Research Article

The effect of *Nigella sativa* and other plants on bacteria isolated from diabetic foot ulcers

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ABSTRACT

Background: Diabetes mellitus is a chronic lifelong disorder distinguishes oneself by chronic hyperglycemia and caused damage of organs. Bad control of diabetes leads to skin infections and non-healing foot ulcers which common in clinical practice. The access to the hospital and mutilation will increase and conversely result in long-term economic, physical, social, and mental disability to the patient. **Methodology:** This study is designed to identified bacteria from diabetic foot ulcer (DFU) patients by tissue specimen and investigates the sensitivity of these bacteria to *Nigella sativa* (NS) oil, other essential oils, and compounds. **Results:** *Staphylococcus epidermidis* 50% was recorded the highest occurrence in patients followed by *Klebsiella pneumoniae* 20%, *Staphylococcus aureus,* and *Escherichia coli* 13.3%. *Pseudomonas aeruginosa* 3.3% was recorded as the lowest occurrence in patients. Polymicrobial growth cultures were present in 6 (100%) of patients and appeared as *S. aureus* with *E. coli and K. pneumoniae* in 2 patient's 33.3%, *S. epidermidis* with *E. coli* in 2 patient's 33.3%, *S. epidermidis* with *P. aeruginosa* in 1 patient 16.6%, and *S. aureus* with *K. pneumoniae* in 1 patient 16.6%. (NS) oil has antibacterial activity against *S. aureus* and *S. epidermidis*. Almond oil, silver sulfadiazine, and hydrogen peroxide 3% were the only showed antibacterial activity. **Conclusion:** Tissue specimen is a good method for bacterial isolation in DFU. (NS) oil, almond oil, and some compounds have antibacterial properties.

KEY WORDS: Bacteria, Diabetic foot infections, Essential oils, Tissue specimen

INTRODUCTION

Diabetes mellitus is a chronic lifelong disorder distinguishes oneself by chronic hyperglycemia and caused damage of organs. Retinopathy, neutralism, nephropathy, diseases of peripheral vascular, and cardiovascular are the main complications related to diabetes mellitus.[1] Persistent hyperglycemic state results in malformation in endothelial cells and distortions of smooth cell in peripheral arteries.[2] Non-healing foot ulcers and skin infections are results from weak control on diabetes which common in clinical practice. The access to the hospital and mutilation will increase and conversely result in long-term economic, physical, social, and mental disability to the patient. Chronic inflammation, host mortality, and impaired healing rates are progressed from damage wounds as a biofilm-associated infection.[3] Treatment for people with diabetes in known pathogens may improve the result. The bacteriology of infections caused by diabetic foot is known over the past 25 years, but the results diversified and different.[4] Specific chemical ingredients that are presented in their tissue including tannins, lipids, alkaloids, and volatile oils are responsible on the effect of herbs on microorganisms,[5] leads to the consideration of these plants as a potent and excessively used alternative to antibiotics that are no longer capable possess the antibacterial effect. In recent years, as a result of increasingly lack consumption of synthetic preservatives, attention to essential oils in food preservation has been magnification.[6]

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MATERIALS AND METHODS

Patients

In our study, 32 diabetic patients are used. All them with an ulcer in different sites of the foot and peripheral neuropathy, 16 were males and 16 were females. The age of patients from 40 to 75 years, 8 with ischemia and 24 without ischemia. Twenty three with moderate infection and 9 with severe infection. Table 1 showed the characteristics of patients.

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Extraction of *Nigella sativa* (NS) Oil

NS was purchased from a local market in Basra, identified and certified by the local pharmacist. Voucher specimens were kept at the Department of Pharmacology and Toxicology, College of Pharmacy, University of Basrah. The seeds were powdered by an ordinary blender. Fixed oil was extracted from the powder of NS using standard Soxhlet method for 18 h, 40°C temperature, petroleum ether (200 ml) was used as a solvent.[7]

Essential Oils and Compounds

Three essential oils (coconut oil, almond oil, and lavender oil) and eight compounds (insulin, activated charcoal, phenytoin, silver sulfadiazine, 3% hydrogen peroxide, sugar, and bread sodium carbonate) were obtained from the local market.

