Level of Salivary Microorganisms after Consumption of Malaysian Tualang Honey: A Preliminary Study

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Abstract

This study was conducted to compare the changes of salivary microorganisms after consumption of tualang honey (TH). A total of 44 USIM dental students (male = 9, female = 35) who fulfilled the inclusion criteria participated in this experimental blinded crossover study. Criteria for subject selection were: subjects with no active caries, no history of antibiotic usage for the past 6 months, no history of antimicrobial mouthwash usage for the past 6 months, no orthodontic appliance worn, and healthy. In phase 1, participants in Group A were not given TH to consume whereas Group B consumed honey. After one month washout period, participants in Group A were given TH to consume and Group B had excluded TH from consumption. The mean differences in the salivary bacterial count (CFU/mL) were analysed using repeated measure ANOVA at *p* value of 0.05. There was not a significant difference in the salivary bacterial count (CFU/mL) at baseline, Day 3, Day 7 and Day 14 during control phase. However, after consumption of tualang honey, the bacterial count was slightly decreased at Day 7, however, the difference was not statistically significant. The increase in CFU count on Day 3 and Day 14 was also not statistically significant. It can be concluded that two weeks consumption of tualang honey did not give any obvious negative effects on the bacterial count. However, further studies will be required to support these preliminary result.

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Introduction

Honey is nectar collected by bees from a wide variety of plants. Honey is primarily composed of fructose (38%), glucose (31%) and other sugars.[1] In Malaysia, the extensively used honey is the tualang honey. Tualang honey is a wild multifloral honey produced by Apis dorsata bees. It is readily available, but its quality and floral origin have yet to be determined and standardized.

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The medicinal and antimicrobial activities of honey have been introduced for thousands of years.[2] It is also based on the belief regarding honey in the Holy Quran in Surah An-Nahl Verse 68 and 69 as stated below:

And thy Lord taught the Bee to build its cells in hills, on trees, and in (men's) habitations; (68) Then to eat of all the produce (of the earth), and find with skill the spacious paths of its Lord: there issues from within their bodies a drink of varying colours, wherein is healing for men: verily in this is a Sign for those who give thought (69).

There has been a marked increase in the number of studies about honey. However, it has been questionable whether honey is harmful to the teeth especially formation of dental caries. Dental caries is the most prevalent infectious disease worldwide. One of the factors that associate with caries occurrence is the interaction of protective and deleterious factors in saliva and plaque. Saliva is a unique oral fluid

produced by major and minor salivary glands. It can be used to provide clinical information about patients and interest in it as a diagnostic medium has increased in the last few years. The use of saliva in diagnosing caries risk status for individual patient is well-accepted.[3]

The demonstration of bacterial specificity in dental caries is difficult. This is due to the complexity and variability of the plaque flora and the fact that the known etiologic bacterial, which is mutans streptococci (MS) and lactobacilli (LB), appear to be present on all dentitions. These bacterial produce metabolic product such as lactic acid and acetic acid. Such acids can reduce the plaque pH below 5.5 and the critical pH for enamel demineralization leading to development of caries.[4] Insufficient information is available regarding the inhibitory effect of honey on oral bacteria.

Ghabanchi *et al.*[5] stated that the antibacterial properties of honey against medically important bacteria have been well documented but little information is available for the oral bacteria and specifically for oral Streptococci. Their study showed that honey had an inhibitory effect on S. mutans. However, further studies on anti S. mutans effect of honey especially in clinical trials are required to determine whether honey can be used as preventive measure for dental caries.

In Malaysia, tualang honey is generally comparable with New Zealand manuka honey in terms of its antibacterial potencies. Zainol *et al.*[6] concluded that all Malaysian honeys possess high non-peroxide antibacterial activity. Gelam, kelulut and tualang honeys have high antibacterial potency of total and non-peroxide activities. The correlations between Minimum Inhibitory Concentration (MIC) and Equivalent Phenol Concentration (EPC) value of Malaysian honey were proven to be dependent on bacteria species and honey origin.

So far, there have been no studies done on the effect of Malaysian honey on salivary microorganisms. However, few studies was done to investigate the effect of honey on dental plaque. Manuka honey was used to assess the effect on the dental plaque levels. They found that both manuka honey and chlorhexidine mouthwash reduced the plaque formation significantly better than the xylitol chewing gum. Result of the study suggested that manuka honey has a potential therapeutic role in the treatment of gingivitis and periodontal disease.[7] Therefore, this study was conducted to compare the changes of salivary microorganisms after consumption of Malaysian tualang honey.

Materials and methods

Study design

This was an experimental blinded crossover study to compare the salivary microorganisms after consumption of Malaysian tualang honey. The study was carried out at the Faculty of Dentistry, Universiti Sains Islam Malaysia (USIM).

Ethical approval

The ethical approval was obtained from the ethics committee at the Faculty of Dentistry, Universiti Sains Islam Malaysia (USIM/FPg-MEC/2013/No(4)).

Study participants

A total of 44 USIM Year 1 and 2 Dental Students who fulfilled the inclusion criteria participated in this study. Participation in the study was voluntary and written and informed consent was received. From 44 participants, 35 were females and nine were males.

Inclusion criteria

Criteria for participant selection were: should have no active caries, no history of antibiotic usage for the past six months, no history of antimicrobial mouthwash usage for the past six months, no orthodontic appliance worn, and healthy. Participants who did not fulfill the inclusion criterias were excluded from the study.

