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To evaluate antimicrobial efficacy of calcium hydroxide against enterococcus faecalis either with or without natural medicaments

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***Abstract*---**Aim: Antimicrobial effectiveness of calcium hydroxide against E. faecalis is the primary goal of this research, which aims to compare the effects of calcium hydroxide with and without the addition of turmeric and ocimum tenuiflorum extract. Ingredients and Procedure: Using calcium hydroxide and herbal extracts, the samples were divided into six groups. Normal saline, turmeric extract, and

tulsi extract are in groups A1, B1, and C1 respectively. Calcium hydroxide and saline are in Group A; calcium hydroxide and turmeric extract are in Group B; and basil extract is in Group C. Enterococcus faecalis may be grown in brain heart infusion broth (BHI). Three equal-sized sets of media are placed in each of three wells with a diameter of 4 mm on each media plate. A sliding calliper is used to identify inhibitory zones after 1-2 days of incubation at 37°Celsius. Results: This study's statistical findings were generated entirely with SPSS version 18. P values of less than 0.04 were deemed significant. ANOVA and post hoc Games Howell tests were used to compare the mean inhibition zone. Conclusion: It was shown that the combination of calcium hydroxide and saline was the most effective method of combating Enterococcus faecalis, whereas turmeric was found to be ineffective.

***Keywords*---**Calcium hydroxide, Turmeric extract, Tulsi extract, E. faecalis, Zone of inhibition.

Introduction

Root canal treatment's major goal is to eradicate bacteria and their products from the root canal system in order to prevent reinfection. Even after root canal instrumentation, certain microorganisms remain in the root canal system. Findings from several studies show that root canals are home to a wide range of microorganisms. In almost 30–75 percent of teeth having endodontic operations, Enterococcus faecalis has been isolated[2]. To disinfect and destroy microorganisms, further procedures such as chemical treatments are required. The root canal system's chemical therapy comprises

- Rinses
- Irrigants
- Interappointment medicaments.

When used as a 7-day dressing, calcium hydroxide is the most often utilized endodontic medicament, because its high pH (12.5) kills most germs[3]. Although calcium hydroxide's antibacterial properties vary depending on the vehicle and route of delivery, it is still effective against germs. E. faecalis has also been discovered to be resistant to calcium hydroxide preparation.[4]

Herbal extracts such as turmeric and tulsi were combined with calcium hydroxide powder in the current investigation to address the inadequacies of current medicaments. Enterococcus faecalis was the microbe employed in the experiment. Calcium hydroxide's antibacterial effectiveness against Enterococcus was examined in this research, both with and without the inclusion of natural antibiotics such turmeric and tulsi extracts.

Preparation Of Calcium Hydroxide: Calcium Hydroxide Mix is made by mixing calciumhydroxide powder with sterile normal saline in a 1:1 (wt/vol) ratio to make paste[5.]

Preparation Of Turmeric Extract: The rhizomes were rinsed with distilled water, and then sliced into irregular big pieces. The aqueous solution of turmeric was prepared as follows: A tray drying procedure was used to dry them in an oven for around 9-10 days at 455° C until they were fully free of water. To make a coarse powder, these uneven bits were pulverized. Maceration was used to remove the coarse powder of Rhizomes. A huge glass chamber was filled with 50 gms of coarse Curcuma longa rhizome powder.
When 1 L of sterilized, purified water was added to a glass container, prepared extracts were created. For seven days, the glass vessel was covered with a glass lid and only sometimes stirred to protect the menstruum from evaporating. The resulting liquid was cooled to 4°C in a beaker.[6]
Preparation Of Tulsi Extract: A sterile disposable cup was used to collect and weigh mature fresh Ocimum Tenuiflorum leaves. Fresh basil leaves were added to 50 ml of distilled water, and the mixture was shaken vigorously. The mixture was macerated for 1-2 minutes at a temperature of 45-50 °C under under supervision. Extract was filtered using muslin cloth to remove any abrasive residue. Using coarse residue and 25 ml ethanol, the procedure was repeated. Afterward, the amber-colored container was put in a hot water bath to speed up the evaporation of the produced extract[6].
Test For Antibacterial Assay: On the prepared agar plates, sterile glass beads were used to disperse a mixture of microorganisms from a stock blood agar plate containing enterococcus faecalis and 2ml dialyzed brain heart infusion broth.
"The samples were divided into 6 groups.

