Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20032 - 20044
Received 10 August 2021; Accepted 25 August 2021.

An Explorative Study on the Effect of Honey on Salivary Parameters among Patients with Chronic Generalised Gingivitis

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ABSTRACT

Honey possesses potential therapeutic roles as an adjunctive treatment for gingivitis and periodontal diseases.In spite of the beneficial effects of honey on chronic gingivitis, its influence on salivary parameters has not been extensively researched. Therefore, the objective of this paper is to investigate the effects of honey on the pH and buffering capacity of saliva among patients with chronic generalised gingivitis. Thirty patients with chronic generalised gingivitis were divided into "honey" and control groups through simple random sampling. Both groups received full-mouth scaling and polishing treatment but the subjects in the "honey" group had their gingivae coated with honey after that. The stimulated saliva of the subjects in both groups was collected before treatment, at the 15th minute, and 30th minute. The pH and buffering capacity of saliva were measured using the GC Saliva-Check BUFFER® (GC Co., Japan).There was no significant difference in the salivary pH between the two groups (p=0.9) but the buffering capacity was significantly lower in the "honey" group than in the control group (p=0.02). Furthermore, there was a significant effect of time on the change of salivary pH (p=0.003).Acaciahoney led to a significant decrease in buffering capacity while affecting saliva pH levels mildly; nevertheless, the effects were transient.

Keywords
Honey; Saliva; Buffer; Gingivitis; Periodontal diseases

Introduction

Recently, there has been growing focus towards the use of natural products for health-related purposes. Honey is one of the most commonly advocated products. Since antiquity, honey has been utilised as an eco-friendly medicine for treating burns, infected wounds [1,2], peptic ulcers, bacterial gastroenteritis [3,4], and ophthalmic infections [5]. Besides its effectivenessin broad-spectrum of antibacterial activities, research works have exhibited that honey demonstrates anti-cancer properties [6].

Different kinds of honey exist, depending on the plants from where it is sourced and the geographic region [7,8]. One of the most researched honey is Acacia, created by *Apis mellifera* honeybees which reap extrafloral nectar from Acaciamangium trees [9]. This honey was found to aid wound contraction from burn harm [10]. Furthermore, it comprises flavonoids and phenolic acids that act as antioxidants [11-13]. A combination of silver nitrate with Acaciahoney was

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found to have antibacterial properties towards both gram-positive and gram-negative bacteria; activity levels were higher than Acacia honey or penicillin used individually [14].

With the growing interest towards the medicinal attributes of honey, a few research works have been carried out for evaluating the positive effects of honey within the domain of dentistry. The possible therapeutic usage of honey for treating gingivitis and periodontal disease was first reported in 2004[6].In a recent article on the antimicrobial potentials of honey against periodontal pathogens, Hbibi*et al*. (2020) noted that the honey tested in the studies, most of which being Manuka and multi-floral honey variants, exhibited significant antimicrobial effect against six microorganisms [15]. A research work by Patel *et al.* (2010) on bacterial isolates acquired from patients going through orthodontic treatment exhibited that honey was a more effective antibacterial agent compared to few of the typical antibiotics tested [16]. Thus, honey was likely effective in inhibiting dental plaque formation and reducing gingivitis associated with orthodontic procedures [16-19].

Anaerobic gram-negative bacteria are the biggest cause of periodontal problems; such microorganisms invade the tooth surface in the proximity of the gingival margin leading to periodontal destruction [20,21]. *Aggregatibacteractinomycetemcomitans*, *Tannerella forsythia*, and *Porphyromonasgingivalis* have been noted as the most common aetiological bacteria. Other organisms related to the development of periodontal ailments encompass *Eubacterium nodatum*, *Campylobacter rectus*, *Prevotella intermedia*/*nigrescens*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Streptococcus intermedius*-complex, *Treponema denticola*, and spirochetes [22]. Two published research works have pointed out that the growth of microorganisms is impacted by pH value; for instance, *P.intermedia* grows at a pH of 5.0-7.0, *P. gingivalis* grows at a pH of 6.5-7.0, whereas *F. nucleatum* grows at a pH of 5.5-7.0 [23,24]. The growth of microorganisms causing periodontal conditions is affected by the pH level in the oral cavity.

