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A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF NEEM, TRIPHALA AND GALLIC ACID AS AN INTRACANAL MEDICAMENT - AN IN VITRO STUDY

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ABSTRACT

Aim: The aim of this study was to determine the antibacterial efficacy of three alternative intracanal medicaments v/s Calcium hydroxide against bacteria of endodontic origin. **Materials and Method:** 100 teeth were selected for the study depending upon selection criteria. Decoronation of the sample was done using carborundum disks. Post decoronation, access opening was done using diamond burs. Cleaning and shaping of the root canal system was done using rotary Pro Taper upto F3.

All samples were divided into 5 groups:

Group A: Neem + Propylene Glycol;

Group B: Triphala + Propylene Glycol;

Group C: Gallic Acid + Propylene Glycol;

Group D: Calcium hydroxide + Propylene Glycol;

Group E: Normal Saline.

Equal amount of the principle ingredients were mixed with Propylene Glycol to achieve a paste like consistency. The cleaned and shaped roots were inserted into Eppendorf tubes and the cold cure acrylic was used to seal the root in the tube. One syringe tip was inserted through the cold cure acrylic to rest inside of the Eppendorf Tube for replenishment of nutrient broth. The entire assembly was autoclaved. ICM was introduced through the root canal orifice to rest into the root canal. The orifice was then sealed using cavit. 0.5 ml of bacterial sample was poured into the tube. Syringes are sealed shut. The assembly was stored at 37^{o}C in a humidor. The bacterial count at the end of 72 hrs as per the group was evaluated by counting the Colony Forming Units. **Result:** Gallic Acid showed least CFU where as Calcium hydroxide performed better than Triphala, Neem and Normal Saline. No significant difference between Calcium hydroxide and Gallic Acid. Triphala showed a better result than Neem but inferior to Calcium hydroxide. Neem had the least antimicrobial effect but better than Normal Saline which was the worst performing group in this study. **Conclusion:** Amongst all the groups, the group with Gallic Acid showed the best antimicrobial efficacy when used as an intracanal medicament. Therefore Gallic Acid can be further studied and used as an intracanal medicament in the near future.

1) INTRODUCTION

The chief objective of performing root canal therapy is to reduce the bacterial load in and around the root canal system. Reduction of endodontic microorganisms has been achieved by a series of antimicrobial strategies that include cleaning and shaping of the root canal, irrigation, placement of intracanal medicaments, and obturation of the root canal.[1-4]

The placement of intracanal medication is greater in those cases where bacteria are resistant to the routine root canal treatment, and where the treatment cannot be successfully completed due to the presence of pain or continuing exudate.[5] Calcium Hydroxide serves as the gold standard, when used with its various vehicles for root canal disinfection.

As it possesses bactericidal properties, calcium hydroxide has been advocated as an intracanal medicament. It can destroy the bacterial cell wall and the protein structure due to its high pH (of about 12.5).[6] However many bacteria have been reported to be resistant to the antimicrobial effect of calcium hydroxide as a result of their ability to enter deep into the dentinal tubules and adapt to the changing environment. Its biggest disadvantage is its inability to overcome the proton pump inhibitor. It also reduces the strength of radicular dentin. Therefore study of the effects of intracanal medicaments remains a relevant issue as the search for potent substances with a wide antimicrobial spectrum and low cytotoxicity continues.[7]

Various herbal medicaments like Neem, Triphala, Gallic Acid, Tannin, *Morinda citrofilia*, Green Tea etc. are continuously studied as intracanal irrigants because of their antibacterial properties. But there are no studies which evaluate them as the intracanal medicament although their efficacy in destroying microorganisms is well known. Evaluation of the capability of alternative intracanal medicament to kill and destroy the microorganism is the need of the hour, particularly as there are hardly any intracanal medicaments used except Calcium hydroxide. This study thus examines the efficacy of Neem, Triphala and Gallic Acid when they are used as intracanal medicament in a unique microbial study model which simulates a periapical lesion.

2) MATERIALS AND METHOD

100 selected roots were included in the study and grouped as follows:

Group A: Neem in addition with propylene glycol (n=20)
Group B: Triphala in addition with propylene glycol (n=20)
Group C: Gallic Acid in addition with propylene glycol (n=20)
Group D: Calcium hydroxide with propylene glycol (positive control, n=20)
Group E: Normal Saline (negative control, n=20)

3) METHODOLOGY

PREPARATION OF TOOTH SAMPLE

Decoronation of the sample was done using carborundum disks.
Post decoronation, access opening was done using diamond burs.
Cleaning and shaping of the root canal system was done using rotary ProTaper upto F3

PREPARATION OF BACTERIAL CULTURE

Bacteriological samples were taken from the periapical lesions of a patient with a chronic periapical abscess. Only patients with no history of antibiotic uptake in the prior month and no history of dental treatment with regard to the tooth in question were considered. The diagnosis of a periapical abscess was confirmed with patient's history, clinical findings and radiographic imaging.

