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Evaluation of the Antimicrobial Effect of Aqueous Extract of Saffron on Streptococcus Mutans in Oral Cavity

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ORIGINAL STUDY

Evaluation of the Antimicrobial Effect of Aqueous Extract of Saffron on *Streptococcus mutants* in Oral Cavity

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Abstract

Purpose: Assessment of the antibacterial impact of liquid essence of Saffron on *Streptococcus mutans* count in saliva and comparing its effect with Chlorhexidine (CHX) mouthwash. **Patients and methods:** A total number of 36 children from both genders were included in this study (18 girls and 18 boys), their age ranged from (5−10) years old. These children were equally divided into two groups regarding the type of mouthwash and were asked to rinse with Saffron stigma extract and CHX mouthwashes (in group A and B, respectively) for 1 min three times daily for 7 days. Saliva samples were collected at 0 (baseline) (S1), after 4 days (S2), and after 7 days (S3). **Results:** In group 20%, the mean colony-forming unit of *Streptococcus mutans*, showed a gradual decrease post 4 and 7 days. In group 35%, the mean colony-forming unit of *Streptococcus mutans*, showed a gradual decrease post 4 and 7 days. In group 50%, the mean colony-forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 days. In CHX group, the mean colony-forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 days. **Conclusion:** Due to the herbal origin of this drug and its nativeness, and as a result, its less side effects, and its more cost-effective compared with CHX and other antibacterial compounds, it may be possible to use this plant as a mouthwash, which requires further studies are in the form of intervention studies.

Keywords: Mouthwash, Saffron, *Streptococcus mutans*

1. Introduction

Missing, discolored or damaged teeth might cause pain, eating, smiling and communication problems, if not treated it may progress to inflammation of tooth surrounding tissues, abscess formation and tooth loss [1].

Dental Caries is a multifactorial disease that leads to hard tissues breakdown by accumulation of plaque on tooth surface then acid production from activities of complex microbes through food fermentation. Bacteria, duration, vulnerable tooth surface, and fermentable carbohydrates are the four key contributing causes. In addition to these, there are other behavioral factors as poor oral hygiene, improper tooth brushing and sugar-containing diet [2].

There are many different types of bacteria in the mouth, but only a small number of them are thought to be responsible for dental caries, including *Streptococcus mutans* and Lactobacillus species. These organisms have characteristics that are exclusively seen in cariogenic bacteria, including the ability to create large amounts of acids during the fermentation of carbohydrates and resistance to the harmful effects of low pH. Lactic, propionic, and acetic acids are among the generated acids that dissolve the minerals in hard tissues [3].

For decades, antimicrobial therapy has been studied as a means of preventing dental caries, at the beginning was through antibiotics administration such as; penicillin. They have been proven to have strong anti-cariogenic effects, but they can seriously affect oral and intestinal flora, and

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prolonged usage can lead to antibiotic resistance. In recent years, using mouthwash has mostly been advised to ease pain, reduce swelling and halitosis, and supply fluoride [4].

The most commonly safe and efficacious antimicrobial agent used is Chlorhexidine (CHX), also has been considered to be the standard measure among antiplaque and anti-gingivitis agents. But, CHX usage has been associated with discoloration of tongue, staining of teeth, resin restorations, transient impairment of taste perception, dryness and soreness of the oral cavity, some bacterial resistance, and oral desquamation in children [5].

As a result of the adverse effects of synthetic chemical substances, humans have been using herbs for the prevention of many diseases, the active phytochemicals derived from plants and herbs that are used in traditional treatments are proving to be a good alternative to synthetic chemicals. Saffron extract may work as an excellent oral hygiene product [6].

Saffron (*Crocus sativus*), also called 'Red Gold' is a member of the Iridaceae category. It is the dried 'stigma' or threads of the flower of the plant. Saffron contains antioxidant, anti-inflammatory, anticoagulant, antibacterial, and analgesic properties, however, there are relatively few clinical studies on the utilization of saffron in the treatment of oral disorders [7].

