Level of Salivary Flow Rate, pH Level, Buffering Capacity and After Consumption of Malaysian Tualang Honey: A Preliminary Study

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Abstract

This study was conducted to assess the changes of salivary pH level, salivary buffering capacity and salivary flow rate after consumption of tualang honey (TH). Fourty-four USIM dental students 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, Group A did not consumed honey whereas Group B consumed honey. After 1 month washout period, subjects in Group A consumed honey and Group B stopped consuming honey. The mean salivary flow rate at baseline before honey consumption was 1.5 ± 0.52 mL/min. There was a significant decreased in salivary flow rate at Day 7 and baseline. At Day 14, the salivary flow rate increased significantly when compared to Day 7. After consumption of tualang honey, there was a significant increased in salivary flow rate at Day 14 when compared to Day 3 and Day 7. There was no significant changes in salivary pH level at control phase and after consumption of tualang honey. After 2 weeks of honey consumption, the percentage of subjects who had normal salivary buffering capacity slightly reduced (38.6%). It can be concluded that 2 weeks consumption of tualang honey did not give any obvious negative effects on the salivary flow rate, saliva pH level and its buffering capacity. 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. 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 in formation of dental caries.

Dental caries is the most prevalent infectious disease worldwide. One of the factor 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.[2] It can be done by measuring the salivary flow rate, pH level and its buffering capacity.

Saliva can be collected as unstimulated and stimulated saliva. The average daily flow varies range between 1 and 1.5 L, in which the accepted range of normal flow for unstimulated

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and stimulated saliva is above 0.1 mL/min and 0.2 mL/min respectively.[3] On average, unstimulated flow rate is 0.3 mL/min while the stimulated flow rate is 7 mL/min.[4,5] The nuclei in the medulla control saliva secretion in which there are three types of triggers for this secretion. The triggers are mechanical, gustatory; with acid is the most stimulating trigger and sweet the least stimulating, and olfactory. In general, the higher the flow rate, the faster the clearance and the higher the buffering capacity.

Stimulated saliva sampling is a good method to determine buffering capacity during a comprehensive oral health assessment as it is more resistant to variation in pH change than unstimulated saliva.[6] The ability of saliva to buffer acids is essential for maintaining pH above the critical pH, thereby protecting the teeth against demineralisation.

As previously stated, honey is a natural supersaturated sugar solution. The mean total sugar content of Malaysian honey was 65.53 g/mL of honey that do not exceeded the highest limit for the total sugar content of honeys established by the European community.[7] 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. Hence, this study was undertaken with the aim of assessing the effect of tualang honey on salivary pH, flow rate and buffering capacity.

So far, there have been no studies done on the effect of honey towards salivary flow rate, pH and buffering capacity. 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 mouth-wash 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.[8]

Another study was conducted to investigate the effect of chewing honey on plaque pH. Result showed that the critical value for decalcification was not reached for in either the honey or sorbitol groups. However, the pH in the sucrose group fell below the critical value.

Materials and methods

Study design

This was an experimental blinded crossover study to evaluate the changes in salivary flow rate, buffering capacity and pH level after consumption of tualang honey. The study was carried out in 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)).

Subjects

Fourty-four 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 fourty-four subjects, 35 were female and 9 were male subjects.

Inclusion criteria

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. Subjects who did not fulfill the inclusion criterias were excluded from the study.

Phases of assessment

Dental screening was done to examine the caries status of the subjects. Then subjects were divided into group A and Group B. In phase 1, Group A did not received TH whereas Group B consumed TH. After 1 month washout period, they started Phase 2 with subjects in Group A consumed TH and Group B stopped consuming TH.

Collection of saliva samples

Stimulated saliva was collected between 9am to 11am from each subject and analysed to establish baseline salivary pH level, salivary buffering capacity and salivary flow rate. Then, another samples were collected on Day 3, Day 7 and Day 14 before the start of the next day experiment.

