

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmyia Abed .Abu-Risha

Nagham Shaker Mohammed Hussein

Dunya Fareed Salloom

Baghdad University - college of science - biology department

Abstract

In this study the effect of *Nigella sativa* seed oil was studied on cytopathic effect which result from injection number of mice intraperitoneal with suspension of *Staphylococcus aureus*, these mice previously orally administrated with concentration 50 % of *N. sativa* oil, another mice group were injected with bacterial suspension without previously administrated with seed oil, the results were showed the cytopathic effect of *S. aureus* suspension on organs of mice that which administrate orally with *N. sativa* seed oil were less than effect of bacterial suspension on organs of animal which don't previously administrated with seed oil.

Introduction

An alarming increase in bacterial strains resistant to existing antimicrobial agents demands a renewed effort to seek agents effective against pathogenic bacteria resistant to current antimicrobials (1). *Nigella sativa* Linn. (Black cumin) essential oil was studied for antibacterial activity against various clinical isolates of bacteria resistant to a number of antibiotics.(2) the *N. sativa* oil proved to be more effective against many strains of bacteria, including those known to be highly resistant to drugs. These included *Vibrio cholera*, *Escherichia coli* (a common infectious agent found in undercooked meats), and all strains of *Shigella* spp., except *Shigella dysenteriae*. Most strains of *Shigella* spp. have been shown rapidly become resistant to commonly used antibiotics and chemotherapeutic agents (3). *N. sativa* seed oil and extracts were tested in varying dilutions against strains of *Pseudomonas aeruginosa* resistant to a number of clinically used antibiotics isolated from patients attending JN Medical College Hospital, Aligarh, using disc agar diffusion technique on inoculated Mueller Hinton agar plates under standard laboratory conditions (4). Both the oil and Methanolic extract showed remarkable dose dependent antibacterial activity against the tested strains up to a dilution of 1:50 as evident from the zones of inhibition. No cross resistance was noticed with any of the tested antibiotics (5). In southeast Asia and the Middle East countries *N.*

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmyia Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

sativa seeds provide treatment for bronchitis, asthma, related inflammatory diseases, rheumatism, promote healthy digestion, increase milk production in nursing mothers, and combat parasitic infections(6). The famous Greek physician Discords used black cumin seeds to treat headaches and toothaches. Neutrophil activity is stimulated by black cumin seeds, these are short-lived immune cells that are normally found in the bone marrow but mobilized into action when there is a bacterial infection (7). Also, the herb used to treat cold symptoms, and skin conditions such as boils, and eczema. Oil (contains fatty oil rich in unsaturated fatty acids (8)from herbal plant seeds contain nigellone, which protects guinea pigs from histamine - induced bronchial spasms (9). Theessential oil, various extracts at different polarity, fractions, and pure compounds obtained from *Nigella damascena* plants and seeds were screenedfor biological activity. Antimicrobial tests showed the essential oil to be active only against Gram positive bacteria. (10)

Material and method:-

1- *N. sativa* seeds were obtained from local market, oil was extracted by methanolic method according to (11) as follow:

The seeds were washed thoroughly with water, to remove dust preparation and impurities, and dried in the air. These were grounded into fine powder and 150 grams of grounded seeds were soaked in 150 ml of HPLC grade methanol in a sterile bottle and kept for 7 days at room temperature with stirring with a sterile rod twice daily. It was then filtered using sterile filter paper under UV lamp. The filtrate was kept in a petri dish at room temperature for 3 days to allow the solvent to evaporate. The extract thus prepared was transferred as aliquots of 1 ml each into sterile vials and stored at -20C°.

2- The isolate of *Staphylococcus aureus* in this study isolated was obtained from higher educational lab. of microbiology at biology department at College of Sciences at University of Baghdad, which isolated from urine sample and re-diagnosed biochemically and by APi staph 20, according to (12).

3- Administration of Mice with *N. sativa* oil:

Two groups of mice ,each group contain five Male Albino mice with age (2-3 weeks),the first group orally administration with 50% of extraction oil for 14 days, the second group was left without administration, beside two mice used as control.

