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## Comparison of efficacy of unheated and heat-treated Sahara honey on wound healing in rabbits

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### ABSTRACT

**Objective:** To investigate the efficacy of unheated and heat-treated Sahara honey on wound healing in rabbits on the basis of macroscopic observation changes.

**Methods:** Eight female rabbits were used. Using aseptic surgical technique, a 3 cm incision was made on the back of each rabbit and two rabbits with injuries in each group were treated daily with a topical application of unheated and heated honey, sulfadiazine and sterile saline, respectively.

**Results:** The unheated honey demonstrated the highest activity on the wound compared to reference ointment silver sulfadiazine, heat-treated honey and sterile saline respectively. Further the present investigation proves that unheated honey is possessing superior wound healing activity than that of heat-treated honey.

**Conclusions:** The result of this study confirms that unheated honey had the best wound healing effect even better than heat-treated honey.

## 1. Introduction

Various agents have been applied topically to treat infected wounds for many years, but their proper roles remain unclear. For millennia, healers have applied various compounds to infected wounds[1-2]. The renewed interest in silver comes as a consequence of the rapid development of bacterial resistance[3]. *In vitro* studies have repeatedly demonstrated that silver is toxic to keratinocytes and fibroblasts[4]. Therefore, some alternative wound healing methods were investigated. A good alternative to silver-based dressings might be honey, which has no adverse effects on wound healing[5]. The therapeutic use of honey in wound care has been applied since ancient times. Honey has several properties that contribute to its wound-healing effects, including antibacterial, debriding and deodorising activity as well as anti-inflammatory and tissue growth-promoting properties[6]. Honey has been shown to have *in vitro*

antimicrobial properties and animal studies have demonstrated that the use of honey accelerated wound healing[7]. Clinical studies on the wound healing properties of honey have found that honey stimulates healing and promotes the re-growth of healthy tissue with no adverse effects[8]. Honey may contain compounds that may lead to toxicity. A compound without naturally present in honey, named 5-hydroxymethylfurfural (HMF), may be formed during the heating of honey. HMF may be a mutagenic, carcinogenic and cytotoxic compound[9]. In Ayurveda, it is quoted that heated honey produce adverse health effects[6]. However, no data have been found on the wound by heated honey. In Algeria, a variety of honey is locally produced. Unfortunately, there is still little evidence to support the potential of honey in Algerian and Sahara on wound healing. *Euphorbia* honey is a type of honey produced locally in Algeria. These studies compare the use of topical honey (unheated and heat-treated) to other treatments including saline and silver sulfadiazine.

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## 2. Materials and methods

### 2.1. Honey sample

*Euphorbia* honey is a local monofloral honey produced by *Apis*

*mellifera* bees from the flora source of *Euphorbia* honey in a water bath was heat treated at 100 °C for 60 min.

**2.2. Animals and experimental procedure**

Rabbits, weighing 1.5-1.8 kg, aged 2-3 month, were distributed into four groups with two animals each. Animals were anesthetized using intramuscular ketamine (0.20 mL/kg) and Acépromazine (CalmivetR) (0.1 mL/kg). The rabbits were kept under standard condition in animal house for one month that had light and dark conditions for each 12 h at 25 °C. Rabbits in Group I received no treatment and saline served as control group. Group II was treated with silver sulfadiazine which is a standard drug ointment. Group III was treated with unheated honey while Group VI with heated honey. All animals in each group were anaesthetized by the open mask method with anaesthetic ether before wound creation.