Determination of salivary flow rate, buffering capacity and pH level

The salivary flow rate of saliva collected from each subjects and pH value of saliva sample were measured. The saliva buffering capacity was measured by examined colour changes of Buffer test strip and calculated by adding the points according to the final colour of each pad. The determination was carried out in triplicates. The mean salivary flow rate and pH value, and saliva buffering capacity score for each saliva samples was calculated and recorded.

Results

Assessment of mean salivary flow rate and pH value at baseline between gender

The mean rank of salivary flow rate and pH value at baseline level was shown in Table 1. There was slightly higher salivary flow rate and pH value in males than in females. However, there was no significant difference between gender on both salivary flow rate and pH value at the baseline level (U = 142, p = 0.651; U = 134.5, p = 0.473 respectively).

	Gender	N	Mean rank	Mann-Whitney U	Sig.
Salivary flow rate	Male	9	24.22	142.000	0.651
	Female	35	22.06		
Salivary pH value	Male	9	25.06	134.500	0.473
	Female	35	21.84		

Table 1. Mean Salivary Flow Rate and Ph Value at Baseline Between Gender.

Assessment of differences in salivary flow rate

Subjects consumed tualang honey for 2 weeks. The mean salivary flow rate at baseline before honey consumption was 1.5 ± 0.52 mL/min. The test is not significant (p > 0.001) so the assumption of sphericity assumption was met, $\chi^2(5) = 20.759$, p = 0.001 (Table 3). The sphericity correction was applied [F(3, 129) = 6.847, p < 0.001]. There was a significant decreased in salivary flow rate at Day 7 and baseline. At Day 14, the salivary flow rate increased significantly when compared to Day 7 (Table 4).

Variable	Mean (SD) (mL/min)			
	Baseline	Day 3	Day 7	Day 14
Level of salivary flow rate at control phase	1.5 (0.5)	1.3 (0.5)	1.2 (0.4)	1.5 (0.5)
Level of salivary flow rate after consumption of tualang honey	1.4 (0.5)	1.3 (0.4)	1.3 (0.4)	1.6 (0.6)

Table 2. Mean Salivary Flow Rate at Control and After Consumption of Tualang Honey.

Within subject effect	Mauchly's W	Approx. Chi-Square	dt	Sig.	Epsilon		
					Green house-Geisser	Huynh-Feldt	Lower-bound
Level of salivary flow rate at control phase	.608	20.759	5	.001	.773	.820	.333
Level of salivary flow rate after consumption of tualang honey	.654	17.694	5	.003	.788	.837	.333

Table 3. Test on Salivary Flow Rate at Control and After Consumption of Tualang Honey.

Level of salivary flow rate at control phase		Mean difference (I-J)	Std Error	Sig.	95% Confidence Interval for Difference	
I	J				Lower bound	Upper bound
Day 7	Baseline	-.211	.066	.015	-.393	-.030
Day 14	Day 7	.277	.071	.002	.081	.473

Table 4. Mean Difference of Salivary Flow Rate at Control Phase.

After consumption of tualang honey, the test is not significant (p > 0.001) so the assumption of sphericity assumption was met, $\chi^2(5) = 17.694$, p = 0.003 (Table 3). The sphericity correction was applied [F(3, 129) = 7.451, p < 0.001]. There was a significant increased in salivary flow rate at Day 14 when compared to Day 3 and Day 7 (Table 5).

Level of salivary flow rate after consumption of tualang honey		Mean difference (I-J)	Std Error	Sig.	95% Confidence Interval for Difference	
I	J				Lower bound	Upper bound
Day 14	Day 3	.295	.087	.009	.054	.537
Day 14	Day 7	.302	.085	.005	.068	.537

Table 5. Mean Difference of Salivary Flow Rate After Consumption of Tualang Honey.

