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The Healing Effect of Mixture of Honey, Putty, Vitriol and Olive oil in *Pseudomonas aeruginosa* Infected Burns in Experimental Rat Model

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ABSTRACT

This study was carried out to investigate the healing effect of mixture of honey, putty and vitriol dissolved in olive oil on burn wounds infected with *P. aeruginosa* in rat as an experimental animal model. From April to July 2008, 60 rats were divided into three equal groups, Group A as control left without any treatment, Group B received mixture of honey, putty and vitriol dissolved in olive oil and Group C received just silver sulfadiazine. A standard 3rd degree burn wound was established and after 24 h, 10^8 colony forming units of toxigenic strains of *P. aeruginosa* were inoculated subcutaneously. Samples from infected areas were cultured to identify the bacteria. After 7, 14 and 21 days, the burn areas were studied histologically. Bacterial count of the wound on 2nd day was 10^5 *P. aeruginosa*. On day 7, 14 and 21, the number of bacteria in groups B and C significantly decreased in comparison to Group A. Regarding epithelialization on day 7, no significant difference was noticed between groups B and C. On day 14, Group B demonstrated better re-epithelialization than groups A and C. On day 21, best results were observed in Group B but the difference was not significant. On day 21, there was poor re-epithelialization in Group A. The mean score of group C was between A and B groups. Our results showed that our mixture for treatment of burn site was safe and effective. However, to use this mixture clinically, more supportive studies seem necessary.

Key words: Burn, healing, honey, silver sulfadiazine, vitriol, putty, rat

INTRODUCTION

Pseudomonas aeruginosa has an important role in infected burned wounds and effectiveness of the tested antibiotics against this bacterium was shown to be 54.0% in hospitalized patients with bloodstream infections in southern Iran (Japoni *et al.*, 2010) and 72.7% in Central Iran (Noorbakhsh Sabet *et al.*, 2010). It is an important cause of severe, acute and chronic nosocomial infection and is generally resistant to numerous antimicrobial agents due to natural resistance in particular impermeability or mutations and acquisition of resistant determinants (Japoni *et al.*, 2009). It may lead to septicemia and death due to exotoxin A as the principal lethal factor of the

bacteria (Manafi *et al.*, 2009). Antiseptic ointments such as silver sulfadiazine are used for treatment of the infected wounds; however the resistance to silver sulfadiazine ointment has been a therapeutic problem of the infected wounds (Japoni *et al.*, 2005).

There are several reports on antimicrobial activities against *P. aeruginosa* as a dressing for infected wounds using experimental animals models (Amini *et al.*, 2010; Hazrati *et al.*, 2010; Hosseini *et al.*, 2007; Nejabat *et al.*, 2009). Honey is a supersaturated sugar solution with approximately 17% water which is obtained when the nectar and sweet deposits from plants are collected, changed and stored in the honeycomb by honeybees (White and Molan, 2005). It was first used in the management of wounds by Egyptians 4000 years ago. It was shown that the ancient Greeks, Romans and Chinese applied honey as a topical antiseptic for skin ulcers and sores (White and Molan, 2005). It has been used for various applications including burn wounds, grafts sites and ulcers (Ahmed *et al.*, 2002; Dumfort *et al.*, 2000; Lusby *et al.*, 2002; Molan, 2002; Namias, 2003; Vandeputte, 2003). Honey has been shown to be bactericidal to several micro-organisms including *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Ahmed *et al.*, 2002; Dumfort *et al.*, 2000; Lusby *et al.*, 2002; Molan, 2002; Namias, 2003; Vandeputte, 2003).

Vitriol has been used since ancient time for treatment of several diseases such as eye infections. Its main constituent is zinc oxide, which is produced during refinement and melting of some quarries containing zinc and/or copper compounds as zinc sulfur, calamine and smitsonit. In Mount Lebanon and Mount Hermon as the origin of this medicine, a notable tendency to use the substances for treating diseases of the skin, the eyes, the sexual organs and hemorrhoids was detected (Lev, 2002).

Putty was shown to have antimicrobial and healing properties, while its major constituents are lead and tin oxide (AgO_2) (www.encyclopaediaislamica.com; <http://dictionary.reference.com>).

As mixture of honey, putty and vitriol dissolved in olive oil was used by migrating nomads as a therapeutic measure in burn wounds for its healing effects especially in infected ones, this study was conducted to determine the healing effect of this mixture on burn wounds infected with *P. aeruginosa* in rat as an experimental animal model.

