



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

International Journal of Current Research
Vol. 11, Issue, 03, pp.1862-1865, March, 2019

DOI: <https://doi.org/10.24941/ijcr.34738.03.2019>

INTERNATIONAL JOURNAL
OF CURRENT RESEARCH

RESEARCH ARTICLE

ELIMINATION OF ORAL PATHOGEN

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ARTICLE INFO

Article History:

Received 20th December, 2018
Received in revised form
15th January, 2019
Accepted 19th February, 2019
Published online 31st March, 2019

Key Words:

Brain heart infusion agar, Diode laser,
Elimination, Enterococcus faecalis,
Miswak.

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Citation: Alaa' Turki Monawer, 2019. "Elimination of oral pathogen", *International Journal of Current Research*, 11, (03), 1862-1865.

ABSTRACT

Background: Root canal treatment has been the main form of treatment for endodontic infections, and it comprises, chiefly, of physical instrumentation coupled with chemical irrigation. **Objective:** To compare the antibacterial effect of a diode laser and a Miswak (*Salvadora persica*) on *Enterococcus faecalis* in deep root canal dentin. **Materials and Methods:** A total of 500 extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city. The samples were collected using sterile red stopper plain plastic tubes. The samples were grouped in to two groups, 250 for the diode laser treatment and 250 for a miswak treatment, and inoculated into (Brain Heart Infusion agar and Blood agar) for the isolation and identification of *Enterococcus faecalis*. **Results:** Thirty (12%) isolates of *E. faecalis* were isolated from 250 single rooted canals, after treatment with diode laser 29 (96.7%) isolates were eliminated, whereas only 1 (3.3%) isolate was resistant to treatment with diode laser, while twenty eight (11.2%) isolates of *E. faecalis* were isolated from 250 single rooted canals, after injection with Miswak 27 (96.4%) isolates were eliminated, whereas only 1 (3.6%) isolate was resistant to Miswak activation. **Conclusion:** Both Diode laser and Miswak methods were very active in the elimination of *E. faecalis* isolated from infected single rooted canals.

INTRODUCTION

Bacterial infection plays an important role in the development of necrosis in the dental pulp and the formation of periapical lesions; therefore, the main goal of endodontic treatment is the elimination of bacterial infection and associated inflammation in the pulpal tissue and also the mechanical removal of damaged tissue found inside the root canal that acts as a growth medium for microbes (Siqueira, 2002). Oral hygiene is one of the most important daily routine practices and keeps the mouth and teeth clean and prevent many health problem. (Halawany, 2012). The 810nm diode laser (Fox, A.R.C., Germany) is specifically a soft tissue laser. This wavelength is ideally suited for soft tissue procedures since it is highly absorbed in hemoglobin and melanin, both of which are prevalent in soft tissues (Zoletti *et al.*, 2006). The use of laser for decontaminating periodontal pockets has been shown to be effective (Grassi *et al.*, 2004; Hayek *et al.*, 2005) and has encouraged research for determining or clarifying its effectiveness in the treatment of peri-implantitis (Giannini *et al.*, 2006; Souza *et al.*, 2006). People have been using different tools and chemicals to maintain their oral health, such as chewing sticks, tooth brushes, gum, mouth wash, and toothpaste (Riggs *et al.*, 2012); chewing-sticks were used by the Babylonians more than 7000 years ago (Dutta and Shaikh, 2012). Currently, in the Muslim world, the use of Miswak as a chewing stick is highly recommended as a Sunnah practiced

by the prophet Mohammad (peace be upon him) and his companions to achieve daily dental care, and the prophet emphasized the importance of using Miswak for oral hygiene. (Abhary and Al-Hazmi, 2016; Alaa', T. 2018). Ahmed, H., and Rajagopal, K., 2013, were succeeded to evaluate the chemical composition of *S. persica* such as flavonoids, salvadorine, cyanogenic glycosides, lignans, saponins, alkaloids, tannins, linoleic acid, stearic acid, salvadorea, vitamin C, silica and different salts are also known to possess significant antimicrobial activity (Ahmed and Rajagopal, 2013). *Enterococcus faecalis* has been the micro-organism most commonly found (Zoletti *et al.*, 2006), due to the specific characteristics such as: ability to colonize the dentin and tubules, which makes its removal by chemical and mechanical means difficult (Duggan and Sedgley, 2007), and may even resist the medication (Saleh *et al.*, 2008) and irrigating solutions (Oliveira *et al.*, 2007). Monawer, A.T. and Abdulkahar, I. M., 2016, showed that *E. faecalis* has proved to be a potentially important microorganism to the colonization or overgrowth in endodontic infections, being the dominant microorganism in post treatment apical periodontitis, and has often been isolated from the root canal in pure culture (Monawer and Abdulkahar, 2016).

