

## Therapeutic Efficacy of Honey and Hydrogen Peroxide in Infected Wounds in Rabbits

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### ABSTRACT

Standard wounds were made in the backs of 20 rabbits , four groups were divided every group contain five animals which were then treated for 7 days as follows : First : sterile wounds without treatment ; Second : infected wounds with no treatment ; Third : infected wounds dressing with honey ; and , Fourth : infected wounds treated with 3 % hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) . Wounds were infected by inoculating *Staphylococcus aureus* .All treated wounds showed reduction of inflammatory exudation , faster wound contraction , granulation tissue formation and epithelization. However, the overall healing was significantly higher in wounds treated with honey as compared to H<sub>2</sub>O<sub>2</sub> treated wounds. Honey treated wounds, showed less neutrophilic infiltration and marked proliferation of angioblasts and fibroblasts in comparison to H<sub>2</sub>O<sub>2</sub> treated wounds. Moreover, infected wounds became sterile within one week of application of honey. It is concluded that honey is effective in treating infected wounds .

### الكفاءة العلاجية للعسل مقارنة بيروكسيد الهيدروجين للجروح الخمجة في الأرانب

#### الملخص

تم عمل جروح (إتباعاً للمبادئ الأساسية للجراحة) في منطقة الظهر لعشرين أرنباً ، ثم قُسمت حيوانات التجربة إلى أربع مجاميع تضم كل مجموعة خمسة حيوانات وكما يلي :- أولاً : جروح نظيفة لم تعامل بأي مادة ، ثانياً : جروح مخمجة بدون معاملة ، ثالثاً : جروح مخمجة عولجت بالعسل ، رابعاً : جروح مخمجة عولجت بـ 3 % بيروكسيد الهيدروجين . وقد تم تخميج الجروح في المجاميع الثانية والثالثة والرابعة بواسطة جرثومة المكورات العنقودية الذهبية. بينت هذه الدراسة أن الجروح المخمجة والمعالجة أظهرت إختزاً في النضجة الإلتهابية وسرعة في تقلص الجروح تبعاً لتكون النسيج الحبيبي فضلاً عن التطهر. وكان الإلتئام في الجروح المعالجة بالعسل متوقفاً عن نظيره في الجروح المعالجة بيروكسيد الهيدروجين ، حيث أظهرت الجروح المعالجة بالعسل قلة في إرتشاح العدلات وتكاثر واضح لكل من الأرومات الليفية والوعائية مقارنة مع الجروح المعالجة بيروكسيد الهيدروجين والجروح غير

المعالجة ، فضلاً عن ذلك فإن الجروح المعالجة بالعسل بينت خلوها من الجرثومة المخمجة خلال الاسبوع الأول من المعالجة . عليه ، فقد تم الإستنتاج بأن مادة العسل كانت فعالة في تحقيق الإنتقام عند الجروح المخمجة .

### INTRODUCTION

Superficial wounds are very often infected with various strains of bacteria. However, in many cases, topical antiseptic treatment of infected wound in patients is necessary (Eaglistein,1990). Recently, we find that honey was effective in treating surgical wounds (Al-Kennany and Allawi,2000). In this paper we report on the efficacy of natural honey dressing in treatment of standard infected skin wounds induced experimentally in rabbits, in comparison with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) treatment.

### MATERIALS AND METHODS

#### Animals

Twenty healthy adult male rabbits of cross breed weighing 1100-2050g were used.

#### H<sub>2</sub>O<sub>2</sub>

3% H<sub>2</sub>O<sub>2</sub> was used (Degussa H<sub>2</sub>O<sub>2</sub> 50 %, Degussa AG merk, Rheinfelden-Belgium).

#### Honey

Pure undiluted sunflower honey (Technical Institute, Agricultural Division-Al-Selamia-Mosul-Iraq) was used in this study.

#### Bacterial suspension

Bacterial suspension of *Staphylococcus aureus* was used. The infected bacteria was isolated from infected udder of ewes (Coagulase positive). The bacterial suspension was prepared by grown the bacteria in nutrient broth for 48 h at 37°c, thereafter centrifugation at 5000 rpm for 20 min. Finally, the bacterial cell pellet was resuspended in phosphate buffer solution (pH 7.4) and used immediately.