Group A– calcium hydroxide + saline
Group B–calcium hydroxide + turmeric extract
Group C – calcium hydroxide + Basil extract
Group A1–Normal saline
Group B1–Turmeric extractGroup C1–Basil extract"

Agar Plate Diffusion Method- The antibacterial action was tested on Enterococcus faecalis ATCC29212 blood agar. In each selected medium plate containing 25 ml agar, three 3 mm diameter wells were punched and filled with each corresponding medicament of equal content. (See Figure 1) All of the groups are combined together until a paste-like consistency is achieved.
Measurements of inhibition zones were made using a sliding calliper after incubation at 37°C for 1-2 days. The mean number of three perpendicular diameters was used to quantify the inhibition area.

Results

Statistical analysis

ANOVA and the post-hoc Games Howell test were used to compare the mean inhibition zone. A summary of results is given in Fig 2, Table-1, Table-2 and chart-1. The zone of inhibition of Group A (16.15mm) against Enterococcus Faecalis was significantly higher than other groups. There was no statistical significance in the difference in zone of inhibition between Group B (15.10mm) and Group C (14.05mm). Amongst all experimental groups, GroupB1 (1.3mm)

showed least zone of inhibition followed by C1 (10.20mm) where the difference was statistically significant when compared with other groups.

Table 1: Mean Zone of Inhibition (in mm) of different experimental groups

GROUPS	ZONE OF INHIBITION (in mm)
GROUP A Calcium Hydroxide+ Saline	16.15
GROUP B Calcium Hydroxide+ Turmeric Extract	15.10
GROUP C Calcium Hydroxide+Basil Extract	14.05
GROUP A1 Normal Saline	0
GROUP B1 Turmeric Extract	1.3
GROUP C1 Basil Extract	10.20”

Table 2: Inter group comparison of zone of inhibition between different groups

INTER- GROUP	MEAN DIFFERENCE	P-VALUE
A vs B	1.05	0.003; Sig
A vs C	2.19	0.001; Sig
A vs B1	14.92	<0.001; Sig
A vs C1	5.90	<0.001; Sig
B vs C	1.13	0.108 ; NS
B vs B1	13.90	<0.001; Sig
B vs C1	4.83	<0.001; Sig
C vs B1	12.76	<0.001; Sig
C vs C1	3.72	<0.001; Sig
B1 vs C1	-9.06	<0.001; Sig

Chart 1: Chart showing zone of inhibition of different groups

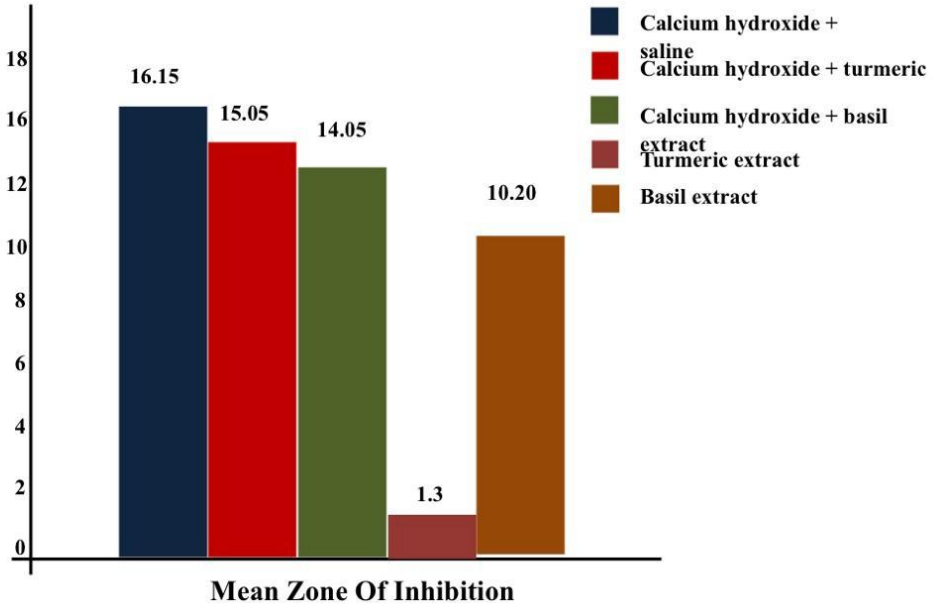
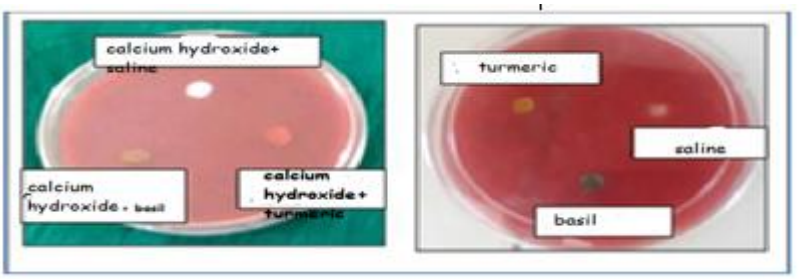


