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Topical Application of Natural Urmia Honey on Experimental Burn Wounds in the Dog: Clinical and Microbiological Studies

[1]Farnood Shokouhi Sabet Jalali, [2]Hossien Tajik,
[1]Siamak Saifzadeh and [3]Bakhtiar Fartash
[1]Department of Clinical Sciences
[2]Department of Food Hygiene
[3]Graduated of Veterinary Medicine, College of Veterinary Medicine,
Urmia University, P.O. Box 57153-1177, Urmia, Iran

Abstract: This study was designed to evaluate the effects of topical application of natural Urmia honey on burn wound healing. The present study is a prospective descriptive trial. Ten adult mongrel dogs with mean weight of 20±4.25 kg were used. Burn wounds were created in dorso-lateral region of each dog, according to Hoekstra model. In treatment group (n = 5), wounds were covered with a thin layer (10 mL) of natural honey. In control group (n = 5); wounds were only washed with the same amount of normal saline. Wound healing was assessed clinically and microbiologically on 1, 7, 14 and 21 days of the experiment. Wounds treated with honey had more improved healing appearance in contrast to the control wounds. Results showed that the wounds treated with honey were approximately complete closed (98.46%) on the 21 day, compared with the control group (86.11%) ($p<0.05$). Also on the 21st day the total number of isolated microorganisms in treatment group was lesser ($3\pm0.5\times10^4$) than control wounds ($3\pm0.5\times10^5$) ($p<0.05$). According to the results of this study, topical application of natural honey has beneficial effect on experimental burn wound from clinical and microbiological points of view. Honey can be considered as a low cost, easily available and potent topical agent in treatment of burn wounds.

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

INTRODUCTION

Annually many thousands of people suffer from different kinds of burn wounds, throughout of the world. People on modern life and technological progress are in more exposed danger of burning (Subrahmanyam, 1998; Subrahmanyam *et al*., 2001). Researches show that the most frequent reason (over 50%) of death resulting from burn is infection (Artz and Reiss, 1975). Burns become infected because the wound area is an ideal medium for the multiplication of the infecting organism. There is a plentiful supply of moisture, nutrients and ideal physical environment (temperature, gaseous requirements, etc.); for growth of microorganisms.

Burn wounds are often protected against infection with temporary covering with xenografts or preserved homografts. These are not available and too costly for use in developing countries. In these countries, most burn units promote early debridement of the wound to encourage healing and covering the burn wound with a routine dressing (Herndon *et al*., 1989). During the past years, the prevalence of certain pathogens causing wound infection has decreased as a result of development in wound management (Monafo and West, 1990; Lawrence, 1994).

Corresponding Author: Department of Clinical Sciences, College of Veterinary Medicine, Urmia University, P.O. Box 57153-1177, Urmia, Iran

In spite of it, there are still a small number of burn units routinely using antibiotics as a prophylactic for prevention of wound infection because of concerns regarding the high incidence of antibiotic resistance, high cost and the risk of adverse drug effects (Edwards-Jones *et al*., 2000).

Increasingly, bacteria are becoming multi-antibiotic resistant, for example methicillin resistant *Staphylococcus aureus* (MRSA) is now the major cause of infections in burns wounds in Europe (Voss *et al*., 1994). Although new antibiotics are still being produced, we need some new strategies for treatment of infections of burn wounds.

The current progress made in the analytical studies and in the field of biotechnology has led to a renewed interest in pharmacotherapy based on biogenic components. More attention has been given to apipharmacotherapy which utilizes the antibacterial properties of active fractions of bee products. Honey has been used as a wound treatment agent for more than 2000 years (Mathews and Binning, 2002; Pierper, 2002). Honey inhibits the growth of gram-positive and gram-negative organisms (Alcaraz and Kelly, 2002; Cooper *et al*., 2002; White *et al*., 1963) and promotes epithelialization and helps in healing of the burn wounds (Cooper, 2001; Fox, 2002; Lusby *et al*., 2002; Misirlioglu *et al*., 2003; Postmes *et al*., 1993; Subrahmanyam, 1993).

