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Experimental Evaluation of Repair Process of Burn-Wounds Treated with Natural Honey

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Abstract: Honey produced by honeybees (*Apis mellifera*) is one of the ancient traditional medicines used for treatment of wounds and various illnesses. This study was an assessment the antimicrobial activity of natural honey on burn wound healing and its properties was compared to silver sulfadiazine. This evaluation was including clinical, microbiological and histopathological examinations. Fifteen adult mongrel dogs, 4 to 5 years old, with mean weight of 21±4.24 Kg were studied. Burn wounds were created in dorsolateral region of each dog, according to Hoekstra model. In honey treated group (n = 5), wounds were cover with a thin layer (5 mL) of natural honey after washing with normal saline once a day and in the SSD treated group (n = 5), instead of honey, 5g of 1% silver sulfadiazine ointment was applied at same frequency and time of day. In control group (n = 5); wounds only were washed with normal saline once a day. Clinical and microbiological examinations of the burn wounds were carried out on 1, 7, 14 and 21days of the experiment. Specimens were taken for histopathological examinations on day 21. Results were shown wounds of honey and silver sulfadiazine groups were healed in significantly shorter period than the control group ($p<0.05$). Also total bacterial count were smaller in wounds of two experiment groups than the wounds of control group ($p<0.05$). On histopathology re-epithelization was more pronounced and the collagen fibers were also more orderly arranged in the honey- treated group. According to results of this study, topical application of natural honey has beneficial effect on experimental burn wound and also there are not significant differences between two treatment groups (honey and silver sulfadiazine groups) from clinical, microbiological and histological aspects.

Key words: Topical application, honey, burn wound, microbiology, histology, dog

INTRODUCTION

In many countries all over the world especially in developing countries, large number of people die daily of preventable and curable injuries related to burn wounds. Superficial burns comprise a spectrum of injury severity depending on the depth of the wound and the proportion of the body affected (Moore *et al*., 2001). Heat injury, with creation of conclusive necrosis in the skin layers, prepares an environment that is suitable for a bacterial infection as a result of low tissue perfusion and high protein content. Wound infection, in addition to changing low degree burn into complete thickness necrosis, facilitates the microbe penetration into vital tissues and infection spreading as a result of skin barrier injury. Thus, infection is the most important impediment topical factor in healing process and causes prolongation of treatment period (Morris and Malt, 1994).

While current approach to burn injury management have improved patient prognosis, increased morbidity and mortality still remains a major challenge for clinicians. Prior to the advent of topical antibacterial agents, the overall mortality rate in a typical burn population was between 38% and 45%. However, introduction of topical antimicrobial therapy reduced the overall mortality to 14 to 24% (Mason *et al*., 1986).

Nonetheless, because of growing concern regarding the high incidence of antibiotic resistance, the risk of adverse drug effects and high costs, using the antibiotics as a prophylactic treatment of wound infection is on the decline (Edwards *et al*., 2000). The development of new strains of drug resistant bacteria is also an important issue. For example Methicillin Resistant *Staphylococcus Aureus* (MRSA) has now become the major cause of nosocomial infections in burn wounds in Europe[5]. Currently three topical antimicrobial drugs (silver sulfadiazine, silver nitrate and acetate mafenid) are available for treatment of burn wounds. Moncrief and Lindberg were the first to employ mafenide acetate on burn wounds infections (Moncrief *et al*., 1966). Silver nitrate entered the therapeutic arena about the same time (Bader, 1966).

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In 1968, Charles Fox introduced a new topical antibacterial called silver sulfadiazine (Fox, 1968). The therapies have drawn the interest of both public and medical communities. Current research has mainly focused on herbal and aromatherapy products. However, a number of other products such as honey have shown therapeutic promise. Honey has been used in wound treatment as long ago as 2000 years (Mathews and Binning, 2002). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans all used honey, in combination with other herbs and on its own, to treat wounds and diseases (Zumla and Lulat, 1989). The use of honey as a medicine has continued into present-day medicine. Philips (1993) mentioned the use of honey in burns and described it as the best natural dressing. Voigtlander (1937) used honey to treat scalds and stressed the relief of pain and the smoothening action of honey. Honey inhibits the growth of gram-positive and gram-negative organisms, promotes epithelialization and helps in healing of the burn wounds (Postmes *et al*., 1993; Subrahmanyam, 1994).