In view of that, salivary parameters such as salivary pH and buffering capacity in patients with periodontal disease are of interest to many researchers. The normal range of salivary pH is 6.2-7.6, with 6.7 being the average pH. Nevertheless, patients suffering from chronic generalised gingivitis typically have higher pH levels, representing higher alkalinity than healthy individuals [25]. In contrast, saliva buffering capacity indicates the degree to which the host can offset plaque pH reduction caused by acidogenic organisms. If buffering capacity is high, the saliva can neutralise acid effectively, thereby resisting demineralisation [26].Hence, such salivary characteristics can represent as biomarkers for chairside diagnosis of periodontal conditions as proposed by Baliga*et al.* (2013). They concluded that chronic generalised periodontitis or gingivitis affected saliva pH levels [25]. Nevertheless, there has been little research concerning salivary parameters specific to periodontal disease patients, explicitly those suffering from chronic gingivitis. Similarly, there is also lack of studies on the association of honey with salivary parameters, despite the well-established benefits of honey for chronic gingivitis patients. Hence, the present study was performed to assess how honey affected saliva pH levels and buffering capacity in patients with chronic generalised gingivitis.

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Methods

Study Population

This was a randomised trial conducted at the dental clinics of Hospital USM in Kelantan, Malaysia. Upon approval from the Human Research and Ethics Committee USM/JEPeM/16030124, patients within the age group of 18 to 55 years with chronic generalised gingivitis were invited to participate in the study. A patient is diagnosed to have chronic generalised gingivitis if they have inflammation of the gingiva without loss of attachment as well as experiencing bleeding on probing in more than 30% of the sites.

Patients were excluded from the study if they were edentulous, smoker or past smoker, allergic to honey, presented with malocclusion, mouth breathers, undergoing or had a history of radiationand chemotherapies, had a history of hospitalisation or intake of medications in the past six months, suffering from diabetes, kidney disease, cancer, fungal or respiratory infections.

Clinical intervention

After written informed consent was obtained, the patients were randomised into the “honey” and control groups. Demographic data such as age, gender, and ethnicity were recorded. All subjects were instructed against eating, drinking, or chewing gum for at least 2 hours, as well as to rinse their mouths at least one hour before the examination [27-29].

The subjects in both groups received full-mouth scaling and polishing treatment. However, the subjects in the “honey” group also received an application of Acacia honey directly onto their gingiva with sterile cotton bud swab stick after the treatment. Following that, they were required to not rinse or drink for 30 minutes.

Saliva collection

The patients were asked to relax and sit upright and have the head bent downwards; they were provided small bits of paraffin wax which they chewed on until it became soft [30]. Subsequently, the first portion of saliva was swallowed. They were then told to refrain from swallowing for five minutes; a plastic bottle was used for collecting spit [31,32]. The stimulated saliva produced was sampled before, after 15 and 30 minutes of full mouth polishing and scaling.

The GC Saliva-Check BUFFER® (GC Co., Japan) was used for evaluating the obtained saliva specimens for pH level and buffering capacity; this was a chairside assessment. A strip was removed from the buffer test foil-sealed container and kept on an absorbent material; the test side was facing upwards. Saliva was extracted using a pipette and one drop each was placed on the three testing pads after which, the strip was rotated by 90° to allow the absorbent material to take up excess saliva. It was a vital step to prevent swelling up of the excess saliva on the test pad, leading to compromised test result accuracy [33]. Immediately, the test pads would display a change of colour, whereby the final colour changes would be recorded after two minutes.

