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Endodontics

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF VARIOUS NOVEL IRRIGATING SOLUTIONS AGAINST ENTEROCOCCUS FAECALIS- AN IN VITRO STUDY

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ABSTRACT **Background:** It is essential to remove all pulpal tissues, dentinal debris, and viable microorganisms from the root canal system during endodontic treatment. The main reason for endodontic failure is the presence of some species of bacteria inside the root canal system such as Enterococcus faecalis. Proper canal instrumentation and adequate irrigation with irrigating solutions can decrease the number of bacteria. **Aim:** The aim of present study was to compare & evaluate the antimicrobial efficacy of 3.8% SDF , 5.25% Sodium hypochlorite,1.6% Calotropis gigantea extract & 0.9% Normal saline against the opportunistic pathogen Enterococcus faecalis. **Material Methods:** The study was done by Agar diffusion test. **Results:** The mean value of the inhibition zone for Group I (3.8% Silver Diamine Fluoride),Group II (5.25% Sodium Hypochlorite), Group III (1.6%Calotropis gigantia Leaf extract) and Group IV (saline) were 18.360,17.566,16.593 and 0 respectively. **Conclusion:** All the endodontic irrigants used in this study showed antibacterial efficacy against E.faecalis.

KEYWORDS : Enterococcus faecalis, Endodontic Irrigants, 3.8% SDF, Agar diffusion test

INTRODUCTION

Successful endodontic treatment necessitates combination of diversity of factors, such as a precise diagnosis, thorough cleaning and disinfection achieved with the help of various intracanal medicaments & irrigation solutions followed by 3-dimensional obturation of the pulp space to obtain a hermatic seal followed by adequate final restoration[1].

Enterococcus faecalis has increased attention in the endodontics, as it can regularly been seen in root canals in cases of failed root canal treatments. There have been limited attempts to eradicate *Enterococcus faecalis* biofilm with commonly used root canal irrigants in the past[2].

Irrigation is presently the best method for lubrication, destruction of microbes, the removal of tissue remnants & dentin during instrumentation. Conventionally used irrigating agents are normal saline, sodium hypochlorite, hydrogen peroxide etc.

Normal saline is isotonic to the body fluids and most common irrigation solution & it has no side effects[3]. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant; it is an antiseptic and inexpensive lubricant with a concentrate ranging from 0.5% to 5.25%[4]. *Calotropis gigantea*, better known as milkweed, this plant is also scientifically reported for its anti-candida activity, cytotoxic activity, antipyretic activity and wound healing activity[5].

Hence the current study has been focused to investigate the antibacterial efficacy of *Calotropis gigantea* leaf extracts as an irrigating solution against clinical isolates of *Enterococcus faecalis* bacteria. Recently Silver diamine fluoride (SDF) of concentration 3.8% is introduced as an irrigating solution. It is very effective agent, especially in pediatric dentistry. Keeping this in mind the goal of this in vitro study is to examine the antibacterial effectiveness of SDF as an irrigating agent in comparison with Sodium hypochlorite and *Calotropis gigantea* against *Enterococcus faecalis*.

MATERIAL METHODS

The present agar well diffusion in vitro study was carried out in in the department of Pediatrics and Preventive Dentistry, D.J College of Dental Sciences and Research, Modinagar, Ghaziabad in collaboration with Dextrose Technologies Pvt. Ltd. Kengeri Satellite Town, Banglore.

Division of samples

A total of 60 wells in 15 Mueller Hinton agar plates were prepared for the study, which were divided in four groups having 15 wells in each group.

- Group I: 3.8% Silver Diamine Fluoride irrigant
- Group II: 5.25% Sodium hypochlorite irrigant
- Group III: 1.6% Calotropis gigantea herbal irrigant
- Group IV: 0.9% Normal saline.

Transport of microbe

The standard bacterial strain of *E.faecalis* (ATCC 29212) was obtained from Himedia Labs, Mumbai, Maharashtra, India. A frozen (-80°C) package was received in thermocol container. The package was freeze dried at 2 - 8°C for 24 hours in Real time diagnostic centre refrigerator.

