

Effect of Khat Chewing on the Salivary pH before and after Using Mouthwashes

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Original Article

Abstract

Background: Saliva is a biological fluid that has multiple protective functions. These functions can be attained only if the saliva keeps certain physio-chemical properties such as pH.

Methods: The main purpose of the current study was to compare the salivary pH among khat chewers and non-khat chewers before and after specific intervals (15, 30, and 60 minutes) of using three commercially available mouthwashes (MWs). Thirty pharmacy student volunteers were divided into three groups of 10 for the three MWs. Each group was then subdivided into 5 volunteers as khat chewer and non-khat chewer. The statistical evaluation was carried out by using paired t-test and analysis of variance (ANOVA) for in vivo study of salivary pH.

Findings: Low salivary pH in all khat-user volunteers before and after using MWs was indicated. There was an increase in the salivary pH after 15 minutes of rinsing with the three selected MWs for both groups of volunteers, after that there was a gradual decrease to the initial pH. The difference between the three groups was not significant at baseline and after 15 minutes, but there was a significant difference after 30 and 60 minutes for the non-khat-chewing and khat-chewing volunteers.

Conclusion: It can be concluded that using MWs results in altering salivary pH and continuous khat chewing lowers the baseline pH of saliva and ability to compensate the change of salivary pH.

Keywords: Mouthwashes; Khat; Saliva

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Introduction

Saliva is a multicomponent biological fluid secreted from salivary gland, containing 99% water and 1% organic and inorganic components with pH of 6.5-7.5. It helps in maintaining the physiological environments of oral cavity through several functions.¹ Oral clearness is defined as a period of time passed between the ingestion of substances in the oral cavity and the instant when its existence no longer be distinguished.² Oral clearness characteristics differ according to individual and depends on many factors, including the salivary flow and enzyme.³ Several salivary factors such as pH, flow rate, oral clearance, the concentration of calcium and phosphate, and others affect the enamel stability.⁴ The pH of the salivary must not be reduced to below 5.5, because this decrease result in demineralization of tooth enamel. The salivary pH must return to the normal pH, which depends on the buffering capacity of the saliva. The main aim of the current study is to evaluate the change of salivary pH after using three mouthwashes (MWs) of different constituents and pH. MWs are considered as pharmaceutical or cosmetic products for oral hygiene, according to their formulation ingredient. They help in reducing the inflammation and dental caries due to their antimicrobial activity.⁵

Yemeni people have unhealthy habit of chewing fresh leaves and twigs of khat (*Catha edulis*), an evergreen plant of the Celastraceae family.⁶ Yemeni people of most age groups and socioeconomic levels consume khat for 4-6 hours per day due to its amphetamine stimulating effects.⁷ This habit was not limited to Yemenis and spread out to the countries of Europe, the United States of America (USA), Australia, and others due to worldwide immigration.⁸ Prevalence of this habit leads to several undesired health effects in general^{9,10} and on dental and oral tissues as well.¹¹⁻²¹ Keeping of khat leaves in the oral cavity for long time results in xerostomia due to extraction of cathinone derivatives which are the essential psychostimulant components of khat.²²⁻²⁴ Long-term chewing also leads to exhaustion, enlargement, and inflammation of the salivary gland²⁵ and a reduction of pH due to increase of salivary uric acid,²⁶ flow rate, and viscosity of saliva.²⁴ Due to the prevalence of

khat-chewing habit and its harmful influences on the oral health, the present study involved the khat chewers in order to get insight about the change of pH before and after using the MWs and measure the buffer capacity of saliva. The literature review revealed that there had been no in vivo study that evaluated and compared the salivary pH changes before and after using MWs of different constituents among khat chewer and non-khat chewer individuals.