Laboratory studies have shown that pure honey has significant antibacterial activities against the major wound-infecting species specially those with the potential to develop antibiotic resistance such as Methicillin-resistant Staphylococcus Aureus (MRSA), beta-haemolytic streptococci, Vancomycin-Resistant Enterococci (VRE) (Cooper *et al*., 2000) and Pseudomonas (Molan, 1999). In two separate studies, beneficial effect of honey in healing the superficial and non-infectious burns outweighed the placental membranes and silver sulfadiazine ointment (Subrahmanyam, 1991; 1994; Adesunkanmi and Oyelami, 1994).

Hence a continuing need exists for a topical treatment for burn wound which is cheaper, easily available and effective in preventing infection. This study was designed to evaluate the efficacy of honey in healing deep burn wounds. Moreover, in the context of wound healing the antibacterial properties of honey was compared to silver sulfadiazine.

MATERIALS AND METHODS

Honey: Natural honey was obtained from beehives in Urmia, (West Azarbaijan province) and no additional procedures were performed. The samples of honey were collected and prepared by one investigator while the experiments were performed blindly by the others. Each honey sample was first filtered with a sterile mesh to remove debris and stored at 2-8°C until used. The average composition of the honey is given in Table 1.

Table 1: Average composition of urmia honey

Average (%)	Component
70.38	Reductant sugars
2.12	Sucrose
0.93	Fructose/Glucose
+	Diastase
-	Commercial glucose
0.05	Mineral components
15.08	Moisture
82.92	Concentration
12.5	Total acid
3.96	pH

Animals: Fifteen apparently healthy adult mongrel dogs of both sexes, 4 to 5 years old and with mean weight of 21±4.24 Kg, were studied. Animals were categorized randomly into 3 groups of control, honey and 1% silver sulfadiazine treated. Five animals were in each experimental group. The animals were kept in individual cages and the investigators adhered to the Animal Welfare Act. The experimental protocol was also approved by the Animal Ethics Committee of the university. The model of the burn wound was produced according to Hoekstra standard (Brans *et al*., 1994). Dogs were premedicated with atropine (Darou pakhsh, Tehran, Iran) (0.04 mg kg^{-1}, intramuscularly) and acepromazine (Hoogsrraten, Belgium) (0.1 mg kg^{-1}, intravenously). Anesthesia was induced with sodium thiopental (Biochemie GmbH, Vienna, Austria) (10 mg kg^{-1}, 2.5% intravenously) and maintained with halothane (ICI Pharmaceuticals, Cheshire, England). Lactated Ringer's solution (Shahid Ghazi Co., Tabriz, Iran) (10 mL/kg/hr, intravenously) was administered during the surgical procedure. Dogs were positioned in ventral recumbency and hair just behind the shoulders was shaved from the backs then, skin was prepared for aseptic surgery.

Experiment protocol: All animal were subjected to the rectangular burn wounds (2×3 cm^2) using a hot (180°C) brass brick weighing 500 g which was pressed against the shaved skin for 10 s on either side of the spine. In the control group, the wounds were washed with normal saline once a day. In the honey treated group, as a daily procedure, wounds were washed with normal saline. Subsequently, 5 mL of pure, unprocessed, undiluted, natural honey was applied in a thin layer to the wounds. However, in the SSD treated group, instead of honey, 5 g of 1% silver sulfadiazine ointment (Darou pakhsh, Tehran, Iran) was applied at same frequency and time of day. All the wounds were bandaged with a nonadhesive dressing, which was held in place with an elastic wrap. No antibiotic was used as a pre- or post-operative prophylaxis. To manage the pain and discomfort Tramadol (KRKA, d. d., Novo mesto, Slovenia) (0.2 mg kg^{-1}, IM) was administered every 3 h after surgery for 24 h and continued as needed.

