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Investigation of some Factors Related to Antibacterial Activity of Saudi Honey

دراسة لبعض العوامل المرتبطة بالنشاط المضاد للبكتيريا في العسل السعودي

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Abstract: Honey was found to have a curing effect on bacterial species known to cause wound infections. The aim of this research was to study some of the factors related to the antibacterial activity of a variety of Saudi honey. Honey samples from different floral sources, geographic locations, and production season were used. The tested organisms represented both gram positive and gram negative bacteria causing wound infection. A zone of complete inhibition of all bacterial species was observed with honey types Samra (S-W), Samra (W-S), Saha (W-S), Samra (S-S), Sidir (S-S), and Sidir (S-W), and a larger zone of partial inhibition of growth was observed in all tested honey types. It can be concluded that osmolarity is involved in the antibacterial activity of the honey. Acidity might be regarded as being of primary importance. On the other hand, hydrogen peroxide was found to play a major role in the bacterial activity of honey.

Keywords: *Honey, antibacterial effect, inhibition zone, wound infections, Saudi Arabia.*

المستخلص: وجد أن للعسل تأثيراً علاجياً على أنواع البكتيريا المسببة لإلتهاب الجروح، وهدف هذا البحث لدراسة بعض العوامل المرتبطة بالنشاط المضاد للبكتيريا في انواع من العسل السعودي. استخدمت عينات عسل مختلفة من حيث المصدر الزهري والموقع الجغرافي ومواسم الإنتاج. اما البكتيريا المختبرة فقد شملت البكتيريا بنوعيها الموجبة والسالبة لجرام، والتي يعتبر بعضها من الانواع الممرضة المسببة لالتهابات الجروح. تمت الدراسة باستخدام طريقة الانتشار في اطباق الاجار السابق تلقيحها بالنوع البكتيري تحت الدراسة. والعوامل التي تمت دراستها هي: التأثير الاسموزي ودرجة الحموضة وفوق إكسيد الهيدروجين، الموجود في العسل كناتج من نواتج تاثير إنزيم الجلوكوز اكسيديز على جلوكوز العسل. اوضحت النتائج ان تاثير العسل المضاد للبكتيريا لا يرجع كلية إلى التاثير الاسموزي للعسل بالرغم من ان الاسموزية تساهم في تاثير العسل النهائي المثبط للبكتيريا، ولكنها ليست المسئولة الوحيدة عن هذا التاثير. اما بالنسبة لدرجة الحموضة فقد دل البحث على ان لها اهمية في التاثير المثبط لنمو البكتيريا. ومن الناحية الاخرى فقد وجد انه عند إضافة إنزيم الكتاليز إلى العسل يختفي تماماً التاثير المثبط التام للعسل في حين يبقى التاثير المثبط جزئياً مما يدل على أن فوق اكسيد الهيدروجين يلعب دورا رئيسيا في النشاط البكتيري للعسل.

كلمات مدخلية : *العسل، مضاد للبكتيريا، منطقة التثبيط، التهاب الجروح، المملكة العربية السعودية.*

Introduction

A zone of complete inhibition of all bacterials species was seen with honey types Samra (S-W), Samra (W-S), Saha (W-S), Samra (S-S), Sidir (S-S), and Sidir (S-W), and a larger zone of partial inhibition of growth was seen with all tested honey types. Three factors have been accepted to play a major role contributing to the antibacterial activity of honey; these are osmolarity, acidity and hydrogen peroxide.

The water activity (*aw*) for honey is a measure of the free water in it that might be available for microorganisms. The high sugar content of honey makes water unavailable for microorganisms, thus no bacteria or fungi can grow in fully ripened honey. Honey, being a saturated to super-saturated solution of sugars, has a water content of only 15-21% by weight, and its remaining solids is a mixture of monoasccharides fructose and glucose. These molecules interact strongly with the available water molecules leaving very small amount of water for microorganisms. Many species of bacteria have their growth completely inhibited by a value of (*aw*) in the range of 0.94 -0.99 (Leistner and Rodel, 1975; Tysset *et al.*, 1980).

