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Physical, Chemical, and Antimicrobial Properties of Chlorhexidine Combine with Calcium Hydroxide as Intracanal Medicament.

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الخلاصة

الأهداف: لمقارَنَة وتَقييم الخواص الفيزيوكيميائية (التدفق، قابلية الذوبان، pH ، ووقت التصلب) والمضادة الميكروبية (ضدّ بكتيريا البرازيلية وفطر المبيّضات) لمعجون هيدروكسيدِ الكالسيوم الذي تم خَلطه مع التراكيز المختلفةِ للكلورهكسدين (١%،١.٥%،٢%). **المواد وطرائق العمل:** معجون هيدروكسيد الكالسيوم تم خلطه مع ٣ تراكيز مختلفة لمعجون الكلورهكسدين (١%،١.٥%،٢%). الخواص الفيزيوكيمائية قُيّمتْ طبقاً لنظام ISO العالمي. التدفق للادوية قيّمَ بطريقةِ الطبقين , قابلية الذوبان قرّرتْ باستعمال تخفيفِ وزن العيّنةِ (%) بعد ٢٤ ساعةِ ، pH قيم بواسطة pH meter، ووقت التصلب قيّمَ بواسطة Gillmore needle. الخواص المضادة للميكروبات ضدّ فطرِ المبيّضات والبكتيريا البرازيلية قُيّما باستعمال اختبار انتشار المادّة المثخّنةِ. وقد تم تحليل البيانات إحصائيا باستخدام طريقة التباين الأحادي واختبارتوكي. **النَتائج:** الكلورهكسدين لم يكن له تأثير معنوي على التدفقَ , قابلية الذوبان ، pH، ووقت التصلب لدواء هيدروكسيد الكالسيوم. المجموعة التي تم خلطها مع ٢% كلورهكسدين كَانَ عِنْدَها تأثيرُ مضاد للميكروبات مختلف بشكل معنوي عن المجاميع التي دمجت مع ١% و١.٥% من الكلورهكسدين ولكن معنويا ما كان هناك اختلاف بين ١% و١.٥% مِنْ مجموعاتِ الكلورهكسدين التي تم خَلطَهم مَع هيدروكسيد الكالسيوم. **الإستنتاجات:** الكلورهكسدين في (١%، ١.٥%، ٢%) وبالتمازج مع هيدروكسيدِ الكالسيومِ عِنْدَهُ خواص فيزيوكيميائية مقنعة وخواص مضادة للميكروبات بحيث من الممكن استعمالهم كدواء داخل القناة. الكلورهكسدين في ٢% نتج عنه التأثير الأفضلِ للمضادة الميكروبية بين المجموعات الأخرى.

ABSTRACT

Aims: To compare and evaluate physicochemical (flow, solubility, pH, and setting time) and antimicrobial properties (against *Enterococcus faecalis* and *Candida albicans*) of calcium hydroxide ($Ca(OH)_2$) paste mixed with different concentrations (1%, 1.5%, 2%) of chlorhexidine gel (CHX).

Materials and Methods: $Ca(OH)_2$ paste was mixed with 3 different concentrations of CHX gel (1%, 1.5%, and 2%). physicochemical properties were evaluated according to ISO internationally. The flow of medicaments were evaluated by two plate method, solubility determined by using sample weight loss (%) after 24 hours, pH determine by pH meter, and setting time was measured with Gillmore needle. Antimicrobial properties against *Candida albicans* and *Enterococcus faecalis* were evaluated using agar diffusion test. The data were analyzed statistically using one way ANOVA and Tukey test.

Results: CHX significantly had no effect on flow, solubility, pH, and setting time of $Ca(OH)_2$. 2% CHX mixed group had antimicrobial effect significantly different from that of 1% and 1.5% of CHX combined groups, but significantly there was no difference between 1% and 1.5% of CHX groups mixed with $Ca(OH)_2$. **Conclusions:** CHX at (1%, 1.5%, 2%) and in combination with calcium hydroxide had satisfactory physicochemical and antimicrobial properties to be used as intracanal medicament. CHX at 2% produce better antimicrobial effect among other concentrations.