Assessment: The clinical evaluation was including the general health conditions and the reaction to environment. The process of burn wound healing was carefully assessed as well as the granulation tissue formation and the progression of scar formation. The time taken for the healing of the wounds was compared in all groups. Clinical and microbiological (quantitative and qualitatively) examinations of the burn wounds were carried out on 1, 7, 14 and 21days of the experiment. Wound specimens of both sides were also taken on day 21 under general anesthesia and fixed in 10% neutral buffered formalin for subsequent histopathological studies. The results of total bacterial count and time for healing were analyzed with an one way Analysis of Variances (ANOVA) test. A P value of less than 0.05 was considered statistically significant. (SigmaStat for Windows, version 2.03, Jandel Corporation, San Rafel, CA).

RESULTS

Wound healing was uneventful during the experiment period. The animals revealed a normal reaction to the environment and showed no symptoms of suffering due to burn wounds at day after operation. The aim of bacteriological examination was to determine the existence of wound surface contamination. On the first day of the experiment, the isolates from control group were *Candida albicans, Streptococcus pyogenes, Staphylococcus aureus* and *Escherichia coli*. On days 3 and 7, same species were isolated. However, *Streptococcus pyogenes* and *Candida albicans did not grow* on day 14. On day 21, *Staph. aureus* was only isolate.

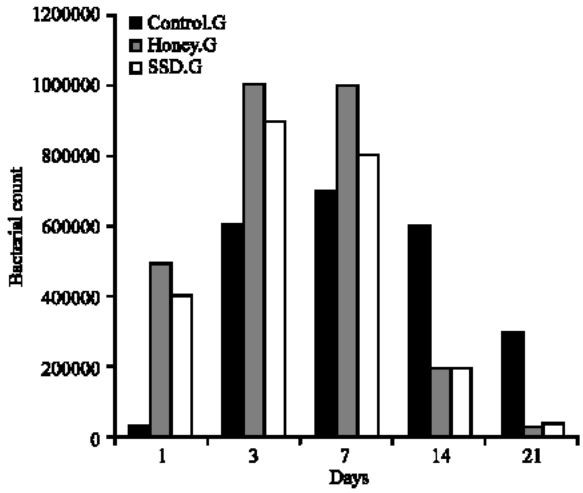

Fig. 1: Total number of microorganisms isolated from burn wounds

In SSD-treated group, on the first day, the growth of *Escherichia coli* and *Streptococcus pyogenes* was observed. These species were also isolated on days 3, 7 and 14. *Escherichia coli* were only isolated on the day 21. In the first 24 h, in honey-treated group, the growth of *Staphylococcus aureus* and *Escherichia coli* were observed. On days 3, 7 and 14, the same strains were isolated, however, on the day 21 a lack of growth of above-mentioned organisms was observed but Staphylococcus *aureus*. Results of quantitative microbiological examinations are shown in Fig. 1. During microbiological examination of the skin before the use of the preparations, *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Candida albicans* strains were isolated and the numbers of all of the strains on the skin were 8.5×10^5 cm^{-2} in the SSD group, 9×10^5 cm^{-2}in the honey group and 2×10^4 cm^{-2} in control group. No difference in cosmetic appearance and clinical signs were noted between all groups on the first and second days. The central of wounds showed necrotic changes and scald. Furthermore, inflammatory reactions were noticed in the surrounding skin. On day 7, in the control group, the skin was cracked, reddened and swollen around of the wounds. The surfaces of some wounds were dried and coated with crusts. But at same time, appearance of wounds of honey and SSD groups were similar. Their surfaces were covered with crust accompanied with the signs of epithelialization and scar at the wound edges and under the crust. In control group,on the day 14, the crusts were still adhered to the central of the wounds while distancing from the edges. At same time, in others groups, the surfaces of wounds

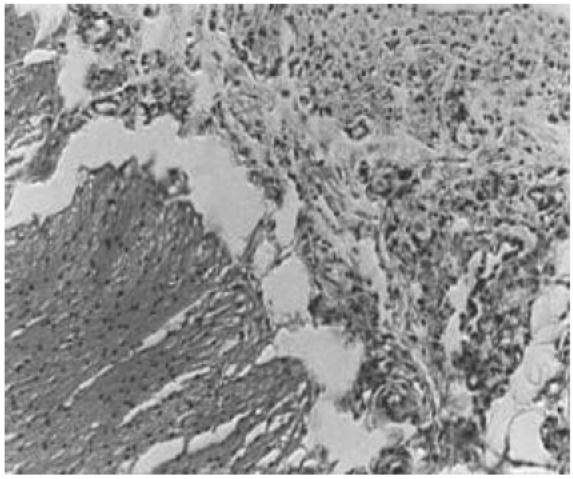

Fig. 2: Photomicrograph of the wound section in control wounds shows loose granulation with leukocytic infiltration (H and E, ×100)

