ARTICLE

COMBINED EFFECT OF IRANIAN PROPOLIS AND HONEY ON HEALING OF INDUCED INCISIONAL WOUND IN RAT

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ABSTRACT

Background: Surgical site infections (SSI), are problematic for the patients. The beehive products can improve wound healing, but whether it can improve healing surgical wounds, has not been well investigated. This experiment was conducted to compare Iranian beehive products with cold cream for healing of incisional cutaneous wound in rats as a model for surgery wounds. **Materials and methods:** 50 male Wistar albino rats were randomly allocated into five equivalent groups, as control, cold cream, propolis cream, honey and a mixture of honey and propolis cream (MHP). A skin incised wound model was induced on the back of all animals. The incision area in the five groups were covered twice-daily with cold cream, propolis cream, honey and a MHP respectively. The percentage reduction of wound size was measured at the start of experiments, some specific days and at day 22. The wound tensile strength was also measured at day 30. **Results:** The results showed that, there were statistically significant differences between the percentage reduction of wound size in treated groups by beehive products compared to the cold cream group on most days of the experiment. Also, the differences between the percentage reduction of wound size in MHP group, compared to honey and propolis groups were statistically significant on days 13 and 19. The differences between the wound tensile strength of treatment groups, compared to cold cream were also significant. **Conclusion:** We concluded that, there may be a synergistic therapeutic effect between propolis cream and honey in the healing of wound in rat. These results provide a rationale for studying the topical application of this mixture in a clinical setting.

KEY WORDS
Propolis, Honey, Cutaneous, Wound, Rat

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INTRODUCTION

The surgery wounds infection with numerous organisms, with additional problems of drug resistance, determines the drug strategy program by the physician for patients. Recently, a summary of data from several hospitals in Europe, Asia, Africa and America found an overall 2.3% incidence of Surgical site infections (SSI) [1, 2]. Besides the morbidity and mortality associated with SSI, there are noteworthy cost implications. A research found that it cost over USD 3000 more to treat a patient with a SSI compare to a non-infected patient [3]. In Britain, a research over a 2-year period found the additional cost attributed to the SSI was £5,239 [4]. So, prevention of SSI has become a priority for health care facilities. The latest studies shows that the remedies of biogenic source with their analgesic, anti-micro-organic and healing properties are becoming more and more important in wound healing [5].

Honey is an ancient remedy that has regained popularity and is widely used as an antimicrobial for treating wounds, ulcers, and burns [6]. On the other hand, propolis has been used in folk medicine for centuries [7]. It has some roles such as immune enhancing [8], antitumor [9], cytostatic [10], anticarcinogenic [11], anti-inflammatory [12], oxygen radical scavenging [13] and antimicrobial activities [14]. Some researchers believe that propolis is the antibiotic of the 21st century for the reason that its synergic action with other antibiotics. In vitro synergy between propolis and antimicrobial drugs has been examined, and preparations combining propolis with antifungal agents and antibiotics are of potential medical interest [15] . Topical application of propolis to several kinds of wounds has been found to be operative in making a fresh granulating bed and controlling infection [16-18].

Skin incisional wounds seem to be an ideal model for drugs antimicrobial properties and laboratory testing of healing and clinical assessment [19, 20] in traumatic wound and surgical procedures [21]. Wound tensile strength is also one of the most significant factors in healing of wound and is a helpful assessment that shows the dermal collagen fibers organization in the recently composed collagen [22].
The goal of the present research was to study the wound healing rate, regarding wound closure time and tensile strength, treated with topically applied 60 % propolis cream, honey and MHP compared to cold cream group in rat.

EXPERIMENTAL DESIGN AND ANIMAL GROUPS

Animals

The trials were done on 50 male Wistar rats, weighing between 200 and 250 g. They were individually housed in a room with natural light cycle and constant temperature (24 ± 2Ċ), where they had free access to water and standard feed.

