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THE EFFECT OF GRAPE SEED EXTRACT MOUTHWASH ON SALIVA BUFFERING CAPACITY AND STREPTOCOCCUS MUTANS COUNT.

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ABSTRACT

Objective: This clinical trial was designed to evaluate the anti-cariogenic effect of Miswak and Grape seed extract (GSE) in comparison to fluoride. **Materials and Methods:** GSE 6.5% and sodium fluoride 0.05% mouthwashes were prepared before beginning of the study. 30 patients were allocated randomly into three groups; (n=10) M1: used Miswak sticks, M2: rinsed with (6.5%) GSE mouthwash, and M3: rinsed with (0.05%) sodium fluoride mouthwash. Patients used their treatments for 2minutes, twice per day for three months. Unstimulated saliva samples were collected at baseline, after one month and after three months. The bacterial count was assessed through streptococcus mutans culturing, and colonies forming units were counted. Saliva buffering capacity (BC) was assessed using a quantitative method using a hand-held pH meter. Data were statistically analyzed, and the significance level was set at $p \leq 0.05$. **Results:** All groups showed a significant decrease in bacterial count by time. There was no significant difference in bacterial count between groups after one month. After three months miswak and GSE significantly decreased bacterial count compared to fluoride. By time, GSE showed a significant decrease in saliva buffering capacity while, miswak and fluoride showed non-significant decrease in saliva buffering capacity. **Conclusion:** The antibacterial effect of the tested materials is time dependent, while having a negative effect on saliva buffering capacity being prominent with GSE.

KEYWORDS: miswak, grape seed extract, antibacterial effect, saliva buffering capacity.

1. INTRODUCTION

Dental caries is a highly prevalent disease all over the world. It encounters a constant problem and a financial burden on public health. It results from the dynamic imbalance that exists in the oral ecosystem resulting from pathogenic biofilm formation. Acids produced by Streptococcus mutans results in pH fluctuation causing, teeth demineralization.[1] Usually, if exposure to acids was brief, saliva neutralizes the pH through its buffering capacity. Salivary Streptococcus mutans count and saliva buffering capacity are critical factors influencing caries onset and progression.[2]

Prevention of dental caries was attempted through different oral hygiene measures such as toothbrushes and mouthwashes. Fluoride mouthwash is one of the most prominent anti-cariogenic agents. However, excess fluoride can be toxic causing enamel stains and dental fluorosis. Emerging of natural products as anti-cariogenic agents has been raised in the last decade. They are characterized by their biological activities, safety, availability, lack of bacterial resistance, and low cost. Miswak (Silvadora persica) is a famous, traditional plant. It has a wide geographic distribution especially in the Middle East and Africa. It contains a variety of organic and inorganic compounds that has anti-cariogenic, anticancer, and antiulcer properties.[3] Grape seed extract (GSE) is a natural product of red grapes. It is rich in proanthocyanidins, which acts as an antioxidant, antibacterial, free radical scavenger, and collagen cross-linking agent.[4,5]

Therefore, our goal is to investigate the anti-cariogenic effect of Miswak as a toothbrush and GSE mouthwash compared to fluoride mouthwash through assessment of salivary Streptococcus mutans count and saliva buffering capacity. The null hypothesis is that Miswak and GSE have no effect, either on Streptococcus mutans count or saliva buffering capacity.

2. MATERIALS AND METHODS

2.1. Sample size calculation

For comparison of the effect of three different materials (miswak, Grape seeds and, fluoride) on Streptococcus

mutans count, the effect size of approximately 0.63 was calculated based on a previous study.[6] A total sample size of 30 patients (10 in each group) was enrolled to detect an effect size of 0.63, with a power (1-β error) of 0.8 and a significance level (α error) of 0.05 for data. Sample size calculation was performed using G*power Program (University of Düsseldorf, Düsseldorf, Germany). G*Power Version 3.1.9.4.

2.2. Trial registration and Ethical approval

This clinical trial was registered online at ClinicalTrials.Gov. All data of this study was submitted including purpose of the study, inclusion, exclusion criteria, number of patients, primary outcome, interventions, start and estimated research completion dates. The ClinicalTrials.gov Identifier number was: NCT04136639. The protocol, patient information and procedures were analyzed and approved by the Research Ethics Committee (REC), Faculty of Dental Medicine, for Girls, Al-Azhar University, Cairo, Egypt. The registration number obtained was (REC 17-082).

