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Effect of grape seeds versus tooth brushing and dental flossing on plaque accumulation in high caries risk participants

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***Abstract*---**Objective the purpose of this study was to assess the effect grape seeds mouthwash on plaque accumulation in high caries risk individuals. Materials and methods: 66 students were recruited in the study. All students were high caries risk patients. They were divided into three groups according to the protocol they followed for one year. Plaque scores were calculated for each patient at baseline and after one year to show the effect of each preventive protocol. Statistical analysis was done using Chi-Square teat to calculate significance between groups. Result: Chi-Square Test showed that there was no statistical significant difference in the plaque score between the three groups at baseline (p value> 0.05). Regarding the plaque scores at 1-year, Chi-Square Test showed that there was no statistical significant difference between group 1, and group 2. There was a statistical significant difference between group 3 and both groups 1 and 2 at 1-year (p value= 0.000). Conclusion: Grape seeds extract mouthwash can improve the plaque score after 1 year. Natural grape seeds extract

is an effective substitute to synthetic agents such as chlorhexidine and fluoride for reducing plaque accumulation.

***Keywords*---**Grape seeds, Grape seeds extract, Dental plaque, caries prevention, proanthocyanidins.

Introduction

Prevention of oral diseases is very important to maintain good oral health condition; in developing countries people usually neglect oral hygiene measures leading to active and uncontrolled dental diseases which subsequently affecting their general health [1]. The mouth is unique in the human body in that it provides non-shedding surfaces (teeth) for natural microbial species. This can result in the accumulation of large masses of bacteria and their products at stagnant sites between teeth (approximal surfaces), in the pits and fissures on the biting (occlusal) surfaces of molars and premolars, and around the gums (gingival crevice) [2]. If plaque accumulated beyond levels that are compatible with health, then disease can occur. Plaque is associated with two of the most prevalent diseases, namely dental caries and periodontal diseases [3]. The widespread nature of these diseases, together with their huge treatment costs, has provided great impetus for research into improved means of controlling plaque formation [4]. The dental diseases are preventable not curable diseases, so dental healthcare workers should focus on preventive dentistry [4]. Various preventable agents developed for self-care at home. Mouth rinses containing antimicrobial agents used as supplements to daily homecare for prevention of bacterial plaque [5]. Synthetic antimicrobial agents usually had several side effects while natural products have been used for thousands of years in medicine. All this directing toward using natural therapeutic agents for prevention of oral diseases such as dental caries [5]. Grape seed extract (GSE) contains high concentration of proanthocyanidin, it represents a variety of polymers of flavanol such as monomeric catechin and epicatechin, gallic acid and polyphenols [6]. Proanthocyanidin (condensed tannin) has several clinical uses and has been used as dietary supplements, it strengthens collagen-based tissues by collagen synthesis and accelerates the conversion of soluble collagen to insoluble collagen. GSE act as antioxidant, antimicrobial and free radical scavenger [6]. It has a remineralizing potential on artificial carious lesions of enamel, inhibits the proliferation of cariogenic bacteria and reduce the synthesis of dental plaque. Also, it has anti-inflammatory action and immune response enhancement, thus, might be used as natural antimicrobial agent in preventive dentistry [7]. The null hypothesis tested was that utilizing grape seed mouth wash will show no significant difference against using tooth brushing and flossing only in reducing plaque accumulation in high caries risk patients.

Materials and Methods

Ethics approval

The study was reviewed and accepted by the Research Ethics Committee (REC) – of Faculty of Dentistry, Cairo University in 2018.

Study Design
This study is a parallel group, two tail and superiority frame with 1:1:1 allocation ratio. It was run at the Cairo University Dental Clinics.

Recruitment
An announcement was made at Faculty of Dentistry to inform young students about the study. Students applied for the study were examined in the Conservative Dentistry clinic to check if they were eligible or not to join the study. They were subjected to clinical examination and a sampling frame was prepared of those who were at an increased risk of dental caries. The first 66 eligible students were registered in the study after signing an informed consent.

Randomization
The 66 participants were then randomly divided and allocated into 3 groups (n=22) by choosing a folded paper containing the number of their assigned group

Grouping

Group 1 (Intervention one):

Each participant in this group had given manual toothbrush (Oral B)**,** fluoridated toothpaste (Signal), dental floss (Oral B), and a bottle containing freshly prepared grape seed extract mouthwash 150ml. They were instructed to follow a certain routine for one year, which was brushing their teeth two times a day, and to floss (oral B) once a day at night. Further, to rinse 15 ml of the grape seeds extract mouthwash for one minute for five days monthly for one year [8].

