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Research Paper

Evaluation of Wound Healing Properties of Natural Honey

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ABSTRACT

Modern medicine has embraced the utilization of natural honey in the management of wounds and burns, showcasing its resurgence in this field. Recent studies have underscored its antioxidant, anti-inflammatory, and antimicrobial properties. In our investigation, we specifically examined the antibacterial effects of acacia honey against 13 bacterial strains isolated from honeybee guts. To assess its wound healing capabilities, we employed mouse injury models. These models involved dividing mice into three groups: one treated with natural honey, another with Neomycin, and a control group receiving no treatment. Wounds were induced on the hind limb skeletal muscles of mice under anesthesia, and daily topical treatments were administered until complete wound healing was achieved. The progression of wound healing was monitored daily, and images were captured for evaluation. Our findings indicate that acacia honey significantly promotes wound healing with its potent antibacterial properties. Honey-treated mice exhibited faster wound closure and re-epithelialization compared to the other groups, experiencing less stress and no mortality. This can be attributed to honey's soothing effect and its effective management of wounds. Our results suggest that honey not only clears infections but also accelerates wound healing and prevents the progression of necrotizing fasciitis more effectively than conventional treatments. This underscores the significance of honey in wound management, affirming its role as a safe and effective natural remedy for healing wounds.

KEYWORDS: Antibacterial Activity, Healing process, Natural Honey, Wounds

INTRODUCTION

Despite recent advances in health care, the rate of morbidity is still increasing due to inadequate wound management and the expansion of secondary infections. Many interventions, including new medications and technologies, are being used to help achieve significant wound healing and eliminate infections. There is a dire need to develop such an intervention that has both therapeutic effects on the healing process and exerts antimicrobial effect with no / less. Honey has been used in traditional medicine since ancient times and has more recently been rediscovered by medical researchers for its clinical & therapeutic uses. There are numerous reports on the antibacterial activities of honey against a wide range of microorganisms and on the acceleration of the wound healing process [1,2]. Honey has antimicrobial activity against both pathogenic and nonpathogenic microorganisms [3].

Honey is extracted from the nectar of plants and then modified by the honeybee. It is a carbohydrate-rich syrup derived from floral and other plant nectars and secretions. Honey may also provide nutrients and other chemicals that speed wound healing. The phytochemical components of honey; flavonoids and phenolic acids play a major role as antioxidants due to their free radical scavenging activities which guard cells from the damage due to free radicals and eventually decrease the inflammatory response [4].

Several reports showed that honey-based gel-treated wounds exhibit abundant mature and

compact collagen compared with other treatments.

Wound healing is a complex dynamic process that involves many cascades of events like hemostasis, inflammation, proliferation, and remodeling of tissues to fill the damaged area and reestablish the skin barrier [5]. Wound healing properties of honey include stimulation of tissue growth, enhanced epithelialization, and minimized scar formation. These effects are ascribed to honey's acidity, hydrogen peroxide content, osmotic effect, nutritional and antioxidant contents, stimulation of immunity, and unidentified compounds. Prostaglandins and nitric oxide play a major role in inflammation, microbial killing, and the healing process. Honey was found to lower prostaglandin levels and elevate nitric oxide end products. These properties help to explain some biological and therapeutic properties of honey, particularly as an antibacterial agent or wound healer [6].

Honey has anti-inflammatory properties as it debrides the wound, inhibits scarring and it also encourages wound healing by stimulating tissue regeneration in addition to reducing the need for skin grafting. The enzyme glucose oxidase of honey provides glucose to leucocytes, which is essential for respiratory bursts to produce hydrogen peroxide leading to the antibacterial activity of macrophages. The acidity of honey further aids in antibacterial activity [7]. Presences of a wide range of amino acids, vitamins, and trace elements also have a direct nutrient effect on regenerating tissues.

Wound management remains an important focus of research. Recently, the interest in using alternative therapies and natural remedies in wound management has rapidly increased. Alternative methods have great potential to improve wound healing for the global population as they reduce the financial burden of modern treatments. One of the natural products of interest in this regard is honey which has attracted the attention of many researchers. The need for effective natural products, such as honey, is essential to overcome the problems of using chemotherapeutics. This study would help to draw attention towards the usage of more economical and natural remedial solutions for wound healing.

