The antibacterial effect of herbal alternative, green tea and Salvadora Persica (Siwak) extracts on *Entercoccus faecalis*

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ABSTRACT

Background: Disinfection and shaping of the canal with a combination of chemical agents and endodontic instruments play an important role in the success of endodontic therapy. Eliminating the microorganisms within the pulp space is a critical and important objective in treating a tooth with apical periodontitis. This study was conducted to evaluate the antibacterial properties of herbal alternatives (Green tea and siwak extracts) as possible irrigants during endodontic treatment compared with the conventional irrigation solutions.

Materials and methods: Salvadora Persica (siwak) and Green Tea solutions were prepared.An agar diffusion test was performed on Mueller-Hinton agar using the well diffusion method. The tested solutions (5.25% NaOCl, 2% Chlorhexidine gluconate, 5% Siwak extract and 5% Green tea extract)were used to fill the wells that were made in the agar media respectively.Plates were left to incubate for 24 hr. at 37°C. Zones of inhibition of the bacterial growth were calculated to measure the antibacterial effect of the tested irrigants.

Results: Sodium hypochlorite had the highest mean value (29.88) followed by Chlorhexidine which had a mean value of (26.13), Siwak with mean value of (11.25) and Green tea being the least with mean value of (8.88). ANOVA test showed a highly statistical difference with a P-value of (0.000).

Conclusions: NaOCl still the superior irrigant than other irrigants. Herbal alternatives (Siwak and Green tea) can be used as possible irrigants solution to disinfect the root canal system from *Enterococcus faecalis* during endodontic treatments.

Key words: Green tea, Siwak, *Enterococcus faecalis.* (J Bagh Coll Dentistry 2015; 27(2):1-5).

INTRODUCTION

Cleaning of the root canal system, as well as proper filling of the canal, are essential procedures for the success of root canal treatment. Even when treatment is adequate, failure may occur within the canal.Therefore, disinfection and shaping of the canal with a combination of chemical agents and endodontic instruments play an important role in the success of endodontic therapy**,** [(1,2)]**.**

Eliminating the microorganisms within the pulp space is a critical and important objective in treating a tooth with apical periodontitis. Studies have shown that recurrent root canal infections can occur even after endodontic treatment and are most commonly associated with Enterococcus faecalis [(3-5)]**.**

Sodium hypochlorite has been used as the irrigant of choice for endodontic procedures, but it has many deleterious effects if pushed beyond the apex. Chlorhexidine gluconate is another commonly used disinfectant. But its activity is pH dependent and it is toxic to human periodontal ligament cells [(2)].

Herbal products have been used in dental and medical practice for thousands of years and now become more popular due to their antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties [(6)]**.**

Tea is the second most commonly drank liquid on earth after water. It is known to possess anticariogenic and antibacterial properties.Green tea has antimicrobial activity which is due to inhibition of bacterial enzyme gyrase by binding to ATP B sub unit**.** Green tea exhibits antibacterial activity on *Enterococcus facealis*. It is also found to be a good chelating agent [(7,8)]**.**

Salvadora persica, commonly known as Siwak, was used as a chewing stick. Extracts of Salvadora persica has been shown to contain trimethylamine, salvadorine, chloride, fluoride, and traces of tannins, saponins, flavonoids and sterol. These components have been shown to have significant antibacterial [(9,10)]**.**

This study was concluded to evaluate the antibacterial properties of herbal alternatives (Green tea and siwak extracts) as possible irrigants during endodontic treatment compared with the conventional irrigation solutions.

MATERIALS AND METHODS

Salvadora Persica (siwak) preparation

Sticks of siwak (S. persica) were incubated at 37^0C for 24 hrs. Each stick was cut with a knife to small pieces then ground into fine powder**,** 250 gm of S. persica powder was put in a beaker to which one litter of sterile distilled water was added. The liquid was boiled at 100^0C for 15 minutes in a closed container. After bench cooling, the liquid was filtered using filter paper

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(No.1), and solution left to dry in an incubator at 37^{0}C for 24 hours to allow complete evaporation of water and obtain powder of siwak. The powder was collected and kept in tightly closed glass container and kept in refrigerator until use [(11)].