Instrumentation and surgical procedure

Experiments were done in the animal house of the Semnan faculty of medical sciences in IRAN in accordance with usual guidelines. The rats were anesthetized by intraperitoneal injection of pentobarbital (75 mg/kg). A linear 3cm incision was made over the skin of animal back, after shaving the back, as described by Okada et al with some modifications [23].

Experimental design

The rats were randomly separated into 5 groups, as control, cold cream, propolis cream, honey and the mixture of honey and propolis. Control group did not take any treatment. Cold cream group received topical cold cream two times a day from the start of experiments to the end of wound closure. The topical treatment was done by 60 % propolis cream, honey and the MHP at the same time. Both researchers and subjects were kept blind about all trials.

60% propolis cream preparation

Propolis and honey sample used in this study was collected by hand from colonies of honeybees located in the northwest area of Mashhad in Iran. The propolis sample was kept at 4°C and stored in a dry place. The sample was ground to a fine powder and extracted with 80% ethanol in a shaker at room temperature for 48 h. After that, the extract solution was filtered through Whatman No. 4 filter paper, and concentrated in a rotating evaporator to obtain the crude extract in paste form, then kept in a dark and dry place.

Ethanolic extracts of propolis were prepared and applied as described by ISLA et al [24] with slight changes. The crude extract was frozen at -20°C, and crushed in a chilled mortar. After that, the prepared powder was pull out with ethanol (15 ml of 80 % ethanol/g of propolis). Three days later, the supernatant of suspension was concentrated in an evaporator and the remainder was added to the same volume of 60 % cold cream (19 % distilled water, cold cream, 12.5 % spermaceti +0.5 % borate of soda + 12 % white wax + 56 % liquid paraffin + Botafarma). The MHP was prepared by adding the round powder of propolis to the same amount of honey.

Measurement and evaluation of wound healing

The size of the wound area was measured on the 1st, 2nd, 4th, 10th, 13th, 16th, 19th and 22nd days from the start of the experiment in all groups, by using a permanent marker and transparencies. The wound sizes of all animals were measured as described previously [25]. After calculating the wound area, wound healing percentages were calculated by walker formula [26]. Wound healing percentages were computed for 4th, 10th, 13th, 16th, 19th and 22nd days after the start of the experiment in all groups as follows:

Percentage of wound area = (Wound area in day X / Wound area on the first day)×100

Percentage of wound healing = 100 – Percentage of wound area

Tensile strength of the wound, which determines the force per unit of cross-sectional area required to discontinuity the wound, was determined on the 30th day by continues, constant water flow technique [27] with some modifications.

Statistical analysis

Data analysis was done blind to avoid bias. All values were presented as Mean ± SEM. The normality of data was checked by the Shapiro–Wilk test via using the SPSS software. All data had a normal distribution. Thus, the obtained data were analyzed by one-way ANOVA followed by analysis of the Tukey test post hoc. If the p-values are less than 0.05, the differences can be considered significant.

RESULTS

The combined effect of Iranian propolis and honey mixture (MHP) on the tensile strength of incisional wound measured in this study, are shown in Table 1. A statistically significant difference was seen between the mean of wound tensile strength of control and honey groups ($F_{4,45}=1.004$, $P=0.005$), cold cream and the MHP groups ($F_{4,45}=21.004$, $P=0.000$), and between honey and the MHP groups ($F_{4,45}=21.004$, $P=0.008$) on the 30th day of the experiment.

The combined effect of Iranian beehive products on wound healing percentages measured in this study are shown in Fig 1. A statistically significant difference was seen between the mean of wound healing percentages of control and cold cream groups on day 7 ($F_{4,45}=12.944$, $P=0.047$). A statistically significant difference was also shown between the mean of wound healing percentages of cold cream and propolis groups on day 7 ($F_{4,45}=12.944$, $P=0.017$). A significant difference was also seen between the mean of wound healing percentages of cold cream and the MHP groups on days 10 ($F_{4,45}=8.452$, $P=0.003$) and 19 ($F_{4,45}=8.452$, $P=0.003$). There was also statistically significant difference between the mean of wound healing percentages of control and honey groups on day 10 ($F_{4,45}=8.452$, $P=0.006$). The

mean of wound healing percentages difference between propolis and the MHP groups was statistically significant on day 13 (F4,45=6.783, P=0.001) and a significant difference was seen between the mean of wound healing percentages of honey and the MHP groups on days 7 (F4,45=12.944, P=0.003) and 13(F4,45=6.783, P=0.044).