These properties might help to explain some biological and therapeutic properties of honey, particularly as an antibacterial agent or wound healer. So the present study is conducted to evaluate the effectiveness of honey hydrogel in wound healing and thus could encourage the use of honey in clinical practice as a natural and safe wound healer.

MATERIALS AND METHODS

Sample Collection

A sample of Acacia honey was collected from a reliable source. A total of 43 bacterial gut isolates of honeybee *Apis mellifera*, were selected for this study. These bacterial isolates were obtained from a previous study [8]. To ascertain the role of honey as an enhancer of wound healing mice injury models were used.

Antibacterial Activity of Natural Honey

The antibacterial activity of natural honey (25 % v/v diluted with distilled water) was examined against 45 bacterial isolates of *Apis mellifera* gut (honeybee gut bacteria) by well diffusion method. For this purpose, Nutrient Agar and broth that contains basic Nutrients for the growth of Nutrient Agar are taken. Both media were autoclaved before use for 45 minutes to 1 hour. As in the present study, the broth was required for the revival and subculturing of the isolates. Initially amongst specific Isolates selected for the study, Target isolated were inoculated in lactose broth for revival and incubated for at least 16hrs at 37°C and the next day these revived bacteria were infused into a nutrient agar plate and left incubated at 37°C for 24hrs. These were then subcultured using lactose broth inoculation for a further 16 hours and finally,

these subcultured isolates were used for the antibacterial activity of acacia honey. Afterward, these bacterial growths were finally steeped over a nutrient agar for evaluation of its inhibitory activity via observing the zone of inhibition.

Anti-bacterial activity of natural honey was examined on a nutrient agar plate. 20-30 µL bacterial cultures were taken and uniformly spread over a nutrient agar with the help of a glass spreader. Borer was used for making a well where 100 µL of 25% acacia honey, freshly diluted with distilled water was poured into that well and evaluated after 24-48hours at 37℃ for its antibacterial activity via observing its inhibitory action as a zone of inhibition against specific bacterial isolates.

Animal/Mice Injury Model

Mice injury models were used to evaluate the role of honey as the enhancer of wound healing and the results were compared with the controlled and conventional treatment.

In this case, the study subjects were mice, they were divided into 3 groups that differed in the application of the regimen. The first group termed C or the Control group comprised Control subjects that were without any treatment. The second group termed the S Group or standard or Conventional treatment subjects, were on Neomycin therapy. The third Group_termed E i.e. Experimental group comprising honey-treated subjects that received natural honey as a treatment regimen.

To assess honey efficacy, these mice were divided into 3 groups as mentioned above, treated similarly under the uniform condition all groups of mice were separately kept, in clean cages under usual experimental conditions of temperature. The injury was induced at hind limb skeletal muscles of the same size in all groups i.e. 4 x 1.5 mm^2 sizes (4 mm in length and 1.5 mm in depth) to rule out any bias, with sterile surgical blade under anesthesia condition (1% lidocaine). Daily treatments were given topically in a rotational manner using a sterile cotton swab until complete wound healing was obtained. The continuous sequential stages of wound healing of each group mentioned were assessed simultaneously daily and images or snaps were taken for results and comparative evaluation.

Assessment Protocol

The comparison between the area at day 0 and at the time-set days was used to calculate the ratio of the wound reduction using the following:

$$Wound\ area\ reduction\ rate = \left(\frac{At}{A0}\right) * 100$$

Where A0 and At are the initial area and the wound area at time t, respectively.

Statistical Analysis

Data was expressed as mean ± SD using SPSS version 24. Statistical differences were measured using one-way ANOVA (analysis of variance), for parametric values. For all statistical tests, results were significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

