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Endodontics

EVALUATION AND COMPARISON OF ANTIBACTERIAL EFFICACY OF THREE DIFFERENT MATERIALS USING AGAR DIFFUSION TEST: AN INVITRO COMPARATIVE STUDY

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ABSTRACT **Aim:** To evaluate and compare antimicrobial efficiency of three different intracanal medicament. **Background:** The principal aim of root canal therapy is to eliminate the pathogenic bacteria located within the anatomically complex canal system. In addition to adequate mechanical instrumentation and frequent irrigation, a resorbable intracanal filling medication with antimicrobial properties is suggested to increase the probability of treatment success. **Methodology:** A total of 10 agar plates were taken for the study. Samples were placed on each agar plate as group A: calcium hydroxide, group B: Acasia Catechu, group C: triple antibiotic paste. These plates were incubated for 72 hours for any bacterial growth of E. faecalis. **Conclusion:** Triple Antibiotic Paste showed significantly higher scores as compared to Ca(OH)2 and Acacia Catechu. Also, to Acacia Catechu showed significantly higher scores as compared to Ca(OH)2.

KEYWORDS : Intracanal medicaments, Calcium hydroxide, Triple antibiotic paste, Acasia Catechu.

INTRODUCTION

The ultimate goals of endodontic treatment are complete removal of bacteria, their byproducts and pulpal remnants from infected root canals and the complete seal of disinfected root canals. Microbial free canal can be obtained by cleaning, shaping, irrigation and intracanal medicaments followed by obturation.

Periapical lesions occur when microorganisms and their byproducts invade the root canal system, causing an inflammatory response. Endodontic therapy aims to remove microorganisms and clean the root canal system. Reducing microbial burden prior to obturation can alleviate symptoms and prevent refractory periapical pathosis. Chemomechanical debridement is used to eliminate microbial growth during endodontic procedures[1].

However, this method alone cannot render the root canal microbial free, particularly when there is a large periapical lesion[2]. Complexities in the root canal system and the lodging of microbes into deep layers of root canal dentin pose a challenge in disinfection and persistence of these microbes can lead to the recurrence of periapical complications[3]. In addition, the polymicrobial nature of endodontic infections makes total disinfection of the canal space difficult. Currently, periapical lesions are managed either with a surgical or nonsurgical approach. In the case of large periapical lesions, nonsurgical endodontic therapy with the use of an antimicrobial intracanal medicament should be the primary line of management[4].

Using an interappointment medicament can greatly increase disinfection after chemomechanical operations[5]. An intracanal drug that is frequently utilised is calcium hydroxide[Ca(OH)2]. Its capacity to dissolve tissue and its antibacterial properties are among its benefits[6]. One example of an intracanal medication is TAP (triple antibiotic paste). It consists of ciprofloxacin, metronidazole, and minocycline. This mixture has been utilised to disinfect the root canal system during root canal treatment and pulpal regeneration[7]. The primary disadvantage of TAP is tooth discolouration due by minocycline[8]. Cefaclor has substituted minocycline in the paste, resulting in a modified triple antibiotic paste (mTAP)[9].

Sato, et al. evaluated the effect of combining metronidazole, ciprofloxacin and minocycline in killing bacteria in the deep layers of the root canal in dentin. Within 24 hours of applying the triple antibiotic paste to the diseased dentin of the root canal wall in situ, no germs were recovered[10].

The extract of Acasia catechu exhibits numerous pharmacological activities, including immunomodulators[11], anti-hyperglycemic[12], antinociceptive, antihyperlipidemic[13], and antiulcer[14]. In a study, A. catechu extracts were used against Escherichia coli, Staphylococcus aureus, Salmonella typhi, Klebsiella pneumonia, and Shigella sonnei through the agar well diffusion method[15].

MATERIALS AND METHODOLOGY

Preparation Of Solution

Three materials like calcium hydroxide, triple antibiotic paste (metronidazole+ciprofloxacin+clindamycin) and A. catechu extract were taken for the comparison.

Group A- calcium hydroxide paste, group B- Acasia catechu, group C- Triple antibiotic paste (metronidazole+ciprofloxacin+clindamycin).

Acacia catechu extract was mixed with saline as a vehicle. The extract was made using heartwood chips (a.k.a katha).

TAP was prepared mixing metronidazole, ciprofloxacin and clindamycin in 1:1:1 proportion with normal saline[16].

A nitro imidazole compound having a broad spectrum of anti-anaerobic and anti-protozoal properties is metronidazole (Flagyl). It has been widely employed in both local and systemic forms due to its powerful effectiveness against a wide range of bacteria and anaerobic cocci. By breaking through the bacteria's membranes, attaching itself to their DNA, and disrupting the helical structure, it swiftly destroys the cells. When it comes to two strains of the required anaerobes under investigation, metronidazole works better than calcium hydroxide at preventing their formation.

