple drug-resistant (e.g. ampicillin, co-trimoxazole and tetracycline) isolates of *Shigella spp.*, *Vibrio cholerae* and *E. coli in vitro* (Ferdous et al., 1992). Our study replicated these antibacterial effects against the standard species of *E. coli in vivo*. Further, our study demonstrated for the chloroform and methanol extracts, as well as the EO of *N. sativa* had showed antibacterial effect against *S. aureus*. In another study, the antibacterial activity of *N. sativa* seed oil was reported against twenty-one pathogenic bacteria such as *S. aureus* and *E. coli in vitro*.²² This study beside that confirmed the antibacterial activity of *N. sativa* with different extracts it also showed its effect on micro-organisms in the body. So now evaluation of black cumin can be more reliable for doing clinical trails and pharmacokinetic parameters such as

metabolism or distribution does not prevent the antibacterial activity of *N. sativa*.

Filter paper discs impregnated with diethyl ether extract of *N. sativa* seeds caused concentration dependent inhibition of *S. aureus* and *E. coli* (Hanafy and Hatem., 1991). This effect is similar to the bacterostatic effect of chloroform and methanol extracts in this *in vivo* study. Thymohydroquinone, a phenolic compound isolated from the essential oil of *N. sativa*, exerted high antimicrobial effect against gram positive microorganisms (El-Fatraty, 1975). Another study showed that the most effective extracts of *N. sativa* seeds were crude alkaloid and water extracts but in our study the aqueous extract showed low antibacterial activity. This may be related to lower accessibility of the aqueous extract to the micro-organism in the *in vivo* study or low extraction of antibacterial components into this extract (Morsi, 2000).

Several compounds such as sterols and phenolic constituents were found in *N. sativa* seed. Thymoquinone or thymohydroquinone are active components of the seed that have different pharmacological activities such as

anti-inflammatory, antioxidant and antihypertensive effects (Khan, 1999). Higher

solubility of these active constituents in organic solvents may contribute to the

Table 4. Effects of intraperitoneal injection of Essential oil (EO) of *N. sativa* seed on infected mice with micro-organisms.

Treatment	Dose	%Activity	
		<i>E.coli</i>	<i>S. aureus</i>
Normal saline	0.1 ml	0	0
Gentamicin	33 mg/kg	100***	100 ***
EO	0.03 g/kg	37.5	50
EO	0.12 g/kg	50	62.5 **
EO	0.21 g/kg	75 **	87.5 **
EO	0.3 g/kg	100***	100***

** P< 0.01, *** P<0.001, compared with normal saline, Fisher's exact test, N=8

observed efficacy of the chloroform and methanol extracts and the reason for low antibacterial activity of the aqueous extract in our study.

In conclusion, this study and previous reports show that *N. sativa* seed has effective antibacterial activities in both *in vitro* and *in vivo* studies. The result showed that the essential oil as well as methanol and chloroform extracts are effective against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. The isolation and purification of the active antibacterial components of plant along with different rout of administration of plant especially oral way are recommended. The results of this research are valuable steps toward doing clinical trials to use this plant in treating human infection.

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