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Original Article

Medical and Chemical Evaluation of the Effectiveness of the New Spray in Preventing the Formation of Dental Plaque

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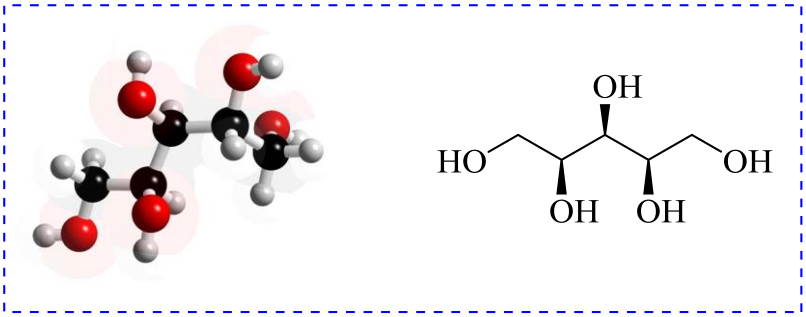
ABSTRACT

To prevent periodontal diseases and dental caries, the person must remove this layer. In this research study, we aimed at assessing the effectiveness of a spray containing silver nanoparticles/ EDTA/MSM/xylitol compared to the standard mouthwash of 0.2% chlorhexidine. This interventional and cross-over clinical trial study was conducted on 40 healthy patients with periodontal diseases and plaque index below 20%. Patients were randomly divided into 2 groups of 20. Then, the patients in each group were asked not to use other mechanical and chemical substances for one week. Instead, the first group used chlorhexidine mouthwash, and the second group used a spray containing silver nanoparticles/ EDTA/MSM/xylitol. In chlorhexidine mouthwash recipients, there were no signs of gingivitis. In spray recipients, 72.5% had no visible signs, 25% had a slight change in the color and surface tissue of the gingiva, 2.5% had visible inflammation and a tendency to bleed from the margin after probing. None of the recipients had severe inflammation and a tendency to bleed spontaneity in both groups. 50% of chlorhexidine mouthwash recipients and 30% of spray recipients had intervention-induced complications. This research revealed that spray effectively reduced plaque and bleeding, and inflammation of the gingiva. However, its effectiveness was not adequate. But the spray was significantly better than chlorhexidine in reducing teeth staining and the other complications related to chlorhexidine.

GRAPHICAL ABSTRACT

Xylotil ($C_5H_{12}O_5$)



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Introduction

As a very advanced technology, Biomaterials play a significant role in preventing, detecting, and improving human diseases. The main goal is to develop natural or synthetic materials in dentistry, orthopedics, maxillofacial, cardiovascular, neurological, and ophthalmic. Today, many advances have been made in applying biomaterials and bioengineering, such as implants, prostheses, and medical and dental instruments. Materials in medicine are part of applied materials sciences, one of the essential critical research fields of applied science research centers, including theoretical to clinical applied sciences. When a bone breaks in the human body, implants are placed in the body so that the two broken heads of the bones can stick together and give life to humans again. Biostructures have been developed to produce medical and dental implants to improve their mechanical and chemical properties and increase their abrasion resistance. Today, medical care is not possible without the use of plastic materials. Nanomedicine is a new subject in science and technology. Bioactive materials are gradually playing a more colorful role in the biomaterials industry, such as synthetic body organs, pharmaceuticals, and more durable and lighter composites in humans. The use of non-magnetic materials in medicine, such as recent developments in imaging, finding the causes of disease, and improving them. The use of graphite materials and ceramics as medical materials in clinical applications is also discussed.

Dental plaque is a biofilm or an accumulation of bacteria that grow on the surfaces inside the mouth and is seen as a white to pale yellow layer on the surface and between the teeth. Although dental plaque is related to oral diseases such as dental caries and periodontal diseases, its formation is a natural and unavoidable process. This formation continuity and its accumulation lead to oral diseases. Therefore, preventing dental plaque accumulation and removing it routinely is essential. Its removal will be accessible using the toothbrush correctly, and dental floss and interdental brush help [2-4]. Experimental and epidemiological studies have clearly shown that dental plaque is the leading cause of gingivitis [5], and actually, plaque-induced gingivitis is the most common form of gingiva disease [5-7]. Plaque control is one of the main components of periodontal treatments, which can be done both mechanically and chemically [5]. Mechanical method, including brushing, is the most reliable way to achieve oral health.