PREPARATION OF THE INTRACANAL MEDICAMENT

The ICM were divided into 4 groups:
NEEM + PROPYLENE GLYCOL
TRIPHALA + PROPYLENE GLYCOL
GALLIC ACID + PROPYLENE GLYCOL
CALCIUM HYDROXIDE + PROPYLENE GLYCOL
Equal amount of the principle ingredients were mixed with Propylene Glycol to achieve a paste like consistency.

PREPARATION OF THE EXPERIMENTAL MODEL

The cleaned and shaped roots were inserted into Eppendorf tubes and cold cure acrylic was used to create an airtight seal for the root in the tube. One syringe tip was inserted through the acrylic resin to rest inside of the Eppendorf tube and this acted as an inlet for the introduction of bacteria into Eppendorf tube. The entire assembly was then autoclaved. As per the group, respective ICMs were introduced through the root canal orifice to rest inside of the root canal. The orifice was then sealed using Cavit™. 0.5 ml of the prepared bacterial sample was poured into the tube. The syringe was then sealed shut using Cavit™. The assembly was stored at 37 C in a humidor for 72 hrs.

Access opening was done under sterile conditions using a rubber dam and the drop into the pulp chamber was taken without water spray. Paper points were introduced into the canal and left for 30 secs. The paper point was then extricated from the canal and kept in an Eppendorf tube along with Robertson's Cooked Meat Media. This procedure was repeated for 5 approved cases and the samples collected were then sent to the laboratory for further culturing. After culturing the samples, the micro organisms found were *Pseudomonas* and *Candida albicans.*

EVALUATION OF THE BACTERIAL COUNT

The bacterial count at the end of 72 hrs as per the group was evaluated by counting the Colony Forming Units. Serial dilution of bacterial samples was done using the sample of the bacterial culture harvested from the experimental models at the end of 72 hours. The Nutrient Agar Plates were inoculated with the bacterial sample. The plates were incubated for 72 hrs. The colonies formed after 72 hrs were counted.

Fig no. 1: EPPENDORF TUBE.

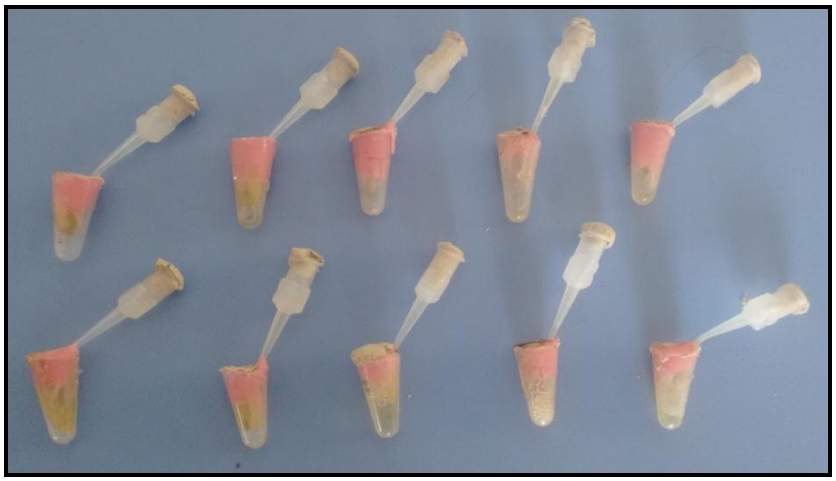
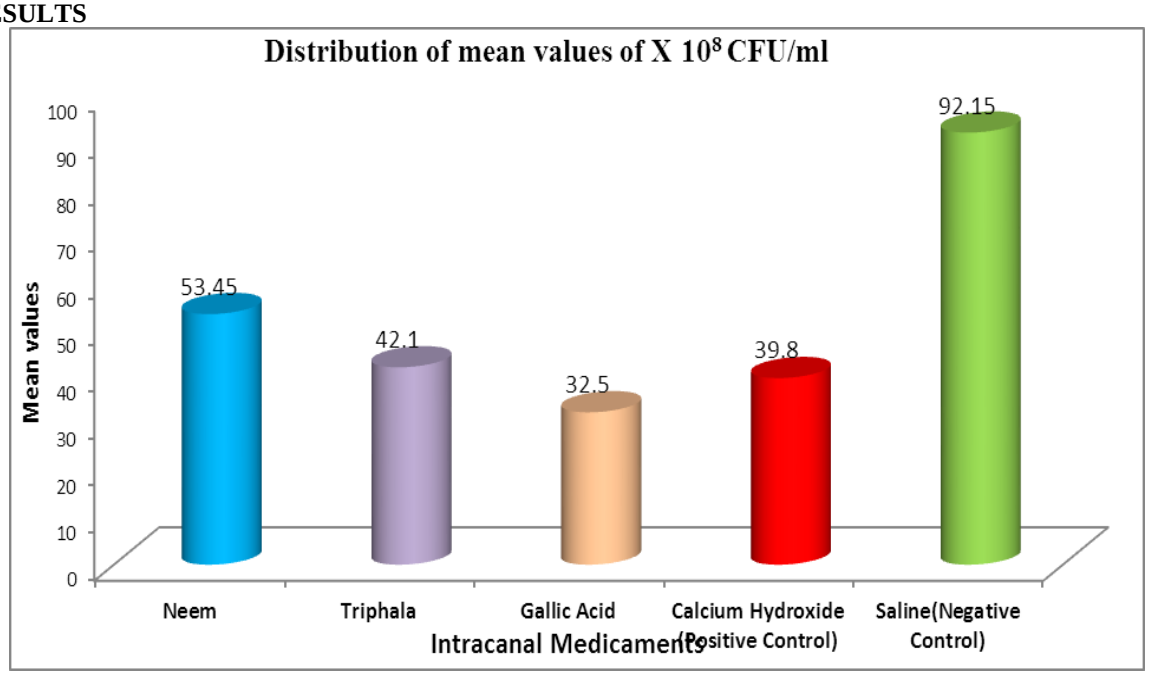


