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Antibacterial Activity of the Aqueous and Methanol Extracts of *Nigella Sativa* Seeds in Mice

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Abstract

The purpose of this research was to determine if *Nigella sativa* (NS) seed extracts have antibacterial properties. In male mice infected intraperitoneally with 0.1 mL of *Staphylococcus aureus* or *Escherichia coli* suspensions (Standard McFarland tube No.0.5), the aqueous (AE) and methanol (ME) extracts of NS seed were compared to gentamycin (positive control) and normal saline (negative control). After 24 hours, the infected mice were given varying doses of AE and ME. After 24 hours, a sample of the intraperitoneal fluid that had been aspirated was cultured on a nutrient agar plate. In mice infected with *S. aureus* and *E. coli*, the methanol extract of *Nigella sativa* showed more antibacterial activity than its aqueous extract, especially against gram-negative bacteria. *Nigella sativa* seed extracts have shown antibacterial activity against both gram-positive and gram-negative bacteria; however this action is dose-dependent.

Keywords: Antibiotic resistance, bacteriostatic, *Nigella sativa*, pharmacokinetic.

Introduction

With the rise in antibiotic resistance in recent decades as a result of improper use, a demand for less expensive and safer alternatives has evolved, and black seed extracts are one of them. The scientific name for black seed or "black cumin" is "*Nigella sativa*" (NS). Black seed is a Ranunculaceae family annual native to North Africa, the Middle East, Europe, and Asia [1]. Black cumin is commonly used for flavour (as a spice) and medicinal purposes. For millennia, these seeds have been used as a natural cure for a variety of ailments and disorders, including asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, dermatitis, fever, dizziness, and influenza [2, 3]. As a diuretic, carminative, lactagogue, and vermifuge, the seeds or their oil are employed [4].

The non-volatile substances flavonoids, phenolic acids, tannins, and a very little amount of volatile substances like terpene chemicals make up the majority of black cumin [5]. Various seed extracts, as well as other bioactive ingredients. It has been shown that the biological effects of NS extracts are closely related to their chemical make up. Thymoquinone, thymohydroquinone, dithymoquinone, thymol carvacrol,

nigellimine-N-oxide, nigellicine nigellidine, and alpha-hederin are all components of NS seeds and oil [6, 7].

A wide range of pharmacological effects, including as immunostimulatory, anti-inflammatory, anti-diabetic, antihypertensive, antiasthmatic, antibacterial, antiparasitic, antioxidative, and anticancer activity, have been reported for NS and its constituents [8].

As interest in herbal medicine rises and bacterial illnesses become more resistant to traditional medications, researchers are looking into the antibacterial properties of plant extracts more and more [9, 10]. Additionally, there have been reports of the NS seeds having a low level of toxicity [11].

Alkaloid and water extracts of NS seeds have been shown to have in vitro antibacterial action [8]. On mice infected with *S. aureus* and *E. coli*, the antibacterial activity of NS seed extracts was investigated in order to prolong this activity and demonstrate this effect in the body.

Materials and Methods

Total of (48) male BALB/C mice were housed in the faculty of pharmacy's animal house at Kerbala University. The mice ranged in age from (4-6) months and weight from (20-26 g). The animals were kept in a colony chamber that was 24°C and had a 12/12-hour light/dark cycle.

A local herbal store sold *Nigella sativa* seeds, which were purchased there. Soxhlet equipment was used to create the methanol extract (ME) of the seeds. Hot water was used to decoct the aqueous extract (AE) for 15 minutes. A distillation device was used to evaporate the extracts after they had been filtered. The resultant extracts were then placed in an oven at 40 °C for a whole night in order to get a totally dry extract. After that, it was then stored in the refrigerator to be tested for antibacterial activity [12].

Different clinical microbiological isolates, including gram-positive (*S. aureus*) and gram-negative (*E. coli*), were employed in this study. At the Al-Zahra Hospital's microbiology lab in Karbala, Iraq, all bacteria were isolated and identified using traditional biochemical tests and the API system. These strains were grown from stock cultures of nutrient agar plate agar, which were already at 4 ˚C, into freshly prepared agar and incubated at 37 ˚C overnight. From these cultures, 0.1mL from the bacterial suspensions (SM tube No.0.5) was made using ordinary saline [13].

Experimental animals and treatment

For this experiment, we used 48 mice. Each of the 16 animal groups consisted of 3 mice. Each group of mice received an intraperitoneal injection of 0.1 ml (SM tube No.0.5) of a suspension of *S. aureus* or *E. coli*. After waiting 24 hours, each group was given a different treatment. The groups were set up as follows:

- Groups 1 and 2 were given 0.5 ml of normal saline as a placebo in an *E. coli* and *S. aureus* infection study.

