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Comparative Evaluation Of Antimicrobial Activity Of Different Root Canal Irrigants With Or Without The Addition Of Etidronic Acid

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Abstract

Aim : The purpose of this study is to evaluate the antimicrobial activity of different root canal irrigants with or without the addition of etidronic acid**.**

Materials and methods: In this experimental study, the samples were divided into four groups as follows: Group I: Sodium hypochlorite, Group II: Green tea extract, Group III: Chitosan, Group IV: Cetylpyridium chloride. All the four groups were then divided into two subgroups each, based on the incorporation of etidronic acid. Antimicrobial activity of the irrigants within the groups as well as with the other groups were evaluated against E.faecalis and compared using Agar Diffusion Test

Results: The study concluded that 9% etidronic acid has potential antibacterial property against E.fecalis. Its antibacterial activity was enhanced when used with chitosan.

Keywords

Antibacterial activity, Agar Well Diffusion Method, Etidronic acid, Chitosan, Green tea extract, Cetylpyridium chloride

Introduction

Most common cause of pulpal and periradicular infection is micro-organism and its by-products which enter the pulp through a break in dentin by caries, traumatic exposure, percolation around a restoration, periodontal pocket, abscess and anachoresis. Hence the ultimate goal of root canal treatment is complete eradication of the microorganism from the root canal system to eliminate infection and prevent reinfection. Mechanical preparation of the root canal may reduce the bacterial load but will not eradicate them completely due to the complexity of the root canal system. The fact that 35 to 50% of the surface area of the canal system remain untouched by endodontic instruments led to appropriate use of root canal irrigants in order to improve disinfection of the root canal system.

In ideal form, irrigants should have antimicrobial action, tissue-dissolution activity, demineralization, lubrication, and ability to remove smear layer and debris[5].

Sodium hypochlorite (0.5% - 6%), Chlorhexidine (2%), Hydrogen peroxide(3%), ethylenediaminetetraacetic acid (17%), Citric acid (10-50%),MTAD, Tetraclean, maleic acid, green tea extract, triphala, morindacitrifolia and recently Electronically activated water, Bis-dequalinium acetate (BDA), Photo-activated disinfection, ozone, lasers are also being used in combinations of two or more solutions for safe and effective irrigation.

The most commonly used endodontic irrigants are NaOCl and EDTA which are effective in the removal of both organic and inorganic debris. But studies have demonstrated that this combination causes an excessively aggressive effect on the canal wall that could lead to erosion and degradation of the peritubular and intertubular dentin and subsequently alteration of its mechanical properties.

Etidronic acid has been introduced as a weak chelating solution, which has the ability to reduce smear layer formation and reduce the accumulation of hard tissue debris. When used in combination with NaOCl, it does not affect the dissolution activity and antimicrobial properties of NaOCl[3].

Though NaOCl is the gold standard antimicrobial agent with tissue dissolving properties this chemical irrigant has several disadvantages like allergic reaction, tissue toxicity, irritation to periapical tissue, inability to remove smear layer and undesirable smell and taste[5].Thus alternative

medicaments have come up which include herbal irrigants. They are becoming popular due to their antibacterial properties, bio-compatibility, anti-inflammatory and anti-oxidant properties.

Green tea extract (GT) is one such product obtained from the leaves of the plant Camellia sinensis which are rich in flavonoids in the form of catechins that are reported to exert antibacterial activity[1].

Chitosan (CT) a natural polysaccharide which is biocompatible, biodegradable, shows bioadhesion and lacks toxicity. It is a cationic biopolymer that possesses lasting antibacterial properties and has low production costs. It is obtained by the deacetylation of citin which is found in crabs and shrimp cells[6].

Cetylpyridium chloride(CPC) are widely used active component of oral antiseptics and has broad antimicrobial spectrum with a strong bacterial effect. These are ionic detergents quaternary ammonium compounds with surface active properties[7,14].

The antibacterial and bio-compatibility properties of these products have encouraged to use them as safe and effective irrigants. The combination of these irrigants with etidronic acid a weak chelating agent has not been studied, used or reported in the literature till now. Thus the aim of the study is to compare and evaluate the antimicrobial activity of the different root canal irrigants with or without the addition of etidronic acid.

