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RESEARCH ARTICLE

Comparison of Antibacterial Efficacy of Combination of Turmeric and Calcium Hydroxide with Three Intracanal Medicaments against Various Endodontic Bacteria: An *in vitro* Study

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ABSTRACT

Introduction: An intracanal medicament have an antibacterial effect on the root canal flora. The effectiveness of different available intracanal medicaments and search for new antibacterial agents to use as intracanal medicament to ensure success of our treatment is today's need. Curcumin (diferuloymthane) main yellow bioactive component of turmeric has wide spectrum of biological actions and this provides a basis for exploring its endodontic applications.

Aim: To determine antibacterial efficacy of Curcuma longa (turmeric) against root canal microorganism and compare its efficacy with calcium hydroxide, calcium hydroxide+ turmeric and metapex.

Materials and methods: For an antibacterial assessment samples were divided into four groups as group 1 calcium hydroxide group 2 calcium hydroxide + turmeric oleoresin group 3 turmeric oleoresin group 4 metapex. Each group was tested against following root canal pathogens *S. aureas*, *E. coli*, *E. feacalis* by agar well diffusion method. Inhibition zone was recorded at 24, 48 and 72 hours. Results were tabulated and sent for statistical analysis.

Result: Maximum inhibition of *E.feacalis*, *E.coli*, *S. aureas* is shown by combination of turmeric oleoresin and calcium hydroxide, i.e., group 2 followed by group metapex and then calcium hydroxide.

Conclusion: Turmeric extract has substantial antibacterial effect and its combination with calcium hydroxide increases its antibacterial efficacy against all important root canal pathogens. So turmeric has great potential to be used as intracanal medicament

Keywords: Antimicrobial efficacy, Intracanal medicament, Turmeric oleoresin,

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INTRODUCTION

Herbology is the study of plants for medicinal purpose, which is of great interest in medicine and dentistry in today's scenario. Turmeric (Curcuma longa), which is commonly and widely used as a home remedy, has been in focus in the field of medicine since long.[1]

Turmeric is a member of the Zingiberaceae family. Curcuminoids are the main component of turmeric which contains curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. Amongst them curcumin is the main component of turmeric responsible for biological properties. Isolation of curcumin was first done by Vogel and Pelletier in 1815, and J Milobedzka and V Lampe in 1910 determined its chemical structure. After extensive research on the therapeutic effect of curcumin, they demonstrated its anti-inflammatory, antibacterial, antiviral, antifungal, antidiabetic, anticoagulant, hepatoprotective, antiulcer, hypotensive, and hypocholestremic effects.[2]

Various intracanal medicaments are used as microorganisms play a key role in pulpal and periapical diseases. Both Gram-positive and Gram-negative bacteria are found to be associated with endodontic infection. These intracanal medicaments help in the elimination or reduction of microorganisms, rendering root canal contents inert, prevention of posttreatment pain, and improvement in the predictability and prognosis of treatment.[3]

Since the introduction of $Ca(OH)_2$ by Herman in 1920, it has been used in endodontics for various purposes and now is currently considered as the first choice for intracanal medicament due to its high antimicrobial and anti-inflammatory properties.[4]

Because of well-documented antibacterial activity of calcium hydroxide, its various combinations are used, among them Metapex, a silicon oil–based calcium hydroxide paste containing 38% iodoform. Iodoform has been used as a disinfectant since ages, and antibacterial properties of calcium hydroxide are well established. So the

combination of these two has been proven to be highly microbicidal because of synergistic effect.[5]

Hypersensitivity, immune suppression, and allergic reactions are some of the adverse effects associated with antimicrobial agents, and the continuous evolution of bacterial resistance has necessitated the search for novel and effective antimicrobial compounds from natural sources like plants and herbs.[6]

So this *in vitro* study was undertaken to evaluate the antimicrobial efficacy of Curcuma longa (turmeric) against root canal microorganisms and to compare its efficacy with calcium hydroxide, calcium hydroxide with turmeric, turmeric alone, and Metapex as an intracanal medicament.

MATERIALS AND METHODS

Materials used were turmeric oleoresin, calcium hydroxide, and Metapex. These were divided into 4 groups:
Group I – Calcium hydroxide [$Ca(OH)_2$] (DPI Pvt. Ltd)
Group II – Calcium hydroxide and turmeric oleoresin
Group III – Turmeric oleoresin
Group IV – Metapex (META BIOMED)

Preparation of Turmeric Oleoresin

Commercially available turmeric powder was extracted with ethanol in a sexhlot extractor for 16 hours as described by Saranya et al.[7] This extract was transferred to a conical flask for drying in a hot air oven overnight. The extract was then dissolved in dimethyl sulfoxide in a ration of 1:2 (w/v). Dimethyl sulfoxide acts as a solvent without changing any property of the ethanolic extract.