**2.3. Percentage wound contraction**

The results of wound measurement on various days were expressed as percentage of wound contraction. The values of Day 0 measurement sand were calculated by Kamath *et al.* formula as

follows[10].

$$\% \text{ Wound contraction} = \frac{\text{Area on day zero (mm)} - \text{Area on day measurement (mm)}}{\text{Area on day zero (mm)}} \times 100$$

**3. Results**

The day when wounds were made was designated as Day 0, and the process of wound healing was observed from Day 1 after wounding. We observed edema, infection, and necrotic tissue on each wound (Groups I, III and VI) shown in Figure 1. The representative wound photographs and wound contraction (%) (Figure 2) on different days show that wound area decreased in a time-dependent manner in the four groups. In group treated with heated honey, 20 days was required for complete healing (Figure 2) while unheated honey reduced the rate of healing to 15 days (Figure 3). Higher mean percentage of wound contraction was obtained in wounds treated with the unheated honey and sulfadiazine followed by heated honey. The silver sulfadiazine group, however, showed better healing time compared to the heated honey group. Also, contraction in sulfadiazine ointment was higher than control (saline group) (Figures 4 and 5).



**Figure 1.** Photographs of rabbits show various phases of wound healing. I: Control group (negative control); II: Control group (positive control); III: Raw honey group; IV: Heated honey group; DAW: Days after wounding.

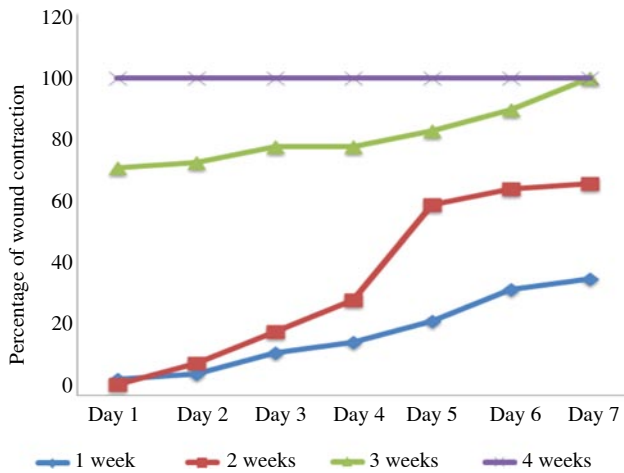


Figure 2. Effect of heated honey on wound contraction.

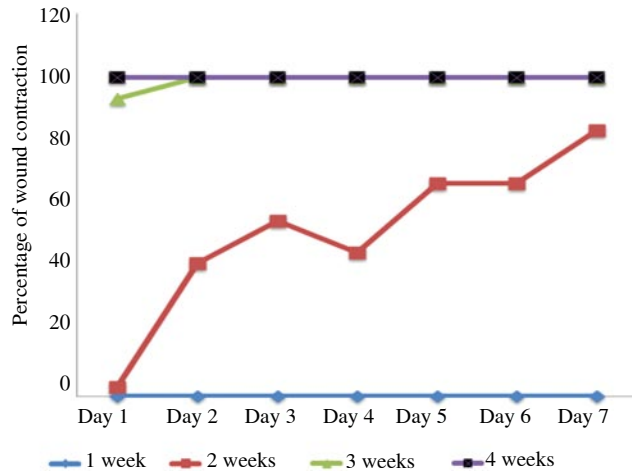


Figure 4. Effect of silver sulfadiazine ointment on wound contraction.

4. Discussion

Honey, known for centuries as a topical treatment for a wide range of wounds, has recently been used in modern wound care. Recent clinical trials have suggested that healing times and incidence of infection after treatment of wounds with honey are reduced compared with conventional treatment, and the results are of clinical significance[10-12]. Algerian honey has been used a lot in topical therapies and traditional medicine for a long time. Topical application of Algerian honey has also been reported to be effective to treat wound in animals[13]. Therefore, we investigated the effectiveness of Sahara honey on wound healing in rabbit. To the best of our knowledge, this study is the first that have used Sahara honey and compared it with heated honey in rabbit. The results of this study showed that topical application of unheated honey significantly accelerated the rate of wound healing compared to the heated honey treated group, indicating an increase in collagen formation. This is similar to findings on the efficacy of topical application of unheated honey in wound management as reported by many researchers[14,15]. The role of honey is mainly due to chemical action on the wound. Hydrogen peroxide, volatiles, organic acids, high osmolarity, beeswax, nectar, pollen and propolis are important chemical factors that have healing properties in honey. Additionally, polyphenolic compounds play an important role in wound healing due to their antimicrobial and antioxidant activities, metal chelating ability and enzyme inhibition properties[16-19]. The temperature at which honeys were stored influenced their total phenolic content.

