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The Effect of Grape Seed Extract Mouth Rinse on the Caries Activity, Dental plaque and Gingivitis

Research Article

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Abstract

Objectives: This study aimed to evaluate the effect of mouth rinse containing grape seed extract (GSE)on caries activity, plaque accumulation and gingivitis.
Material and Methods: A total of ninety-five healthy children participate in a clinical trial, they divided into two groups, group 1: grape seed extract mouth rinse (prepared for this study) (test group) and group 2: chlorhexidine mouth rinse (positive control group). The evaluation of caries activity, dental plaque accumulation and gingivitis were done prior to and on weekly basis for 3 weeks. The effect of mouth rinses was evaluated using one-way ANOVA test to compare baseline scores with different follow up scores. Multiple comparisons within each group were done using Post-hoc test. Comparison between the two groups was done using Un-paired t test. p values less than 0.05 were considered statistically significant.
Results: The efficacy of GSE mouth rinse was comparable to that of chlorhexidine in reducing the dental caries activity, dental plaque accumulation and gingivitis.
Conclusion: GSE mouth rinse may be used as a self-applied preventive measure to maintain healthy oral and dental tissues in the future.

Keywords: Caries Activity; Grape Seed Extract; Oratest; Dental Plaque; Gingivitis.

Introduction

Prevention of oral diseases is very important to maintain good oral health condition; children usually neglect oral hygiene measures leading to active and uncontrolled dental diseases which subsequently affecting their general health [1]. Dental decay and gingivitis are the most prevalent dental disease in children, oral micro-organisms and bacterial plaque attached to tooth surface are responsible for initiation and progression of these diseases [2, 3].

The dental diseases are preventable not curable diseases, so dental healthcare workers should focus on preventive dentistry. Various preventable agents developed for self-care at home [4]. Mouth rinses containing antimicrobial agents used as supplements to daily homecare for prevention of bacterial plaque. 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, 6].

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 [7, 8]. 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 [6, 9, 10].

GSE act as antioxidant, antimicrobial and free radical scavenger [11, 12]. It has a remineralizing potential on artificial carious lesions of enamel, inhibits the proliferation of cariogenic bacteria and reduce the synthesis of dental plaque [13, 14]. Also, it has anti-inflammatory action and immune response enhancement, thus, might be used as natural antimicrobial agent in preventive dentistry [15].

Oratest is diagnostic test used to determine microbial load in sa-

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liva. It is used to assess periodontal disease as there is high correlation between the plaque and gingival indices and Oratest scores [16]. It is simple, inexpensive and rapid caries activity test helps in identification of high caries risk individuals [17]. The Oratest make the management of dental disease easier by determine and customize the most suitable therapeutic preventive measures, it used as an index to check the effectiveness of treatment instilled [18].

The literature indicated that, GSE is effective against streptococcus mutans *in vitro* but limited information is available regarding its effect *in vivo*. The comparative effect of these active agents on caries activity, dental plaque and gingivitis using Oratest is not studied yet. So, this trial was designed to evaluate their efficacy *in vivo*.

Aim Of The Work

This trial designed to:-

1- Evaluate the efficacy of GSE mouth rinse on the caries activity, dental plaque and gingivitis.
2- Compare the efficacy of GSE with that of commercially available chlorhexidine mouth rinse.

Material and Methods

Study design and ethical approval

The study design is clinical trial which carried out at the dental clinic of Pediatric, Dental Public Health and Preventive Dentistry Department, Faculty of Dentistry, Mansoura University, Egypt. After obtaining the ethical approval from institutional ethical committee. Written informed consents were obtained from the children's parents after understanding the aim of the study.

Sample size calculation

The target population was children aged from 9-12 years old. By calculating the sample size with confidence interval 95% and marginal error of 5 % and expected percentages of caries activity of 50%. The calculated sample size was (90 subjects) which was increased to 95 subjects.

Sample inclusion criteria

- Children with an age range from 9 - 12 years.
- Children with high caries risk detected by dental caries scores (DMFT + dft≥ 3).
- Children not brushing their teeth or brushing once before the bedtime and had fair to poor plaque accumulation according to Silness and LÖe Index.
- Moderate to severe gingivitis according to LÖe and Silness Index.