Methods

A total of 30 undergraduate students (aged 20-26 years) were involved in the study; the selection was based on being healthy and free from any oral or other diseases. After that, the volunteers were divided into khat users and non-khat users. These individuals were divided into three groups of five volunteers for each MW. Before conducting the study, ethical approval (Coded REC-50-2019) was attained from the Ethics Research Committee of the School of Medicine and Health Sciences, Aden University, Yemen. The purpose and methodology of the study was clarified to each of the volunteers and informed consent was obtained.

Three commercially-available and mostly-used MWs were selected that had different constituents and pH value. The first MW (non-herbal) contained: chlorhexidine gluconate, sorbitol, propylene glycol, methyl and propyl parabens, peppermint flavor, menthol, and ethanol. The second MW (herbal and non-herbal) contained: thymol, benzoic acid, eucalyptol, menthol, and methyl salicylate. The third MW (herbal) contained: menthol oil, peppermint oil, rose oil, and clove oil. The laboratory-measured pH of these MWs was 6.85, 3.74, and 6.16, respectively.

Before the examination, the volunteers obtained the guideline about way of collection of saliva. About 1 ml of unstimulated saliva was collected in the morning before having breakfast. The volunteers were informed to allow a saliva pool in the floor of the mouth for at least 1 minute before using MWs and then expectorate into a disinfected disposable container.²⁷ Then, the volunteers rinsed with the selected MWs for 30 seconds by properly stirring the complete content in the oral cavity at once and then expectorated, after which the unstimulated salivary pH was again measured at 15, 30, and

60 minutes, respectively.²⁸

The salivary pH was measured using a digital pH meter (inoLab, WTW, Germany) calibrated using buffers of pH 4, 7, and 9. The sensing electrode was cleaned after every sample and the accuracy of the pH meter was verified by dipping the electrode in a standard solution of pH 7 at the systematic period to certify that measurement was exact.

The measured pH values during the progression of the study were systematically introduced in Microsoft Excel sheet. Then statistical data analysis was performed using GraphPad Prism 6. Paired t-test was used for intragroup comparison, while analysis of variance (ANOVA) and Tukey's test were used for intergroup comparison.

Results

The current study was carried out to estimate and compare the pH of the saliva in healthy volunteers before and after using three MWs. Since most of the Yemeni population were khat chewers, the volunteers were divided into two groups (khat chewing and non-khat chewing) to evaluate the pH differences between these two groups. The salivary pH plays an important role in oral health and new researches proposed its significance in dental caries.²⁹ The pH of MWs may affect the salivary pH. The low pH of MWs may affect the enamel and mineral structure on the tooth's surface;³⁰ also it has dental erosion potential.^{31,32} An in vitro study indicated that prolonged use of MWs under study influenced the dentine smear layer, particularly if used in combination with mechanical tooth brushing.³³

The current study results showed a gradual increase in the salivary pH from the baseline value to 15 to 30 minutes after using MWs. This finding specifies that saliva tries to compensate the effect of MW pH due to its dynamic system. Comparable result was obtained by other studies carried out in India.^{33,34} The result of changing salivary pH with time intervals is represented in figure 1.

Statistical evaluation of the pH differences before and after using MWs: Intragroup comparison was done by paired t-test whereas repeated measure ANOVA and Tukey's test were used in intergroup comparison.

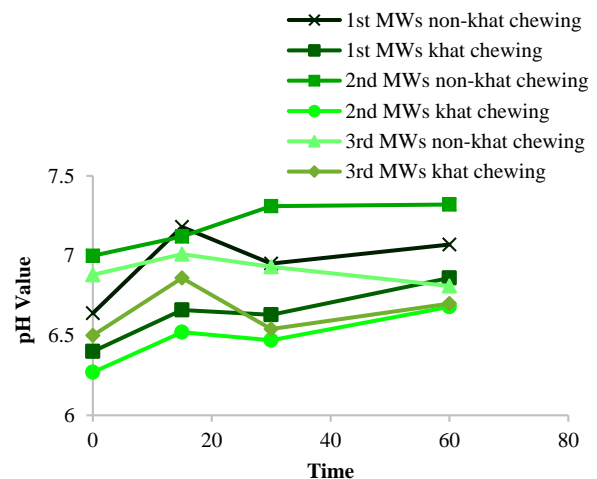


Figure 1. The salivary pH changes at different time intervals in all groups

Intragroup comparison of first MW (non-khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were 0.61 ± 0.24 , 0.35 ± 0.20 , and 0.48 ± 0.14 , respectively (Table 1). The overall $P < 0.05$ in 1st MW (non-khat chewing) group was statistically significant.

