



The Effect of Aqueous Extract of *Crocus sativus* L. (Saffron) on Periodontal Indices of Patients with Generalized Periodontitis

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Abstract

Many therapeutic properties have been reported for saffron (*Crocus sativus* L.), including anti-inflammatory effects due to its strong antioxidant properties and the ability to accumulate oxygen free radicals by components such as crocin and crocetin. The aim of this study was to evaluate the anti-inflammatory effect of saffron on the treatment of patients with periodontitis. 40 patients with moderate to severe generalized periodontitis were selected and divided randomly into the intervention and control groups. The intervention group was instructed to use a mouthwash containing aqueous saffron extract twice a day for 2 weeks; while the control group members were asked to apply chlorhexidine as the gold standard mouthwash. The participants' gingival probing depth index, modified gingival index, and gingival bleeding index were measured before and one month after the intervention. The patients' level of satisfaction on the taste of mouthwash was also measured. T-test and Mann-Whitney test were run for statistical analysis. A significant difference was observed in terms of the gingival indices before and four weeks after using both saffron and chlorhexidine mouthwashes (p value: 0.001). However, no statistically significant difference was found between two groups. Patients reported higher levels of taste satisfaction after using saffron aqueous extract mouthwash than the chlorhexidine mouthwash (p value: 0.021). Saffron mouthwash showed comparable therapeutic effect to the gold standard mouthwash, chlorhexidine, and may be helpful as an adjunct treatment for management of periodontitis.

Keywords: Periodontitis; Periodontal index; *Crocus sativus*; Mouthwash

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Introduction

Periodontitis, a common inflammatory disease, affects the teeth-supporting tissues [1]. In this complex disease, dental biofilm interacts with inflammatory immune system, which may lead to an imbalance between bacterial invasion and the intensity of the host immune response [2]. Scaling and root planning (SRP) is a common major method used as the gold standard for periodontal treatment. However, SRP alone has limited effects on some bacterial species and does not remove subgingival bacteria completely. This may be related to the fact that some species may exist in soft tissue, dentinal tubules, or root surface irregularities, which are associated with the treatment failure [3]. Therefore, to help the healing process, both chemical and herbal therapeutic mouthwashes were added to the treatment. In this way, herbs such as turmeric with its potent anti-inflammatory active ingredient, curcumin, or ginger have been used as adjunctive treatment for periodontitis along with SRP [4-6].

In traditional Persian medicine, saffron and its extracts are used to improve digestion, increase appetite, relaxation, and treat liver diseases, spasm, toothache, rhinitis, pharyngitis, insomnia, depression, cough, asthma, bronchitis, fever, nausea, scarlet fever, urinary tract infections, cardiovascular disorders, and even cancers and immune system modulation [7-11]. According to the modern studies, saffron has antioxidant, anti-inflammatory, anticoagulant, antibacterial and analgesic effects [7,10,12]. However, clinical

studies are very limited about the use of saffron in the field of oral diseases.

A laboratory study reported that ethyl acetate extract of saffron has strong antimicrobial effects against various microorganisms such as both gram-positive (g+) and gram-negative (g-) bacteria [13], but it is obvious that alcoholic compounds themselves may have an antibacterial effect, so to avoid errors, we chose the aqueous type from two types of alcoholic and aqueous extracts. Considering the anti-inflammatory effect of saffron and due to the dissatisfaction of some patients with the taste of conventional mouthwashes, especially the gold standard chlorhexidine, this study was conducted to investigate the effect of mouthwash containing saffron aqueous extract on periodontal indices of patients with moderate to severe generalized periodontitis.

Materials and Methods

This double-blind, randomized clinical trial was approved by ethics committee of Shahid Sadoughi University of Medical Sciences (IR.SSU.REC.1397.169) and was registered in IRCT (Iranian Registry of Clinical Trials) by IRCT20190402043155N1 code. The duration of intervention in this trial was considered to be two weeks and then the evaluation of variables was recorded after 4 weeks. In order to conduct the study, 40 patients with moderate to severe generalized periodontitis who referred to the periodontology department of Dental School were selected. Considering a test power of 80% and a signifi-

cant difference of 0.5 points in the mean of the bleeding index (BI) and after including the possible sample loss, 20 patients in each group, were recruited. Individuals in the age range of 35 to 65 years were selected to avoid the influence of age on the status of periodontal disease. Patients had at least 16 natural teeth and more than 3 mm of attachment loss was observed in at least 30% of dental sites. Other inclusion criteria were lack of a history of allergies and systemic diseases such as diabetes, no pregnancy and lactation, no use of tobacco or other related products, no intake of antibiotics during the past 3 months, and no use of drugs that affect the periodontal condition such as anticonvulsants, calcium channel blockers, immunosuppressant or oral contraceptives.