Fig no. 2: MODELS PREPARED.

4) RESULTS



POST HOC ANALYSIS

Table No. 1: Comparison of mean and SD values of values of X 10^8 CFU/ml.

		Unpaired 't' test value	'p' value and Significance
Group I V/s	**Group II**	**6.16**	**0.0012, highly significant**
	Group III	**3.06**	**0.0058, highly significant**
	Group IV	**4.30**	**0.0021 highly significant**
	Group V	**12.13**	**0.0001, highly significant**
Group II V/s	**Group III**	**3.86**	**0.0022, highly significant**
	Group IV	**0.75**	**0.547, not significant**
	Group V	**15.99**	**0.0001, highly significant**
Group III V/s	**Group IV**	**2.45**	**0.0067, highly significant**
	Group V	**19.64**	**0.0001, highly significant**
Group IV V/s	**Group V**	**14.83**	**0.0001, highly significant**

Statistical analysis

Statistical analysis was done by descriptive statistics as mean, and SD.

Student's Unpaired't' test of difference between two samples are used to compare all experimental groups and control groups.

One-way ANOVA test (Tukey-Kramer multiple comparison test) was applied to compare all groups together. Probability $p<0.05$, considered as significant Post hoc multiple comparison test was also used.

Statistical analysis software SYSTAT version 12 (By Cranes software's, Bangalore) was used to analyse the data.

5) SUMMARY

Pulpal and periapical inflammation is an immunological self defense reaction against sustained bacterial stimuli; therefore, strategies for endodontic treatment are directed toward the removal or at best the reduction of these bacteria and their by-products from the root canal system. Intracanal medicaments have been thought to be an essential step in killing the bacteria in root canals; however, in modern endodontics, shaping and cleaning may be assuming greater importance than intracanal medicaments as a means of disinfecting root canals. This is particularly true with the increasing popularity of Single Visit Endodontics as and when indicated.