Given that the mechanical methods are not 100-percent effective and many patients also have problems with its acceptance and commitment, chemical substances such as an auxiliary method in controlling plaque have been recommended [6]. Recently, chewing gum containing xylitol has been used to control chemical plaque [7]. Xylitol is a 5-carbon alcohol sugar that prevents plaque formation due to its incremental effect on saliva flow and its bacteriostatic property [8]. Of course, it is worthy of mentioning that chlorhexidine mouthwash is now known as a gold standard for chemically preventing dental plaque [9].

Antibacterial properties of silver ions and their compounds have been proved by their effect on the bacterial cell wall and creating cavities and pores in it and have recently been considered and used abundantly in medicine. Antibacterial property effectiveness can be increased by using silver nanoparticles because of the increase in the contact level at the same dose [10]. EDTA or ethylene diamine tetraacetic acid is a carboxylic acid amino acid that is solid, colorless, and water-soluble. EDTA as a chelating agent can bind with calcium and iron ions. As a result, it can remove these two ions necessary for plaque formation and establishment [11]. Recently, this substance has also been used in toothpaste composition to prevent plaque formation [12]. The ability of EDTA to chelate to the bacterial cell wall and destabilize biofilms by absorbing calcium, magnesium, zinc, and iron has made it a suitable agent for use in biofilms [13]. EDTA can be used as an independent antimicrobial and anti-film agent or with other anti-microbes to care for wounds [13]. MSM or methyl sulfonyl methane with a chemical formula of $C_2H_6O_2S$ is an organic chemical compound with a PubChem ID 6213 that molar mass is 94.13 g/mol. It is an anti-inflammatory composition that, together with vitamin C, can play a crucial role in forming tissues

collagen and increases cell permeability to foods and liquids. This substance effectively uses an EDTA transmission amplifier to increase its local performance [14]. Recently, MSM, combined with EDTA has been used in a commercial toothpaste called Livionex, which has had very acceptable results in controlling plaque. In some studies, this toothpaste has been compared with other common toothpaste that, based on their findings, has had a better performance in plaque control [12, 15]. MSM, EDTA, Xylitol are classified in the collection of the Generally regarded as safe (GRAS) by FDA organization [15, 19]. For this reason, in this work, we want to use synergistic effects of silver nanoparticles, xylitol (Figure 1), EDTA, MSM in the form of a spray to control plaque and compare its effectiveness with 0.2% chlorhexidine mouthwash [20-23].

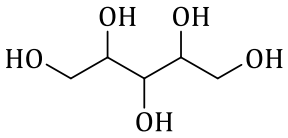

Figure 1: Xylitol, a promising allied for oral heath

Materials and Methods

This study was approved by the University Ethics Committee with IRUMSHA.REC.1399.533 code was conducted based on the statement of Ethics in Helsinki research, and conscious consent was obtained from all participants participating in this plan [24-26].

Making spray solution

First, to make nano-silver, silver nitrate is solvated in hydrogen peroxide for compound oxidation and formation of silver nitrate particles. To stabilize these particles and prevent these particles from reconnecting, we mixed this solution with the combined solution of distilled water and polyvinyl pyrrolidone, which leads to encapsulation and stabilization of the solution. We added and dissolved xylitol, MSM powder, and EDTA of silver nanoparticles. Then, the resulting powder is thoroughly stirred. Then menthol flavor is added to the solution. Compounds and their related percentage are shown in the Table 1:

Table 1: Compounds and their related percentage

Substance	Weight percentage
Distilled water	54.97
Sodium citrate	1
Silver nitrate	0.03
Poly vinyl pyrrolidone	1
EDTA	4
MSM	8
Xylitol	30
Caramel flavor	1

Chlorhexidine 0.2% made by Behsa Company is used for the control group (Figure 2).

The study was conducted as an interventional clinical and cross-over trial (eliminating confounding agents) in 2021 in Hamedan faculty of dentistry. The studied population of this research included 40 dentistry students and volunteer staff of The Faculty of Dentistry at Hamedan University of Medical Sciences. The participated individuals were randomly divided into 2 groups.

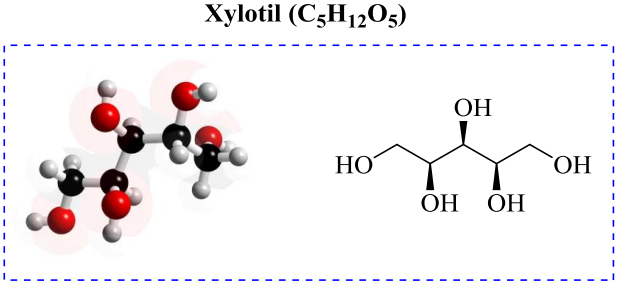

Figure 2: Structural chemical formula and molecular model of xylitol

They were asked not to use the other mechanical and chemical methods of controlling plaque during the study. The first individuals Oleary plaque index and staining index were taken and recorded. Then, each group was asked to act as below:

Group 1: using chlorhexidine mouthwash twice a day and 10 mL, each time for 1 min, and avoid eating, drinking, and washing their mouth until 1-hour later [27-29].

