

Original Research

To compare the influence of various intracanal calcium hydroxide-herbal pastes on diffusion of hydroxyl ions through dentinal tubules at various time intervals – an in vitro study

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ABSTRACT

Aim: The aim of this study was to compare the diffusion ability of hydroxyl ions through dentinal tubules of different calcium hydroxide-herbal pastes and compare it with the calcium hydroxide paste prepared with saline.

Methods: A comparative study regarding the diffusion of hydroxyl ions through dentinal tubules using (1) calcium hydroxide-saline paste (2) calcium hydroxide-tulsi paste (3) calcium hydroxide-ginger paste, conducted on 60 extracted mandibular premolar teeth with a single root canal. After decoronation with a diamond disc and working length determination, the canals were prepared upto 40 k file. Teeth were distributed into three experimental groups and kept in deionised water. Change in pH was evaluated using digital pH meter at 24,72 and 168 hours. Data were analysed by ANOVA test and Post- hoc Tukey's test using IBM SPSS-20 software.

Result: Statistical analysis indicated that group I had the highest pH values at 24 and 72 hours, at 168 hours highest pH values was by group III followed by group II and group I respectively. Group I showed no change in pH values after 72 hours.

Conclusion: Herbal preparations allow the maximum ionization of calcium hydroxide and promote sustained release of the ions for a longer duration of time.

Introduction

One of the most important factors for successful endodontic therapy is complete root canal cleaning which includes removal of necrotic pulp tissue, micro-organisms, their byproducts, inorganic and organic debris.¹ One of the major causes of endodontic failure is persistent microbiological infection.² Studies also showed that the bacteria remaining in the root canal will grow and rapidly increase in number at inter-appointment duration if no antibacterial medicament is placed in the root canal.³

The role of bacteria in periradicular infection has been well known and endodontic treatment will be at higher chances of failure if microorganisms persist in the canals at the time of root canal obturation. Bacteria harboured in root canal areas such as isthmuses, ramifications and dentinal tubules may evade disinfectants. Therefore the aim of the treatment should be complete eradication of the bacteria from the root canal.²

Reduction in the bacterial count in infected root canals is achieved by a combination of measures such as mechanical cleansing and irrigation with various medicaments.³ Unfortunately, the bacteria cannot be completely eliminated by instrumentation alone and often requires supporting action of intracanal medicaments.⁴

Calcium hydroxide is the most commonly used intracanal medicament in Endodontics.⁵ This material was introduced by **Hermann in 1920** and has been largely employed in different clinical situations.⁵ The material is chemically classified as a strong base (pH approximately 12.5–12.8), its main actions come from the ionic dissociation of Ca^{2+} and OH^- ions and their effect on vital tissues, induction of hard-tissue deposition and being antibacterial.⁶ The root canal medicament should have an antimicrobial potential,⁷ ability to diffuse through dentinal tubules, biocompatibility, and ability to stimulate the process of repair on periapical

tissue.^{4,8} **Simon et al.** have demonstrated that the vehicle can exert a great influence on the release of ions.⁹ Ca(OH)₂ powder for root canal medicament has been mixed with different vehicle such as distilled water, camphorated monochlorophenol, normal saline, cresatin, glycerin, and propylene glycol. Anaesthetic solutions as well as other liquids such as herbal extract, have been frequently employed as vehicles in calcium hydroxide pastes.^{9,10}

Ocimum sanctum or Tulsi has been the pillar of holistic health system in Ayurveda, India. Various parts of the Tulsi plant have been used extensively in the treatment of several systemic diseases. The antimicrobial property of Tulsi has been tested against various microorganisms.¹¹ *Ocimum sanctum* leaves have antibacterial agents mainly in the form of essential oils. They stimulate leakage of cellular potassium and exert membrane damaging effect against gram positive and gram negative bacteria.¹¹

Zingiber officinale commonly known as **ginger** is also an antimicrobial agent which is effective, safe and economical, and has been shown to possess promising inhibitory effect on many of the oral microorganisms.¹² Its pungent oil component is polyphenolic ketone called **gingerols** which have been found to possess antibacterial and antifungal properties.¹³

Considering the antimicrobial potential of herbal components combined with calcium hydroxide, it becomes important to determine how well such pastes can promote the diffusion of ions through dentinal tubules.