Until recently, formocresol and its relatives as well as Calcium Hydroxide were frequently used as intracanal medicaments, but it was pointed out that such bactericidal chemicals used in the root canal system permeated to the whole body from the root apex and so might induce various harmful effects running the entire gamut from cell mediated immunities, allergies to carcinogenic potential. Furthermore, as these medicaments could be potentially carcinogenic, there is no indication for these chemicals in modern endodontic treatment.[8]

The more modern meaning of intracanal dressing is for a blockade against coronal leakage from the gap between filling materials and cavity wall. Calcium hydroxide has been used in dentistry for almost a century now. Calcium hydroxide has been determined as suitable for use as an intracanal medicament as it is stable for long periods, harmless to the body, and is bactericidal albeit in a limited area. It also induces hard tissue formation and is anti-inflammatory. The high pH of $Ca(OH)_2$ due to the release of hydroxyl ions in an aqueous environment causes damage to the bacterial cytoplasmic membrane and disrupts both protein structure and DNA of bacterial cells.[9] The antimicrobial action of calcium hydroxide is also related to its capacity to react with carbon dioxide in the root canal[10] and thus some CO_2-dependent bacteria do not survive in this environment. $Ca(OH)_2$ also denatures a potent endotoxin,[11] lipopolysaccharide, thereby rendering it less antigenic.[12]

In vitro and *in vivo* studies have clearly demonstrated that intracanal calcium hydroxide fails to eliminate *E. faecalis* from the infected dentine.[13] It is well documented that *E. faecalis* is the dominant microbe in persistent apical periodontitis (retreatment).[14-17] On the other hand, no other medicament has shown better *in vivo* effectiveness against *E. faecalis* either. Other microbes frequently found in retreatment cases include Gram positive facultative organisms such as *Streptococcus spp., Lactobacillus spp., Actinomyces spp., Propionibacterium spp.,* Gram-negative coliform rods, and the yeast *Candida albicans.*[18-20]

$Ca(OH)_2$ is also known to have a deleterious effect on dentin. The flexural strength of dentine might, in part, depend on an intimate link between 2 main components of dentine, the hydroxyapatite crystals and the collagenous network. The organic matrix is composed of acid proteins and proteoglycans containing phosphate and carboxylate groups (Andreasen et al. 2002). These substances act as bonding agents between the collagen network and the hydroxyapatite crystals (Andreasen et al. 2002). Rosenberg et al. (2007) measured the effect of $Ca(OH)_2$ on the microtensile fracture strength (MTFS) of teeth and found a reduction of almost 50% in the strength.

White et al. 2002 reported a 32% decrease in strength, whilst Kawamoto et al. (2008) concluded that exposure

to $Ca(OH)_2$ paste led to an increase in the mean elastic modulus of bovine dentine, thereby making it more susceptible to fracture. Grigoratos et al. (2001) too reported that treatment with $Ca(OH)_2$ reduced the flexural strength of dentine.[21]

The role of chemical disinfectant inside the root canal is to pass to and fro through the canal to the apex and thereby raise the immunity and helps in repair. $Ca(OH)_2$, due in part to its high alkalinity has the property to make the immunity go haywire and thereby may prevent repair. $Ca(OH)_2$ will lead to Cell Mediated Immunity, formation of multinucleated giant cells and granuloma formation which is one aspect of immunity. If this persists then autoimmune disease may occur. Therefore, the search for a new medicament which will do only good and no harm is always on the forefront of the cutting edge of research.

Also in order to prevent re-infection of the Root Canal System, it is imperative that the canal space continue to resist the onslaught of bacteria from either end of the continuum. Thus for the Intracanal Medicament to have a fair amount of substantivity would then be of immense help. The property of substantivity possessed by calcium hydroxide is very less as compared to chlorhexidine substantivity. This substantivity is very important as it helps in a better long term prognosis of the treatment. Thus just like in every other avenue of medicine and healing research in this arena too has headed in the direction of alternative or herbal intracanal medicaments.

For the most part, the use of systemic antibiotics is not a routine part of endodontic treatment. Administration of systemic antibiotics should be considered when infection appears to be spreading, indicating failure of local host responses, or in immune-compromised patients.[22,23] Therefore, the focus must be on local antimicrobial measures, namely chemo-mechanical preparation and disinfection.

The rationale for using Neem, Triphala and Gallic Acid as intracanal medicaments was that they showed sufficient antibacterial activity as an irrigant and at the same time there is no concrete evidence that a reduction in the hardness of the tooth tissue occurs. So we attempted with this study to evaluate their efficacy when they were used as intracanal medicaments. Propylene Glycol was used as a vehicle for Neem, Triphala and Gallic Acid.

