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### RESEARCH ARTICLE

#### COMPARATIVE EVALUATION OF DIFFUSION OF FOUR DIFFERENT COMMERCIALY AVAILABLE CALCIUM HYDROXIDE THROUGH DENTINAL TUBULES OF RETREATED ROOT CANAL: AN IN VITRO STUDY

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#### Abstract

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#### Introduction:-

The ultimate intent of a root canal therapy is directed towards the prevention and control of pulpal and periradicular infection. The curtailment of endodontic microbiota during root canal therapy is accomplished by various sequences of antimicrobial approaches in the form of various canal preparation and irrigation methods. Nevertheless it is recognized that chemo-mechanical instrumentation alone is unable to completely disinfect the root canal system<sup>1</sup>. In study done by Tang G et al it was stated that the bacteria which remains in the root canal after instrumentation proliferates in between appointment<sup>2</sup>. So to deal with such situation antimicrobial agents such as Calcium Hydroxide could be used as an intracanal medicament also breach in the treatment protocol can lead to endodontic failures with noticeable periapical changes in such situation intracanal medication in the form of calcium hydroxide is to be given.

Calcium hydroxide was introduced to dentistry by Herman in 1920<sup>3</sup>. Today it is used widely as an intracanal medicament in the field of Endodontics<sup>4,5</sup>. Its antimicrobial property is directly related to diffusion of hydroxyl ion (OH<sup>-</sup>) ion resulting in an increase in the pH<sup>6</sup>. According to the study done by Estereia C et al there is a direct relation among concentration and velocity of ionic liberation with antimicrobial action<sup>7</sup>.

There are basically three types of vehicles that have been used namely (1) Viscous vehicle formed by using either saline or ringer solution (2) Viscous vehicle like glycerine or poly ethylene glycol and (3) Oil based vehicle that contains olive oil and camphor<sup>8</sup>.

Calcium hydroxide is considered to be gold standard due to its bacteriocidal activity neutralizing effect on remaining tissue debris in root canal system and its continuous release of (OH<sup>-</sup>) ions to promote an alkalizing osteogenic environment in surrounding tissue. According to various studies it has been shown that the effectiveness of calcium hydroxide and the pH are always directly related to each other. So higher the pH more effective the material is.

Different formulations have different chemical composition and alkalizing potential, Hence the purpose of this study was to comparatively evaluate the hydroxyl ion diffusion of four different commercially available calcium hydroxide through dentinal tubules of retreated root canals.

### Materials and Methods:-

After obtaining permission from the institutional scientific and ethical committee of BVDUDCH 50 freshly extracted premolars having single root were obtained from the department of oral and maxillofacial surgery from our institute. The teeth collected were thoroughly cleaned of any calculus or periodontal tissue; they were also checked for any defect or any microcracks and stored in the saline for the further experimental use.

#### Inclusion criteria:

Premolar extracted for orthodontic purpose from patient 18-25 years of age ,having single root with vertucci type I canal configuration were selected for this study. And the type of canal configuration was confirmed by using a radiograph.

#### Exclusion criteria:

Out of the collected samples those premolars having any sort of restoration, cracks or defects were excluded from the study.

#### Specimen preparation:

Samples were first mechanically cleaned using an ultrasonic scaler and were carefully examined under the microscope (Zumax 12.5X ) magnification to check for the presence of any cracks or defect if any. Radiograph was taken both in mesiodistal and buccolingual direction to confirm the presence of single and straight canal. Access opening was carried out using (BR 47) Round diamond point, followed by deroofting of the pulp chamber using (EX-24) diamond point .The canal orifice was enlarged using the Gates Glidden drill. After that the sample teeth were decoronated using Diamond Disc (Bosh) along the cement enamel junction.Next canal patency was checked using the number 10 K file, proper glide path was established and working length was determined using digital radiograph by keeping working length 1mm short of the radiographic apex and standardized to nearly the same length (13± 1mm ) for all the specimen.

#### Canal preparation:

Root canal were instrumented using crown down technique with Protaper universal NiTi rotary instrument (Densply Tulsa Dental) till # F3 using a X-SMART Densply electric motor with a 16:1 reduction contra –angled hand piece Master apical filling was done upto apical ISO size 30 for all the canals and in between each subsequent filing proper lubrication with RC Help (Prime Dental) and irrigation was carried out using 5ml of 5% of sodium hypochlorite solution to remove all the debris present in canals for all the samples.

