Original Article

Effect of Arginine, Protamine, and Aqueous Extracts of Green Tea and Aloe Vera Against *Enterococcus faecalis*

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Abstract

Background and Aim: Because of complexity in root canals, irrigating solutions are needed in addition to mechanical instrumentation for thorough cleansing of the root canal system. This in-vitro study was designed to determine the inhibitory effect of arginine, protamine, and aqueous extracts of green tea and aloe vera against *Enterococcus faecalis* (*E. faecalis*), which causes endodontic failure.

Materials and Methods: In this experimental study, aqueous extracts of green tea and aloe vera and protamine at a concentration of 400 mg/ml and arginine at a concentration of 160 mg/ml were used. *E. faecalis* was cultured on Mueller-Hinton broth, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the test materials against these microorganisms were determined using serial dilutions and according to the microdilution test. The positive control was 2.5% sodium hypochlorite (NaOCl).

Results: Aqueous extract of aloe vera has high antibacterial activity against *E. faecalis* (MIC=12.5 mg/ml and MBC=100 mg/ml), and protamine had the least effect (MIC=400 mg/ml and MBC>400 mg/ml) compared to other test materials. NaOCl, with MIC and MBC of 0.25 mg/ml against *E. faecalis,* showed higher antibacterial activity compared to other test materials.

Conclusion: Among the tested materials, except for NaOCl as a positive control, aqueous extract of aloe vera showed better antibacterial properties against *E. faecalis*.

Key Words: Arginine, Protamines, Green Tea, Aloe vera, Enterococcus faecalis, Minimum Inhibitory Concentrations, Microbial Sensitivity Tests

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Introduction

Premature loss of primary teeth can cause disruptions in the occlusion, aesthetics, and eruption of the permanent teeth [1,2]. Sometimes, to avoid early loss of primary teeth, root canal treatment is indicated [1,2]. Complete removal of microorganisms is the key to increasing the success rate of root canal treatment [2]. Mechanical instrumentation alone cannot completely eliminate microorganisms; therefore, irrigation plays a major role in the complete removal of microorganisms from the root canal system [2-5].

Enterococcus faecalis (*E. faecalis*), a gram-positive coccus, is able to foray the dentinal tubules and grow without the help of other microorganisms in the root canals; also, it is resistant to most antibiotics. Therefore, it plays a serious role in the failure of root canal treatment [6,7].

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Sodium hypochlorite (NaOCl) is commonly used as a root canal irrigation solution because it has antimicrobial and tissue-dissolving properties [7]. However, it has some unpleasant characteristics such as cytotoxicity, corrosion of instruments, irritation of periapical tissues, inability to remove the smear layer, and undesirable smell and taste; these disadvantages limit its use in some conditions [7-10].

L-arginine is a necessary amino acid present in the saliva. Arginine is converted into nitric oxide (NO) in the saliva and then into nitrite and nitrate by nitric oxide synthase (NOS) [11]. NO plays an important role in antimicrobial activity [12]. Many bacteria catabolize arginine to ammonia, carbon dioxide (CO_2) and Adenosine triphosphate (ATP) via the arginase and arginine deiminase (ADI) pathways [12]. The ADI pathway is important for bacterial virulence and bacterial survival in an acidic environment [12,13]; in addition, the arginase of bacteria can prevent the production of NO and facilitate evasion of the host defense system by competing with inducible NO synthase (iNOS) of host cells for a common substance i.e. arginine [11,12]. Some studies have found that arginine decreases bacterial counts by improving the phagocytic activity of macrophages [13].

Protamine is a polycationic protein with antimicrobial activity against various microorganisms [14]. However, the mechanism of action of protamine has not been completely revealed. It is believed that its antimicrobial activity is related to its capacity to disrupt the cell wall of bacteria by the electrostatic interaction between the positive charge of this protein and the negative charge of the bacterial cell wall, which results in leakage of K^+, ATP, and intracellular enzymes [14-16].