Assessment of differences in salivary pH value

Table 6 shows a mean in pH value at Day 3, Day 7 and Day 14 both at the control and intervention phase. The test is significant ($p < 0.001$) at control phase and after consumption of tualang honey. Therefore, the assumption of sphericity assumption was not met, $\chi^2(5) = 389.764$, $p < 0.001$, $\chi^2(5) = 24.246$, $p < 0.001$, respectively (Table 7). The Greenhouse-Geisser correction was applied [$F(1.008, 43.337) = 1.086$, $p > 0.001$ at control phase] and [$F(2.144, 92.186) = 1.922$, $p > 0.001$ after consumption of tualang honey]. There was no significant changes in salivary pH level at control phase and after consumption of tualang honey.

Variable	Mean (SD)			
	Baseline	Day 3	Day 7	Day 14
Level of salivary pH at control phase	7.6 (0.2)	8.2 (3.6)	7.6 (0.1)	7.6 (0.1)
Level of salivary pH after consumption of tualang honey	7.6 (0.1)	7.6 (0.1)	7.5 (0.3)	7.5 (0.2)

Table 6. Mean Salivary Ph at Control and After Consumption of Tualang Honey.

Within Subject effect	Mauchly's W	Approx. Chi-Square	dt	Sig.	Epsilon		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Level of salivary pH at control phase	.000	389.764	5	.000	.336	.336	.333
Level of salivary pH after consumption of tualang honey	.559	24.246	5	.000	.715	.753	.333

Table 7. Test on Salivary Ph at Control and After Consumption of Tualang Honey.

Assessment of salivary buffering capacity score

About 59.1% in which 13.6% were males and 45.5% were females subjects had normal salivary buffering capacity score (Figure 1). The highest percentage of subjects who had normal buffering capacity was after Day 7 before honey consumption (70.5%). Then the percentage of subjects who had normal buffering capacity became consistent even after tualang honey consumption. After 2 weeks of honey consumption, the percentage of subjects who had normal salivary buffering capacity slightly reduced (38.6%) (Figure 2).

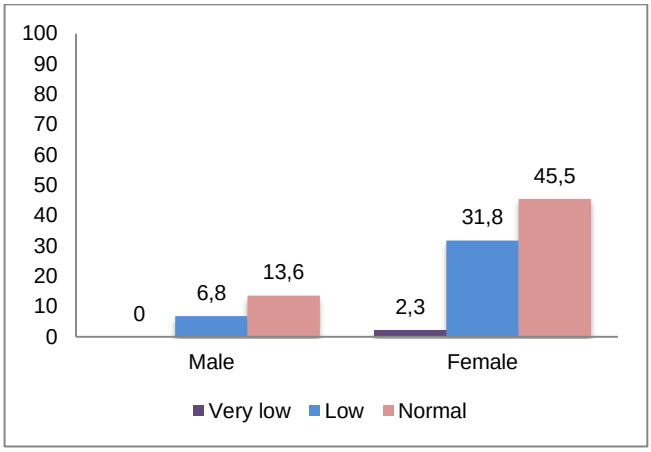

Figure 1. Percentage of subjects salivary buffering capacity score at baseline.

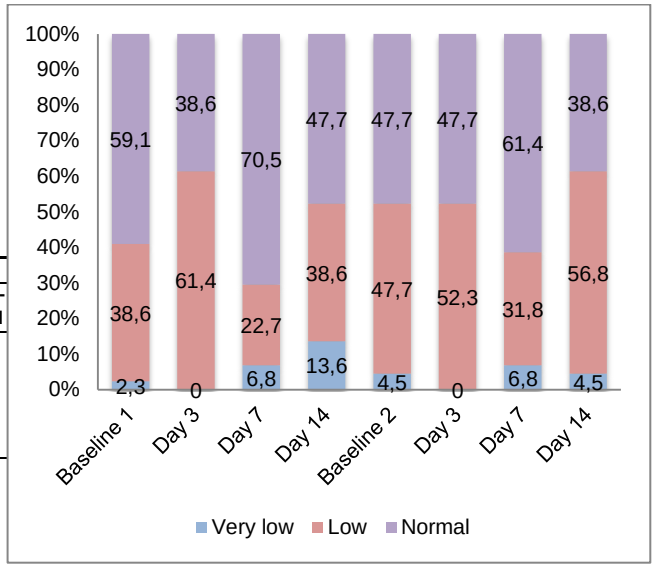

Figure 2. Differences of salivary buffering score at baseline, day 3, 7 and 14.