#### Root canal filling:

Then the sample were dried using the paper point, and were coated with Zinc Oxide Eugenol sealer with lentilospiral and obturation was carried with # F3 Gutta percha using the cold lateral condensation technique, each specimen was then placed in separate container containing cotton wool soaked in unbuffered isotonic saline placed in oven at 37degree Celsius with 100% humidity for 7 days to allow the sealer to set.

#### Sample/ Group preparation:

After allowing the sealer to set for 7 days obturated gutta percha was removed using RC solve and Pro Taper universal Retreatment file with # D3 along with small amount of dentine To maintain the uniformity among the samples the dentinal thickness in apical middle and coronal third of all the samples were measured by taking radiograph in mesiodistal and buccolingual direction and the samples were randomly distributed into 5 group containing 10 teeth each.

Group I	Root canal not filled with any material
Group II	Root canal filled with calcium hydroxide powder mixed with distilled water
Group III	Root canal filled with RC Cal
Group IV	Root canal filled with Metapex
Group v	Root canal filled with Vitapex

The access cavities and the apical opening of all the teeth were sealed with amalgam restoration and kept in small vials containing 30ml of distilled water at 37degree Celsius with 100% humidity for 7, 10, 14, 30 days.

**pH measurement:**

The change in pH of the distilled water was determined using pH meter at different time interval of 7<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> day respectively and for each measurement first the pH meter is calibrated and after each use it is dried using the blotting paper.

**Result:-**

The pH of the sample were recorded using ph meter. The pH of all the samples were calculated at the interval of 7, 10, 14 and 30 days and from the obtained data it was noted that there was gradual increase in the pH for Metapex and Vitapex and on the other hand Calcium Hydroxide power mixed with saline and RC cal showed gradual increase in pH upto 10<sup>th</sup> day after which from 14<sup>th</sup> day there was gradual decrease in the pH

The data was described for all the groups on 7<sup>th</sup>, 10<sup>th</sup>, 14<sup>th</sup> and 30<sup>th</sup> day in form of mean, median, mode and standered deviation in Table 1. and post hoc comparison Table 2

**Discussion:-**

It is a white odourless powder with chemical formulation of  $\text{Ca}(\text{OH})_2$ . It shows high degree of solubility when it comes in direct contact with fluid, leading to rapid release of calcium and Hydroxyl ions. This property of rapid release of Calcium and Hydroxyl ion is considered to be desirable in the clinical situation making it effective for the short inter appointment disinfection as root canal medicament<sup>9</sup>. These Hydroxyl ion are highly reactive to several bio molecules, they have a lethal effect on these bio molecules /bacteria by damaging their cytoplasmic membrane which causes denaturation of protein leading to the damage of nucleic acid and ultimately damage of DNA<sup>10</sup>

Along with its antimicrobial property Calcium Hydroxide have high remineralizing potential. This is mostly attributed to its elevated pH which is responsible for the activation of enzyme Alkaline Phosphatase which is a hydrolytic enzyme. It is basically responsible for liberation of inorganic phosphates which once free tends to react

with Calcium ions present in the blood stream to form a precipitate of calcium phosphate in the organic matrix, now this formed precipitate is considered to be the molecular unit of Hydroxyl appetite<sup>11</sup>. According to Maisto and Capurro (1964) alteration in the mineralization potential occurs when calcium ions come into contact with carbon dioxide or carbonate ions leading to the formation of calcium carbonate<sup>12</sup>. Studies have shown that it takes around 7<sup>th</sup> day for Calcium Hydroxide for the formation of mineralizing tissue after contact with the connective tissue.

One of the cardinal factors in relation for determining the therapeutic actions of the Calcium hydroxide is the time. In the present study it was noted that there was a gradual increase in the pH for all groups except the control group which did not contain any formulations. On 7<sup>th</sup> and 10<sup>th</sup> day the highest pH was noted for RC Cal with the mean of (12.6) on 14<sup>th</sup> day the highest pH was noted for Metapex and Vitapex with the mean value of (10.49 and 10.58) respectively and there was gradual decrease in pH noted among the various comparative groups on 30<sup>th</sup> day. Mustafa et al did a study comparing diffusion of various Calcium hydroxide formation through dentinal tubules in similar retreatment condition and found the similar results<sup>13</sup>

The difference in the rate of diffusion could be explained by the fact that various types of substances are added to Calcium Hydroxide to enhance properties like flow, antimicrobial activity, radio opacity. These substances which act as vehicles play an important role in the velocity of ionic dissociation. According to Fava (1991) ideal vehicle should have no adverse effect on hard tissue deposition, have gradual and slow ionic release, slow diffusion in the tissue with low solubility. Generally there are three types of vehicles used including Aqueous, Viscous and oily<sup>14</sup>

