WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 1431-1438. Research Article ISSN 2277– 7105

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF SUDANESE HONEY AGAINST TYPHOIDAL& NON-TYPHOIDAL SALMONELLAE

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Article Received on
13 September 2014,

Revised on 07 Oct 2014,
Accepted on 31 Oct 2014

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ABSTRACT

Back ground: Typhoid fever remains a serious public health problem throughout the world, with an estimated 16–33 million cases and 500 000 to 600 000 deaths annually. Honey has been used by humans to treat a variety of ailments, from gastric disturbances to ulcers, wounds and burns, through ingestion or topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained. Few studies that have studied the antibacterial activity of Sudanese honey were reported but none of them evaluated the antibacterial activity of honey on certain *Salmonella* species. This study was therefore performed to study the effect of three types of honey on selected *Salmonella* species. **Materials and methods:** the antibacterial activity of three mono floral Sudanese honey (Acacia, Sidr and Sunflower honey) were evaluated using agar well diffusion susceptibility method in different concentrations(undiluted honey,75%, 50% and 25%v/v) against nineteen salmonella isolates in addition to reference strain (*S.typhimurium* NCTC12023, ATCC14028) compared to the commercial antibiotics (ciprofloxacin). **Results:** All clinical isolates and the reference strain were observed to be susceptible to undiluted honey, 75% and 50% v/v dilutions of the three types of honey used according to Clinical and Laboratory Standards Institute (CLSI) formerly NCCLS. Variations in susceptibility of some isolates were observed at dilution of 25%v/v of the three honey types.

KEYWORDS: Honey, Antibacterial susceptibility, Salmonella species.

INTRODUCTION

Typhoid fever, also known simply as typhoid, is a common worldwide bacterial disease. It is transmitted by the ingestion of food or water contaminated with the feces of an infected person, which contain the bacterium *Salmonella typhi*, serotype *typhi* [1,2]. The disease is almost exclusively transmitted by food and water contaminated by the faeces and urine of patients and carriers. Polluted water is the most common source of typhoid transmission. In addition, shellfish taken from sewage contaminated beds, vegetables fertilized with night-soil and eaten raw, contaminated milk and milk products have been shown to be a source of infection [3]. People can transmit the disease as long as the bacteria remain in their body; most people are infectious prior to and during the first week of convalescence, but 10% of untreated patients will discharge bacteria for up to 3months [3].

Typhoid fever remains a serious public health problem throughout the world, with an estimated 16–33 million cases and 500 000 to 600 000 deaths annually. In the last outbreak in the Democratic Republic of Congo, Between 27 September 2004 and early January 2005, not less than 42.564 cases of typhoid fever were reported, including 214 deaths and 696 cases of peritonitis and intestinal perforations. In virtually all endemic areas, the incidence of typhoid fever is highest in children from 5–19 years old [3].

Antimicrobial susceptibility testing is crucial for the guidance of clinical management. Isolates from many parts of the world are now multidrug-resistant (MDR). Isolates are usually resistant to ampicillin, chloramphenicol, sulfonamide, trimethoprim, streptomycin and tetracycline. Alternative drugs that are used for treatment include: fluoroquinolones (e.g. ciprofloxacin), third-generation cephalosporins (e.g. ceftriaxone, cefotaxime), a monobactum beta-lactam (aztreonam) and a macrolide (azithromycin). Even though resistance to the first two has been noted they nevertheless remain useful. Reduced susceptibility to fluoroquinolones is indicated by in vitro resistance to nalidixic acid [4]. Many natural materials have been tried for the treatment of typhoid fever among these is a bee honey.

Honey has been used by humans to treat a variety of ailments, from gastric disturbances to ulcers, wounds and burns, through ingestion or topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained. Different honeys have different properties, which were known since ancient times. Much scientific research

has been done, with emphasis of late on fighting infections in wounds [5]. The antibacterial mechanisms known to date are H_2O_2, methyl glyoxal (MGO), bee defensin-1, the osmotic effect and the pH [6]. According to the Muslim holy book "The Holy Hadith", dating back to the 8th century AD the prophet Mohamed recommended honey against diarrhea. Also, the Roman physician Celsus (ca. 25 AD) used honey as a cure for diarrhea. The use of honey for prevention and treatment of gastro-intestinal disorders such as peptic ulcers, gastritis, and gastroenteritis has been reported in various books and publications from Eastern Europe, and from Arab countries [7-13].