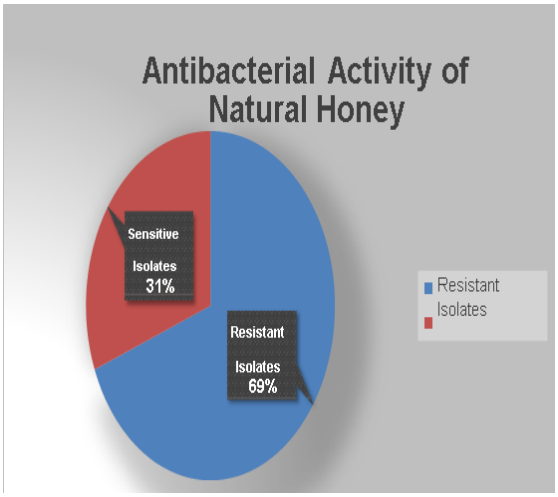
Antibacterial Activity of Honey

Antibacterial properties of natural honey and its effects on wound healing have been recently investigated [24-27]. There are numerous reports on the antibacterial activities of honey against a wide range of microorganisms [9,10]. Some recent Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antibacterial agent and could play a role in wound healing [11, 12]. There are numerous reports on the antibacterial activities of honey against a wide range of microorganisms [13]. The antibacterial activity of honey can differ to some extent considerably and different microorganisms have different susceptibilities to different types and concentrations of honey.

During the present study, the antibacterial activity of 25 % (v/v) honey was screened out by well diffusion method against 43 bee guts isolates. 13 isolates were found sensitive to 25 % (v/v) natural acacia honey in this study. Various investigations have revealed that most of the bacteria in the gut are anaerobic [14]. They can survive in an acidic environment and can ferment sugar and make acetic and lactic acids, making them valuable to humans and other animals [15]. They serve as a defensive system against other intestinal pathogens while also allowing the colonization of healthy microbial families. These bacteria can also reduce nitrate and are involved in nitrogen metabolism [16]. The present study utilized 25 % diluted honey without considering light and heat effects on the efficacy of honey as the former study reported the role of light and heat that can affect the antibacterial activity of honey [17].

Moreover, the enzyme glucose oxidase is a heat and light-sensitive enzyme, secreted from the hypopharyngeal gland of the honeybee and is found to be in an inactive state in undiluted honey. This enzyme converts water and sugar into hydrogen peroxide when honey is diluted [18]. In the present study, the dilution factor might be one of the reasons for this reduction in efficacy to some extent besides other factors. A recent study showed that undiluted honey therefore inhibits the growth of bacteria due to its sugar content, which exerts osmotic pressure on bacterial cells [19]. One another study reported that most of the isolates in the gut of honeybees belong to the genus Bacillus [15]. The antibacterial activity of natural honey against different strains of *Bacillus subtilis* is well-reported. Further susceptibility is confirmed by previous studies which also reported that Bacillus licheniformis is susceptible to natural honey due to hydrogen peroxide, non-peroxide, pH, osmotic pressure as well as proteins [20].

Figure 1: Antibacterial activity of different bacterial isolates against natural honey tested during this study.



In our study, 13 (31%) bacterial isolates showed susceptibility to acacia honey observed as a zone of inhibition on Nutrient agar while most of the isolates showed a resistant pattern (Figure 1). These factors as mentioned above, reported by different studies could have contributed such as the light, heat, dilution factor, and anaerobic nature of the bee guts bacterial isolates that have the potential to survive in an acidic environment. Despite all these factors, the results of our study reveal acacia honey as a useful antibacterial agent. As it has shown more than thirty percent antibacterial activity, these outcomes are satisfactory and adequate considering the above aspects.

In the present study wounds, the healing properties of acacia honey have been characterized and its role as wound healer was evaluated and compared using a mice injury model with control and conventional neomycin therapy. The results show the mean value of days taken for wound healing was five with honey as compared with control i.e. eight days and Neomycin i.e. 6.3 days as depicted in Table 1. Furthermore,

mortality during follow-up in control and standard groups was observed in the form of death of mice, which was not in the case of honey. Similar results without harmful effects are also reported in a previous study [21].

Honey is an effective broad-spectrum antibacterial agent that provides quick autolytic debridement and stimulates wound tissue growth to speed healing and initiate the healing process in dormant wounds. Its anti-inflammatory properties reduce pain and exudate and prevent scarring [21].

The present study established honey as a better regimen considering wound closure, re-epithelialization, and collagen deposition, as wounds in honey-treated mice groups significantly healed faster than wounds in the other two groups, with less stress and no mortality because of the soothing effect and better wound management in the case of honey. The Statistical evaluation as depicted in Table 2 shows more significant outcomes and enhancement of wound healing in honey-treated mice as compared to the control (P=0.000) and the standard group (P=0.017). Comparative evaluation of both honey and the conventional treatment with controls separately also indicates honey with a more significant enhancer of the wound healing process (Figure 2).

