

Evaluation of Preventive Antibacterial Properties of a Glass-Ionomer Cement Containing Purified Powder of *Salvia officinalis*: An *In vitro* Study

Abstract

Background: In this study, the anti-*Streptococcus mutans* and anti-*Lactobacillus casei* properties of a restorative glass-ionomer cement (GIC) modified with extract powder of *Salvia officinalis* as a safe and effective herbal extract at weight concentration levels of 0.5%, 0.75%, 1%, and 1.25% are investigated. **Methods:** The *S. officinalis* extract powder is provided by doing a multistep laboratory procedure and is filtered to obtain particles smaller than 50 μ . The GIC powder is modified by adding extract powder in weight concentrations of 0.5% (Group II), 0.75% (Group III), 1% (Group IV), and 1.25% (Group V) to form experimental groups, each of 1 g. Five disk-shaped samples, 1 cm in diameter and 2 mm height, of each group (including control group [Group I]) are prepared for each bacterial category of *S. mutans* and *L. casei*. The twenty-five samples for each category are tested *in vitro* against strains of *S. mutans* and *L. casei*. Following Agar diffusion tests, the inhibition zone diameters are recorded. The data are tested for normality by means of Kolmogorov–Smirnov procedure. The Kruskal–Wallis and Mann–Whitney tests are used to perform a one-way ANOVA and to do pair-wise comparisons, respectively, at 5% significance level. **Results:** The mean diameter of the inhibition zones are significantly different among the test groups and also the test groups and the control group except for the group with 0.5% in *L. casei* category which shows no significant difference with the control group. **Conclusions:** The present study revealed direct inhibitory activities of *S. officinalis*-containing GIC against *S. mutans* and *L. casei* in a dose-response manner.

Keywords: Antimicrobial agents, dental material, glass ionomer, *Salvia officinalis*

Introduction

Dental caries is still a major concern in public health management.^[1] Establishment of a productive dental health philosophy, especially for the low socioeconomic levels of the society, where the lack of development endangers the dental health, even more, seems to be necessary.^[2] Microbial nature of caries and the contemporary concept of treatment based on the medical model necessitate the consideration of chemical control plans along with the traditional mechanical means of caries control.^[3] Innovations in dental material science are come to the point of developing smart materials.^[4] glass-ionomer cement (GIC), a tooth-colored acid-base material, with the capability of fluoride release in an aqueous environment is the first of this category.^[3,4] It can be used as base, liner, or direct restorative material and is also considered as the material of choice in atraumatic restorative technique (ART). The

ART is a minimal intervention approach, particularly beneficial for pediatric and elderly patients as well as those with dental anxiety or learning difficulties.^[3]

While the bulk of evidence supports the protective nature of fluoride in public water and oral health products; however, the data available does not endorse the anti-caries ability of GIC or any useful outcome of fluoride-releasing restorative materials.^[3,5] To address the issue, multiple modifications are being under investigation to develop a GIC with direct antimicrobial effect based on its ability to participate in ion-exchange reactions with the oral environment.^[3,5] Such a material would be of tremendous clinical benefits. Providing antibacterial seal leads to suppression of remaining bacteriae under the restoration, reduced postoperative sensitivity, improved pulpal and periodontal health, and finally reduction in recurrent caries. Different studies are designed attempting to develop a modified GIC by incorporation of antimicrobial agents such as chlorhexidine hydrochloride,

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cetylpyridinium chloride, cetrimide, and benzalkonium chloride.^[6]

Hook *et al.* proposed a GIC functionalized with chlorhexidine-hexametaphosphate particles. The suggested material released chlorhexidine in a dose-dependent manner and showed to have antimicrobial properties.^[3] In another study, de Castilho *et al.* added doxycycline hyclate as an antibiotic with local antimicrobial and antimatrix metalloproteinase activities into the GIC. The modified material showed inhibitory effects on caries producing bacteria.^[7] Deepalakshmi *et al.* incorporated chlorhexidine and cetrimide as wide-spectrum antiseptics into GIC and developed an antibacterial material.^[6]

Herbal extracts have recently gained even more attention as active agents to be incorporated into oral care products and dental materials. Many of them have potential antimicrobial activities which could lead to the production of safe, economical, and efficient alternative materials for use in caries management.^[8] The Iranian herbal medicine with a long history of effectiveness and safety is a rich source of such agents.