4- Injection of mice:-

S. aureus was streaked on nutrient agar and incubation at 37C° for 24 hr. After that some colonies was taken and inoculated on nutrient broth, incubation at same condition, bacterial suspension on nutrient broth having turbidity which equivalent to McFarland tube No. 0.5, this suspension used for injection the two groups of mice intraperitoneal with 1 ml of suspension, two mouse injected with saline and consider as control, all mice were left for week, after that was killing and dissected and taken the organs (intestine, liver and spleen) and put on 10% formalin until preparation histological

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmiya Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

section.

5-Preparation of histological section:

The histological sections were made according (13) as follow:

The organs were fixed by 10%formalin,then washed by tap water for several min. passing through a serial concentration of alcohol (50%, 70%, 80%, 90% and 100%) for 2hr. in each concentration, then cleared by xylol, saturated with paraffin at 60C° for 3hrs.embedded in pure paraffin, the blocks were then cut into sections with 5 µg in thickness by using microtome these sections were held on glass slides using Myers albumin, they wereleft for drying at 37C°, hematoxylin stain was used for 5-10 min, washed by tap water then with acidic alcohol then washed by tap water.After that Eosin stain was used for 15-30 sec. and then washed with distil water . Serial concentration of alcohol were then used (70%, 90% and 100%) for 2 min. in each concentration, dry with xylol for 10 min., then Canada balsam was used, covered by slide cover and examined by light microscope.