Antimicrobial activity of honey is thought to be the result of its physicochemical properties and phytochemical agents. The exact composition of natural honey depends on the region, mainly the botanicals, from where they are derived (Felsner, 2001).

Because of a continuing need to a topical agent for wound treatment which is cheaper, easily available and effective in preventing infection, decreasing fluid loss and enhancing re-epithelialization, we designed this experimental study in order to assess the clinical and antimicrobial effects of topically applied natural Urmia honey on burn wounds.

MATERIALS AND METHODS

Honey samples used in this study were harvested during spring 2005, from Targavar Bee Keeping Corporations, Urmia. Each 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.

Ten apparently healthy adult mongrel dogs of both sexes, 4.5±0.5 years old and with mean weight of 20±4.25 kg, were used in this study. Dogs were considered to be healthy on the basis of physical and laboratory examination including CBC and serum biochemistry results. The animals were kept in individual cages and had access to water and food *ad libitum*. The experimental protocol was approved by the Veterinary Clinical Sciences Committee at Urmia University. The animals were randomly assigned to two experimental (n = 5) or control (n = 5) groups. Dogs were premedicated with atropine (Darou pakhsh, Tehran, Iran) (0.04 mg kg^{-1}, intramuscularly (IM)) and acepromazine (Hoogsrraten, Belgium) (0.1 mg kg^{-1}, IM). Anesthesia was induced with sodium thiopental (Hoogsrraten, Belgium) (10 mg kg^{-1}, 2.5% intravenously) and maintained with halothane after

Table 1: Average composition of Targevar honey

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

intubation. Dogs were positioned in ventral recumbency and the dorso-lateral back area was shaved just behind the shoulders, followed by skin preparation for aseptic surgery.

The rectangular-shapedwounds (2×3 cm^2) were created with hot (180°C) brass brick (College of Engineering, Urmia University, Urmia, Iran) weighting 500 g and applied for 10 sec. The model of the burn wound was prepared according to Hoekstra standard (Brans *et al*., 1994).

In the experimental group, 10 mL pure, unprocessed and undiluted honey was applied once a day to the surface of the burn. In the control group, the wounds were treated with normal saline, applied once a day. All the wounds were bandaged with a nonadhesive dressing, held in place with an elastic wrap.

The wounds were observed for evidence of infection, excessive exudates or leakage until the wound healed. Time elapsed for wound healing was considered in both groups. Clinical and microbiological (quantitative (total plate count) and qualitative (using specialized microbial medias) examinations of the burn wounds were done on 0, 7, 14 and 21 days of the experiment.

Neither prophylactic nor postoperative antibiotic therapy was done. Tramadol (KRKA, d. d., Novo mesto, Slovenia) (0.2 mg kg^{-1}, IM) was administered every 3 h after surgery for 24 h and as needed thereafter to control pain and discomfort.

The wounds were photographed on days 0, 7, 14 and 21. All the photographs were scanned and wound areas were measured using digital scanning software (Sigma Scans Pro 5.0, SPSS Science, Chicago, IL).

Variable analyzed included the rates of wound contraction (percent decrease of wound area) (wound area on day 0 minus wound area on day n, divided by the wound area on day 0 expressed as a percentage).

The results of total bacterial count and time taken for healing were analyzed with an unpaired Student's t-test. Differences were considered significant if $p<0.05$ (SigmaStat for Windows, version 2.03, Jandel Corporation, San Rafel, CA).

RESULTS

During the days after the experiment the animals showed a normal reaction to the environment and revealed no symptoms of suffering due to burn wounds. Wound healing was uneventful during the experimental period. The rates of wound contraction (percent decrease of wound area) in experiment and control wounds are shown in Table 2.

