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ANTIBACTERIAL EFFECTIVENESS OF GINGER EXTRACT AND CHLORHEXIDINE AS ROOT CANAL IRRIGANTS IN PRIMARY TEETH CONTAMINATED WITH ENTEROCOCCUS FAECALIS (AN IN VITRO STUDY)

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ABSTRACT

Objective: Irrigation of the root canals with antibacterial solutions is a mandatory step to reduce or eliminate micro-organisms or their byproducts from the root canal system. Considering potential side effects and safety concerns of synthetic drugs, herbal alternatives may prove to be advantageous for endodontic usage.

Aim: To compare the antimicrobial effect of 20% ginger ethanolic extract versus 2% chlorhexidine solution as root canal irrigants in primary teeth roots contaminated with Enterococcus faecalis (E.faecalis).

Materials and methods: A total of 75 palatal roots of extracted primary molars were randomly divided into five groups (n=15/group): Group I (negative control group): roots were neither contaminated nor irrigated, Group II (positive control): roots were contaminated with E.faecalis and irrigated with sterile saline, Group III: irrigation with 20 % ginger ethanolic extract solution, Group IV: irrigation with2% chlorhexidine (CHX) solution, GroupV: irrigation with 95% Ethanol solution. After mechanical preparation of the root canals with manual K-files, the roots were infected with E. faecalis for 21 days. Thereafter, the root canals were irrigated for five minutes with the assigned irrigant . Samples were taken from each canal using sterile paper points which were transferred to Brain Heart Infusion (BHI) culture medium and incubated at 37ºC for 48 hours. Bacterial counts were expressed as colony forming units/ml (CFU/ml).

Results: CHX showed the highest antibacterial activity, followed by 95% ethanol and 20% ginger ethanolic extract solution. While statistically there was no significant difference between ginger ethanolic extract solution and 95% ethanol.

Conclusion: 20% ginger ethanolic extract solution did not show a significant antibacterial effect on E.faecalis when compared to 95% ethanol or 2% CHX.

KEYWORDS: Ginger, Enterococcus faecalis, Chlorhexidine.

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INTRODUCTION

The aim of root canal treatment is to maintain teeth with compromised pulp in the oral cavity in order to avoid more complex treatments. This reaches more importance in children, as the early loss of deciduous teeth can compromise the development of the stomatognathic system and the installation of the permanent dentition as well as can lead to emotional, psychological and behavioral problems. The primary objective of pediatric pulp therapy is to maintain the deciduous teeth in their form and function and to facilitate the proper eruption of permanent successors.[1]

Deciduous teeth with primary infected root canals are characterized by the predominance of obligatory anaerobic and aerobic microorganisms. However, in secondary infected root canals, there is a predominance of facultative anaerobic microorganisms. Enterococcus faecalis (E.faecalis) is one of the most predominant bacteria found in root canal failures and persistent infections of endodontic treatment.[2]

The success of endodontic treatment in primary teeth strongly relies on achieving an adequate level of disinfection within their root canals. Mechanical instrumentation cannot effectively eliminate the micro-flora from the root canals of the teeth due to their anatomical complexity, thus the dependence on irrigating solutions for endodontic success becomes more crucial.[3]

Among the phases of endodontic treatment, the choice of instrumentation and irrigating solution that permit bacterial neutralization and toxin inactivation without negative interference with the healing process is fundamental to the success of treatment.[4] In the recent years, the use of herbal products as root canal disinfectants and irrigants has been widely investigated in endodontics because of their efficiency, safety and accessibility.[5]

Ginger is one of the classic examples of a herb used for not only culinary preparations but also for unique therapeutic significance owing to its anti-inflammatory, analgesic, antimicrobial, antifungal, antioxidant, anticancer and hypoglycemic activities.[6]Most studies in the literature investigated the antibacterial effect of ginger extract as root canal irrigant solution in permanent teeth. For this reason; the aim of the current study was to evaluate the antibacterial effect of ginger extract as an irrigant in extracted primary teeth.

MATERIALS AND METHODS

Ethical approval:

The Ethical approval for this research was obtained from the Ethics Committee, Faculty of Dentistry, Ain Shams University. The approval number was: FDASU-RecE011940.