Intragroup comparison of first MW (khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were 1.00 ± 0.57 , 1.23 ± 0.65 , and 1.22 ± 0.69 , respectively (Table 1). The overall $P < 0.05$ in 1st MW (khat chewing) group was statistically significant.

Intragroup comparison of second MW (non-khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were -0.11 ± 0.15 , -0.31 ± 0.08 , -0.31 ± 0.19 , respectively. The overall $P < 0.05$ in 2nd MW (non-khat chewing) group was statistically significant except for baseline to 15 minutes with $P > 0.05$ (Table 1).

Intragroup comparison of second MW (khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were -0.25 ± 0.82 , -0.20 ± 0.82 , and -0.41 ± 0.92 , respectively (Table 1). The $P > 0.05$ in 2nd MW (khat chewing) group was not statistically significant.

Intragroup comparison of third MW (non-khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were -0.14 ± 0.39 , -0.05 ± 0.32 , and 0.07 ± 0.21 , respectively (Table 1).

Table 1. Intragroup comparison of three groups (paired samples t-test)

Duration (minute)	First MW (non-khat chewing)				
	Paired differences		t	df	P
	Mean ± SD				
Pair 1	0-15	-0.60 ± 0.23	5.746	4	0.0045
Pair 2	0-30	-0.35 ± 0.19	3.993	4	0.0162
Pair 3	0-60	-0.47 ± 0.13	7.667	4	0.0016
First MW (khat chewing)					
Pair 1	0-15	-1.04 ± 0.57	4.092	4	0.0149
Pair 2	0-30	-1.23 ± 0.65	4.236	4	0.0133
Pair 3	0-60	-1.22 ± 0.69	3.000	4	0.0167
Second MW (non-khat chewing)					
Pair 1	0-15	-0.11 ± 0.14	1.744	4	0.1562
Pair 2	0-30	-0.30 ± 0.08	8.161	4	0.0012
Pair 3	0-60	-0.31 ± 0.19	3.652	4	0.0217
Second MW (khat chewing)					
Pair 1	0-15	-0.25 ± 0.82	0.864	4	0.5315
Pair 2	0-30	-0.20 ± 0.81	0.557	4	0.6072
Pair 3	0-60	-0.41 ± 0.92	0.994	4	0.3763
Third MW (non-khat chewing)					
Pair 1	0-15	-0.13 ± 0.38	0.783	4	0.4772
Pair 2	0-30	-0.05 ± 0.31	0.365	4	0.7334
Pair 3	0-60	0.07 ± 0.20	0.748	4	0.4956
Third MW (khat chewing)					
Pair 1	0-15	-0.35 ± 0.22	3.586	4	0.0230
Pair 2	0-30	-0.04 ± 0.28	0.335	4	0.7543
Pair 3	0-60	-0.19 ± 0.15	2.842	4	0.0468

SD: Standard deviation; df: Degree of freedom; MW: Mouthwash

The overall $P > 0.05$ in 3rd MW (non-khat chewing) group was not statistically significant.

Intragroup comparison of third MW (khat chewing) group: The mean pH differences from baseline to 15 minutes, baseline to 30 minutes, and baseline to 60 minutes were -0.36 ± 0.22 , -0.04 ± 0.28 , and -0.20 ± 0.16 , respectively. The $P < 0.05$ in 3rd MW (khat chewing) group was statistically significant except for baseline to 30 minutes with $P > 0.05$ (Table 1).