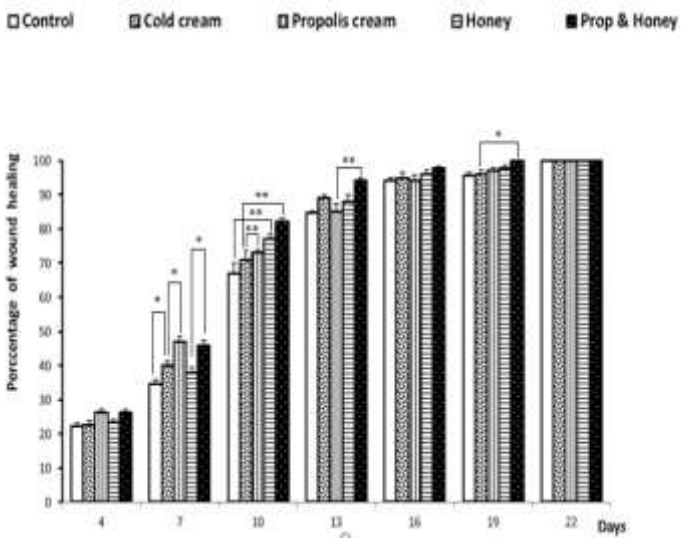

Fig. 1: Changes in wound healing percentage (Mean ±SEM) of the rats following the induction of wound at the time of wounding (day 0) and at termination (day 22).
* P <0.05 as compared with the control group
** P <0.01 as compared with the cold cream group

Table1: Effect of honey bee products on the tensile strength of incisional wound model on rats compared to cold cream treatment

Groups	Treatment twice a day	Tensile strength
1	No	615.9±1.3
2	Cold cream	622.8±2.4
3	60 % propolis cream	635.7±7.1*
4	Honey	663.5±7.4&
5	Mixture of honey and propolis	702.4±13.5*#$

*P<0.01 vs cold cream group
#P<0.001 vs propolis cream group
$P<0.01 vs honey group
&P<0.01 vs control group

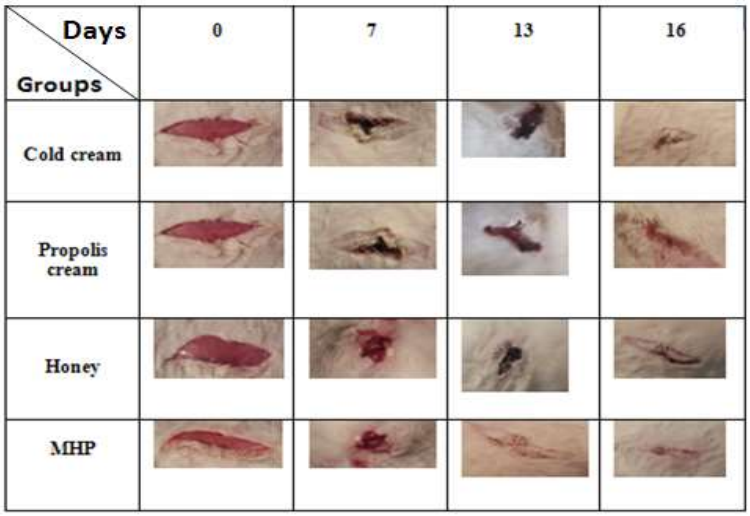

Fig. 2: Representative photos of each treatment on rats subjected to skin incision wounds, during experiments. Wounds were generated at day 0 and photos were taken as on **Fig. 2:** Each row shows the same wound as it progresses for 16 days. **Notes:** The animals received 1 g of local applied cream consisting of cold cream, 60 % propolis cream, honey and a mixture of honey and propolis cream twice a day. **Abbreviation:** MHP, mixture of honey and propolis cream

DISCUSSION

On recent years, the development of antibiotic-resistant bacteria is a great challenge facing human and veterinary medicine concerning to the infection of surgery wounds. Indeed, studies have been focusing on the last few decades on an alternative therapy and natural medicinal products that can potentially cease and/or remove the threat of microbial resistance, and stimulate tissue repair for the wound local treatment, due to the high costs of traditional treatments [28, 29].