Participants were simulated in terms of age and gender using stratified randomization to make our groups more homogeneous. Then they were divided into two groups by the random table of numbers.

Before the start of the study, all patients were asked to sign an informed consent form to enter the study. Moreover, comprehensive

periodontal examinations for the purpose of gingival indices measurement such as Modified Gingival Index (MGI) and BI [14] and Probing Depth (PD) [15] were performed for all the participants.

Saffron powder (purchased from Ghaenat Saffron Company, prepared from the stigma of *Crocus sativus* L.), was authenticated in the herbarium of Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran (voucher number: ssu0075). Total of 10 mg of saffron powder was added to 100 ml of distilled water. Then, it was incubated on a shaker for 24 h. The material was passed through several layers of muslin cloth. The extracts were then poured into a round bottom balloon and placed in the freezer at a temperature of -80°C . After freezing, the extracts were placed in a freeze-dryer. In vacuum, the solvent was removed and saffron powder was obtained. After preparing the initial concentration, serial dilution with ratios of one-half and one-third was performed in sterile vials and one-half of the final concentration was discarded (Figure 1).

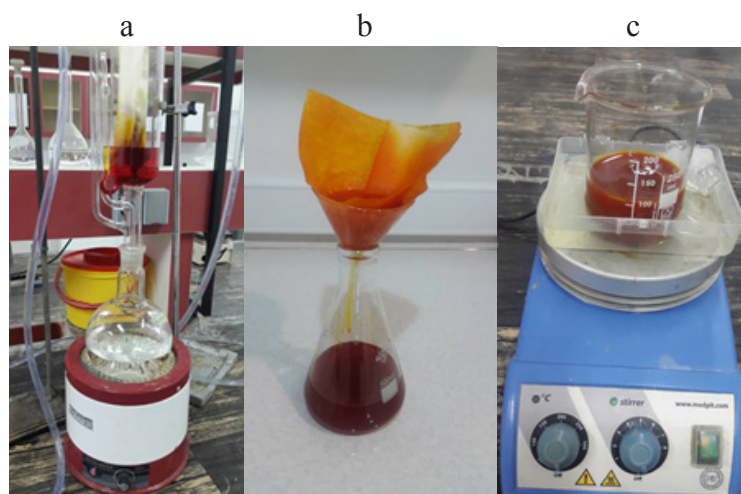


Figure 1. Aqueous saffron extract preparation a) Using shaker incubator b) Crossing the muslin cloths c) Serial dilution step

After the preparation of extract, the 0.2% saffron mouthwash was prepared by mixing the following ingredients: 1. propylene glycol (5 g) 2. saffron extract (0.2 g) 3. polysorbate 80 (1.8 g) 4. sodium benzoate (0.2 g) 5. water (91 g).

The participants were required to use a mouthwash containing aqueous extract of saffron or a commercial product of 0.2% chlorhexidine mouthwash (Najo Company, Iran) twice a day for two weeks, according to their groups. Chlorhexidine may cause tooth-staining after more than 2 weeks, so we considered this duration for usage of both mouthwashes. Patients were advised to gargle 20 mL of mouthwash for one minute at each use and to refrain from eating or drinking for at least half an hour after using the mouthwash. All patients were also instructed to brush twice a day in the morning and at night, each time for 5 minutes using the Modified Bass method.

The patients and the oral examiner were not informed about the types of mouthwashes and both were poured in same amber glass bottles in laboratory and labeled as A and B (double-blinded study) (Figure 2).



Figure 2. Same amber bottles used for mouthwashes

The participants' MGI, PD, and BI were measured again after 4 weeks. Furthermore, the participants underwent a detailed examination to determine possible teeth discoloration after using mouthwashes. In case of discoloration, the teeth were polished. It should be mentioned that patients were excluded from the study if they did not attend the follow-up sessions, had poor oral hygiene, or had not used the mouthwash according to the given instructions.

Patients' satisfaction with the taste of mouthwashes were evaluated after 4 weeks using Visual Analog Scale (VAS).

Finally, the data were analyzed by statistical package for the social sciences (SPSS) 23 software using paired and independent T-tests, as well as Mann-Whitney test.

Results

As the participants were matched in terms of age and gender using the stratified randomization method, no comparison of demographic characteristics was performed between the two groups.

At the end of the study, PD values decreased significantly in both groups compared to the beginning of the study (p value: 0.001); though, no statistically significant difference was observed between the intervention and control groups in this regard (p value: 0.051). The BI values decreased significantly in both groups prior to and after the intervention (p value: 0.001). However, the BI mean changes were not significant between the intervention and control groups (p value: 0.47) (Table 1).