Green Tea preparation

Five gram of the selected dry green teas leaf, steeped for 1.5- 2 minutes in 100 ml of distilled water. The coolest brewing temperature was below 70˚C.The mixture was purified to obtain the 5% concentration solution of green tea [(12)].

Sample grouping

Group A: 5.25% NaOCl irrigant was used to fill the wells that were made in the agar media.

Group B: 2% Chlorhexidine gluconate irrigant solution was used to fill the wells that were made in the agar media.

Group C: 5% Siwak extract irrigant solution was used to fill the wells that were made in the agar media.

Group D: 5% Green tea extract irrigant solution was used to fill the wells that were made in the agar media.

Test Microorganism and Growth Conditions

Antibacterial activities of the irrigants were evaluated against the *Enterococcus faecalis*. An agar diffusion test was performed on Mueller-Hinton agar using the well diffusion method. Mueller-Hinton agar was freshly prepared after which, the surface was inoculated with 0.1 ml. of brain heart infusion (BHI) broth culture of *Enterococcus faecalis*. A well was punched in the agar in the center of each Petri dish and the tested materials were added to these wells. Plates were left to incubate for 24 hr. at 37°C. Zones of inhibition of the bacterial growth were calculated to measure the antibacterial effect of the tested irrigants.

RESULTS

The results obtained from this study were as follow (table 1).

Table 1: Bacterial inhibition zone in mm. using 5.25% Sodium hypochlorite, 2% Chlorhexidine gluconate, 5% Siwak extract and 5% Green tea extract.

Samples	NaOCl Bacterial inhibition mm	Chlorhexidine Bacterial inhibition mm	Siwak Bacterial inhibition mm	Green Tea Bacterial inhibition mm
1	30	25	12	9
2	32	23	11	9
3	35	27	10	8
4	26	25	13	10
5	33	25	13	8
6	24	26	11	9
7	29	28	10	10
8	30	30	10	8

Statistical analyses of the obtained results were performed using SPSS program version 19. The mean values and standard deviation of the tested irrigants were shown in Table 2 and Figure 1.

Table 2: Descriptive statistics

Groups	Mean	±S.D.	Min.	Max.
A	29.88	3.60	24	35
B	26.13	2.17	23	30
C	11.25	1.28	10	13
D	8.88	0.83	8	10

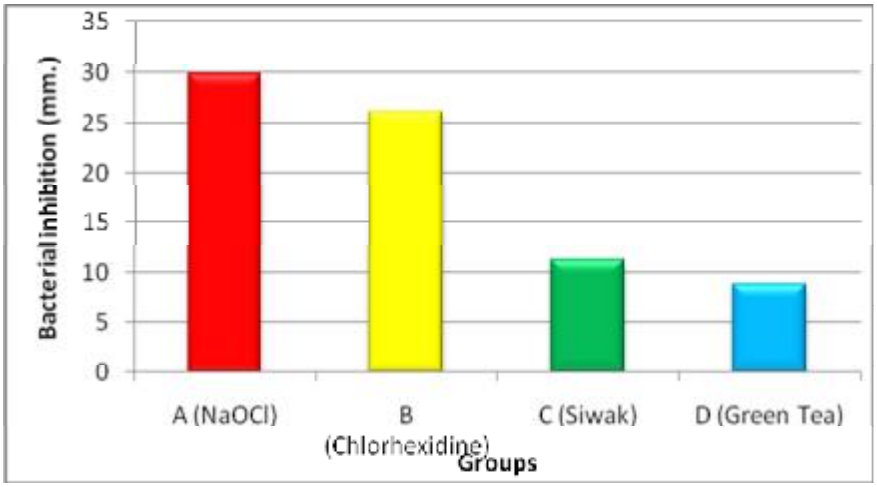

Figure 1: Bacterial inhibition in mm.

From table 2, Sodium hypochlorite had the highest mean value (29.88) followed by Chlorhexidine which had a mean value of (26.13), Siwak with mean value of (11.25) and Green tea being the least with mean value of (8.88). Groups' comparison between the four groups and within the groups were made by using ANOVA test which showed as highly statistical difference with a P-value of (0.000) as shown in table 3.