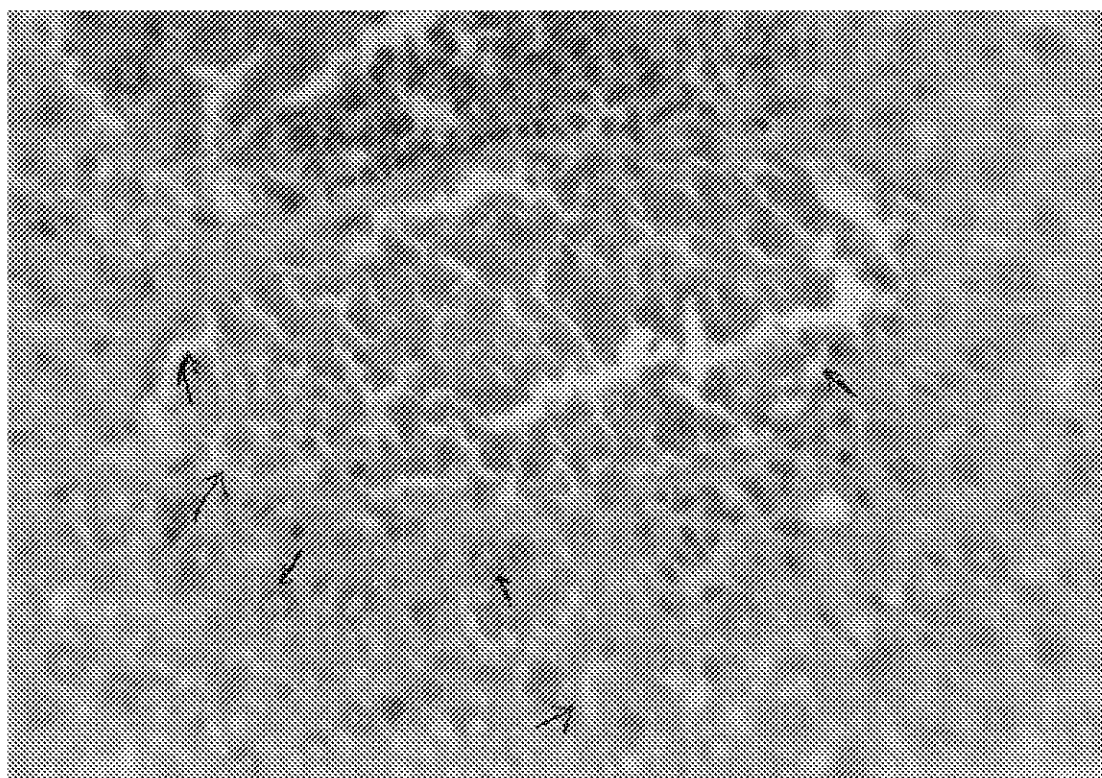
Result and discussion:-

- 1- Diagnosis of *S. aureus* strain: The re-diagnosis of *S. aureus* strain was showed positive result for coagulase, manitol sugars fermentation, oxidase, catalase, the first two tests were distinguished species *aureus* from other species of *Staphylococcus*, also the species was diagnosed and gave positive result by using Apistaph strep.
- 2- Histopathological results: Black seed extract has been extensively studied for its antimicrobial activity against *S. aureus* suspension cytopathic effect on animals model, the result was showed this black seed extract have efficient effect through examination histological section of mice organs, the effect of bacterial suspension on liver of mice which was injected with bacterial suspension without previously administration with seed oil, represented with dilation of hepatic sinusoid, hemorrhagic and aggregation of inflammatory cells (fig. 1), while the effect on intestine was included picnotic necrosis and degeneration villus of intestine (fig. 2), but no effect was found in spleen tissue (fig. 3), and when examined the tissue of organs which belong to mice which was previously administrated with black seed oil, no effect had been observed no liver and intestine tissue (fig 4,5), the anti-bacterial effect of the phenolic fraction of *N. sativa* oil was first reported by Topozada *etal* (14). Thymohydroquinone was later isolated by El-Fataty (15) from the volatile oil of *N. sativa* and found to have high activity against gram-positive microorganisms. Hanafi and Hatem (16) studied the antimicrobial effect of diethyl-ether extract of *N. sativa* and reported that it had a concentration dependent inhibition of gram-positive bacteria (represented by *Staphylococcus aureus*) and gram-negative bacteria also showed synergistic effect with streptomycin and gentamycin and additive effect with spectinomycin, erythromycin, tobramycin, doxycycline,

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmyia Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

chloramphenicol, nalidixic acid, ampicillin, lincomycin and co-trimoxazole. In addition, the extract was found to have a concentration dependent inhibitory effect against pathogenic yeast *Candida albicans* and bacterial species represented by *P. aerogenosa* and *E. coli* (17) recently, crude extracts of *N. sativa* were reported to have a promising gram- negative bacteria (18). Intra-peritoneal administration of *N. sativa* oil strikingly inhibited the virus titer in spleen and liver of mice infected with murine *cytomegalo virus* (19) This action was possibly mediated by increasing the number and function of M & phi as well as IFN- γ production (20), also exhibited potent antibacterial activities against all bacterial isolates tested. The results of the present study were similar to those reported by Khanna and Nag (21) that constituents of *E. officinalis* have been found to be active against a range of bacteria including *S. aureus*, *E. coli* and *Mycobacterium tuberculosis*, another study the researchers tested methanolic black seed



extract against *S. aureus* MIC56 (22)

Fig (1): cytopathic effect of bacterial suspension on liver tissue of mice without previously administrated with seed oil. (40X Stained with Eosin and Haemotoxin)

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmyia Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

