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Original Article

Dentistry Section

Comparative Evaluation of Fracture Resistance of Root Dentin Treated with *Calendula Officinalis L.* and Calcium Hydroxide as Intracanal Medicaments- An In vitro Study

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ABSTRACT

Introduction: Intracanal Medicaments (ICMs) play a major role in disinfection of root canal system. The use of interappointment ICM during endodontic treatment may affect the mechanical properties of dentin which results in decreased fracture resistance of teeth. The use of synthetic medicament is associated with many limitations such as antibiotic overdose, side effects and cytotoxic reactions. In order to overcome this, recent research has been directed towards herbal ICMs with better efficacy and lesser side effects.

Aim: To evaluate the effect of a novel herbal ICM *Calendula officinalis L.* (CO) on fracture resistance of root dentin in comparison to Calcium Hydroxide (CH).

Materials and Methods: This in vitro study was conducted in the Department of Conservative Dentistry and Endodontics, SRM Dental College and Hospital, Ramapuram, Chennai, Tamil Nadu. Thirty freshly extracted single rooted human premolar teeth were selected for the in vitro study and randomly assigned into three groups: Group 1: No medication (Control group) (n=10), Group 2: CH (n=10), Group 3: CO (n=10). The samples were decoronated and biomechanical preparation was done followed by placement of respective ICMs in the root canal space, sealed with glass ionomer cement and immersed in saline for a storage period of 7 and 14 days. Each group was subdivided into 5 teeth, depending on the storage period. After each storage period, ICMs were removed and samples were subjected to fracture resistance test using universal testing machine. Data were analysed using One-way Anova followed by Tukey HSD post-hoc test with level of statistical significance set at $p<0.05$.

Results: On evaluation of compressive strength, CO group showed more fracture resistance compared to CH group on 7th day and no statistical significant differences were seen between CO and CH groups on 14th day.

Conclusion: CO can be efficiently used as an alternative to CH because of its low toxicity and increased resistance to fracture.

Keywords: Disinfection, Endodontic treatment, Universal testing machine

INTRODUCTION

The primary objective of endodontic therapy is to remove microorganisms from the root canal system. Chemomechanical preparation removes most of the irritants; however, total elimination is not achieved due to complex root canal anatomy, accessory and lateral canals [1]. In situations where infections are resistant to regular treatment and therapy fails due to presence of pain or weeping canals, the need for medication arises [2]. A wide range of ICMs have been used and classified on the basis of chemical composition. These are classified into CH, phenolic compounds which include eugenol and camphorated monochlorophenol, aldehydes, halides which include potassium iodide, antibiotics and other combinations [3]. CH, the widely used ICM with a high pH destroys and changes the lipopolysaccarides of bacteria present in the cell wall. But it was proven to be ineffective in killing *E.faecalis*, as the beneficial effects of its alkaline pH could not be maintained in the presence of this microbe. Further, studies have proven that it causes root resorption and decreases fracture resistance of roots when kept as medicament for longer periods [4].

Recent trend towards the use of biologic medication extracted from natural plants has gained momentum to negate the cytotoxic reactions of most of the commercial ICMs. CO, commonly known as 'Pot Marigold' belongs to the family Asteracea [5]. The parts of the plant and their dried flowers which are commonly used as spices are recognised to be safe by the Food and Drug Administration (FDA) and Flavours and Extracts Manufacturers Association (FEMA) [6]. Traditionally, it is used topically as a natural anti-inflammatory medicine for poorly healing wounds, leg ulcers, burns and scalds, bruises, boils, and rashes. CO exerts several therapeutic effects such as antibacterial, antifungal, antiviral, anti-HIV, antioxidant, anti-inflammatory, analgesic, hepatoprotective, cardioprotective, gastroprotective and wound healing properties [7]. Singh M Bagewadi A, showed the efficacy of CO gel as a therapeutic agent for oral leukoplakia and concluded that it can be an effective alternative to conventional treatment modality [8]. Babaee N et al., evaluated the effect of CO mouthwash in oral mucositis of head and neck cancer patients undergoing radiotherapy and proved that CO decreases inflammation [9].