A number of reports have demonstrated quite clearly that there is much more than an osmotic effect involved. *Staphylococcus aureus*, which is known to have an exceptionally high tolerance at low (aw), yet is one of the Bacterial species most sensitive at the antibacterial activity of honey. Pothman (1950) found that 13 different species were substantially or completely inhibited by 17% honey in nutrient agar, but were not inhibited by artificial honey at the same concentration.

Bacteriostatic effect was reported with artificial honey up to 20% concentration only against gram positive species, whereas natural honey showed both bacteriostatic and bactericidal activity against all species tested (Christian and Mladenov, 1961). Ali *et al.* (1991) reported that artificial honey up to 30% did not inhibit the growth of 10 different organisms, whereas honey of 10% concentration inhibited the growth at most of these organisms. Honey is characteristically quite acidic, and such acidity has been thought earlier as one of the possible explanations at the antibacterial activity of honey (Prica, 1938; Sackett, 1919). However, a number of studies found that there is no correlation between antibacterial activity and the pH of the honeys studied (Leistner and Rodel, 1975; Daghie *et al.*, 1972; Boganov *et al.*, 1987). Roth *et al.* (1986) reported that inhibition by 5% honey in agar diffusion assay was lost if phosphate buffer was added to bring the pH to 6.1 - 6.5.

White and Subers (1963) showed a direct relationship between the hydrogen peroxide produced and the inhibine number of various honeys. The inhibine number describes the degree of dilution to which a honey will retain its antibacterial activity. However, complications in determining the inhibine number arise from the fact that in such a case the bacteria are responding to a secondary substance (hydrogen peroxide), and not to the substance being diluted. Since White and Subers (1963) work, the term inhibine has in many cases been used interchangeably with hydrogen peroxide in the literature of honey. However, other factor beyond acidity and osmolarity have not been considered to be involved (Molan, 1992).

Materials and Methods

a. Properties of selected honey samples and standard test organisms

Some physical and chemical tests were performed on all honey samples through the Honey Quality Laboratory (Riyadh, Saudi Arabia) to identify some of their properties. The standard test organisms used through the study were obtained from (Difco Laboratories, MI, USA). They were selected as representative of gram positive and gram negative pathogenic bacteria causing primarily wound infections except for *Bacillus subtilis*. Clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from different patients at the Riyadh Armed forces Hospital, Saudi Arabia.

b. Assay of the antibacterial activity of honey

The antibacterial activity of the honey samples was assayed by the agar well diffusion method adapted from Stokes (1975) and Anon (1982). The honey samples were tested crude and undiluted against all the standard organisms.

Osmolarity: The inhibitory effect of all natural bee honey samples against *S. aureus* and *E. coli* was compared to that of artificial honey each used at dilutions of 50, 30, 25, 20, 15, 10 and 5% W/V. Artificial honey was a glucose-fructose-sucrose-maltose (GFSM) mixture prepared by mixing these sugars together in the same proportions as they are found in natural honey.

Hydrogen peroxide: The technique outlined by (Allen *et al.*, 1991) was used to assess the contribution of hydrogen peroxide to the antibacterial activity of all honey samples under the study. The test organism used in this experiment was *S. aureus*. Proper controls were tested to ensure that the amount of catalase added was enough to destroy all the hydrogen peroxide in the honey.

Acidity: To test the contribution of acidity to the antibacterial effect of honey, the method described by Molan *et al.* (1988) was used The acidity of three selected honey samples, Samra (W-S), Samra (S-S) and Sidir (S-S), showing higher levels of antibacterial effect was assessed. The antibacterial activity of the gluconic acid solutions and the honey solutions were assessed by the agar well diffusion technique in plates seeded with *S. aureus*.

