Antibacterial Activity of *Origanum vulgare* on *Staphylococcus aureus* in a Rat Model of Surgical Wound Infection

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Received: 16 Jan. 2015 **Accepted:** 12 Jan. 2016

Abstract

Background: Wound infection is one of the frequent complications in patients with surgical operations. *Staphylococcus aureus* is the most common cause of surgical wound infections. *Origanum vulgare*, a common culinary herb, has been shown to have strong antimicrobial activity, especially against Gram-positive pathogens.

Objective: This study was designed to investigate the antibacterial effects of *O. vulgare* on *S. aureus* in surgical wounds using a rat model.

Methods: Twenty male Sprague-Dawley rats were divided randomly into two groups of treatment and control rats (1:1). A circular incision was made on the dorsal inter-scapular region of each rat. Then, rats were inoculated topically with 1×10^4 CFU of *S. aureus* at the site of skin wounds. *O. vulgare* extract was applied to wounds twice a day during the experiment. Animals of the control group were left untreated.

Results: The load of bacteria in untreated rats was $7 \times 10^6 \pm 6$ CFU/wound while this was $2 \times 10^5 \pm 1$ CFU/wound for the treatment animals, which was significantly lower.

Conclusion: Results have showed that topical application of *O. vulgare* extract on the infected wounds included significant antibacterial activity against *S. aureus*.

Keywords: ***Origanum vulgare*, *Staphylococcus aureus*, Surgical wound**

Introduction

Wound infection remains a common and serious complication of surgical operations. It is the second most frequent nosocomial infection in most hospitals. Patients with such infections experience pain, inconvenience and economy loss. Regarding health services, patients with surgical infections stay in hospitals on average twice as long as uninfected patients [1]. However eradication of wound infections in surgeries seems impossible, decreasing the number of infection victims will result in significant benefits for the medical societies such as higher patient reliefs and lower medical costs [2]. Gram-positive bacteria are the predominant organisms on the skin, from which, *Staphylococcus aureus* is the most common agent of surgical wound and nosocomial infections [3]. *S. aureus* is a part of the normal flora and can be isolated from the nose of up to 60% of the healthy individuals. It is readily transmitted from person to person by contaminated hands, clothes, objects and via the room air. In recent decades, the bacteria has been reported to show increased multiple antimicrobial resistance, especially in methicillin-resistant *S. aureus* [4].

Research on herbal drugs and dietary supplements which can be effective on antimicrobial-resistant microbes have been facilitated in recent years [5]. One of these candidate herbals, oregano, is a common culinary herb used as spice in various countries. Oregano is a genus of Laminaceae family with many species. Two species *Origanum majorana* and *O. vulgare* have therapeutic properties. *Origanum* spp. includes strong antimicrobial effects against common human pathogens. Phenolic compounds, especially phenolic acids and flavonoids, are possibly the responsible agents. *O. vulgare* is an aromatic plant with a wide distribution in European, Mediterranean and Asian countries, especially Iran. Extracts of the plant have been shown to exhibit strong antimicrobial activity, especially against Gram-positive pathogenic bacteria [6, 7]. This study presents the first detailed investigation of *in vivo* antibacterial properties of *O. vulgare* against surgical wounds infected by *S. aureus*.

Experimental Procedures

The plant and extract preparation

Leaves of of *O. vulgare* were collected from Chalus, province of Mazandaran, Iran. Voucher specimens have been deposited at the Herbarium of the faculty of agricultural science, Islamic Azad University, Garmsar branch, Garmsar, Iran. Leaves of the plant were dried and grounded into fine powder using an electric blender. Extract was prepared by cold maceration with alcohol for 24 h. Briefly, 50 g of powder were suspended at 100 ml ethanol for 24 h at a room temperature. The mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1). The extract was concentrated using vacuum distillation.

Animal model

20 male rats (Sprague Dawley) weighing 238 ± 14.8 g were purchased from Razi Vaccine and Serum Research Institute (Karaj, Iran). The animals were housed in Center for Laboratory Animal Care at the Faculty of Veterinary Medicine, Islamic Azad University,

Garmsar Branch. Before the experiment, rats were maintained for seven days at room conditions for acclimatization. Animals were fed on standard pellet diet and water *ad libitum*. All animals received sufficient care according to "Guide for the Care and Use of Laboratory Animals" published by the National Institute of Health.

Bacteria and preparation of inoculum

S. aureus (ATCC 29213) was used as positive standard for inoculation and assessment of antibacterial activity of *O. vulgare*. 1×10^4 inoculum was used to cause wound infection as described previously by Stratford *et al.* [8] and Barker *et al.* [9].

Minimum inhibitory concentration

Minimum inhibitory concentration (MIC) of the extract was assessed using broth macrodilution assay. A 30,000 ppm stock solution of *O. vulgare* extract was prepared in phosphate buffer saline (PBS). The MIC was assessed by the preparation of two-fold dilutions (up to 1875 ppm) of the extract in nutrient broth, and *S. aureus* inoculum (1×10^4 CFU/ml) was subsequently added to each tube. Tubes were vortexed and incubated at 35°C for 18–20 h and then turbidity of the tubes was checked. The last dilution with no bacterial growth was reported as MIC of the extract [10].

