

Osmotic Effect of Honey on Growth and Viability of *Helicobacter pylori*

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Honey from New Zealand and Saudi Arabia at concentrations approximating 20% (v/v) inhibit the growth of *H. pylori in vitro*. The anti-*H. pylori* effect involves both hydrogen peroxide- and non-peroxide-mediated killing mechanisms. This study was designed to determine whether the anti-*H. pylori* activity of honey differed regionally (honey from Texas, Iowa, and New Zealand) and to determine whether this activity was due to the presence of hydrogen peroxide. Broth dilution susceptibility tests were performed using solutions of honey prepared in BHI broth ranging in concentration from 5 to 35% (v/v) in 5% increments. Control solutions containing glucose, fructose, and combined glucose/fructose solutions in ratios of 1:1.23 were also prepared. Paired catalase controls were included in all tests. Twenty-eight clinical isolates of *H. pylori* were tested. Growth was determined on the basis of a plus/minus grading score. All of the solutions containing either fructose, glucose, glucose and fructose combinations, or honey were equally effective in inhibiting the growth of *H. pylori*. All of the isolates were inhibited by solutions containing 15% (w/v) carbohydrate. Honey solutions, with or without catalase, inhibited 24/28 isolates at a concentration of 10%, and 28/28 isolates at a concentration of 15%. In conclusion, regional differences in honey activity against *H. pylori* were not detected, nor was the effect of killing related to the presence of hydrogen peroxide in the honey samples. Osmotic effects were shown to be the most important parameter for killing *H. pylori* as all carbohydrate solutions $\geq 15\%$ (v/v) inhibited 100% of the *H. pylori*.

KEY WORDS: *Helicobacter pylori*; honey; antibacterial activity; hydrogen peroxide; osmolarity.

Honey has been used as a folk remedy for various ailments, but its heterogeneous composition has limited scientific evaluation of this material. Recently, minimal criteria necessary for classifying a product as honey have been published (1). The observation that honey produced in New Zealand and Saudi Arabia at concentrations approximating

20% (v/v) can inhibit the growth of *H. pylori in vitro* (2, 3) prompted this investigation to evaluate other honey varieties for antimicrobial effect. There are multiple mechanisms of the anti-*H. pylori* effect of honey involving both hydrogen peroxide- and non-peroxide-mediated killing. Honey varieties higher in hydrogen peroxide content are more effective in killing *H. pylori* as compared to honey varieties with lower levels of hydrogen peroxide (3, 4). This study was designed to determine whether honey from different places (Texas, Iowa, New Zealand) differed with regard to the anti-*H. pylori*-inhibiting activity and to determine whether this activity was due to the presence of hydrogen peroxide in the honey.

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TABLE 1. COMPOSITION OF HONEY AS DEFINED IN FLORIDA
EXTENSION BEEKEEPING NEWSLETTER

	Average	Range	Standard deviation
Fructose-glucose ratio	1.23	0.76-1.86	0.126
Fructose (%)	38.38	30.91-44.26	1.77
Glucose (%)	30.31	22.89-40.75	3.04
Minerals (ash, %)	0.169	0.020-1.028	0.15
Moisture (%)	17.2	13.4-22.9	1.46
Reducing sugars (%)	76.75	61.39-83.72	2.76
Sucrose (%)	1.31	0.25-7.57	0.87
Total acidity (meq/kg)	29.12	8.68-59.49	10.33
True protein (mg/100 g)	168.6	57.7-567	70.90

MATERIALS AND METHODS

Solutions of honey (Roy Freese, Glen Flora, Texas, Ray and Pat's Wildflower Honey, Houston, Texas, SueBee Clover Honey, Souix City, Iowa, Creamed Manuka Honey, Airborne Honey Ltd., Leeston, New Zealand) were prepared in supplemented brain-heart infusion (BHI) broth (Difco Laboratories, Detroit, Michigan) containing 0.25% yeast extract (Difco) and 10% horse serum (HyClone Laboratories, Orem, Utah) ranging in concentration from 5 to 35% (v/v) in 5% increments. Control solutions containing glucose (10-30%), fructose (10-40%), and combined glucose-fructose solutions in ratios of 1:1.23 were also prepared. The 1:1.23 ratio of glucose-fructose reflected their composition in honey as defined by the Florida Beekeeping Extension Service (Table 1). Paired catalase controls, ie identical samples of honey with and without catalase, were also included in the tests to ascertain the effect of hydrogen peroxide content on the growth and viability of *H. pylori*. To each 1 ml honey solution, 100 μ l catalase (Worthington Biochemical Corp., Freehold, New Jersey, 50,514 units/mg P, 0.98 mg P/ml) were added (~ 5000 units). Broth dilution assays were then performed. All wells in the cluster dish (Corning Glass Works, Corning, New York) contained 1 ml of BHI-sugar and/or honey solution and were inoculated with 20 μ l of a cell suspension equivalent in density to a No. 2 McFarland standard (6×10^8 bacteria/ml). Twenty-eight isolates of *H. pylori* recovered from patients with duodenal ulcer were tested. The culture dishes were gently agitated following the addition of the inoculum, then placed at 37°C under 12% CO₂ for three days. At the end of incubation, 10 μ l aliquots from each well were transferred to Mueller-Hinton agar plates (BBL, Cockeysville, Maryland) and incubated under 12% CO₂ for an additional three days. Growth was determined on the basis of a plus/minus grading scheme.