Calcium Hydroxide Preparation

The calcium hydroxide mixture was prepared by mixing the pure powder (DPI Pvt. Ltd., Mumbai) with distilled water to form a paste. This paste could be expressed through a syringe.

Combination of Turmeric Oleoresin and Calcium Hydroxide

Combination of turmeric oleoresin and calcium hydroxide is prepared by mixing turmeric oleoresin and paste of calcium hydroxide in the ratio of 1:1. This combination is easily expressed through a syringe.

Bacterial strains used for the study were as follows:
Enterococcus faecalis – ATCC 29212
Escherichia coli – ATCC 35218
Streptococcus aureus – ATCC 25923

MEDIA PREPARATION AND ANTIMICROBIAL ACTIVITY

Agar well diffusion method was used to perform antimicrobial assay. The cultured reference strains were streaked on the plates of Muller Hilton agar. Three separate agar plates were prepared for different microorganisms. Four wells were cut measuring 5×5 mm each in each agar plate. About 60 µL of the test agent was pipetted into their respective wells in each agar plate and then incubated at 37°C for 24 hours. After 24 hours, the diameter of the inhibition zones around each well in all the plates were measured, and then the same measurement was done after 48 and 72 hours (Figs 1 to 3). The experiment was done three times, and the mean values were recorded.

The results were tabulated and statistically analyzed using the Kruskal–Wallis test to identify the p-value and the Mann–Whitney U test to identify the significant groups (Table 1). Intracanal medicament that exhibited the maximum zone of inhibition was considered to have the most efficient activity against that organism.

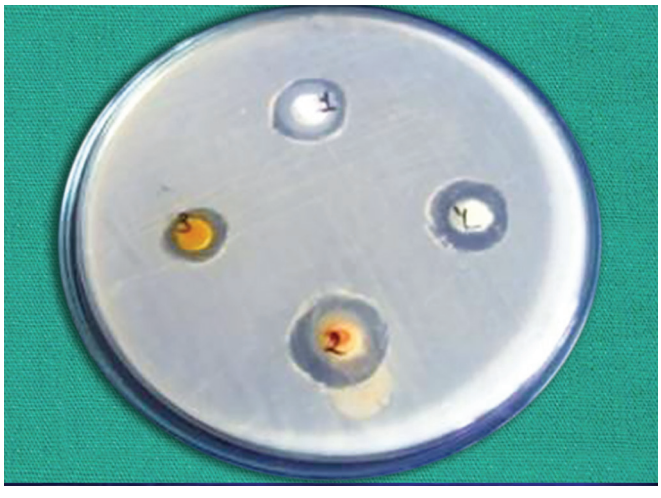

Fig. 1: Zone of inihibition for *E. coli*

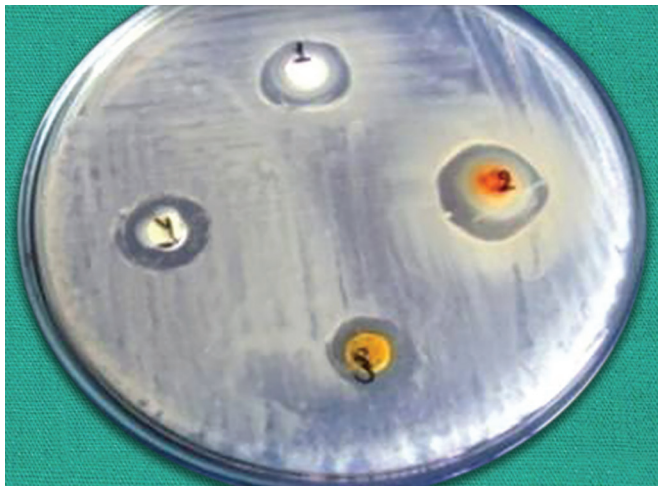

Fig. 2: Zone of inihibition for *E. feacalis*

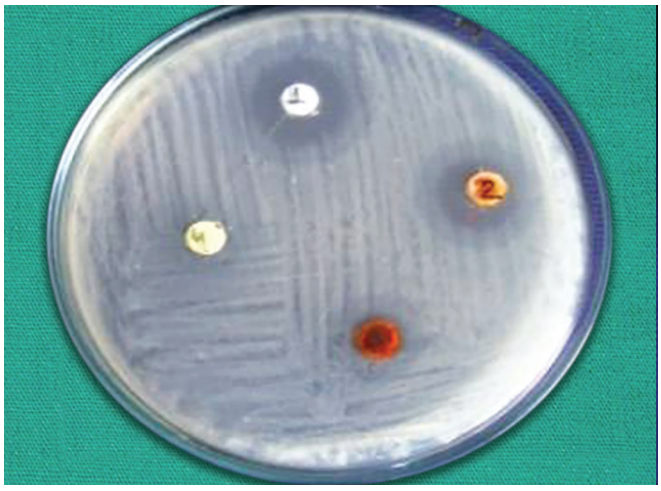

Fig. 3: Zone of inihibition for *S. aureas*

RESULTS

The results show a statistically significant difference in the antimicrobial action of the medicaments and with time ($p < 005$).