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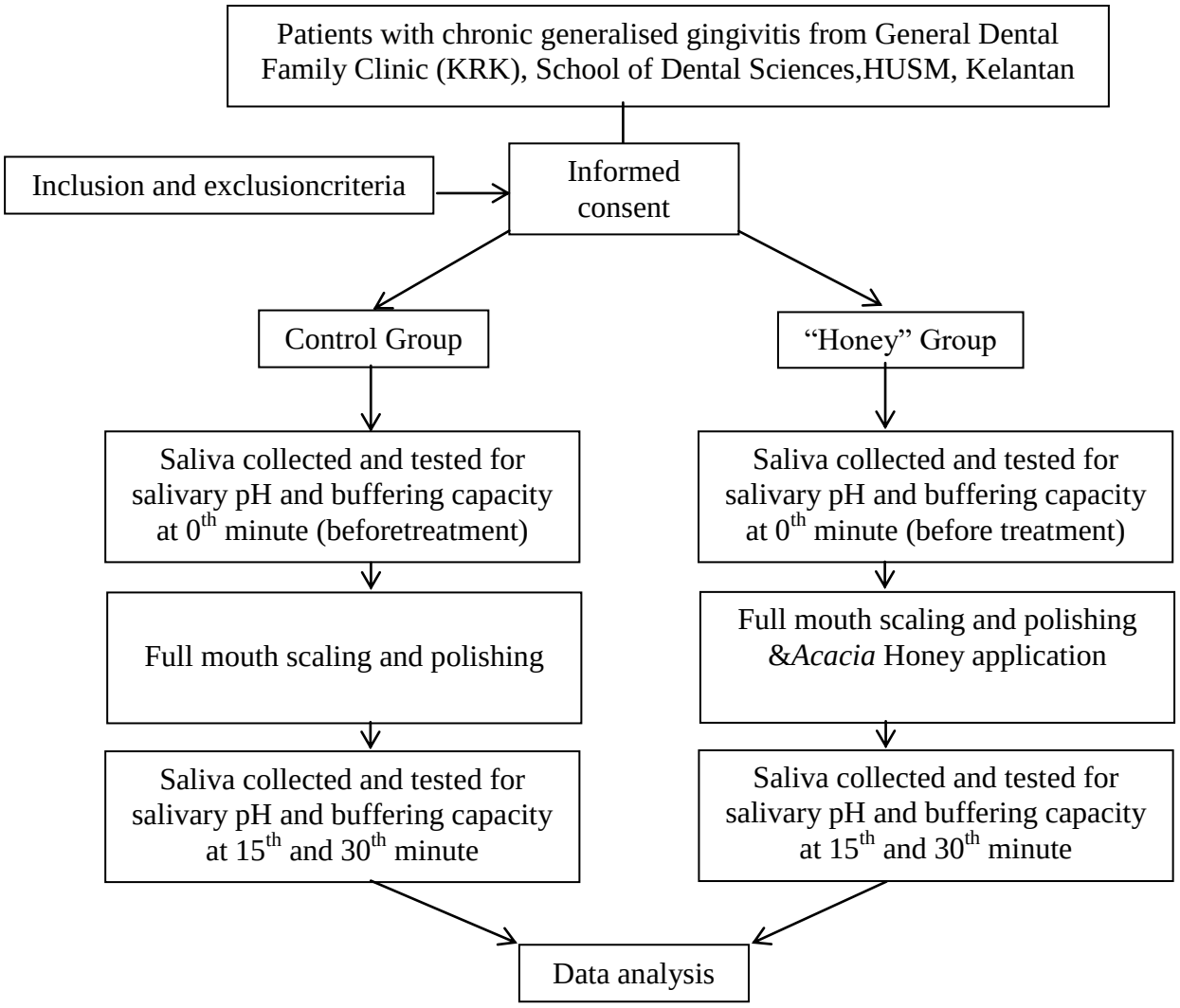
The result was calculated by aggregating points as per the final colour reflected on each pad, i.e., 4, 3, 2, 1, and 0 points for green, green-blue, blue, red-blue, and red, respectively. The sum of the aggregated points indicated the buffering capacity: 10-12 suggests normal or high buffering level, 6-9 points suggest low capacity and 0 to 5 indicates very low buffering capacity [34].

Saliva pH evaluation was conducted by placing the test strip into the saliva sample for ten seconds. Subsequently, the colour transformation was contrasted against the reference chart, and its pH was determined. Result interpretation was done as: pH 5-5.8 indicates very acidic saliva, 6.0-6.6 indicates moderate saliva acidity, while the 6.8-7.8 indicates healthy pH [33].

Flow chart of study



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Data Analysis

Data was entered and analysed using Statistical Package for Social Sciences (SPSS) version 22.0. The descriptive assessment was performed for the two groups. A two-way ANOVA repeated measures test was performed to contrast the mean difference in buffering capacity and salivary pH at the three intervals and between the "honey" and the control groups. This test was performed at the α=0.05 significance level.