2.3. Trial design and patient enrollment

This study was a single-blind randomized clinical trial performed in the Faculty of Dental Medicine, for Girls, Al-Azhar University, Cairo, Egypt from March, to October 2019. Thirty Patients were chosen from the out clinic according to specific inclusion and exclusion criteria. Inclusion criteria were Male or female medically free patients with medium or high caries risk (20-45 years). Exclusion criteria were Medically compromised patients, or under antibiotic, or anticariogenic mouthwashes in the last 30 days, patients wearing removable appliance or undergoing orthodontic treatment. Written informed consents were taken from eligible participants before starting the trial.

2.4. Clinical Procedures

Patients' preparation

Thirty patients were divided randomly into three groups (n=10) Group 1; patients were asked to use miswak sticks with a Pen-like grip. Group 2; patients were asked to rinse with 10 ml of 6.5% GSE mouthwash. Group 3; patients were asked to rinse with 10 ml of 0.05% sodium fluoride mouthwash (positive control). Instructions were given to all patients to avoid using any source of fluoride (supplements, professional or other dental products) during the study period.

Group 1; patients used Miswak sticks

Miswak sticks were purchased from (local market of Makka Al Mokaramah, KSA). They were distributed to the patients and instructed how to use sticks with Pen like grip. Patients were asked to peel off 1 cm of the sticks bark from the tip then to chew the tip gently until it becomes soft like brush. Patients brushed their teeth using sticks for 2 minutes, twice daily (in the morning and in the evening) for three months. They held the stick in one hand and moved it from cervical to coronal direction. Patients cut the brush end of the sticks daily using a knife.

Group 2; patients used 6.5% GSE mouthwash

Sixty-five grams of GSE (Bulk Powders, Vegan, UK.) were weighted on a sensitive balance. They were mixed with 8 grams of glycerol, 8 grams of propylene glycol, 2 drops of peppermint oil (Chemajet, Alexandria, Egypt) as a flavoring agent, and 1-liter distilled water in a mixer (Vertical colloid mill, Karishma Pharma Machines, India). They were transferred into a clean, sterile glass bottle and labeled. Patients rinsed with 10 ml of the mouthwash for 2 minutes, twice daily (in the morning and in the evening) for three months.

Group 3; patients used (0.05%) sodium fluoride mouthwash

Sodium fluoride powder (0.5 gm by weight) (Al Gomhouria Company, Cairo, Egypt) was measured on a sensitive balance. It was added to 8 grams of glycerol, 8 grams of propylene glycol, and 2 drops of peppermint oil, all were mixed with 1-liter of pure distilled water in the mixer. They were transferred into a clean, sterile glass bottle and labeled. Patients were asked to rinse with 10 ml of fluoride mouthwash for 2 minutes, twice daily (in the morning and evening) for three months.

Patients avoided eating or drinking 1 hour after using interventions. All mouthwashes were freshly prepared before use in the Faculty of Pharmacy, Cairo University, Drug Manufacturing Unit (DMU), Cairo, Egypt. Table (1) shows description of interventions.

Table 1: Description of interventions.

Intervention	Miswak	GSE	Fluoride
Concentrations	Miswak sticks	(6.5% GSE mouthwash)	(0.05% sodium fluoride mouthwash)
Dosage / regimen	Brush for 2 minutes twice daily (in the morning and evening)	Rinse with 10 ml for 2 minutes twice daily (in the morning and evening)	Rinse with 10 ml for 2 minutes twice daily (in the morning and evening)
Duration	3 months	3 months	3 months
Refrain from drinking or eating for 1 hour after interventions use			
Color	Light brown sticks	Dark reddish-brown solution	White solution

Saliva samples collection.
Unstimulated saliva samples were collected from patients (at baseline, after one month and after three months) for bacterial count and saliva buffering capacity assessment. Samples were collected in the morning from 10 to 12 am by spitting method.[7] They were collected 2 hours after breakfast or using interventions. Patients sit relaxed in an upright position. They spit 2 ml of resting unstimulated saliva in a sterile 15 ml falcon tube and closed tightly with a screw. Samples were free from blood or any food debris. Each sample was divided immediately (using sterile plastic disposable syringe) into two parts: 1 ml for saliva buffering capacity measurement and 0.5 ml for antibacterial assessment. Samples were transferred to a cool ice box immediately after collection.