Circular excision wound model

To evaluate the antibacterial activity of *O. vulgare* extract, a circular excision wound model was used. Rats were anaesthetized intraperitoneally with a combination of 10% ketamine hydrochloride (50 mg/kg) and 2% xylazine hydrochloride (5 mg/kg). Then, back hair of the animals were shaved. Site of the surgery was scrubbed with povidone iodine followed by 70% ethanol solution. A 15-mm circular incision was made on the dorsal inter-scapular region of animals and the local skin was carefully removed. Then, rats were inoculated topically with 1×10^4 CFU of *S. aureus* at the site of skin wounds (Figure 1). The extract was applied topically twice a day throughout the experiment. No treatment was carried out on the animals in control group.

Tissue preparation and colony count

Preparation of tissues and antibacterial screening was carried out as described previously by Stratford *et al.* in 2002. Tissue specimens were weighed and homogenized in broth. The mixture was used to make 1:10 serial dilutions in Mueller-Hinton broth and transferred to sterile test tubes at 4°C. Dilutions (each 100 µl) were transferred to tryptone soy agar plates and incubated at 35°C for 24 h to form colonies. Colony counts were carried out for 30 to 300 colonies per plate. The following formula was used to calculate the bacterial colony forming units per gram:

$$\text{CFU/g} = \frac{\text{Colony counts} \times 50 \times \text{Dilution Factor}}{\text{Specimen weight (g)}}$$

Figure 1- Preparation of animals for the experiment. A circular incision was made on the dorsal inter-scapular region of each rat. Rats were topically inoculated on their skin wounds with *S. aureus*

Statistical analysis

Statistical analysis was performed using SPSS® software v16.0 (SPSS Inc, USA) and independent *t*-test and analysis of variance. Data were expressed as mean ±standard deviation (SD). Data were considered significant when $P \leq 0.05$.

Results

The average bacterial count in untreated animals was $7 \times 10^6 \pm 6$ CFU/wound eight days after infection. This was $2 \times 10^5 \pm 1$ CFU/wound in animals treated with extract of *O. vulgare* ($P \leq 0.05$).

Discussion

The results of the present study have revealed that topical application of *O. vulgare.* Extract at the infected wound site produced significant antibacterial activity against *S. aureus*. These findings support findings by Saeed and Tariq [6] and Ortega-Nieblas *et al.* [7], who have studied the antibacterial potency of oregano against Gram-positive bacteria *in vitro*. The antibacterial activity of the extract possibly linked to its major components or synergy between the major and minor compounds. The main chemical compounds of *O. vulgare* include carvacrol, thymol and p-cymene [11, 12]. Researchers have reported that carvacrol and thymol (the major compounds) has a strong antibacterial activity against Gram-positive bacteria [13, 14, 15]. Karuppusamy *et al.* [16] and Mishra *et al.* [17] studied the antibacterial activity of some essential oils on Gram-positive bacteria and showed that the p-cymene (the minor compound) has a significant antibacterial effect on *S. aureus*. Further research have been carried out on antimicrobial activity and mechanism of action of oregano essential oils (OEO) [18, 19, 20, 21, 22]. This antimicrobial property has been linked to the presence of hydroxyl groups in phenolic components [23]. In 2002, Ultee *et al.* showed that the presence of a free hydroxyl group and delocalized electron system is essential for the antibacterial activity [24].

The antibacterial mechanism of *O. vulgare* extract has been explained by the alteration of bacterial membrane integrity. Lambert *et al.* (2001) found that OEO damaged bacterial in-membrane integrity, which affected pH homeostasis and equilibrium of inorganic ions [20]. In 1999, Ultee *et al.* showed that carvacrol decreased bacterial intracellular potassium and membrane potency and increased the extracellular potassium [19]. Antibacterial effects of OEO are not limited to the bacterial membrane. Transfer of monoterpene to the bacterial cell throughout the lipid bilayer and its interaction is also suggested [22]. Furthermore, Vattem *et al.* (2007) found that OEO inhibited the quorum sensing (QS) of the bacteria. QS is a mechanism; by which the bacteria modulate the expression of their genes involved in processes of survival and pathogenesis [26].

Conclusion

Literature review shows that hydroalcoholic extract of the Iranian *O. vulgare* includes antimicrobial activity. In the current study, *O. vulgare* extract has been demonstrated to include strong antibacterial activity against *S. aureus*, which can be linked to the major (carvacrol and thymol) or minor (p-cymene) compounds of the plant or synergy between these compounds.

Acknowledgements

The authors are grateful to Dr. Koochakzadeh, Khosravi, Yazdani and Keshmiri for excellent technical assistance.

