

Antibacterial activity of Iraqi Honey against *Staphylococcus aureus* isolated from a wound in vitro and vivo

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ABSTRACT

Honey is among many of the natural products that have been used to treat various ailments such as burns and wound since ancient times. So this study was aimed to achieve to the effectiveness of various constration of Iraqi honey against the growth of *Staphylococcus aureus* by using of well agar diffusion technique in comparison with Gentamycin. Results showed a difference in the rate of zones inhibition by using Iraqi honey against *staphylococcus* where he scored concentration (60 mg / mm) (28 ± 0.57) while the Gentamycin a concentration (60 microgram / ml) on the growth of *Staphylococcus aureus* (27.33 ± 0.33). (15) rabbits were used, divided into 3 groups ($n = 5$ / group). All animals in all groups experimentally induced skin incision (30 mm) in the flank area of the body, and infected with *S. aureus* suspension, and treated after infected the wound (24 hrs.) according to the experimental design. The groups were treated with diluted honey at concentration (60 mg/ml), appear complete healing in 14 days of treatment and returning of hair in the wound area, in comparison with control group and the group when treated with Gentamicin ointment that required (21) days to recovery . So the honey is good as an anti -bacterial effectiveness against bacterial infections that infect wounds.

Introduction

Honey is a sweet food made by bees using nectar from flowers. Honey produced by other bees and insects has distinctly different properties. Honey bees from nectar into honey by a process of regurgitation and store it as a food source in wax honeycombs inside the beehive. Beekeeping practices encourage overproduction of honey, so that the excess can be taken without endangering the bee colony. Honey gets its sweetness from the monosaccharide fructose and glucose and has approximately the same relative sweetness as that of granulated sugar (74% of the sweetness of sucrose, a disaccharide) .

The reasons for the antibacterial activity of honey are controversial. A laboratory demonstration of its antibacterial activity was first carried out by (1) who gave the name 'inhibine' to the substance which inhibited bacteria. (2) first suggested the possibility that hydrogen peroxide was responsible for the antibacterial activity of honey for both the antibacterial activity of honey and hydrogen peroxide were destroyed by light. (3) and (4) reported that hydrogen peroxidase which is produced by the glucose oxidase of honey could be the inhibitory substance against bacteria.

Honey has been demonstrated in many studies to have antibacterial effects, attributed to its high osmolarity, low pH, hydrogen peroxide content and content of other, uncharacterized compounds. The low water activity of honey is inhibitory to the growth of the majority of bacteria, and too many yeasts and moulds. When applied topically to wounds, osmosis would be expected to draw water from the wound into the honey, helping to dry the infected tissue and reduce bacterial growth. Even when diluted with water

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absorbed from wounds, honeys would be likely to retain a water activity sufficiently low to inhibit growth of most bacteria. *Staphylococcus aureus* is one bacterium that is tolerant of low water activities, being observed to grow when water activity is as low as 0.86. It has been found to survive treatment on infected skin treated with concentrated solutions of pure sugars, but to be sensitive to the other antibacterial components of honey of the same water activity (2).

• ***Staphylococcus aureus***: -It is Gram positive spherical cells arranged in irregular grape-like clusters "bunch of grapes", single cocci, diplococci or quadrates also seen under a light microscope, the average diameter of the cocci was 1.0 μm . They are facultative anaerobes (fermentative), and grow readily on most ordinary media but not MacConkey, typical colonies appear yellow to golden yellow in color, smooth, slightly raised and hemolytic on blood agar from 5-7% sheep RBCs (various degrees of hemolysis α , β , γ , and even double zone of hemolysis), the golden appearance is the etymological root of the bacteria's name "Latin: aureus=golden" *S.aureus* can be purified on mannitol salt agar (contain 7.5% NaCl that inhibit all other bacteria except *S.aureus* can tolerate salt up to 20% and grow in) so it could be used as selective agar for *S.aureus*, also *S.aureus* grow in temperature ranging from 10-45 °C and pH (4-10)(5).