Samples and Bacterial Isolation

A total of 32 patients used in our study, from March 2018 to January 2018. The clinical data taken were the type of diabetes, duration of diabetes, previous ulcer, site of tissue taken, ischemic, peripheral neuropathy, and severity of the infection. Collection of data from the hospital record section in special form is shown in Table 1. Tissue specimens were obtained at the time of admission, after the surface of the wound had been washed vigorously by saline, and followed by debridement of superficial exudates with a sterile curette. After that, specimens were placed in thioglycolate solution tubes and sent to the laboratory for bacterial isolation. Isolation and identification of bacteria were accomplished by standard methods.[8] In this study, tissue specimens samples were cultured on MacConkey agar, Mannitol salt agar, and nutrient agar media.

Antibacterial Activity

The effect of (NS) oil, plant oils, and compounds against bacteria isolated from diabetic foot ulcer (DFU) was determined by well diffusion agar.[9] Inhibition zones were measured depending on.[9] Analyzing the results was done according to *t*-test; significant value was $P \leq 0.05$.[5]

RESULTS

Isolation of Bacteria

Bacteria that isolated from tissue specimens involved *Staphylococcus epidermidis* 15 (46.9%), *Klebsiella pneumoniae* 6 (18.8%), *Staphylococcus aureus* 6 (18.8%), *Escherichia coli* 4 (12.5%), and *Pseudomonas aeruginosa* 1 (3.2%) are shown in Table 2.

Polymicrobial growth cultures were found in 6 (18.8%) of patients, as shown in Table 3. The most commonly isolated organisms from polymicrobial growth was *S. aureus* associated with *E. coli* and *K. pneumoniae* 2 (33.3%) and *S. aureus* associated with *K. pneumoniae* 1 (16.6%). *S. epidermidis* associated with *E. coli* 1(16.6%) and *S. epidermidis* associated with *P. aeruginosa* 1 (16.6%).

Antibacterial Activity of Antibiotics and NS

In well diffusion agar method significantly, NS and antibiotics were documented a good activity against *S. epidermidis* and *S. aureus,* but no showed NS oil effective against other isolates, as shown in Table 4 and Figure 1.

Antibacterial Effect of Plant Oils and Compounds

Some of the plant oils have an antibacterial effect against bacteria in our study. The effect of three essential oils and seven compounds against isolates is shown in Table 5. Almond oil, silver sulfadiazine, and hydrogen peroxide 3% were the only showed antibacterial activity against some tested isolates with the highest activity (27 mm) that recorded with hydrogen peroxide 3% against *S. epidermidis* and the

Table 1: Patients features used in this study

Features	Number of patients (*n*=32)
Age	
40–50 years	8
51–61 years	14
63–75 years	10
Sex	
Male	16
Female	16
Type of DM	Type 2 (no insulin) 23
Duration of DM	
Less than 10 years	9
10 years	13
Above of 10 years	10
Previous ulcer	
Present	22
Absent	10
Site of tissue taken	
Foot	8
Heel	4
Ankle	5
Big toe	12
Meatiest	3
Ischemia present	8
Ischemia absent	24
Peripheral neuropathy	32
Severity of infection	
Moderate	23
Severe	9

DM: Diabetes mellitus

Table 2: The number and percentage of bacteria isolated by tissue specimens

Bacterial isolates	Number	Percentage
Staphylococcus epidermidis	15	46.9
Klebsiella pneumoniae	6	18.8
Staphylococcus aureus	6	18.8
Escherichia coli	4	12.5
Pseudomonas aeruginosa	1	3.2
Total	32	100

lowest (15 mm) that recorded with silver sulfadiazine against *E. coli,* as in Figure 2. Coconut oil, lavender oil, insulin, activated charcoal, phenytoin, sugar, and bread sodium carbonate showed no activity against all isolates.