Results

Osmolarity: Comparison of the effect of the same concentrations of natural honey types with that of artificial honey on *S. aureus* and *E. coli* has revealed that the nature of the inhibitory effect of natural and artificial honey is different. A partial inhibitory effect caused by various dilutions of both natural and artificial honey on the growth of *S. aureus* and *E. coli* has been detected (Tables 1 and 3). Artificial honey caused partial inhibition of growth of *S. aureus* and *E. coli* up to a concentration of 15%. On the other hand, all the natural honey types except Samra (W-S) and Katada (W-W) for *S. aureus* and Samra (W-S) for *E. coli* caused partial inhibition of growth up to a concentration of 5%. However, zones of complete inhibition of growth of *S. aureus* and *E. coli* were seen only for natural honey types Samra (S-W), Samra (W-S), Samra (S-S), Sidir (S-S) and Sidir (S-W) up to a concentration of 25% as shown in Tables 2 and 3.

Hydrogen peroxide: Results of the experiments performed to assess the contribution of hydrogen peroxide to the antibacterial activity of the different honey types are summarized in Table 4. The different honey types when tested at 50% concentration caused partial inhibition to the growth of *S. aureus*. Honey types Samra (S-W), (S-W), (S-S), Saha (W-S), Sidir (S-S) and (S-W) caused also zones of complete inhibition to the growth of *S.aureus*. Upon addition of catalase to all honey types, only partial inhibitory effect was seen. Proper controls were tested to insure that the amount of catalase added was enough to destroy all the hydrogen peroxide in the honey. Results are indicative that hydrogen peroxide is strongly contributing to the antibacterial activity of some of the honey types tested.

Table 1. Partial inhibitory effect of various dilutions of natural and artificial honey on *staphylococcus aureus*.

Honey Sample	Diameter of zone of inhibition (mm) ± S.E* at different honey concentrations						
	50%	30%	25%	20%	15%	10%	5%
Artificial Honey	30.00 (1.00)	28.67 (1.15)	24.67 (2.08)	22.33 (2.08)	19.33 (2.31)	-	-
Samra (S–W)	32.67 (1.15)	31.00 (1.00)	30.33 (6.81)	29.00 (3.00)	21.00 (2.65)	16.00 (5.20)	10.67 (1.15)
Samra (W–S)	31.00 (1.00)	30.00 (0.00)	27.00 (1.00)	25.33 (1.15)	19.67 (1.53)	11.67 (2.08)	-
Saha (W–S)	32.67 (1.15)	31.00 (1.00)	29.33 (0.58)	24.00 (1.00)	19.00 (1.00)	17.33 (2.52)	11.00 (1.00)
Samra (S–S)	31.33 (1.15)	29.33 (0.58)	26.00 (2.00)	23.00 (1.73)	16.33 (2.52)	14.33 (1.53)	7.00 (1.00)
Magra (W–W)	31.00 (1.00)	29.33 (0.58)	26.33 (1.15)	21.33 (1.53)	18.33 (0.58)	15.33 (0.58)	10.67 (1.15)
Katada (W–W)	26.33 (1.53)	25.67 (1.15)	22.33 (2.52)	21.67 (1.53)	20.67 (0.58)	19.00 (1.00)	-
Astra Tabook (N–W)	27.33 (1.15)	24.00 (1.73)	20.67 (1.15)	18.67 (1.58)	16.00 (1.00)	14.67 (2.08)	11.67 (2.08)
Astra tabook (N–S)	28.00 (1.00)	24.33(1.53)	20.00 (2.00)	19.67 (1.53)	16.00 (5.29)	13.33 (1.53)	12.67 (1.15)
Sidir (S–S)	30.00 (0.00)	26.33 (1.15)	24.33 (0.58)	21.33 (1.15)	19.33 (1.15)	12.33 (1.73)	8.20 (2.52)
Sidir (S–W)	32.67 (1.15)	29.33 (1.15)	22.33(2.52)	21.00 (1.73)	20.33 (1.53)	18.67 (1.53)	14.33 (1.15)

* Diameter of zone of inhibition in mm (average of four replicates) ± Standard Error.