Phases of assessment

Oral examination was done to examine the caries status of the participants. They were then divided into group A and Group B. In phase one, Group A was not given TH to consume whereas Group B consumed TH. After one month washout period, they proceeded with Phase two with participantss in Group A were given TH to consume and Group B stopped consuming TH.

Collection of saliva samples

Stimulated saliva was collected between 9am to 11am from each study participant and

analysed to establish baseline salivary viridans streptococci. Then, another collection of samples were carried out on Day 3, Day 7 and Day 14 before the start of experiment on the next day.

Determination of salivary viridans streptococci in Colony Forming Units (CFU/mL)

Each saliva sample was vortexed for 30 seconds. The sample was then pipetted out and serial dilutions were prepared. Each diluted salivary sample was pipetted onto separate agar plates and spread using sterile spreaders. Mitis Salivarius (MS) agar with 1% potassium tellurite were used in this study for culturing salivary viridans steptococci. All of the plates were incubated at 37 °C for 48 hours.

Colonies of *Streptococcus mutans* were identified as round, raised, convex, pale-blue colonies that are granular "frosted glass" appearance. Colonies may exhibit a glistening bubble on the surface due to excessive synthesis of glucan from sucrose. *Streptococcus salivarius presented* as large, pale- blue, mucoid colonies that are glistening "gum-drop" in appearance. *Streptococcus mitis* was identified as small, flat, hard colonies, blue in color with a domed center. Enterococci was identified by blue-black, shiny, and slightly raised colonies. The colony count of each plate was recorded and the mean Colony Forming Units (CFU/mL) was determined. Then the mean differences in the salivary bacterial count (CFU/mL) were analysed using repeated measure ANOVA at *p* value of 0.05.

Results

There was no significant difference in the salivary bacterial count (CFU/mL) at baseline, Day 3, Day 7 and Day 14 during control phase (Table 1). However, after consumption of tualang honey, the bacterial count was slightly decreased at Day 7. It was not significantly increased at Day 3 and Day 14 (Table 2).

		Mean (CFU/mL)	Std. deviation	Mean difference (I-J)	Sig.
Control phase	Baseline 1	$39.2x10^4$	$31.8x10^4$		
	Day 3	$47.8x10^4$	$43.9x10^4$	$8.6x10^4$	1.000
	Day 7	$34.1x10^4$	$26.6x10^4$	$-5.1x10^4$	1.000
	Day 14	$56.8x10^4$	$57.0x10^4$	$17.7x10^4$	1.000

CFU = Colony Forming Unit

Table 1. Mean Salivary Bacterial Count at Baseline and Control Phase.

		Mean (CFU/mL)	Std. deviation	Mean difference (I-J)	Sig.
Control phase	Baseline 1	$39.2x10^4$	$31.8x10^4$		
Intervention phase	Day 3	$50.4x10^4$	$35.1x10^4$	$11.2x10^4$	1.000
	Day 7	$28.7x10^4$	$30.2x10^4$	$-10.5x10^4$	1.000
	Day 14	$39.3x10^4$	$25.7x10^4$	$0.1x10^4$	1.000

CFU = Colony Forming Unit

Table 2. Mean Salivary Bacterial Count at Baseline and After Honey Consumption.

Discussion

Foods that have anticariogenic properties are referred to as cariostatic factors. Consumption of foods with cariostatic properties that are also healthy in terms of the diet in general should be encouraged. Therefore, research on antibacterial activity of honey against cariogenic bacteria in vitro and in vivo should be recommended.

Honey is mainly composed of a complex mixture of carbohydrates. It also contains water, proteins, enzymes, amino acids, organic acids, lipids, vitamins, phenolic acids, flavonoids and minerals.[8, 9, 10] Two enzyme contribute to the biological activities of honey namely bee-origin glucose oxidase and floral-origin catalase. These enzymes are important in determining the level of peroxide activity in honey that underlies antibacterial potency.[6] Active glucose oxidase in a high amount will hydrolyze glucose to produce hydrogen peroxide (H_2O_2) resulting in oxidative stress that is useful in controlling bacterial colonization.

In undiluted honey, glucose oxidase is inactive thus the H_2O_2 level is said to be minimized. In theory, therefore the antibacterial potency is low in undiluted honey. At this stage, very high osmotic pressures with high acidity are

the two main contributing factors to the antibacterial properties.[11, 12] Honey such as manuka honey from New Zealand possesses high non-peroxide antibacterial activity that can retain antibacterial potency even after removing the peroxide component from diluted honey.[13]

Other than the two factors stated above, physical properties, low water activity, low pH, acidic environment, low protein content, high carbon to nitrogen ratio, low redox potential due to the high content of reducing sugars are factors that involve in antimicrobial activity of honey.[14, 15, 16] Other study also reported that the antibacterial activity of honey increased when the honey was diluted.[17]

The results of this study showed that after consumption of tualang honey, there was an insignificant decreased in the bacterial count at Day 7. However, it is inconclusive if the decrease in bacterial count was caused by the consumption of tualang honey. This study tested honey with one concentration without dilution.

Conclussion

Within the limitations of the study, it can be concluded that two weeks consumption of tualang honey did not give any obvious negative effects on the bacterial count. However, further studies will be required to support these preliminary result. The immediate effect of tualang honey should be assess to prove that honey can prevent dental caries in the future.

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Declaration of Interest

Authors have declared that they have no conflict of interest in this study.

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