Intergroup comparison of salivary pH at baseline, 15, 30, and 60 minutes between three groups (non-khat chewing): In the case of 1st MW, there was an increase in the salivary pH after 15 minutes, then there was a small reduction in the salivary pH after 30 minutes and 60 minutes; in the case of 2nd MW, the salivary pH started to increase after 15 minutes and remained almost constant after 30 and 60 minutes. While in the case of 3rd MW, there was a gradual increase in the salivary pH and then it restored to the initial pH (Table 2).

The difference between the groups at the

baseline was not statistically significant ($P = 0.1313$); after 15 minutes, it was also found to be statistically not significant ($P = 0.3016$); however, there was a significant difference between groups after 30 minutes ($P = 0.0350$) and after 60 minutes ($P = 0.0346$).

Table 2. Intergroup comparison with relation to all times for non-khat chewing volunteers (n = 5)

Duration (minute)	Groups (non-khat chewing)	Mean ± SD
0	1 st MW	6.70 ± 0.25
	2 nd MW	7.00 ± 0.13
	3 rd MW	6.87 ± 0.16
15	1 st MW	7.31 ± 0.35
	2 nd MW	7.12 ± 0.18
	3 rd MW	7.01 ± 0.25
30	1 st MW	7.05 ± 0.20
	2 nd MW	7.31 ± 0.14
	3 rd MW	6.92 ± 0.20
60	1 st MW	7.18 ± 0.19
	2 nd MW	7.32 ± 0.22
	3 rd MW	6.80 ± 0.19

SD: Standard deviation; MW: Mouthwash

Intergroup comparison of salivary pH at baseline, 15, 30, and 60 minutes between three groups (khat chewing): In case of 1st MW, there was an increase in the salivary pH after 15 and 30 minutes, then there was a small reduction in the salivary pH after 60 minutes; in case of 2nd MW, the salivary pH started to increase after 15 and 30 minutes and remained almost constant after 60 minutes. While in case of 3rd MW, there was a gradual increase in the salivary pH after 15 minutes, then it restored to the initial pH after 30 minutes and started to increase again after 60 minutes (Table 3).

Table 3. Intergroup comparison with relation to all times for khat-chewing volunteers (n = 5)

Duration (minute)	Groups (khat chewing)	Mean ± SD
0	1 st MW	5.68 ± 0.53
	2 nd MW	6.26 ± 0.92
	3 rd MW	6.50 ± 0.41
15	1 st MW	6.73 ± 0.16
	2 nd MW	6.51 ± 0.51
	3 rd MW	6.85 ± 0.54
30	1 st MW	6.92 ± 0.18
	2 nd MW	6.47 ± 0.49
	3 rd MW	6.54 ± 0.64
60	1 st MW	6.90 ± 0.29
	2 nd MW	6.67 ± 0.21
	3 rd MW	6.69 ± 0.45

SD: Standard deviation; MW: Mouthwash

The difference between the groups at the baseline was not statistically significant (P = 0.2049) and after 15, 30, and 60 minutes, it was also found to be statistically not significant (P = 0.4345, P = 0.2768, and P = 0.4801, respectively).

Discussion

After 60 minutes, the salivary pH starts to return back to the near baseline value in the non-khat chewer volunteers specifically in the case of using herbal MWs. While in the case of khat-chewer volunteers, the difference between the baseline and after 60 minutes pH value is slightly higher than non-khat users. It was noticed that the average baseline salivary pH of khat-chewing volunteers (6.15) was low in comparison to the normal non-khat chewing volunteers (6.5). This finding is in line with the parallel study carried out to compare the salivary parameters among

khat chewers and non-chewers,²⁴ which can be attributed to the high uric acid level in saliva as mentioned before.²⁶