Honey is the worlds' oldest known wound dressing .Its use being recorded over 4000 years ago, Researchers started to document the wound healing properties of honey in the early 20th century, but the introduction of antibiotics in 1940 temporarily halted its use. Now concerns about antibiotic resistance, and a renewed interest in natural remedies, have prompted resurgence in the antimicrobial and wound healing properties of honey (6) .

rovides a nonstick barrier by its osmolarity .Honey has an anti-inflammatory action, it also reduces edema, thus improves circulation and reduces pain and exudation in wound. Honey stimulates angiogenesis, granula-tion and epithelialisation by stimulate the growth of fibroblasts, and thus it helps skin regeneration .Honey has anti-bacterial effects. Honey prevents scarring and hypertro-phyication (7).

Material and Method

Api Staph Kit , (bioMe'rieux – France)

It was an identification system for the genera *Staphylococcus* which was performed by using standardized and miniaturized biochemical test with especially adapted database. The identification obtained on the classification of (8) as in bioMe'rieux manufacture (France).

Experimental Bacteria

- *Staphylococcus aureus* was obtained from, patients in Ramadi Hospital, *S aureus* isolated from patients with serious wounds and burns , the identification was done by biochemical test and API Staph test in Ramadi Laboratory shown in figure (1).



Figure (1):Positive results of *S.aureus* on API Staph reading stripe.

Honey: Honey which were obtained from in the downtown (Anbar, Heet city), in the season of Spring (April, May and June) were used. The stock solutions of a honey were prepared by dissolving 10 gm. of a Honey in 100 ml of distal water (w/v), for getting 100 mg/ml.

Antibacterial agents

Gentamicin, ampoule 80 mg /2 ml , Meheco company, china.

Experimental animals:- Rabbits: Iraqi local breeding, male, age range between 4-6 months, and weights ranged between 1.5 - 2.0 kg.

Preparation the antibiotics: Gentamicin 80 mg /2ml : taken the 0.25 ml (10 mg-10000 μg) of antibiotic solution and then completed to 100 ml distal water in order to getting 100 μg /ml (stock solution) ,and from it premeditated the different concentration : 20, 40 ,60, and 80 μg /ml .(17)

Bacterial count: *Staphylococcus aureus* :is counted in indirectly by:

1. Spectrophotometer :was used to measure the turbidity of *Staphylococcus aureus* suspension to reach an appropriate optical density (O.D), whereas

the percent of focus transmission has been 25% at a Wave length of 450 nanometer.

2. McFarland Solution (tube No .0.5) Standard McFarland solution No.0.5 was prepared according to (9)

Bacterial inoculation of media

Nutrient agar has been prepared after sterilized by autoclave for 15 minutes at 121 C and 15 lb/in², then followed:

Left the medium to cooling to reach 48 C by using of water bath, from the bacterial suspension 0.2 ml of *Staphylococcus aureus* was taken and that compared to the McFarland solution No.0.5 , using sterile micropipette, and added to each 20 ml of the medium.

20 ml of the medium which contained bacteria were added to each sterile Petri- dish and left for 10 minutes to allow solidifying of the media.

Activity of 100µl/well of different concentration of honey and antibiotics to the experimental bacteria (*S. aureus*) performed by Wells of 6 mm in diameter in the solid medium . The plates containing bacteria were incubated at 37°C for 24 h. After incubation, the diameter of inhibitory zones was measured in mm (10).

In vivo study

A total number of 15 rabbits (local breed) were used in this study . They were divided into 3 groups (n= 5 / group), and kept at a temperature between 23-28C. The animals were housed in metal cage (30 x 70 x 60 cm) individually in an air-conditional room.

3. Result and Discussion

Wound (Open wound)

All Animals in all groups will make skin incision (30 mm) by scrable above the first layer of muscle in the flank area of the body.

Group A: skin incision (30 mm) in flank area immediately above the first layer of muscle, and infected with 1 ml of *Staphylococcus aureus* suspension which contain 1.5×10^8 cell/ml, and after following the infection wounds (24) were treated with pure honey 5gm, once daily for (14) days.

