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Research Article

ANTIMICROBIAL EFFICACY OF CHLORHEXIDENE, TRIPHALA AND GREEN TEA POLYPHENOLS AS IRRIGATING SOLUTION AGAINST E.FAECALIS:- A COMPARATIVE IN VITRO STUDY

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ABSTRACT

As on today the endodontists are facing more and more retreatment cases. So in this study we are comparing antimicrobial efficacy of Triphala solution and Green tea polyphenols (GTP) in relation with Chlorhexidene (CHX) against *Enterococcus faecalis (E.faecalis).* In this study root canals of 30 human permanent single rooted teeth with roughly round canals were prepared with hybrid technique and sterilized. Then the samples were contaminated with *E.faecalis* for 7 days to form a biofilm. After 7 days, the infected samples were irrigated with following irrigants i.e. CHX (Gp I), Triphala (Gp II) & GTP (Gp III) and assessment of antimicrobial efficacy was done. On applying ANOVA AND Tukey test the differences between each group was found to be non - significant as the value for p was found to be <0.05 for all the comparisons. All the 3 irrigants showed acceptable antimicrobial efficacy against *E.faecalis* and the difference between the three is found to be non- significant.

Key words:- Chlorhexidene, Triphala, Green tea polyphenols, *E.faecalis*.

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INTRODUCTION

It's a well-known fact that success of endodontic therapy is dependent on the endodontic triad. The first and the most important step is the achievement of a sterile canal i.e total elimination of micro-organisms with biomechanical preparation.

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This cannot be achieved using mechanical instrumentation alone due to extremely complex root canal anatomy. Hence, the irrigating solutions are an important adjunct for decrease or elimination of microorganisms from the root canal system [1].

The endodontic irrigants are generally selected on the basis of their antimicrobial properties and ability to remove organic tissues along with being nontoxic to periapical tissues.

According to Grossman, amongstthe commonly found micro-organisms in the canal, *E. faecalis* despite making up a small proportion of the flora plays a major role in the aetiology of persistent peri-radicular lesions after root canal therapy. As it is an organism which is able to survive as a single organism or as a major component of the flora of endodontically treated teeth with chronic apical periodontitis [2].

The list of irrigants which have been traditionally used areWater, Organic acids (Citric, Maleic, Tannic, Phosphoric acid, Tublicid and Lactic acid), Inorganic acids (Sulphuric and Hydrochloric acid), Ethylenediamine tetraacetic acid(EDTA), Iodine potassium iodide(IPI), Sodium hypochlorite, Hydrogen peroxide, Chlorhexidine and many other recent irrigating solutions such as Ozonated water, Superoxide, Glyoxide, Electrolytically precipated water, MTAD, Tetraclean, PAD (photoactivated disinfection), HEBP (1-hydro xyethylidene-1,1-bisphosphonate) and BDA(bis-dequalinium acetate). Most of these irrigants are non-specific. Amongst the commonly used irrigants, Sodium hypochlorite and EDTA are specifically effective against *E.faecalis*, but the major drawbacks if extruded into periapical tissues are inflammation, ecchymosis, and hematoma sometimes even necrosis and paresthesia [1].

Whereas, Chlorhexidine gluconate (CHX) being devoid of all these complications and having increased specificity against *E.faecalis* becomes the irrigant of choice.

There is no doubt that most of the synthetically manufactured drugs have deleterious effects on health. This has prompted us to look for herbal drugs like Triphala, Green tea polyphenols, Propolis, Orange oil and Tea tree oil which have no deleterious effects as such. Studies have shown that Triphala&Green tea polyphenols are proven to be safe & contain active constituents that have beneficial physiologic effect apart from its antimicrobial properties [3].

Triphala is an Indian ayurvedic herbal formulation consisting of dried and powdered fruits of three medicinal plants *Terminalia bellerica, Terminalia chebula,* and *Emblica officinalis* which are effective against enteric pathogens. Green tea polyphenols is the traditional and most widely consumed beverage of China and Japan, obtained from the young shoots of tea plant *Camellia sinensis*. The cathechins and the flavins are considered as microbiologically active ingredients [3].