Preparation of experimental material

a) Preparation of 3.8% SDF solution

1:10 dilution of 38% SDF solution was prepared

b) Preparation of ethanolic extract of Calotropis gigantean

Fresh Calotropis gigantea leaves were collected from the wasteland of Modinagar, Ghaziabad. Then leaves were washed in running water and shade dried at room temperature for 3-4 days. Then dried leaves were powdered, then three hundred grams of powdered calotropis was separately macerated with 100% ethanol. Then extracts were filtered with Whatman filter paper to obtain a clear filtrate. The filtrates were reduced at 60°C to obtain a solid residue.

One gram of the extract was taken and added to 4ml of sterile saline. This will give 25% dilution of the extract. Then 1ml of this solution was added to another 1ml of sterile saline to create 12.5% dilution of the extract. In this solution, another 1ml of sterile saline was added to make it 6.25% to which 1ml of sterile saline solution was added to make it 3.125% dilution. Then 1ml of 3.125% solution was added into another 1ml of sterile solution this gave us 1.6% dilution.

c)Preparation of 5.25% Sodium hypochlorite & 0.9% normal saline

Commercial preparation used.

Agar-diffusion test

E. faecalis strains were inoculated in brain–heart infusion and incubated at 37°C for 24 h. The density of selected organisms was adjusted equal to that of the 0.5 Mc Farland standards (1.5x 10^8 CFU/ml) by adding them to nutrient broth for Enterococcus faecalis. A 24 hour old culture was used for the preparation of bacterial suspension. For the agar diffusion test, Petri plates with 20 ml Mueller-Hinton Agar were inoculated with 0.1 ml of the microbial suspensions, were spread onto the agar plates. Fifteen cultivated agar plates were taken and four holes (4 mm in depth, 6 mm in diameter) were punched. Around 80µl of the sample (3.8% SDF, 5.25% NaOCl, 1.6% Calotropis gigantean extract & o.9% normal saline) were filled separately in these four holes of all the fifteen plates. These plates were reincubated aerobically at 37°C for 24 h. Then, the diameter of microbial inhibition zones (Fig 1) around each well was measured and recorded in millimeters.

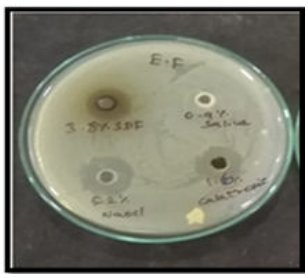

Fig 1. Microbial Inhibition zone

STATISTICALANALYSIS

The data for the present study was entered in the Microsoft Excel 2007 and analyzed using the SPSS statistical software 23.0 Version. The descriptive statistics included mean, standard deviation . The level of the significance for the present study was fixed at 5%.

The intergroup comparison for the difference of mean scores between independent groups was done using the one way Anova and Post Hoc analysis. The intergroup comparison was done using the Indpendent t test.

RESULTS

1. Mean Value Of Inhibition Zones Of various groups

The mean value of the inhibition zone for Group I, Group II, Group III and Group IV were 18.360,17.566,16.593 and 0 respectively. It was found that Group I had highest mean value followed by Group II and III. The control group (saline) had the least value among the four groups. (Table 1)

Table 1 : Mean values of inhibition zone in different groups

Group	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Group I (3.8% Silver Diamine Fluoride)	18.360	0.282	0.072	18.00	18.80
Group II (5.25% Sodium Hypochlorite)	17.566	0.448	0.115	16.00	17.00
Group III (1.6%CalotropisgigantiaLeaf extract)	16.593	0.433	0.111	13.00	14.00
Group IV (saline)	0.000	0.000	0.000	.00	.00

2. Inter group comparison of mean values of inhibition zone of Group I, Group II , Group III and Group IV

Table 2: Intergroup Comparison of means of inhibition zone by Tukey's (2sided) Post-hoc Test

*Groups		Mean Difference	Std. Error	Sig.
Group I	Group II	0.794	.12496	0.237 (Non- Sig)
	Group III	1.767	.12496	0.062 (Non-Sig)
	Group IV	18.360	.12496	0.001 (Sig)
Group II	Group III	0.973	.12496	0.104 (Non- Sig)
	Group IV	17.566	.12496	0.001 (Sig)
Group III	Group IV	16.593	.12496	0.001 (Sig)

* Group I (3.8% Silver Diamine Fluoride), Group II (5.25% Sodium Hypochlorite), Group III (1.6%CalotropisgigantiaLeaf extract) and Group IV (saline)

The intergroup comparison between the individual groups was done using post hoc analysis (Post Hoc Tukey analysis). The intergroup comparison between all the groups were non-significant except Group IV (control) was compared with experimental groups (Group I – III).(Table 2).

