

HYDROGEN PEROXIDE OF HONEY ANTIBACTERIAL EFFECT AGAINST COAGULATES-NEGATIVE STAPHYLOCOCCI

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ABSTRACT

The aim of this study was to determine the minimum active dilution of two standardized type, representative honeys from two bees farm for 7 clinical isolates of coagulates-negative staphylococci.

An agar incorporation technique was used to determine the minimum active dilution, with dilution steps for 1% (v/v) honey and 5% (v/v) for sugar syrup .The plates were inoculated with 10 μ L spots of cultures of the isolates.

the pasture honey inhibited at mean $SD3.2 \pm 0.7\%$ (v/v), $3.3 \pm 0.5\%$ (v/v) for the Manuk honey and $30.9 \pm 1.9\%$ (v/v) for the sugar syrup. Typical honeys are about eight times more potent against coagulase-negative staphylococci than bacterial inhibition were due to their osmolarity alone, but also the activity of honey by having its content.

INTRODUCTION

Honey is very useful against infection and diseases so that ancient used for its medical properties. Honey is carbohydrate-rich syrup produced by bees, primarily from floral nectars. Fructose and glucose are the major components but a large number of other chemical compounds are present in small quantities.¹ Moisture content and water activity are low¹. Four aspects of the composition of honey have been identified to contribute to its antibacterial activity. The low water activity inhibits microbial growth, particularly bacterial growth. Its low pH, a result of the formation of gluconic acid, also has a mild antibacterial effect^{2,3}. Until the 1960s coagulated-negative staphylococci were regarded as saprophytic commensally of low pathogenicity that normally inhabit human skin and mucosal membranes. However, increased recovery rates from clinical specimens prompted a re-evaluation of their clinical status to opportunist pathogens and they are now among the five most frequently isolated causative agents of hospital-acquired infection, often associated with the use of temporary and permanent invasive medical devices (e.g. intravenous catheters, continuous ambulatory

peritoneal dialysis catheters, urethral stents, because of their ability to adhere to synthetic polymeric biomaterials and form biofilms^{4,5}. The reasons for the antibacterial activity of honey are controversial. A laboratory demonstration of its antibacterial activity was first carried out by Dock *et al* who gave the name inhibine to substance which inhibited bacteria. Dock first suggested the possibility that hydrogen peroxide was responsible for the antibacterial activity of honey since both the antibacterial activity of honey and hydrogen peroxide were destroyed by light⁶. White reported that hydrogen peroxidase which is produced by the glucose oxidase of honey could be the inhibitory substance against bacteria. However, it is known that honey itself, as well as bacteria produce a catalase that eliminates hydrogen peroxide, but although catalase is active with high concentration of hydrogen peroxide, it is of low activity with physiological levels. Hydrogen peroxide rapidly breaks down into water and oxygen and its production and decomposition are continuous. The hydrogen peroxide concentrations remain stable under given set of conditions of temperature, sugar concentration etc, and is sufficiently high to give good protection against some harmful microorganisms by a biochemical mechanism which disrupts their metabolism. The same system is thought to operate when honey is diluted with water and for this reason, honey has been successfully used as a microbicidal wound dressing^{7,8}. Non-dissociated organic acids also play a role in the antimicrobial activity of honey since they are very soluble in cell membranes and induce alterations in the cellular permeability and in oxidative phosphorylation^{9,10}. The floral sources of honey may also be responsible for some of the antibacterial activity of honey^{11,12}. Antibacterial activity of honey varies not only between floral sources but even within one floral source representative honeys with median levels of activity were used. Most commercial honey is produced by the species, *Apis mellifera*. The antimicrobial activity in most honeys is due to the enzymic production of hydrogen peroxide, but honey from some *Leptospermum* species, such as manuka, can also have a high antimicrobial activity due to an unidentified phytochemical component, honey from other species, also have antibacterial activity by content hydrogen peroxides but in low level such as pasture honey¹³, we studied the activities of two types of honey and the activity of syrup stimulating honey (as control) and any osmotic inhibition of bacteria.