The study aimed at comparing the efficacy of these three solutions Triphala solution and Green tea polyphenols (GTP) and Chlorhexidene (CHX) against E.faecalis when used as root canal irrigant

MATERIAL AND METHODOLOGY

Thirty extracted intact human permanent single rooted teeth with roughly round canals were collected from the Department of Oral and Maxillofacial Surgery of SDDHDC, Panchkula and the study was approved by the ethical committee of the college. All the selected teeth were cleaned, washed and stored in distilled water. Ideal access cavity was prepared, working length was determined and the root canal was prepared using hybrid technique upto the measured working length with #30 Kfile as the master apical file. The canals were kept wet by repeated irrigation with normal saline.

Samples thus prepared were autoclaved at 121^{o}C and 15 Psi for 20 minutes and sterility of the sample was checked by dipping each sample in a test tube containing 5 ml of sterile brain heart infusion broth which was incubated at 37^{o}C for 48 hrs (fig.1).

If no turbidity appeared the samples were considered as sterile. However in case of turbidity, the samples were resterilized.Enterococcus faecalis in the freeze dried form obtained from Institute of microbial technology, Chandigarh was dissolved in distilled water which was inoculated on Tryptone soy agar + 5% blood agar and the colonies were allowed to grow for 24 hrs, which were then grown on bile esculin azide agar for confirmation.

30 test tubes containing 5ml of sterile Brain heart infusion broth were infected with E.faecalis (fig.2) and the samples were dipped in it and incubated at 37oC for 7 days. The infected samples were taken out from the turbid brain heart infusion broth and rinsed with freshly prepared distilled water. The external surface of the sample was wiped with gauze dipped in alcohol. The samples were then dipped into fresh sterile brain heart infusion broth, incubated at 37oC and checked for turbidity after every 24 hrs for maximum period of 72 hrs. Appearance of turbidity indicated that the samples were infected with Enterococcus faecalis. In cases where no turbidity appeared they were re-infected till the turbidity appeared.

Preparation of the irrigating solutions

Triphala solution

Six gms of Triphala powder (Dabur pharmaceuticals) was dissolved in 10% DMSO (Dimethyl sulfoxide solution of Nice company) by shaking the beaker and keeping it on hot plate so that the particles of triphala solution were dissolved easily and the solution was filtered.

Green tea solution

Six gms of Green tea powder (Health aid company) was made by crushing the tablets of green tea and dissolving in 10% DMSO (Dimethyl sulfoxide). Then the beaker was shaken and keept on

hot plate so that the particles of green tea solution were dissolved easily and then the solution was filtered.

2% Chlorhexidene solution

Readymade solution of 2% Chlorhexidene of Amndent company

The samples thus prepared were randomly divided into 3 experimental groups having ten samples each.

Irrigation of the infected samples

Each of the 10 samples of Group I were irrigated with prepared 10 ml Triphala solution by holding the tooth in wet sterile Gauge and using 24 gauge needle. Similarly each sample of Group II was irrigated with prepared 10 ml Green tea solution and in Group III irrigation was done with 10 ml of 2% Chlorhexidene solution for each sample. (fig.3).

The sample was wrapped in dry sterilized gauze and the canals were dried with the sterile paper points then dipped in sterile Brain heart infusion broth and incubated for 72 hrs at 37^{O}C. Serial dilution were done for all the groups and 1ml of suspension from the last dilution was inoculated on Bile esculin azide agar which were incubated at 37^{0}C for 24 hrs after which the colony forming units were counted and observations were recorded and data was put to statistical analysis.

RESULTS

The number of colonies for all three groups are shown in table no1. Comparison of the three groups for potency of antimicrobial efficacy by Independent samples ANOVA test revealed no statistically significant difference between the groups (P= 0.89) (table no.2)

Inference: The three agents used were equally efficacious without any significant difference between their potency of antimicrobial efficacy.

When frequency of colony formation was compared between the groups (table no.3), Group A showed colony formation in 4 out of 10 samples, while group B & C had formed colonies in 5 out of 10 samples. By analysing the frequency of colony formation statistically by Chi square test, no significant difference was found (P = 0.951).

Inference: Frequency of bacterial colony formation was similar with all the three agents, thus implying similar antimicrobial efficacy.