The goal of this experimental research was to assess the wound curing activity of honey bee products on a rodent incisional skin wound as a suitable model for clinical surgery wounds. In this study we used Wistar rats to assess the cold cream, propolis cream, honey and a MHP on a randomized controlled study.

As the wound tensile strength indicates the organization of subdermal collagen fibers on the recently composed collagen, it is a critical measure [30]. It shows how much the healed tissue resists to rupture under strain and may partly show the quality of the repaired tissue [31]. Clinical and experimental trials designed to effect wound healing rates or strength have been showed with varied success [32].

According to our results, wound tensile strength measurements showed that 60 % propolis cream significantly increased treatment of wounds compared to cold cream group on day 30 of experiment [Table 1]. Also, the honey treatment showed better healing of wounds significantly compared to control group and the healing effect of MHP on regard to wound tensile strength, was better than propolis cream or honey group separately [Table 1].

Wound healing percentage measurements showed that cold cream significantly increased treatment of wounds compared to control group on day 7 of the experiment [Fig. 1], which was reported and discussed by the author previously [33]. Also 60 % propolis cream increased treatment of wounds significantly compared to cold cream on day 7 of the experiment [Fig. 2]. As the solvent applied for the extraction of propolis may influence the potency of its antimicrobial activity, we prepared ethanolic extract and the results of propolis ethanolic extract healing effect were consistent with the earlier studies [17, 34]. Despite the propolis extract on this study supplied via ethanol showed a high percentage of wound closure time, the propolis aqueous extract used by author significantly hadn't improved wound healing previously [35]. So we suggested that on addition to the kind of preparation, the inconsistency seen between studies may be due to the dosage, form and geographic origin of propolis. Although the adverse reactions related to the usage of propolis on wounds are poorly documented on the literature, a well-documented allergic reaction to propolis is contact dermatitis [36]. It has been found that if very large quantities of propolis are administered, it is not toxic to mammals or humans [37]. As we expected, no mortality was seen on all five groups of our study and the rats gained weight on a normal way (data not shown), which confirmed that the doses of honey bee product used, were not toxic.

Propolis is a complex mixture consisting of at least 230 different natural substances including aminoacids, flavanoids, phenolic acids esters, phenolic acids, cinnamic acid, terpens, caffeic acid, and trace minerals such as iron and zinc [38]. As the antioxidant, anti-inflammatory and antimicrobial activities of the natural product constituents are the most important properties for wound healing and different natural substances on propolis may have all these actions, the wound healing effect of propolis is explicable.

One special feature of propolis is its anti-inflammatory properties. Flavonoids and caffeic acid decrease the inflammatory response by inhibiting the production of prostaglandins via blocking the lipoxygenase activity leading to phagocytes and immune cells stimulation and make effective analgesic and anti-inflammatory mechanisms similar to aspirin and with fewer side effects [39, 40]. Antibacterial effect of propolis is related to its flavonoids, esters, and circular acids [39]. Bioflavonoids on propolis stop an exodus of inflammatory mediators from mast cells and thus inhibit the allergic reaction and inflammation [41]. Flavonoids also have antioxidant [42] and antimicrobial effects [43], while coumaric acids, lignan and diterpen have antibacterial effects [44].

Wound healing includes inflammatory, proliferative and remodeling phases. An earlier work has shown that anti-inflammatory action of propolis is more effective than dexamethasone [45]. So, it is probable that the most important mechanism of propolis action on wound healing is through its anti-inflammatory properties and its contribution to the production of collagen.