So aim of this study is to compare efficacy of different calcium hydroxide pastes prepared with Saline, Tulsi extract and Ginger extract on diffusion of hydroxyl ions through dentinal tubules at different time intervals.

Material and Methods:

Sixty extracted human permanent mandibular premolars with fully formed apex with single root having single canal were collected from Oral and Maxillofacial surgery department of our college. Teeth with presence of previous endodontic treatment, pre-existing fracture or cracks, developmental anomalies or anatomical variation, external resorption, internal resorption and calcified pulp chamber or root canals were excluded from this study. Selected teeth were sterilized according to Occupational Safety and Health hazards norms (OSHA Norms) and stored in 10% formalin

until further use. The presence of single canal was confirmed by taking radiographs in mesiodistal and buccolingual directions. The entire samples were marked to standardize the root length at 12 ± 1 mm and were decoronated using a straight hand piece and tooth sectioning disc at low speed under water spray.

The patency of the canal was determined by inserting size 10 K-hand file. After seeing the tip of file in the apical foramen, working length was established by subtracting 1 mm from the length of file and was confirmed with the help of digital radiographs. Each canal was enlarged sequentially upto 2% hand 40 K-file. Periodic recapitulation was done by returning to smaller sizes to avoid blockage. Between each instrument changeover, the canal was irrigated with 1 ml of 5% NaOCl for 1 min followed by normal saline. The canals were then irrigated with 1 ml of 17% EDTA. The root canals were finally irrigated with 5 ml of normal saline to remove chemical residues, and then were dried internally with absorbent paper points.

The teeth were randomly divided into three groups according to the following calcium hydroxide pastes used as an intracanal medicament:

- Group I (n = 20): Calcium hydroxide–saline paste, prepared by mixing calcium hydroxide powder with saline in 1:2 P/W ratio.
- Group II (n = 20): Calcium hydroxide–Tulsi paste, prepared by mixing calcium hydroxide powder with Tulsi extract in 1:2 P/W ratio.
- Group III (n = 20): Calcium hydroxide–Ginger paste, prepared by mixing calcium hydroxide powder with Ginger extract in 1:2 P/W ratio.

Freshly prepared Tulsi extract and Ginger extract was mixed with Calcium hydroxide powder (1:2 P/W ratio) on mixing pad using cement spatula. The placement of the intracanal medicaments was performed by lentulospiral filler. (Fig. 1)

After the complete filling of the root canal, their openings were sealed with temporary cement. The apical foramen and root canal orifice (over the temporary cement) was sealed with epoxy resin. The teeth were placed in containers filled with 50 ml deionized water with predetermined pH (7.00) were kept in an incubator at 37°C.

Group	Time Interval	N	Mean	Std. Deviation	Minimum	Maximum
Group I	24 Hours	20	8.21	0.11	8.0	8.4
	72 Hours	20	8.34	0.10	8.2	8.5
	168 Hours	20	8.37	0.11	8.2	8.6
Group II	24 Hours	20	7.91	0.08	7.8	8.1
	72 Hours	20	8.27	0.07	8.2	8.4
	168 Hours	20	8.50	0.08	8.4	8.6
Group III	24 Hours	20	8.08	0.09	7.9	8.2
	72 Hours	20	8.26	0.10	8.1	8.4
	168 Hours	20	8.51	0.08	8.4	8.6