The present study showed similar and compatible results with a recently conducted study where honey showed remarkable improvement in wound closure [9]. Topical application of honey in diabetic mice model showed significant wound closure, re-epithelialization, and collagen deposition and honey promotes sequential stages of wound healing in diabetic mice model. In our study, honey was found to be a fast healer that enhances the healing process better than the conventional treatment. Another study showed the beneficial effect of honey on the healing of infected wounds that were even unresponsive to conventional antibiotic treatment [22].

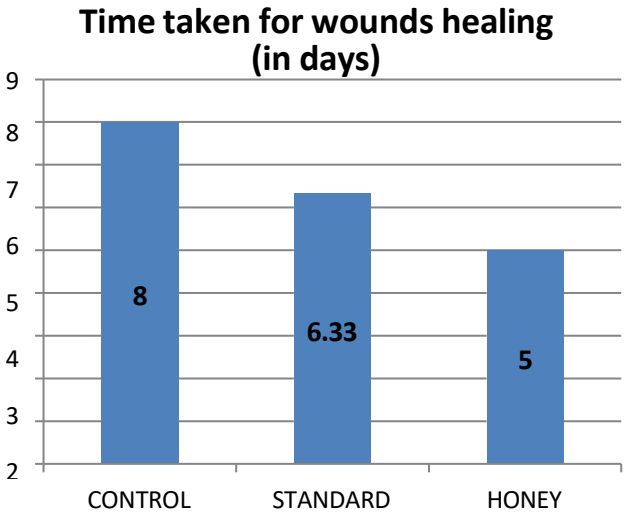


Figure 2: Wound Healing Time Taken by Different Regimens.

In one case of knee amputation in a young boy, who was heavily infected with *Pseudomonas species* and *Staphylococcus aureus* and non-responsive to conventional treatment, the application of sterilized active Manuka honey dressing led to complete healing in ten weeks. Similar results were reported with burns. Honey dressing speeds up the healing process, sterilizes wounds, and reduces pain. Studies on Fournier's gangrene showed rapid improvement with decreased edema and discharge, rapid regeneration and little or no scarring, effective wound debridement, and a decrease in mortality [23].

The honey-induced activation of both types of immune cells could promote the debridement of a wound and speed up the repair process. Similarly, human keratinocytes, fibroblasts, and endothelial cell responses (e.g., cell migration and proliferation, collagen matrix production, chemotaxis) are affected positively in the presence of honey; thus, honey may accelerate reepithelization and wound closure. The immunomodulatory activity of honey is highly complex because of the involvement of multiple quantitatively variable compounds. The identification of these individual compounds and their contributions to wound healing is crucial for

a better understanding of the mechanisms behind honey-mediated healing of chronic wounds.

CONCLUSION

The present study reveals natural acacia as a better regimen in the wound healing process and a useful antibacterial agent. In this study, honey appeared to be an enhancer for the wound healing process considering its closure and re-epithelization, as wounds in honey-treated mice groups healed faster than wounds in the other two groups, with less stress and no mortality, probably because of the soothing effect and better wounds management in case of honey.

These outcomes showed honey to be capable of clearing infection, healing wounds, and thwarting the progression of necrotizing fasciitis comparatively better than the conventional treatments indicating its significance for wound management thus the study concluded the effectiveness of honey as an effective natural remedy and a safe wounds healer.

Table 1: Statistical Representation of different regimens and comparative evaluation of their effectiveness in wound healing process.

	Control Days taken for healing	**Standard.** Days taken for healing	**Honey** Days taken for healing
N (Mice)	3	3	3
Mean	8.0000	6.3333	5.0000
Median	8.0000	6.0000	5.0000
Minimum	7.00	6.00	4.00
Maximum	9.00	7.00	6.00

Table 2: Paired t-test showing honey as a better regimen when compared to other regimens.

Pair t-test for comparison		**Mean diff**	**t value**	**Sig. (2-tailed)**
Pair 1	Control vs Standard	1.66667	3.371	0.020
Pair 2	Standard vs Honey	-1.50000	-3.503	0.017
Pair 3	Honey vs Control	3.16667	10.304	0.000

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