DISCUSSION

Irrigation is the only way to clean those areas of root canal wall that are not touched by mechanical instrumentation. The root canal system is complex with structures, such as fins, cul de sacs, and intercanal communications, are occupied by microorganisms once the tooth becomes infected[6]. The simple act of irrigation flushes away loose, necrotic, contaminated materials[3]. According to Cogulu et al[7], the most prevalent microorganisms found in the root canal system of primary teeth are E. faecalis, Porphyromonas gingivalis and Treponema denticola . E faecalis has high prevalence in secondary endodontic infections[8].

In the present study, the highest zone of inhibition was seen with 3.8% SDF endodontic irrigant against E. faecalis which was higher but comparable to other irrigants used in this study. It has been demonstrated by Tanaka M 1970[9] that a liquid solution of AgF has strong antibiotic and protein-coagulating properties, and also has a substantially potent antibacterial action, which chokes up the dentinal pipettes of root canal wall with respect to the electric resistance. Hiraishi et al. studied the effect of 3.8% SDF and NaOCl on E. faecalis biofilm. They reported that 3.8% SDF showed 100% efficiency against E. faecalis after a direct 60-min exposure[10]. Silver interacts with the sulphydryl and thiol groups present in the bacterial amino acids and nucleic acids. This inhibits cell division, cellular respiration, metabolism, and biofilm formation. Several investigators have studied the cellular mechanisms that are affected by silver, thus proving its effectiveness as an anti-bacterial agent[11]. Antibacterial cationic nanoparticles such as silver nanoparticles (AgNP) show substantial antibacterial activity against biofilms. AgNPs show multiple antibacterial mechanisms such as adherence and penetration into the bacterial cell wall, leading to a loss of integrity of the bacterial cell membrane and cell wall permeability[12].

The antimicrobial efficacy of 5.25% NaOCl irrigant was found to be lower but comparable than that of 3.8% SDF irrigant, and was higher but comparable to 1.6% Calotropis gigantea herbal irrigants in reduction of E.faecalis. NaOCl acts on the albumins denaturing them and turning them soluble in water. It acts on microbial cells disrupting their vital functions leading to cell death. The NaOCl alkali contacting with organic products in decomposition liberates chlorine and nascent oxygen that promote bactericidal action[13]. Gomes et al.2001[14] concluded that chlorhexidine gluconate in the liquid form at all

concentrations tested (0.2%, 1% and 2%) and NaOCl (5.25%) were the most effective irrigants in killing E. faecalis.

The antimicrobial efficacy of 1.6% Calotropis gigantea herbal irrigant was found to be lower but comparable than 3.8% SDF and 5.25% NaOCl irrigant. Calotropis leaves contain alkaloids, glycosides, mudarine[15]. Calotropis gigantea reported as antimicrobial, antibacterial and cytotoxic effect[16]. Sharma M et al 2015[17] stated that C. gigantea has shown antimicrobial activity against S. mutans and lactobacilli. It was found to be effective at as low as 1.25% concentration also.

Normal saline showed no antibacterial efficacy against E. faecalis, thereby authenticating the validity of the present study. 3.8% SDF & 1.6% Calotropis gigantean are recommended as irrigating solutions owing to their comparable antibacterial efficacy & lesser toxicity than 5.25% NaOCl. We recommend further studies to authenticate these results. Clinical trials should be made so as to check other properties in the intraoral environment.

CONCLUSION

- All the endodontic irrigants used in this study showed antibacterial efficacy against E.faecalis.
- 3.8% SDF endodontic irrigant showed maximum antibacterial efficacy against E. faecalis among experimental groups.
- 5.25% NaOCl showed lesser antibacterial efficacy than 3.8% SDF but better antibacterial efficacy than 1.6% Calotropis gigantean herbal irrigant against E. faecalis.
- 1.6% Calotropis gigantean herbal irrigant showed least antibacterial efficacy against E. faecalis than 3.8% SDF and 5.25% NaOCl irrigant.
- Antibacterial efficacy of all experimental groups were comparable.
- 0.9% Normal saline has no effective against E. faecalis of all the tested irrigants.

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