The aim of this study is to compare the antibacterial effect of a Diode laser and a Miswak (*Salvadora persica*) on *E. faecalis* in deep root canal dentin.

MATERIAL AND METHODS

A total of 500 extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city. The samples were collected using sterile red stopper plain plastic tubes. The samples were grouped in to two groups, 250 for the diode laser treatment and 250 for Miswak treatment.

Diode laser 810 nm treatment

250 extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city, Kurdistan province, Iraq. The study was carried out in the Department of Microbiology, School of Medicine, Faculty of Medical sciences, University of Duhok. A reamers of suitable sizes (#15-30) were used to enlarge the canal and remove the pulp tissues. Instrumentation was followed by irrigation with normal saline. The initial microbiological samples were obtained by inserting sterile paper point into the prepared canal. The laser needle was introduced into the canal, which should be reaching the apex and one to two impulses were emitted and then the second sample was taken by inserting sterile paper point into the canal. Following the manufacturer recommendation, the current applied was 3watts for 30 seconds. The sterile paper points were cultured into brain heart infusion broth. After incubation at 37°C for 24 hours, each sample was sub-cultured on brain heart infusion agar, and blood agar, After incubation at 37°C for 24 hours, colony morphology were noted and diagnosed by catalase test and gram stain. The suspected colonies were sub-cultured onto Bile Esculin agar and incubated at 37°C for 24 hours (Monawer and Abdulkahar, 2016).

Miswak treatment

Dried plant samples were used in this study. Dry stems of *S. persica* from a local markets of Duhok city. Miswak samples were used. Extraction was made with three different solvent: a mixture of acetone, water, and ethanol; then the extracts of the plant species were prepared by adding 4 g of small particle fresh plant material powder with a commercially available coffee blender to 40 ml solvent and allowing the mixtures to stand overnight at room temperature,

after which the supernatants were filtered and dried under a controlled temperature (40°C) and then antibacterial activity was evaluated (Noumi *et al.*, 2011). 250 extracted teeth with single rooted canal were collected randomly from different private dental clinics in Duhok city, Kurdistan province, Iraq. Reamers of suitable sizes (#15-30) were used to enlarge the canal and remove the pulp tissues. Instrumentation was followed by irrigation with normal saline. The initial microbiological samples were obtained by inserting sterile paper point into the prepared canal (Alaa',T. 2018). The *S. persica* (Miswak) extract was injected into the canal, which should be reaching the apex, and then the second sample was taken by inserting sterile paper point into the canal. The sterile paper points were cultured into brain heart infusion broth. After incubation at 37°C for 24 hours, each sample was sub-cultured on brain heart infusion agar, and blood agar, after incubation at 37°C for 24 hours, colony morphology were noted and diagnosed by catalase test and gram stain. The suspected colonies were sub-cultured onto Bile Esculin agar and incubated at 37°C for 24 hours (Alaa',T. 2018).

RESULT

A total of 500 single rooted canals were collected, Gram stained, cultured and treated with Diode laser and Miswak in which only 58 samples showed positive isolates for *E. faecalis*, in two separate treatments as demonstrated in table (1). Effect of Diode Laser on *Enterococcus faecalis* growth. In the Diode laser treatment, only 30(12%) isolates of *E. faecalis* were isolated and 220(88.8%) showed negative cultures for *E. faecalis*. 29(96.7%) isolates of *E. faecalis* were eradicated by Diode laser whereas only 1(3.3%) isolate was resistant to Diode laser as demonstrated in table (2). Effect of Miswak on *Enterococcus faecalis* growth Among the 250 samples treated with Miswak, only 28(11.2%) isolates of *E. faecalis* were isolated and 222(88.8%) showed negative cultures for *E. faecalis*. 27(68.75%) isolates of *E. faecalis* were eradicated by Miswak whereas only 1(3.125%) isolates were resistant to Miswak as shown in table (3). Comparison of *Enterococcus faecalis* response to Diode Laser treatment and Miswak 5 treatment. The Chi-Square test showed no significant differences between the two treatments (Diode laser and Miswak) in suppressing *E. faecalis* growth in single rooted canals as in table (4).