Salvia officinalis, which is also called Sage, is a perennial evergreen plant native to Mediterranean region^[8] and is also naturalized in Iran.^[9] It has woody stems, grayish leaves with blue-to-purplish flowers. A long ancient and contemporary history of medicinal and pharmacological applications supports its use. *S. officinalis* extract has antimicrobial, analgesic, anti-inflammatory and antioxidant properties.^[8,10] The essential oil of *S. officinalis* consists of alpha- and beta-thujone, borneol, camphor, and cineole. In contemporary European herbal medicine, a type of *S. officinalis* tea is commonly advocated to control a sore throat, inflammatory oral lesions, and gingivitis.^[8,10] Kermanshah *et al.* showed Sage mouthwash to have an inhibitory effect on caries-producing oral microbial flora.^[9] In another study, Beheshti-Rouy *et al.* proved the *S. officinalis* extract to be effective in reducing the number of *Streptococcus mutans* colonies in bacterial plaque.^[11] Different other studies also reported the inhibitory effect of *S. officinalis* extract on collagenolytic activity of *Porphyromonas gingivalis*,^[12] and also on the attachment of *Candida albicans* to denture surfaces.^[8]

In an attempt to develop a GIC with direct antimicrobial properties, this study is conducted to investigate any possible inhibitory effects *S. officinalis* modified GIC may have on *S. mutans* and *Lactobacillus casei* as the main bacteria involved in caries initiation and progression process. The experiment is performed for *S. officinalis* weight concentration levels of 0.5%, 0.75%, 1%, and 1.25%.

Methods

Some high-quality dried *S. officinalis* is purchased from a local herbal medicine retail store (Parand

herbal products, Isfahan, Iran). Leaves of the plant are chopped and fragmented into small pieces and filtered through a #40 mesh (Sina Lab. Inst., Tehran, Iran). Each 50 g of leaves are soaked in 1500 ml of solvent (50% water, 50% ethanol [96%]) in a shaker apparatus (Heidolph Unmax, Schwabach, Germany) at 90 rpm for 48 h. Thereafter, the solution is passed through a strainer and then transferred to a rotary evaporator apparatus (Heidolph WD2000; Schwabach, Germany) to separate the solvent from the extract.^[11] The purified extract is then dried by applying freeze-drying technique in three stages over a 1-week period. The final extract powder is stored in sealed vial at low temperature to be used in the next steps. To filter the particles, the same size as range of GIC powder which is to be <50 μ, the powder is grinded and again filtered through a #270 laboratory mesh (Sina Lab. Inst., Tehran, Iran). The procedure is performed under the supervision of a pharmacology professor at the main laboratory of the School of Pharmacy, Isfahan University of Medical Sciences and Health Services, Isfahan, Iran.

A conventional powder and liquid Fuji IX GIC (GC Corporation, Tokyo, Japan) is used as control group named Group I. Experimental GIC samples are prepared by incorporating *S. officinalis* extract powder into the powder component of Fuji IX GIC (GC Corporations, Tokyo, Japan) at 0.5% (Group II), 0.75% (Group III), 1% (Group IV), and 1.25% (Group V) weight concentration levels using a digital weight scale (CAS MWP Micro Weighing Balance, CAS Scales, New Zealand). Thus, four groups of experimental powder formulations are obtained, each sample weighs 1000 mg according to the procedure outlined in.^[6] The concentrations used in this research are based on the minimal inhibitory concentration and minimal bacterial assays suggested in.^[9] The groups are presented in Table 1.

Five specimens are prepared from each experimental group by a specialist in restorative dentistry. Powder/liquid is

Table 1: Test groups assigned to each bacterial category based on the weight concentration of *Salvia officinalis* extract powder

Test group number*	Weight percentage concentration of <i>S. officinalis</i> extract powder [^]
I (control)	0
II	0.5%
III	0.75%
IV	1%
V	1.25%

*These groups are assigned to each bacterial category (*S. mutans* and *L. casei*), [^]Weight concentration of *S. officinalis* extract powder added to the powder component of Fuji IX Glass-ionomer cement (GC Corporation, Tokyo, Japan) to form test groups. *S. mutans*=*Streptococcus mutans*, *L. casei*=*Lactobacillus casei*, *S. officinalis*=*Salvia officinalis*

dispensed on a mixing pad according to the manufacturer recommendations and mixed for 30 s with a sterile plastic spatula, and then inserted into the stainless steel molds of 10 mm in diameter and 2-mm thickness (Abzar Iran, Arak, Iran) within 1 min with a sterile dental instrument and allowed to set for 30 min at room temperatures, same as the procedure in.^[6]