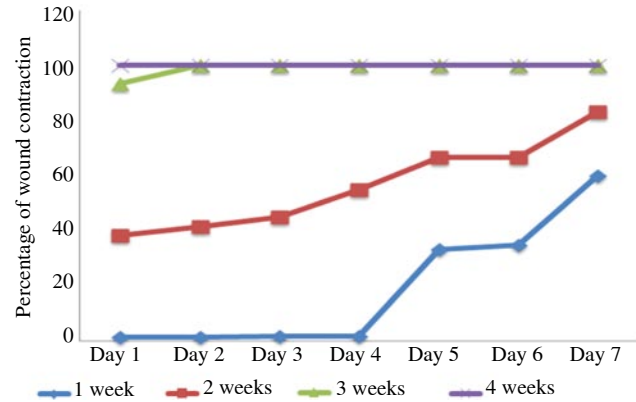


Figure 3. Effect of unheated honey on wound contraction.

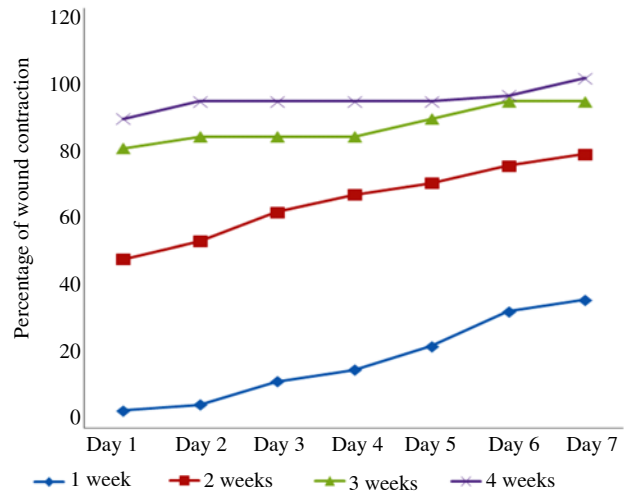


Figure 5. Effect of saline wound contraction.

Also, heating honey is contraindicated according to Ayurveda as it causes deleterious effects. Hence, to evaluate this concept, honey has been heated beyond the permissible temperature, i.e., 140 °C for 2 min. Silver sulfadiazine is commonly used to manage burns. However, evidence of their effectiveness remains poorly defined. Topical silver might delay wound healing, because of its toxicity to keratinocytes, fibroblasts, and possibly leukocytes[1]. In addition, silver sulfadiazine can cause a transient leukopenia. Also, inhibition of wound contraction by the commonly used antimicrobial silver sulfadiazine was demonstrated in a rodent excisional wound model[20]. Another problem associated with silver sulfadiazine remains bacterial resistance to sulphadiazine component[21]. Wound healing is a complex process, coordinated by various mechanisms, such as coagulation, inflammation, fibroplasia, epithelization, contraction and remodelling[22]. Honey promotes the formation of clean healthy granulation tissue[23], epithelialization[24], growth of fibroblasts[25], and increased angiogenesis[26]. Also, honey has an anti-inflammatory influence even when there is no infection present, this being seen as a reduction in the number of inflammatory cells infiltrating the wound tissue[27]. According to Ayurvedic medicines the honey should not be heated or consumed warm as it causes toxic effect. Furthermore, not only does heating honey make it toxic, but new research also indicates that most of honey benefits such as a variety of amino acids, enzymes, minerals, fatty acids and carbohydrates are destroyed by the application of heat.

This study demonstrated the efficacy of unheated honey in wound

healing. Topical application of unheated honey accelerated the rate of wound healing by increasing the wound contraction. But heated honey has been found to be less active, as evidenced by the decrease in the rate of wound contraction. These findings justify the inclusion of this natural product in the management of wound healing in Algerian folk veterinary medicine.

### Conflict of interest statement

The authors declare that there is no conflict of interests in this research.

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