Group B: skin incision (30 mm) infected with 1 ml *Staphylococcus aureus* suspension which

contain 1.5×10^8 cell/ml and after 24 hrs. of infection treated with Gentamicin 5 gm, once daily for (14) days.

Group C: skin excision (30 mm) and infected with *Staphylococcus aureus* suspension which contain 1.5×10^8 cell/ml, without treatment for (14) days.

Effect of honey on Different Concentration of Bacterial Growth

The growth inhibitory effect of different concentrations of Honey on *Staphylococcus aureus* was illustrated in tables (1), (2) and figure(2).

Results showed that the concentration (10 mg/ml)of honey has not any affect antibacterial effect against *S.aureus* while 20mg/ml showed the superiority f activity with inhibition zone 16.0 ± 3.21 ($p < 0.05$) and this may due t the increase in sugar contant.This study wasn't in agreement with (15), who found Minimum inhibitory concentration of honey for 58 strains of coagulase -positive *Staphylococcus aureus* isolated from infected wounds 30 – 40mg/ml for pasture honey. The mean zones of inhibition of honey against *S aureus* at (60mg/ml) are (28.00 ± 0.57) . The concentration 60 mg/ml for honey showed significance effect at ($p < 0.05$) compared with their 20 & 40 mg/ml concentrations.

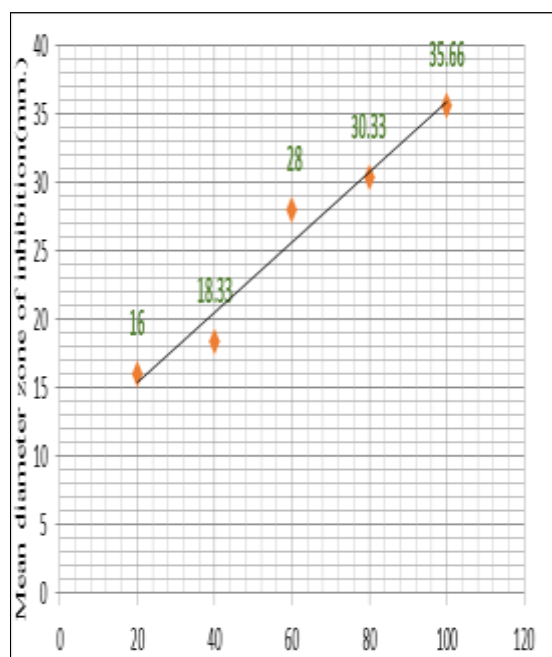


Figure (1) shown Concentration of Honey mg/ml

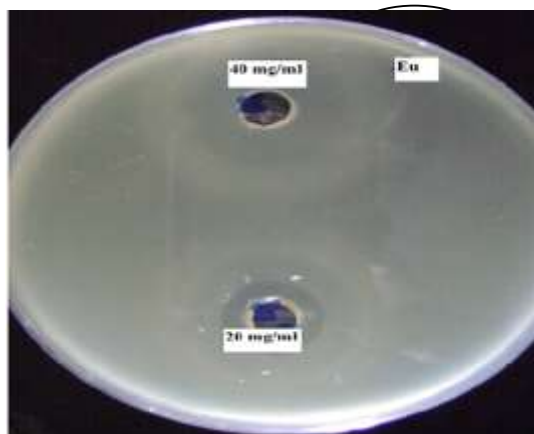


Figure (2). concentrations of honey mg/ml and mean diameter zone of inhibition (mm.) against *S. aureus*

Table (2). Diameter zone of inhibition (mm.) of Honey against <i>S. aureus</i>						
Concentration Honey	10 mg/ml	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100mg/ml
Honey	00.00±0.00	16.00±3.21	18.33±3.33	28.00±0.57	30.33±0.88	35.66±1.20
Gentamicin	20.00±0.57	22.66±0.88	25.33±0.33	27.33±0.33	29.00±0.57	33.31±0.26

The effect of Gentamicin at concentration 20 µg /ml on bacterial growth and honey against *Staphylococcus aureus* are listed in table (2).