Table 1. Number & percentage of single rooted canals before Diode laser treatment and miswak treatment

Treatment	No. of positive cases of <i>E. faecalis</i>	No. of negative cases of <i>E. faecalis</i>	Total
Diode laser	30 (12%)	220 (88%)	250
Miswak	28 (11.2%)	222 (88.8%)	250
Total	58 (11.6%)	442 (88.4%)	500 (100%)

Table 2. Number & percentage of *E. faecalis* response before & after treatment with Diode laser

No. of samples	Positive cases of <i>E. faecalis</i>	Before treatment with Diode laser	After treatment with Diode laser	
			Eradicated	Non eradicated
250	30 (12%)	30 (100%)	29 (96.7%)	1 (3.3%)

Table 3. Number & percentage of *E. faecalis* response before & after treatment with Miswak

No. of samples	Positive cases of <i>E. faecalis</i>	Before treatment with Miswak	After treatment with Miswak	
			Eradicated	Non eradicated
250	28 (11.2%)	28 (100%)	27 (96.4%)	1 (3.6%)

Table 4. Comparison of *E. faecalis* response to Diode laser & Miswak treatments in single rooted canals

Treatment	No. of resistant isolates	No. of eradicated isolates	Total
Diode laser	1 (3.3%)	29 (96.7%)	30 (100%)
Miswak	1 (3.6%)	27 (96.4%)	28 (100%)

DISCUSSION

E. faecalis was chosen as a test organism because it is a facultative organism that is non-fastidious, easy-to-grow, and rapidly colonizes tubules (Hancock *et al.*, 2001). It has been used extensively in endodontic research because it has been found to be present in 63% of teeth with post-treatment disease (Monawer and Abdulkahar, 2016). For the first time diode laser is used in Iraq and Middle East using wave length 810 nm for the elimination of *E. faecalis* in root canal (infected pulp) (Monawer and Abdulkahar, 2016). Miswak is a common name for *S. persica*, which is commonly used in Saudi Arabia and the Arab world. Miswak wicks clean between the teeth and do not break, regardless of the amount of pressure applied, as they are flexible and strong (Al-bayati and Sulaiman, 2008). *S. persica* has a wide geographic distribution ranging from India, Nepal, Malaysia, Pakistan, Iran, Iraq, Saudi Arabia, Egypt, and Africa (Alaa',T. 2018; Kshirsagar and Jareen, 2017). In the present study, only 11.6% cases of *E. faecalis* were isolated and 88.4% were negative for *E. faecalis*. This is similar to a study from Turkey (Ercan *et al.*, 2006). A study from Duhok city, Kurdistan, Iraq showed that the prevalence of *E. faecalis* in root canal (infected pulp) was 37/400 (8%) positive cases (Noumi *et al.*, 2011).

Diode laser treatment

In the present study, out of 250 sample only 30 (12%) cases of *E. faecalis* were isolated and 220(88%) were negative for *E. faecalis*, and after treatment with Diode laser 29 (96.7%) cases of *E. faecalis* were eradicated, while only 1(3.3%) was resistant. An Iraqi study showed that Diode laser 810 nm eliminated *E. faecalis* in 95.2% isolates, and these results similar to the results that obtained in this study (96.7%) (Noumi *et al.*, 2011). Other in vitro studies performed on roots of extracted human teeth described that an average of bacterial reduction 90.3% to 99.91% by laser (Gouw-Soares *et al.*, 2000).

Miswak treatment

In the present study, out of 250 sample only 28 (11.2%) cases of *E. faecalis* were isolated and 222 (88.8%) were negative for *E. faecalis*, and after treatment with Miswak extract 27 (96.4%) cases of *E. faecalis* were eradicated, while only 1(3.6%) was resistant. The data from a Saudi Arabia's study done by Researchers from the College of Medicine at Saudi Arabia's Najran University tested Penicillin-resistant *E. faecalis* against extracts of Miswak twigs suggested that Miswak successfully killed the bacteria, also the same study reported that the Miswak twigs not only fight bacteria that infect the mouth, they also fight the most lethal strains of antibiotic resistant bacteria (Al-Ayed *et al.*, 2016). A study by Alaa' illustrated that the Miswak showed the highest antibacterial effect against *E. faecalis* (91% of the isolates were eradicated) (Alaa',T. 2018). In this study the results obtained were so similar to the Alaa' study in which Miswak showed the higher antibacterial effect against *E. faecalis*.

Conclusion

The diode laser showed high effectiveness in eliminating *E. faecalis* from single rooted canals. We recommend Diode laser to be used as a replacement to classical method used in the dental clinics for treating single rooted canals for the complete

eradication of *E. faecalis*. The study showed new natural antibacterial agent that can replace the synthetic ones to be used in oral hygiene; thus, the effective source of *S. persica* could be employed in all medicinal preparations to combat a wide variety of bacteria. So Miswak strongly recommended to be used as daily teeth cleaner.

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