MATERIAL AND METHODS

Honey

The activity of two type of honey were tested against *Staphylococcus aureus*:⁽⁹⁾ a honey A(PASTURE) with non-peroxide activity equivalent to 16.8% (w/v) phenol and a honey B(MANUKA) with hydrogen peroxide activity equivalent to 17.5% (w/v) phenol. The simulated honey was prepared by combining 38.4 g of fructose, 30.3 g of glucose, 1.3 g of sucrose, 8.6 g of maltose and 1.4 g of maltodextrin with 17.2 mL of distilled water.

Bacterial isolates

coagulate-negative staphylococci were obtained from Cultures were isolated from midstream and catheter urines, catheter tip and blood cultures. The isolates were identified using a range of biochemical and morphological techniques. The isolates were stored at 37°C.

Microbiological materials

1. Tryptic soy broth (TSB).
2. Nutrient agar was obtained .
3. Blood agar base was obtained.
4. 5% sterile sheep's blood was added.

Determination of minimum active dilution of honey

Prior to testing, each isolate was cultured from preserver beads by inoculating two beads into 9 mL of TSB and incubating for 16 h at 37°C. The obtained culture was diluted with TSB to obtain 2–3 x 10⁷ cfu/mL, the minimum to produce confluent growth at inoculation positions.

The minimum active dilution of each honey for each of the clinical isolates was determined by an agar incorporation technique. Nutrient agar was made up at double strength, measured out into 25 mL aliquots and autoclaved. To prepare the plates it was melted and tempered in a 50°C water-bath until poured. Solutions of the two natural honey samples (at a concentration of 20% v/v) and the simulated honey (at a concentration of 70% v/v) were prepared in sterile de-ionized water immediately prior to performing an assay and diluted with different volumes of sterile de-ionized water to give double the final concentration required in a volume of 25 ml. These solutions were then also tempered at 50°C, then each mixed with one of the 25 ml lots of double-strength nutrient agar. The various agar–honey mixtures were then poured into duplicate Petri dishes.

A dilution series with honey concentrations in the range 1–10% (v/v) final honey concentration, in 1% increments, was used for the susceptibility assays for the natural honeys, and in the range 5–35% (v/v) final honey concentration, in 5% increments, for the simulated honey. Duplicate control plates of nutrient agar with no honey were included in each susceptibility assay to confirm the viability and density of the cultures.

Samples (10 µl) of each culture were inoculated onto the agar plates in three rows of three spots using channel auto-pipettor with tips attached to channels, to obtain two strains inoculated per plate as evenly spaced spots. Duplicate plates were inoculated and assays were repeated on two subsequent days, with fresh subcultures on each occasion.

The inoculated plates were incubated at 37°C for 16 h , partial inhibition or complete inhibition of grow was recorded at each inoculation position. The minimum active dilution was taken to be the lowest concentration of honey at which bacterial growth was completely inhibited, and the mean value for the minimum active dilution was calculated.

RESULTS

In (table 1), it can be seen that the growth of 7 coagulase-negative staphylococcus isolates was inhibited by manuka and pasture honeys at concentrations of 3-3.7% (v/v). By contrast, simulated honey inhibited the various isolates at concentrations of 30-32.3% (v/v), showing that the antibacterial activity observed with the natural honeys was greater than that of stimulating honey due to the osmotic effect of the sugar content of honey.

Table 1:- Minimum active dilution of representative honeys determined for (7) isolates of coagulates-negative staphylococci

Isolated No.	Species	Antibiotic Resistance	Honey Pasture minimum active dilution (%v/v)		Honey Manuka aminimum active dilution (%v/v)		Stimulation honey minimum active dilution (%v/v)	
			Mean	SD	Mean	SD	Mean	SD
119	Staphylococcus epidermidis	FL,MET	3.2	0.5	3	0.0	31.0	0.0
118	S.epidermidis	FL,GEN,MET	3	0.0	3	0.0	31.8	2.6
125	S.epdermidis	FL,GEN,RIF	3	0.0	3.7	0.5	32.3	2.6
128	S.epidermidis		3.7	0.5	3.5	0.5	30	2.6
126	S.epidermidis		3	0.5	3	0.9	30	0.0
102	S.haemolytices	FA,FL	3.4	0.5	3.5	0.9	31.7	2.6
123	S.haemolytices		3.2	1.0	3.7	0.5	30	0.0

FA= fusidic acid resistant.