The salivary pH control is based on the salivary flow rate. The salivary pH becomes more acidic with slow rate and more alkaline with faster rate.³⁵ The above-mentioned study also showed that the khat chewers might have lower salivary flow rate. The underlining factors may be due to salivary gland exhaustion with regard to chewing for several hours daily, lack of stimulus to the salivary gland, or a problem with the salivary gland itself.²⁴ Also, another study showed a correlation between khat chewing and salivary gland enlargement and inflammation.²³ This study has some limitations: the result cannot be generalized since the study was limited to a small group of society; though, additional studies are suggested taking more advancement with higher number of volunteers. The duration of study is recommended to be increased because the return of the salivary pH to the baseline was not complete in 60 minutes. Other salivary parameters such as buffering capacity, flow rate, and constituents are suggested to be evaluated. Oral health related education, programs, training, and rising awareness about the suitable oral habits and hygiene and avoiding unhealthy habits (khat chewing) should be carried out to enhance oral health, not only among the college students but also in primary schools.

Conclusion

The current study showed that there was a gradual increase in the salivary pH after 15 minutes of using the selected MW; then, the salivary pH started to return to the baseline value after 30-60 minutes in case of non-khat users. The khat-chewing volunteers showed lower baseline salivary pH than non-khat chewing volunteers which can be related to the decrease in the flow rate of saliva. In addition, the khat chewers exhibited a slight delay in returning to the baseline pH in comparison to the non-khat chewers which can be attributed to insufficient salivary buffering capacity. There were statistically significant intergroup differences between non-khat users especially after 30 and 60 minutes. These differences can be explained by individual variation. While in the case of khat users, there was no statistically significant

intergroup differences. This can be explained by presence of mutual factor which khat-chewing.

Conflict of Interests

The Authors have no conflict of interest.

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تأثیر جویدن قات بر pH بزاق قبل و بعد از استفاده از دهان شویه

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مقاله پژوهشی

چکیده

مقدمه: بزاق یک مایع بیولوژیک می‌باشد که عملکردهای حفاظتی متعددی دارد. این عملکردها در صورتی تداوم خواهد داشت که خواص فیزیوشیمیایی خاص آن مانند pH حفظ شود.

روش‌ها: هدف از انجام پژوهش حاضر، مقایسه pH بزاق بین کسانی که قات می‌جویدند و آن‌هایی که قات استفاده نمی‌کردند، قبل و بعد از استفاده از سه دهان شویه موجود در بازار در بازه‌های زمانی خاص (۱۵، ۳۰ و ۶۰ دقیقه) بود. ۳۰ دانشجوی داروسازی به صورت داوطلبانه در سه گروه ۱۰ نفره (هر گروه برای یک دهان شویه) قرار گرفتند. سپس هر گروه به دو گروه ۵ نفره استفاده‌کنندگان از قات و کسانی که قات مصرف نمی‌کردند، تقسیم شدند. داده‌ها با استفاده از آزمون Paired t و ANOVA به منظور بررسی درون تنی pH بزاق تجزیه و تحلیل گردید.

یافته‌ها: میزان pH کم بزاق در مصرف‌کنندگان قات قبل و بعد از استفاده از دهان شویه‌ها مشخص شد. پس از ۱۵ دقیقه شستشو با سه دهان شویه انتخابی برای هر دو گروه از داوطلبان، افزایش pH مشاهده گردید و پس از آن یک کاهش تدریجی نسبت به pH اولیه وجود داشت. تفاوت معنی‌داری بین سه گروه در ابتدا و ۱۵ دقیقه بعد مشاهده نشد، اما بعد از ۳۰ و ۶۰ دقیقه، بین مصرف‌کنندگان قات و آن‌هایی که قات مصرف نمی‌کردند، تفاوت معنی‌داری وجود داشت.

نتیجه‌گیری: استفاده از دهان شویه‌ها منجر به تغییر pH بزاق می‌گردد و جویدن مداوم قات، pH اولیه بزاق و توانایی جبران تغییر pH بزاق را کاهش می‌دهد.

واژگان کلیدی: دهان شویه‌ها، قات، بزاق

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