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The antibacterial effect of honey on diarrhoea causing bacterial agents isolated in Lagos, Nigeria

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Summary

The antibacterial effect of local honey on local isolates of bacterial agents of diarrhoea was determined by an *in vitro* method involving the impregnation of filter paper discs in undiluted honey and different honey concentrations ranging from 10%-50%. The discs were later placed on plates that have been seeded with the different bacteria and zones of inhibition of growth were measured after a 48 hr. period of incubation. Results presented show that undiluted honey and honey at concentrations of 40% and above were inhibitory to all enteropathogens tested. Zones of inhibition of growth around the disc margin of the various enteropathogens tested ranged from 16-18mm in diameter for the local undiluted honey and 7-12mm in diameter for concentrations of honey at 40% and 50%. The possible mechanisms of this inhibitory effect of local honey are discussed.

Résumé

L'effet antibactérien du miel pur sur l'isolé local d'agent de b bactérie de la diarrhée était déterminé par une *in vitro* nécessitant la poussée des concentrations variées du miel et aussi par la méthode de disque nécessitant l'impregnation de la disque de filtre-pressé dans la concentration divers du miel. Les disques ont placés plus tard sur les assistées, qui ont été planté avec les bactéries différentes et les zones de l'inhibition de poussé ont mesuré après une période de 48H de l'incubation. Les résultats présentés montrent que le miel non-dilué et du miel sur les concentrations de 40% et au-dessus étaient bactérien aux tous entre pathogènes testées. Les zones d'inhibition de poussée autour la marge de disque des entropathogènes varié va de 8mm à 18mm. Une tentative a été fait pour

expliquer les mécanismes possibles de cet effet bactérien du miel pur.

Introduction

Honey is the nectar and saccharine exudation of plants; gathered, modified and stored as honey in the comb by honey bees, *Apis mellifera* [1]. Honey is a pale yellow, thick, sweet liquid made in the stomach of worker-bees and poured back into the cells. It acts as a food store. Two enzymes are secreted into honey by bees, invertase and glucose oxidase [1]. Honey is a supersaturated solution and has a high osmotic pressure and capable of killing many forms of bacteria [2]. It is a readily available source of glucose and fructose [3].

The antibacterial effects of pure honey have been reported by a handful of workers [2,4-6]. Pure honey has been shown to be bactericidal to many pathogenic organisms, including enteropathogens such as *Salmonella* spp; *Shigella* spp., Enteropathogenic *Escherichia coli*, *Vibrio cholera* and other Gram-negative or Gram-positive organisms in other parts of the world [5,6]. The significance of this finding has been applied in the post operative management of patients undergoing radical vulvectomy for vulval carcinoma. Cavanagh *et al.* [4] reported the quickening of wound healing by local application of honey in patients who developed post operative wound breakdown. Honey also accelerates the healing of infected surgical wounds and bed sores [7]. The use of honey in the treatment of infantile gastroenteritis has also been reported [8].

In Nigeria, apart from the well known bacterial agents of diarrhoea (*Salmonella* species, *Shigella* species, *Vibrio cholera*, *Yersinia enterocolitica*) newer agents have emerged. These newer agents include *Campylobacter jejuni*, *Aeromonas*

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hydrophilia and *Plesiomonas shigelloides*[9,10].

However, in spite of the reported bactericidal effect of honey against enteropathogens in other parts of the world[5,6,8], this has received only a little attention in Nigeria.

This study was therefore undertaken at the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, to determine the antimicrobial effect of honey on local isolates of bacterial agents of diarrhoea.

Materials and methods

Bacteria: Strains of *Salmonella typhi*, *Shigella boydii*, *Yersinia enterocolitica*, *Vibrio cholerae*, *Escherichia coli*, *Plesiomonas shigelloides* and *Aeromonas hydrophilia* were obtained from Professor Tolu Odugbemi, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos. *Campylobacter jejuni* was obtained from Dr. A.O. Coker, of the same Department. Standard Control Strains comprising *E. coli* NCTC 25922; *A. hydrophilia* NCTC, *P. shigelloides* NCTC 2339 and *C. jejuni* NCTC 11168 were included in the study.