Table 1. Mean and standard deviation of PD, BI and MGI before and after using mouthwash (T-test)

| Mouthwash | PD | | BI | | MGI | |
|--------------------------|-----------|-----------|----------|---------|-----------|-----------|
| | Before | After | Before | After | Before | After |
| Chlorhexidine | 2.30±0.39 | 2.06±0.36 | 68±0.17 | 33±0.20 | 1.15±1.14 | 0.4±0.51 |
| P value | 0.001 | | 0.001 | | 0.001 | |
| Aqueous saffron extract | 2.37±0.46 | 1.95±0.42 | 61±0.021 | 28±0.17 | 0.97±1.05 | 0.35±0.54 |
| P value | 0.001 | | 0.001 | | 0.001 | |
| Comparison of two groups | 0.23±0.24 | | 2.6±3.6 | | 0.47±0.77 | |
| P value | 0.051 | | 0.47 | | 0.478 | |

PD: Probing Depth, BI: Bleeding Index, MGI: Modified Gingival Index

Comparison of the MGI values between the two groups showed that the changes were not significant (p value: 0.478) (Table 1).

After measuring the study variables, patients were asked to state their level of satisfaction

with the mouthwash taste on a scale ranging from 0 to 10, using VAS diagram. Patients were more satisfied with the taste of mouthwash containing aqueous saffron extract (Table 2).

Table 2. Median and interquarter range of the patients' satisfaction with regard to the taste of mouthwashes (Mann-Whitney test)

| Rate of satisfaction | Median | Interquarter range | P value |
|-------------------------|--------|--------------------|---------|
| Chlorhexidine | 5 | 3 | 0.021 |
| Aqueous saffron extract | 4 | 2 | |

Discussion

Various studies reported the effect of non-surgical periodontal treatments including oral health instructions plus SRP as the standard treatment in patients with generalized periodontitis [15]. In the present study, PD values changed significantly after the intervention compared to the beginning of the study in both groups (p value: 0.001), which indicates the major role of scaling in the intervention and control groups apart from the mouthwash effects. Based on the findings of this study, PD changed significantly in both groups (p value: 0.001). A study by Frouzanfar et al. showed that a toothpaste containing aqueous

extract of saffron did not significantly improve PD [16], which is not consistent with our results. However, their method of saffron application (toothpaste vs mouthwash) was different and as they studied on the marginal gingivitis patients instead of periodontitis, they could not evaluate PD changes.

In the current study, MGI had also a significant decrease after treatment in both groups, but the changes were not significantly different between chlorhexidine and saffron (p value: 0.478). A significant decrease in Gingival Index (GI) was also reported by Rahimi et al. [17] who used saffron effervescent tablets.

Our findings also showed a significant de-

crease in BI after the treatment in the study groups (p value: 0.001). Forouzanfar et al. [16] also concluded that a significant decrease in both GI and BI indices happened after using toothpaste containing saffron extract.

Generally, the participants' gingival indices improvement in the saffron group, can be occurred due to the anti-inflammatory, antibacterial, and antioxidant properties of this herbal mouthwash. As mentioned before, among saffron compounds, crocin and crocetin, as well as phenolic and flavonoid derivatives, have antioxidant properties. In this regard, crocin is the most important factor in the pharmacological properties of saffron such as the accumulation of oxygen free radicals and pro-inflammatory cytokines [18,19].

At the end of the study, the patients' level of satisfaction with the taste of mouthwashes was investigated by VAS scale. The participants reported higher levels of satisfaction with the taste of mouthwash containing aqueous saffron extract (p value: 0.021). According to the similar anti-inflammatory effect of chlorhexidine and saffron aqueous extract on improving the gingival indices, the better taste of saffron aqueous solution can increase the patients' tendency to continue using mouthwash. Apart from its unpleasant taste, chlorhexidine mouthwash may cause tooth discoloration and other unwanted side effects such as imbalance in oral microbial flora and burning sensation in mouth [20]. So it is recommended to use other substances without such side effects.

It should be noted that studies on the use of saffron derivatives in the management of oral diseases are very rare. On the other hand, among these few studies, most of them used different saffron products other than mouthwashes such as toothpaste or effervescent tablets. Therefore, it was not easy to compare this study with previous ones.

Limitations

The high price of materials required for preparing saffron mouthwash and attracting the cooperation of patients to follow the instructions for using mouthwash and returning for a follow-up session, were some of the limitations of this research.

Conclusion

Considering the limitations of this study, it seems that aqueous extract of saffron may have comparable anti-inflammatory effect with gold standard mouthwash, chlorhexidine, and can help improving periodontal indices.

Conflict of Interests

The authors declare that there is no conflict of interest.

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None.

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