Participant exclusion criteria

- Children with systemic disorders.
- Children taken antibiotic within one month before the study.
- Baseline Oratest score more than 25 minutes.

Sample selection

107 participants meeting inclusion criteria were selected from children seeking treatment in Pediatric Dentistry Department. 101 subjects from the selected 107 who their parents signed the informed consents were included in this study. Later, 6 participants have baseline Oratest score more than 25 minutes were excluded and the remaining 95 children formed the study sample.

Materials

- Chlorhexidine (Hexitol, The Arab Drug Company, Cairo, Egypt) (Group I = Positive control). Commercially available chlorhexidine mouthwash contains 0.12% chlorhexidine gluconate.

- Grape Seed Extract (GSE) mouthwash (Group II = Test group). It was prepared for this study and contains 2 % Grape Seed Extract and other ingredients like that found in chlorhexidine mouthrinse.

- Oratest armamentarium:-

1. Sterile beakers & Screw cap test tubes.
2. Sterilized milk & 0.1% Methylene blue solution.
3. 10ml disposable syringes & sterile pipettes.
4. Mirror & test tube stand.

Randomization

The study sample was stratified into three groups according to dental caries scores, from (3-5), (6-10) and (11 or more). Each stratum subsequently divided according to the gender into males and females substrata, from the final substrata the two study groups were allocated randomly, group I [47(25 female + 22 male)] children and group II [48(25 female + 23 male)] children.

Blinding

Both participants and researcher who carried out caries activity test, plaque and gingivitis scoring were blind to the study materials used. The collected data was also analysed by an external statician.

Validity of the data

This trial was carried out by two researchers. One of them perform randomization and the other one performs clinical examination and Oratest procedure all over the study. To ensure the consistency of the measurements during the study period, intra examiner consistency was obtained by training and calibration before starting the study and during it. The intra examiner consistency was tested by Kappa test and weighted kappa test was 90 %.

Clinical interventions

Demographic characters, frequency of tooth brushing/day, Number of dental visits/years, dental plaque and gingival indexes scores, dental caries experience and caries activity (Oratest scores) were recorded as baseline data.

Oratest scores

0.1% methylene blue was prepared by mixing 100 mg of methyl-

ene blue in 100 ml of distilled water. Every child was instructed to rinse his mouth thoroughly with 10 ml of sterilized milk for 30 seconds then expectorate in a sterile beaker. 3 ml of expectorate was added to a screw cap test tube containing 0.12 ml of 0.1% methylene blue. Then content of the tube mixed thoroughly, and a mirror was used to detect any color change (blue to white) at the bottom of the tube every 5 minutes, the time taken for the initiation of color change was recorded [19].

Mouth washes usage

The children in both groups were instructed to rinse their mouths with (5 ml) of mouth rinse for five minutes in the morning before school time and at evening before the bedtime (daily for one week). To ensure the children compliance, a bottle contains 100ml of mouth washes enough for one-week use was supplied to each childand the extra return mouth rinses were calculated. Parents were instructed to supervise their children during the rinsing procedures and fill the supplied checklist.

Follow up intervals

Plaque Index and Gingival Index scoring and Oratest procedure were carried out at the end of 1st, 2nd, and 3rd weeks from the beginning of the mouth washes usage to evaluate and compare their effect.

Statistical analysis

Data was collected and analyzed using SPSS version 21(IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp). Independent t test was used to compare between the two groups at baseline and different follow up intervals. One-way ANOVA and post Hoc Tukey tests were used to evaluate the effect of each mouth wash and compare among the scores of the different follow up intervals in the same group. The p values less than 0.05 were considered statistically significant.

Results

At baseline; there were no significant differences between the 2 groups as regards age (p=0.138), brushing frequency/day (p=0.339), number of dental visits/year (p=0.080) and DMFT + dft scores (p=0.068) (Table 1), Oratest scores (p=0.451), dental plaque (p=0.302) and gingivitis (p=0.984)(Table 2).

Both chlorhexidine and GSE mouthrinses produce statistically significant reduction in; caries activity (increased time required for colour change by Oratest) (p= 0.000 for both), dental plaque scores (p=0.050 and p=0.010 respectively) and gingivitis scores (p= 0.010 for both) (Table 2).