Ciprofloxacin (Ciplox, Cipla) belongs to a class of second-generation fluoroquinolone antibiotics used to treat bacterial infections that cause stomach pain, diarrhoea, and urinary tract infections. Ciprofloxacin and other fluoroquinolones are used for various purposes, in both oral and intravenous forms, because of their high tissue penetration.

Clindamycin is used to treat certain types of bacterial infections, including infections of the lungs, skin, blood, female reproductive organs, and internal organs. Clindamycin is in a class of medications

called lincomycin antibiotics. It works by slowing or stopping the growth of bacteria

The antimicrobial activity was carried out against standard strains of American Type Culture Collection (ATCC) Enterococcus faecalis (ATCC 35550). Agar well diffusion method was used to determine the antibacterial activity of endodontic medicaments. A 50 µL of respective microbial inoculum was taken using a micropipette in order to provide an even lawn of cells, and loaded onto the agar plates evenly. The agar plates were inoculated with the respective microorganisms by even streaking of the swab over the entire surface of the plate three times rotating the Petri plates at 60°C approximately after each applications. Finally, it was swabbed all around the periphery of the agar surface. Three wells of 3 mm size and 4 mm depth were made at an equal distance. The plates were then incubated at 37°C for 24 hours in an aerobic environment. Petri plates were observed for zone of inhibition, which were measured using a scale in millimeters. The tests were repeated ten times to minimize errors.

RESULTS

- Group C (Triple antibiotic paste) shows the highest average inhibition zone (27.1 mm), indicating it has the strongest antibacterial effect among the three substances tested.
- Group B (Acacia catechu) follows with an average inhibition zone of 10 mm, showing moderate antibacterial activity.
- Group A (Calcium hydroxide) has the lowest average inhibition zone (7.7 mm), suggesting the weakest antibacterial effect among the tested substances.

Table No.1: Zone Of Inhibition In Milimeters

	Group A (calcium hydroxide)	Group B (Acasia Catechu)	Group C (Triple Antibiotic paste)
1	8mm	9mm	26mm
2	9mm	10mm	27mm
3	7mm	10mm	28mm
4	7mm	11mm	28mm
5	6mm	8mm	30mm
6	9mm	9mm	27mm
7	7mm	10mm	27mm
8	8mm	12mm	26mm
9	7mm	11mm	25mm
10	9mm	10mm	27mm

Table No.2: Comparision Of Intracanal Medicament

	Group	Mean	SD	Difference	p-value
Pair 1	Ca(OH)2	7.70	1.06	-19.40	<0.001*
	TAP	27.10	1.37		
Pair 2	Acacia Catechu	10.00	1.15	-17.10	<0.001*
	TAP	27.10	1.37		
Pair 3	Ca(OH)2	7.70	1.06	-2.30	<0.001*
	Acacia Catechu	10.00	1.15		

Independent t test; * indicates significant difference at p≤0.05

- The statistical analysis shows that in all three comparisons (Pair 1, Pair 2, and Pair 3), the difference in mean inhibition zones between the groups is highly statistically significant ($p < 0.001$).
- Triple antibiotic paste (TAP) consistently exhibits significantly larger inhibition zones compared to both Calcium hydroxide [Ca(OH)□] and Acacia Catechu.
- Acacia Catechu shows larger inhibition zones compared to Calcium hydroxide, but the difference is smaller compared to TAP.

In summary, based on the statistical analysis, Triple antibiotic paste (TAP) demonstrates significantly stronger antibacterial activity compared to both Calcium hydroxide and Acacia Catechu in this experimental setup.

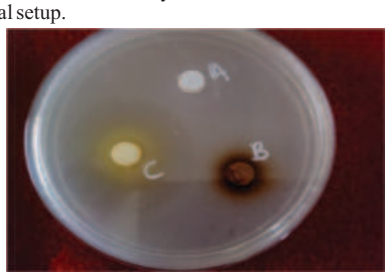
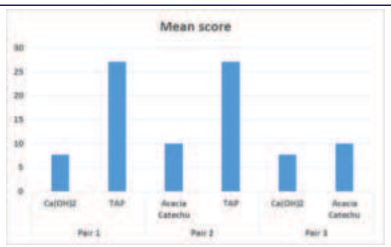


Fig: A- Calcium hydroxide paste; B- Acasia catechu (katha); C- Triple Antibiotic Paste



Graph No.1

Discussion

Intracanal medications can be used in regenerative endodontics or multiple visit endodontics following trauma, among other endodontic disorders[17]. These medications are applicable for a range of durations, from one week to several months[18]. The American Association of Endodontists advises clinical regeneration techniques should be administered with intracanal medication for a minimum of one week. Moreover, a 7-day $Ca(OH)_2$ interappointment dressing has been reported to be adequate in reducing intraradicular bacteria to a level that yielded a negative culture[19]. Nonetheless, it has been proposed that prolonged use of appointment-only medications may alter the mechanical characteristics of root dentin by causing either excessive demineralization in the case of antibiotic pastes or collagen degradation in the case of $Ca(OH)_2$. This might potentially compromise the root fracture resistance[20].