DISCUSSION

The skin represents the main protection against microorganisms, and infection simply indicates the presence of commensal, pathogen, and opportunist microbes. There are many factors that cause skin infections such as organism adherence, environmental factors, virulence, and host immunity. *Streptococcus pyogenes* and *Staphylococcus aureus* are among the important causing factors of primary and/or secondary skin infections in human.[10-12] These infections may lead to serious topical and organized involvements.[13] Debridement from infected tissue culture is an excellent technique of bacterial diagnosis[14] and deeply tissue sample is the gold standard in culturing technique.[15] Removing external debris before taking specimen will remove surface contaminants and provide accurate results.[16] Therefore, in this study, many important species of bacteria are isolated in different sites of the body using tissue specimen [Table 2]. *S. aureus* was considered the main microorganism isolated by these techniques.[17] Wounds are colonizing by diverse microorganisms that in some cases proliferate in it, causing damage to the tissues and inflammation that is clinical infection.[18,19]

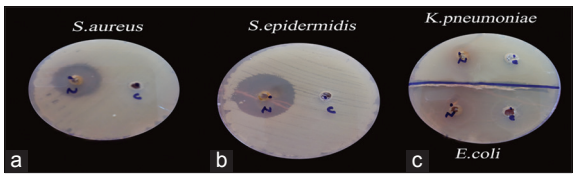

Figure 1: The antibacterial activity of NS oil against bacteria in our study (N: NS oil and C: Control). (a) *Staphylococcus aureus*, (b) *Staphylococcus epidermidis*, (c) *Klebsiella pneumoniae* and *Escherichia coli*

In this study, polymicrobial cultures found in some patients are shown in Table 3. This result is an agreement with previous studies.[20,21] However, the most common organism found in Gram-positive cocci. In a multicentric clinical trial conducted in ulcer, Citron *et al*. have shown, 83.8% of the cultures are polymicrobial in nature with 48% only aerobic bacteria, 43% mixed with aerobe and anaerobe, and only 1.3% shows anaerobic bacteria.[22] Wound represented the prevalence of mixed cultures with

Table 3: Polymicrobial growth isolated by tissue specimens

Polymicrobial growth cultures	Number	Percentage
Staphylococcus aureus + *Escherichia coli* + *Klebsiella pneumoniae*	2	33.3
Staphylococcus epidermidis + *Escherichia coli*	2	33.3
Staphylococcus epidermidis + *Pseudomonas aeruginosa*	1	16.6
Staphylococcus aureus + *Klebsiella pneumoniae*	1	16.6
Total	6	100

Table 4: Inhibition zones (mm) of bacteria against NS oil and antibiotics by well diffusion method

Extract oil and antibiotics	Inhibition zone (mm)				
	Staphylococcus aureus	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
NS oil	39	43	0	0	0
Ciprofloxacin	43	44	-	-	-
Metronedazole	49	48	-	-	-
Cefotaxime	18	0	-	-	-

Table 5: Inhibition zones (mm) of bacteria against essential oils and compounds by well diffusion method

Essential oil and compounds	Inhibition zone (mm)				
	Staphylococcus aureus	***Staphylococcus epidermidis***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***
Coconut oil	0	0	0	0	0
Almond oil	0	0	0	25	0
Lavender oil	0	0	0	0	0
Insulin	0	0	0	0	0
Activated charcoal	0	0	0	0	0
Phenytoin	0	0	0	0	0
Silver sulfadiazine	0	0	15	0	25
Hydrogen peroxide 3%	25	27	21	16	0
Sugar	0	0	0	0	0
Bread sodium carbonate	0	0	0	0	0