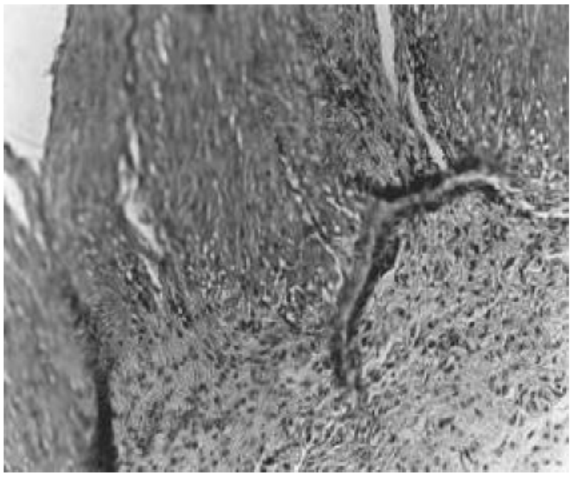

Fig. 3: Photomicrograph of histology of burn wound treated with silver sulfadiazine showing advanced proliferation of granulation tissue and lack of reactive changes. (H and E, ×100)

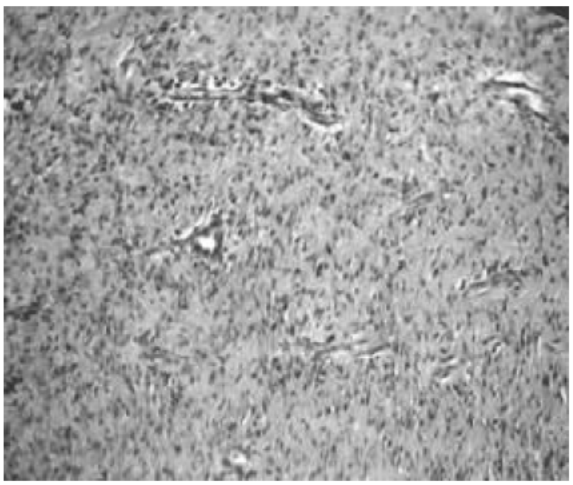

Fig. 4: Photomicrograph of histology of burn wound dressed with honey showing more advanced maturation of granulation tissue with respect to collagen formation (H and E, ×100)

were considerably decreased and the healing appearances were identifiable. However, in honey group the wounds surfaces were covered with thicker scar though it was approximately less than those of SSD wounds. On day 21, wounds of SSD and honey groups almost healed and the wound gap completely closed. But in control group, the surface of wounds was not diminished.

In the control group, there was still presence of inflammatory cells (Fig. 2) where as in the experimental (honey and SSD treated) groups there was a compact granulation with least amount of inflammatory cells (Fig. 3 and 4). Not much of differences seen compare to 21th days in both the experimental groups histologically.

DISCUSSION

The aim of this study was to evaluate the efficacy of honey on the healing of burn wounds compared to that of silver sulfadiazine. Whether the antimicrobial properties of honey were well-matched with silver sulfadiazine and if honey was able to accelerate wound healing in infectious environment burn's wound was not known.

There are some reports in well respected medical literatures suggesting the curative role of honey in healing of wounds such as burn wounds. Some investigators have found that honey possess some antibacterial activities which current antibiotics lack (Subrahmanyan, 1991; Somal *et al*., 1994; Efem, 1993). In 1998, Efem tried to use honey in the treatment of wounds and ulcerations in 59 patients that conventional therapies had proved to be ineffective. When honey was applied, already infected wounds became sterile after about one week (Efem, 1998). In another study the possible therapeutic effect of topical crude undiluted honey in the treatment of severe acute postoperative wound infection was studied. After using honey 84.4% patients showed complete wound healing (10.73±2.5 days) without wound disruption or need for re-suturing and only 4 patients showed mild dehiscence. Nonetheless in control group 50% patients showed complete wound healing (22.04±7.33 days) and 12 patients showed wound dehiscence, six of them needed re-suturing under general anesthesia (Waili and Saloom, 1999). A total of seven randomized trials were performed on the application of honey in treatment of burn wounds (Subrahmanyam, 1991; 1994; 1996; 1999; Adesunkanmi and Oyelami, 1994). Two of the studies were involved in superficial burns, three partial thickness burns (Subrahmanyam, 1991; 1994; 1996), one moderate to severe burns that included full thickness injury (Subrahmanyam, 1999) and one infected postoperative wounds (Subrahmanyam, 1991).