Grape seed extract preparation

120 gm of air dried powdered grape seeds (Vitis vinifera L.) were extracted by percolation in aqueous ethanol (70% v/v) for three consecutive times (3 x 150 ml) to ensure complete extraction. The collected filtered extracts were combined and dried under reduced pressure (45 °C) till complete dryness by means of a rotary evaporator (Heidolph VV 2000, Heidolph Instruments GmbH, Germany). A dark brown residue was obtained and reduced to fine powder by the means of a mortar and pestle. The dry powdered extract was kept in an airtight container till further use [7].

Mouthwash preparation

Grape seed extracts were dissolved in sterile distilled water with gentle heating and various dilutions were made by doubling the dilutions and was kept in separate beaker. The fine powder of alum was mixed in peppermint oil. Copper sulfate was dissolved in demineralized (DM) water separately. Both the above groups were mixed well (alum and copper sulfate) and were filtered through a filter press and the solution could cool off. Later V. vinifera were added to the above solution. This was mixed using a stirrer until a uniform color was obtained. The solution was left for overnight. On the next following day, the solution was filled in a bottle of 150 mL and packed [9].

Group 2 (Intervention two):

Participants in this group were given a manual toothbrush (Oral B), fluoridated toothpaste (Signal), dental floss (Oral B) and mouthwash bottle that contains chlorhexidine (CHX) and fluoride (Emofluor 250ml, Value pharmacueutical, Egypt).

Group 3 (Control Group):
Participants in this group were given only a manual toothbrush (Oral B), fluoridated toothpaste (Signal), dental floss (Oral B)

Follow up period:
All participants were scheduled to follow up every 3 months for one year from their initial visit.

Outcome measured:
Plaque score recording:

Participants plaque scores were calculated and recorded by Plaque Index (PI). It evaluates the amount of plaque in the cervical part of the tooth. Four surfaces on each tooth were recorded, buccal, lingual and proximal surfaces [10].

For each participant six teeth: 16, 12, 24 and 36, 32, 44 were used for obtaining the plaque index score. A disclosing solution was used to visualize plaque to the patient, and it also makes it easier to record the score[11]. The index for each participant is calculated by using 6 teeth while for each tooth four records are registered that reflect the four surfaces Buccal, Lingual, Mesial and Distal. Therefore, the index for each participant was calculated as follows for each tooth B+L+M+D= (1+2+2+1) / 4 = 1.5. For the full mouth if maxillary right first molar (16) score was 1.5, maxillary right lateral incisor (12) 1.3, maxillary left first bicuspid (24) 1.2, mandibular left first molar (36) 1, mandibular left lateral incisor (32) 1.6 and mandibular right first bicuspid (44) 1.3. The index for the patient = (1.5 + 1.3 +1.2 + 1 + 1.6 + 1.3) / 6 = 1.4. This index was used for determining the plaque score Table (1).

Table (1)
Plaque score

plaque score	PI
0	Plaque Index, PI < 0.4
1	PI = 0.4-1.0
2	PI = 1.1- 2.0
3	PI > 2.0

Results

The plaque score of each participant was recorded from (0 to 3) according to their plaque index where 0 denotes plaque index less than 0.4 and 3 denotes plaque index greater than 2. The numbers and percentages of participants in each score at baseline and at one year are shown in Table (2 and 3). At baseline, only 4

participants had score 0 and they represent 6.1 % of the whole participants while 8 participants had score 1 (12.1%), 16 participants had score 2 (24.2%) and 38 participants had score 3 (57.6%) Table (4). After one year the percentage of plaque scores 0,1,2 and 3 were 4.5%, 40.9%, 34.8% and 19.7% respectively for all participants indicating improvement in the plaque score for most of the participants Table (4). For group 1 the percentage of plaque scores 0,1,2 and 3 at baseline were 13.6%, 18.2%, 27.3% and 40.9% respectively, whereas at one year the percentages were 4.5%, 72.7%, 22.7% and 0% respectively Table (5), denoting group improvement. Regarding group 2 the percentage of plaque scores 0,1,2 and 3 at baseline were 4.5%, 9.1%, 22.7% and 63.6% respectively, whereas after one year the percentages were 9.1%, 40.9%, 50% and 0% respectively Table (6). As for group 3 the percentage of plaque score 0,1,2 and 3 at baseline were 0%, 9.1%, 22.7% and 68.2% respectively, whereas after one year the percentages were 0%, 9.1%, 31.8% and 59.1% respectively Table (7). Chi-Square Test showed that there was no statistical significant difference in the plaque score between the three groups at baseline (p value> 0.05) Table (8). Regarding the plaque scores at 1-year, Chi-Square Test showed that there was no statistical significant difference between group 1, and group 2 Table (9). There was a statistical significant difference between group 3 and both groups 1 and 2 at 1-year (p value= 0.000) Table (10 and 11).