Globally some studies were conducted to test the antimicrobial activities of some types of honey against certain bacteria including *Salmonella* [14,15]. Despite these, the literature reported no studies from Sudan that evaluated the antibacterial action of honey on typhoidal or non typhoidal *Salmonellae*. This study was therefore performed to evaluate the antibacterial susceptibility of three types of honey against twenty typhoidal *Salmonellae*.

MATERIALS AND METHODS

In this study the antibacterial action of three different floral sources honey (Acacia, Sidr and Sunflower honey) were tested against nineteen clinical isolates of *Salmonella* species in addition to a reference strain (S. *typhimurium* NCTC12023, ATCC14028). These isolates included *S.typhi (1)*, *S.paratyphi* A (2), *S.paratyphi* B(6), *Salmonella* group C1(2), *Salmonella* groupC2-C3(2), *Salmonella* groupD1(2), *Salmonella* groupE1(2), and *Salmonella* group H(2). All of them were obtained from the microbiology laboratory of Khartoum State directorate of the laboratories. The three types of honey were purchased from the local beekeeper namely the bee world company and kept in sterile containers in a dark area away from the direct sunlight.

The physicochemical properties were estimated for the three honey types at National Research Center in the Khartoum State (table 4). All the isolates were subcultured and the serotypes confirmed based on biochemical tests and specific antisera. Then purity culture of each isolate was done for the purpose of antimicrobial sensitivity against the used types of honey. Then suspension for the each isolate was prepared in a sterile saline and it's turbidity matched with 0.5 Mc Farland turbidity standards [16, 17]. The following dilutions of the different three types of honey were prepared: undiluted honey (100%), 75%, 50% and 25%v/v using sterile distilled water. The antimicrobial activity was performed by agar well diffusion method using Mueller-Hinton agar [18].

Inoculation of the Media by the Different Isolates and Addition of the Different Honey Concentrations.

For each *Salmonella* isolate and the control strain two Mueller-Hinton agar plates were used. The prepared suspension of the isolate was first inoculated into corresponding plate. There after four wells (each with a diameter of 8mm) were punched on each agar plate. 100µl of undiluted honey, 75%, 50% and 25%v/v were added into the corresponding well on the agar plate [19].

The inoculated plates were then incubated aerobically overnight (18-24hour). By the end of the incubation the plates were examined for bacterial growth and inhibition zone around each well containing honey concentrations. The diameters of the inhibition zones were then measured in mm using a ruler.

The in vitro antibacterial activity were determined by measuring the inhibition zone diameter which due to the antibacterial activity of honey and interpreted according to Clinical and Laboratory Standard Institute (CLSI) as sensitive, intermediate and resistant in compare with inhibition zones of ciprofloxacin as, sensitive ≥21mm, intermediate16-20mm and resistant ≤15mm.

RESULTS

The three honey types (Acacia honey, Sidr honey, and Sunflower honey) which were used for the evaluation of antibacterial activity showed slight variation in their physicochemical constituents but all of them were within the normal values (Table 1).

Table (1): Physicochemical Properties of the Honey Used.

Types of honey	pH	Refractive index	Moisture	Free acidity	Total acidity	Lactone	Reducing sugars	Specific gravity
Acacia	5.11	1.5030	13.4	37.00	45.50	8.50	64.38	1.4391
Sidr	5.91	1.5038	13.2	33.50	39.50	6.00	62.29	1.4274
Sunflower	4.51	1.5028	13.6	39.00	46.50	7.50	64.10	1.4310
Reference values	3.4-6.1	-	Not more than 20%	Not more than50 meq/kg	Not more than50 meq/kg	–	Not less than 60%	Not less than 1.37

All isolates and the control strain were found to be sensitive to neat honey, 75% and 50%v/v for the three types of honey used. The variations in susceptibility were observed at the dilution 25%v/v for all honey types (Table 2: A, B, C). Four isolates were found to have an

intermediate susceptibility to ciprofloxacin 5µg: *S.paratyphi* A, *S.paratyphi* B, *Salmonella* group D1, and *Salmonella* group E1.