Stock cultures of *S. mutans* (UA159) and *L. casei* (ATCC #193) obtained from the Microbiology and Immunology Laboratory of the School of Medicine, Isfahan University of Medical Sciences and Health Services, Isfahan, Iran are used by an expert laboratory technician. For each bacterial category, cells are cultured freshly from frozen stock on brain–heart infusion broth (BHI; DIFCO Laboratories, Detroit, MI, USA) for 24 h at 37°C in a 10% CO₂ incubator. The viability and absence of contamination is confirmed by plating in a specific medium and using Gram procedures. Cultures are again grown in BHI for 20 h at 37°C and the inoculums for subsequent testing are obtained according to.^[7]

In each sterilized Petri dish (15 mm × 90 mm), a base layer containing 15 ml of BHI agar mixed with 300 ml of each inoculums (*S. mutans* and *L. casei*) was prepared according to.^[7] The set disk-shaped specimens are placed on a BHI agar plate inoculated with bacterial strain and left at 37°C for 48 h. Zones of inhibition are then measured in millimeters using a digital caliper at three different points. Size of the inhibition zones is calculated by subtracting the diameter of the specimen from the average of the three measurements of the halo according to.^[6]

Statistical analysis

Data are analyzed using Statistical Package for the Social Sciences, SPSS software version 22 for Windows (IBM Inc., NY, USA). SPSS is a software package used in statistical analysis of data. This software package was developed by SPSS incorporation and acquired by IBM in 2009. In 2014, the software was officially renamed IBM SPSS statistics.

Kolmogorov–Smirnov test is used to test the normality of the data. The test is rejected at significance level of 5%, and the data follow distributions different than normal. Thus, nonparametric Kruskal–Wallis, Mann–Whitney, and Wilcoxon signed-rank sum tests are performed to compare the test groups and also the test groups with the control group.

Data are analyzed using Statistical Package for the Social Sciences (SPSS, version 22) for Windows

(IBM Inc., NY, USA). Kolmogoro v–Smirnov test is used to test the normality distribution of the data. The nonparametric Kruskal–Wallis and Mann–Whitney tests are then employed to compare the test groups and also the test groups with the control group. The statistical analyses are all performed at significance level of 5%.

Results

According to Kruskal–Wallis test, there was a significant difference between the mean diameter of the inhibition zone for *Streptococcus mutans* ($P < 0.001$) and *Lactobacillus casei* ($P < 0.001$) in different concentrations of salvia extract [Table 2].

According to Mann–Whitney test, the mean diameter of the inhibition zone for *Streptococcus mutans* was significantly different between all groups with each other and with the control group ($P < 0.05$) and the mean diameter of the inhibition zone for *Lactobacillus casei* was significantly different between all groups with each other and with the control group ($P < 0.05$), except between the control group and the group with the concentration of 0.5% ($P = 0.134$). [Tables 3 and 4].

Pairwise comparison of the mean diameter of inhibition zones of samples with similar concentrations in the *Streptococcus mutans* and *Lactobacillus acidophilus* showed that only the inhibitory effect of the concentration of 0.5% on *Streptococcus mutans* was significantly higher than that of *Lactobacillus acidophilus* [Table 5].

Discussion

In this research, the possibility of producing an herbally modified GIC with direct antibacterial properties is investigated. A distinguishing feature of this study is the use of an herbal extract with a long history of safety and effectiveness instead of chemical agents to produce a modified GIC. The results showed that the incorporation of *S. officinalis* extract into the powder component of a routinely used GIC produces a dental material with direct inhibitory properties against the most important bacterial causes of dental caries. It is also shown that this ability has a dose-response nature.

The statistically nonsignificant difference between Group I and Group II (control group) in the *L. casei* category shows a lower potential in *S. officinalis* to inhibit the *L. casei* at its lowest concentration. The statistically significant higher value of the mean inhibition zone diameter in Group II of the *S. mutans* category compared to the Group II of the

Table 2: The mean diameter of the inhibition zone for *Streptococcus mutans* and *Lactobacillus casei* in different concentrations of salvia extract

Concentration	Mean±SD					P
	Group 1	Group 2	Group 3	Group 4	Group 5	
<i>Streptococcus mutans</i>	0	0.26±0.05	0.02±0.34	0.02±0.62	0.07±1.1	<0.001
<i>Lactobacillus casei</i>	0	0.05±0.04	0.02±0.47	0.05±0.56	0.05±1.06	<0.001

Table 3: Comparison of the mean diameters of the inhibition zones between the test groups in the *Streptococcus mutans* category*

Test group	Group I	Group II	Group III	Group IV	Group V
Group I	-				
Group II	0.005	-			
Group III	0.004	0.014	-		
Group IV	0.005	0.007	0.006	-	
Group V	0.005	0.007	0.006	0.007	-