Efstratiou E et al., showed the efficacy of CO against certain clinical microbes and it was proved to be effective against *E.faecalis* and *Candida albicans*, which are most predominantly seen in root canal system [10]. However, the effect of CO as ICM on fracture resistance of root dentin is unknown. Hence, this in vitro study was undertaken to compare the efficacy of CO on the fracture resistance of root dentin with CH at 7 and 14 days.

MATERIALS AND METHODS

This in vitro study was carried out in January 2019 in the Department of Conservative Dentistry and Endodontics, SRM Dental College and Hospital, Ramapuram, Chennai for a period of one month. The study protocol was approved by the Institutional Ethical Committee. (SRMU/M&HS/SRMDC/2020/PG/006).

Calendula officinalis L. (CO) Medicament Preparation

The dried flower petals of CO were procured (Organic Bioherbs, USA) and powdered using a blender. The powder was added to 100 mL of sterile water in a 250 mL conical flask. The flask was then covered with aluminum foil and kept in a reciprocating shaker for 24 hours with continuous agitation (150 rpm) for thorough mixing. It was then filtered using muslin cloth followed by Whatman No. 1 filter paper in a micro-fluid filtration unit [11]. The resultant extract was lyophilised using Freeze drier (Martin Christ, Germany) at -40°C for 24 hours. Lyophilisation was done to remove the water and stabilise the extract so that it can retain satisfactory pharmacological activity during long-term storage [12]. The lyophilised powder was stored at 4°C and brought to room temperature before application. The powder was later mixed with saline and used as ICM for the study.

Evaluation of Fracture Resistance

Grouping and Sample Preparation

Thirty freshly extracted single rooted mature human premolar teeth with fully formed apices, and without root caries, resorption or fracture, were selected for the study and were stored in normal saline. The teeth were sorted and randomly assigned to three groups of 10 teeth each; group 1- No medicament, group 2- CH (ApexCal, Ivoclar Vivadent, Schaan, Liechtenstein) and group 3- *Calendula officinalis L. (CO)*.

The teeth were sectioned so as to obtain a standardised root length of 14 mm using a diamond saw under coolant. Working length was then determined using 15 no. K file (Mani Inc., Japan) extending just beyond the apical foramen and by subtracting 1 mm from the length of the file. Biomechanical preparation was done till 20 no. K file followed by ProTaper rotary instrumentation upto a master apical file F3 using a torque and speed-controlled electric motor. Irrigation was done using 3 mL of 3% Sodium hypochlorite (NaOCl) (TRU LON, Jayna industries, Ghaziabad, India) between each successive files. Root canals were rinsed with saline as a final flush and dried using paper points. In group 2 and 3, the respective ICMs were applied to the root canal spaces using a sterile lentulospiral (Dentsply Maillefer, Ballaigues, Switzerland) in a slow speed hand piece upto the cementoenamel junction level. Later, the access openings of all teeth were sealed with glass ionomer cement (GC Company, Europe) and apical sealing was done with flowable composite (Tetricflow, Ivoclar Vivadent, Schaan, Liechtenstein). Then the samples were immersed in saline. Each group was subdivided into 5 teeth in each, depending on storage period for 7 days and 14 days, respectively. At the end of the storage period, the ICM was removed and finally irrigated with 0.2% saline. The apical root ends were embedded in 7 mm acrylic resin blocks exposing 7 mm of the coronal end of each root [Table/Fig-1]. Fracture resistance of each 7 mm root cylinder was tested using Universal Testing Machine (UTM) (TSI TECH SOL, Chennai, India) [Table/Fig-2]. A loading fixture was lowered until the tip rested on root cylinder. Then, a vertical loading force was applied at a crosshead speed of 1 mm/min until the root cylinder was fractured. The load at fracture was measured and expressed in Newton units [1].

[Table/Fig-1]: Representative samples.

[Table/Fig-2]: Fracture resistance test using Universal testing machine.