Neem extract has been shown to have a wide spectrum of antibacterial activity against both gram positive and gram negative micro organisms. These effects have been seen against *S. mutans and E. feacalis*. High levels of antimycotic activity have been reported with extracts from different parts of Neem. It is extremely effective against *Candida*. Nimbidin a major crude bitter principle extract of Neem has been shown to be the key reason for its antibacterial and anti-inflammatory activity. The phytochemical components of *Azadirachta indica*(neem) contains saponin and phlobatanin and shows an absence of alkaloids, tannins, phenolics, glycosides, flavonoids and triterpenes. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) are 5mg and 50mg respectively for *Pseudomonas aeruginosa, Kl. ozanae, Staphylococcus aureus and Escherichia coli.*[24]

Triphala is a mixture of 3 fruits composed of dried fruits of *Emblica officinalis Gaertn(Euphorbiaceae), Terminalia belerica Linn (Combertaceae) and Terminalia chebula (Combertaceae)* in equal proportions (1:1:1) as described in Ayurvedic Formulary of India.[25] Triphala controls dental plaque from baseline, gingival inflammation and microbial growth caused by *Streptococcus mutans* and *Lactobacillus*. Triphala's activity is comparable to commonly available mouthwash Chlorhexidine.[26]

Triphala and its individual components showed antibacterial effect on both gram –positive and gram-negative bacteria, suggesting the ingress of active phytochemicals through both the bacterial cells walls.[27]

Triphala showed more potency on the endodontic micro organisms.

Gallic Acid (GA), the main compound of gallo-tannins in Galla Rhois inhibit the proliferation of oral bacterial and the *in vitro* formation of *Streptococcus mutans* biofilms. GA has inhibitory effects on the growth of cariogenic (MIC<8 mg/ml) and periodontal bacteria (MIC=1 mg/ml). Moreover, Gallic Acid significantly inhibit the *in vitro* formation of *S.mutans* biofilms (MG, 1 mg/ml; GA, 4 mg/ml; $P<0.05$).[28]

The major advantages of using herbal alternatives are affordability, accessibility, increased shelf life, low toxicity, and lack of microbial resistance reported so far.

In this study, bacteriological samples were taken from the periapical lesions of a patient with a chronic periapical abscess. Access opening was done under sterile conditions and paper points were introduced into the canal and left for 30 secs. The paper point was then extricated from the canal and kept in an Eppendorf tube along with Robertson's Cooked Meat Media and the samples collected were then sent to the laboratory for further culturing. After culturing the samples, the micro organisms found were *Pseudomonas* and *Candida albicans*

The uniqueness of this study was the bacterial model that was created in which the collection of bacteria at the end of the model simulated periapical lesion and then the effect of the intracanal medicament of the respective group was evaluated on the number of bacterial colonies present.

In this study the CFU counting method was used because in this study a periapical infection model has been replicated, in which a periapical area of bacterial growth has been created around a root apex simulating a periapical lesion. Thus using the chemical which has a proven MIC in such a case it is better to use a CFU because the bacteria found in the periapical lesion are multi-talented bacteria which have a combination of aerobic and anaerobic both, so a Direct Contact Test using a single agar plate would not have worked and hence the decision was to use CFU counting.

As expected the best performing group was the one with Gallic acid. Calcium hydroxide which is the gold standard did better than Triphala, Neem and Saline. There appeared to be no significant difference between Calcium hydroxide and Gallic Acid. There appeared to be a statistically significant difference between Gallic Acid and Triphala. Though, no difference could be found between Triphala and Calcium hydroxide, there was a statistically significant difference between the groups containing Gallic Acid and Neem and also between Calcium hydroxide and Neem. Triphala showed a better result than Neem but inferior to Calcium hydroxide. Neem failed to do as well as Calcium hydroxide, Calcium hydroxide showed a lower number of CFUs than the group containing Triphala and Neem. As expected, Normal Saline performed poorly amongst them all.

So it can be concluded that Gallic Acid could be considered as an intra canal medicament because it gave the same if not better level of disinfection as did $Ca(OH)_2$. However the deleterious effect of Gallic Acid upon radicular dentin has to be further investigated to ascertain that it is not reducing the strength of the roots. Also the bacteria that were a part of this investigation were limited and an expansive review of the action of Gallic Acid on the bacteria inhabiting the root canal is therefore necessary. How the Gallic Acid deteriorates beyond a period of 72 hours is also worthy of exploration in order to determine if this could be used as a long term Intracanal Medication.

Removal of the Intracanal Medicament often poses a challenge to the clinician and may require a special effort for its removal. Hence it should be removed in its entirety and leave no remnants behind. This aspect needs to be evaluated for Gallic Acid. A good intracanal medicament doesn't interfere with the subsequent Irrigants or even the bonding of sealers to the radicular dentin, whether or not Gallic Acid does this; needs to be found out.

A future line of action would involve Animal Testing and Clinical Trials to test the safety of Gallic Acid.

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