#### Surgical Technique

Rabbits were anaesthetised with atropine sulphate at dose of 1.0 mg/kg B.W. intramuscularly as a premedication followed within 10-15 min. by an intravenous administration via the ear vein with a mixture of 2.5 % thiopentone sodium at a dose of 10 mg/kg B.W. and 20 % pentobarbitone sodium at a dose of 40 mg / kg B.W. The whole back of rabbits was prepared under aseptic condition. Standard wound (3 cm in length) was made in the back skin of all rabbits. The wound was allowed to pass through the epidermis, dermis and subcutaneous fat but not the subcutaneous muscle.

#### Model of wound infection and treatment

All wounds, except that in group one, were infected by topical application of 0.05 ml of bacterial suspension containing 10<sup>3</sup> bacterial cells. Wounds in group 2 received no treatment (sterile normal saline). In group 3, wounds treated by a local dressing of honey

twice daily for 7 days. Wounds in group 4 were treated by local application of 3 ml H<sub>2</sub>O<sub>2</sub> for 7 days.

#### Histopathological examination :

After inducing local anesthesia by 2 % lignocaine, specimens were collected from wounds in all groups at the time of admission, and on days 3,7,14 and 21. Biopsies were taken from the wound surface to include 3 mm depth and a 3 cm<sup>2</sup> area, transferred to be fixed in 10 % buffered neutral formaline, paraffin embedded, cut into 5 µm thick sections and stained with hematoxylin and eosin stains (Luna,1968).

#### Bacteriological examination :

Bacterial cultures were made from swabs taken from the wounds area at the time of admission and on days 3,7,14 and 21 in all infected groups.

## RESULTS

#### General observation

Table 1. shows the clinical and macroscopical findings in all experimental groups. The development of granulation tissue was earlier in G3 (7 days) as compared with other infected wounds (groups 2 and 4), as well as the non infected one (G1). Moreover, infected wounds treated with honey (G3) revealed negative bacterial isolation starting at 7 days post infection (PI), while the non treated infected wounds (G2) showed positive bacterial results till 21 days PI. Infected wounds treated with H<sub>2</sub>O<sub>2</sub> (G4) persist the positive bacterial isolation up to 14 days PI.

Table1: Time required for healing of wounds in rabbits.

| Clinical observation | Time of healing in days |                |                |                |
|----------------------|-------------------------|----------------|----------------|----------------|
|                      | G <sub>1</sub>          | G <sub>2</sub> | G <sub>3</sub> | G <sub>4</sub> |
| Exudation            | 5                       | 1-21 pus       | 1-3            | 1-10           |
| Granulation tissue   | 7-10                    | Over 21        | 7              | 14-21          |
| Epithelization       | 10                      | < 21           | 7              | 14-21          |
| Foul smelling        | -                       | 1-21           | 1-3            | 1-10           |
| Swab Culture         | -                       | 21 positive    | 7 negative     | 14 negative    |

#### Histopathological findings

In G1, the histological picture of the wound biopsies revealed the development of granulation tissue at 7 days and complete healing achieved at 14 days. Conversely, the microscopic features of the infected wound without treatment, (G2), characterized by abscessation of the epidermal and dermal layer with infiltration of mononuclear cells (Fig.1) till the 14 days PI. At 21 days PI, granulation tissue was start to be formed (Fig.2).

In infected wounds and treated with honey (G3), the granulation tissue and epithelization of epidermis and dermis was seen at 7 days PI. Slight reactive changes with

normal maturation of horny layer have been observed (Fig.3and5). In G4 ( $H_2O_2$  treated), although the reparative activity was noted at 7 days PI, granulation tissue formation appeared at 14 days PI. The later changes have been infiltrated with neutrophils, macrophages and plasma cells (Fig.6and7) .

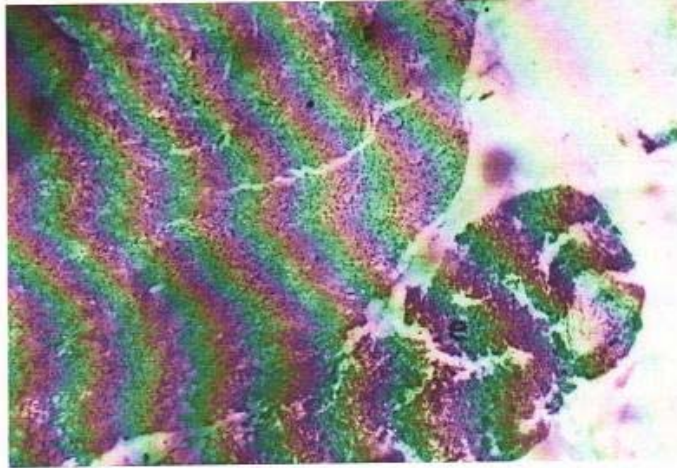


Fig1: Photomicrograph of wound at 7 days in G2 showing purulent exudate (abscess) in subcutaneous tissue.( e ). H & E. 10X.