Post hoc test revealed that, both chlorhexidine and GSE mouthrinses exhibited statistically significant effect on caries activity, dental plaque and gingivitis at all time intervals compared to each other or compared to baseline scores except dental plaque score for week three in group I compared to baseline. Student's t test revealed that, there were no significant differences between the two groups at all time intervals (Table 2, graph 1, 2 and 3).

Table 1. Baseline data of the study groups.

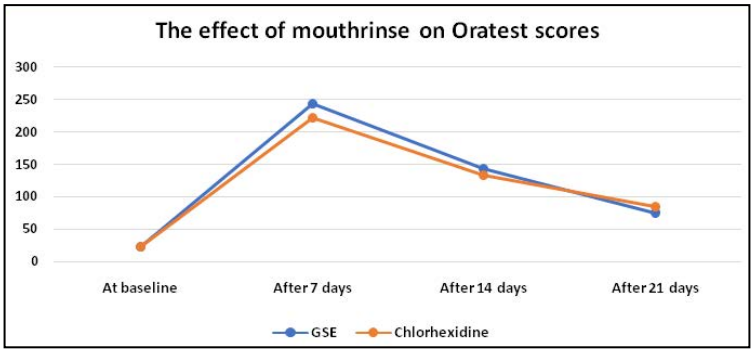
Groups Variables	Group I Mean ± SD	Group II Mean ± SD	p value
Age	10.590 ±0.332	10.550 ± 0.429	(0.138)
Brushing frequency/day	0.725 ± 0.167	0.741 ± 0.139	(0.339)
No. of dental visits /year	1.790 ± 0.174	1.820 ± 0.177	(0.080)
DMFT+ dft	5.959 ± 1.891	5.675 ± 1.979	(0.068)

Group 1= GSE mouthrinse, Group II=chlorhexidine mouthrinse,SD= Standard Deviation., p = level of significance calculated by independent student t test. DMFT+ dft= Decayed, Missed and Filled Permanent teeth + decayed and filled deciduous teeth.

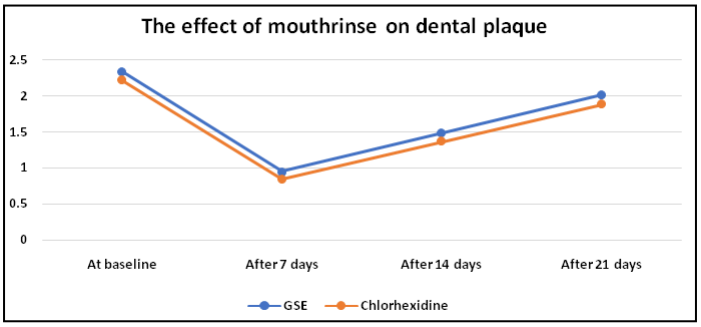
Table 2. The effect of chlorhexidine and GSE on Oratest scores, dental plaqueand gingivitis.

Variables	Follow up Groups	At baseline Mean ± SD	After 7 days Mean ± SD	After 14 days Mean ± SD	After 21 days Mean ± SD	P1
Caries activity	Group I	23.07 ± 5.499	243.04 ± 8.034	143.35 ± 7.501	75.39 ± 7.271	0.000
	Group II	22.39 ± 4.429	221.98 ± 9.017	132.78 ± 7.448	84.46 ± 7.194	0.000
	p	0.451	0.399	0.276	0.404	
Dental plaque	Group I	2.341 ± 0.528$	0.952 ± 0.024	1.491 ± 0.089	2.02 ± 0.211$	0.050
	Group II	2.218 ± 0.632	0.844 ± 0.023	1.368 ± 0.086	1.883 ± 0.131	0.010
	p	0.302	0.1	0.204	0.306	
Gingivitis	Group I	2.173 ± 0.612	1.062 ± 0.025	1.362 ± 0.078	1.933 ± 0.213	0.010
	Group II	2.281 ± 0.598	0.954 ± 0.022	1.241 ± 0.073	1.899 ± 0.209	0.010
	p	0.948	0.107	0.178	0.343	