Currently, because of the increasing resistance of bacteria to antibiotics and the complications of synthetic drugs, researchers are looking for herbal medicines with strong antibacterial properties and few side effects [10]. Green tea is a popular drink with antimicrobial activity against a large number of oral microbes [17]. The antimicrobial activity of green tea is mediated through disruption of the bacterial cytoplasmic membrane, prevention of fatty acid synthesis, prevention of enzymatic activity, etc. [17,18].

Another medicinal herb is aloe vera with different pharmacological properties such as antibacterial, antifungal, anti-inflammatory, hypoglycemic, and immune strengthening properties [7]. Aloe vera gel is released by the parenchymatous cells in the leaf pulp. It has also been applied in dentistry for management of oral lesions, including oral lichen planus and candidiasis, and for management of xerostomia. It is also placed over extraction sockets, has been used as an endodontic medicament, and has been incorporated into various dentifrices [7,19-21].

The aim of the present study was to determine the effect of arginine, protamine, and aqueous extracts of green tea and aloe vera against *E. faecalis,* which causes endodontic failure. Based on the results, an appropriate compound can be proposed for root canal irrigation

Materials and Methods

This experimental study has been approved by the Ethics Committee of Babol University of Medical Sciences (MUBABOL.REC.1396.5). Sampling was performed non-randomly.

Preparation of aqueous extracts of green tea and aloe vera:

Green tea leaves were purchased in the spring from Lahijan city, Gilan Province, Iran. After rinsing and drying, the leaves of green tea were washed and dried at room temperature; then, its powder was prepared. Aqueous extract of green tea was prepared by mixing 100 g of dry powder of the plant's leaves with 500 ml of sterile distilled water in an Erlenmeyer flask placed on a shaker for 72 hours. After this period, the solution was passed through a Whatman filter paper and then placed in an oven at 46°C for 72 hours to allow the solvent to evaporate. Then, the extract was collected and kept at 4°C.

Aloe vera leaves were purchased in April from the Sari Agricultural Sciences and Natural Resources University, Mazandaran Province, Iran. The fully expanded leaves of aloe vera were selected and washed with fresh water; then, their epidermis was removed, and the gel was extracted. The gel was air-dried, and its powder was prepared. The same steps described above for the preparation of aqueous extracts of green tea were performed for the preparation of aqueous extracts of aloe vera.

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Microorganism preparation:
The standard suspensions of *E. faecalis* (ATCC 29212) were obtained from the Pasteur Institute of Iran. They were inoculated into Mueller-Hinton broth and then in blood agar and MacConkey agar plates. The plates were incubated at 37°C for 24 hours. A 0.5 McFarland solution holding 1.5×10^8 colony-forming units per milliliter (CFU/ml) was prepared from the microorganisms.
Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of arginine, protamine, and aqueous extracts of green tea and aloe vera:
The MIC of arginine, protamine, and aqueous extracts of green tea and aloe vera for *E. faecalis* and *Escherichia coli* (*E. coli*) was determined using the microdilution test. A total of 100 µl of brain heart infusion (BHI) broth was added to all the wells of 96-well microplates, except for the first column of the test materials. Arginine and protamine were sterilized and filtered through a 0.2-µm membrane filter. Then, 200 µl of the test materials (arginine=160 mg/ml, protamine=400 mg/ml, aqueous extracts of green tea=400 mg/ml, aqueous extracts of aloe vera=400 mg/ml) were poured into the first row of the microplate. Then, for twofold serial dilutions, 100 µl of the first well contents were transferred to the second well, and after mixing thoroughly, 100 µl of the solution was transferred to the third well. The same procedure continued up to the twelfth well, and 100 µl of the mixture was discarded from the last row. The 0.5 McFarland turbidity standard was prepared (1×10^8 CFU/ml) for each bacterial species; then, 1 µl of the bacterial suspension was separately added to all the wells, except for the last row, serving as the negative control. The positive control was 2.5% NaOCl, and the negative control was a normal saline solution. The microplates were placed in an incubator at 37°C for 24 hours. After the incubation periods, the wells of the microplate were checked for turbidity, and the lowest sample concentration showing no turbidity was determined as the MIC. In order to determine the MBC, 100 µl of the samples were taken from each well that did not show any growth and were spread on sterile agar plates. After the incubation period, the lowest concentration of test materials showing no colonies of bacteria on agar plates was determined as the MBC. The experiments were performed in triplicate to ensure the accuracy of the MIC and MBC results.

