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Evaluation of the Antibacterial Effect of Grape Seed Extract on Streptococcus Mutans in Children

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ABSTRACT

Purpose: To evaluate the effect of grape seed extract on streptococcus mutans in children **Materials and methods:** Forty children were selected for this study with ages ranged from (7-12). The participants were divided into two equal classes at random; A& B (n= 20). Participants were asked to rinse with 15 ml of either grape seed extract or 0.125% CHX mouthwashes (in group A & B respectively) twice daily (after breakfast and dinner) for 60 seconds and not to rinse with water thereafter. Saliva samples were collected at 0 (baseline) (S1), after 2 days (S2) and after a week (S3). All collected saliva samples were submitted to a microbiology laboratory for total bacterial counting at the three intervals for the two groups, the data were then collected, tabulated, and statistically analyzed **Results:** The comparison between grape seed extract and CHX group showed that there was no statistically significant difference in the mean CFU between the two groups at different time intervals.(P-value=0.708,0.202,0.056) **Conclusion:** Grape seed extract mouthwash is considered a successful antimicrobial agent compared to a potent antiseptic as chlorhexidine when used in children.

KEYWORDS

Grape Seed Extract;
Chlorohexidine Mouthwash;
Antibacterial Mouthwash.

INTRODUCTION

Dental caries has multi-factorial etiology factors which are: The host (the saliva & teeth), the microflora, and the substratum. Preventive measures were placed in place at all levels to track caries lesion progression. Good oral hygiene and dietary modification may help in hindering this disease in infants. Resident oral microbiota is natural and profits the host

- Paper extracted from Master thesis titled "Evaluation of the Antibacterial Effect of Grape Seed Extract on Streptococcus Mutans in Children."

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by impeding colonization with exogenic micro-organisms. The oral diseases with the most prevalence such as dental caries are microbiota-associated [1-5].

The most virulent oral bacteria are Streptococcus mutans, which have been found to be the initiator of most dental caries. The pathogenicity of *S. mutans* depends on their ability to form biofilms on the teeth surface, create an acidic state for a wide variety of sugars and to survive this acidic environment. Preventive dentistry has a role in minimizing the risk of caries in highly vulnerable individuals by using antimicrobial mouthwash once a day for 30 s as an addition to daily tooth brushing can minimize the occurrence of gingivitis and caries in children within 6 months [6, 7].

Chlorhexidine is a highly cationic polybiguanide (bisbiguanide) with a broad spectrum of antimicrobial activity. Chlorhexidine's cationic nature enables its adherence to the structure of the tooth, and significantly decreases the formation of the pellicle and its increase through limited release of the agent. It greatly inhibits the regrowth of plaque and avoids gingivitis. Because of its strong antioxidant capacity, limited adverse effects, and economic affordability, medicinal plants are gaining great attention. They contain a wide range of chemical constituents, which may function individually, or synergistically to cure diseases associated with oxidative stress and improve health [7-9].

There are many phytochemicals that can be found in plants as tannins and flavonoids that have medicinal and biological activities. These phytochemicals can be obtained from different sources of plants and plant parts such as bark, leaves, fruits, seeds. Among the different types of fruit, Grape seed extract (GSE) derived from the seeds of Vitis vinifera is rich in polyphenol compounds and contains free monomeric flavanols, i.e. proanthocyanidins (PAs). Catechin (tannis), epicatechin, and epicatechin 3-O-gallate, which are the structural construction blocks of GSE, are examples of PAs found in GSE.

Some researchers have shown that extracts from the seed are more effective than other sections of grapes in terms of antibacterial activity. Multiple mechanisms of antibacterial activity have been identified in phenolics: they interact with bacterial proteins and cell wall structures, may cause damage to cytoplasmic membranes, decrease membrane fluidity, suppress nucleic acid synthesis, cell wall formation, or energy metabolism [10-12]. Therefore, this study was conducted to evaluate the antibacterial effect of grape seed extract on streptococcus mutans.

MATERIAL AND METHODS

This study has been approved by the Research Ethics Committee (REC18-046), Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt. Also, informed consent forms were signed by the parents of the participants before conducting the research.

Case Selection

Forty children of both sexes were involved in this study (11boys and 29girls). Their age varies between seven and twelve years old. Children involved in this study are healthy, with no history of oral prophylaxis for at least 3 months before the study, no history of recent administration of antibiotic (previous 2 weeks), no history of using antimicrobial mouth rinse (previous 12 hrs.) [13].