Materials and Methodology

Preparation of Green tea

Green tea extract was supplied by Tetrahedron Beverages Pvt, Kakkaular, Tamil Nadu, India. It was supplied in the form of powder which is readily soluble in water. 3% Green tea solution was prepared by weighing the powder in an electronic weighing machine and mixed with 100 ml of sterile boiling distilled water for 5mins and filtered using a syringe filter. The dilution was kept for sterility check overnight at 37° C.

Preparation of Chitosan

For preparation of the 0.2% of chitosan (Mahtani Chitosan Pvt. Ltd Veraval, India) solution, 0.2g of chitosan was diluted in 100 mL of 1% acetic acid and the mixture was stirred for 2 hr using a magnetic stirrer until a crystallinehomogeneous solutions with 3.2 pH was obtained.

Preparation of Cetylpyridium chloride

Cetylpyridium chloride was supplied by Kem Colours International(Ankleshwar, India). It was supplied in powder form which is readily soluble in water. Concentration of 0.2% was prepared by weighing the required quantity and dissolving it in

100 ml of sterile distilled water.

Etidronic acid (9%)is readily availableas Chloroquick, zodenta and 5% NaOCl was readily available.

Test for Antibacterial activity was evaluated using Agar Well Diffusion test

Micro-organism ATCC 29212-Enterococus fecalis were obtained from American type culture collection and maintained in the microbiology department in BHI agar. The colonies were inoculated into 5ml BHI broth and incubated at 37°C for 4 hrs. The culture was adjusted to MacFarland 0.5 opacity std to obtain a culture equivalent to 1.5x 10^8 CFU/ml. 25 µl of the adjusted culture was added to 25ml of freshly prepared BHI agar, cooled to 50°C, mixed well & poured into a sterile petri plate (90 mm) and the medium was allowed to set and wells of 6mm were made using sterile templates.

Meanwhile the materials were prepared and assigned into four groups:Group I: NaOCl, group II: green tea extract, group III: chitson, group IV: cetylpyridium chloride. Each group was further divided into two subgroups based on the inclusion of etidronic acid, ie:Group IA: NaOCl, group IIA: green tea extract, group IIIA: chitson, group IVA: cetylpyridium chloride and group IB: NaOCl + Etidronic acid, group IIB:GT + etidronic acid, group IIIB: CT + etidronic acid, group IVB: CPC + etidronic acid.

Due to the presence of 8 groups, two petri plates were prepared, Plate 1 contained: chlorhexidine (positive control), saline (negative control), etidronic acid alone, GT alone and with etidronic acid, CT alone and with etidronic acid. Plate 2: along with positive and negative control, it contained NaOCl and CPC alone and with etidronic acid.

For group A: 50µl of the test reagents were inoculated into wells under aseptic conditions and for group B: 25µl of each reagent were inoculated so that the combination equals to 50µl. The plates were then incubated at 37°C for 24hrs and the zone of inhibition of growth around the wells were measured using a measuring scale.

Results

The results showed that the 9%etidronic acid alone had the highest zone of inhibition compared to all other agents in Group A followed by Group IA:5% NaOCl, group IIA: 0.2% chitosan, group IIIA: 0.2% CPC and the least with Group IVA:3% GT.

In group B, 25µl of the reagents were used which is half of group A, so the zone of inhibition were smaller compared to group A. In this group the highest zone of inhibition was seen in Group IIIB: CT+ etidronic acid, indicating chitosan has a synergistic effect. Whereas no other irrigants of Group B showed any increase or decrease in antibacterial activity in combination.

When comparing both the subgroups: Group IIIB showed an increased zone of inhibition than group IIIA where as no other group showed any increase or decrease in zone of inhibition.

Discussion

An ideal irrigant should possess tissue dissolving property, ability to remove smear layer and antibacterial activity. At present no single irrigant combines all these ideal characteristics[8].Thus newer irrigants have come up having lesser disadvantages and better smear layer removal and antibacterial activity. In the present study the irrigants used were green tea extract, chitosan, cetylpyridium chloride and etidronic acid.

Enterococcus fecalisis the most common microorganism found in failed root canal treatment. It's a grampositive cocci and facultative anaerobe that is non fastidious, easy to grow, ability to form monospecies biofilms and rapidly form colonies in the tubules. It exhibits strong adhesion to collagen and display resistance to chemomechanical preparation. It also survives in quiescent phase with low metabolic activity for long period of time[9].Thus it is important to eliminate such microorganism with the use of potent irrigating solutions.