STATISTICAL ANALYSIS

The data were analysed statistically using Statistical Package for Social Sciences (SPSS) software version (V.2.2). They were assessed for normality and homogeneity of variances using Shapiro Wilk test and Levines test, respectively. Since the data was normal in distribution and variances were homogenous in nature, standard parametric tests were performed (One-way ANOVA followed by Tukey HSD post-hoc test). A p-value of 0.05 or less was considered statistically significant.

RESULTS

Fracture Resistance

Results showed that the mean peak load of fracture of control group (group 1) was higher in both 7 days and 14 days specimen. In group 2, i.e., the CH group the mean peak load of fracture was 270.97±24.76 on day 7, whereas in the CO group it was 374.37±36.44. On Day 14, the mean peak load of fracture in group 2 and 3 decreased to 227.05±13.07 and 330.35±10.82, respectively. The intergroup comparison showed a significant difference between the groups at day 7 and 14 [Table/Fig-3]. The mean peak load of fracture for both the experimental groups were statistically significant at 7 days time interval ($p<0.05$) [Table/Fig-4]. On Day 14, there was no statistically significant difference seen between CO and CH groups. Both the experimental groups showed decrease in fracture resistance on 14th day ($p>0.05$) [Table/Fig-5].

Groups	At 7th day		p-value (ANOVA)	At 14th day		p-value (ANOVA)
	Mean	Standard deviation		Mean	Standard deviation	
Group 1 (No ICM)	443.11	33.77	<0.001	382.50	21.10	<0.001
Group 2 (CH)	270.97	24.76		227.05	13.07	
Group 3 (CO)	374.37	36.44		330.35	10.82	

[Table/Fig-3]: The Mean fracture resistance values for each group at 7 and 14 days. No ICM: No Intracanal medicament; CH: Calcium hydroxide; CO: *Calendula officinalis L*; p-value <0.05 is considered significant

DISCUSSION

For complete eradication of bacterial toxins, biomechanical preparation alone is not sufficient. Antimicrobial agents in the form of irrigants and ICMs are needed in disinfecting the root canal system

Type of medicament (i)	Type of medicament for comparison (j)	Mean difference (i-j)	Standard error	Significance (p-value)
Group 1 (No ICM)	Group 2 (CH)	172.13	20.273	0.000 (S)
	Group 3 (CO)	68.73	20.273	0.014 (S)
Group 2 (CH)	Group 1 (No ICM)	-172.13	20.273	0.014 (S)
	Group 3 (CO)	-103.40	20.273	0.001 (S)
Group 3 (CO)	Group 1 (No ICM)	-68.73	20.273	0.000 (S)
	Group 2 (CH)	103.40	20.273	0.001 (S)

[Table/Fig-4]: Descriptive statistics showing intergroup comparison at Day 7 using Tukey's post-hoc analysis. (p-value <0.05 is considered significant).
S: Significant difference; No ICM: No Intracanal medicament; CH: Calcium hydroxide; CO: *Calendula officinalis L*

Type of medicament (i)	Type of medicament for comparison (j)	Mean difference (i-j)	Standard error	Significance (p-value)
Group 1 (No ICM)	Group 2 (CH)	135.54	25.517	0.001 (S)
	Group 3 (CO)	72.05	25.517	0.038 (S)
Group 2 (CH)	Group 1 (No ICM)	-135.54	25.517	0.038 (S)
	Group 3 (CO)	-63.49	25.517	0.068 (NS)
Group 3 (CO)	Group 1 (No ICM)	-72.05	25.517	0.001 (S)
	Group 2 (CH)	63.49	25.517	0.068 (NS)

[Table/Fig-5]: Descriptive statistics showing the intergroup comparison at Day 14 using Tukey's post-hoc analysis. (p-value <0.05 is considered significant).
S: Significant difference; NS: No significant difference; No ICM: No Intracanal medicament; CH: Calcium hydroxide; CO: *Calendula officinalis L*