Fig 1. Sterile tooth dipped in BHI broth

Fig 2. Contamination of BHI broth with E.faecalis

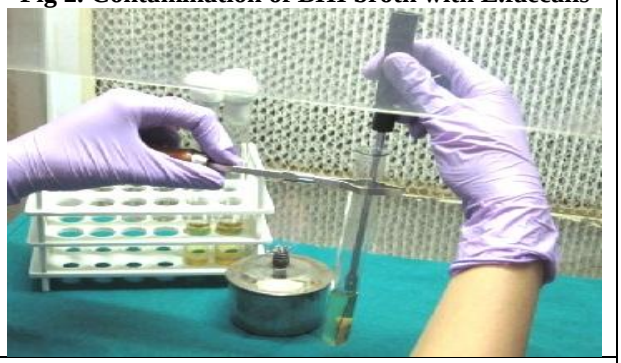

Fig 3. Irrigation with the test irrigant

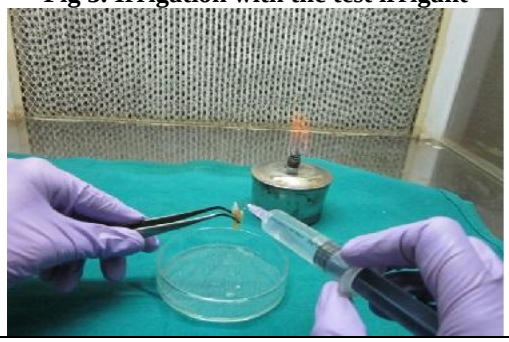

Table 1. Bacterial count from each sample (bacterial count in CFU/ml)

Triphala (Gp I)	0	0	1	0	1	0	0	5	1	0
Green tea(Gp II)	0	1	1	0	0	2	1	1	0	0
Chlorhexidene (Gp III)	1	1	0	0	0	0	1	2	1	0

Table 2. Anova Test

	Group 1	Group 2	Group 3	Total
N	10	10	10	30
$\sum X$	8	6	6	20
Mean	0.8	0.6	0.6	0.667
$\sum X^2$	28	8	8	44
Variance	2.4	0.489	0.489	1.057
SD	1.549	0.699	0.699	1.028
SE	0.489	0.221	0.221	0.187

Table 3. Comparison between the three groups for antimicrobial efficacy by analysing mean number of colonies formed

	Sum of Squares	Degree of freedom	Mean Square	F	P value
Comparison between groups	0.267	2	0.133	0.12	0.8873
Error	30.4	27	1.125		

Table 4. Comparison of the frequency of colony formation between the groups

	No. of samples with colonies	Total	Chi square	df	P value
Group 1	4	10	0.1	2	0.951
Group 2	5	10			
Group 3	5	10			

DISCUSSION

Microorganisms and their end products are considered the main cause of pulpal and periapical pathosis [4]. The ingress of irritants from an infected root canal system through tubules, lateral or accessory canals, furcation canals, and the apical foramina is very well known to directly affect the surrounding attachment apparatus and vice-versa. This is possible because the diameter of dentinal tubules is large enough to allow bacterial penetration. The number of microorganisms within an infected root canal system may vary anywhere from 10^2 to more than 10^8 as said by Sjogren [5]. Horiba studied that the microbes are present in all parts of the root canal system, including fins, and anastomoses and may be found at varying depths of up to 300 μm within the dentinal tubules, from the pulpal end [6].

The microorganisms involved in secondary infections may be introduced during treatment, between appointments or after treatment because of faulty obturations or pre-existing infected apical periodontitis. Pseudomonas aeruginosa, Staphylococcus sp., Escherichia coli, Candida sp. and E.faecalis are commonly found in such infections. *E. faecalis* is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment [2,7]. E.faecalis was selected for the purpose of the present study because it is believed to be one of theintracanal bacteria which are most resistant to elimination by disinfecting agents.

A chemomechanical preparation technique is advocated to disinfect root canals because it allows a greater number of root canals to be rendered bacteria free. Bystrom & Sundqvist in 1981 have reportedthatbetween 20% of the canals can be disinfected without the use of an antimicrobial irrigant whereas around 80% with the use of an antimicrobial irrigant [8].