In the present study antimicrobial activity against E.fecalis was conducted with the use of Agar Well Diffusion Test. It is a commonly used method for antimicrobial activity assessment and is widely accepted as an adequate way of comparing antibacterial affect. In this test the antibacterial activity is assessed by the formation of the zone of inhibition. The diffusion capacity of the solutions in the agar depends on several factors like contact between the solutions and the agar, molecular weight, concentration of the test material and agar gel viscosity, incubation temperature of plates and reading points of inhibition haloes[5].

In this study the zone of inhibition for etidronic acid is the highest (24mm) indicating that etidronic acid has good antimicrobial activity followed by5% NaOCl (IA-15mm),0.2% chitosan (IIIA-12mm),cetylpyridium chloride (IVA- 9mm) and least antibacterial activity was seen in 3% green tea extract (7mm).

Etidronic acid is a soft chelating agent and an alternative to EDTA to removes smear layer. It acts by calcium chelation and it does not affect the properties of other materials added along with it. Ana Morago et al[3], Maria Teresaetal[10] demonstrated that use of a mixture of 2.5% NaOCl/ 9% etidronic acid effectively removed the smear layer and showed good antibacterial properties without altering the action of each irrigant. In this study etidronic acid in combination with other irrigants like group IB: NaOCl (19mm), group IVB: cetylpyridium chloride (17mm) and group IIB: green tea extract(14mm) did not increase or decrease their antimicrobial activity. Combination with chitosan (Group IIIB- 20mm) gave a synergistic effect indicating that on addition with

chitosan its antimicrobial activity was enhanced. Other studies by Vidya N et al[8], Srinidhi Surya et al[11] showed that antibacterial activity of 18% etidronic against E faecalis is significantly superior to that of 0.2% chitosan.

The results indicated that, the next best zone of inhibition was noted in group IA: NaOCl (15mm), its antibacterial effect depends on the formation of hypochlorous acid (HOCl), which reacts with organic debris, it exerts its effect by oxidation of sulphydryl groups within bacterial enzyme systems disrupting the microbial metabolism.

The third best zone of inhibition was noted in group IIIA: Chitosan, produced synthetically by the de-acetylation of chitin which is the structural element in the exoskeleton of crustaceans. It has broad spectrum of activity against microbes which acts by the ionic surface interaction resulting in cell wall leakage, inhibition of mRNA, protein synthesis, formation of external barrier, chelating metals and provoking the suppression of essential nutrients to microbial growth[8,9]. The molecular weight of chitosan potentially affects its antibacterial activity i.e. lower the molecular weight, higher will be the effectiveness on reducing microorganism growth and multiplication. A study by Vidya N et al[8]showed that preparation of 0.2% chitosan was by 90.84% de-acetylation. The study also concluded that different molecular weight and or degree of de-acetylation show better antibacterial properties.

The next biggest zone of inhibition after chitosan was group VIA: 0.3% cetylpyridium chloride. Its combination with etidronic acid did not show any positive or negative results. A study by E. Gjorgievska et al[7] showed that increase in concentration of cetylpyridium chloride may have better antimicrobial properties.

The least antimicrobial activity was shown by group IIA and group IIB, green tea extract, its action is due to the presence of catechins but it has mild antimicrobial activity which may not kill strong microorganism like E.fecalis. Moghbel et done by done by Sardari et al[1] investigated and found that antibacterial effect of green tea extract can increase by increasing the concentration of green tea. Its has good antioxidant property.

In the present study the concentrations used for different groups are taken in relation to their Minimum Inhibitory Concentration (MIC). It is the lowest concentration of an antimicrobial agents that will inhibit the visible growth of a microorganism after overnight incubation[5]. The MIC for green tea extract is 3.5%[5], cetylpyridium chloride 0.2%[12,13], and chitosan 4.5%[9,15].

This study showed that etidronic acid alone has good antibacterial properties compared to all other groups, along with smear layer removal property and when used in combination with chitosan demonstrated a synergistic effect. Studies showed that etidronic acid and chitosan both had better smear layer removal properties than EDTA. A study Surya et al[11] concluded that use of etidronic acid helps to optimize bonding quality of resin based sealer during obturation. Thus etidronic acid may be

use as a single irrigating solution. Future studies are needed to determine cytotoxicity and other properties of etidronic acid.