Materials

The materials used in this study: prepared grape seed extract as a mouth-wash for the experimental group. Prepared by mixing 1.563 gram of grape seed extract powder with 100 ml of distilled water, thereafter the beaker was placed on hot plate magnetic stirrer at 600 ºC to dissolve the extract in solvent till a homogenous solution was obtained, thus obtained solution was mixed with 900 ml distilled water to make a final volume of 1000 ml in a clean sterile measuring cylinder. The solution was then transferred to sterile plastic bottle. The final extract was stored in amber colored bottle under refrigeration for further use [14].

Chlorohexidine (0.12%) mouthwash for the control group which is commercially available as Hexitol (Arab Drugs Co. for Pharmaceuticals & Chemical Industries, Cairo, A.R.E.)

Forty children were equally divided into two groups in accordance with mouthwash used

Group A:

Twenty children were asked to rinse with 15 ml of grape seed extract mouthwash for one minute twice a day, once before breakfast and once after dinner.

Group B:

Twenty children were asked to rinse with 15 ml of CHX mouthwash for one minute twice a day, once before breakfast and once after dinner.

Three saliva samples were collected from each child, once before starting the mouthwash, the second after 48 hours, and the third after 7 days of using the mouthwash. The samples were sent to the laboratory to determine the bacterial count (Fig. 1 & Fig. 2).

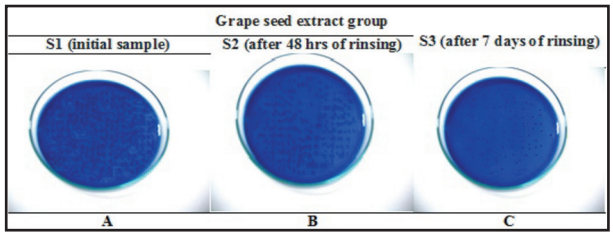

Figure (1): A photograph showing (S1) initial sample of S.mutans on mitis salivaris agar, (S2) after 48 hrs, (S3) after 7 days " in Grape seed group ".

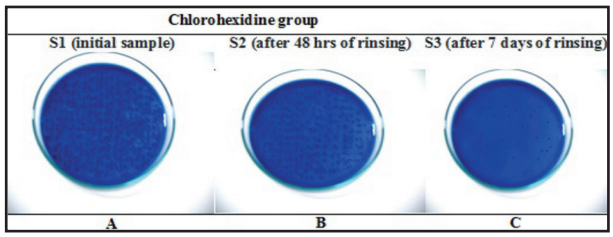

Figure (2) A photograph showing (S1) initial sample of S.mutans on mitis salivaris agar, (S2) after 48 hrs ,(S3) after 7 days " in chlorohexidine group ".

Statistical Analysis

In order to compare the mean bacterial count within and between categories, statistical analysis was then carried out using a commercially available software programme (SPSS 19; SPSS, Chicago, IL USA). As data were parametric, significance of the difference between groups was evaluated using paired t test; the mean values for pre-treatment and post-treatment were compared.

The percentage of reduced S.mutans was calculated by the following formula ×100. The significance level has been set at $P \leq 0.05$

RESULTS

In Chlorohexidine (CHX) group the colony forming unit (CFU×10^5) significantly decreased from 1338 in pre samples to 2.72 after 48 hours and to 0.00052 after one week. The result of *P* value = 0.008 ($P \leq 0.05$) which indicates that there was a statistically significant differences by time within chlorohexidine group. (Table 2, Fig. 4)

In grape seed extract group, the colony forming unit (CFU×10^5) significantly decreased from 1055 in pre samples to 8.99 after 48 hours and to 0.0144 after one week. The result of *P* value = 0.046 ($P \leq 0.05$) which indicates that there was a statistically significant differences by time within chlorohexidine group. (Table2, Fig. 4)

Regarding comparing grape and chlorohexidine groups at different time intervals might conclude that there was no statistically significant difference in the mean CFU between the two groups at different time intervals.

(*P*-value = 0.708, 0.202, and 0.056 for times Pre, post 48 hr., and Post 7 days, respectively) which indicated that the grape seed extract almost has the same efficiency to eradicate the streptococcus colony as chlorohexidine at different time intervals. (Table 1, Fig. 3)

Table (1) *Comparison of descriptive statistics and P-values for chlorohexidine and grape groups at different time intervals*

Time	Chlorohexidine(x10^5)		Grape(x10^5)		P-value*
	Mean	SD	Mean	SD	
Pre (S1)	1338.04	2036.71	1055.31	2212.02	0.708
Post 48 hr (S2)	2.72	10.47	8.99	17.13	0.202
Post 7 days (S3)	0.00052	0.00026	0.0144	0.03	0.056

**P-value significant at P ≤ 0.05.*

Table (2) *Descriptive statistics and results of one paired sample T test for grape seed extract and chlorohexidine groups (pre, post 48 hr, and post 1 week).*