Therefore, irrigation is an essential part of root canal debridement because it allows for cleaning beyond what might be achieved by root canal instrumentation alone. Among the steps of endodontic treatment, the choice of an ideal instrumentation technique and effective irrigating solutions that permit bacterial neutralization and toxins inactivation without negative interference with the healing process is fundamental for the success of root canal treatment [4].

Most of the recent irrigating solutions such as Ozonated water, Superoxide, Glyoxide, Electrolytically precipitated water, Bardac-22, 1% Pentanedial, MTAD and Tetraclean, most of these irrigants are non-specific and the efficacy of most of the irrigants is pH dependent i.e either the irrigant is caustic or acidic. CHX is the only irrigant to have optimal antimicrobial action ranging from pH 5.5 to 7.0 that seems to act by adsorbing onto the cell wall of the microorganism and causing leakage of intracellular components [9]. Therefore, Chlorhexidine gluconate (CHX) which is devoid most of the complications and has increased specificity against E.faecalis makes it an irrigant of choice.

At low chlorhexidine concentrations (0.2%), small molecular weight substances will leak out, specifically potassium and phosphorous, resulting in a bacteriostatic effect. At higher concentrations (2%), chlorhexidine has a bactericidal effect due to precipitation and/or coagulation of the cytoplasm, probably caused by protein cross-linking [10-12].

Chlorhexidene is known to be effective against E.faecalis as stated by Rosenthal et al with the advantage of substantivity extended up to 12 weeks and is effective against gram-positive organisms and gram-negative organisms [13]. According to Schafer and Bossman, 2% CHX is more effective compared to its lower concentrations, manifesting its influence in a shorter period of time and with a proper antimicrobial influence on E. Faecalis [14]. Therefore, 2% CHX was used in the present study.

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Plants have been used for centuries in the treatment of medical illness. Throughout the history of mankind, many infectious diseases have been treated with herbs. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. Triphala controls plaque from baseline and its activity is comparable to commonly available mouthwash Chlorhexidine [15].

Next thing which is important is the size of canal. Khademi et al concluded that minimum instrumentation size is needed for the penetration of irrigants to the apical third of the root canal is a 30#file [16, 17]. Hence, in the present study apical preparation was done upto 30#K file for better penetration of the irrigant into the canals.

There is a risk of false results due to growth potential of other bacterial contaminants which might occur during handling. So Bile esculin azide agar was used in the present study because it allows the growth of selective microflora such as E.faecalis which gives black colonies for E.faecalis.[3]This agar was developed by Isenberg in 1970. He found that the Enterococci and streptococci poses the ability to hydrolyse esculin to esculetin and dextrose which reacts with ferric citrate producing brownish black colonies and blackening of the agar [18, 19].

The results of the present study are similar with the study of Madhu Pujar who compared the antimicrobial efficacy of NaOCl, Triphala and Green tea polyphenols and found Triphala and Green tea to be effective against E.faecalis. Tannic acid is the major constituent of the ripe fruit of T.chebula, T.belerica and E. officinalis that is bacteriostatic or bactericidal to some gram positive and gram negative pathogens. Triphala & Green tea polyphenols are proven to be safe, containing active constituents that have beneficial physiologic effect apart from its curative property such as antioxidant, anti-inflammatory, and radical scavenging activity that may have an added advantage over the traditional root canal irrigants. DMSO was used as a solvent for Triphala. DMSO is a clean, safe, highly polar solvent that helps in bringing out the pure properties of all the components of the herb being dissolved [3].

The results of the present study are in concurrence with the study of Zohreh. He also found that Chlorhexidene showed acceptable antimicrobial efficacy against E.faecalis along with MTAD and Sodium hypochlorite [1].

From the results of the present study it can be deduced that the type of irrigant is important for reduction of bacteria inside a canal. Ten ml of the irrigant is sufficient for bacterial reduction as this is the minimum amount needed to flush the canal.

CONCLUSION

Based on the results of this study, it seems that all the 3 irrigants showed acceptable antimicrobial efficacy against E.faecalis and the difference between the three is found to be non-significant. Further in vivo studies are needed with greater sample size to determine the effect of these findings in clinical settings.

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DECLARATION OF INTEREST

None declared.

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