In the present study all the type vehicles are compared for their Hydroxyl ion diffusion through the dentinal tubules. Where Group II containing Calcium Hydroxide mixed with saline showed increase in pH from 9.17 on the 7<sup>th</sup> day to 10.13 on the 10<sup>th</sup> day, followed by gradual decrease in pH on 14<sup>th</sup> and 30<sup>th</sup> day with the mean value of (9.89 and 7.34) respectively. This could be explained by the fact that Aqueous vehicles tend to show higher degree of solubility and dissociation making it possible for the aqueous vehicle to achieve high pH values in the initial periods. Our results were consistent with the result of study done by Grover et al who noted that there was a rapid increase in pH from 6.5 to 11.8 at 24 hrs, 10.4 on 7<sup>th</sup> day, 9.4 on 15<sup>th</sup> day and 7.6 on 30<sup>th</sup> day. However since the alkalinity of these type of vehicles are not maintained for a period of 14 days, these medications require frequent changing.

The Viscous vehicles being water soluble substances tend to slowly release the Calcium and Hydroxyl ions and the time taken by their dissociation is extended. This is mostly due to their high molecular weight which minimizes the rate of dispersion, because of this prolonged nature of dissociation and low solubility it stays in the canal for a longer period of time. Hence the number of appointments is reduced. According to the results obtained in our study RC Cal showed the highest pH on the 7<sup>th</sup> day (12.6) on 10<sup>th</sup> day (10.15), 14<sup>th</sup> day (9.8) and on 30<sup>th</sup> day. Our results were in favour of the study conducted by Punit et al who found that release of Hydroxyl ion was more for RC Cal in comparison to that of Calcium Hydroxide with Saline this is mainly attributed as there is sudden rise in the pH after 24<sup>th</sup> hour on the other hand for RC Cal there is gradual increase in the pH with decrease in pH after 15 days to 30<sup>th</sup> days<sup>15</sup>

Metapex and Vitapex both are oil based formulations. They are formed from Calcium Hydroxide with Idoform with silicon oil. These type of oil based vehicles are said to be non water soluble hence promoting low solubility and diffusion within the tissue, such type of formulations may remain in the root canal for a longer time. Various oils are used for the formation of such types of vehicles they include Olive oil, Silicon oil, Camphor, Metacresylacetate and some fatty acids such as Oleic, Linoleic and Isostearic acid. These formulations tend to reach their highest pH after 7 days. According to the results obtained in our study. Metapex and Vitapex lowest pH on the 7<sup>th</sup> day with the mean of (10.04 and 10.33) respectively which was found to gradually increase and highest pH values were noted on 14<sup>th</sup> day with the mean of (8.75), statistically there was no significant difference found in the release of hydroxyl ions, our results were consistent with study conducted by Mustafa et al who checked for the diffusibility of Calcium Hydroxide powder and Metapex. The gradual decrease in pH was seen in all the comparable formulations and no statistical difference was found in hydroxyl ion release of both Metapex and Vitapex<sup>16</sup>

Very few studies to our notice have been conducted to evaluate the dissolution capacity of Calcium Hydroxide in the retreated cases, in which the remaining gutta percha and sealer could impact the penetration and diffusion of Hydroxyl ions. Hence we intended to remove sound dentine during the removal of sealer and gutta percha. We were not able to standardize the method in terms of dentinal tubule size, length of tubules, diameter and density of tubules

which are considered to be influential in determining permeability and diffusibility of Calcium Hydroxide<sup>17,18</sup>. Many different methods namely atomic absorption, spectrophotometer, potentiometry and colorimetric can be used for evaluation of Calcium Hydroxide diffusibility<sup>19,20</sup>. However no comparison among results obtained by using these methods should be done with ours as there could be great variability in experimental conditions. The pH measurement method was used in our study because it is easy to perform, easy to replicate and obtained results are equally reliable.

### Conclusion:-

Both freshly mixed Ca(OH)<sub>2</sub> powder with saline and RC Cal has higher pH than Metapex and Vitapex on both 7<sup>th</sup> and 10<sup>th</sup> day. For Metapex and Vitapex highest pH was observed on the 14<sup>th</sup> day. There was gradual decrease in the pH of freshly mixed Ca(OH)<sub>2</sub> and RC Cal after 7<sup>th</sup> day. Metapex and Vitapex showed gradual increase in pH and highest pH was noted on 14<sup>th</sup> day. The gradual decrease in pH was noted in all the groups on 30<sup>th</sup> day statistically no difference was found among Metapex and Vitapex.