Table (2)
Showing numbers and percentages of participants in each plaque score at baseline

			Group			Total
			1	2	3	
Plaque Score	0	Count	3	1	0	4
		% within plaque Score	75.0%	25.0%	0.0%	100.0%
		% within Group	13.6%	4.5%	0.0%	6.1%
	1	Count	4	2	2	8
		% within plaque Score	50.0%	25.0%	25.0%	100.0%
		% within Group	18.2%	9.1%	9.1%	12.1%
	2	Count	6	5	5	16
		% within plaque Score	37.5%	31.3%	31.3%	100.0%
		% within Group	27.3%	22.7%	22.7%	24.2%
	3	Count	9	14	15	38
		% within plaque Score	23.7%	36.8%	39.5%	100.0%
		% within Group	40.9%	63.6%	68.2%	57.6%
Total		Count	22	22	22	66
		% within plaque Score	33.3%	33.3%	33.3%	100.0%
		% within Group	100.0%	100.0%	100.0%	100.0%

Table (3)
Showing numbers and percentages of participants in each plaque

			Group			
			1	2	3	Total
Plaque Score-1y	**0**	Count	1	2	0	3
		% within plaque Score-1y	33.3%	66.7%	0.0%	100.0%
		% within Group	4.5%	9.1%	0.0%	4.5%
	1	Count	16	9	2	27
		% within plaque Score-1y	59.3%	33.3%	7.4%	100.0%
		% within Group	72.7%	40.9%	9.1%	40.9%
	2	Count	5	11	7	23
		% within plaque Score-1y	21.7%	47.8%	30.4%	100.0%
		% within Group	22.7%	50.0%	31.8%	34.8%
	3	Count	0	0	13	13
		% within plaque Score-1y	0.0%	0.0%	100.0%	100.0%
		% within Group	0.0%	0.0%	59.1%	19.7%
Total		Count	22	22	22	66
		% within plaque Score-1y	33.3%	33.3%	33.3%	100.0%
		% within Group	100.0%	100.0%	100.0%	100.0%

Table (4)
Showing percentage of each plaque score in all participants at baseline and after 1 year

All participants	Plaque score			
	0	1	2	3
Baseline	6.1%	12.1%	24.2%	57.6%
1 year	4.5%	40.9%	34.8%	19.7%

Table (5)
Showing percentage of each plaque score in group 1 at baseline and after 1 year

Group 1	Plaque score			
	0	1	2	3
baseline	13.6	18.2	27.3	40.9
1 year	4.5	72.7	22.7	0

Table (6)
Showing percentage of each plaque score in group 2 at baseline and after 1 year

Group 2	Plaque score

	0	1	2	3
baseline	4.5	9.1	22.7	63.6
1 year	9.1	40.9	50	0

Table (7)
Showing percentage of each plaque score in group 3 at baseline and after 1 year

Group 3	Plaque score			
	0	1	2	3
baseline	0	9.1	22.7	68.2
1 year	0	9.1	31.8	59.1

Table (8)
Chi-Square Test showing no significance between groups regarding plaque score at baseline

	Value	df	p value
Pearson Chi-Square	6.257(a)	6	0.395
Likelihood Ratio	7.078	6	0.314
Linear-by-Linear Association	5.297	1	0.021
N of Valid Cases	66		

df: degree of freedom, Significant at $p \leq 0.05$

Table (9)
Chi-Square Test showing no statistical significance between group 1 and 2 regarding plaque score at 1-year

	Value	df	p value
Pearson Chi-Square	4.543(a)	2	0.103
Likelihood Ratio	4.632	2	0.099
Linear-by-Linear Association	1.612	1	0.204
N of Valid Cases	44		

df: degree of freedom, Significant at $p \leq 0.05$

Table (10)
Chi-Square Test showing statistical significance between group 1 and 3 regarding plaque score at 1-year

	Value	df	p value
Pearson Chi-Square	25.222(a)	3	0.000
Likelihood Ratio	32.138	3	0.000
Linear-by-Linear Association	24.254	1	0.000
N of Valid Cases	44		

df: degree of freedom, Significant at $p \leq 0.05$

Table (11)
Chi-Square Test showing statistical significance between group 2 and 3 regarding plaque score at 1-year

	Value	df	p value
Pearson Chi-Square	20.343(a)	3	0.000
Likelihood Ratio	26.509	3	0.000
Linear-by-Linear Association	17.641	1	0.000
N of Valid Cases	44		

df: degree of freedom, Significant at $p \leq 0.05$

Discussion

This randomized clinical trial was performed to obtain the best evidence, since the gold standard for examining causation or treatment effect is the randomized clinical trials (RCTs). RCTs have many advantages like prospective data collection, standardization of the intervention, investigation of adverse events, masked outcome measures and examining the effective treatment on secondary outcomes [12].