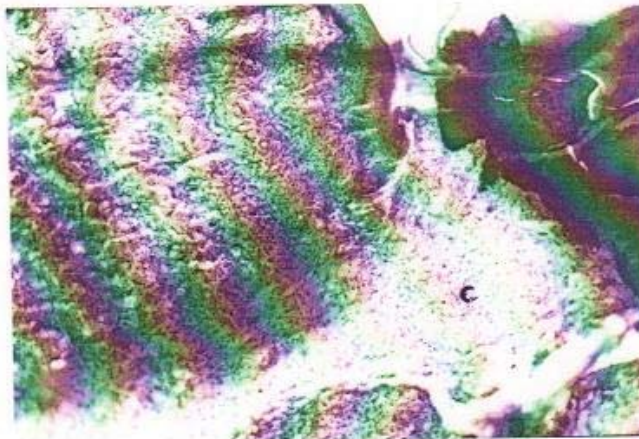


Fig.2: Photomicrograph of infected wound with no treatment (G2). Note serofibrinous exudate with severe neutrophilic infiltration (c).H & E. 10X .

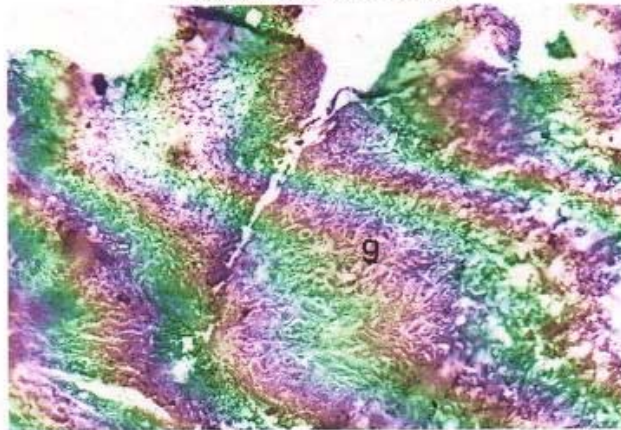


Fig.3. Photomicrograph of wound at 7 day in G3 showing granulation tissue formation with little perivascular infiltration of inflammatory cell (g). H & E. 10X.

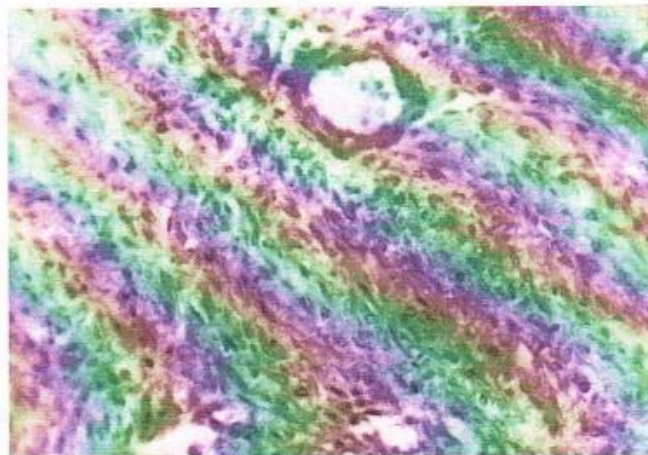


Fig.4: Photomicrograph of wound at 7-14 days in G3 showing mature granulation tissue with absence of reactive change. H & E. 40X.

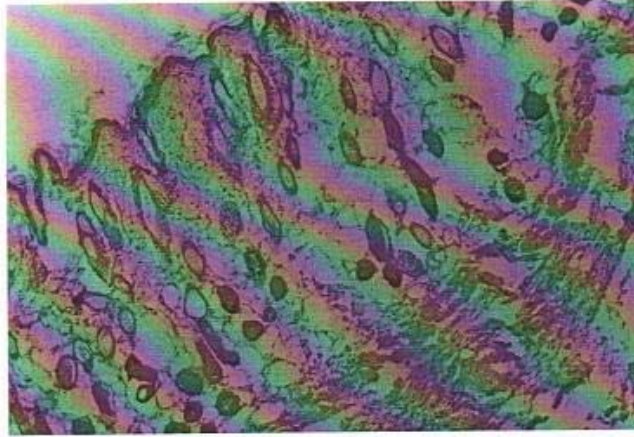


Fig.5: Photomicrograph of wound at 7-14 days in G3 showing epithelization with complete healing (normal horny layer) . H & E. 40X..