Table 2. Complete inhibitory effect of various dilutions of natural and artificial honey on *Staphylococcus aureus.*

Honey Sample	Diameter of zone of inhibition (mm) ± S.E at different honey concentrations						
	50%	30%	25%	20%	15%	10%	5%
Artificial Honey	-	-	-	-	-	-	-
Samra (S–W)	11.33 (0.58)	10.00 (1.15)	9.33 (0.58)	-	-	-	-
Samra (W–S)	15.33 (1.53)	14.33 (1.53)	11.67 (0.58)	-	-	-	-
Saha (W–S)	10.33 (0.58)	-	-	-	-	-	-
Samra (S–S)	15.00 (1.00)	13.67 (0.58)	10.33 (0.58)	-	-	-	-
Magra (W-W)	-	-	-	-	-	-	-
Katada (W–W)	-	-	-	-	-	-	-
Astra Tabook (N–W)	-	-	-	-	-	-	-
Astra tabook (N–S)	-	-	-	-	-	-	-
Sidir (S–S)	19.00 (1.73)	17.33 (1.53)	9.33 (1.15)	-	-	-	-
Sidir (S–W)	15.33 (2.08)	14.67 (1.53)	7.33 (1.15)	-	-	-	-

* Diameter of zone of inhibition in mm (average of four replicates) ± Standard Error.

Table 3. Partial inhibitory effect of various dilutions of natural and artificial honey on *E.coli.*

Honey Sample	Diameter of zone of inhibition (mm) ± S.E at different honey concentrations						
	50%	30%	25%	20%	15%	10%	5%
Artificial Honey	27.33 (1.15)	24.33 (0.58)	22.33 (1.15)	20.67 (1.15)	15.67 (1.15)	-	-
Samra (S–W)	27.67 (2.08)	27.67 (0.58)	24.00 (1.00)	22.33 (1.53)	21.00 (1.00)	19.67 (0.58)	18.67 (0.58)
Samra (W–S)	29.67 (1.53)	26.67 (1.15)	24.33 (2.08)	23.67 (0.58)	21.33 (1.15)	19.00 (1.73)	-
Saha (W–S)	30.33 (0.58)	26.67 (1.15)	25.33 (1.53)	23.00 (1.00)	22.33 (1.15)	21.33 (1.15)	18.33 (1.53)
Samra (S–S)	28.00 (2.00)	27.33 (1.15)	25.33 (0.58)	24.33 (0.58)	19.00 (1.15)	20.00 (1.00) 16.00	13.00 (1.73)
Magra (W-W)	29.00 (1.00)	23.33 (2.89)	21.00 (1.00)	20.33 (0.58)	21.00 (1.00)	(1.00)	11.00 (1.00)
Katada (W–W)	30.00 (1.00)	28.67 (0.58)	26.00 (1.73)	25.00 (0.00)	22.67 (1.53)	22.33 (2.52)	20.33 (0.58)
Astra Tabook (N–W)	29.33 (0.58)	25.00 (1.00)	24.00 (0.58)	23.33 (1.15)	24.00 (0.00)	20.00 (1.15)	18.67 (1.53)
Astra tabook (N–S)	27.33 (1.15)	26.00 (2.00)	25.00 (2.00)	24.00 (2.00)	23.00 (2.00)	20.67 (1.53)	14.33 (0.53)
Sidir (S–S)	30.00 (2.00)	25.33 (1.15)	23.33 (1.15)	21.33 (1.15)	20.33 (1.53)	18.00 (1.73)	13.00 (1.73)
Sidir (S–W)	32.00 (0.00)	27.33 (1.15)	23.33 (1.53)	23.33 (0.58)	21.33 (1.15)	19.67 (0.58)	16.33 (1.53)

* Diameter of zone of inhibition in mm (average of four replicates) ± Standard Error.

Table 4. Antibacterial activity of different honey samples tested with and without catalase on *staphylococcus aureus*.