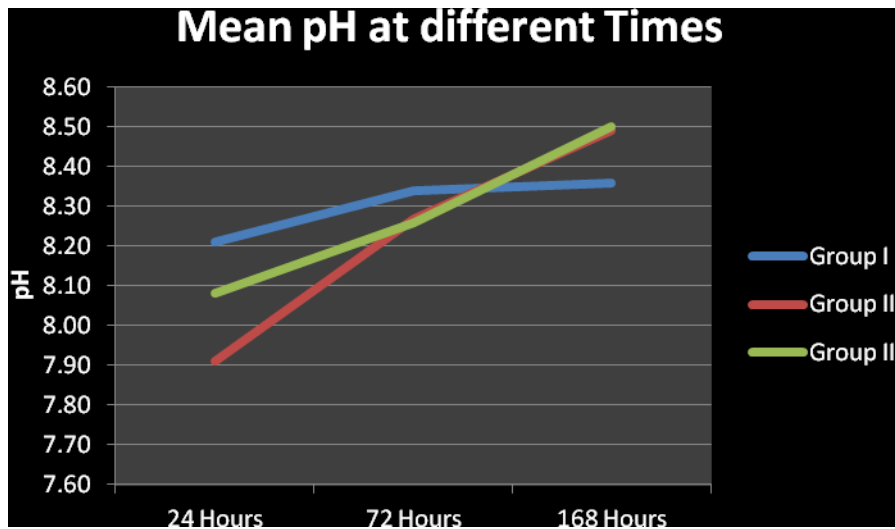
Table 1 The mean, standard deviation, minimum and maximum values of all groups

Variable	Group I	Group J	Mean Difference (I-J)	Std. Error	Sig.	Result
Group I	24 Hours	72 Hours	-.1300*	.0338	.000	Significant
		168 Hours	-.1550*	.0338	.000	Significant
	72 Hours	168 Hours	-0.025	.0338	.463	Not Significant
Group II	24 Hours	72 Hours	-.3600*	.0233	.000	Significant
		168 Hours	-.5850*	.0233	.000	Significant
	72 Hours	168 Hours	-.2250*	.0233	.000	Significant
Group III	24 Hours	72 Hours	-.1800*	.0281	.000	Significant
		168 Hours	-.4250*	.0281	.000	Significant
	72 Hours	168 Hours	-.2450*	.0281	.000	Significant

Table 2 Multiple Comparisons within each group

Time	Group I	Group J	Mean Difference	Std. Error	Sig.	Result
24 Hours	Group I	Group II	.3000*	.0299	.000	Significant
		Group III	.1300*	.0299	.000	Significant
	Group II	Group III	-.1700*	.0299	.000	Significant
72 Hours	Group I	Group II	.0700*	.0283	.017	Significant
		Group III	.0800*	.0283	.007	Significant
	Group II	Group III	.0100	.0283	.726	Not Significant
168 Hours	Group I	Group II	-.1300*	.0279	.000	Significant
		Group III	-.1400*	.0279	.000	Significant
	Group II	Group III	-.0100	.0279	.722	Not Significant

Table 3 Multiple Comparisons between the groups at different time intervals (Post Hoc Tukey Test)



Graph 1 Line diagram showing intergroup-comparison of Mean pH values at different time interval



Fig. 1 Placement of intracanal medicament using Lentulospiral filler



Fig. 2 Digital pH meter

After 24,72 and 168 hours the pH values of the solutions in the containers were measured using a digital pH meter (Fig. 2). pH meter was calibrated and standardized with standard buffer solutions at pH 4.01 and 6.86.

Data collected was entered into computer and analyzed using SPSS software. Level of significance was fixed at $p=0.05$ and any value less than or equal to 0.05 was considered to be statistically significant.

Result

In the present study, 60 single-rooted premolar teeth were used and divided into three groups, based on different vehicles used to place calcium hydroxide. At regular intervals of 24, 72 and 168 hours, the pH of the deionised water was recorded for each group.

All the values obtained from the study were tabulated and subjected to the statistical analysis using ANOVA test and Post- hoc Tukey's test using IBM SPSS-20 software, at the significance level of 0.05 (**$P \leq 0.05 = \text{Significant}$**).

Group I (calcium hydroxide-saline) showed highest mean pH value up to 72 hours. No statistically significant difference was observed in the pH value after 72 hours till 168 hours ($p < 0.05$).

Group II (Calcium hydroxide-Tulsi) and group III (Calcium hydroxide- Ginger) showed continued rise in pH values even after 72 hours, with mean pH of group III being highest at 168 hours followed by group II and group I respectively. [table 1,2,3][graph 1]

All the herbal preparations showed an increase in the pH of the solution upto 168 hours, both Tulsi and Ginger paste did not allow the decrease of the pH after 72 hours, compared to calcium hydroxide-saline paste.