In both groups clinical signs were similar in first and second days and no difference in appearance of wound was noted between treatment and control wounds. Surrounding skin showed inflammation and large quantity of exudation. Necrotic changes and scald were noted in central part of the wounds.

On day seven, crust covered the surfaces of wounds in honey group. Under the crust, scar could be seen and re-epithelialization was developed at wound edges. At the same time, the surrounding skin of the control wounds was cracked, reddened and swollen. Some wounds were dried and coated with crusts. On day 14, wounds of treatment group were covered with scar and considerably reduced in size. The appearances of wounds were recognized as healed. In other group, in spite of crusts came off from edges of wound but they were still adhered to the surface of wounds. The surfaces of wounds

Table 2: Comparison of wound area (mm^2) (mean±SD) and percent decrease in the experiment and control wounds (n = 5 dogs)

Day	0	7	14	21
Group experiment	688.6±42.22	364.4±16.26* (47.08%)	62.24±24.44* (90.97%)	10.64±8.64* (98.46%)
Control	644.8±64.86	486.6±44.82 (24.54%)	206.86±48.26 (67.92%)	89.58±12.84 (86.11%)

*Significant difference ($p<0.05$)

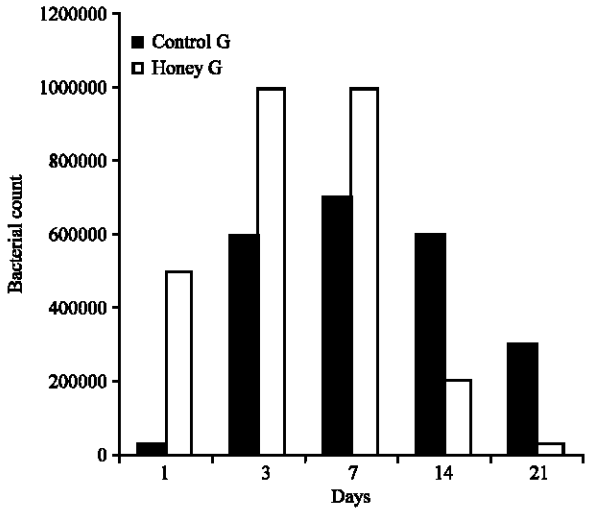

Fig. 1: Results of quantitative microbiological examinations

were not decreased. On day 21, the wounds gaps of treatment group were approximately closed but in control group, crusts came off from the surface of the wounds. The area of wounds was not diminished.

During the microbiological examination of the skin before commencement of the study, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Candida albicans* strains were isolated and the number of all the strains on the skin was $9\pm0.5\times10^5$ cm^{-2} in the honey group and $2\pm0.6\times10^4$cm^{-2} in control group.

In honey-treated group, the growth of *Staphylococcus aureus* and *Escherichia coli* were observed in the first day. Also on days 3, 7 and 14 of the experiment these microorganisms were isolated, but on day 21 just *S. aureus* was isolated.

In the control group, after the first 24 h of the experiment, *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes* and *Candida albicans* were isolated. These species were also isolated on days 3 and 7 of the experiment. On the 14th day the growth of *Streptococcus pyogenes* and *Candida albicans* was not observed. On day 21, only *Staph. aureus* was isolated.

At the same time quantitative examinations of bacterial flora were conducted in the burn wounds. In the control group, the number of microbes existing on 1 cm^2 of the wound in the first day, were $3\pm0.8\times0^4$cm^{-2} and gradually increased during the following days to the value of $7\pm0.6\times10^5$ on day 7. In the 14th day the number of microbes imperceptibly to decrease until day 21, when reached to $3\pm0.5\times10^5$cm^{-2}.