Table 2(A, B, C): The antibacterial activity for the three honey types used at (25%v/v) dilution against different *Salmonella* isolates.

Table 2(A): Acacia Honey.

***Salmonella* Species**	**No tested**	**Percentage of Sensitive**	**Percentage of Intermediate**	**Percentage of Resistant**
S.paratyphi A	2	100%	0%	0%
S.paratyphi B	6	50%	33%	17%
Salmonella group C1	2	100%	0%	0%
Salmonella group C2-C3	2	100%	0%	0%
Salmonella group D1	2	100%	0%	0%
S. typhi	1	100%	0%	0%
Salmonella group E1	2	100%	0%	0%
Salmonella group H	2	100%	0%	0%
S.typhimurium ATCC(14028)	1	0%	100%	0%

Table 2(B): Sidr Honey.

***Salmonella* Species**	**No tested**	**Percentage of Sensitive**	**Percentage of Intermediate**	**Percentage of Resistant**
S.paratyphi A	2	100%	0%	0%
S.paratyphi B	6	67%	33%	0%
Salmonella group C1	2	100%	0%	0%
Salmonella group C2-C3	2	100%	0%	0%
Salmonella group D1	2	100%	0%	0%
S. typhi	1	100%	0%	0%
Salmonella group E1	2	100%	0%	0%
Salmonella group H	2	50%	50%	0%
S.typhimurium ATCC(14028)	1	0%	100%	0%

Table2 (C): Sunflower Honey.

***Salmonella* Species**	**No tested**	**Percentage of Sensitive**	**Percentage of Intermediate**	**Percentage of Resistant**
S.paratyphi A	2	100%	0%	0%
S.paratyphi B	6	100%	0%	0%
Salmonella group C1	2	100%	0%	0%
Salmonella group C2-C3	2	100%	0%	0%
Salmonella group D1	2	100%	0%	0%
S. typhi	1	100%	0%	0%
Salmonella group E1	2	100%	0%	0%
Salmonella group H	2	50%	0%	50%
S.typhimurium (ATCC14028)	1	0%	100%	0%

DISCUSSION

All isolates and control strains were observed to be sensitive to all undiluted honey types. This is in agreement with Raied Taha AL-Naama, 2009 who found the potency of neat honey (100%concentration) to be superior against all bacteria tested [20]. All isolates and control strains were observed to be sensitive to 75% and 50%v/v dilutions of all honey types which is in disagreement with study done by Shahedur Rahman and his colleagues in 2011 who found that the same dilutions had no effect against *Salmonella* species [21]. The 25%v/v dilutions of all honey types used, showed variation in the susceptibility of some species (*S.paratyphi* B, *Salmonella* group H and *S.typhimurium* ATCC14028, NCTC12023) which is in agreement with Abdul Hannan and his colleagues 2009 who observed significant variations among the median minimum inhibitory concentration (MIC) of black seed honey against *S.typhi*, *S.paratyphi* A and *S.paratyphi* B [14].

The variation of antibacterial activity may be due to different storage conditions, processing procedure, concentration of hydrogen peroxide, plant derived non peroxide factors and plant derived factors which are unique to each plant species. These may contribute significantly in the variation between honeys of different plant sources [22, 23, 24]. There was no relationship between honey color and its antibacterial activity because lighter Sunflower honey was found to have more potent antibacterial activity than Acacia and Sidr honey. This is in agreement with Eman Halawani and Mohamed Shohayeb's study that showed no relationship between color and antibacterial activity of honey [25]. There was variations in susceptibility of some clinical isolates to the tested antibiotic (ciprofloxacin 5µg) as some of isolates were found to have an intermediate susceptibility and this is in disagreement with Abdul Hannan and his colleagues 2009[14].

CONCLUSION

Our results revealed that the honey types used from different floral sources have a clear antibacterial activity against the different *Salmonella* species under test. This indicated the susceptibility of *Salmonella* species to the Sudanese honey. Based on the results of this study, honey can be recommended to be introduced as a natural product for treatment of infections caused by *Salmonella* species.

ACKNOWLEDGMENTS

The authors would like to thank the director and staff member of microbiology lab of Khartoum State directorate of the laboratories for providing the *Salmonella* isolates.

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