Fig. 1: Agar plates filled with experimental groups



Protein denaturation and DNA damage are all caused by Ca(OH)2's antibacterial action, which may be traced to the compound's high pH (12.5-12.8) and the capacity to dissociate into hydroxyl ions (OH-ions). Although $Ca(OH)_2$'s antibacterial activity reduced with time, this might be due to a variety of variables.[8]:

1) "Buffering action on the alkalinity of $Ca(OH)_2$ by dentin and its components.
2) Colonization of E. faecalis within dentinal tubules.
3) Decreased diffusibility of hydroxyl ions in dentinal tubules."

An antibacterial activity against facultative and anaerobic bacteria has been shown using the agar diffusion test in studies. [9]. As a polyphenolic molecule, curcumin (Turmeric) is well-known for its robust antibacterial action against a wide range of pathogenic bacteria by blocking the assembly dynamics of Z-ring, which is required for bacterial cell division. To keep curcumin from degrading, it has to be kept in a pH range between 6.5 and 7.4.[10]

When fatty acids or basic liquids like $Ca(OH)_2$ come into contact with turmeric (yellow colour owing to xanthophylls), the carotene pigment becomes more active and turmeric turns red[11].

Basil is an antioxidant that is biocompatible. Because of its anti-adherence function, it affects oral microbiota by modifying bacterial adhesion. Nimbidin and Nimbolide in basil have also been discovered to have antibacterial and antifungal activities, causing bacterial cell wall lysis[12]. Turmeric and Basil have a neutralizing effect on Calcium hydroxide, which has a high alkaline pH ranging from 12.5 to 12.8 and is stable at pH 6.1-6.5.

Discussion

Instrumented, unobturated canal bacteria may proliferate and approach their pre-treatment levels in only three days, according to research1. Preserving periapical lesions is a major factor in the persistence of Enterococcus faecalis, an antibiotic-resistant bacterium. The ideal pH range for Enterococcus faecalis is 6.5-7.5, however it may thrive in a broad pH range (4.6–9.9). When it comes to fighting Enterococcus, calcium hydroxide and saline combination proved to be the most effective, with a statistically significant difference. Curcumin extract was shown to have poor anti-bacterial efficacy when used on its own.
In the comparative analysis between the groups,

- Group A showed highest zone of inhibition than Group B, C, B1, C1 which was statisticallysignificant.
- Group B showed no statistical significant difference with Group C and higher zone of inhibition than Group B1, C1which was statistically significant.
- Group C showed higher zone of inhibition than GroupB1, C1 which was statisticallysignificant.

Study by Hegde and colleagues found that the aqueous extract of turmeric has modest efficacy against Enterococcus faecalis[14]. In contrast a study by Anurag Singhal et al. Showed that calcium hydroxide and basil combination is highly effective against E. faecalis[15].

Conclusion

Under the limitations of the study, the following can be concluded:

- They all have antibacterial properties against Enterococcus faecalis.
- Although turmeric had the least effect, a mixture of calcium hydroxide and saline had the highest results against Enterococcus faecalis ($P>0.001$)

A clinical trial is also needed to suggest the best techniques for employing these materials in clinical trials.

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