- Gentamicin (33 mg/kg) was used as a positive control against *S. aureus* and *E. coli* in Groups 3 and 4.
- *Nigella sativa* seed methanol extract (ME) was administered at 0.1, 0.2, and 0.3 g/kg against *S. aureus* in Groups 5-7, and at 0.1, 0.2, and 0.3 g/kg against *E. coli* in Groups 8-10 .
- Groups 11-13 were treated with 0.1, 0.2, and 0.3 g/kg of the aqueous extract for *S. aureus*, while groups 14-16 were treated with 0.1, 0.2, and 0.3 g/kg of the aqueous extract for *E. coli*.

After 24 hours, cultures were grown from 0.1 ml aspirated samples of intraperitoneal fluid on a nutrient agar plate surface. The number of colonies on each plate was counted after being incubated at 37 ˚C for an entire night [13].

Statistical analysis

The colonies that emerged were counted and expressed as a percentage of colonies generated from each sample. With one-way analysis of variance (ANOVA) test, this value was compared to the positive and negative control groups. Statistical significance was assigned to the p values that were less than 0.01.

Results and Discussion

Colony formation of both microorganisms was entirely halted by the addition of gentamicin (positive control) (Figure 1-2).

Significant antibacterial activity was shown in the methanol extract against both microorganisms. It was clearer in the case of E. coli that this extract had an effect (Figure 1). The aqueous extract's substantial antibacterial activity against both microorganisms was lower than that of the other extract, even at high doses (0.3 g/kg) (Figure 2).

This study's findings demonstrated the potency of the black seed's alcoholic and aqueous extracts against both negative and positive bacteria. However, the effect of the two extracts is greater efficacy against negative one (*E.coli*).

Antibacterial activity against *S. aureus, E. coli, Shigella* spp., and *Vibrio chloerae* has been demonstrated in *in vitro* studies of NS seeds [8]. Several drug-resistant (e.g., ampicillin, co-trimoxazole, and tetracycline) isolates of *Shigella spp., Vibrio chloerae*, and *Escherichia coli* were killed *in vitro* by the alkaloid and water extracts of NS seeds [14].

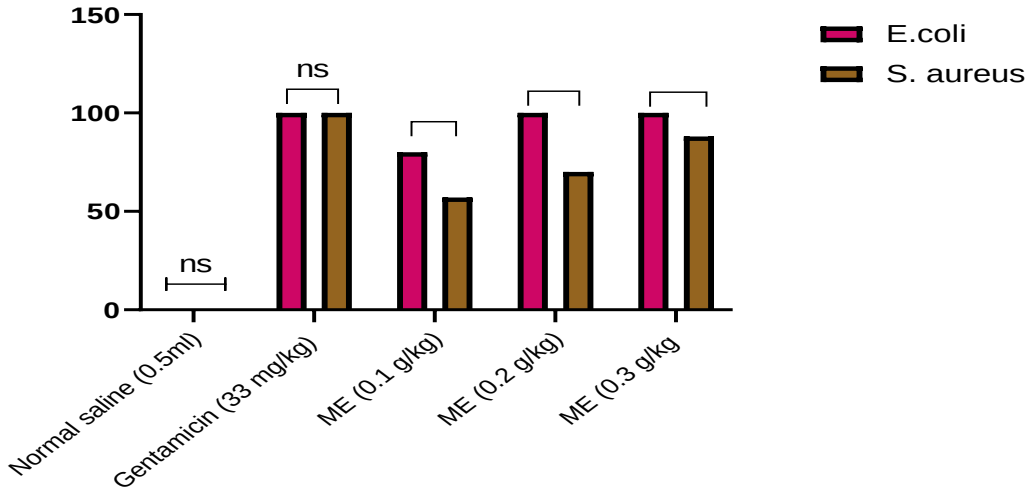

Figure (1): the activity percent of NS seeds (ME) against gram + and – bacteria.
P< 0.01, compared with normal saline and gentamicin, ANOVA test.