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Nil

### Conflict of Interest:

No conflict of interest

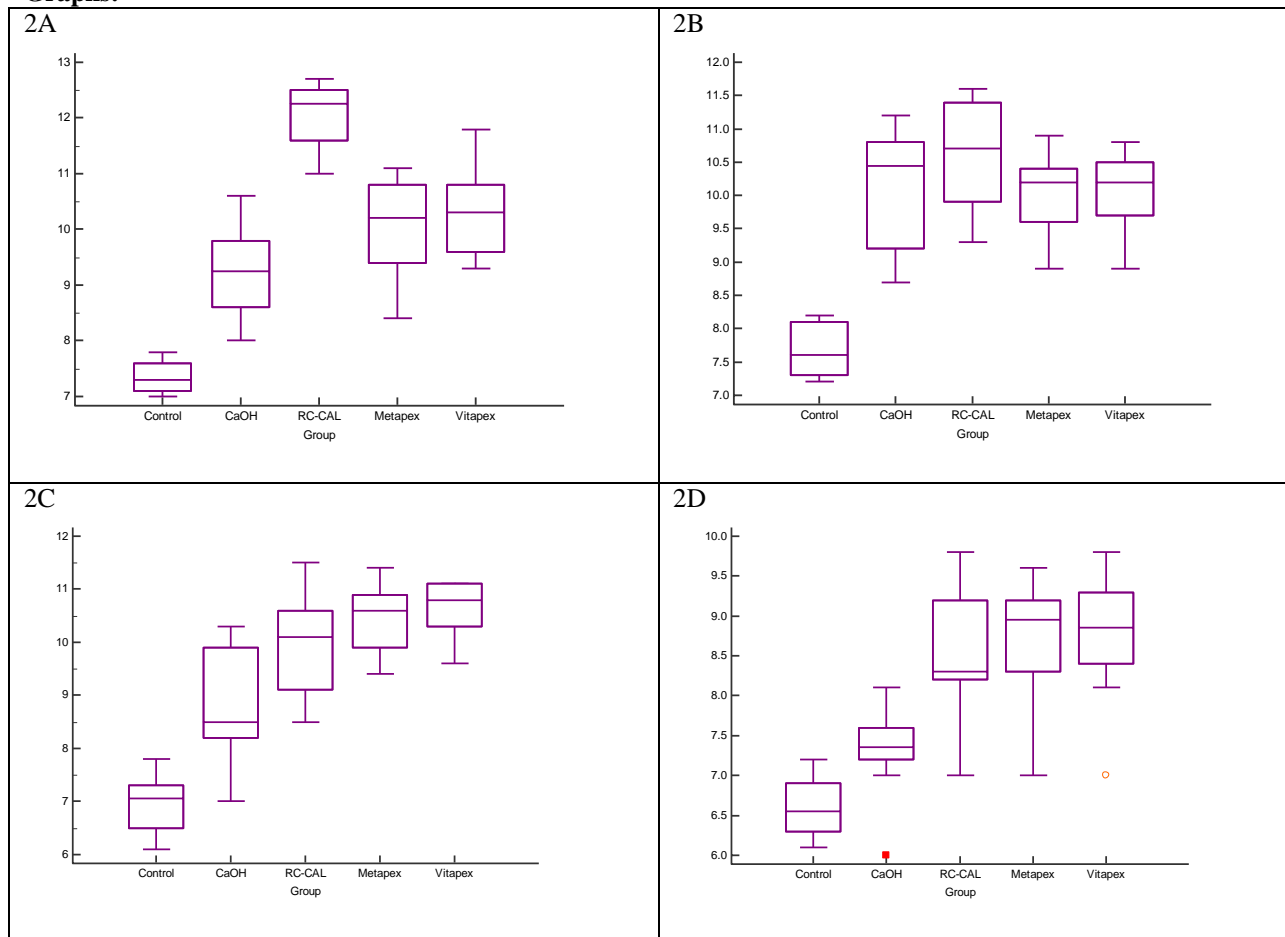
**Table 1:-**

		Group					
		Control	CaOH	RC-CAL	Metapex	Vitapex	Total
7 days	N	10	10	10	10	10	50
	Median	7.30	9.25	12.25	10.20	10.30	9.80
	Mean	7.35	9.17	12.06	10.04	10.33	9.79
	SD	0.26	0.85	0.59	0.95	0.82	1.71
	SEM	0.08	0.27	0.19	0.30	0.26	0.24
10 days	N	10	10	10	10	10	50
	Median	7.60	10.45	10.70	10.20	10.20	10.15
	Mean	7.68	10.13	10.64	10.12	10.08	9.73
	SD	0.38	0.91	0.78	0.60	0.58	1.24
	SEM	0.12	0.29	0.25	0.19	0.18	0.18
14 days	N	10	10	10	10	10	50
	Median	7.05	8.50	10.10	10.60	10.80	9.85
	Mean	6.96	8.73	9.89	10.49	10.58	9.33
	SD	0.52	1.17	0.98	0.62	0.54	1.57
	SEM	0.16	0.37	0.31	0.20	0.17	0.22
30 days	N	10	10	10	10	10	50
	Median	6.55	7.35	8.30	8.95	8.85	8.15
	Mean	6.58	7.34	8.51	8.75	8.75	7.99
	SD	0.39	0.59	0.78	0.77	0.83	1.11
	SEM	0.12	0.19	0.25	0.24	0.26	0.16

Original:  
Table 2:-

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
7 days	Control	CaOH	-1.82	0.33	<0.0001	-2.48	-1.16
		RC-CAL	-4.71	0.33	<0.0001	-5.37	-4.05
		Metapex	-2.69	0.33	<0.0001	-3.35	-2.03
		Vitapex	-2.98	0.33	<0.0001	-3.64	-2.32
	CaOH	Control	1.82	0.33	<0.0001	1.16	2.48
		RC-CAL	-2.89	0.33	<0.0001	-3.55	-2.23
		Metapex	-0.87	0.33	0.011	-1.53	-0.21
		Vitapex	-1.16	0.33	0.001	-1.82	-0.50
	RC-CAL	Control	4.71	0.33	<0.0001	4.05	5.37
		CaOH	2.89	0.33	<0.0001	2.23	3.55
		Metapex	2.02	0.33	<0.0001	1.36	2.68
		Vitapex	1.73	0.33	<0.0001	1.07	2.39
	Metapex	Control	2.69	0.33	<0.0001	2.03	3.35