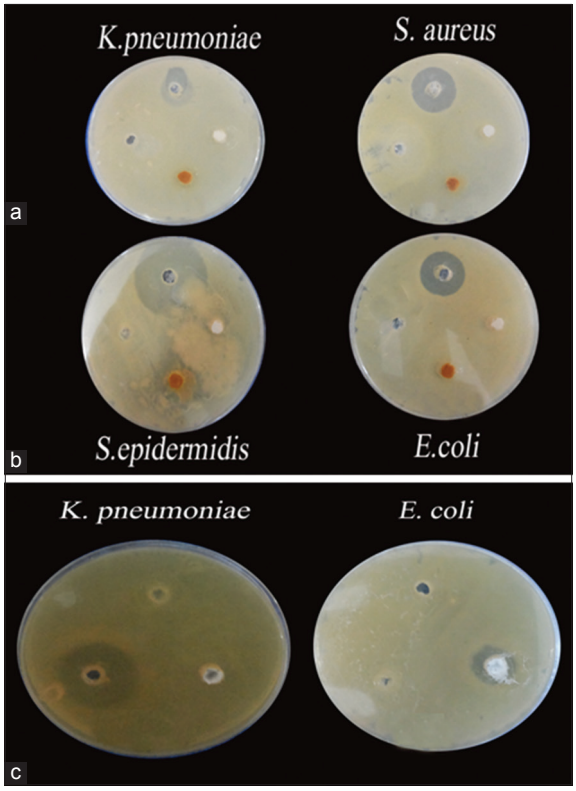

Figure 2: The antibacterial activity of essential oils and compounds against bacteria from diabetic foot ulcer patients. (a) Hydrogen peroxide 3% against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae* and *Escherichia coli*, (b) Silver sulfadiazine against *Escherichia coli*, (c) Almond oil against *K. pneumonia*

Pseudomonas, *Staphylococci aureus*, *E. coli,* and *Klebsiella*.[23] The polymicrobial infection was found to be lower than monomicrobial infection.[24,25] *Pseudomonas* spp., *S. aureus*, *Proteus* spp., *E. coli*, and *Enterobacter* spp. are the main isolates from deep tissue and bone tissue samples.[15] *E. coli*, *S. aureus*, *S. epidermidis*, *P. aeruginosa,* and *Klebsiella* spp. are the main pathogens in our study and this result agreement with.[16,26] The prevalence of bacteria was coagulase-negative Staphylococcus species, oxacillin-susceptible S. aureus, oxacillin-resistant S. aureus, *P. aeruginosa*, *and* Enterobacteriaceae.[22] *S. aureus* and *S. epidermidis* are the most common in wound infection.[27] Oils of plants are volatile and aromatic liquids[28] and have a broad range of secondary compounds that active against microorganisms and a variety of targets, especially the membrane, cytoplasm or alternative the cell morphology. Plant oils have a good role in plant protection.[29] NS is a medicinal plant that has proven to be cured in many diseases.[30]

The data of the preliminary assessment of antibacterial activity of NS seeds revealed that fixed extract contains antibacterial constituents against *S. epidermidis* and *S. aureus,* but no showed effective against other isolates, as in Table 4, which is an agreement with other studies on the same plant species. NS oil exhibits highly antibacterial effect on *S. epidermidis* and *S. aureus* due to the presence of the major components of its oil includes thymoquinone and carvacrol.[31] The aqueous and oil extracts of the seeds showed that have antioxidant, anti-inflammatory, anticancer, analgesic, and antimicrobial activities. Thymoquinone considered the main content in black seed oil and the active principle responsible for many of the seed's beneficial effects.[32]