For *E. coli*, a combination of turmeric and calcium hydroxide (group II) shows the maximum mean value for the zone of inhibition for all three time intervals followed by Metapex (group IV) and then calcium hydroxide (group I) and the least mean value for the zone of inhibition by turmeric alone (group III). There was a statistical difference between group II (calcium hydroxide with turmeric) and the other three groups at all three time intervals except group IV (Metapex) at 48 hours ($p \leq 0.002$).

For *E. faecalis*, the same result was repeated as that of *E. coli*, and there was a statistical difference between group II (calcium hydroxide with turmeric) and other three groups at all three time intervals ($p \leq 0.003$).

For *S. aureus*, a combination of turmeric and calcium hydroxide (group IV) showed the maximum mean value for the zone of inhibition for all three time intervals followed by Metapex (group IV) and then calcium hydroxide (group I) and the least mean value for the zone of inhibition by turmeric alone (group III) except at 24 hours when calcium hydroxide (group I) had more zone of inhibition than Metapex (group IV). There was a statistical difference between groups ($p \leq 0.003$).

DISCUSSION

Chemomechanical preparation of the root canal system is the most important step for successful endodontic therapy. Despite the antimicrobial properties of the intracanal medicaments and irrigants, because of the varying vulnerabilities of the involved organisms, elimination of microorganisms may not be uniform.[8]

Most studies evaluate only the immediate antimicrobial activity of the materials. This does not have clinical relevance, because it is commonly in practice to leave the intracanal medicament in place for a time period of 7 to 10 days. It would be more relevant if the effective duration of antimicrobial action is determined. To achieve this, we determine the zone of inhibition of different intracanal medicaments after 24, 48, and 72 hours.

The agar diffusion method has been widely used to test the antimicrobial activity of dental materials and medicaments. The advantage of this method is that it allows direct comparison of the materials against the organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the disadvantage of this method is that the result not only depends on the toxicity of the material for the particular organism but

Table 1: Comparison of antibacterial efficacy of combination of turmeric and calcium hydroxide with three intracanal medicaments

Groups		*24 Hours*	*48 Hours*	*72 Hours*
Calcium hydroxide (group I)	Mean	14.80	13.40	10.40
	Std. deviation	0.84	1.14	0.55
	Median	15.00	13.00	10.00
Calcium hydroxide + Turmeric (group II)	Mean	18.40	17.60	13.60
	Std. deviation	1.14	1.14	1.14
	Median	18.00	18.00	14.00
Turmeric (group III)	Mean	11.80	11.80	9.60
	Std. deviation	0.84	0.84	0.55
	Median	12.00	12.00	10.00
Metapex (group IV)	Mean	15.40	16.20	11.40
	Std. deviation	2.30	0.84	0.55
	Median	16.00	16.00	11.00
Kruskal-Wallis test value		14.862 p = 0.002 (sig.)	16.194 p = 0.001 (sig.)	16.335 p = 0.001 (sig.)
Mann-Whitney U test results (significant differences)		1&2, 1&3, 2&3, 2&4, 3&4	1&2, 1&4, 2&3, 3&4	1&2, 2&3, 2&4, 3&4

is also influenced by the ability of the material to diffuse across the medium.[9]

According to this study, a combination of calcium hydroxide and turmeric produced the largest inhibitory zones against all the microorganisms, even after 72 hours. The antimicrobial activity of $Ca(OH)_2$ is related to the release of hydroxyl ions in an aqueous environment. Hydroxyl ions are highly oxidant-free radicals that show extreme reactivity with several biomolecules. This reactivity is high and indiscriminate, so this free radical rarely diffuses away from sites of generation. The lethal effects of hydroxyl ions on bacterial cells are probably due to the following mechanisms:

- Damage to the bacterial cytoplasmic membrane;
- Protein denaturation; and
- Damage to the DNA.[10]

A study demonstrated that a 7-day application of a $Ca(OH)_2$ medicament was sufficient to reduce canal bacteria to a level that gave a negative culture.[11] One more study found that aqueous $Ca(OH)_2$ paste and silicone oil-based $Ca(OH)_2$ paste were effective in the elimination of *E. faecalis* in dentinal tubules.[12] Another study showed that the placement of $Ca(OH)_2$ for at least 1 week rendered 92.5% of canal bacteria free.[13]