MATERIALS AND METHODS

From April to July 2008, 60 rats with the weight range of 180-220 g were randomly divided into three equal groups, control (Group A), mixture of honey, putty, vitriol and olive oil (Group B) and silver sulfadiazine (Group C). Ketamine (15 mg kg^{-1}) and xylocain (1.1 mg kg^{-1}) were injected intramuscularly to sedate the animals. The back hairs of all animals were shaved and their skin was cleansed with povidone iodine solution and wiped with sterile water. A standard 3rd degree burn wound was established by a hot plate with an identical size about 20% of Total Body Surface Area (TBSA) and at similar temperature as described by Manafi *et al.* (2009).

After 24 h of burn production, 10^8 Colony Forming Units (CFU) of toxigenic strains of *P. aeruginosa* (PA 103) were inoculated subcutaneously into the burned area. All groups were supervised in their cages for 21 days. Samples were obtained from the infected areas using sterile swabs and saline and checked for the presence of *P. aeruginosa* after 48 h and 1, 2 and 3 weeks of experimental production of burns.

All swabs were cultured on blood and Muller-Hinton agar plates. They were incubated at 37°C under ambient conditions for 24 h. *P. aeruginosa* was identified by colony morphology, odor, a zone of hemolysis and oxidase, methyl red, Voges Proskauer, citrate and TSI tests (Forbes *et al.*, 1998).

Animal selection, all experiments, subsequent care and the sacrifice procedure were all adhered to the guidelines of Animal Care Committee of Iran Veterinary Organization. All experiments were undertaken under aseptic conditions in Laboratory Animal Center of Shiraz University of Medical

Sciences. The protocol of anesthesia, procedures, postoperative care and sacrifice were similar for all animals. During the experiments, the animals were housed one per cage, maintained under controlled environmental conditions (ambient temperature of 21±2°C, relative humidity of 65-70% and a balanced diet with free access to food and water).

For treatment of wounds in Group B, a dressing of the mixture of honey, putty, vitriol and olive oil (2 cm each) was used and in Group C, 1% silver sulfadiazine ointment was applied on the burn wounds and in the control group (Group A), it was left without any treatment.

In group B, the mixture of honey, putty, vitriol and olive oil was produced in the paste form as follows: (1) Honey wax as base of 35 g (35% of the compound), (2) Powder of putty (Soranj) as 15 g (15% of the compound), (3) Powder of vitriol (Totia) as 25 g (25% of the compound) and (4) Olive oil as 25 mL (25% of the compound).

After 7, 14 and 21 days of treatment, the animals were sacrificed with an overdose of anesthetics and the burn areas were removed for histological studies. The samples were fixed in 10% buffered formalin overnight at room temperature. The 5 µ thick sections of paraffin-embedded tissues were prepared and then stained with hematoxylin and eosin and examined at ×10-20 magnification for presence or absence of reepithelialization, crusting blistering, spongiosis, granulation tissue and collagen matrix organization, inflammation, congestion, edema, etc.

Histological scorings were made at magnification of ×40 from 20 random fields per section from each specimen as described by Mehrabani *et al.* (2009) including degree of epithelialization (absence or presence of epithelial covering, number of epithelial cell layers, crusting, spongiosis, intraepithelial inflammatory cells and blistering), granulation tissue and collagen matrix organization (adipose tissue substitution as an index of impaired wound closure, loose connective tissue or dense eosinophilic collagen matrix, edema, hemorrhage, degree of inflammation, number and organization of fibroblasts as well as their morphologic characteristics such as a plumped, spindle, or stellate-morphology as indexes of activity), inflammatory infiltrates (number of inflammatory cells and their localization such as perivascular or intravascular inflammatory infiltration or diffuse tissue inflammation of both neutrophils and lymphocytes) and angiogenesis (number of capillary lumens, congestion, fibrin deposition, hemorrhage). The histological scoring system ranged between 1 and 5 (Table 1).

Table 1: Scoring system of the histological changes in burn wound healing

Score	Reepithelialization	Granulation	Inflammatory cells	Angiogenesis
0	Absence of epithelial proliferation in = 70% of tissue	Immature and inflammatory tissue in = 70% of tissue	13-15 inflammatory cells per histological field	Absence of angiogenesis, presence of congestion, hemorrhage, edema
1	Poor epidermal organization in = 60% of tissue	Thin immature and inflammatory tissue in = 60% of tissue	10-13 inflammatory cells per histological field	1-2 vessels per site, edema, hemorrhage, congestion
2	Incomplete epidermal organization in = 40% of tissue	Moderate remodeling in = 40% of tissue	7-10 inflammatory cells per histological field	3-4 vessels per site, moderate edema, congestion
3	Moderate epithelial proliferation in = 60% of tissue	Thick granulation layer and well formed collagen matrix in = 60% of tissue	4-7 inflammatory cells per histological field	5-6 vessels per site, slight edema, congestion
4	Complete epidermal remodeling in = 80% of tissue	Complete tissue organization in = 80% of tissue	1-4 inflammatory cells per histological field	More than 7 vessels per site vertically disposed toward the epithelial surface

All wounds were examined daily for any changes in appearance, discharge, color, smell of wounds and time of scar separation. Animal activity was also recorded if they were lethargic.