Results

The 30 subjects in this study had a mean age of 26.4 years (SD 8.03). Most of the subjects were females (76.7%) and of Malay ethnicity (83.3%). Table 1 summarises the demographic characteristics of all the subjects diagnosed with chronic generalised gingivitis.

Table 1: Demographic characteristics of the study subjects (n=30)

Characteristics	Frequency (%)		Mean (SD)	
	Honey group	Control group	Honey group	Control group
Age (years)			24.20 (6.405)	28.67 (9.045)
Gender				
Male	3 (10.0)	4 (13.3)		
Female	12 (40.0)	11 (36.7)		
Ethnicity				
Malay	12 (40.0)	13 (43.5)		
Chinese	1 (3.3)	1 (3.3)		
Indian	1 (3.3)	0 (0.0)		
Others	1 (3.3)	1 (3.3)		

Salivary pH

Table 2 shows the mean salivary pH for both the "honey" group and control group at baseline, 15th, and 30th minute. All values indicate healthy saliva pH. The "honey" group had a significantly higher mean salivary pH compared to the control group at baseline (mean difference=0.4, SE=0.13, p=0.002). In the "honey" group, there was a decrease in the salivary pH at the 15th minute after the application of Acaciahoney. An increase in the salivary pH was noted at the 30th minute.

The control group was observed to have a gradual saliva pH increase at the 15th and 30th minute (Figure 3). Overall, saliva pH differences were not statistically significant for the two groups (p=0.9).

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Table 2: Result of analysis comparing the changes in mean salivary pH for "honey" group and control group at 0th minute, 15th minute, and 30th minute

Time	Control Group	Honey Group	Total salivary pH	Time	Honey Group vs Control Group
	Mean (SD)	Mean (SD)	Mean (SD)	F (*p*-value)	F(*p*-value)
0th minute	6.840 (0.3225)	7.267 (0.3677)	7.053 (0.4032)		
15th minute	7.280 (0.3278)	7.040 (0.4356)	7.160 (0.3979)	6.99 (0.003)	0.023(0.9)
30th minute	7.320 (0.3189)	7.187 (0.3815)	7.253 (0.3521)		