Our results also showed that honey increased wound closure time significantly compared to the control group on day 10 of the experiment. Surprisingly the MHP not only could accelerate wound healing significantly compared to cold cream on more days of the experiment but also its healing effect was significant compared to propolis cream on day 13 and compared to honey on days 7 and 13 of the experiment.

Honey contains moisture absorption properties that can decrease edema of the wound. Our results on faster wound closure time by honey treatment is consistent with the other's works [5, 46]. Honey causes hydrogen peroxide production with its insulin-like effects and induces stimulation, cell proliferation and angiogenesis on the wound area. Moreover, it has an essential role on the removal of microbial agents [47]. Additionally, some researchers have shown that honey can accelerate granulation tissue formation

and angiogenesis on the wound region [48]. Honey supplies oxygen and available nutrient to fibroblasts via increasing angiogenesis, and oxygen supplied from hemoglobin for its acidic PH and leads to improved activity of fibroblasts and collagen development. As angiogenesis is accelerated, recovery of wound occurs earlier [48, 49].

Our study concern to the combined effect of honey and propolis on wound healing had some similarities to the work of Takzaree et al and the results had consistency. They proposed that as angiogenesis is an essential factor on wound healing process, the blood vessels growth on propolis and honey combined groups on comparison with control group had an increasing trend to nutrition, oxygenation, cell proliferation and ultimately accelerate wound healing [49]. Better wound healing acceleration on MHP compared to propolis or honey groups was shown on our study as we expected. On spite of the difference between the models of wound, the origon of honey bee products and different kinds of applied methods, the novelty of our work compare with the work of Takzaree et all was assessing wound healing on regard to wound tensile strength on addition to wound closure time measuring.

Propolis also has the potential to establish synergic effects with synthetic antibiotics, leading to a development on its antimicrobial effects on both in vitro [15] and in vivo [50]. Flavonoids on honey and propolis stimulate the immune system and enhance its antibiotic properties, reducing the wound size and restoring it, caused to decrease inflammation, infection and stimulation of fibroblasts to produce collagen fibers. This finding is consistent with the hypothesis that the antimicrobial properties of MHP may have prevented the wound healing delay that would otherwise happen on the wounds infection.

So, reduction of wound size and increasing of wound tensile strength on our MHP treatment group [Fig. 3 and Table 1] may be due to a synergistic effect that increased collagen fiber due to promoted fibroblasts and has consistency with the other's works [47, 51] [Fig. 3 and Table 1]. It suggests that the MHP group may be able to increase fixation and compaction of the collagen fibers. Nevertheless, on the future, more researches require to be done to approve it.

CONCLUSION

This short experimental study concludes that the combined propolis honey (MHP) has a significant incisional wound curing activity on the animal model. This synergism between honey and propolis may contribute to the decreasing the of synthetic drugs administration and the management of antibiotic resistant microorganisms' production, may open new windows for the synthesis of novel drugs. Patients management with cutaneous wound continue to struggle surgeons and physicians on dermatology and surgery area [22] and some medications such as a combined propolis honey preparation may be usable to accelerate the healing of full thickness skin wounds. More works are also essential to be done on combined propolis and honey component stability on different preparations, safe, and bioavailability and effective doses for the management of diseases. So, we suggest further studies of combined propolis honey preparations to be used on man whom surgical wounds show an increasing therapeutic clinical and clinical challenge.

LIMITATIONS OF THIS STUDY

We request the reader to bear with us on this study as we focus on the macroscopic results, i.e., the end outcome of wound healing, rather than the details of the healing mechanism. Although there is much to be done, our work has found important results on the field of the effect of Iran's honey bee product on wound healing. Our wound healing research was also done only on regard to wound closure time and tensile strength measurement and samples for this study were collected from only one geographical site.

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COMPETING INTEREST

The authors confirm that the study wasn't done on any financial or commercial associations that could be explained as a possible competing interest.

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