2.5. Assessment of bacterial count
Streptococcus mutans count was assessed (colony forming units/ml: CFU/ml) at baseline, after one month, and after three months from interventions use. It was assessed using Selective Mitis Salivarius Agar Base (MSB) method. Sterile Mitis Salivaris agar plates were prepared with 15% Sucrose and 0.2 U/ml medium Bacitracin. It was inoculated at 35°± 2°C in Laminar flow. We diluted 0.5 ml of saliva in a ten-fold saline solution. A sterile inoculum spreader was used to evenly distribute the inoculum over the surface of the agar media. Spreading inoculum completely to the edges of the plate was avoided. All plates were incubated in an incubator for 24 h at 35°± 2°C**.** After incubation, colonies were identified by their morphological characteristics. Streptococcus mutans were identified by their raised, convex, opaque, blue colonies having a granular appearance. Colony-forming units (CFU) were counted manually by colony counter. The number of colonies were determined and expressed as CFU. It was estimated by multiplying the average of colonies per plate by the dilution factor. CFU/ml = No. of colonies per plate X dilution factor.

2.6. Assessment of saliva buffering capacity
The buffering capacity of saliva samples was measured by a quantitative method using a Hand-Held pH meter (Waterproof Ph Testers- AD11&AD12, Adwa Kit, Europe),[8] at baseline, after one month, and after three months from interventions use. The pH meter has a measuring range from 0 – 14 pH. The pH meter was calibrated at regular intervals using standard buffers of pH 4 and 7 to ensure the accuracy of readings. Immediately, after saliva collection, 3ml of 0.005 M Hydrochloric acid (HCL) was added to 1ml of saliva, then they were gently shaken to homogenously mix saliva and HCL. The pH meter tip was immersed into the sample and the saliva buffering capacity was recorded for all samples.

2.7. Statistical analysis
Numerical data were explored for normality by checking the distribution of data using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Logarithmic transformation of bacterial count data was performed due to the high range of bacterial counts. Log_{10} Colony Forming Unit (Log_{10} CFU) of bacterial count data showed non-parametric distribution. Data were presented as mean, standard deviation (SD), median and range values and 95% confidence interval (95% CI). Kruskal-Wallis test was used to compare between the three groups. Friedman's test was used to study the changes by time within each group. For comparison of saliva buffering capacity in the three groups ANOVA test was used. The significance level was set at $P \leq 0.05$

3. RESULTS

3.1. Bacterial count
Table (2) and figure (1) show changes in bacterial count within each group by time. Results revealed that all groups, showed a statistically significant decrease in Log_{10} CFU of bacterial counts after one month as well as after three months compared to baseline. In Miswak group, bacterial count mean values were (5.17± 0.65) at baseline. It showed a statistically significant decrease in bacterial count mean values after one month (1.65±1.81). Then, bacterial count mean values significantly decreased after three months (0.27±0.85) with (P-value <0.001, Effect size = 0.929). In GSE group bacterial count mean values were (5.58±0.81) at baseline. It showed a statistically significant decrease in bacterial count mean values after one month (2.3±1.61). After three months, bacterial count mean values significantly decreased (0.68±1.43) with (P-value <0.001, Effect size =0.933). In Fluoride group bacterial count mean values were (5.59±0.9) at baseline. It showed a statistically significant decrease in bacterial count mean values after one month (2.45±1.77). After three months, bacterial count mean values significantly decreased (1.81±1.67) with (P-value <0.001, Effect size = 0.844).

Table (3) and figure (1) show comparison of bacterial count between the three groups at different time intervals. Results showed that there was no significant difference in bacterial count mean values between the three groups at baseline (P-value = 0.418, Effect size = 0.389) and after one month (P-value = 0.547, Effect size = 0.113). After three months miswak and GSE groups showed statistically significant decrease in bacterial count mean values compared to fluoride group at (P-value = 0.048, Effect size = 0.585). There was no significant difference in bacterial count mean values between miswak and GSE.

Table 2: Descriptive statistics and results of Friedman's test for comparison between Log_{10} CFU of bacterial counts within each group at different time periods.