Solution Tested		Diameter of Inhibition zone Growth, mm(average of four replicates) ± Standard Error									
		Samra (S-W)	Samra (W-S)	Saha (W-S)	Samra (S-S)	Magra (W-W)	Katada(W-W)	Astra Tabook (N-W)	Astra Tabook(N-S)	Sidir (S-S)	Sidir (S-W)
Honey[1]	p.I.[2]	32.33 (1.53)	30.67 (0.58)	32.33 (0.58)	30.67 (0.58)	30.00 (1.00)	26.67 (1.53)	26.67 (2.52)	29.00 (1.00)	30.67 (1.53)	31.67 (0.58)
	C.I.[3]	8.33(1.4)	10.67 (1.15)	8.60 (0.52)	10.00 (-)	-	-	-	-	18.00 (2.00)	11.33 (0.58)
Honey + Catalase	P.I.	33.33 (1.15)	31.00 (1.73)	32.67 (0.58)	33.33 (0.58)	29.33 (1.15)	27.00 (1.73)	27.00 (2.00)	29.33 (1.15)	31.33 (1.15)	31.67 (2.08)
	C.I.	-	-	-	-	-	-	-	-	-	-
Honey + H_2O_2	P.I.	33.00 (1.00)	31.33 (0.58)	33.33 (0.58)	33.67 (1.58)	31.33 (1.15)	30.67 (1.15)	29.67 (2.52)	29.33 (2.08)	32.00 (1.00)	31.67 (0.58)
	C.I.	11.33 (1.15)	10.67 (0.58)	13.00 (5.20)	10.33 (1.00)	23.00 (4.00)	23.67 (3.21)	10.00 (1.73)	11.00 (2.65)	21.67 (0.58)	16.33 (0.58)
Honey + Catalase + H_2O_2	P.I.	30.67 (1.15)	30.67 (1.53)	31.00 (1.00)	34.00 (1.00)	28.67 (0.58)	27.00 (1.73)	26.00 (3.00)	31.33 (4.16)	31.00 (1.00)	32.33 (1.00)
	C.I.	-	-	-	-	-	-	-	-	-	-
Catalase	P.I.	-	-	-	-	-	-	-	-	-	-
	C.I.	-	-	-	-	-	-	-	-	-	-
H_2O_2	P.I.	-	-	-	-	-	-	-	-	-	-
	C.I.	22.33 (0.58)	23.67 (2.31)	24.00 (1.00)	23.33 (0.58)	24.00 (2.65)	22.33 (2.08)	23.67 (2.89)	22.67 (2.08)	22.33 (1.15)	22.00 (1.00)

1= Honey tested at 50% dilution (W:V); 2= Zone of partial inhibition of growth; 3= C.I Zone of complete inhibition of growth

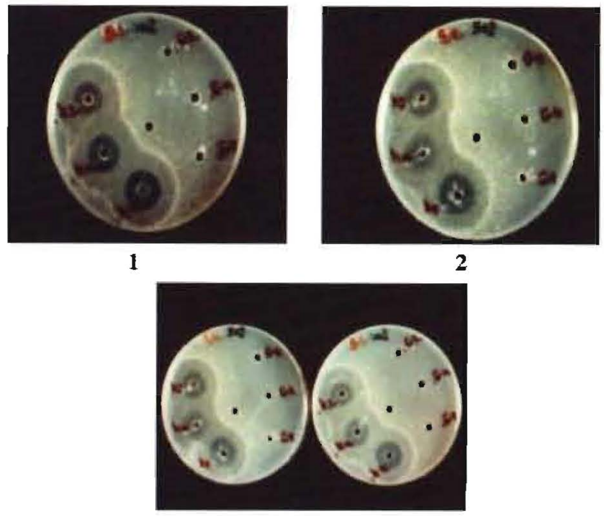

Fig. 1. Development of complete and partial inhibition zones for S. aureus growth in honey types Samra (W-S) (#1), Samra (S-S) (#2), and Sidir (S-S) (#3) at 100% and 50% concentrations.