A study demonstrated that $Ca(OH)_2$ decreased the numbers of *E. faecalis* at all depths within dentinal tubules up to 24 hours and that less-viscous preparations of $Ca(OH)_2$ were more effective in the elimination of *E. faecalis* from dentinal tubules than viscous preparations.[14] A study found that *E. faecalis* cells in the exponential growth phase were the most sensitive to $Ca(OH)_2$ and were killed within 3 seconds to 10 minutes.[15]

By contrast, several studies have attested to the ineffectiveness of $Ca(OH)_2$ in eliminating bacterial cells.

A study found that $Ca(OH)_2$ in infected dentinal tubules had no antimicrobial effect on *E. faecalis*, *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, or the bacterial mixture used throughout the experiment.[16] Another study found that $Ca(OH)_2$ dressing between appointments did not have the expected effect in terms of disinfection of the root canal system nor the treatment outcome.[17] A study concluded that the viability of *E. faecalis* in infected root dentine was not affected by $Ca(OH)_2$.[18] In a systematic review to assess the antibacterial efficacy of $Ca(OH)_2$, eight clinical trials including 257 cases were evaluated. They concluded that $Ca(OH)_2$ had limited effectiveness in eliminating bacteria from human root canals when assessed by culture techniques.[19]

In summary, although some clinical studies have supported the efficacy of calcium hydroxide as an intracanal medicament, other studies have questioned its efficacy and indicated CHX instead of calcium hydroxide. So we planned mixing turmeric to calcium hydroxide to increase its antibacterial efficacy as turmeric is considered a disinfectant since ages.

The stability and assembly of FtsZ protofilaments as a crucial factor for bacterial cytokinesis are introduced as a possible drug target for turmeric as an antibacterial agent. Curcumin suppressed the cytokinetic Z-ring formation without significantly affecting the segregation and organization of the nucleoids markedly suppressed the cytokinetic Z-ring formation. It was demonstrated that curcumin reduces the bundling of FtsZ protofilaments associated with the binding ability to FtsZ with a dissociation constant of 7.3 µM. It was shown that curcumin via inhibition of assembly dynamics of FtsZ in the Z-ring can possibly suppress the bacterial cell proliferation as one of the probable antibacterial mechanisms of action. The study on *E. coli* and *B. subtilis* demonstrated that curcumin by the inhibitory effect against FtsZ polymerization could suppress the FtsZ assembly leading to disruption of prokaryotic cell division.[20]

A study found that curcumin applied at high doses has been demonstrated to have strong antibacterial activity against methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *E. faecalis*, *B. subtilis*, *P. aeruginosa*, *E. coli*, and *Klebsiella pneumonia*.[21]

A study concluded that *C. longa* extracts showed definite antimicrobial effectiveness against *S. aureus, E. coli*, and *E. faecalis.* The present study shows clearly that *C. longa* (turmeric) in a diffusible form has definite antimicrobial effectiveness. This is higher than the antimicrobial action of eugenol, which provides a wider horizon for using *C. longa* (turmeric) as a sealer or an intracanal medicament in endodontics.[7] A study found 54% inhibition of *E. faecalis* by aqueous extract of turmeric. In our study, due to the synergistic effect of antibacterial activities of turmeric and calcium hydroxide, the zone of inhibition of a combination of the two is larger than either of the two.[22]

Metapex is a combination of calcium hydroxide and iodoform in a silicone-based oil. Iodoform has long been advocated as an antiseptic in the treatment of pulpless teeth. In 1928 Walkoff introduced iodoform as a root canal filling material in primary teeth. Iodoform proved to be a potent bactericidal, nonirritant radiopaque material. A study reported that when combining pure iodoform with calcium hydroxide powder, excellent results were obtained based on clinical, radiographic, and histological evaluations.[23]

Metapex contains silicone oil as its vehicle and has a pH below that which is effective to kill *E. faecalis*. The superior antimicrobial effects of Metapex may be due to the combination with iodoform and to the viscous and oily vehicle, which may prolong the action of the medicament. Accordingly, a study showed that oily vehicles increase the antimicrobial effects of calcium hydroxide against *E. faecalis* and other bacteria. This is in accordance

with our study where a wider zone of inhibition was seen with Metapex than with calcium hydroxide.[24]

CONCLUSION

Turmeric extract has a substantial antibacterial effect and its combination with calcium hydroxide increases its antibacterial efficacy against all important root canal pathogens. So, within the limitation of our study, we can conclude that turmeric has a great potential to be used as an intracanal medicament.

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