Time	Miswak (n = 10)	GSE (n = 10)	Fluoride (n = 10)
Baseline			
Median (Range)	5.07 (4.08 – 6.23)	5.73 (4.39 – 6.4)	5.7 (3.8 – 7.38)
Mean (SD)	5.17 (0.65)	5.58 (0.81)	5.59 (0.9)
1 month			
Median (Range)	1.15 (0 – 3.98)	2.98 (0 – 3.78)	3.02 (0 – 4.26)
Mean (SD)	1.65 (1.81)	2.3 (1.61)	2.45 (1.77)
3 months			
Median (Range)	0 (0 – 2.7)	0 (0 – 3.6)	2.15 (0 – 4.26)
Mean (SD)	0.27 (0.85)	0.68 (1.43)	1.81 (1.67)
***P*-value**	<0.001*	<0.001*	<0.001*
Effect size (w)	0.929	0.933	0.844

*Significant at $P \leq 0.05$

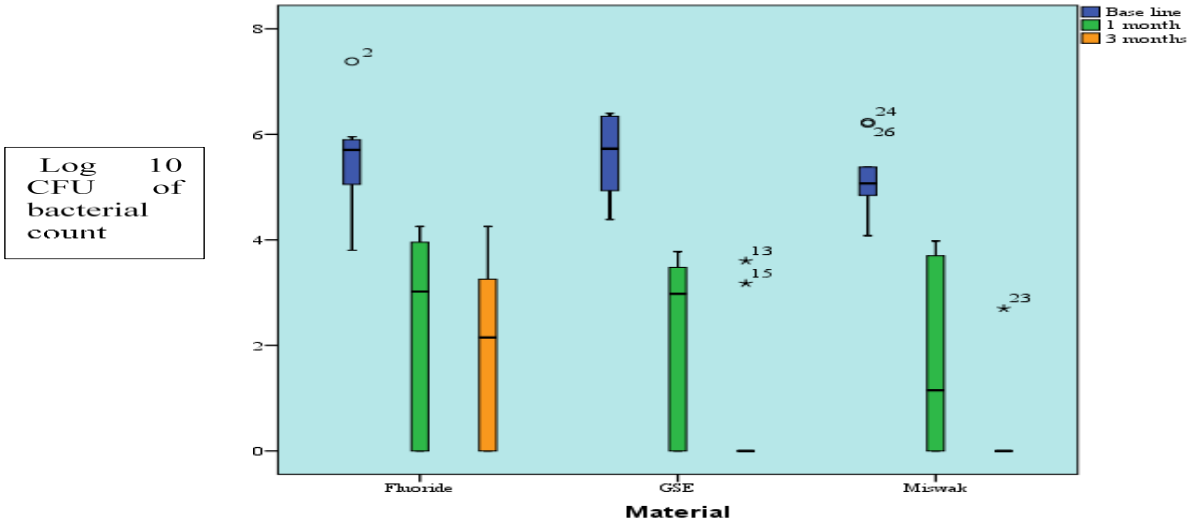

Figure 1: Box plot representing median and range values for Log_{10} CFU of bacterial counts in the three groups by time.

Table 3: Descriptive statistics and results of Kruskal-Wallis test for comparison of Log_{10} CFU of bacterial counts between the three groups.

Time	Miswak (n = 10)	GSE (n = 10)	Fluoride (n = 10)	P-value	Effect size
Baseline				0.418	0.389
Median (Range)	5.07 (4.08 – 6.23)	5.73 (4.39 – 6.4)	5.7(3.8-7.38)		
Mean (SD)	5.17(0.65)	5.58 (0.81)	5.59 (0.9)		
1 month				0.547	0.113
Median (Range)	1.15 (0 – 3.98)	2.98 (0 – 3.78)	3.02 (0 – 4.26)		
Mean (SD)	1.65 (1.81)	2.3 (1.61)	2.45 (1.77)		
3 months				0.048*	0.585
Median (Range)	0 (0 – 2.7)	0 (0 – 3.6)	2.15 (0 – 4.26)		
Mean (SD)	0.27 (0.85)	0.68 (1.43)	1.81 (1.67)		

*: Significant at $P \leq 0.05$.