Discussion

There were age-related and gender differences in certain aspects of salivary gland function. The flow rates of unstimulated whole saliva, stimulated parotid saliva as well as unstimulated and stimulated submandibular-sublingual saliva all decreased with increasing age. In term of gender differences, females had significantly lower flow rates than males for unstimulated whole saliva.[10] In this study, females had slightly lower stimulated flow rate than males.

The range of mean stimulated salivary flow rate was between 1.2 mL/min to 1.5 mL/min. The accepted range of stimulated salivary flow rate is above 0.2 mL/min.[3] However, it is accepted that hyposalivation is characterized by a salivary flow of less than 0.7 mL/min.[11, 12] It was clear from the results that the subjects fall into normal flow rate category. It was reflected to the clinical caries presentation of the subjects. Subjects were presented with no active caries using 'The International Caries Detection and Assessment System (ICDAS)' score. Studies showed that chronically low salivary flow rate was found to be the strongest indicator of an increased risk for caries prevalence or incidence.[13, 14, 15]

The normal pH of saliva ranges from 6.7 to 7.4. When the pH level in the mouth goes below critical pH value (below 5.5), the acids begin to break down the enamel on teeth. The longer the teeth are exposed to a low salivary pH, the more likely the development of dental caries is.[16] The baseline mean pH in this study was found to range between 7.6 and 8.2 which is slightly higher than the normal pH of saliva.

Generally, the accuracy of pH measurements depends on the method of saliva collection and on the time interval between collection and analysis. The pH of saliva, if exposed to air, will slowly rise with time due to continuous loss of carbon dioxide (CO_2) from the saliva sample.[17] However, in this study, there was slight delayed between saliva collection and analysis.

Salivary buffering capacity has been identified as one of the factors that may affect an individual's caries risk.[18] The ability of saliva to buffer acids is essential for maintaining pH above critical pH. It is not surprising that impaired salivary buffering capacity may well be clinically important influence in relation to caries development during young age.[19]

The bicarbonate (HCO_3^-) buffer system is the major buffer system present in saliva in which the concentrations vary from about 3-5mmol/L in resting saliva. The concentration of salivary bicarbonate depends on flow rate. However, in this study, there was no association analysis done between buffering capacity and salivary flow rate. Clearly, majority of subjects had normal salivary flow rate (1.5 ± 0.52 mL/min) and buffering capacity (59.1%) at baseline.

Buffer test strip was able to quantitatively test saliva buffering capacity at the chairside that will aid dentists and patients to know more about patients' caries risk. However, this method sometimes give inconclusive results as the colour reaction depends on the time saliva remains in contact with the strip and variation in individual visual interpretation.[20]

From the result, there was a significant decreased in stimulated salivary flow rate after consumption of tualang honey compare to baseline. However, after two weeks the flow rate was slightly increased. Therefore, it is inconclusive if the increase in salivary flow rate was caused by the consumption of tualang honey in which can stimulate saliva production. For pH, there was a decreased at Day 7 and Day 14. However, it was statistically not significant changes. After 2 weeks consumption of tualang honey, majority of subjects had low and very low buffering capacity. However, from observation, there was no major changes in the level of buffering capacity before and after tualang honey consumption. The reasons for this may be due to extrinsic factors such as dietary habit as well as intrinsic factors such as bicarbonate content.[21]

Conclusion

Within the limitations of the study, it can be concluded that 2 weeks consumption tualang honey did not give any obvious negative effects on the salivary flow rate, saliva pH level and its buffering capacity. However, further studies will be required to support these preliminary result. The immediate effect and the salivary clearance 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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