Table 3: Groups' comparison by ANOVA test

	Sum of Squares	d.f.	Mean Square	F-test	p-value
Between Groups	2652.84	3	884.28	176.69	0.000 (HS)
Within Groups	140.13	28	5.00		
Total	2792.97	31			

LSD test was performed to compare among the mean difference between each paired groups, all showed highly significant P-value except between group C and D which only showed significant difference with P-value of (0.043) as shown in table 4

Table 4: LSD test

Groups		Mean Difference	p-value
A	**B**	3.75	0.002 (HS)
	C	18.63	0.000 (HS)
	D	21.00	0.000 (HS)
B	**C**	14.88	0.000 (HS)
	D	17.25	0.000 (HS)
C	**D**	2.38	0.043 (S)

DISCUSSION

The irrigant solutions are very important during the root canal preparation, because they aid in the cleaning of root canal, lubricate the files, flush out debris, and have an antimicrobial effect and tissue dissolution, without damaging the periapical tissues. The microorganism tested in this study is a part of the endodontic microbial flora**.**

NaOCl solution is, to date, the most commonly employed root canal irrigant, but no general agreement exist regarding its optimal concentration, which ranges from 0.5% to 5.25% [13].

In the present study, 5.25% NaOCl was effective to considerably reduce *Enterococcus faecalis* creating the largest inhibition zones of bacterial growth in the agar media. This result was in agreement with several studies [14,15].

Chlorhexidine gluconate has been recommended as an alternative irrigating solution to NaOCl. The antimicrobial effect of Chlorhexidine gluconate is related to the cationic molecule binding to negatively charged bacterial

cell walls, thereby altering bacterial osmotic equilibrium [16].

Within the results of the present study regarding the inhibition of *Enterococcus faecalis* by Chlorhexidine gluconate which was found to be effective but less than the inhibition effect of NaOCl. These results were in agreements with the results obtained by many studies, as Murad *et al.* found that most effective irrigants in eliminating *E. faecalis* biofilms were 2.5% and 5.25% NaOCl and the Chlorhexidine liquid and MTAD were less effective than 2.5% and 5.25% NaOCl. [17], Spratt *et al.* and Dunavant *et al.*, reported that different concentrations of NaOCl (varying from 1% to 6%) were more effective than 0.2% Chlorhexidine and 2% Chlorhexidine and MTAD in the elimination of *E. faecalis* biofilms [23,24].

Salvadora Persica (Miswak-Siwak), Its chewing sticks contain trimethyl amine, salvadorime chloride and fluoride in large amounts [18], showed some antimicrobial activity which make it possible to be used as irrigant solution in endodontic treatment against the endodontic pathogens, It can be used as a substitute for sodium hypochlorite and chlorhexidine as root canal irrigant [19,20].

Green tea is a traditional drink of Japan and China and is prepared from the young shoots of tea plant Camellia Sinensis [8]. The leaves from the tea plant contain polyphenolic components with activity against a wide spectrum of microbes [21].

In the present study 5% Green tea extract was used to evaluate its effectiveness against *Enterococcus faecalis*, it obtained a mean of 8.88 which was the least of the tested groups. This finding indicates that it is less effective than NaOCl, CHX and Miswak. The results of the this study were similar to another studiesas Prabhakar *et al.* concluded that 5% sodium hypochlorite showed maximum antibacterial efficacy against *Enteroccus faecalis* biofilm while triphala, green tea polyphenol and MTAD showed statistically significant antibacterial activity [8]

Anurag *et al.* concluded that although conventional irrigants are more frequently used, have shortcomings in antibacterial efficacy. It was concluded that neem leaf and green tea extract has a significant antimicrobial effect against *E. faecalis*. As the American Medical Association shows that green tea has excellent medicinal values. It is also observed that green tea has antibacterial effect against *Enterococcus faecalis*. Green tea polyphenols antioxidant potential is directly related to the combination of aromatic rings and hydroxyl groups that make up their structure, and is a result of binding and neutralization of free radicals by the hydroxyl groups leading to destruction and dissolution of bacterial cell wall [22].

As conclusion; NaOCl still the superior irrigant than other irrigants. Herbal alternatives (Siwak and Green tea) can be used as possible irrigants solution to disinfect the root canal system from *Enterococcus faecalis* during endodontic treatments.

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