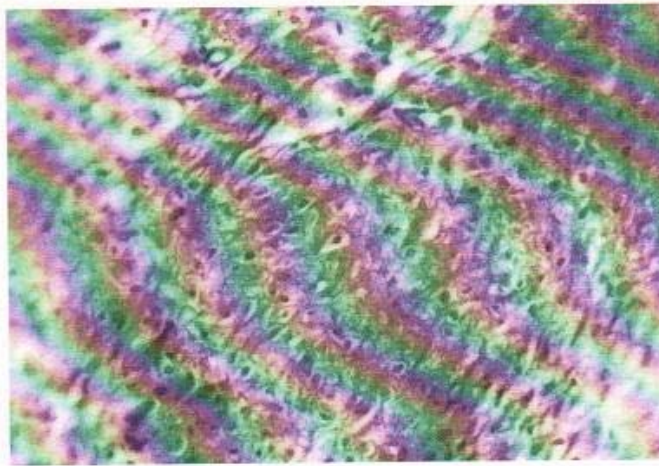


Fig.6: Photomicrograph of wound at 14 days in G4 showing start formation of granulation tissue. H & E. 40X.

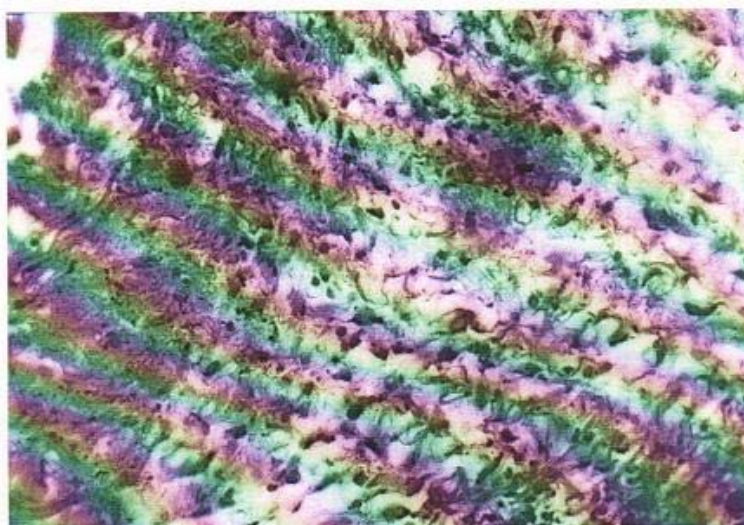


Fig.7: Photomicrograph of wound at 14 days in G4 showing infiltration of mononuclear inflammatory cell (macrophage , lymphocyte and plasma cell). H & E. 40X.

#### DISCUSSION

This study was recognized that topical application of honey can be effective in treating infected wounds. Therefore,our results may be in accordance with other reports (Subrahmanyam,1996). Swabs culture made from infected wounds that treated with honey revealed negative bacterial isolation since 7 days PI (Tab.1). The antibacterial activity of honey may be claimed to its low pH (3.6) and hygroscopic properties, leading to dehydration of bacteria which became inactive (White et al ., 1963 ). Moreover the viscosity of honey is high (Efeme,1991) that may forms a physical barrier which prevents bacterial colonization in wounds.

On the other hand, the infected wounds which was treated with honey (G3) showed earlier signs of healing (less exudation,and rapid epithelization (7 days PI)), as compared with other experimental groups. This indicated that honey promot healing through the release of fibulin-1 and tenascin-c from epidermal epithelial cells to accelerate granulation tissue formation this agreement with other workers (Fassler et al.,1996).

In regards to infected wounds and treated with  $H_2O_2$ , our results revealed retarded wound healing till 14 days PI. Its well documented, that  $H_2O_2$  as wound antiseptic lead to inhibition of the migration and proliferation of keratinocytes especially at the first 48 hours after application (O'Toole et al.,1996). Furthermore,  $H_2O_2$  it self generate lipid peroxidation that leads to induction of hydroxyl radicals causing an inhibition in the antioxidant status of the epithelial cells which is necessary in healing processes (Lubec et al.,1996).

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