This study examined the efficiency of grape seeds extract (GSE) in the form of mouthwash for caries prevention. The novel idea for utilizing natural products for the remineralization processes of dental hard tissues and dental caries prevention [5]. GSE was also selected as it has revealed promise for the manufacturing of innovative generation of antibacterial products for dental use without any unwanted effect on the ecosystem in the oral cavity. we decided to formulate its mouthwash to check its efficacy and substantivity [13]. Simultaneously, we wanted to know its comparison with chlorhexidine.

Results of this study showed there was no statistical significant difference in the plaque score between the three groups at baseline (p value> 0.05). Regarding the plaque scores at 1-year, Chi-Square Test showed that there was no statistical significant difference between group 1, and group 2. There was a statistical significant difference between group 3 and both groups 1 and 2 at 1-year (p value= 0.000). This could be explained as Vitis vinifera contains many phenolic compounds. The most important phenolics extracted from grape seeds are catechins (catechin, epicatechin, and procyanidins) and their polymers [14]. The process behind polyphenolic toxicity against microbes is due to the suppression of hydrolytic enzymes or other interactions to inactive microbial adhesions and nonspecific interactions with carbohydrates [15]. This infers the fact that phenolic antibacterial activity is due to enzymatic inhibition by the oxidized compounds which occurs through a reaction with sulfhydryl groups with the proteins, additionally, flavonoids and tannins can bind to form precipitates with various proteins [16]. A study by Aurelia et al. [17] found that GSEs show a bacteriostatic effect on the anaerobic group that significantly diminishes the formation of biofilm. Zhao Wet al. [18] concluded that GSE has substantial antibacterial activity against mutans. In addition to that, increased antioxidant products like proanthocyanidins and other antibacterial properties of V. vinifera might have played a role in the lowest plaque deposition [19]. This was in accordance with

Mishra et al. [20] who found that on comparison of plaque deposition, grape seeds extract mouthwash showed the least deposition after 15 days and that was due to the increased antioxidant products like proanthocyanidins and other antibacterial properties of grape seeds extract that might have played a role in the lowest plaque deposition. Abdulkareem et al. [21] examined the antiplaque and antigingivitis efficacy of chlorhexidine, antioxidant and hyaluronic acid mouthwashes, and concluded that chlorhexidine still superior to other mouthwashes in term of plaque control. Therefore, the null hypothesis was rejected since grape seed extract mouthwash was a successful protocol in reducing dental plaque accumulation in high caries risk participants while standard preventive measures wasn't enough alone.

Conclusion

Grape seeds extract mouthwash and the combined chlorohexidine and fluoride mouthwashes can improve the plaque score after 1 year. Natural grape seeds extract is an effective substitute to synthetic agents such as chlorhexidine and fluoride for reducing plaque accumulation.

References

1. Twetman S. Prevention of dental caries as a non-communicable disease. *European journal of oral sciences.* 2018;126:19-25. https://doi.org/10.1111/eos.12528
2. Huang R, Li M, Gregory R L. Bacterial interactions in dental biofilm. *Virulence.* 2011;2:435-44. https://doi.org/10.4161/viru.2.5.16140
3. Valm A M. The structure of dental plaque microbial communities in the transition from health to dental caries and periodontal disease. *Journal of molecular biology.* 2019;431:2957-69. https://doi.org/10.1016/j.jmb.2019.05.016
4. Kassebaum N J, Bernabe E, Dahiya M, Bhandari B, Murray C J, Marcenes W. Global burden of untreated caries: a systematic review and metaregression. *J Dent Res.* 2015;94:650-58. https://doi.org/10.1177/0022034515573272
5. Elkwatehy W M, Salama R I. The Effect of Grape Seed Extract Mouthrinse on the Caries Activity, Dental plaque and Gingivitis. *Int J Dentistry Oral Sci.* 2020;7:1148-52. http://dx.doi.org/10.19070/2377-8075-20000228
6. Palombo E A. Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. *Evid Based Complement Alternat Med.* 2011;2011:1-15. https://doi.org/10.1093/ecam/nep067
7. Nagi S M, Hassan S N, Abd El-Alim S H, Elmissiry M M. Remineralization potential of grape seed extract hydrogels on bleached enamel compared to fluoride gel: An in vitro study. *J Clin Exp Dent.* 2019;11:e401-e07. https://doi.org/10.4317/jced.55556
8. Mazhari F, Boskabady M, Moeintaghavi A, Habibi A. The effect of toothbrushing and flossing sequence on interdental plaque reduction and fluoride retention: A randomized controlled clinical trial. *J Periodontol.* 2018;89:824-32. https://doi.org/10.1002/JPER.17-0149
9. Mishra P, Marwah N, Agarwal N, Chaturvedi Y, Suohu T. Comparison of punica granatum, Terminalia chebula, and Vitis vinifera seed extracts used