3.2. Saliva buffering capacity

Table (4) and figure (2) show changes in saliva buffering capacity mean values within each group by time. Results revealed that, in miswak group, the saliva buffering capacity mean values decreased after one month (2.61±0.71), then slightly increased after three months (2.68±0.94) compared to baseline (3.08±0.88), with no statistically significant difference between them (P value=0.42, Effect size=0.062). In GSE group, the saliva buffering capacity mean value showed a significant decrease after one month (2.34±0.11) & after three months (2.15±0.24) in comparison to baseline (2.67±0.39), with a significant difference (P value=0.001, Effect size=0.412). In fluoride group, the saliva buffering ca-

pacity mean value showed a gradual decrease after one month (2.55±0.33) & after three months (2.47±0.28) in comparison to baseline values (2.93±0.76), with no significant difference (P value=0.114, Effect size=0.148). Comparison of saliva buffering capacity mean values between groups is shown in table (4) and figure (2). Results revealed that, there was no significant difference in saliva buffering capacity mean values between the three groups at different time intervals. At baseline, the highest saliva buffering capacity mean values were recorded in Miswak group, followed by fluoride group then GSE group showed the least saliva buffering capacity mean values with no significant difference between groups (P value=0.435, Effect size=0.059). After one month and three months, the highest saliva buffering capacity mean values were recorded in Miswak group followed by fluoride group then GSE group that showed the least mean values with no statistically significant difference between groups (P value=0.392, Effect size=0.067) and (P value=0.142, Effect size=0.134).

Table (4): The mean, standard deviation (SD) values and results of one-way analysis of variance (ANOVA) test for comparison of saliva buffering capacity in the three groups.

Time	Miswak (n = 10)		GSE (n = 10)		Fluoride (n = 10)		P-value between groups)	Effect size *(Eta squared)*
	Mean	SD	Mean	SD	Mean	SD		
Baseline	3.08	0.88	2.67	0.39	2.93	0.76	0.435	0.059
1 month	2.61	0.71	2.34	0.11	2.55	0.33	0.392	0.067
3 months	2.68	0.94	2.15	0.24	2.47	0.28	0.142	0.134
P-value within group)	0.42		0.001*		0.114			
Effect size *(Eta squared)*	0.062		0.412		0.148			

*: Significant at $P \leq 0.05$

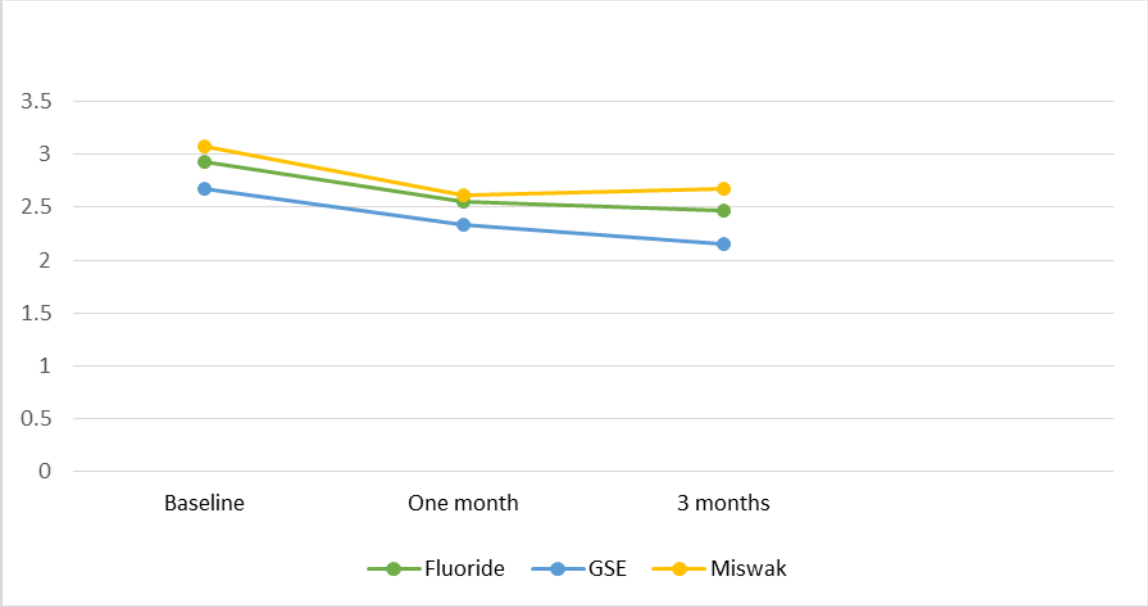

Figure (2): line chart representing mean and standard deviation values for saliva buffering capacity of the three groups at different time intervals.