*Wilcoxon signed-rank sum test as a nonparametric test was used for comparing the means. The numbers show *P* - value within groups. Significant level was considered at 5%

Table 4: Comparison of the mean diameters of the inhibition zones between the test groups in the *Lactobacillus casei* category*

Test group	Group I	Group II	Group III	Group IV	Group V
Group I	-				
Group II	0.134	-			
Group III	0.005	0.007	-		
Group IV	0.005	0.007	0.02	-	
Group V	0.005	0.007	0.007	0.007	-

*Wilcoxon signed-rank sum test as a nonparametric test was used for comparing the means. The numbers show *P* - value within groups. Significant level was considered at 5%

Table 5: Comparison of the mean diameters of the inhibition zones of the same group between two bacterial categories of *Streptococcus mutans* and *Lactobacillus casei**

Test group	Mean diameter of the inhibition zones		<i>P</i>
	<i>S. mutans</i>	<i>L. casei</i>	
Group II	0.26 mm	0.04 mm	0.006
Group III	0.34 mm	0.47 mm	0.058 [^]
Group IV	0.62 mm	0.56 mm	0.339 [^]
Group V	1.10 mm	1.06 mm	0.99 [^]

*Wilcoxon signed-rank sum test as a nonparametric test was used for comparing the means. Significant level was considered at 5%, [^]*P*-value indicates no statistically significant difference. *S. mutans*=*Streptococcus mutans*, *L. casei*=*Lactobacillus casei*

L. casei category points also to the same finding which should be considered cautiously.

The findings of this study challenge the traditional idea that the dental materials must be passive without any interactions with surrounding oral environment^[4] and also recommends the use of materials with active capabilities as a possible approach in the management of different carious states. It seems that the ability of such a modified GIC to inhibit the bacterial activity depends on its interactions with the surrounding aqueous environment.^[3,5]

It is shown that bacteria responsible for caries may survive up to 2 years even under regular GICs, which many believe to have anticariogenic properties.^[13] This means that any intentional (in case of close proximity to pulp) or

unintentional residual caries under the restorations may progress and encroach on pulp and produce discomfort for patient and finally endanger the pulp vitality.^[14-16] This also questions the accuracy of ART, in which multiple carious teeth are only excavated with a hand instrument and filled with a regular GIC, especially in low socioeconomic areas of society.^[2,16,17] A modified GIC with direct antimicrobial properties addresses the situation here.

Different investigators incorporated different antimicrobial agents in different concentrations into GIC in an attempt to find a novel solution.^[1,3,6,7,18] Wide-spectrum antimicrobials such as chlorhexidine,^[1,3,18] cetrimide,^[6] and antibiotics^[7,6] were at the center of the scope of those studies. The agent(s) were added into the powder or liquid component of the GIC, utilizing different incorporation techniques. However, none of them are incorporated any herbal agent as a safer source with fewer or no possible complications such as mucosal or dental staining seen in chlorhexidine products.^[19]

The reasons for selecting the *S. officinalis* in this research are the long history of successes and no history of any negative side effect documented for, in traditional and contemporary medical literature.^[12] It is also reported that *S. officinalis* extract has superior efficiency compared to the antibiotics and has also less chance of drug resistance when used in high concentrations.^[20] In addition, researchers are recently investigating possible applications of *S. officinalis* essential oil extract in oral care products. The studies reported inhibitory effect of the extract on many oral bacteria and fungi responsible for carious and periodontal infections.^[8,9,11,12] They also reported positive effect on reducing the number of plaque bacterial colonies,^[11] impairing plaque formation,^[21,22] and fungi attachment to prosthetic surfaces.^[8]

Based on the available evidence, which recognizes the anti-inflammatory properties in *S. officinalis* extract,^[9,12,20,22,23] one may suggest the use of the *S. officinalis* modified GIC may alleviate and control the inflammatory status of the pulp or periodontium. It is recommended to conduct experiments to investigate such abilities.

While the presence of *S. officinalis* extract as an antibacterial agent in GIC is effective against oral pathogens, investigations to evaluate other important properties such as mechanical, physical, and biocompatibility properties of the material are highly recommended before any routine clinical applications. Considerations should be taken to improve the color of *S. officinalis* modified GIC since the current product has an intense yellow color.

Conclusions

Within the limitations of this study, it is concluded that a *S. officinalis* modified GIC has a direct antibacterial property against *S. mutans* and *L. casei*. This, in turn, provides many advantages for use as a base, liner, or restorative material in

operative procedures and management of the complications of carious teeth. It also emphasizes the importance of the herbal agents in developing new dental materials with astonishing properties.

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Conflicts of interest

There are no conflicts of interest.

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