Conclusion

The study concluded that 9% etidronic acid has potential antibacterial property against E.fecalis. Its antibacterial activity was enhanced when used with chitosan.

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Groups	Zone of inhibition - Total diameter
Positive control – Chlorhexidine 0.2%	**13 mm**
Negative control – Saline	
9% Etidronic acid	**24 mm**
Group IA - 5% Sodium hypochlorite	**15 mm**
Group IIA - 3% Green tea extract	**7 mm**
Group IIIA - 0.2% Chitosan	**12 mm**
Group IVA - 0.3% Cetylpyridium Chloride	**9 mm**

Table 1 : Antibacterial activity of Groups without the addition of Etidronic acid

Table 2: Antibacterial activity of Groups with the addition of Etidronic acid

Groups	Zone of inhibition -Total diameter
Group IB – NaOCl + etidronic acid	19 mm
Group IIB – GT + ertidronic acid	14 mm
Group IIIB – CT + etidronic acid	20 mm
Group IVB – CPC + etidronic acid	17 mm

Figure 1: Micro-organism ATCC 29212-Enterococus fecalis

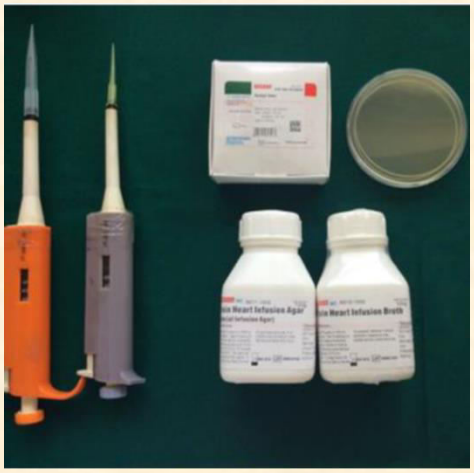

Figure 2: Materials used – BHI broth

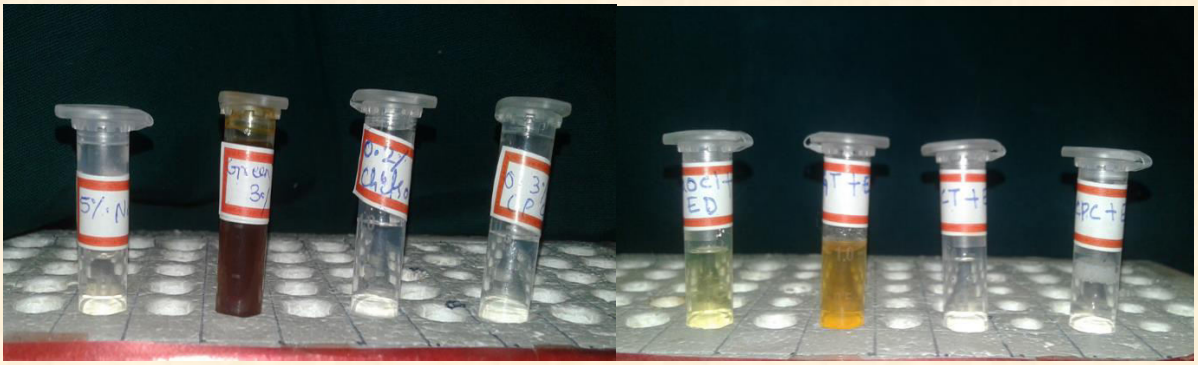

Figure 3: Grouping of the samples with and without addition of etidronic acid

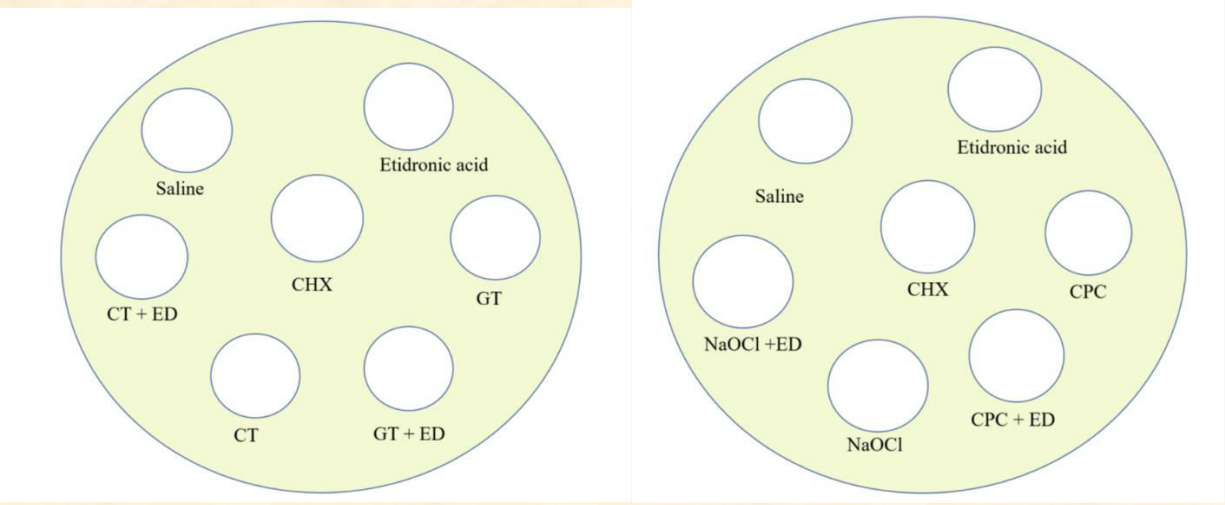

Figure 4: Preparation of pertiplates for the groups

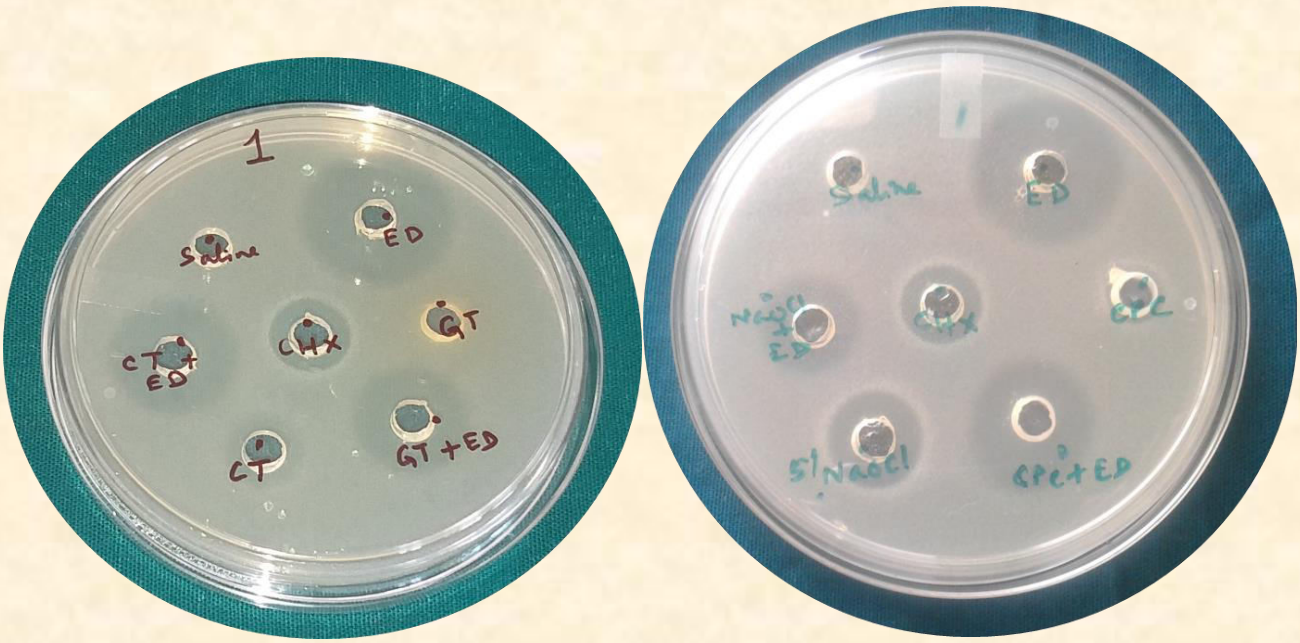

Figure 5: Agar Well Diffusion results – Zone of Inhibition