Although it is obvious that alcoholic compounds themselves may have an antibacterial effect against various microorganisms such as both gram-positive (g+) and gram-negative (g−) bacteria, we chose the aqueous type to evaluate its antibacterial effect on *Streptococcus mutans* count and compare its effect to that of CHX mouthwash to avoid errors [8].

2. Patients and method

Research Ethics Committee approval was obtained from Faculty of Dental Medicine for girls, Al-Azhar University (REC-PE-22-04) for this study. Comprehensive treatment plan was illustrated to children's parents and informed consents for treatment were signed.

2.1. Case selection

This investigation was conducted on 36 children from both genders, that were included in this study (18 girls and 18 boys), their age ranged from 5 to 10 years old. All 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 or fluoride treatment (previous 2 weeks), no history of using antimicrobial mouth rinse (previous 12 h) [9].

2.2. Sample size calculation

Based on previous study [10], the calculation was estimated utilizing CDC Epi Info program version 7.2.0.1 (Atlanta, USA) assuming a power of 80% and alpha = 0.05 to detect significant difference in antimicrobial effect on *Streptococcus mutans* count between aqueous extract of Saffron and CHX mouthwash.

A total of 36 children were needed based on detection of Mean bacterial concentration (MBC) of *Streptococcus mutans* of 133.3 ± 25.82 mg/ml in experimental group aqueous extract of Saffron compared with 39.6 ± 16.61 mg/ml in control group alcoholic Extract.

2.3. Materials used in this study

(1) Prepared saffron stigma (Shana for Natural products) extract as a mouthwash for the experimental group (Fig. 1).
(2) CHX (0.12%) mouthwash (Hexitol, the Arab Drug Co.) for the control group (Fig. 2).
(3) Prepared saffron was put into hermetically sealed plastic bottles.
(4) Mitis salivarius agar: base for use with potassium tellurite supplement and bacitracin to selectively isolate *streptococcus mutans* and inhibit any other microorganism. (Hi−Media laboratory, Mumbai, India) (Fig. 3).

2.4. Preparation of saffron extract

Based on previous investigation [10], Saffron was purchased from the Shana for Natural products. It was ground into fine powder in an electrical mixer.

Fig. 1. Powdered saffron.

Fig. 2. Chlorhexidine mouthwash.

A 10 mg of finely powdered saffron were mixed with 100 ml boiling distilled water. The mix was incubated for 24 h then filtered through sterile filter paper then poured into a bottom-round balloon and stored in a freezer of 80 °C till further experimenting. For preparation of aqueous extract 10 mg of saffron powder, 100 ml of distilled water was used for the extract to be considered as 100% in concentration. Different concentrations were made respectively by diluting the concentrated extract. These concentrations were 20, 35, and 50%. Saffron mouth rinses were put into hermetically sealed plastic bottles. Figure 4.

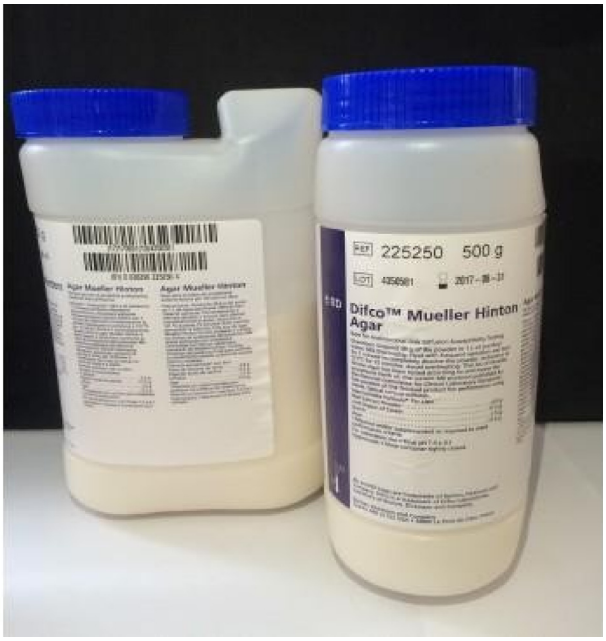

Fig. 3. Mitis salivarius agar base.

