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

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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 dysentriae. 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 IN 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.
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*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). The essential oil, various extracts at different polarity, fractions, and pure compounds obtained from *Nigella damascena* plants and seeds were screened for 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 -20°C.

2- The isolate of *Staphylococcus aureus* in this study isolated was obtained from higher educational lab. of microbiology at biology departmentat 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 37°C 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 intraperitonial 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
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Rasmyia Abed, Abu-Risha, Nagham Shaker, Mohammed Hussein, Dunya Fareed, Salloom

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 2 hr. in each concentration, then cleared by xylol, saturated with paraffin at 60°C for 3 hrs. 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 were left for drying at 37°C, 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 distilled 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 necrotic 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 et al (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 - Nagaam 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 Haemotoxlin)
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-Risa - Nagham Shaker Mohammed Hussein - Dunya Fareed Salloom

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

Fig (5): effect of bacterial suspension on intestine tissue 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 and 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 product 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
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Rasmyia Abed, Abu-Risha - Nagham Shaker Mohammed Hussein – Dunya Farعد Salloom


الخلاصة

درس الورقة الواقفي لزيت بذور الحبوب السوداء من التأثيرات المرضية السيجية التي يسببها عالم S. aureus بكثرة مع تأثير قوي على فروع فازات لكل مجموعة (بعلاج البكتيريا). أدى المجموعتين مجزرة مسبيقاً بزيت الحبوب السوداء بتركيز 50% أما المجموعة الأخرى غير مجزرة، كما استخدمت فازات سيدة حقنها بالمحلول المائي الفلوري البليني بلاستي في أداء دراسة التأثيرات المرضية على الجهاز وأيضاً الحيوانات المحروقة غير المجزرة بعلاق البكتيريا أقل من التأثيرات السيجية في أعضاء الحيوانات المجزرة مسبيقاً بزيت الحبوب السوداء.