All the controls were active comparisons, though these included potato peelings (Subrahmanyam, 1996) and amniotic membrane (Subrahmanyam, 1994) as well as conventional treatments. The effects of honey in comparison to controls on healing time and infection rate, though antibiotic use and hospital stay have better outcomes in all these studies. In one experimental study comparisons were made between honey and silver sulfadiazine and between honey and sugar, on standard deep dermal burns, 7×7cm^2, made on Yorkshire pigs. Epithelialisation occurred within 21 days with honey and

sugar whereas it took 28-35 days with silver sulfadiazine. Granulation was clearly seen to be suppressed initially by treatment with silver sulfadiazine. In all honey-treated wounds the histological appearance of biopsy samples showed less inflammation than those treated with sugar and silver sulfadiazine and a weak or diminished actin staining in myofibroblasts suggested a more advanced stage of healing (Postmes *et al*., 1997). In our study, the wounds treated with honey and SSD were completely healed on the day 21. At the same time, in control wounds, crusts came off from the surface of wounds but the area of wounds was not diminished. According to the results of this experiment, on the day 21, the total number of isolates was lesser 3×10^4 when honey and SSD were applied compared to the control wounds (3×10^5) ($p<0.05$). In an experimental report, the common pathogenic isolates from burn wounds were *Staphylococcus aureus, Pseudomonas aeruginosa and Streptococcus pyogenes*. Various coliform bacilli, streptococci and anaerobic organisms can also cause infection (Lawrence, 1994). Similar organisms were isolated from burn wounds of our experiment, with the exception of *Pseudomonas aeruginosa*.

Microscopically, re-epitheliazation of the wound area was accelerated by the topical treatment with honey, the epithelial thickness was uniform and normal compared with the SSD wounds. The collagen accumulation in both sets of wounds was equal, but in the honey treated wounds was more orderly arranged. Furthermore, the cellularity and inflammatory reaction of the scar were no-significantly greater in the SSD treated wounds (Fig. 3 and 4).

Antimicrobial activity of honey is thought to be due to physicochemical properties (high content of reducing sugars, high viscosity, high osmotic pressure, low pH, low water activity (AW), low protein content) and hydrogen peroxide (Snowden and Cliver, 1996; Molan and Cooper, 2000). Also Radwan attributed the antibacterial activity to the specific chemicals in honey. The nature of these chemicals and the mechanisms of their action are not fully understood even though Thin Layer Chromatography (TLC), Polyacrylamide Gel Electrophoresis (PAGE) or High Performance Liquid Chromatography (HPLC) have shown that honey contains fatty acids, lipids, amylases and ascorbic acids (Radwan *et al*., 1984). Taking into account that the antimicrobial property of honey is thought to be due to non-specific mechanisms (physicochemical properties). Thus, this feature puts honey under the category of disinfectants rather than antibiotics. Hence, it is expected that honey possess broad-spectrum antimicrobial potency with a low microbial resistant. Researches relating honey show that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria (Ceyhan and Ugur, 2001; Al-Jabri *et al*., 2003). This study indicates that there were not major differences between two experimental groups (honey and SSD treated groups) as regards the population of microorganisms, dimension of scar tissue and shortening of healing duration. Furthermore, it indicates that honey has beneficial antibacterial properties which in process of healing are comparable to that of SSD.

CONCLUSION

In conclusion, honey could be considered as a low cost, easily available and potent topical agent in preventing wound infection. However, it seems that more research needs to be done on pharmacological activity of honey in order to decipher its antimicrobial and wound healing attributes.

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