Group 2: using spray twice a day and each time 3 puffs (almost the entire mouth area) and avoid eating, drinking, and washing their mouth until 1 hour later [30-33].Plaque index (PI), gingival index (GI) and staining index (SI), and bleeding on probing (BOP) were measured in the studied people, and the date was recorded. Brusage was done to reach below 20% of the plaque index. Individuals returned to their common way of controlling plaque after two weeks (using a toothbrush and dental floss). Then the method of controlling two groups was changed as below:

Group 1: Using new spray twice a day and each time 3 puffs (almost the entire mouth area) and avoid eating, drinking, and washing their mouth until 1 hour later [34-36].

Group 2: Using chlorhexidine mouthwash twice a day and each time 10 ml for 1 min and avoid eating, drinking, and washing their mouth until 1 hour later. After 7 days, plaque, gingival, staining, and BOP indexes in the studied people wear measured, and the date was recorded. The data were compared with the previous data. Finally, Brusage was done to bring the plaque index below 20%.

Inclusion criteria

1. BOP or plaque indices 20% or below 20%.
2. Having at least 20 normal teeth.
3. Lack of plaque retentive factors, including overhang or calculus

Exclusion criteria

1. Individuals with systemic problems (smokers and pregnant women were also considered systemic problems).
2. People who have used mouthwash or chemical agents during the last 3 months.
3. People with a history of sensitivity to mouthwash or toothpaste and the probe depth more than 3 mm.

Data were analyzed using SPSS software version 22 and descriptive statistical methods and statistical tests such as t-test and paired t-test.

Results and Dissection

11 of the participants were males (27.5%), and 29 (72.5%) were females. The age range of the participants was 8-52 years, and the average age was 32 years (SD=8.66). Plaque index mean after using chlorhexidine mouthwash was 69%, and after using a spray, it increased by 5.49%. The paired t-test results revealed that this increase in chlorhexidine mouthwash recipients was insignificant (p value=.237). While it was significant in spray recipients (p-value< .001). The difference in the plaque index mean of chlorhexidine mouthwash and spray recipients had increased by 4.79%, and the statistical results of paired t-test showed that this increase was statistically significant (pvalue<.001) (Table 2).

Table 2: Comparing plaque index percentage before and after in chlorhexidine mouthwash and spray recipients

	Number	Previous mean (SD)	Next mean (SD)	Previous mean next mean	Statistic	p-value
Plaque index of the group receiving chlorhexidine mouthwash	40	(3/73) 5/38	(3/93) 6/07	(3/65) -0/69	Paired t = -1/20	0/237
Plaque index of the group receiving spray	40	(3/47) 5/31	10/80 (7/15)	(6/49) -5/49	Paired t = -3/41	<0/001
Difference between the two groups 'plaque index	40			(5/33) -4/79	Paired t = -5/68	<0/001

After receiving chlorhexidine mouthwash, the BOP index mean had increased by 0.04%, and after receiving spray, it increased by 2.07%. The paired t-test showed that this increase was not significant in chlorhexidine mouthwash recipients (p-value=.819) while in spray recipients (p value=.001). The difference in the average BOP index in chlorhexidine mouthwash and spray recipients had increased by 2.95%. The paired t-test revealed that this increase was statistically significant (p value=.005) (Table 3).

Table 3: Comparing BOP index percentage (bleeding) before and after in chlorhexidine mouthwash and spray recipients

	Number	Previous mean (SD)	Next mean (SD)	Previous mean next mean	Statistic	p-value
BOP index of the group receiving chlorhexidine mouthwash	40	(1/06) 0/30	(1/01) 0/34	(1/16)- 0/04	paired t = -0/231	0/819
BOP index of the group receiving spray	40	(1/23) 0/44	(4/66) 2/51	(3/77)-2/07	Paired t = -3/46	0/001
Difference between the two groups' BOP index	40			(4/36) -2/03	Paired t = -2/95	0/005

No signs of gingivitis were observed in chlorhexidine mouthwash recipients. In spray recipients, 72.50% had no visible symptoms, 25% had a slight change in the surface tissue and color of the gingiva, 2.50% had visible inflammation and a tendency to bleed from the margin after probing. None of the participants had severe inflammation and a tendency to bleed spontaneously in both groups. In chlorhexidine mouthwash and spray recipients, 100% and 72.50% were respectively without visible symptoms, which had the most frequency in both groups (Table 4).