4. DISCUSSION

Prevention is the key component for management of dental caries. It was attempted through several arms: antimicrobial agents, or modulation of the host factors. The most efficient way to prevent caries is by using fluoridated dental products. However, it meets some adverse effects like enamel staining, mottled teeth, fluorosis, and toxicity in some cases.[9] Using plant extracts and natural products in caries prevention increased in the past decade as an emerging trend replacing fluoride. The main advantages of herbal substitutes are low toxicity, lack of microbial resistance, increased shelf life, and cost-effectiveness.[10]

Miswak is a part of (Arak tree). It is traditionally used in the middle east and most of the Arabian communities around the world as an anticariogenic natural toothbrush substitute long time ago. It contains a wide variety of (organic and inorganic) compounds: flavonoids, minerals, and volatile oils that possesses many medicinal values.[11] Grape Seed extract (GSE) is a natural extract of Vitis vinifera seeds. It consists of Proanthocyanidins (PAS) that have been used as, antioxidant, anti-inflammatory, crosslinking agent, and anti-bacterial substances.[12] Few studies provided data about the effect of GSE mouthwash on salivary bacterial count.[13]

Therefore, the present study was conducted to investigate the anticariogenic effect of Miswak as a toothbrush and GSE mouthwash compared to fluoride mouthwash in vivo, via the assessment of salivary streptococcus mutans count and saliva buffering capacity. The null hypothesis was that Miswak and GSE have no effect either on streptococcus mutans count or saliva buffering capacity.

In this study, miswak was used in its natural form (sticks). Sticks were recommended by the World Health Organization (WHO) as an efficient tool for maintenance of oral health. The Miswak sticks edge acts as a brush while their active ingredients (organic and inorganic compounds, calcium, phosphate, and fluoride ions) are released in the oral cavity.[14] The stick end was cut daily as bristles were shredded, and the ingredients were lost and become ineffective as well as to avoid sticks bacterial contamination.[15] In this study, GSE mouthwash (6.5%) was prepared for its reported anticariogenic properties.[16] GSE, is a rich source of polyphenolic compounds that contains 95% oligomeric proanthocyanidins (PAS) (monomeric catechin, epicatechin, gallic acid and tannins) that have a beneficial pharmacological and antibacterial properties. It was reported to inhibit bacterial growth.[10]

Sodium fluoride (0.05%= 250 ppm F) mouthwash was used as a positive control group. It was recommended to include a (250 ppm F) to obtain a "dose-response group "Gold standard" for its anticariogenic properties.[17]

Mouthwash formulation preparation is generally much simpler than dentifrices. In this study, the preparation of GSE and sodium fluoride mouthwashes used distilled water as it is safer than alcohol that may cause irritation to buccal and labial mucosa and gingiva. Also, excessive use of products that contain alcohol weaken the natural ability of the immune system to fight pathogens. Non active ingredients (surfactants) were included in the mouthwash formulations; Glycerol and Propylene glycol as binding and emulsifying agents that prevent the solid and liquid substances from separation. Also, no preservatives were added to avoid its misleading antibacterial effect.[18] Peppermint oil was added as a flavoring agent in mouthwash formulations to get rid of the unpleasant smell and taste of the raw materials and to give a cold, refreshing taste. It was solubilized and dispersed through the solution via the surfactants (glycerol and propylene glycerol).[19]

Unstimulated saliva was collected to assess the bacterial count and saliva buffering capacity as it represents the basal salivary parameters and the oral load of microorganism. It was collected with spitting method, that appeared to be highly convenient and reproducible method**.** To decrease the diurnal variations in the flow of saliva and its composition, samples were collected 2 hours after breakfast.[7] Also, fluctuation in salivary microbial count were avoided through sample collection at the same hour every time.[20]

In this study, Mitis Salivaris Bacitracin (MSB) media was selected for detection and counting salivary streptococcus mutans colonies.[1] Streptococcus mutans is a gram-positive coccus that are mainly facultative anaerobic, acidogenic, aciduric bacteria. They are considered the main cariogen in the oral cavity and the most pathological bacterial strain in dental caries. Bacitracin was added to the media, that allowed only streptococcus mutans to grow and form colonies while inhibited the growth of most other oral bacteria. This method is easy, simple, valid, accurate and reliable culturing method. Colony forming unit (CFU) is a measurement of only living viable bacterial cells. That method is an effective method to determine the bacterial load and amplitude of infection in saliva samples.