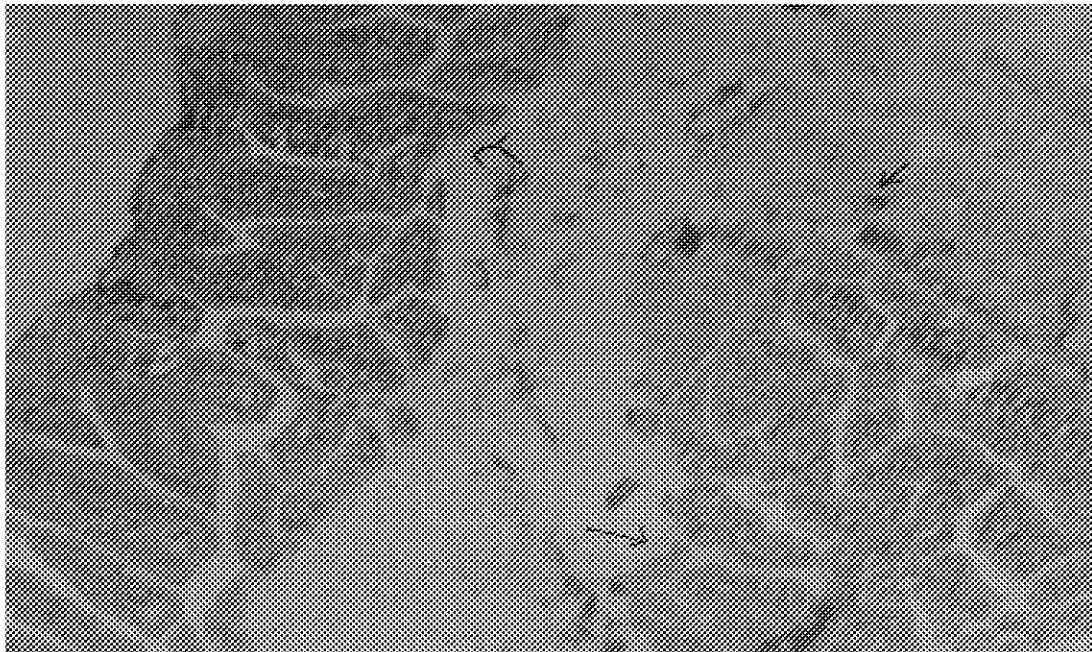


Fig (2): cytopathic effect of bacterial suspension on intestine tissue of mice without administrated with seed oil. (40X)

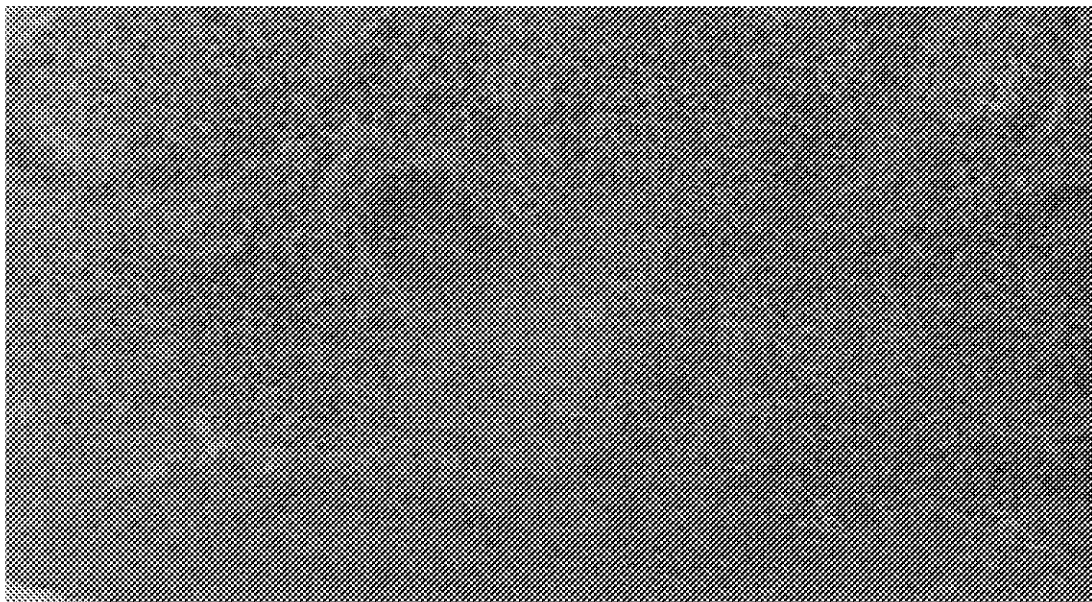


Fig (3): cytopathic effect of bacterial suspension on spleen tissue of mice without administrated with seed oil. (40X)

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmyia Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