Table 4: Comparing the frequency of gingivitis in chlorhexidine mouthwash and spray recipients

Intervention type	Without visible symptoms	A slight change in the gingival color and surface tissue	Visible inflammation and tendency to bleed from the margin after probing	Severe inflammation and tendency to bleed spontaneously	Total
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
Chlorhexidine recipient	(100) 40	(0) 0	(0) 0	(0) 0	(100)40
spray recipient	29 (72/50)	(25/00)10	(2/50) 1	(0) 0	(100) 40

Staining intensity index in chlorhexidine mouthwash in order of the highest frequency was less color (95%), colorless (2/5%), bright orange to brown (2.5%). The intensity index in spray recipients in the highest frequency was less color (95%) and colorless (5%). For intensity index, 95% of the chlorhexidine mouthwash recipients had less color, and 95% of the spray recipients were colorless. Therefore, the intensity index in spray recipients was less than chlorhexidine mouthwash recipients (Table 5). The buccal and lingual intensity index for chlorhexidine mouthwash recipients was more than spray recipients.

Table 5: Comparing the frequency of the staining intensity in chlorhexidine mouthwash and spray recipients

Intervention type	Colorless	Less color	Bright orange to brown color	Dark brown to Black	Total
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
Chlorhexidine recipient	(2/50) 11	(95/00) 38	(2/50) 1	(0) 0	(100)40
spray recipient	38 (95/00)	(5/00) 2	(0) 0	(0) 0	(100) 40

50% of chlorhexidine mouthwash recipients and 30% of spray recipients developed a complication caused by the intervention. The complication frequency in chlorhexidine mouthwash recipients was more than spray recipients. Tooth staining and tongue color change in chlorhexidine mouthwash had the most and the lowest frequency, respectively. And increasing plaque and other complications in spray users have had the most and the lowest frequency, respectively (Figure 3).

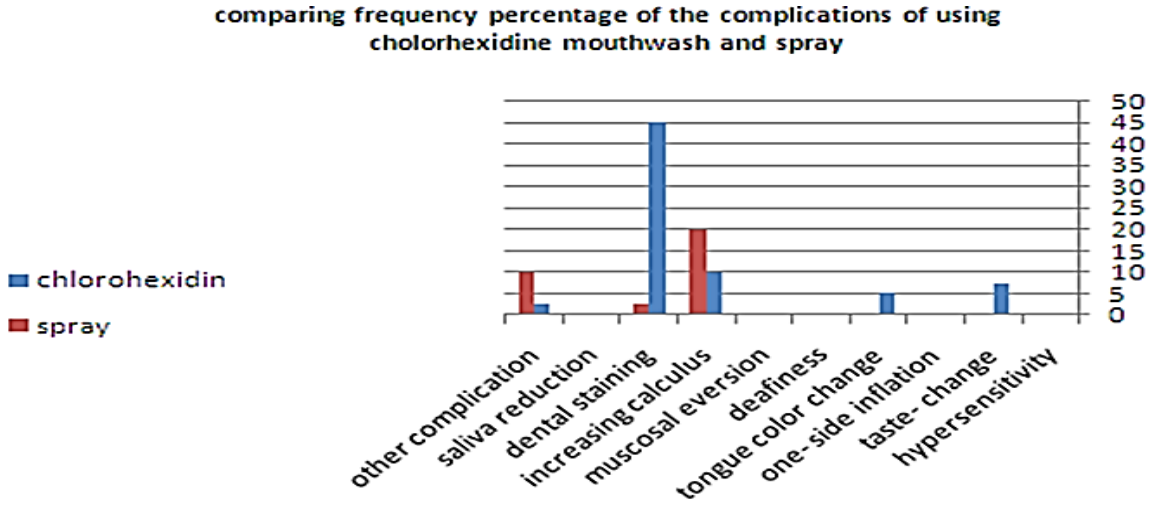

Figure 3: Increasing plaque and other complications in spray users have had the most and the lowest frequency, respectively

The purpose of this study was to study the anti-plaque, anti-inflammation, and staining effects of silver nanoparticles/EDTA/MSM/xylitol compared to chlorhexidine mouthwash as a standard sample. In the current study, periodontal indexes of 40 patients were studied before and after using spray and chlorhexidine mouthwash.