		CaOH	0.87	0.33	0.011	0.21	1.53
		RC-CAL	-2.02	0.33	<0.0001	-2.68	-1.36
		Vitapex	-0.29	0.33	0.383	-0.95	0.37
Vitapex	Control	2.98	0.33	<0.0001	2.32	3.64	
	CaOH	1.16	0.33	0.001	0.50	1.82	
	RC-CAL	-1.73	0.33	<0.0001	-2.39	-1.07	
	Metapex	0.29	0.33	0.383	-0.37	0.95	
10 days	Control	CaOH	-2.45	0.30	<0.0001	-3.06	-1.84
		RC-CAL	-2.96	0.30	<0.0001	-3.57	-2.35
		Metapex	-2.44	0.30	<0.0001	-3.05	-1.83
		Vitapex	-2.40	0.30	<0.0001	-3.01	-1.79
	CaOH	Control	2.45	0.30	<0.0001	1.84	3.06
		RC-CAL	-0.50	0.30	0.099	-1.12	0.10
		Metapex	0.01	0.30	0.974	-0.60	0.62
		Vitapex	0.05	0.30	0.869	-0.56	0.66
	RC-CAL	Control	2.96	0.30	<0.0001	2.35	3.57
		CaOH	0.51	0.30	0.099	-0.10	1.12
		Metapex	0.52	0.30	0.092	-0.09	1.13
		Vitapex	0.56	0.30	0.071	-0.05	1.17
	Metapex	Control	2.44	0.30	<0.0001	1.83	3.05
		CaOH	-0.01	0.30	0.974	-0.62	0.60
		RC-CAL	-0.52	0.30	0.092	-1.13	0.09
		Vitapex	0.04	0.30	0.895	-0.57	0.65
Vitapex	Control	2.40	0.30	<0.0001	1.79	3.01	
	CaOH	-0.05	0.30	0.869	-0.66	0.56	
	RC-CAL	-0.56	0.30	0.071	-1.17	0.05	
	Metapex	-0.04	0.30	0.895	-0.65	0.57	
14 days	Control	CaOH	-1.77	0.36	<0.0001	-2.50	-1.04

**Original:  
Graphs:**



**Legends:**

- 1A: Sample
- 1B: sample soaked in saline after performing root canal
- 1C: Sample soaked in distil water with intracanal medicament
- 1D: Measurement of pH using pH meter
- 2A: Median graph on 7<sup>th</sup> day
- 2B: Median graph on 10<sup>th</sup> day
- 2C: Median graph on 14<sup>th</sup> day
- 2D: Median graph on 30<sup>th</sup> day

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