Sample size estimation:

A power analysis was designed to have adequate power to apply a 2-sided statistical test of the research hypothesis (null hypothesis) that there is no difference in the antimicrobial effect of the ginger solution versus 2% CHX solution when used as root canal irrigants in primary teeth roots contaminated with E.faecalis. According to the results of Radwan et al., (2015) [7] assuming an alpha (α) level of 0.05 (5%), a Beta (β) level of 0.05 (5%) i.e. Power=95%, and an effect size (f) of (0.62); the predicted sample size (n) was a total of (75) samples i.e. (15) for each group. Sample size calculation was performed using G*Power version 3.1.9.2.[8]

Teeth selection:

Seventy-five extracted maxillary primary molars teeth were collected from the Outpatient's Clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Ain Shams University according to the following criteria:

- At least two-thirds of the root length is present.

- Absence of root caries.
- No previous endodontic treatment.
- Teeth with patent canals.

A rotary double-sided diamond disc mounted on a low-speed handpiece with water coolant was used to de-coronate the crown of multi-rooted teeth below the cement-enamel junction. Then, the roots were separated.[9]

Study groups:

Seventy-five primary teeth palatal roots were randomly distributed among three experimental groups, one positive control group and one negative control group as follows,(n=15/group):

Group I (negative control): Roots were not contaminated nor irrigated.

Group II (positive control): Roots were contaminated with E.faecalis and irrigated with sterile saline.

Group III: Roots were contaminated with E.faecalis and irrigated with 20% Ginger ethanolic extract solution.

Group IV: Roots were contaminated with E.faecalis and irrigated with 2% Chlorhexidine solution.

Group V: Roots were contaminated with E.faecalis and irrigated with 95%ethanol.

Preparation of 20% Ginger ethanolic extract solution:

The fresh Ginger rhizomes were washed, peeled, sliced and air dried at room temperature for 14 days.[10] After drying, ginger slices were ground into a fine powder in a mechanical grinder. Ginger extract was prepared according to Park et al.,(2008) [11] where five hundred grams of the dried ginger rhizomes powder were weighed using a citizen digital electronic scale. Then the dried powder was macerated into 3 liters of (95%) ethanolin a sterile beaker. The beaker was placed in the Ultrasonic Cleaner machine at 50°C for 30 minutes to enhance extraction of the active compounds from ginger. The beaker was covered and incubated at room temperature for 48 hours. The extract was then filtered through Whatman No.1 filter paper. To concentrate the extract, a rotary evaporator was used to separate ethanol from the extract at 50 °C. The resultant ginger ethanolic extract was left to dry by keeping it in a desiccator overnight to convert the extract to powder. The final concentrated ethanolic extract powder was diluted to bring a final concentration of 20% ginger ethanolic extract solution by adding 10 ml of 95% ethanol to every 2 grams of ginger ethanolic extract powder.

Mechanical preparation of the root canals:

K-file #15 was placed in the root canal of each tooth until its tip appeared at the apical foramen to ensure patency of the canal. The length of the file was measured and the working length was then calculated and recorded by subtracting 1mm from the anatomical root length. Root canals were then mechanically prepared using hand files (k-type), employing the step back preparation technique reaching a master apical file size #40 to standardize the diameter of the root canal. Irrigation of the root canals with 2 ml of normal saline was performed after using each file to prevent blockage of the canal. The apical foramina of all roots were then sealed with glue to prevent bacterial micro-leakage.[12,13]

Sterilization of the samples

All samples were packed in sterilization pouches and autoclaved at 121 °C and 15 PSI pressure for 15 minutes in Andromeda vacuum xp autoclave.[13] After sterilization 15 samples were kept to serve as a negative control group, (group I).

Biofilm Development:

The microbiological culturing was carried out in the Department of Microbiology and Immunology

Faculty of Medicine, Ain Shams University. The prepared roots of groups II, III, IV and V were immersed in a 24-hour pure culture suspension of E.faecalis grown in Brain Heart Infusion broth (BHI) and adjusted to No. 1 MacFarland turbidity standard. All teeth were incubated at 37°C in sealed vials. This procedure was repeated every 72 hours using a 24-hour pure culture to avoid saturation and confirm the viability of E.faecalis. The negative control roots were immersed in sterile BHI broth which was replenished with sterile saline every 72 hours. All the teeth were maintained in a humid environment at 37°C for 21 days.[14,15]

Irrigation of the specimens

After contamination, roots were randomly allocated into three experimental groups (III, IV, V) and one positive control group (II). Each root was irrigated with 5 ml of the assigned group irrigant using a 5 ml (24G) sterile plastic syringe for 5 minutes.[13]

Bacterial sampling and bacterial count:

Two consecutive sterile absorbent paper points size 20 were inserted inside each root for one minute. Then the paper points were transferred to a test tube containing 1.0 ml of saline and vortexed for twenty seconds. After 10-fold of serial dilution, aliquots of 0.1 ml were spread plated onto BHI agar plates and incubated at 37°C for 48 hours. Visible colonies of E.faecalis were counted in every plate and the number of colonies/plate was multiplied by the corresponding dilution factor and by 10 to determine the total colony forming units (CFU) per ml.[16]