References

1. Paocharoen V, Mingmalairak C and Apisarnthanarak A. Comparison of surgical wound infection after preoperative skin preparation with 4% chlohexidine and povidone iodine: a prospective randomized trial. *J. Med. Assoc. Thai.* 2009; 92: 898 - 902.
2. Nandi PL, Soundara Rajan S, Mak KC, Chan SC and So YP. Surgical wound infection. *Hong Kong. Med. J.* 1999; 5: 82 - 6.
3. Onche I and Adedeji O. Microbiology of post-operative wound infection in implant surgery. *Nige. J. Surg. Res.* 2004; 6: 37 - 40.
4. Naik G and Deshpande S. A study on surgical site infections caused by Staphylococcus aureus with a special search for methicillin-resistant isolates. *J. Clin. Diagnostic. Res.* 2011; 5: 502 - 8.
5. Essawi T and Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharmacol.* 2000; 70: 343 - 9.
6. Saeed S and Tariq P. Antibacterial activity of Oregano (Oreganum vulgare Linn.) against gram positive bacteria. *Pak. J. Pharm. Sci.* 2009; 22 (4): 421 - 4.
7. Kaurinovic B, Popovic M, Vlaisavljevic S and Trivic S. Antioxidant Capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. Extracts. *Molecules* 2011; 16: 7401 - 14.
8. Stratford AF, Zoutman DE and Davidson JS. Effect of lidocaine and epinephrine on Staphylococcus aureus in a guinea pig model of surgical wound infection. *Plast. Reconstr. Surg.* 2002; 110: 1275 - 79.
9. Barker W, Rodeheaver G, Edgerton M and Edlich RF. Damage to tissue defenses by a topical anesthetic agent. *Ann. Emerg. Med.* 1982; 11: 307 - 10.
10. Coyle MB. Manual of Antimicrobial Susceptibility Testing. American Society for Microbiology. 2005, pp: 53 - 62.

11. Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T and Arsenakis M. Antimicrobial and Cytotoxic Activities of Origanum Essential Oils. *J. Agric. Food. Chem.* 1996; 44: 1202 - 5.
12. Derwich E, Benziane Z, Manar A, Boukir A and Taouil R. Phytochemical analysis and in vitro antibacterial activity of the essential oil of Oreganum vulgare from morocco. *Am-Euras. J. Sci. Res.* 2010; 5 (2): 120 - 9.
13. Özkalp B, Sevgi F, Özcan M and Özcan MM. The antibacterial activity of essential oil of oregano (Origanum vulgare L.). *J. Food. Agr. Environ.* 2010; 8 (2): 272 - 4.
14. De Martino L, De Feo V, Formisano C, Mignola E and Senatore F. Chemical composition and antimicrobial activity of the essential oils from three chemotypes of *origanum vulgare* L. ssp. hirtum (Link) Ietswaart growing wild in campania (Southern Italy). *Molecules* 2009; 14: 2735 - 46.
15. Nostro A, Roccaro A, Bisignano G, Marino A, Cannatelli MA and Pizzimenti FC. Effects of oregano, carvacrol and thymol on Staphylococcus aureus and Staphylococcus epidermidis biofilms. *J. Med. Microbiol.* 2007; 56: 519 - 23.
16. Karuppusamy S, Muthuraja G and Rajasekaran K. Chemical composition and antimicrobial activity of essential oil from fruits of Vanasushava pedata (Apiaceae). *Advan. Biol. Res.* 2009; 3: 196 - 200.
17. Mishra D, Joshi S, Bisht G and Pilkhwal S. Chemical composition and antimicrobial activity of Solidago Canadensis Linn. Root essential oil. *J. B. Clin. Pharm.* 2010; 1: 187 - 90.
18. Hammer KA, Carson CF and Riley TV. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* 1999; 86 (6): 985 - 90.
19. Ultee A, Kets E and Smid E. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. *Appl. Environm. Microbiol.* 1999; 65 (10): 4606 - 10.
20. Lambert RJ, Skandamis PN, Coote P and Nychas GJ. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* 2001; 91 (3): 453 - 62.
21. Nostro A, Blanco A, Cannatelli M, Enea V, Flamini G, Morelli I, Roccaro A and Alonzo V. Susceptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. *Fems. Microbiol. Lett.* 2004; 230 (2): 191 - 5.
22. Trombetta D, Castelli F, Sarpietro M, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G and Bisignano G. Mechanisms of antibacterial action of three monoterpenes. *Antimicrob. Agents. Chemother.* 2005; 49: 2474 - 78.
23. Ben A, Combes S, Preziosi-Belloy L, Gontard N and Chalier P. Antimicrobial activity of carvacrol related to its chemical structure. *Lett. Appl. Microbiol.* 2006; 43 (2): 149 - 54.
24. Ultee A, Bennink M and Moezelaar R. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. *Appl. Environ. Microbiol.* 2002; 68 (4): 1561 - 68.
25. Dorman H and Deans S. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* 2000; 88 (2): 308 - 16.
26. Vattem D, Mihalik K, Crixell S and Mclean R. Dietary phytochemicals as quorum sensing inhibitors. *Fitoterapia* 2007; 78 (4): 302 - 10.