2.5. Samples grouping

Children were randomly divided into two groups of 18.

2.6. Group A (experimental group)

18 patients were divided into three subgroups 6 children per each. Each group was instructed to rinse with specific concentration of Saffron stigma extract mouthwash (20%, 35%, 50%).

2.7. Group B (control group)

18 patients were instructed to rinse with specific effective amount of chlorohexidine mouthwash. Every child in both groups was instructed to rinse for 1 min three times daily for 7 days under their parent's supervision. Children were adjusted not to eat or rinse for the next 30 min and frequent reminders were given to their parents to insure compliance. Tooth brushing and mouth rinsing techniques to be maintained.

2.8. Samples collection

To determine the salivary concentration of *streptococcus mutans* prior to the experiment, samples of unstimulated saliva were obtained before breakfast or at least 1 h after a meal and before washing (S1) [11]. On 5th day, the second salivary sample was collected post rinsing (S2). On 8th day, the third salivary sample was collected also postrinsing (S3). Each child provided three samples in total. The

Fig. 4. Saffron extraction.

samples were transported as soon as possible in ice box to the microbiological lab at Microbiology and Immunology Department, Faculty of Medicine for Girl's, Al-Azhar University.

2.9. Statistical analysis

Excel 2016 and SPSS 20 were used for the statistical analysis. Shapiro Wilk and Kolmogorov–Smirnov normality tests [12] were employed to assess if the data were normal, and the results showed that values were parametric data while % change was non-parametric. Quantitative information was displayed as median, range, confidence intervals, means, and standard deviation. The One-Way analysis of variance test and Bonferroni's Post Hoc test for multiple comparisons were used to compare parametric data. Repeated measures ANOVA was used to compare various observation periods within the same group. The chi square test was used to compare qualitative data that were presented as numbers and percentages. The percent change was calculated by the formula: [(Value after-value before)/value before] X 100. Statistical significance was established as P less than or equal to 0.05.

3. Results

3.1. Demographic data

The highest mean age was recorded in 50% group, followed by 20% group, then CHX group, with the least mean age in group 35%. The difference in age was not statistically significant ($P = 0.452$). Regarding gender distribution, all patients of group20% were females, in comparison to 66.7% females in group 35% and group 50%, while in CHX group 55.6% were females i. The difference in sex distribution was not statistically significant ($P = 0.261$).

3.2. Colony forming unit of Streptococcus mutans (CFU)

3.2.1. Comparison between groups

As shown in Fig. 5, There was no significant difference between groups in pre-treatment ($P = 0.797$). Post 4 days treatment a significantly higher value was recorded in group 20% (5.604 ± 0.77), followed by group 35%, then group 50%, with the least mean value recorded in CHX group (3.929 ± 0.87). ANOVA test and Bonferroni post hoc test revealed that group 20% was significantly higher than all other groups ($P = 0.000$), while group 35%, group 50% and CHX were not significantly different. Post 7 days treatment a significantly higher value was recorded in Group 20% (5.051 ± 0.73), followed by group 35%, then group 50%, with the least mean value recorded in CHX group (2.781 ± 1.27). ANOVA test and Bonferroni post hoc test revealed that group 20% was significantly higher than all other groups ($P = 0.000$), while group 35%, group 50% and CHX were not significantly different.

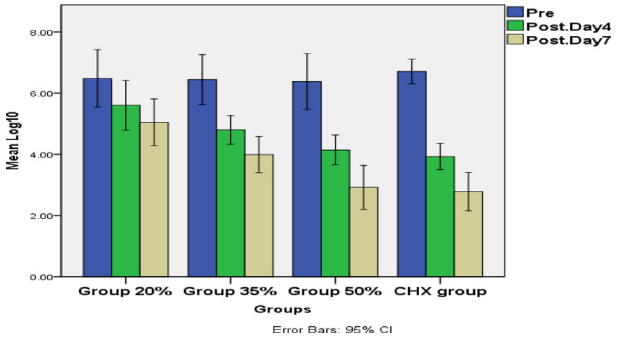

Fig. 5. Line chart illustrating mean Colony forming unit (CFU) of Streptococcus mutans (log10) in different groups.