Culture media: Nutrient agar plate (Mueller-Hinton media; Oxoid Ltd.) Blood agar base (Oxoid) supplemented with 5% sheep blood were used as control plates. The media were prepared according to the manufacturers' instructions. *V. cholerae*, *S. boydii*, *S. typhi*, *Y. enterocolitica*, *A. hydrophilia*, *P. shigelloides* were grown on nutrient agar plates. The bacteria tested on blood agar plates were *C. jejuni* and *Y. enterocolitica* [6].

Bacterial inoculum: Bacterial cultures were grown following standard procedures on nutrient agar and blood agar plates. Colonies were inoculated into 5ml of tryptic soy broth and incubated as already reported[6]. The turbidity of the culture was then adjusted to Mcfarland 0.5 turbidity standard and 1:20 dilution was prepared in sterile saline for inoculation. Nutrient agar and blood agar plates were inoculated. The inoculum was 10^4 Colony Forming Units (C.F.U.) in each case and plates were incubated as already reported for the different organisms[6,9,10].

Disk method: Six-mm filter paper disks were sterilized and impregnated into local undiluted honey, different concentrations of honey (10-50%) and later placed on nutrient agar and blood agar plates seeded with the different organisms. Impregnation was done as previously reported[11] and diameter of zones of inhibition of growth measured.

Results

The growth inhibitory effect of local undiluted honey using the disk method showed that all the enteropathogens tested (*E. coli*, *S. typhi*, *Shigella boydii*, *Vibrio cholerae*, *A. hydrophilia*, *C. jejuni*, *P. shigelloides* and *Yersinia enterocolitica*) were inhibited with zones of inhibition of growth ranging from 16mm-18mm in diameter.

There were no zones of inhibition of growth of the enteropathogens tested around filter paper disks impregnated in 10%, 20% and 30% concentrations of honey. At 40% and 50% concentrations of honey, zones of inhibition of growth for the same group of organisms ranged from 7mm to 12mm. (Table 1).

Table 1: Inhibition of growth of the enteropathogens tested by the disk method using undiluted honey and varying concentrations of honey on all media

Bacteria tested	Diameter of zone of inhibition of growth (mm \pm S.D.)					
	Undiluted honey	Varying concentrations of honey				
		50%	40%	30%	20%	10%
<i>E. coli</i>	18 \pm 2	10 \pm 1	8 \pm 1	—	—	—
<i>S. boydii</i>	17 \pm 1	10 \pm 1	9 \pm 0	—	—	—
<i>S. typhi</i>	17 \pm 2	11 \pm 0	9 \pm 0	—	—	—
<i>V. cholerae</i>	17 \pm 3	9 \pm 0	8 \pm 2	—	—	—
<i>A. hydrophilia</i>	16 \pm 2	8 \pm 0	8 \pm 1	—	—	—
<i>P. shigelloides</i>	16 \pm 1	9 \pm 2	7 \pm 0	—	—	—
<i>Y. enterocolitica</i>	16 \pm 2	12 \pm 2	11 \pm 1	—	—	—
<i>C. jejuni</i>	18 \pm 3	10 \pm 1	8 \pm 0	—	—	—

Discussion

This study has shown the inhibitory effect of pure honey against our local isolates of bacterial agents of diarrhoea and confirms the work of other workers[5,6]. Local honey in its undiluted form or when used at a concentration of 50% and above excellently inhibited growth of *E. coli*; *V. cholerae*, *Y. enterocolitica*, *P. shigelloides*, *A. hydrophilia*, *S. typhi*, *Shigella boydi* and *C. jejuni*. This result harmonises with a previously reported observation[6]. In our environment, this study is immensely significant because the above mentioned enteropathogens are responsible for a considerable degree of morbidity and mortality[10,12]. Honey is taken frequently among inhabitants because of its sweet taste[1]. The currently recommended therapy for treatment of diarrhoea is oral rehydration therapy and even in cases requiring antimicrobial therapy, resistant strains of the different organisms to a wide range of antibiotics have been reported[13,14]. The use of honey will therefore be of immense importance in our environment; both as an inhibitory agent and its richness in sugars for replacement of lost electrolytes.