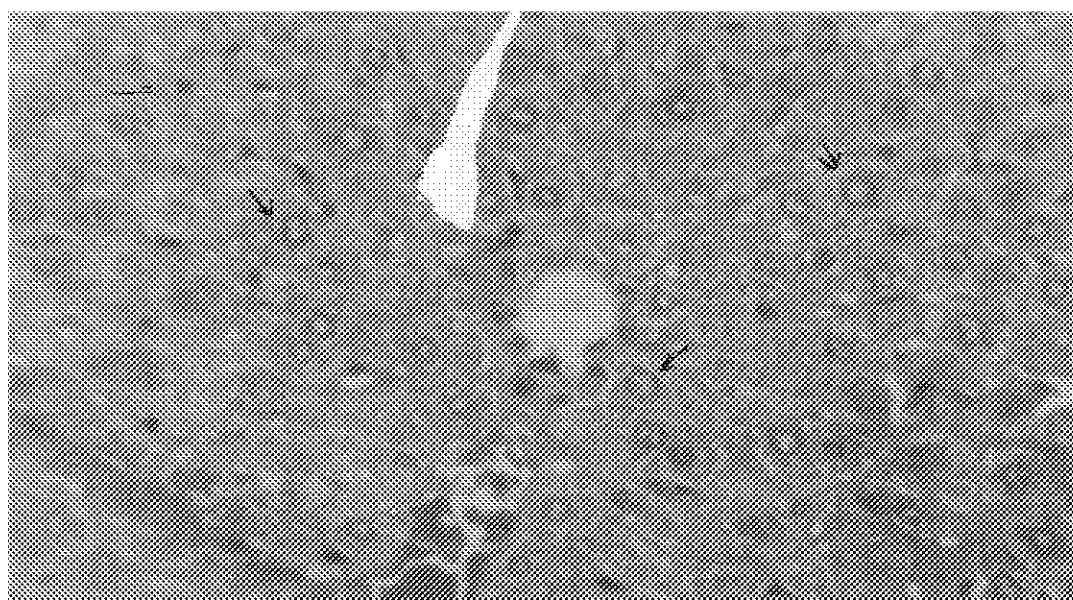


Fig. (4): effect of bacterial suspension on liver tissue of mice which administered with seed oil. (40X)



Fig (5): effect of bacterial suspension on intestine tissue administered with seed oil. (40X)

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmiya Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

Conclusion

It may be concluded from this study that *N. sativa* seed extract had antimicrobial activity against *S. aureus* and protect from bacterial infection. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

References

1. Jones, M.E.; Karlowsky, J.A.; Draghi, D.C.; Thornsberry, C.; Sahm, D.F.; Nathwani, D.(2003). Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. *Int. J. Antimicrob. Agents*, 22:406-19.
2. Mashhadian, N.V. and Rakhshandeh, H. (2005). Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *Ps. aeruginosa* and *C. albicans*. *Pak. J. Med. Sci.*, 21: 47-52.
3. Salman, M. T.; Khan, R. A. and Shukla, I. (2009). A study of *Nigella sativa* Linn, seeds for antimicrobial activity against multidrug resistant clinical strains of *Pseudomonas aeruginosa*. *Hippocratic Journal of Unani Medicine*, 4 (4):.95-104.
4. Ali B.H.; Blunden, G. (2003). Pharmacological and toxicological properties of *Nigella sativa*. *Phytother. Res.*, 17:299-305.
5. Abdel-Salam, I.; Abdel-Wahab, S.; Aaser, A.; and El-Merzabani, M. (1992). Biochemical and cytotoxic effects of *Nigella sativa* L.. *The Egyptian Journal of Biochemistry*, 12(2): 348-355.
6. Chakravarty N. (1993). Inhibition of histamine release from mast cells by Nigellone. *Annals of Allergy*, 70 (3): 237-242.
7. Ansari, M.A.; Ahmed, S.P.; Haider S. and Ansari.N.L. (2006). *Nigella sativa*: A non-conventional herbal option for the management of seasonal allergic rhinitis. *Pakistan Journal of Pharmacology*, 23(2): 31-35.
8. Kahraman, A.; Erkasap, N.; Koken, T.; Serteser, M.; Aktepe, F. and Rkasap, S. (2003). The anti-oxidative and anti-histaminic properties of quercetin in ethanol-induced gastric lesions. *Toxicology*, 183: 133-142.
9. El-Gazzar, M.; El-Mezayen, R.; John, C.; Mark, R.; Andrew, C. and Stephen, C. (2006). Anti-inflammatory effect of thymoquinone in a mouse model of allergic lung inflammation. *Int. Immunopharmacol.*, 6: 1135-1142.
10. Agarwal, R.; Kharya,M.D. and Shrivastava, R. (1979). Antimicrobial and anti-helminthic activities of the essential oil of *Nigella sativa* Linn. *Indian J. Exp. Biol.*, 17: 1264-1265.
11. Doraiswamy, L.K.; Ferreira, S.R.S.; Meireles, M.A.A.; Nikolov Z.L. and Petenate, A.J.(1999). Supercritical fluid extraction of black pepper (*Piper nigrum* L.) essential oil. *J. Supercrit. Fluids*, 14: 235-245.
12. Holt, J.G.; Krieg, N.R.; Sneath,P.H.; Staely, J.T. and Williams, S.T.(1994). *Bergeyes*

