The influence of catalase on the antibacterial activity of honey

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ABSTRACT

Honey has high sugar content, low water content and it is acidic. These properties restrict the growth of micro-organisms and explain why honey rarely spoils in storage. Some honeys generate hydrogen peroxide on dilution which contributes to antibacterial activity[1], whereas others additionally possess plant-derived components[2]. In clinical use honey will be diluted by body fluids. This study was designed to determine whether a selection of honeys retained the ability to inhibit six cultures of bacteria on dilution in the presence and absence of catalase (an enzyme associated with human tissue that degrades hydrogen peroxide). Ten table honeys and one medical grade honey* were tested against two reference bacteria (*Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571) and four clinical isolates from infected wounds (*Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus pyogenes*). An artificial honey solution was also used to establish whether inhibition was due to the sugars contained in honey. Using a serial dilution in isosensitest broth (ISB) the lowest concentration of honey (MIC) capable of inhibiting each culture was determined in the presence and absence of 1 mg/ml catalase. The medical grade honey was more effective at inhibiting bacteria than the table honeys. Only four honeys retained activity that was significantly higher than the artificial honey solution in the presence of catalase. Since catalase is present in plasma and human tissue, honeys chosen for clinical use must be carefully evaluated in the laboratory for activity that is retained in the presence of catalase.

* Comvita manukacare 18+

INTRODUCTION

Honey is an ancient topical treatment for wounds that has been re-introduced into contemporary practice. Modern formulations use medical grade honey (MGH) and are sterilised prior to use. Honey is universally recognised and it is readily accessible. Patients and practitioners may consider using table honey from supermarkets as a cheap, readily available alternative to more expensive, regulated, honey-based wound care products. This study was designed to compare the antibacterial action of 10 table honeys to a representative sample of a MGH (manuka honey) with a view to exploring their therapeutic potential.

METHODS & MATERIALS

Nine honeys were purchased from British supermarkets and one honey came from a French market stall. A medical grade Active Leptospermum Honey (Manukacare 18+™) was provided by Comvita UK Ltd; this honey is marketed in Canada and US as Medihoney™. An artificial honey solution was also tested to determine activity attributable to sugars (100 g was prepared by dissolving 1.5 g sucrose, 7.5 g maltose, 40.5 g fructose and 33.5 g glucose in 17 ml sterile deionized water). It's activity in the presence of catalase would not alter because the enzyme that generates hydrogen peroxide was not included. Using serial doubling dilutions in isosensitest broth (ISB) the lowest concentration of honey capable of preventing the growth of each of 6 bacterial cultures was determined in the presence and absence of 1 mg/ml catalase in microtitre plates incubated at 37°C for 24 hours. Organisms tested were *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571, EMRSA-15 NCTC 13142 and three clinical isolates from infected wounds (*Pseudomonas aeruginosa, Staphylococcus epidermidis and Streptococcus pyogenes*). NB. Contrary to the abstract *Enterococcus faecalis* failed to grow from stock culture and was replaced by MRSA.

DISCUSSION

The results of this study demonstrate varying antibacterial activity associated with different honeys and illustrate the importance of selecting a honey of high potency when attempting to limit the growth of bacteria capable of causing wound infection. Seven of the samples tested exhibited diminished activity in the presence of catalase, indicating that their activity in the absence of catalase involved the generation of hydrogen peroxide. Since catalase is present in plasma and human tissue, it is probable that the antibacterial activity of these honeys would be rapidly inactivated in clinical use. The MGH tested here, however, is not likely to be readily inactivated by catalase.

RESULTS

In the absence of catalase, the medical grade honey (MGH) was found to be most effective by preventing growth of test organisms at the lowest concentrations (Table 1). In the presence of catalase,the activity of 7 of the honey samples *(shaded blue)* was no more effective than that of the artificial honey solution, demonstrating that their activity on dilution was due exclusively to the generation of hydrogen peroxide. Four honey samples retained activity in the presence of catalase *(shaded orange)* demonstrating that their activity on dilution was due to factors additional to hydrogen peroxide generation and sugar content. MGH exhibited the greatest activity.

TABLE 1

ANTIBACTERIAL ACTION OF HONEYS EXPRESSED AS THE LOWEST CONCENTRATION (G/ML) TO INHIBIT GROWTH

Honey	*S. aureus* NCTC 6571		*E. coli* NCTC 10418		MRSA NCTC 13142		*Streptococcus pyogenes*		*Staphylococcus epidermidis*		*Pseudomonas aeruginosa*	
	MIC	MIC + cat	MIC	MIC + cat	MIC	MIC + cat	MIC	MIC + cat	MIC	MIC + cat	MIC	MIC + cat
Artificial	1		0.5		1		0.25		1		0.5	
Clover	0.5	1	1	1	0.5	1	0.5	1	0.5	1	0.5	1
Heather	0.125	1	0.25	1	0.5	1	0.5	1	0.5	1	0.5	1
Manuka	0.25	0.25	0.5	0.5	0.25	0.5	0.25	0.25	0.25	0.25	0.5	0.5
Eucalyptus	0.25	1	0.5	1	0.25	1	0.25	0.5	0.25	1	0.5	0.5
Sunflower	0.5	1	0.5	1	0.5	1	0.25	1	0.5	1	0.5	1
Lavender	0.5	1	0.5	1	0.5	1	0.25	0.25	0.5	1	0.5	0.5
Heather	0.5	1	0.5	0.5	0.5	1	0.25	0.5	0.5	1	0.5	0.5
Tasmanian leatherwood	0.25	1	0.5	0.5	0.25	1	0.25	0.25	0.25	1	0.5	0.5
Manuka	0.25	0.25	0.5	0.5	0.25	0.5	0.125	0.25	0.25	0.25	0.5	0.5
Buckwheat	0.5	0.5	0.5	0.5	0.5	0.5	0.063	0.5	0.5	0.5	0.5	0.5
MGH	0.125	0.125	0.25	0.25	0.125	0.125	<0.03	0.063	0.125	0.125	0.25	0.5

References:

1 White JW, Subers MH, Schepartz AI. (1963). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. Biochim. Biophys. Acta 73: 57-70

2 Molan PC. (1992). The antibacterial nature of honey: 1. The nature of the antibacterial activity. Bee World 73(1): 5-28

*MEDIHONEY™ Absorbent Calcium Alginate Dressing with *Leptospermum* Honey, Derma Sciences, Inc., Princeton, New Jersey.
Funding for costs associated with this poster provided by Derma Sciences.