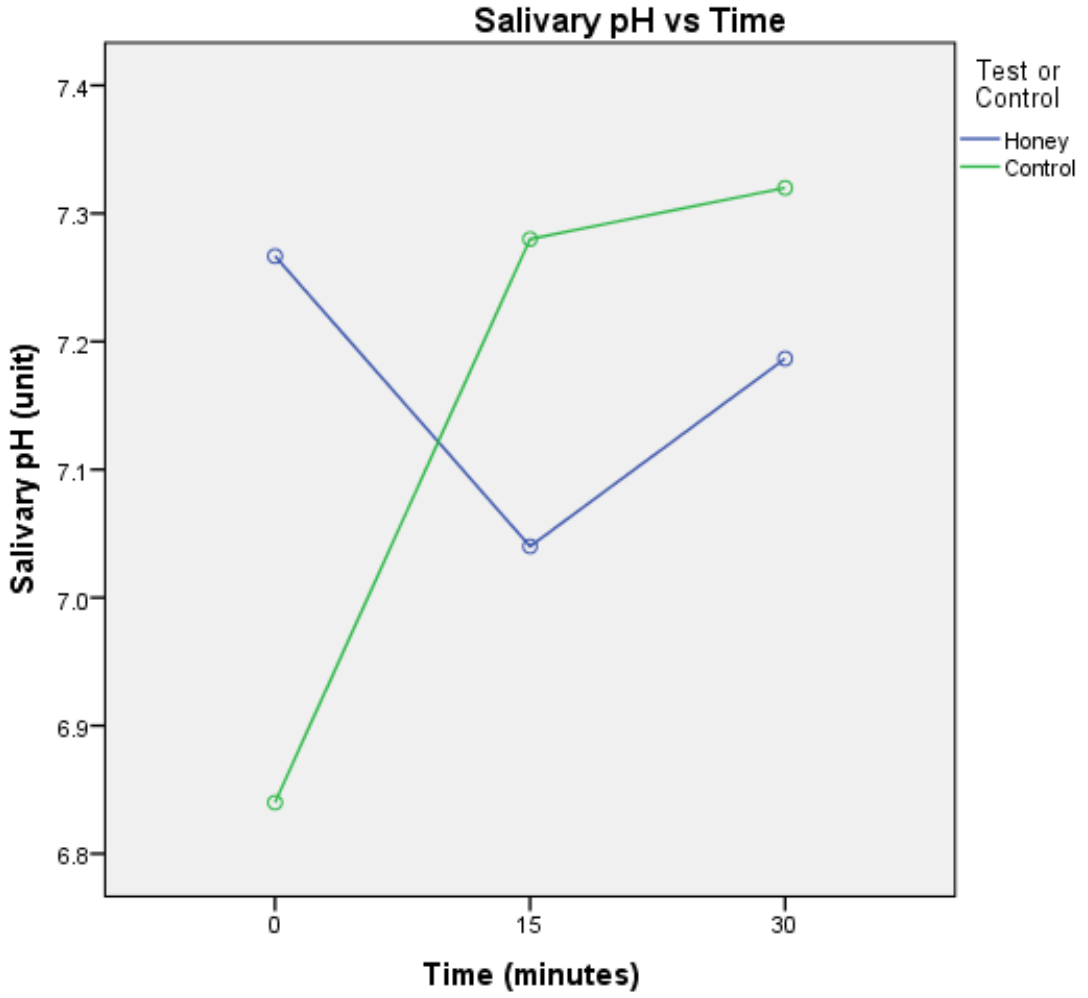

Figure 3: Salivary pH at 0th minute, 15th minute, and 30th minute in "honey" group and control group

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The Mauchly Sphericity test used in the two-way repeated ANOVA analysis suggested that the sphericity assumption was not violated ($\chi2(2) = 5.782$, p=0.06). Time affected salivary pH significantly (p=0.003). Post hoc assessment suggested that initial saliva pH (0^{th} minute) was significantly higher than the 30^{th} minute reading after the treatment (p<0.001). However, there was no statistical significance between the readings at the 0^{th} and 15^{th} minutes (p=0.11) and 15^{th} and 30^{th} minutes (p=0.09). These values are specified in Table 4.

Table 4: Comparison of salivary pH between different duration of time

Comparison of Salivary pH between time	**Difference**	**95% Confidence Interval**		***p*-value**
		Lower Bound	**Upper Bound**	
0^{th} minute vs 15^{th} minute	-0.11	-0.23	0.02	0.095
0^{th} minute vs 30^{th} minute	-0.20	-0.28	-0.12	<0.001
15^{th} minute vs 30^{th} minute	-0.09	-0.21	0.02	0.105

Buffering capacity

Table 5 shows the mean buffering capacity for both groups. Saliva from the "honey" group had normal buffering capacity before treatment; nevertheless, there was a significant drop at 15^{th} minutes after the honey was applied.

For the control group, the saliva buffering capacity rose steadily from baseline and became normal at the 15^{th} minute and 30^{th} minute after the completion of periodontal treatment (Figure 6).

The buffering capacity of saliva was significantly lower in the "honey" group compared to the control group (p=0.02) (Table 5).

Mauchly's Sphericity Test was used as part of the two-way repeated ANOVA assessment; the test suggested that sphericity assumption could be held ($\chi2(2) = 1.125$, p=0.57). Statistics are indicated in Table 5; time did not significantly affect saliva buffering capacity (p=0.3).

Table 5: Comparison of the changes in mean buffering capacity of saliva for "honey" group and control group at 0^{th} minute, 15^{th} minute, and 30^{th} minute

Time	**Control Group**	**Honey Group**	**Total buffering capacity**	**Time**	**Honey Group vs Control Group**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**F (*p*-value)**	**F (*p*-value)**
0^{th} minute	**9.07 (1.831)**	**10.13 (1.767)**	**9.60 (1.850)**	**1.23 (0.3)**	**6.047(0.02)**

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15th minute	**10.53 (1.187)**	**8.07 (2.154)**	**9.30 (2.120)**
30th minute	**10.93 (1.033)**	**8.93 (2.404)**	**9.93 (2.083)**