Statistical analysis:

Numerical data were presented as mean and standard deviation values and were explored for normality by checking the data distribution, calculating the mean and median values and using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed non-parametric distribution and extreme positive skewness. Log transformation of the data was carried out to correct for the skewness. Leven's test showed a violation of variance homogeneity assumption so robust one-way ANOVA followed by Games Howell post hoc test was used for the analysis. The significance level was set at $p \leq 0.05$ within all tests. Statistical analysis was performed with IBM® SPSS® Statistics Version 26 for Windows.

RESULTS

Table (1) shows that the highest antibacterial activity was observed in 2% CHX solution with a total number of colonies (1.46±1.08), followed by 95% ethanol (2.81±0.20), and then the 20% ginger extract solution (3.19±0.12). There was no statistically significant difference between ginger ethanolic extract solution and ethanol. The number of detected colonies of E.faecalis in the positive control group in which the roots were irrigated with sterile saline was (5.30±0.07), while no colonies were detected in the negative control group which was kept sterile (without bacterial contamination nor irrigation).

Percent of reduction from positive control

Mean and standard deviation (SD) values of percent of bacterial count difference from positive control for different groups were presented in table (2) .

There was a significant difference between different groups ($p<0.001$). The highest difference was found in Chlorhexidine (99.94±0.05), followed by 95% Ethanol (99.64±0.21), while the lowest value was found with ginger solution (99.21±0.22). Pairwise comparisons showed values of different groups to be significantly different from each other ($p<0.001$).

TABLE (1): Mean ± standard deviation (SD) of bacterial count (CFU/ml) for different groups

Log bacterial count (mean±SD)					p-value
Chlorhexidine solution	**Ginger solution**	**95% Ethanol**	**Positive control**	**Negative control**	
1.46±1.08^{C}	3.19±0.12^{B}	2.81±0.20^{B}	5.30±0.07^{A}	0.00±0.00^{D}	**<0.001***

Means with different superscript letters are statistically significantly different*; significant (p≤ 0.05)

TABLE (2): Mean ± standard deviation (SD) of bacterial count difference from positive control (%)

Bacterial count reduction(mean±SD)			p-value
Chlorhexidine solution	**Ginger solution**	**95% Ethanol**	
99.94±0.05^{A}	99.21±0.22^{C}	99.64±0.21^{B}	**<0.001***

Means with different superscript letters are statistically significantly different*; significant (p ≤ 0.05)

DISCUSSION

The current study investigated the antibacterial effect of 2% CHX, 95% Ethanol and 20% ginger ethanolic extract solution as intracanal irrigants in roots of primary teeth against E.faecalis.

The isolation of E.faecalis from the root bacterial ecology is considered a sophisticated process that might lead to inaccurate data and results.[17] Therefore, the current study was designed as an in-vitro investigation.

Single-rooted teeth are usually used in studies assessing different irrigants to exclude the possibility for re-infection from lateral and accessory canals that are hard to reach by mechanical instrumentation and irrigation. However, primary single-rooted teeth with minimal root resorption are usually hard to collect. Thus, palatal roots of maxillary primary molars were used in the current study.[7]

In the current study, the positive control group was used to ensure contamination of the samples and to act as a reference against which comparisons with other active groups were made. On the other hand, the negative control group was used to emphasize the sterilization of the samples.[18]

The study used ginger plant extract as a root canal irrigant seeking to find a natural alternative to chemical irrigation. Moreover, scholars have been studying ginger plant widely for its pharmacological properties which showed antibacterial, antifungal, antivirus, anti-inflammatory, and anti-tumor properties. The ginger plant also has been reported to have beneficial effects in the medical field. [19,20]

Maceration was used as a method of choice for ginger extraction to avoid the effect of heat on the content of the ginger rhizomes. Maceration extraction is counted as a crude extraction; solvents diffuse into solid plant material and solubilize compounds with similar polarity. This process also has high absorption effectiveness of the active substances contained in the ginger rhizomes.[21]

Furthermore, the study chose ethanol as the solvent to extract the ginger rhizomes because it proved that it is more effective than aqueous extracts. [22] It was also found ethanolic extract has a higher zone of inhibition of Gram-positive (S.aureus)

and Gram-negative bacteria (E.coli). [23] Ethanolic extract yields more flavonoid content than aqueous extracts that are known to have antibacterial activity. This difference in the composition of ethanoic and water extracts can be attributed to the difference in solubility of various components of ginger rhizomes in water and organic solvents.[22-24]