The exact mode of inhibitory action of honey on bacteria has not been fully elucidated. However, evidence abound to show that honey may destroy bacteria because of its high sugar content which causes shrinkage of the bacterial cell, low pH (pH 3.2-4.2) and also because of the presence of an inhibitory substance called inhibin in honey[15]. Also, honey contains an enzyme, glucose oxidase, which converts a sugar in honey (dextrose) to hydrogen peroxide and gluconic acid prevents or slows down the growth of bacteria[1].

The clinical and therapeutic significance of honey is further exemplified by the work of Cavanagh *et al*[4]. They reported that wounds after treatment with honey became bacteriologically sterile within 3-6 days and rapidly healed and found such treatment to be more effective than the use of expensive antibiotics. Armon[7], recommended the use of honey for speeding the healing of surgical wounds.

Haffejee and Moose[8] reported that honey given with oral rehydration therapy shortens the duration of bacterial diarrhoea and may safely be used as a substitute for glucose provided the solutions contains electrolyte. In some parts of Nigeria (undocumented data) honey is used to cure ear-ache. Honey has another advantage of being safe, non-allergic and easily available in most communities.

In conclusion, we recommend a clinical trial to evaluate the effect of orally administered honey in the treatment of diarrhoea in Nigeria.

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References

1. Bassey IH. In Beckseping, Digest of selected literature on beckseping, honey and beewax processing. Libriservice Limited, Lagos, Nigeria. 1986.
2. Bose D. Honey or sugar in the treatment of infected wounds (letter) *Lancet* 1982; 24: 963.
3. WHO. Treatment and prevention of dehydration in diarrhoea diseases. A guide for use at the primary level, Geneva, WHO 1976.
4. Cavanagh D, Beazley J, Ostapowicz F. Radical operation for carcinoma of the vulva: A new approach to wound healing. *J. Obstet. Gynaecol. Br. Cwlth.* 1970; 77: 1037-1040.
5. Ibrahim AS. Antibacterial action of honey. *Bull. Islam. Med.* 1985; 1: 363-365.
6. Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Haffejee IE, Moosa A. The antibacterial action of honey. *South Afr. Med. J.* 1985; 67: 257-258.
7. Armon PJ. The use of honey in the treatment of infected wounds. *Trop. Doc.* 1980; 10: 91.
8. Haffejee IE, Moosa A. Honey in the treatment of infantile gastroenteritis. *Bri. Med. J.* 1985; 290: 1966-1967.
9. Obi CL, Coker AO. Production of antisera against campylobacter species in Lagos. *Nigeria. Cent. Afr. J. Med.* 1988; 34: 139-141.
10. Agbonlahor DE. Studies on the pathogenicity of *Yersinia enterocolitica* and *Yersinia enterocolitica* like bacteria isolated in Nigeria. Ph.D thesis submitted to the University of Lagos. 1984.
11. Kazmi SU, Roberson DA, Stern NJ. Cadmuim chloride susceptibility: A characteristic of *Campylobacter* species. *J. Clin. Microbiol.* 1985; 21(5): 708-710.

12. Obi CL, Coker AO. The role of macrophages in experimental campylobacter infections of mice in Lagos, Nigeria. *Trop. Geog. Med.* 1989; 41(2): 154-159.
13. Coker AO, Olaiya B, Obi CL, Alabi SA. Characterization and antibiotic sensitivities of *Campylobacter jejuni* and *Campylobacter coli* isolated from children at the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria. *J. Trop. Med. Hyg.* 1989; 92: 104-107.
14. Agbonlahor DE, Coker AO, Odugbemi TO. Antimicrobial susceptibilities of diarrhoeagenic *Campylobacter jejuni* and *Yersinia enterocolitica* isolated in Nigeria. *Cent. Afr. J. Med.* 1987; 33(6): 149-153.
15. White JW, Subers MM, Schepartz AI. The identification of inhibin: The antibacterial factor in honey as hydrogen peroxidase and its origin in a honey glucose-oxidase system. *Biochem. Biophys. Acta* 1963; 73: 57-70.

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