3.3. Comparison within the same

In group 20%, the mean Colony forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 Days, the difference between observation times was statistically significant ($P = 0.002$). In group 35%, the mean colony-forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 Days, the difference between observation times was statistically significant ($P = 0.002$). In group 50%, the mean Colony-forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 Days, the difference between observation times was statistically significant ($P = 0.002$). In CHX group, the mean Colony-forming unit of *Streptococcus mutans*, showed a gradual decrease at post 4 and 7 Days, the difference between observation times was statistically significant ($P = 0.000$).

3.4. Percent change in colony forming unit of Streptococcus mutans

As shown in Fig. 6, from pre to Post Day 4: a significantly higher percent decrease was recorded, in CHX groups (−41.46 ± 10.34%), followed by group 50%, then group 35%, with the least mean percent decrease recorded in group 20% (3.929 ± 0.87%). ANOVA test and Bonferroni post hoc test revealed that the percent decrease in CHX group and group 50% was significantly greater than

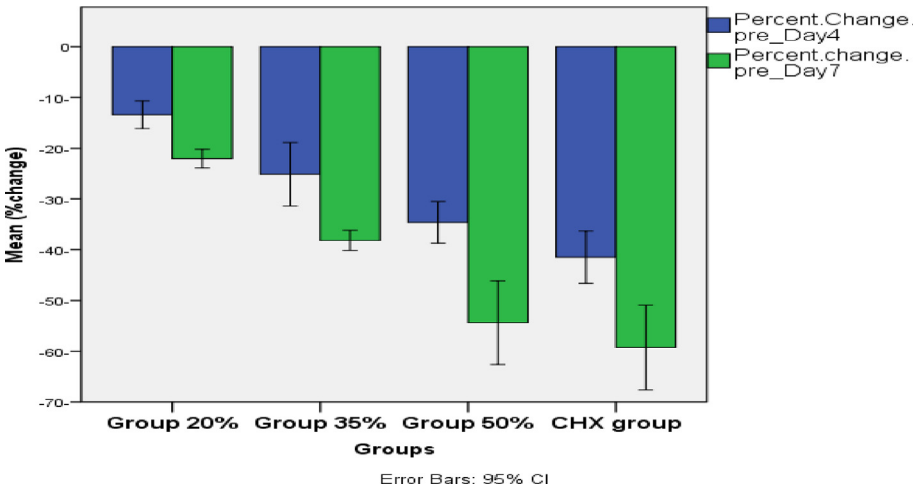

Fig. 6. Bar chart illustrating mean percent change in Colony forming unit (CFU) of Streptococcus mutans (%) in different groups.

other groups ($P = 0.000$). Moreover, group 35% and group 20% were not significantly different.

From pre to Post Day 7: a significantly higher percent decrease was recorded in CHX groups ($-59.23 \pm 16.84\%$), followed by group 50%, then group 35%, with the least mean percent decrease recorded in group 20% ($-22.05 \pm 1.75\%$). ANOVA test and Bonferroni post hoc test revealed that the percent decrease in CHX group and group 50% was significantly greater than other groups ($P = 0.000$). Moreover, group 35% and group 20% were not significantly different.

4. Discussion

Dental caries is one of the most frequent oral diseases in children [13]. While the frequency of caries has decreased in many developed nations over the past few years, it has grown in some developing nations. Micro-organisms present in dental plaque accumulated on tooth surface make contribution to initiation, and progression of dental caries, *Streptococcus mutans* (SM), Lactobacilli (LB) and *Candida albicans* are the predominant cariogenic micro-organisms associated with carious lesions [14].