Key words: calcium hydroxide, chlorhexidine, flow, solubility, pH, setting time, *Candida albicans*, and *Enterococcus faecalis.*

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INTRODUCTION

Medicaments are used as an aid to improve predictability and prognosis of endodontic treatment. The need for intracanal medicament is greater in those cases where bacteria are resistant to routine treatment, and where the therapy cannot be completed successfully due to the presence of pain or continuing exudates.[1,2]

Calcium hydroxide ($Ca(OH)_2$) has been used extensively in dentistry. Today, it is still the most commonly used endodontic medicament throughout the world. The reason for its application is based on its antimicrobial and mineralizing properties.[3]

Enterococcus faecalis and *Candida albicans* are the most common isolates from root treated teeth with persistent per-

iapical disease. These microorganisms appear to be highly resistant to $Ca(OH)_2$. So, there is a need for supplementary agents to effectively treat persistent periapical lesion.[1,2,4]

Different vehicles have been added to $Ca(OH)_2$ in an attempt to enhance its antimicrobial activity. These vehicles may be water soluble, aqueous, viscous, inert or with an antiseptic action. $Ca(OH)_2$ has low water solubility, good flow, and inherently high pH. Therefore, the vehicles for calcium hydroxide must not affect its physicochemical properties and should enhance its antimicrobial activity.[3]

Chlorhexidine (CHX) has been suggested to be useful endodontic irrigant and intracanal antiseptic due to its low toxicity and excellent antibacterial and antifungal activities.[5,6]

The purposes of current study were to investigate physicochemical properties (flow, solubility, pH, and setting time) and antimicrobial properties against *Enterococcus faecalis* and *Candida albicans* for combination of CHX gel at different concentration (1%, 1.5%, and 2%) with $Ca(OH)_2$ paste.

MATERIALS AND METHODS

Preparation of Chlorhexidine Gel:

CHX (15% chlorhexidine gluconate, Croatch company) at 1%, 1.5%, and 2% were used in this study, 2 gm of orabase was add to 10 ml of one of the concentration of CHX to obtain a gel (pH 7.0).[7] Orabase checked in previous study that it had no antimicrobial effect.[8]

Tested Medicaments:

The medicaments tested in this study were divided into four groups as follow:

Group I: $Ca(OH)_2$) paste (Metapex, Germany).

GroupII: $Ca(OH)_2$ paste mixed with 1% CHX gel (2:1).

GroupIII: $Ca(OH)_2$ paste mixed with 1.5% CHX gel (2:1).

GroupIV: $Ca(OH)_2$ paste mixed with 2% CHX gel (2:1).

$Ca(OH)_2$ paste mixed with CHX gel in a ratio of (2:1) for about 10±1 sec at 37°C until complete homogenization of mixture was obtained.

Physicochemical Properties:

The following tests were performed according to international standard ISO 6876 /2001 for dental root canal materials.[9]

Flow:

A volume of 0.05 ml of the mixture of each tested medicament dispensed on the center of glass slab (using a syringe), which covered by another glass slab (70 g) with an additional weight to achieve a total of 120±2 g on application. After 10 minutes, the diameter (mm) of resulting nearly circular disc was measured twice (along perpendicular lines) using digital vernia.[10-12] This was shown in Figure (1).

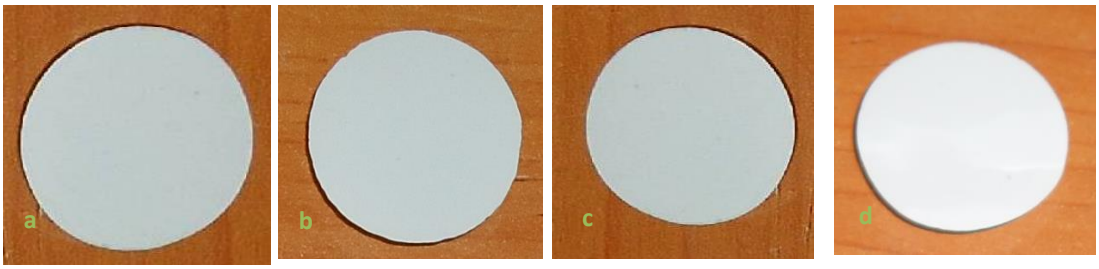

Figure (1): Flow rate among tested medicaments.
a= $Ca(OH)_2$. b= $Ca(OH)_2$& 1%CHX. c= $Ca(OH)_2$ & 1.5%CHX. d= $Ca(OH)_2$ & 2%CHX

Solubility:

Ring mould with an internal diameter of 20 mm and a height of 1.5 mm was placed on the glass slide with larger dimensions than the mould; the mould was then filled with one of the tested medicament. Another glass slide lined with plastic sheets (polyethylene) was then placed over prepared sample with 100g weight on it. The filled mould was placed in an incubator with 37±1°C temperature and 95% humidity for 24 hours. Sample was removed from the mould and the weight of sample was measured to the nearest 0.001 g (Figure 2).

Figure (2): Prepared sample and mould for solubility test.