Honey has been found to be the most effective agent against *S.aureus*, compare with Gentamicin at concentration (60) µg /ml with inhibition zone 27.33±0.33 while honey was 28.00±0.57 .

In group treated with honey After 14 days showed complete healing and presence of hair, without scar tissue (Figure 6). While in group treated with Gentamicin ,was healing with formation of scar tissue as shown in (Figure 4) .After 14 days in control group the wound has been found signs of inflammation (swelling, redness and hotness) and exiguity of food consumption. These results are in agreements with previous reports (16 ,11), the finding of the present study indicate that honey increased the formation of granulation tissue, density and activation of

fibroblasts, keratinisation in the surface of wound, thickness of epidermis and thickness of collagen fibers. Moreover, keeping with earlier reports,(14,12) honey decreases infection, inflammation, edema and dehiscence. As well, honey increases the rate of wound healing, which confirms previous reports (13) .

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Figure (4). Skin of rabbit, after 14 days in control group the wound has been found signs of inflammation (swelling, redness and hotness)



Figure (3). Skin of rabbit, after 24 hours of infection with *S aureus* Showed inflammation and presence of pus



Figure (5). Skin of rabbit, after 24 hours of treatment with honey showed the wound is clean and without any signs of inflammation



Figure (6). Skin of rabbit, after 14 days of treatment with pure honey, showed complete healing and presence of hair



Figure (7). Skin of rabbit, after 24 hours of infection with *S aureus* in control group, showed the wound is inflamed and presence of pus, swelling, redness, hotness



Figure (8). Skin of rabbit, after 14 days of treatment with Gentamicin showed healing and presence of scar tissue

الفعالية ضد البكتيرية للعسل العراقي ضد البكتيريا *Staphylococcus aureus* المعزولة من الجروح خارج وداخل الجسم الحي.

اشير عبد الحميد الهيتي نجم الدين عبد الله طه

الخلاصة:

إن العسل من النواتج الطبيعية الذي أستعمل منذ قديم الزمان لعلاج مختلف الأمراض وكذلك الجروح والحروق والقروح. حيث هدفت هذه الدراسة التحري عن الفعالية ضد نمو بكتريا المكورات العنقودية الذهبية *Staphylococcus aureus* للعسل العراقي وبمختلف التراكيز (10، 20، 40، 60 ، 80 ملغم /مل) وبواسطة استعمال تقنية التخفيف في الحفر مع مقارنته بالجنتاميسين. سجلت النتائج اختلاف في معدل تثبيط باستخدام العسل العراقي ضد جرثومة المكورات العنقودية حيث سجل تركيز (60 ملغم/مل) (28 ± 0.57) بينما بلغ قطر التثبيط عند استخدام مقارنة الجنتاميسين عند تركيز (60 مايكرو غرام /مل) عند استخدام العسل العراقي (27.33 ± 0.33). استخدم في هذه التجربة (15) أنبياً وقسمت إلى (3) مجاميع حيث تم أحداث جروح في منطقة الخاصرة من الجسم وخمجت بالعالق البكتيري للمكورات العنقودية *S. aureus* ومن ثم عولجت المجاميع بعد (24) ساعة من الخمج وظهور الالتهاب وحسب تصميم التجربة. أظهرت المجاميع المعالجة بالعسل المخفف بتركيز (60 ملغم/مل) سرعة الالتئام ، وفي اليوم (14) من العلاج لوحظ التئام كامل وعودة نمو الشعر في منطقة الجرح، مقارنة مع مجموعة السيطرة وتلك المعالجة بمرهم الجنتاميسين (Gentamycin) حيث احتاج فترة أطول (21) يوم لحصول الالتئام إضافة إلى أنه لم يُلاحظ عودة نمو الشعر في منطقة الجرح ،لذا فان للعسل فاعلية جيدة كمضاد جرثومي ضد الاصابات البكتيرية التي تصيب الجروح.