Discussion:

A medicament is an antimicrobial agent that is placed inside the root canal between treatment appointments in an attempt to destroy remaining microorganisms and prevent reinfection (**Weine 2004**).¹⁴

Thus, they may be utilized to kill bacteria, reduce inflammation (and thereby reduce pain), help eliminate apical exudate, control inflammatory root resorption and prevent contamination between appointments.¹⁵ Calcium hydroxide paste is one of the main root canal medicament used in endodontics.⁷ The vehicle required to make the paste of calcium hydroxide may well increase its suitability as a medicament by allowing maximum ionisation and diffusion of hydroxyl ions.¹⁶

The present study was conducted to evaluate the diffusion of hydroxyl ions through dentinal tubules by using calcium hydroxide-saline, calcium hydroxide-tulsi and calcium hydroxide-ginger extract paste as an intracanal medicament on human extracted teeth in an in-vitro condition as the extent and measuring the diffusion of hydroxyl ions through dentinal tubules was not possible in vivo due to practical and moral limitations.

In the present study, the diffusion of hydroxyl ions through dentinal tubules was calculated as change in pH values of deionized water in which sample were kept.

In the present study, the Mean pH value of control group i.e.

Calcium hydroxide-saline paste (group I) was more significant than Calcium hydroxide-Tulsi paste (group II) and Calcium hydroxide- Ginger paste (group III) at 24 and 72 hours, but at 168 hours Mean pH value of Calcium hydroxide-Tulsi paste (group II) and Calcium hydroxide-Ginger paste (group III) was significantly higher than Calcium hydroxide-saline paste (group I). Similar results were observed in study of **Victor Eduardo de Souza Batista et al. (2014)**¹⁷ who found that, in Group Calcium hydroxide-saline, the highest pH value was observed at periods of 3 hours and 24 hours. There was a significant decrease in pH between 24 and 72 hours, yet after this period the pH values showed no change and remained stable up to 30 days.¹⁷ However A gradual reduction of the pH was observed throughout the test period in the study of **Semra Çalt et al. (1999)**¹⁸ and **Fuss Z et al. (1996)**¹⁹

Priyanka Dausage et al. (2017)²⁰ compared diffusion of ions from Calcium Hydroxide with various herbal pastes through dentin and observed that hydroxyl ions from Calcium hydroxide-Tulsi paste diffused through the dentinal tubules and presented a better ability to alkalize external root surface after 72 hours when compared with saline. **Valera MC et al. (2014)**²¹ compared antimicrobial activity of Calcium Hydroxide-saline solution with Calcium Hydroxide associated with glycolic ginger extract as intracanal medicament for 14 days. They observed that calcium hydroxide, pure or associated with glycolic ginger extract was effective on the micro-organisms tested and however the peak action of ginger occurs at nearly 7 days (168 hours), with a loss of activity after this period.

In the present study, the mean pH value of Calcium hydroxide- Ginger paste (group III) was significantly higher than Calcium hydroxide- Tulsi paste (group II) at 24 hours, but at 72 hours mean pH value of Calcium hydroxide-Tulsi paste (group II) was more than Calcium hydroxide- Ginger paste (group III). At 168 hours mean pH value of Calcium hydroxide- Ginger paste (group III) was higher than Calcium hydroxide-Tulsi paste (group II). There was no statistically significant difference between pH values of group II and group III at 72 and 168 hours.

The prolonged release of hydroxyl ions from Calcium hydroxide-Herbal pastes in our study may be due to the viscous nature of the vehicle, which releases calcium and hydroxyl ions more slowly because of their higher molecular weights.

Conclusion:

Herbal calcium hydroxide preparations showed an increase in the pH of the solution up to 168 hours, inferring that they allow ionization of calcium hydroxide and promote sustained release of the ions for longer duration.

These calcium hydroxide herbal medicaments can be used in cases where medicaments are meant to be placed for longer duration to be effective. As a result, number of appointments is reduced.

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