The results were expressed as mean±SD. The data were analyzed using SPSS software (Version 11.5, Chicago, IL, USA) by t and Chi-Square tests. A $p < 0.05$ was considered statistically significance.

RESULTS AND DISCUSSION

After first induction of general anesthesia 9 out of 60 rats did not recovered from anesthesia and died, so they were excluded from the control group and number of rats in the control group reduced to 11 animals.

Bacterial count of the wound culture on the 2nd day after burn production revealed a mean bacterial count of 10^5 *P. aeruginosa*. The bacterial number after 1 week decreased significantly in Group B and C in comparison to Group A ($p = 0.001$). After 3 weeks, the difference was significant between Group A and B ($p = 0.001$) but was not significant between Group A and C. The difference was significant between Group B and C after 1, 2 and 3 weeks too ($p = 0.001$).

On day 7, histological findings demonstrated no significant difference between treatment groups for re-epithelialization, granulation, inflammatory cells and angiogenesis. On day 14, in Group B less inflammatory cell infiltration was noticed, the number of capillaries increased and re-epithelialization was better when compared with groups A and C. The best results were observed on day 21 in Group B but the difference was not statistically significant (Fig. 1). After 3 weeks, the epidermal re-epithelialization was poor in Group A (Fig. 2). In group C, the mean score was between groups A and B (Fig. 3).

In clinical practice, antibiotic-resistant *P. aeruginosa* and its associated costs are still important in burn patients (Al-Waili *et al.*, 2005). The advent of topical antimicrobials in 1960s reduced the mortality from burn wound sepsis from 60 to 28%. However, burn wound sepsis remains an important and potentially remediable cause of significant mortality and morbidity. The advent of multi-resistant bacteria has hastened the almost exclusive use of silver-based topical antimicrobial agents for the prevention of burn wound sepsis. The mainstay of burn wound topical antimicrobials in most countries is the preparation of 1% silver sulphadiazine (SSD) (Fraser *et al.*, 2004; Paddle-Ledinek *et al.*, 2006).

Silver sulfadiazine was shown as the gold standard in topical burn therapies which is also a useful antibacterial agent. It may delay the wound healing process (Cho *et al.*, 2005) and may

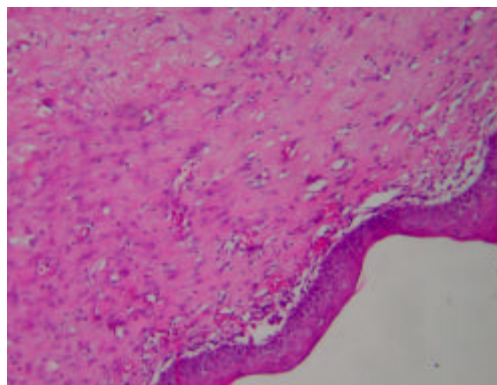


Fig. 1: Scar tissue formation and Epithelialization in group B (200x)

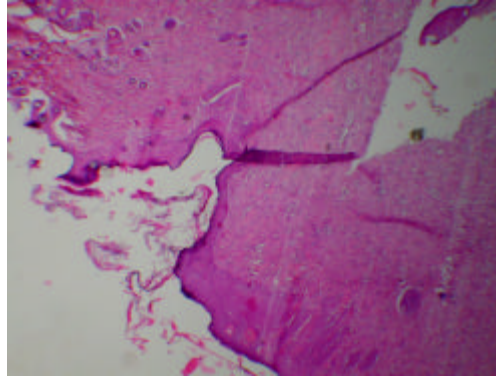


Fig. 2: Healing with scar formation in group A (200x)