Group	Time	Mean(×10^5)	SD (x10^5)	Number	P value *
Grape	**Pre**	1055.31	2212.02	20	0.046 *
	Post48hr	8.99	17.13		
	Post 7days	0.0144	0.03		
Chx	**Pre**	1338.04	2036.71	20	0.008*
	Post48 hrs	2.72	10.47		
	Post 7 days	0.00052	0.00026		

**P-value significant at P ≤ 0.05.*

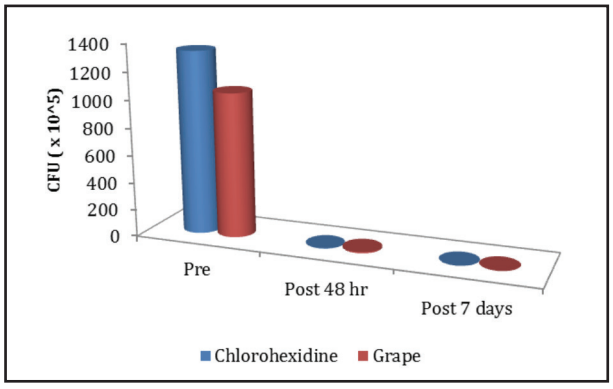

Figure (3) 3D cylindrical chart comparing the mean of CFU for two groups at different time intervals

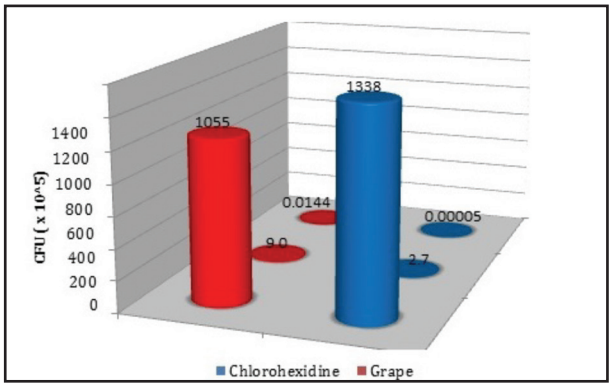

Figure (4) 3D cylindrical chart comparing mean of CFU of pre, post 48 hr and post 1 week for chlorohexidine group and grape seed extract group.

DISCUSSION

The most common bacterial infections in humans are dental caries. It is widespread both in children/adults, it is one of the most substantial and predominant oral diseases that affect the majority of people in the world, and the cost of care puts place a huge burden on health services. It is caused by a variety of cariogenic microbes that form plaque biofilms on the tooth surfaces, including Lactobacillus and streptococcus [15].

In the present study, streptococcus mutans were selected because they are known to be the main causative agents of dental caries. They are capable of forming a biofilm on the tooth surface, producing organic acid from different carbohydrates (acidity), they have the ability to thrive at low pH (acidity), the exceptional ability to outperform other bacteria through the development of bacteriocin, and their adaptability to rapidly evolving environments can be attributed as the major virulence factor [16].

In the present study, the aqueous extract of grape seed was used to determine the antibacterial effect on Streptococcus mutans. This is in accordance with a previous study that analyzed and compared the effect of pomegranate, grape seed and guava extract mouthwash on salivary streptococci levels. the results showed that aqueous extracts from the selected herbal plants had sufficient antibacterial efficacy against oral streptococci [14]. This agreement may be due to the fact that grape seed extract (GSE) is rich in proanthocyanidins (PAS) which play a role in the prevention of caries due to their particular structure, PACs have hydrophobic and hydrophilic properties which enhance their ability to bind irreversibly to a variety of compounds, in particular minerals, proteins and carbohydrates. Biofilm formation on the tooth surface may be interrupted by binding of GSE to carbohydrate substrates which is necessary for the spread of bacteria as *S.mutans* [17].

When comparing grape seed extract and chlorohexidine groups at different time intervals, the results revealed that at different time intervals, there was no statistically significant difference between the mean CFU of the two groups which indicated that the grape seed extract almost have the same efficient to eradicate the streptococcus colony like Chlorohexidine.

The results of the present study were in disagreement with a previous study [1] that stated that Grape seed extract was not that much effective as an antibacterial agent compared to chlorhexidine Gluconate. This difference in result may be due to using grape seed aqueous extract in the present study while this previous one used grape seed alchoholic extract and these results in accordance with another study [18] that stated that alcoholic and acetonic grape seed extracts had no antibacterial effect.

CONCLUSION

Grape seed extract mouthwash is considered a successful antimicrobial agent compared to a potent antiseptic as chlorhexidine when used in children. Since it is natural, safe, and has no side effects, it can be used as a preventive therapy at home to maintain oral hygiene.

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