After that, the mass of petri dish (diameter of 90 mm and minimum volume of 70 ml) was weighted three times in an analytical scale. Two samples were placed inside the dish and approximately 50±1 mL of distilled water (pH 7.0) was then added and covered the dish. The dish was kept in an incubator for 24 hrs; the samples were then removed and washed with 2 to 3 mL of distilled water. Petri dish was placed inside a lyophilizer (EDWARDs, Moduly, England) for water evaporation and drying at high vacuum. Subsequently, new measurement of the petri dish was performed, and difference between the two measurements was used to determine the amount of lost mass which express as a percentage.[10,11,13]

pH:

Tested materials was placed in rubber mould (3 mm height X 10 mm diameter), and the pH was determined using a digital pH meter (J, Morita corporation, Japan).[10,14]

Setting Time:

Stainless steel ring shape moulds with an internal diameter of 10 mm and 2 mm height were prepared. Stainless steel moulds were placed on the glass plate; subsequently moulds were filled with one of the tested materials. Another glass plate lined with plastic sheets (polyethylene) wasplaced on the filled material with100g weight on it, and carefully removed in order to remain a flat uniform surface. The filled mould was placed in an incubator with 37±1^{o}C temperature and 95% humidity. The setting of each sample was tested using Gillmore needle (MARUTO, Japan) (rod of 100±0.5 g having a flat end 2.0±0.1 mm in diameter, with the needle cylindrical for a distance of 5 mm from its end) that was carefully lowered vertically onto flat surface of the tested material (Figure 3), the tip is cleaned and the operation is repeated until no indentation can be seen. Test was performed 40 minute after filling the mould and was repeated every 10 minutes. The time of "no indent" is recorded from the end of mixing. The average value is known as the setting time.[12,15]

Figure (3): Measuring "set" with Gillmore needle.

Antimicrobial Properties:

The microorganisms tested in this study were *Candida albican* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*) which were isolated from clinical acute periapical lesion. The antimicrobial activity was performed using agar diffusion test. An over night mixed broth culture of each microorganisms (10^{8} cfu/ml) on brain heart infusion broth (Oxiod LTD, Basingstoke, Hants/ England) were prepared. 1µl of one inoculums were inoculated into Sabouraud agar plate (Oxiod LTD, Basingstoke, Hants/ England) for *C. albican*, and into *E. faecalis* plate (Difco Laboratories Detroit Michigan, USA) for *E. faecalis*. For each agar plate 5 sterile filter paper discs were placed on it, in which four of discs were impregnated in one of the tested medicament and one remain without coating (negative control). All plates incubated into incubator (37±1ºC) for 24h, and the diameter (mm) of the growth inhibition zone were then measured.[1-3,13]

Every physicochemical and antimicrobial test was repeated ten times.

RESULTS

One way analysis of variance and Tukey post Hoc multiple range tests ($P<0.05$) were conducted on all physicochemical and antimicrobial properties. This was shown in Tables (1) and (2).

Results revealed that flow, solubility, pH, and setting time of $Ca(OH)_2$ mixed with CHX gel at different concentrations were significantly not different from those of $Ca(OH)_2$ paste.

Results also found that $Ca(OH)_2$ paste and control negative groups shown no inhibition zones against any tested microorganisms. While $Ca(OH)_2$ mixed with 2% CHX had antimicrobial effect against two tested microorganisms significantly different from that of 1% and 1.5% of CHX combined with $Ca(OH)_2$, but significantly there was no difference between 1% and 1.5% of CHX groups mixed with $Ca(OH)_2$. As shown in Tables (1) and (3) and Figure (4).

Table (1): One way analysis of variance for the differences on the physiochemical and antimicrobial properties of the tested medicaments.

		Sum of Squares	df*	Mean Squares	F-value	*P*-value**
F low	Between Groups	19.767	3	6.589	2.951	0.063
	Within Groups	80.371	36	2.233		
	Total	100.138	39			
Solubility	Between Groups	0.074	3	0.025	2.073	0.121
	Within Groups	0.427	36	0.012		
	Total	0.501	39			
pH	Between Groups	0.417	3	0.139	3.124	0.055
	Within Groups	1.602	36	0.044		
	Total	2.,019	39			
Setting time	Between Groups	1.149	3	0.383	1.908	0.146
	Within Groups	7.227	36	0.201		
	Total	8.376	39			
Antimicrobial	Between Groups	110.586	2	55.293	63.677	0.000
E.faecalis	Within Groups	23.445	27	0.868		
	Total	134.031	29			
Antimicrobial	Between Groups	185.158	2	92.579	39.025	0.000
C.albicans	Within Groups	64.051	27	2.372		
	Total	249.209	29			

*df=degree of freedom. **$P\leq0.05$ mean significant different exist. **$P>0.05$ mean no significant different exist.