RESULTS

All three honey samples inhibited the growth of *H. pylori* at concentrations >15% (v/v) (Figure 1). The Manuka honey from New Zealand demonstrated the greatest inhibitory activity, inhibiting 100% of the isolates at a concentration of \leq 10% (v/v). The US-produced honey samples inhibited 14/18 isolates at a concentration of \leq 10% (v/v), and 100% at a concen-

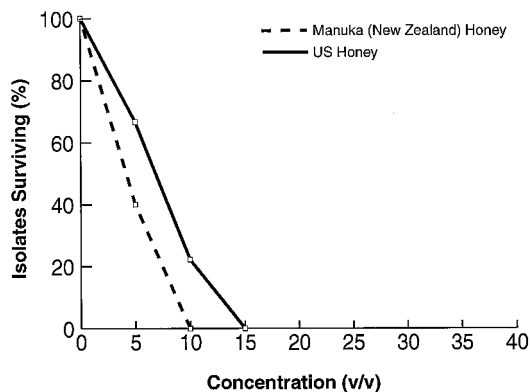


Fig 1. Percent of *H. pylori* isolates surviving after exposure to honey or carbohydrate solutions for three days. Growth of all isolates was inhibited at a concentration of \geq 15% honey or carbohydrate with or without catalase.

tration of \geq 15% (v/v). These values were not statistically significantly different as determined by the Fisher exact test ($P = 0.243$ at 5% honey and $P = 0.265$ at 10% honey).

All solutions containing either glucose, fructose, or glucose-fructose combinations \geq 15% inhibited growth of 18/18 *H. pylori* isolates. These results were identical to those of the US produced honeys at all concentrations (Figure 1). However, the inhibitory effect was not the result of hydrogen peroxide content. Treatment of honey with catalase did not alter the *in vitro* *H. pylori* inhibitory effect as compared to honey alone. In only three instances did the result vary between catalase-treated honey and the effect of honey alone. In all three cases, inhibition of growth was recorded in the next higher concentration of honey (10% with catalase vs 5% without catalase).

DISCUSSION

Honey is a folk remedy for a number of ailments. Studies have shown natural honeys to have significant inhibitory activity against a number of bacterial genera including *Streptococcus*, *Bacteroides*, *Staphylococcus*, *Prevotella*, and various enteropathogens (4-9). Recently, honey from non-US sources (Saudi Arabia, New Zealand) has also been shown to inhibit *H. pylori* in concentrations of 20-30% (v/v) (3, 4). While the mechanism by which honey facilitates microbial killing involves multiple pathways, the hydrogen peroxide content of honey seems to be an important component in the killing (3, 4). Nonperoxide and osmotic effects have also been postulated to effect microbial killing of honey (3, 4, 6).

The US-produced honeys inhibited growth of 18/18 test isolates at a concentration \geq 15 percent (v/v);

78% (14/18) were inhibited by $\leq 10\%$ (v/v) honey, and 6/18 (33%) at a concentration of $\leq 5\%$ (v/v) honey. In comparison, the New Zealand manuka honey inhibited 6/10 (60%) test isolates at a concentration of $\leq 5\%$ (v/v) and 10/10 isolates at a concentration of $\leq 10\%$ (v/v) honey with and without catalase.

Our data suggest that the mechanism for growth inhibition in the US-produced honeys is unrelated to the presence of hydrogen peroxide. Addition of catalase to test plates containing paired samples of honey resulted in inhibitory activity identical to that of honey alone in all but three instances. These data confirm the findings of Allen et al (6), Willix et al (4), and al Somal et al (3), who showed that the antimicrobial killing effect of manuka honey was due to nonperoxide mechanisms. While regional differences may influence the amount of hydrogen peroxide in honey samples, our data suggest that nonperoxide mechanisms are equally important in effecting microbial killing.

Using a document from the National Honey Board's Product Research and Development Committee as a basis (Table 1) (1), we prepared and tested comparable carbohydrate solutions separately and in combinations to ascertain whether osmotic shock could be responsible for the antibacterial effect of honey. Control solutions containing glucose, fructose, and glucose-fructose (1:1.23 ratio) in concentrations from 10 to 35% (v/v) were evaluated. All 18 *H. pylori* isolates were inhibited by solutions containing $\geq 15\%$ (v/v) carbohydrate, an identical inhibitory pattern as seen with the US-produced honeys alone, suggesting that osmotic effects were paramount in effecting *H. pylori* growth inhibition.

In conclusion, US- and New Zealand-produced

honeys were effective in inhibiting the growth of *H. pylori* at concentrations of $\geq 15\%$ (v/v). Osmotic effects, not hydrogen peroxide content, were shown to be responsible for the anti-*H. pylori* effect of the US-produced honeys. Because it is difficult to maintain 15% honey or carbohydrate solutions at the gastric mucosa for extended periods, use of honey as an adjunctive treatment of *H. pylori* gastritis may not be feasible.

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