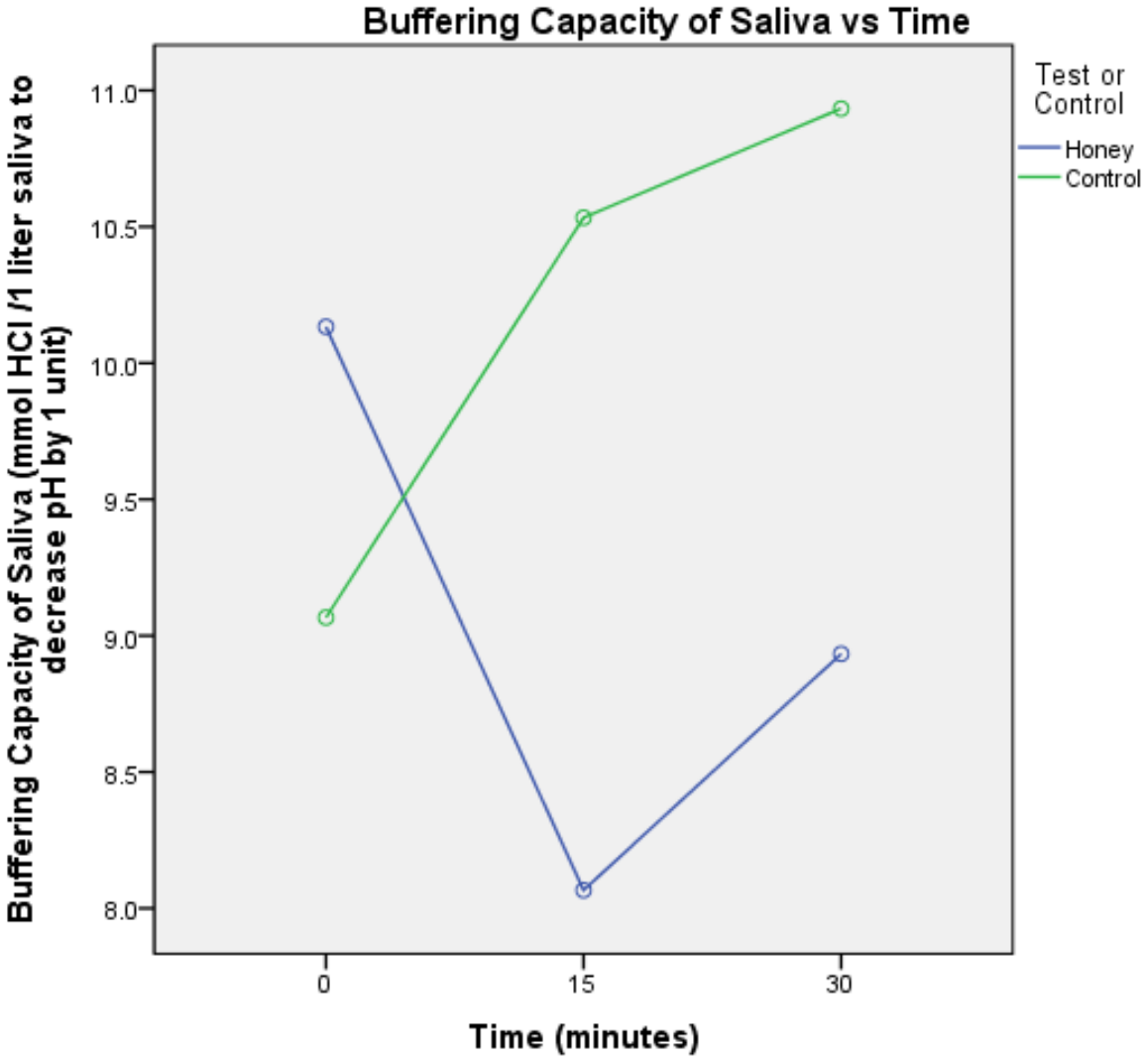

Figure 6: Buffering capacity at 0th minute, 15th minute, and 30th minute in the "honey" group and control group

Discussions

Saliva is critical for oral health maintenance. Saliva acts as an acid buffer and regulates oral pH above threshold requirement, thereby preventing tooth demineralisation [35]. In this study, the subjects in the "honey" group had a mean saliva pH of 7.27 ± 0.37, which is close to the 7.24 ± 0.10 determined by Baliga*et al.* (2013). Saliva pH of 7.0 represents healthy oral condition with a less likelihood of caries, while calculus is minimal or zero. On the other hand, pH higher than 7.0

indicates alkalinity [25];alkaline condition can predispose plaque formation among patients with chronic generalised gingivitis, hence accounting for the moderate alkaline saliva pH for the subjects in this study.