The fixed oil of organic solvents had more effective against Gram-positive than Gram-negative bacteria. Gram-negative bacteria are impenetrable to antimicrobial agents according to an outer membrane. This reason explains that efficiency of NS organic extracts was largely due to this permeability barrier. Furthermore, Gram-positive bacteria are only composed of the peptidoglycan cell wall, making it sensitive to antimicrobial agents.[33,34] Although petroleum ether extract was a crude one, it proved superior over the standard antibiotic, chloramphenicol, when testing against *S. aureu*s, *S. epidermidis,* and *Bacillus subtilis*. This activity demonstrated by the presence of potent antibacterial constituents in high concentrations in the extract. Moreover, the effect of active chemicals against bacteria is attributed to their chemical composition and morphology.[34] NS has an antibacterial effect against various resistant pathogens of both Gram-positive and Gram-negative bacteria.[35]

Table 5 appeared the susceptibility modality of isolates to three essential oils and seven compounds. Almond oil, silver sulfadiazine, and hydrogen peroxide 3% were the only showed antibacterial activity against some tested isolates with the highest activity (27 mm) that recorded with hydrogen peroxide 3% against *S. epidermidis* and the lowest (15 mm) that recorded with silver sulfadiazine against *E. coli,* as shown in Figure 2. Coconut oil, lavender oil, insulin, activated charcoal, phenytoin, sugar, and bread sodium carbonate showed no activity against all isolates. Hydrogen peroxide has an antibacterial effect against bacterial spores and used on the skin wounds. The mixture of chemicals and peroxide will stimulate the antibacterial effect in lesser concentrations. Hydrogen peroxide significantly reduces any risk of the development of resistance to the biocide over time.[36] Hydrogen peroxide in honey of Mount Olympus has antibacterial effect on *P. aeruginosa* and *E. coli*.[37] Almond oil has antibacterial effect with big inhibition zone diameter and less MIC and MBC values.[38] Almond oil has shown an antibacterial effect on Gram-negative bacteria, this agreement with the result of Table 5.[39]

Silver sulfadiazine is an antiseptic agent and inserted into different wound dressing products. Most

pathogenic organisms found in the wound including fungi, MRSA, and vancomycin-resistant enterococcus are killed *in vitro* by nanochemistry silver.[35] The absorption of silver by cells of the skin stimulates metallothionein production that leads to raises absorption of copper and zinc, that raises DNA and RNA synthesis.[40] After that, tissue repair and cell proliferation were done.[41] Burning skin linked with wounds of rat is healing when treated with silver sulfadiazine and NS cream.[42] Silver sulfadiazine is effective against both *Pseudomonas*, *S. aureus,* and *E. coli*.[43] Studies have shown that bacteria will produce extracellular enzyme lipases that degradation of triglyceride in coconut oil making it not have any bacteriological activity against tested isolates.[44]

Topical insulin has been used for decade's dates back to the 1960s.[45] The mixture of zinc and topical insulin has shown faster to wounds healing.[46] Insulin organizes the response to wounding inflammation by stimulating the spread and migration of macrophages and keratinocytes in neighboring tissues.[47] These previous studies not agreements with the result of this study that documented insulin not have any bactericidal effect against bacteria, which causing skin infection. Phenytoin is anticancer, antifungal, antimicrobial, anti-inflammatory, antitumor, and antiviral[48] and effective on *E. coli*, *Klebsiella,* and *S. aureus*.[49]

Sugar distillation in the wound appears to have a local genital effect that promotes the formation of granular tissue, reduces edema in the wounds, reduces the pH of the pelvis, promotes antibacterial effect, promotes small blood vessel expansion, promotes bacterial decomposition, and prevents bacterial growth by reducing the water activity available that is required for the growth of most bacterial organisms.[50] Therefore, the treatment of post-operative wounds, burns, mediastinitis, and diabetic ulcers by this technique.[51] According to the previous references that documented the potent effect of several oils, isolates in this study may be resistance to these oils and compounds.

CONCLUSION

Tissue specimen is a good method for bacterial isolation in DFU. (NS) oil, almond oil, and some compounds have antibacterial properties.

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