[13]. CH has been advocated as the gold standard material of choice as an ICM. Commonly used as a short-term or long-term intracanal dressing material, CH dissociates into calcium and hydroxyl ions on contact with aqueous fluids. Hydroxyl ions are believed to be responsible for the highly alkaline nature of the CH [14]. However, its low solubility and difficulty in removing the medicament from the root canal could interfere with the subsequent penetration of root canal sealers into the dentinal tubules [15,16]. Guiotti FA et al., showed that CH residues adversely affected the bond strength of AH Plus sealer by acting as a physical barrier between root dentin and the sealer [17]. It is also ineffective in killing *E.faecalis* [18]. Evans M et al., demonstrated that the proton pump activity of *E. faecalis* offers resistance to the high pH of CH. The antimicrobial efficacy of CH could not be increased when it is kept for an extended period of time in the root canal because the hydroxyl ions do not pass through patent dentinal tubules to alkalise the medium surrounding the teeth. Due to the buffering action of dentin, the high pH cannot be maintained within the dentinal tubules [19].

Fracture resistance of teeth plays a major role to resist masticatory forces during biting. Studies have shown that long-term CH therapy was found to have a negative effect on the strength of root dentin, which makes the tooth more susceptible to root fractures. Larger periapical lesions necessitate placement of ICM for a longer period of time and CH is shown to lower the resistance of teeth to fracture when used in such conditions [20]. Doyon GE et al., have shown that there is a decrease mechanical properties of radicular dentin when CH is used as ICM for more than 5 weeks and stated that there may be disruption in collagen fibres and hydroxyapatite crystals interaction at alkaline pH due to CH, which may negatively influence the mechanical properties of radicular dentin [21].

Herbal agents are potential sources of new antimicrobial compounds especially against bacterial pathogens. They are also having hydrophobic characteristics and they can degrade the lipids of the bacterial cell wall and the mitochondria and subsequently destroy the bacterial structures [22]. Another advantage of using herbal medicaments comparing to their synthetic counterparts is their lower toxicity [5]. According to Muley BP et al., CO is shown to have high antimicrobial, antioxidant, anti-inflammatory, analgesic, and wound healing properties. [7]. Roopashree TS et al., showed the antibacterial activity of CO using different extracts against both gram positive and gram negative organisms and found out that the aqueous extracts were found to be more effective against all the bacteria. Hence, aqueous extract of CO was used in the present study [23].

The results revealed that no medicament (group 1) showed highest fracture resistance at both 7 and 14 days compared to group 2 and 3. There was a statistical significant difference seen between CO and CH at 7th day. This shows the process of dissolution, denaturation and hydrolysis of the organic structure of radicular dentin due to medicaments. CO showed slightly higher resistance to fracture compared to CH. On 14 days examination, there was no statistically significant difference seen between CO and CH groups. The results for CH showed less fracture resistance which may be explained by the disruption of the link between the hydroxyapatite crystals and the collagenous network in dentin. According to Andreasen JO et al., the disruption could take place due to neutralisation, dissolution, or denaturing of the acid proteins and proteoglycans in dentin which might serve as bonding agents between the collagen network and the hydroxyapatite crystals [24]. Herbs are richest sources of phenolic acids and flavonoids [25]. Phenolic acids are weak acids that could be adsorbed on hydroxyapatite molecules. After adsorption, there might be a chemical reaction between hydroxyapatite and the solution which is called surface complexation [26]. This might be the reason for CO to show less fracture resistance on 14 days compared to 7 days.

Limitation(s)

One of the limitations of this study is the lack of radiopacifier in CO, the addition of which would have been helpful in radiographic examination of this material.

CONCLUSION(S)

Within the limitations of this in vitro study, it can be concluded that CO can be efficiently used as an alternative to CH because of its low toxicity, bactericidal activity, low side effects, low costs and easy availability. It can be effectively used as an ICM. This study will pave way for many such herbal ICMs for its effective use in endodontics with maximum therapeutic action and least toxicity.

Future scope involves the cytotoxic evaluation of methanolic and ethanolic extract of CO on human cell lines with higher concentrations. Long-term influence of CO on the fracture resistance of teeth with large sample size needs to be evaluated for more accurately extrapolating the results of this in vitro study to clinical situation.

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