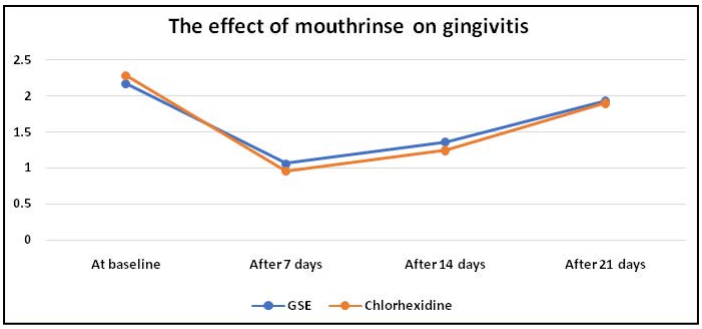
Group 1= GSE mouthrinse, Group II=chlorhexidine mouthrinse, SD= Standard Deviation, p1= level of significance calculated by One-way ANOVA test, p = level of significance calculated by independent student t test, $ = no significant difference between corresponding follow up scores in the same row and calculated by post Hoc Tukey tests.



Graph 1. Shows the effect of GSE and chlorhexidine mouthwash on Oratestscores.



Graph 2. Shows the effect of GSE and chlorhexidine mouthrinse on dental plaque scores.



Graph 3. Shows the effect of GSE and chlorhexidine mouthrinse on gingivitis.

Discussion

The prevalence of dental diseases was increased among children and to prevent the initiation of such diseases and overcome its sequelae advocated the use of natural product with antimicrobial potential. This study was carried out to evaluate the effect of GSE mouthrinse on the caries activity, plaque accumulation and gingival inflammation in comparison with chlorhexidine mouth rinse. Chlorhexidine was used as positive control because several studies proved its antimicrobial activity and use it as gold standard for evaluating the antimicrobial efficacy of other antimicrobial agents [20-22].

Oratest was used in present trial to evaluate the efficacy of GSE compared to chlorhexidine because it is simple non-invasive test,needs minimal armamentarium, acceptable for children assuring greater patient compliance [23]. Oratest is effective and reliable in assessment of caries activity and gingivitis, there were inverse relation between salivary bacterial load and time taken for the change in the color of methylene blue and vice versa [24, 28, 16].

The significant increase in time of colour change in the present study (Oratest scores) may be due to the antimicrobial effect of GSE and chlorhexidine which reduce the bacterial load in saliva and subsequently reduced rate of oxygen depletion [25].

Ourc results indicated a significant reducing effect of both mouth rinses on caries activity, plaque and gingival indexes when comparing the scores at baseline with the scores at different time intervals (Table 2). The effect of GSE and chlorhexidine mouth rinses was comparable to each other where there were no significant differences between them as regards caries activity, gingival and plaque indexes scores at different follow up intervals (Table 2, graph I, 2 and 3). The reduction in caries activity was in accordance with previous studies concluded that, GSE inhibits enamel caries *in vitro* due to its ability to suppress growth of cariogenic bacteria and plaque formation as well as promoting dental remineralization [13, 26].

The reduction in dental plaque and gingivitis scores were in accordance with the other studies used GSE mouth rinse and produced significant reduction in the gingivitis score, the improved gingival condition may be due to antimicrobial effect of GSE (reduction in dental plaque biofilm) [22], anti-inflammatory properties of bioactive antioxidants in GSE (polyphenols, catechin, and flavanol) [27-29] and increases collagen synthesis and accelerates the conversion of soluble collagen to insoluble collagen as GSE is a rich source of proanthocyanidin [6].

The present findings support results of another study evaluate the antimicrobial, anti-gingivitis, and anti-caries effect of GSE and suggested the future possibility of its utilization in preventive and restorative materials to preserve the dental health [30].

Conclusion

We can conclude that,GSE mouthrinse have anti-caries, anti-plaque and anti-gingivitis effect like that of 0.12% chlorhexidine gluconate mouthrinse. GSE can be used as safe mouth rinse for short term maintenance therapy with an advantage of having no side effects as it was used as dietary supplement and antioxidant.

Recommendation

Further research is recommended to test the efficacy of GSE mouth rinse on larger population as the present study is the first one evaluating the GSE as a mouth rinse to prove anti-caries, anti-gingival and anti-plaque effects.

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