



Short Communication

Honey-an antimicrobial therapy for common microbial pathogens

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Abstract

Honey is a product obtained by processing of nectar collected by bees from various plant sources. It has been used for its antimicrobial role for more than a century. The pharmaceutical industries are producing novel antibiotics for sale in market; but simultaneously various pathogenic bacteria are developing resistance against these antibiotics. This is because of transfer of antibiotic resistant plasmids within bacterial populations. The presence of immunosuppressed patients in hospitals, which are more susceptible to new infections by antibiotic resistant pathogens, is a matter of concern. This multidrug resistance acquired by pathogens, prevents the effective action of the antibiotic. In this study an attempt was made to demonstrate use of honey as an antimicrobial agent against various human pathogens and wound infecting bacteria e.g. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. Laboratory investigation demonstrated effectiveness of honey as an antimicrobial at various concentrations of honey.

Keywords: Honey, antimicrobial property, antibiotic resistant microorganism.

Introduction

Honey has been known for its nutritive and antimicrobial value since ancient times. The recognition of antibacterial activity of honey was first reported by Dustmann in 1892¹. Honey is reported to be composed of 15-20% of water, and 80-85% of sugar with the remaining component which includes of proteins, enzymes and nonessential amino acids²⁻⁴. This antimicrobial nature was found to be, due to a component known as inhibine.⁵ Later on this component was identified as hydrogen peroxide, an inhibitory agent⁶. The osmotic effect of sugars in honey is also responsible for inhibition of bacterial growth⁷. Flavonoids and phenolic acids isolated from honey further contribute to its antimicrobial activity⁸. Honey has been well established as an agent for wound dressing in ancient and traditional medicine⁹. There are many reports of widespread use of honey for effective treatment of wound¹⁰, burn¹¹ and skin ulcer¹². Honey is effective as moist dressing, on wound or ulcer, promoting healing process. Effective treatment of wound infection may be due to its antimicrobial activity along with some other additional factors. In addition to its antimicrobial properties, honey can effectively treat infection in various ways *in vivo*, like boost up the immune system, anti-inflammatory, and antioxidant activities and via stimulation of cell growth¹³. Honey has a stimulatory effect on production of B-lymphocytes, T-lymphocytes and phagocytes at a concentration of 0.1%¹⁴. At 1% concentration it stimulates monocytes leading to release of cytokines, tumor necrosis factor, interleukin which activate the immune system in response to infection¹⁵. The bactericidal action of the

macrophages may be due to combined action of glucose and acidic pH¹⁶. Honey can also be used to treat the infection caused by multidrug resistant strains of bacteria¹⁷.

In this study an attempt was made to detect and confirm honey as an antimicrobial agent against pathogenic microorganisms e.g. *Pseudomonas aeruginosa* – causing blue pus in wounds, *Staphylococcus aureus* – causing acute pyogenic lesion, *P. mirabilis* an opportunistic pathogenic for urinary and septic infection, *Klebsiella pneumoniae* causing pneumonia, *Salmonella typhi* causing typhoid and *E. coli*- a common intestinal opportunistic pathogen causing diarrhea, gastroenteritis etc.

Material and methods

Active cultures of pathogenic bacteria (Isolated from hospital):
i. *Salmonella typhi*, ii. *Staphylococcus aureus*, iii. *Escherichia coli*, iv. *Proteus mirabilis*, v. *Klebsiella pneumoniae*.

Preparation of inoculums: Active cultures of various pathogenic microorganisms were made from stock culture isolated from hospital and maintained at 4°C in refrigerator on nutrient agar slants. Active culture for the experiments i.e. detection of honey as an antimicrobial was prepared by transferring a loop full of inoculum from stock cultures to test tubes of Muller-Hinton broth and incubated at 37°C for 24 hours. Turbidity of the inoculums for different test organisms after incubation was adjusted to 0.5 McFarland standard¹⁵. This approximately is equivalent to 10⁵-10⁶ cells/ml of the inoculum.

Preparation of different concentration of honey: Raw (unprocessed) honey sample was purchased from local vendor. Different concentrations of honey sample 25%, 50%, 75% and 100% (undiluted) were prepared using sterile distilled water.

Antimicrobial susceptibility test of pathogens for honey: Gel diffusion method was used to assess the sensitivity of isolated human pathogens. Sterile Muller Hinton agar plates were prepared and used for the antimicrobial susceptibility test of honey. The 0.1ml inoculum of pathogen suspension was spreaded uniformly on Muller Hinton agar. Wells were prepared in the center of the agar plate using sterile cork borer. Prepared wells were filled with solutions of different dilutions of honey and plates were kept in refrigerator for 10-15 minutes for diffusion of honey into the agar medium. After proper diffusion, plates were incubated in the incubator at 37°C for 24 hours and then examined for zone of inhibition around the well for different test organisms. A control plate was kept for every honey sample and test organism. Classification of the degree of susceptibility or resistance of the test isolate to each dilution of honey sample was tested, based on predetermined guidelines and standardized reference procedures based on Kirby-Bauer method¹⁸.

Results and discussion

Various common pathogen and wound infecting microorganisms demonstrated significant sensitivity against different concentrations of honey 24%, 50%, 75% and 100% (undiluted honey), demonstrated zone of inhibition varying in its size (Table-1).

Table-1: Antimicrobial activity of honey against pathogens.

Bacterial test culture	Concentration of honey sample used			
	25%	50%	75%	100%
	Zone of inhibition (in mm)			
<i>Staphylococcus aureus</i>	4.6	7.8	13.6	19.8
<i>Escherichia coli</i>	2.1	4.7	8.3	12.9
<i>Pseudomonas aeruginosa</i>	--- no zone	3.3	6.1	7.9
<i>Salmonella typhi</i>	3.2	4.6	7.4	12.0
<i>Klebsiella pneumoniae</i>	2.0	3.8	6.5	11.2
<i>Proteus mirabilis</i>	-- no zone	2.1	5.2	8.1

Out of all the organisms tested *S.aureus* demonstrated highest inhibition with all dilutions of honey and least inhibition was demonstrated by *Pseudomonas aeruginosa*. The cultures of *Pseudomonas aeruginosa* and *Proteus mirabilis* demonstrated

no zone of inhibition at the least concentration of honey sample i.e. 25%. It was observed that the inhibition efficiency of the honey samples on the growth of the test organisms increased with increase in concentration from 25 to 100%. The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent¹⁹. Similar antimicrobial activity of honey was also detected to restrict growth of *Salmonella* and *Escherichia coli*²⁰. For all the six tested bacterial cultures, the zone of inhibition differed significantly at various concentrations of the honey samples.

Conclusion

The studies done supported the earlier observations that honey can be used as an effective antimicrobial agent against common pathogens and wound infecting agents, which are becoming resistant to routine antibiotics. The concentrations of honey inhibiting the pathogens could be exploited clinically against antibiotic resistant microorganisms. The antimicrobial activity of honey is multifactorial (due to significant acidity, high sugar concentration, hydrogen peroxide etc) and hence microorganisms are unlikely to develop resistance towards them. Additionally, honey is hygroscopic, it can pull moisture out of the environment and desiccate bacteria, and its high sugar content in addition to low level pH can also avoid the microbial growth²¹. Microbial resistance to honey has never been informed, which makes it a very encouraging relevant antimicrobial agent to counter the infection of antibiotic-resistant bacteria and in the cure of prolonged wound infections that do not respond to antibiotic remedy.

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