Acidity: The contribution of acidity to the antibacterial effect of honey was tested through comparing the effect of gluconic acid of equivalent acidity and pH to that of 3 natural honey types on the growth of *S. aureus*. As shown in Figure 1, honey types Samra (W-S), Samra (S-S) and Sidir (S-S) at concentrations of 100% and 50% caused the production of both zones of complete and partial inhibition of growth of *S. aureus*. On the other hand, when a solution of gluconic acid of equivalent acidity and pH to each of the 3 honey types tested, was applied on the same agar plate seeded with *S. aureus*, it had no effect on its growth. This indicates that the acidity of gluconic acid is not one of the factors contributing to the antibacterial effect of the tested honey types.

Discussion

In order to explain the antibacterial activity of honey, the first factor investigated was osmotic effect. The use of artificial honey of the same sugar proportions as that of typical honey has caused the formation of zones of partial inhibition of growth of *S. aureus* and *E.coli*, but it did not cause the formation of zones of complete inhibition of growth against either organism. The use of natural honey at 50% sugar concentration has caused the formation of zones of complete inhibition of growth for honey types Samra (S-W), Samra (W-S), Saha (W-S), Samra (S-S), Sidir (S-S) and Sidir (S-W). These findings are in agreement with those of Pothmann (1992) who found only a very low degree of inhibition of *E. coli* and *S. aureus* with artificial honey compared with that from natural honey.

The bacteriostatic effect of Saudi natural honey was maintained up to 10% for most honey types and even up to 5% concentration for others. Artificial honey showed similar bacteriostatic effect up to 15% concentration but no bacterial effect. This is similar to the findings of christou and Mladenov (1961) who found bacteriostatic effect with 20% artificial honey, but both bacteriostatic and bactericidal effects with 20% down to 0.6% natural honey. In a study by Ali *et al.* (1991) on natural Saudi honey, artificial honey had no effect on any of 10 bacterial species tested including *S. aureus* and *E.coli* even at 30% concentration. Natural honey inhibited the growth of both organisms at 10% and 30% respectively. Unfortunately they did not mention the origin, type or any characteristics of the honey which could have allowed better comparison with our results. Thus, it can be concluded that osmolarity is involved in the antibacterial activity of honey.

Investigating the contribution of the acidity of the honey has indicated that such a factor might not be regarded as being of primary importance. Gluconic acid which is the main acid in honey (Stinson *et al.,* 1960) had no detectable antibacterial effect when tested in an agar diffusion assay in comparison with the 3 honey types selected as representing the more active types. Exactly the same finding was reached by Molan and Russell (1988) where natural honey was effective at 12.5% but not buffered glucolactone/gluconic acid solution made to match the most acidic honey samples. Although this finding may indicate that the acidity of honey is not important, it does not mean that acidity does not contribute to the antibacterial activity of honey.

With respect to the hydrogen peroxide effect, all honey types included in the study were tested in presence or absence of the enzyme catalase known to destroy hydrogen peroxide (Adcock 1962, Allen *et al.,* 1991). The results clearly indicate that hydrogen peroxide is playing a major role in the bacterial activity of honey. For honey types showing higher levels of antibacterial activity in the form of zones of both partial and complete inhibition of growth, the zones of complete inhibition of *S. aureus* disappeared upon the addition of catalase, but not those of the partial inhibition. For other honey types where the effect is only partial inhibition of growth such effect was not altered upon the addition of catalase. Thus, the results could be explained by the assumption that the zone of partial inhibition of growth could primarily be due to the osmotic effect of honey. The formation of zone of complete inhibition of growth could primarily be due to the hydrogen peroxide in honey. A similar finding has been reached by other workers (White and Subers; 1963; Russell *et al.*; 1985; Molan and Russell, 1988). White *et al.* (1963) reported that gluconic acid and hydrogen peroxide are produced by the action of glucose oxidase enzyme. Such enzyme being present in the hypopharyngeal glands of the honey bee (Gauhe, 1941).

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