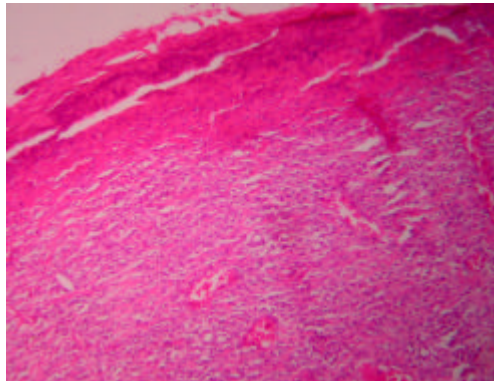


Fig. 3: Granulation tissue formation in group C (200x)

produce serious cytotoxic effects on host cells (Braydich-Stolle *et al.*, 2005; Hussain *et al.*, 2005). The silver released from its commercial products is very toxic to both fibroblasts and keratinocytes (Poon and Burd, 2004). It may lead to transient leukopenia due to bone marrow suppression (Homann *et al.*, 2007). Its anti-infective products may not also provide the moisture to promote a rapid wound healing (Homann *et al.*, 2007). There are also many reports on resistance of silver sulfadiazine (Wasiak *et al.*, 2008). So, there is a need for new products for therapy of burn wounds in the healthcare industries (Van Den Plas *et al.*, 2008).

Honey was reported to be effective in wound healing since ancient times (Cooper *et al.*, 2002). Hazrati *et al.* (2010) reported more epithelialization in honey treated group in comparison with silver sulfadiazine ointment and the control group with a significant decrease in bacterial count in the wound area. Similar result was demonstrated by Nejabat *et al.* (2009) while honey was more effective in reduction of *P. aeruginosa* induced keratitis in comparison to the control group. In present study, honey was part of our therapeutic mixture in group B revealing more significant re-epithelization on days 14 and 21 in comparison to groups A and C. On days 7, 14 and 21, the number of bacteria in group B was significantly less than group A, but this difference was not significant between groups A and C which are identical to previous reports.

Several researchers showed that honey can help wound healing when used topically in several rat models of wound healing. Its acidic pH and hyper-tonicity are regarded as major factors in accelerating the healing in burns. Honey can enhance wound healing by accelerating glycolytic enzyme activity and supplying enough energy for cellular repair. Formation of collagen, wound

contraction and epithelization are important stages in wound healing while inflammation is interlinked. Thus an intervention in any one of these phases may result into either promotion or depression of the collagen formation stage of healing. The healing effect of honey was shown to be due to its unique properties including the pH, high osmolarity, osmotic effect, hydrogen peroxide content and some phytochemicals (Ahmed *et al.*, 2002; Dumfort *et al.*, 2000; Lusby *et al.*, 2002; Molan, 2002; Namias, 2003; Vandeputte, 2003).

Its antimicrobial activities are related to its high osmolarity, ability to produce hydrogen peroxide when diluted, acidity and direct action of antimicrobial chemicals with anti-inflammatory properties (Ahmed *et al.*, 2002; Dumfort *et al.*, 2000; Lusby *et al.*, 2002; Molan, 2001, 2002; Namias, 2003; Vandeputte, 2003; White and Molan, 2005).

In RCTs, Iftikhar *et al.* (2010) showed that treatment with honey increased both granuloma tissue weight and the breaking strength. Pieper (2009) reported that honey-based dressings facilitate healing in chronic wounds and burns. Gethin and Cowman (2008) compared honey and hydrogel in treatment of 104 patients. During 12 weeks, the percentage of patients healed was more in honey-treated (44%) than the hydrogel group (33%). Slough reduction was also more noticed in honey group at 4 weeks, although the percentage of the venous leg ulcer covered in slough significantly decreased in both groups from the baseline to week 4 (from 85 to 29% in the honey group and from 78 to 43% for the hydrogel group). Smith *et al.* (2009) evaluated application of honey in 11 patients with venous incompetence and non-healing venous leg ulcers failing to respond to an assortment of different therapeutic measurements including 4 layer compression, non-adherent dressings, topical silver and antibiotic therapy. A complete wound closure was visible within 3 to 4 weeks.

They demonstrated that the effects of honey increased the velocity of healing when used together with multilayer compression bandages. These reports confirm the healing effect and antimicrobial activity of honey which were also visible in our study as part of the mixture.

Vitriol which was part of our mixture has been used since ancient time for treatment of several infections in the Levant in the Medieval and early Ottoman periods (Lev, 2002). This property against infection may partially explain the decrease in our microbial count in the site of injury.

Putty as the other part of our mixture was also shown to have antimicrobial and healing properties (Neiva *et al.*, 2008; Turner *et al.*, 2003; Vastardis *et al.*, 2005; Wilkins and Kelly, 2003). These findings also are in agreement with our results demonstrating the healing effect and antimicrobial property of the mixture.

In this study, olive oil was the solvent part of the mixture which was shown to have healing effects (Suntar *et al.*, 2010).

Present findings show that the mixture of honey, vitriol and putty dissolved in olive oil can be a safe therapeutic method for treatment of burn site when infected with *P. aeruginosa*. So, it can be recommended in burn patients effectively as a topical therapeutic measurement. However, to use this mixture clinically, more researches seem necessary.

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