Table (2): Tukey test for the differences on the physicochemical properties of the tested medicaments.

Tested Medicaments	Mean±SD			
	Flow (mm)	Solubility (%)	pH	Setting time (hrs:min)
$Ca(OH)_2$	28.35±1.97 A*	1.65±0.10 A	11.85±0.18 A	4:54±0.44 A
$Ca(OH)_2$ & 1%CHX	26.66±1.06 A	1.61±0.10 A	10.87±0.82 A	5:00±0.45 A
$Ca(OH)_2$ & 1.5%CHX	26.65±1.10 A	1.58±0.12 A	10.89±0.22 A	5:03±0.46 A
$Ca(OH)_2$ & 2%CHX	26.62±1.63 A	1.54±0.10 A	10.9±0.23 A	5:05±0.42 A

*The similar letters vertically mean no significant difference exist.

Table (3): Tukey test for the differences on the antimicrobial effect of the tested medicaments.

Tested Medicaments	Inhibition Zone Mean(mm)±SD	
	E. faecalis	***C. albicans***
Control -ve	0.0±0.0 A*	0.0±0.0 A
$Ca(OH)_2$	0.0±0.0 A	0.0±0.0 A
$Ca(OH)_2$ &1%CHX	23.66±0.78 B	18.79±1.26 B
$Ca(OH)_2$ & 1.5%CHX	24.01±0.71 B	19.78±1.12 B
$Ca(OH)_2$ &2%CHX	27.9±1.21 C	24.49±2.06 C

* The different letters vertically mean significant difference exist.

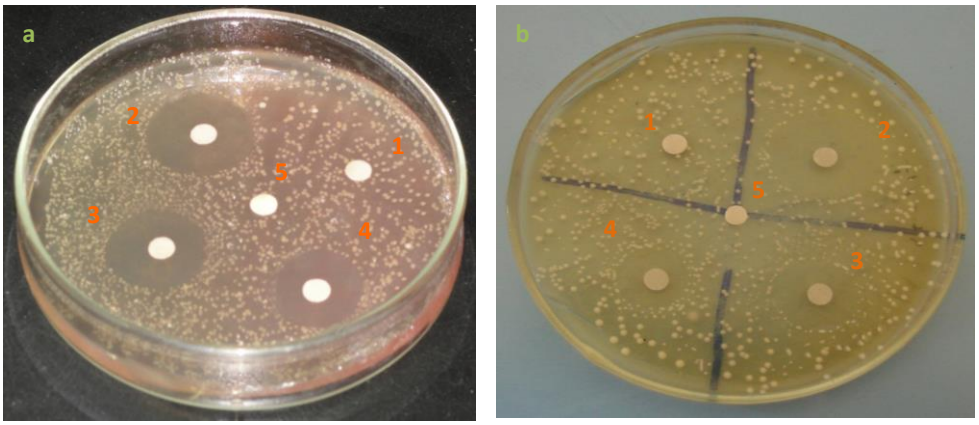

Figure (4): Inhibition zones of the tested medicaments against tested microorganisms.

a= *E. Faecalis*. 1= $Ca(OH)_2$. 2= $Ca(OH)_2$& 2%CHX. 3= $Ca(OH)_2$ & 1.5%CHX. 4= $Ca(OH)_2$ & 1%CHX. 5= -ve control.

b= *C. albicans*. 1= $Ca(OH)_2$. 2= $Ca(OH)_2$& 2%CHX. 3= $Ca(OH)_2$ & 1.5%CHX. 4= $Ca(OH)_2$ & 1%CHX. 5= -ve control

DISCUSSION

Intracanal medicament has been unequivocally shown to contribute to favorable outcome when treating endodontic infections. [(1)]

$Ca(OH)_2$ containing materials have widely used in endodontic therapy to stimulate apexification, to repair perforation, to promote healing by hard tissue formation in root fracture, to control external and internal inflammatory root resorption, and had antibacterial action. $Ca(OH)_2$ was also the main component in root canal sealer and in several pastes that are used as intracanal dressings in case of periapical lesions.[(3)]

Many reports showed that *E. faecalis* and *C. albicans* are the common isolates from infected root canals and in cases of therapy resistant periapical lesions. They had been also used in several studies of antibacterial properties of materials because of there relative resistance.[(2,4)]