While, the final concentration of the investigated ginger solution was 20% because this was the common percent to test its antibacterial activity in the literature.[25]

A 95% of ethanol was used as an irrigant in group V to ensure that the antibacterial activity that was shown in the ginger group was attributed to the compounds in the Ginger extract itself and not to the ethanol only.[26]

The five minutes duration of exposure to irrigating solutions was selected as it was found to be the optimum time for the irrigation material to do its antibacterial effect based on studies by **Gomes et al., (2001)**[27] **and Torabinejad et al.,(2003).**[28]

According to the results of the present study, CHX, Ginger, as well as 95% Ethanol irrigants showed an antibacterial effect in comparison to the positive control that was irrigated with saline. Moreover, the statistical comparison proved that CHX had the highest antibacterial activity followed by the ethanol group then the Ginger group while the positive control group had the lowest antibacterial effect, table(2).

CHX is well known for its potent antibacterial activity which is related to its ability to alter the osmotic equilibrium of bacterial cell walls leading to leakage of intracellular components.[27,29]As an intracanal irrigant, studies confirmed CHX efficiency against E.feacalis and other intracanal bacteria both in-vitro and in-vivo with the 2% concentration showing the highest effect.[4,27,30,31]

Results also showed that 95% ethanol had a better antibacterial effect compared to 20% ethanolic ginger extract implying that adding Ginger to 95% ethanol in a 20% concentration did not have a synergistic antibacterial effect.

The present study results agreed with **Valera et al., (2013)**[32] who evaluated the antibacterial efficacy of 20% glycolic ginger extract as an irrigating solution compared to 2% CHX against C.albican and E.faecalis. It was found that CHX eliminated the microorganism from the roots canal completely. While 20% glycolic Ginger extract caused minimal reduction in the number of microorganisms. This supports the results of the current study and shows that ginger itself has a minmal antibacterial effect and that the antibacterial effect that was found in the current study can be attributed to ethanol.

Another study evaluated the antimicrobial activity of 100mg/ml ethanolic extract of ginger on S.aureus and E.faecalis. The researchers found that ethanolic extract of ginger was more effective against S. aureus when compared with E. feacalis. However, in this study ginger ethanolic extract was not used as an irrigating solution but the antibacterial effect was tested directly on bacterial cultures using the agar disc diffusion method. [33].

Giriraju & Yunus (2013)[34] evaluated the antimicrobial activity of 10% ginger ethanolic extract compared to 0.2% CHX against S. mutans, C. albicans, and E.faecalis using agar disc diffusion method. It was found that 10% ethanolic ginger extract had an antimicrobial effect against all the three pathogens used in the study which was also lower than 0.2% CHX.It is worth to mention that, the stock solution of ethanolic ginger extract powder was dissolved in 100 ml of Dimethyl sulphoxid which is also an antibacterial agent to obtain 10% ginger extract.[35]

The present study results also agreed with **Azhar et al., (2018)**[36] who evaluated the antimicrobial activity of 40% ginger ethanolic extract compared to

2% CHX against E.faecalis cultures. The researchers also reported that 2% CHX had a significantly higher antibacterial effect against E.feacalis compared to 40% ethanolic ginger extract which showed a minimal effect.

Although that the previous studies agreed with our result that ginger extract whether ethanolic or glycolic had an antibacterial effect against E.feacalis, none of these studies investigated the antibacterial effect of the solvent by itself.

However, the present results were not in line with the study carried out by **Alrazhi et al., (2014)** [25] who evaluated the antibacterial efficacy of 20% ethanolic ginger extract as an irrigating solution compared to 2% CHX. It was found that the ginger extract had a higher antibacterial activity compared to 2% CHX solution against E.faecalis. However, in this study the used CHX was prepared by the researchers. While in our study a commercially available CHX was used.

In the current study, different concentrations of ginger extract were not tested. Additionally, E. feacalis was used in an isolated form, which does not resemble clinical infection where multiple species are found in the infected root canals in a biofilm form which can increase bacterial resistance to antimicrobials. These factors constitute the limitations of this study.

CONCLUSIONS:

Within the limitations of the present study, we concluded that:

1- Twenty percent ethanolic extract of Ginger solution did not show a significant antibacterial effect when compared to 95% ethanol or 2% CHX.

2- Two percent chlorhexidine irrigation was superior to 95% ethanol and 20% Ginger ethanolic extract irrigation when used against E.faecalis bacteria.

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