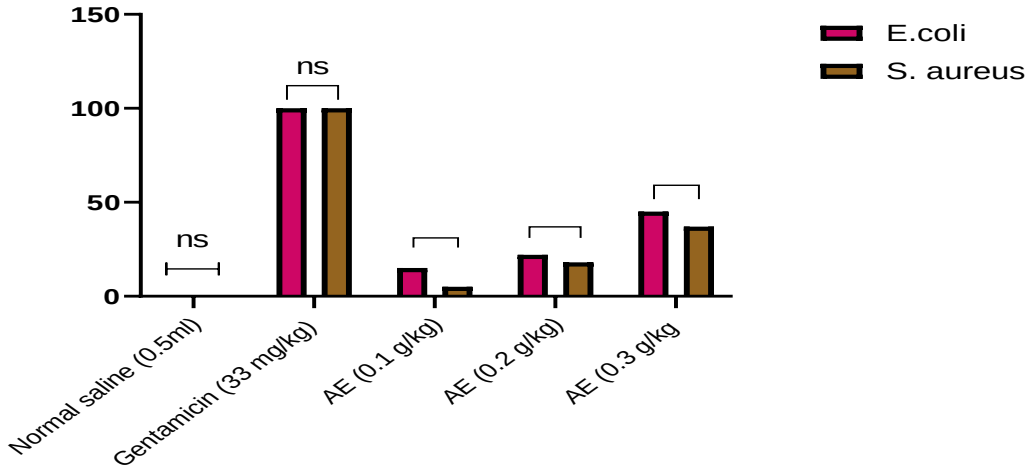

Figure (2): the activity percent of NS seeds (AC) against gram + and – bacteria.
P< 0.01, compared with normal saline and gentamicin, ANOVA test.

In this work, NS seeds as a methanol extract (ME) (Figure 1) showed more antibacterial activity *in vivo* than the aqueous extract (AC) (Figure 2). On the other hand, the aqueous extract had little significance in the statistical analysis even at high concentrations. Moreover, the antibacterial activity of both the methanol extract (ME) and the aqueous extract (AC) was higher against gram- negative bacteria than gram- positive one. This *in vivo* study's results are consistent with those of the methanol extract's bacteriostatic efficacy.

Previous *in vitro* studies, such as the one conducted by Samsam and Moatar, had revealed that "NS seed extract was shown to have antibacterial activity *in vitro* against twenty-one harmful bacteria, including *S. aureus* and *E. coli*" [15]. The results of the

in vivo study mirrored those if the *in vitro* study. According to Hanafy and Hatem[16], " filter paper discs coated with diethyl ether extract of NS were inhibiting *S. aureus* and *E. coli* in a concentration dependent manner".

Chemicals including sterols and phenolic compounds could be found in NS seed. Two active components of the seed, thymoquinone and thymohydroquinone, have been shown to have anti-inflammatory, antioxidant, and antihypertensive effects [17, 18].

Alcohol is more effective than water for phytochemical extraction because most phytochemicals are more soluble in alcohol than in water. Antibacterial activity against gram-positive pathogens was shown by thymohydroquinone and other phenolic components isolated from NS methanol seed extract [19, 20]

Contrary to what was concluded in the present investigation, where the aqueous extract exhibited little antibacterial action, another study indicated that the crude alkaloid and aqueous extracts of NS seeds were the most effective. This may be due to the low accessibility of the aqueous extract to the microorganisms *in vivo* investigation or The duration of the aqueous solution's presence in the body is brief, due to a poor bioavailability (slight absorbency across the cell membrane and a fast excretion rate), which results its effectiveness *in vivo* studies is low [10, 21]. Since these active ingredients are more soluble in organic solvents than water, this may help to explain why the methanol extract was so effective while the aqueous extract was so ineffective.

Although the mechanism of action of NS extracts as an antibacterial was still unknown, its effectiveness as a broad range antibiotic has been demonstrated [22] .This research not only shown that NS has an effect on microorganisms in the body, but it also proved that it has antibacterial action when used with other extracts. This means that pharmacokinetic aspects like metabolism or distribution do not compromise NS's antibacterial action, and therefore the evaluation of black cumin in clinical studies may now be more reliable.

The potential for pharmacological effect of such a combination of NS with other plants has also been revealed. While the synergistic effects of clinically used medications have been demonstrated to be beneficial, this is something that may significantly benefit in the development of successful therapies.

Finally, this study and earlier findings reveal that NS seed has excellent antibacterial activity in both *in vitro* and *in vivo* tests. The results revealed that methanol extract (ME) is more efficient against gram-negative (*E. coli*) bacteria and gram-positive (*S. aureus*) than aqueous extract (AE). It is suggested that the active antibacterial components of the plant be isolated and purified, Due to the exacerbation of bacterial resistance to most antibiotics currently in existence, therefore, the findings of this study are important steps toward conducting clinical trials to use this plant to treat human and animal infections.

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