Given that this study was done cross-over, the effect of mouthwash being the first or second was eliminated in the results. The statistical analysis showed that the average plaque index (O'Leary plaque index) before and after using chlorhexidine mouthwash had no significant difference. This finding correlates with previous research, which revealed the effectiveness of chlorhexidine in reducing dental plaque [5]. In the study of Alsharani *et al*. (2018), the anti-plaque effect of the mouthwash containing nono silver and chlorhexidine were compared with each other. Both types of mouthwash reduced plaque in patients, but chlorhexidine mouthwash showed more plaque reduction [16]. Jose *et al*. (2015) studied the effect of chlorhexidine mouthwash with or without alcohol on the amount of gingival bleeding. They found that both types of mouthwash decrease gingival bleeding scores [17]. In this study, silver nanoparticles/EDTA/MSM/xylitol spray was prepared by Chista Sazan Notash Fartak Company of Qazvin. The mean of plaque index after receiving chlorhexidine mouthwash increased by 69%, and after receiving spray, it increased by

5.49% that this difference in recipients was statistically significant. The spray-controlled plaque in patients who did not use other mechanical and chemical methods of controlling plaque. However, it was not as effective as chlorhexidine standard mouthwash. BOP index means after receiving chlorhexidine mouthwash increased by 0.04% and after receiving spray increased by 2.07% that this difference in recipients was statistically significant. As a result, spray-controlled bleeding in patients, but it was not as effective as chlorhexidine mouthwash.

In a systematic review of Sadeq *et al*. (2020), the effect of aloe Vera mouthwash on the amount of plaque and gingivitis was investigated. The researchers concluded that aloe Vera mouthwash is comparable to chlorhexidine in reducing gingivitis, but it is lower than chlorhexidine mouthwash [18]. In the current study, no symptoms of gingivitis were observed in chlorhexidine mouthwash recipients and spray recipients, 72.5% were without visible symptoms, 25% had a slight change in the color and surface tissue of the gingiva, and 2.50% had visible inflammations and a tendency to bleed from the margin after probing. The silver nanoparticle/EDTA/MSM/xylitol spray effectively reduced patients' gingivitis, but it was not as effective as chlorhexidine mouthwash.

One of the most common problems of the chlorhexidine mouthwash is staining the teeth and mucous membrane, which upset patients [19]. In recent years, different methods have been performed to reduce chlorhexidine staining, such as anti-staining systems. The results of the studies are contradictory. However, most studies have shown that adding such substances reduces chlorhexidine efficacy [20, 21]. In the current study, the results of Lobene staining index were such that 95% of the silver nanoparticle/EDTA/MSM/xylitol spray were colorless, and 5% of them had less color. In comparison, 95% of chlorhexidine recipients had less color, and 2.5% were colorless, and 2.5% had bright orange to brown color. Our study shows that spray is more effective in reducing teeth staining than chlorhexidine mouthwash.

Chlorhexidine mouthwash effectively prevents gingival plaque in the absence of other oral health methods [22]. But, it is always associated with side effects such as hypersensitivity reaction, taste changes (especially saltiness and bitterness tastes, but these changes are transient and disappear after stopping consumption), one-sided or two-sided carotid inflation, mucosal erosion, repair process changes, increasing plaque formation, staining teeth, the back surface of the tongue and restorations [23].

Conclusion

In this study, the occurrence of chlorhexidine mouthwash was 50% and for silver nanoparticle/EDTA/MSM/xylitol spray was 30% which shows much fewer side effects of spray than chlorhexidine. Complications such as taste change, tongue color change, teeth staining that were not highly observed in chlorhexidine mouthwash users were not observed in silver nanoparticle/EDTA/MSM/xylitol spray users. The results of this study revealed that silver nanoparticle/EDTA/MSM/xylitol spray is effective in reducing plaque-bleeding and gingivitis, but it was not effective as chlorhexidine standard mouthwash. However, the silver nanoparticle/EDTA/MSM/xylitol spray is significantly better than chlorhexidine in reducing the number of teeth staining and other side effects related to chlorhexidine. Based on the results obtained from this study and the non-toxicity of these substances on the Vero cell line [1], it can be a good alternative for controlling the chemical plaque of the patients. Therefore, the results obtained from this study and the non-toxicity of this substance on the cell line of Vero can be a good alternative for controlling patients' chemical plaques.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

The authors have no conflicts of interest relevant to this article.

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