FL=flucoxacillin resistant.

GEN=gentamicin resistant

MET=methicillin resistant.

RIF=rifampin resistant.

The values show are the mean values obtained from duplicated determinations on 3 separate days with fresh subculture

Table 2. Mean values for grouped results (data from Table 1).

Group	Honey Pasture minimum active dilution (% v/v)		Honey Manuka minimum active dilution (% v/v)		Stimulated honey minimum active dilution (% v/v)	
	Mean	SD	Mean	SD	Mean	SD
All isolated no. =7	3.2	0.3	3.3	0.4	30.9	1.48
Antibiotic resistance isolated =4	3.15	0.25	3.3	0.35	31.25	1.95
Antibiotic susctible isolated =3	0.6	0.0	3.4	0.6	30	0.8
Staphylococcus epidermis =5	3.18	0.3	3.24	0.38	31.02	1.5
Staphylococcus haemolytics =2	3.3	0.75	3.6	0.7	30.85	0.0

The mean values for the results in various groupings of the data are shown in (table 2). There was no significant difference between the two types of natural honey for all 7 isolates ($P = 0.44$), or between the antibiotic-resistant and antibiotic-susceptible isolates ($P = 0.35$), or between any of the species of bacteria ($P = 0.66$)

DISCUSSION

The results of this study clearly show that honey has the potential to be used as an antibacterial agent to prevent and control infection with coagulase-negative staphylococci. The lack of significant difference in susceptibility to honey between any of the isolates tested ($P = 0.13$) indicates that other isolates are likely to be equally as susceptible. The similarity in susceptibility to honey between antibiotic-resistant and antibiotic-susceptible strains was also seen with *S. aureus*^{14,5,16}.

These findings show that coagulase-negative staphylococci are very similar to *S. aureus* in their susceptibility to honey of similar antibacterial potency and more susceptible than *Pseudomonas aeruginosa* and *Enterococcus* species¹². Thus, they can be expected to be

controlled by honey in vivo since there are many reports of honey rapidly healing wounds infected with *S. aureus* and *pseudomonads*. The results show that honey could be diluted by exudates up to 20-fold and still inhibit the growth of coagulase-negative staphylococci. Honey, therefore, would be suitably active for both therapeutic and prophylactic application¹³. There are other advantages in applying honey to the traumatized tissue around medical devices^{17,18,19}. Its anti-inflammatory activity can be expected to prevent serous exudates, which can provide a medium for bacteria to colonize. Also, its physical properties provide moist conditions ideal for healing and it has a stimulatory action on growth of wound repair tissues. Furthermore, unlike other antiseptics it has no harmful effects on tissues, the slow enzymic production of hydrogen peroxide giving about one thousandth of that in a 3% hydrogen peroxide solution.^{20,21}

The development of honey in the form of a rubbery gel that can be moulded to conform to any shape will further increase the practicality of use with medical devices beyond that with the honey-impregnated dressings currently available^{22,23}. It remains for further clinical evaluation to be tried.

فعالية بيروكسيد الهيدروجين في العسل ضد المكورات العنقودية السالبة للتجلط

رنا عدنان فائز

فرع الإحياء المجهرية - كلية الطب البيطري - جامعة البصرة - البصرة - العراق

الخلاصة

هدف هذه الدراسة هو تحديد التخفيف الأدنى الفعال من نوعين قياسييين من مزرعتين لنحل العسل لاختبار سبعة عزلات سريرييه للمكورات العنقودية السالبة للتجلط. تقنية طعن الاكار استعملت لتحديد التخفيف النشط الأدنى، بخطوات تخفيف 1% (v/v) عسل أو 5% (v/v) من شراب السكر يرجع إلى التأثير التنافي للعسل. الإطباق تحقن بـ 10 µl من عزلات البكتريا. العسل يثبط الجراثيم في التخفيف (3.2±.7)% (v/v) للعسل PASTURE، (3.3±.5)% (v/v) للعسل (Manuk) و(30.9 ±1.9)% (v/v) شراب السكر. العسل المثالي حوالي ثمان مرات أكثر فعالية ضد المكورات العنقودية السالبة للتجلط بسبب ضغطها التبادلي لوحده، لكن أيضاً نشاط العسل بامتلاك محتواه.

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