Results
As shown in Table 1, the lowest MIC and MBC values for *E. faecalis* were achieved with 2.5% NaOCl, which were equal to 0.62%. This result indicates that aqueous extracts of aloe vera were found to be most effective in inhibiting the growth of *E. faecalis* (MIC=12.5 mg/ml and MBC=100 mg/ml), and protamine had an antimicrobial effect against *E. faecalis* at high concentrations (MIC=400 mg/ml and MBC>400 mg/ml).

Discussion
Complete elimination of pathogenic microorganisms from the root canal system can increase the success rate of endodontic treatment, but mechanical instrumentation alone cannot entirely eliminate the microorganisms. Therefore, the use of irrigant solutions is essential to ensure complete cleanliness of the canal system [19]. Various irrigants are used to clean the root canal system. In general, ideal irrigant solutions should have strong antimicrobial efficacy and few adverse effects. To achieve this aim, recently, researchers have examined various materials for better results. NaOCl is the gold standard of irrigant solutions [22]. NaOCl has adverse effects such as toxicity and foul smell and taste; it decreases dentinal flexural strength and elastic modulus [1,23]. In the present study, NaOCl showed better antibacterial property against *E. faecalis* compared to arginine, protamine, and aqueous extracts of green tea and aloe vera.
Arginine (MIC=80 mg/ml) and aqueous extracts of green tea and aloe vera (MIC=25 mg/ml and 12.5 mg/ml, respectively) exhibited distinct antibacterial activity against *E. faecalis*, but protamine showed poor antibacterial activity against this microorganism. As far as we know, few studies have been done on the antibacterial effect of arginine against *E. faecalis*.
Darouiche et al [24] in 2008 concluded that protamine alone does not have antibacterial activity against *E. faecalis*, which is consistent with our results. However, Kim et al [14] in 2015 showed that protamine had antimicrobial activity

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Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested solutions against *Enterococcus faecalis* (*E. faecalis*)

Tested solutions \ Microdilution	MIC	MBC
Arginine	80 mg/ml	160 mg/ml
Protamine	400 mg/ml	>400 mg/ml
Aqueous extracts of green tea	25 mg/ml	100 mg/ml
Aqueous extracts of aloe vera	12.5 mg/ml	100 mg/ml
Positive control (2.5% NaOCl)	0.25 mg/ml	0.25 mg/ml

NaOCl=sodium hypochlorite

against a wide range of oral pathogenic microbial species, including *E. faecalis*. These findings are not consistent with our results. This difference in the results might be due to differences in the bacterial species, differences in the methods for assessing the sensitivity of microorganisms and bacterial culture; therefore, further studies are recommended on different species of *E. faecalis*.

Jose et al [19] in 2016 showed that aqueous extracts of aloe vera had antibacterial activity against *E. faecalis* but this activity was lower than that of 2.5% NaOCl. Prabhakar et al [25] in 2010 compared the antimicrobial effects of green tea, Triphala, MTAD, and 5% NaOCl against *E. faecalis* and showed that 5% NaOCl was the most effective antibacterial agent. Triphala, green tea, and MTAD also showed significant antibacterial effects [25]. These results are consistent with our conclusion.

NaOCl, as the positive control, showed higher antibacterial activity against microorganisms compared to the tested materials because NaOCl is a well-refined industrial product; therefore, its antibacterial activity will be higher compared to crude materials and extracts. If the test materials used in the present study were refined, higher and more efficient activities might have been observed.

Conclusion

Among the tested materials, except for NaOCl as a positive control, aqueous extract of aloe vera showed better antibacterial properties against *E. faecalis*. However, protamine did not exhibit good antibacterial activity against *E. faecalis*.

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