In this study, Group A (Calcium hydroxide) has the lowest average inhibition zone (7.7 mm), suggesting the weakest antibacterial effect among the tested substances.

Acacia catechu is valued in India for making two commercially important products from its heartwood: Katha and cutch. Katha is used as an ingredient of paan (betel leaf masticatory) which is used traditionally and as well as chewed after meals in South Asia[21]. Cutch is used as a tanning agent for leather, dye, mail bags, etc., and in oil-well drilling as a viscosity modifier of drilling mud. Flavonoids are one of the major secondary compounds which are important for humans due to their antioxidative and anticancer activities. Quercetin, present in the A. catechu plant, was found to have anti-inflammatory, antioxidant, and anticancer properties. Catechin and epicatechin present in Katha are used as food supplements.

Epicatechin 3 gallate is used to treat dental caries and periodontal infections. Although, there are not many research studies (in vivo and clinical trials) clarifying the safety and utilization of A. catechu as a food supplement[22]. A variety of ailments can be treated with Acacia catechu in both conventional and alternative medical systems. A. catechu exhibits a high concentration of bioactive substances and varies in the amount of polyphenolic and flavonoid content, such as epicatechin, quercetin, lupeol, kaempferol, and catechin, in its heartwood, leaves, bark, and seed. The plant's pharmacological properties have been thoroughly investigated, and the findings indicate that it has rich antioxidant, antimicrobial, antidiabetic, hepatoprotective, antidiarrheal, antipyretic, antinociceptive, antihyperlipidemic, antiulcer, antiproliferative, antibacterial, antifungal, hemolytic, and anti-inflammatory qualities that make this plant extremely valuable[23].

According to Verma K. and et al., the application of A. oleracea and A. catechu gel together with scaling and root planing improved clinical parameters in a way that suggested improved anti-inflammatory and healing qualities[24].

Group B (Acacia catechu) in this investigation showed a modest antibacterial activity against E. faecalis, with an average inhibition zone of 10 mm.

In order to facilitate local response and guarantee the healing of damaged tissues, the sterilisation and disinfection of the root canal systems entails a decrease in microorganisms'[25]. Research on the microbial diversity of root canal infections show that facultative anaerobes proliferate when root canal infections persist longer and that the relative proportions of anaerobes rise with time. S. mutans is the most often isolated organism, followed by facultative microorganisms, which are the most resistant species and include E. faecalis and S. aureus. One of the potential causes of root canal failure

is E. faecalis. E. faecalis has also been mostly detected in primary endodontic infections in recent times[26].

Strong antibacterial agent TAP is employed in a variety of ways and for a range of functions. It attempts to "regenerate and revascularize" the damaged pulp in an effort to maintain its vitality. TAP is essential for revascularization and regeneration in addition to being a strong disinfectant[27].

As an intracanal medication for periapical lesions, external inflammatory root resorption, root fractures, and treatment flare-ups—a frequent endodontic treatment complication—TAP is an essential application. A flare-up occurs when an abrupt worsening of an existing problem necessitates an unforeseen treatment session. It is characterised by the beginning of severe pain and swelling following an endodontic therapy appointment[28].

Nakornchai et al. reported that TAP and Vitapex® (J. Morita Corporation, Kyoto City, Japan) demonstrated significant outcomes in the root canal treatment of primary teeth that were infected[29]. The impact of TAP on the clinical results of tissue healing and lesion sterilisation in primary teeth with peri-radicular lesions was investigated by Takushige et al. Following treatment, clinical complaints included sinus tracts, gingival edoema, dull discomfort, and other symptoms vanished. The teeth that had received successful treatment showed up and normally erupted on radiographs[30,31].

Tooth discoloration represents a significant disadvantage of TAP. Research has indicated that, in comparison to other antibiotic pastes such as Ledermix, polyantibiotic paste, and Septomixine Forte, TAP was more associated with discoloration. Consequently, the use of double antibiotic pate (DAP), which solely contains ciprofloxacin and metronidazole, has been recommended in certain situations[32].

Minocycline is associated with drug induced lupus like syndrome, autoimmune hepatitis and vasculitis, Tissue hyperpigmentation. It causes drug resistant bacteria.

In the present study, Group C (Triple antibiotic paste) shows the highest average inhibition zone (27.1 mm), indicating it has the strongest antibacterial effect among the three substances tested.

CONCLUSION

TAP showed significantly higher scores as compared to $Ca(OH)_2$ and Acacia Catechu. Also, to Acacia Catechu showed significantly higher scores as compared to $Ca(OH)_2$. Further in vivo studies are needed to evaluate osteoinductive and nociceptive properties of Acacia Catechu.

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