Though both groups comprised of patients having chronic generalised gingivitis, baseline mean buffering capacity and saliva pH levels were significantly lower for the control group as compared to the "honey" group. In this study, caries-free was not pre-identified as an inclusion criterion. Hence, it could be an explanation for the lower baseline mean salivary pH and mean buffering capacity among some of the subjects in the control group. Numerous studies have established an association between saliva buffering capacity with dental caries. Ericsson (1959) indicated that patients with and without caries presented variations in saliva buffering capacity [35]. Moreover, the Vipeholm study suggested that individuals free of caries had higher buffering capacity compared to those with carious lesion [36].

Apart from that, the present study indicated that the application of Acacia honey led to a noteworthy reduction in buffering capacity against the control group (p=0.02). Saliva pH decreased after 15 minutes; however, the reduction was not statistically significant. It is probable that the high acidity of Acacia honey led to a reduction in saliva pH and affected buffering capacity. Previous research suggests that Malaysian Acaciahoney has a pH range of 3.53 ± 0.06, while the honey freshness threshold is pH 3.4-6.1. The Acaciavariety honey is more acidic than any other honey found in Malaysia; this includes Borneo honey (pH value of 4.03 ± 0.06), Tualang honey (pH value of 3.80 ± 0.0), and Pineapple honey (pH value of 3.73 ± 0.06) [9]. Recent research by Fuad *et al.* (2020) suggests that Acacia honey stored at room temperature has a pH range of 3.15-3.46. Nevertheless, it gains acidity at low temperatures (4 and -20^{0}C). Hence, it can be concluded that temperature affects honey pH level [37].

Moreover, the results indicated that in spite of salivary pH and buffering capacity reduction for the "honey" group at the 15th minute, baseline pH and buffering capacity steadily improved by the 30th minute. It might be caused by the effective buffering activity of the saliva leading to acid neutralisation for both periods after application. High concentration of sugar content (68.40± 0.80) present in Acacia honey might be the reason for the initial pH reduction [9]. The particular trend in saliva pH changes for the subjects might be understood using the Stephen curve. A noticeable pH decrease was observed five to ten minutes after coming in contact with sugar. Nevertheless, fifteen to thirty minutes after the sugar challenge, pH levels stabilised to the baseline values. Considering these observations, it is vital to gather more data concerning saliva pH directly after the honey is used; moreover, data should then be recorded within shorter intervals, for example, at the 5th, 10th, 15th, and 30th minutes. It is recommended to have a larger sample to counter possible confounding aspects that may interfere with study results. Collectively, these recommendations are expected to provide enhanced understanding concerning the correlation between honey and saliva pH.

The present study demonstrated a substantial rise in salivary pH level (p=0.003) after scaling and polishing treatment. The outcome is aligned with a study concerning Turkish children with gingival inflammation in which a significant increase in saliva pH was reported after scaling treatment ($p< 0{\cdot}001$) [38].

The present study assessed the direct application of honey using a sterile cotton bud swab stick on the gingivae of the participants. The outcome indicated that honey caused no considerable impact on the pH of saliva. Nevertheless, the disadvantage of the technique is that honey does not persist in the oral cavity for long. For future research, honey in the form of diluted mouth rinse can be considered as an alternative method of application due to ease of delivery and broader coverage of the gingival surface area. Moreover, diluted honey should have better antibacterial properties than its concentrated form because of the presence of glucose oxidase that prolongs hydrogen peroxide production [39,40], a known antimicrobial agent present in honey [41].

Conclusion

To the best of our knowledge, the present study is the first to investigate the influence of Acacia honey on salivary pH and buffering capacity in patients with chronic generalised gingivitis. Acaciahoney led to a significant decrease in buffering capacity while affecting saliva pH levels mildly; nevertheless, the effects were transient.

Acknowledgement

The authors would like to express gratitude towards the patients involved in this research work, staff and nurses at the trauma centre dental clinic for their assistance in providing treatment for the patients and Human Research Ethics Committee of USM (JEPeM) for granting approval for the research.

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