Saliva buffering capacity was defined as saliva ability to neutralize acids and its resistance to pH changes. It was measured by a quantitative method using Hand-held pH meter as it is easy and simple method. Hand-held pH meter was used for its accuracy and convenience. It measures some levels of buffering capacity difficult to classify using color code strips.[21]

In this vivo study, the inclusion criteria constituted patients with moderate or high caries risk according to the ADA caries risk assessment, as they are the target population to control and prevent caries development. In addition, participants age group was (25-40 years) to standardize the salivary flow rate that differ with age. Participants under medication, antibiotics or using anticariogenic products were excluded from this clinical trial to avoid their effect on oral microbiome, salivary flow, and salivary composition. Using these criteria, 30 patients were selected for this study and divided according to the intervention used into three groups (n=10); miswak, GSE and fluoride. Saliva samples were collected for bacterial count and saliva buffering capacity assessment at baseline, after one month and after three months.

Results of this clinical trial revealed that, all tested materials significantly decreased bacterial count by time. There was no significant difference between all tested groups after one month. However, miswak and GSE groups significantly decreased bacterial count after three months compared to fluoride.

The decrease in bacterial count observed with miswak by time agreed with previous studies.[15, 22] They found that, miswak content of Sulphur, N-benzyl-2-phenylacetamide, butanediamide and alkaloids have an antimicrobial effect through disruption of the bacterial cell membrane and its transport system. Also, those components inhibited streptococcus mutans uptake of oxygen leading to oxidative stress and immediate bacterial cell toxicity and death. In addition, the mechanical cleaning effect of sticks combined with its potential release of biologically active compounds and ingredients in saliva could have also played role in this respect.

Similarly, GSE decreased streptococcus mutans count by time. This agreed with previous study,[13] which reported an antibacterial activity of GSE against streptococcus mutans through its polyphenolic compounds, mainly (monomeric catechin and epicatechin, gallic acid and polymeric, and oligomeric procyanidins, bioflavonoids) collectively known as grape seed proanthocyanidins. GSE polyphenols inhibit streptococcus mutans glycosyltransferases production and suppress bacterial growth as well as its possible acid production. In addition, polyphenols are toxic to bacteria through destruction of its cell membrane transport protein system.

The decrease in bacterial count observed with fluoride, was documented by another study.[23] They found a direct antibacterial effect of fluoride through disruption of cell membrane permeability. Also, it inhibits the essential enzymes required for cell growth. Fluoride decreases the bacterial metabolic activity through inhibition of the enolase enzyme, which is a glycolytic enzyme, responsible for sugar uptake by bacteria. This results in suppression of acid production from bacterial glycolysis. Also, fluoride caused intracellular acidification through inhibition of proton-translocating ATPase.

The significant difference between the test groups and fluoride after three months may be attributed to, the synergetic effect of different constituents in each test group rather than fluoride. Moreover, each test group possess different mechanisms for decreasing streptococcus mutans count.

Regarding saliva buffering capacity, results revealed that, GSE significantly decreased saliva buffering capacity by time. This effect is not completely understood. It could be attributed to two theories. First: Unstimulated saliva buffering system depends mainly on salivary phosphate and proteins systems.[7] Proanthocyanidins and polyphenols content of GSE have been reported to bind to salivary proteins (salivary amylase, lactoferrin, and mucins) and non-salivary proteins, such as, gelatin, bovine serum albumin and casein,[24, 25] hence decreasing the saliva buffering capacity. Second: there is a direct relation between salivary flow and saliva buffering capacity, where the concentration of saliva buffering systems increases with increased salivary flow.[26, 27] GSE tannins have been reported to develop powerful astringent sensations and this astringency decrease the salivary flow.[28] The combined effect of both theories may induce the significant decrease in saliva buffering capacity by GSE.

5. CONCLUSION

All tested materials are effective antibacterial agents, though natural agents could successfully replace fluoride. In addition, the antibacterial effect of the tested materials is time dependent, while having a negative effect on saliva buffering capacity being prominent with GSE.

Ethical statement

The protocol, patient information, and procedures of this study were analyzed and approved by the Research Ethics Committee (REC), Faculty of Dental Medicine, for Girls, Al-Azhar University, Cairo, Egypt. The registration number obtained was (REC17-082).

Credit authorship contribution statement

Maha Ahmed Niazy: Conceptualization, Project administration, Writing review & editing, Validation, Supervision. **Doaa AbdEllatif El Sharkawy:** Conceptualization, Methodology, Project administration, Writing review &editing, Validation, Supervision. **Hala Khoriba:** Formal analysis, Funding acquisition, Investigation, Resources, Data curation, Writing original draft, Visualization.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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