as mouthrinse on salivary Streptococcus mutans levels in children. *The Journal of Contemporary Dental Practice.* 2019;20:920-27. https://doi.org/10.5005/jp-journals-10024-2617

10. Badri S M, Felemban E H, Alnajjar G K, Alotaibi F M, Aljahdali S T, Maher Y A, et al. Effectiveness of probiotic lozenges and Chlorhexidine mouthwash on plaque index, salivary pH, and Streptococcus mutans count among school children in Makkah, Saudi Arabia. *The Saudi Dental Journal.* 2021;33:635-41. https://doi.org/10.1016/j.sdentj.2020.05.006
11. Bratthall D, Petersson G H, Stjernswärd J. Cariogram MANUAL. *Internet version.* 2004;2:1-51.
12. Wallace D K. Evidence-based medicine and levels of evidence. *Am Orthopt J.* 2010;60:2-5. https://doi.org/10.3368/aoj.60.1.2
13. El-Adawi H. Inhibitory effect of grape seed extract (GSE) on cariogenic bacteria. *J Med Plant Res.* 2012;6:4883-91. https://doi.org/10.1055/s-0032-1321341
14. Zhao W, Xie Q, Bedran-Russo A K, Pan S, Ling J, Wu C D. The preventive effect of grape seed extract on artificial enamel caries progression in a microbial biofilm-induced caries model. *J Dent.* 2014;42:1010-18. https://doi.org/10.1016/j.jdent.2014.05.006
15. Silva A P, Goncalves R S, Borges A F, Bedran-Russo A K, Shinohara M S. Effectiveness of plant-derived proanthocyanidins on demineralization on enamel and dentin under artificial cariogenic challenge. *J Appl Oral Sci.* 2015;23:302-09. https://doi.org/10.1590/1678-775720140304
16. Swadas M, Dave B, Vyas S M, Shah N. Evaluation and comparison of the antibacterial activity against Streptococcus mutans of grape seed extract at different concentrations with chlorhexidine gluconate: An in vitro study. *Int J Clin Pediatr Dent.* 2016;9:181-85. https://doi.org/10.5005/jp-journals-10005-1360
17. Furiga A, Lonvaud-Funel A, Badet C. In vitro study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. *Food Chemistry.* 2009;113:1037-40.
18. Zhao W, Xie Q, Bedran-Russo A K, Pan S, Ling J, Wu C D. The preventive effect of grape seed extract on artificial enamel caries progression in a microbial biofilm-induced caries model. *J Dent.* 2014;42:1010-18. 10.1016/j.jdent.2014.05.006
19. Singla S, Malhotra R, Nd S, Saxena S. Antibacterial Efficacy of Mouthwash Prepared from Pomegranate, Grape Seed and Guava Extracts against Oral Streptococci: An in Vivo Study. *J Clin Pediatr Dent.* 2018;42:109-13. https://doi.org/10.17796/1053-4628-42.2.5
20. Mishra P, Marwah N, Agarwal N, Chaturvedi Y, Suohu T. Comparison of Punica granatum, Terminalia chebula, and Vitis vinifera Seed Extracts used as Mouthrinse on Salivary Streptococcus mutans Levels in Children. *J Contemp Dent Pract.* 2019;20:920-27. https://doi.org/10.5005/jp-journals-10024-2617
21. Abdulkareem A A, Al Marah Z A, Abdulbaqi H R, Alshaeli A J, Milward M R. A randomized double-blind clinical trial to evaluate the efficacy of chlorhexidine, antioxidant, and hyaluronic acid mouthwashes in the management of biofilm-induced gingivitis. *Int J Dent Hyg.* 2020;18:268-77. https://doi.org/10.1111/idh.12432