In the honey group, the number of microbes were $5\pm0.6\times10^5$ cm^{-2} in the first 24 h of the experiment. On the 7th day of the experiment this number was unchanged; however, on day 14 it decreased significantly to the value of $2\pm0.5\times10^5$ cm^{-2}. This value decreased significantly on the 21st day and reached to the $3\pm0.5\times10^4$ cm^{-2} Fig. 1.

DISCUSSION

The purpose of this study was to highlight the beneficial effects of honey in treatment of the burn wounds. Some workers found that honey possesses antibacterial activity where current antibacterial substances were ineffective (Subrahmanyam, 1991; Efem, 1993; Al-Somal *et al*., 1994). Therapeutic efficiency of apitherapeutics, which pharmacological activity results from physicochemical properties

of honey or propolis, have been confirmed in clinical research by Molan (2001). In 1998 Efem tried to use honey in the treatment of wounds and ulcerations in 59 patients where conventional methods of therapy did not bring awaited effects. When honey was applied, wounds, formerly infected, became sterile after about a week (Efem, 1998). In another study the possible therapeutic effect of topical crude undiluted honey in the treatment of severe acute postoperative wound infection (50 patients having caesarean sections or total abdominal hysterectomies) was studied. Complete wound healing was evident after 10.73±2.5 days in Honey treated group and after 22.04±7.33 in control group ($p<0.05$) (Al-Waili and Saloom, 1999).

The results in Table 2 showed that the decrease in wound area was significantly greater from day 7 on in the experiment wounds compared to the control ($p<0.05$). Furthermore, although contraction was steadily moved on in both groups, the rate of contraction (percent decrease of wound area) was obviously greater in the experiment wounds.

In this study, the wounds treated with honey were approximately complete closed (98.46%) on the 21day, compared with the control group (86.11%).

There were seven randomized trials on application of honey in treatment of burn wound. From these studies six were performed in India by the same researcher and one in United Arab Emirates (Adesunkanmi and Oyelami, 1994, Subrahmanyam, 1991, 1994, 1996 and 1999). Two of the studies involved superficial burns (Subrahmanyam, 1991), three partial thickness burns (Subrahmanyam, 1991, 1994 and 1996) one moderate to severe burns that included full thickness injury (Subrahmanyam, 1999) and one infected postoperative wounds (Subrahmanyam, 1991). The results of these studies revealed the promising effect of honey on wound healing and diminishing the infection rate (Moore *et al*., 2001).

According to the results of our experiment, on the 21st day the total number of isolated microorganisms in treatment group was less ($3\pm0.5\times10^4$) than control wounds ($3\pm0.5\times10^5$) ($p<0.05$).

In an experimental report, the common pathogens isolated from burn wounds were *Staphylococcus aureus, Pseudomonas aeruginosa*, *Streptococcus pyogenes*, various coliform bacilli, streptococci and anaerobic organisms (Lawrence, 1994). Similar organisms were isolated from burn wounds of our experiment, with exception of *Pseudomonas aeruginosa*.

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 (a_w), low protein content) and hydrogen peroxide (Bergman *et al*., 1983; Hyslop *et al*., 1995; Molan, 2000; Oka *et al*., 1987; Radwan *et al*., 1984; Snowden and Cliver, 1996).

These properties are thought to be due to non-specific mechanisms (physicochemical and peroxidial properties), which is more similar to the disinfectants than the antibiotics. Thus it can be assumed that honey should possess broad-spectrum antimicrobial potency and may provoke slight microbial resistance.

Researches working on honey, show that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria (Al-Jabri *et al*., 2003; Ceyhan and Ugur, 2001; Haffejee and Moosa, 1985; Obi *et al*., 1994). This experimental study shows that use of topical honey in treatment of burn not only accelerates the healing procedure but has valuable antimicrobial effect in promoting the recovery of this kind of wounds. Honey can be considered as a low cost, easily available and potent topical agent in preventing infection wounds.

It is supposed that further researches on pharmacological activity of honey will reveal their antimicrobial mechanisms and its encouragement of wound healing.

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