The prophylactic role of *Nigella sativa* oil on cytopathic effect of *Staphylococcus aureus*

Rasmia Abed .Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Fareed Salloom

- Manual of Determinative Bacteriology. 9th ed., Williams and Wilkins, P.:1063.
13. Humson, C.L.(1972). Animal tissue Techniques. 3rd ed., W.H. Freeman company, p.:641.
 14. Topozada, H.H.; Masloun, H.; El-Dakhakhany, M. (1965). The anti-bacterial properties of *Nigella sativa* seeds: Active principle with some clinical application. J. Egypt. Med. Assoc., 48(suppl): 187-202.
 15. El-Fataty.(1975). Isolation and structure assignment of an anti-microbial principle from the volatile oil of *Nigella sativa* L. seeds. Pharmazie, 30 (2): 109-111.
 16. Hanafi, M.S. and Hatem, M.E. (1991). Studies on the anti-microbial activity of the *Nigella sativa* seed (Black Cumin). J. Ethnopharmacol, 34 (2-3): 275-8.
 17. Morsi, N.M. (2000). Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotic resistant bacteria. Acta. Microbiol. Pol., 49 (1): 63-74.
 18. O'Mahony, R.; Al-Khtheeri, H.; Weerasekera, D.; Fernando, N.; Vaira, D.; Holton J. and Basset C.(2005). Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori*. World J. Gastroenterol., 11(47): 7499-7507.
 19. Salem, M.L. and Hossain, M.S. (2000). Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus. Int. J. Immunopharmacol., 22 (9): 729-40.
 20. Swamy, S.M. and Tan, B.K. (2000). Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. J. Ethnopharmacol., 70 (1): 1-7.
 21. Khanna, P. and Nag, T.H. (1973). Isolation, identification and screening of phyllembliss from *Emblica officinalis* Geartin tissue culture. Indian Journal of Pharmacy, 35(1): 23-28.
 22. Tenover, F.C.; Biddle, J.W. and Lancaster, M.V.(2001). Increasing Resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. Emerg. Infect. Dis., 7:327-32.

الخلاصة

درس الدور الوقائي لزيت بذور الحبة السوداء من التأثيرات المرضية النسيجية التي يسببها عالق بكتيريا *S. aureus* حيث تم حقن مجموعتين (وبواقع خمس فأرات لكل مجموعة) بعالق البكتيريا . أحدى المجموعتين مجرعة مسبقاً بزيت الحبة السوداء بتركيز 50 % أما المجموعة الأخرى غير مجرعة ، كما استخدمت فأرتين سيطرة حققت بالمحلول الملحي الفسيولوجي السلائين ، ووجد من خلال دراسة التأثيرات المرضية على أمعاء واكباد الحيوانات المحقونة غير المجرعة بعالق البكتيريا أقل من التأثيرات النسيجية في أعضاء الحيوانات المجرعة مسبقاً بزيت الحبة السوداء .