$Ca(OH)_2$, although suitable as an intracanal medication, cannot be considered a universal intracanal medication, because it is not equally effective against all microorganisms found in the root canal especially *E. faecalis* and *C. albicans*. However, the association of antimicrobial agents with calcium hydroxide should be avoided, especially those that have been shown to be irritating to periapical tissues. Another medication, such as CHX gel which has a wide spectrum of antimicrobial activity with prolonged action, is biocompatible with periapical tissues, stays longer in contact with the microorganisms, and diffuses through the dentin tubules, should be considered to be used in combination with $Ca(OH)_2$ to enhance its antimicrobial efficacy.[(2,5)]

CHX in gel form was used in this study because many authors believed that CHX gel was able to clean the root canal walls and their anatomic complexities effectively due to the viscosity of the gel.[(5,9)] Also in this study a paste containing calcium hydroxide was used so its more predictable to combine it with gel rather than solution in order to maintain as possible as the physiochemical properties of this paste.

A number of tests have been developed to assess the physical and technological properties of root canal medicament. Such tests serve a number of purposes: They ensure that the materials are presented in a consistency so that they are practical to use in a clinical situation; they provide a physical characterization of the materials when mixed and set; and they may in some instances be helpful in anticipating how the material will perform clinically.[(10)] Therefore, assessment of the physicochemical properties of $Ca(OH)_2$ mixed with CHX were very necessary before it is used.

In this experimental study, it was found that CHX at different concentration when combined with $Ca(OH)_2$ did not signifi-

cantly effect it is flow, solubility, pH, and setting time.

The flow behaviour of a $Ca(OH)_2$ was one of the most important handling properties. Firstly, favourable flow behaviour results in easy mixing. Secondly, the filling material must be able to be introduced easily into the root canal and exhibit a certain stability.[1,12] According to this study, mixing $Ca(OH)_2$ with CHX at different concentrations were decrease its flow, but significantly not different, this might result from the fact as CHX gel was viscous material [6], therefore it will increase the viscosity of $Ca(OH)_2$[14], so that, as the viscosity of $Ca(OH)_2$ increase its flow decrease because the viscosity is defined as the resistance of material to flow or its a measurement of the inner friction of a fluid. Thus, if a solution flows easily it has a low viscosity and the interactions between the particles are very small and the reverse is true.[16]

$Ca(OH)_2$ had a low water solubility and an inherently high pH. Its low water solubility was a useful characteristic because a long period was necessary before it becomes soluble in tissue fluids when direct contact with vital tissues. Also, low solubility was important to provide the hermetic seal of root canal system to avoid the bacterial proliferation and increase the success of the endodontic treatment [10,13]. As well as, maintained the pH of $Ca(OH)_2$ was important because its kill most of bacteria by high pH.[4] In this study, it was found that solubility and pH of $Ca(OH)_2$ were maintained when mixed with CHX. This might related to that CHX had pH (7.0) and it had low water solubility when it use in gel form.[6] Therefore the mixture of $Ca(OH)_2$ and CHX was effective to occupy the apical regions in a sufficient quantity to permit its biological effect to be exerted in close proximity to the appropriate tissues.

Materials needed to be introduced into the root canal must have sufficient working time for appropriate clinical application in root canals. Also, unset material may cause harmful tissue reactions.[12] In this study, the addition of CHX to $Ca(OH)_2$ slightly increase its setting time which significantly result in no differences.

It was revealed by this study, $Ca(OH)_2$ paste alone was not effective against *C. albicans* and *E. faecalis*. This result from the fact that antimicrobial effect of $Ca(OH)_2$ attributed to its high pH, several studies [1,2,14] demonstrated that *E. faecalis* and *C. albicans* had inherent ability to resist its high pH, therefore $Ca(OH)_2$ alone unable to kill this two microorganisms. While mixing $Ca(OH)_2$ with 2% CHX had antimicrobial effect against two tested microorganisms significantly different from 1% and 1.5% CHX groups mixed. This may related to that CHX is bactericidal at higher concentrations (1.8%-2% and above) which causing precipitation of bacterial cytoplasm and cell death, while its bacteriostatic at lower concentrations causing increased cell permeability with leakage of important intercellular components.[1,5,6]

On the basis of the results obtained and of the experimental conditions used in this study, this study was concluded an additive benefits achieved by combining $Ca(OH)_2$ with CHX. Therefore, this combinations can be consider as an effective intracanal medicament in the treatment of root canal infection. Further researches made both in vitro and in vivo are necessary for better understanding the antibacterial efficiency of this combinations as intracanal medicaments.

CONCLUSIONS

CHX at different concentrations and in combination with $Ca(OH)_2$ paste maintain its physicochemical properties to be used as an intracanal medicaments. $Ca(OH)_2$ was ineffective against *C. albicans* and *E. faecalis* especially when it used alone without combination with CHX.

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