The prevention strategy of dental caries mainly involves approaches to reduce the microbial load. Several antimicrobial agents have been introduced aiming of suppressing the cariogenic micro-organisms. The gold standard is CHX, which has broad antibacterial action and may inhibit cariogenic microorganisms. However, it can stain teeth and resin restorations, therefore using herbal agents can be a good option [15,16].

Crocus sativa L, also known as Saffron, is one of the most significant medicinal herbs. Saffron and its extracts are employed to treat numerous conditions in traditional Persian medicine, including cancers and immune system modulation. They also help with digestion, appetite, relaxation, and the treatment of liver diseases, spasms, toothaches, pharyngitis, rhinitis, insomnia, depression, cough, asthma, and bronchitis [17].

A trial of toothpaste containing aqueous saffron extract did not conclude a remarkable decrease in the streptococcus count in saliva. However, because they applied the saffron differently (using toothpaste as opposed to mouthwash), they were unable to assess changes in the streptococcal count [18].

Another used saffron effervescent tablets, likewise observed a significant drop in gingival index (GI). Generally speaking, the anti-inflammatory, antibacterial, and antioxidant capabilities of this herbal mouthwash can be linked to the participants' gingival indices improving in the saffron group. As previously mentioned, the antioxidant effects of saffron components include crocin and crocetin as well as phenolic and flavonoid derivatives. In terms of the pharmacological effects of saffron, such as the buildup of oxygen free radicals and pro-inflammatory cytokines, crocin is the most crucial component [19].

This result was supported by previous authors who attributed this inhibitory effect to the hydrophobic activity of the aqueous extract which may lead to destruction of lipid content of the bacterial cell membrane with disturbing the cellular structure that became more permeable with the result leakage of intracellular components, inhibition of energy production of the bacterial cell and finally cell death [20].

The findings of this investigation also revealed that saffron significantly prevents any increase in the count of *Streptococcus mutans* after 4 days. This result was in agreement with those who had previously stated that there was an inhibitory zone of

the aqueous saffron extract against *Streptococcus mutans*, which may be linked to the mono-hydroquinone's antibacterial action against gram + ve pathogens [21].

Another opinion was reported in previous studies, who suggested that the presence of high content of protein in saffron extract which were proved to be involved in the defense mechanism against the microorganisms especially a protein called crocin. Although this great inhibitory effect of saffron against *Streptococcus mutans* after 4 days, the result showed no inhibitory effect after 7 days contact which means that saffron extract had its effect at the beginning of contact, but this effect decreases and disappears by time [22].

A chemical examination of more than 150 distinct compounds found in saffron stigma revealed that carotenoids and monoterpene aldehydes are the strongest saffron constituents [23].

The point to be noted in this study is that the effective amount of aqueous extract against these microbes has been obtained *in vitro* and this concentration may not have such an effect in clinical conditions. The reason for this is the difference between the oral environment and the laboratory environment.

Another interesting point is that while applying antimicrobials topically in the mouth and mouthwashes, generally after gargling for a few seconds, they come into continual touch with the microbe in tubes and plates holding the growth media. By removing the drug from the oral environment, the mouth's natural elements counteract its effects. Saffron extracts were used in the current study in their purest form; they were not used in the creation of a mouthwash that also contains other ingredients [24].

4.1. Conclusions

(1) Saffron extract mouthwash was successful as an antimicrobial agent. It significantly reduced the salivary level of S.M which are the main etiologic factor in dental caries when compared with CHX 0.1% mouthwash.
(2) Saffron extract is natural and it was safe and effective alternative to chlorohexidine with no side effect in its long term use.

4.2. Recommendations

Due to the herbal origin of this drug and its nativeness, and as a result, its less side effects compared with CHX and other antibacterial compounds, it may be possible to use this plant as a mouthwash, which requires further studies are in the form of intervention studies.

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No funding was received for this study.

Conflicts of interest

There are no conflicts of interest.

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