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AN ALTERNATIVE THERAPEUTIC STRATEGY FOR ROOT CANAL DISINFECTION

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ABSTRACT

Aim of the study: This study was performed to evaluate the antibacterial effectiveness of Boswellic acids (BA) as root canal irrigation solution with Sodium hypochlorite, Chlorhexidine.

Materials and Methods: forty five patients having single rooted teeth with single canal diagnosed as necrotic pulps with chronic apical periodontitis were included in the study. Bacterial samples were taken from the root canal before preparation (S1) , Post instrumentation sample S2 after using the tested irrigants. All samples collected were transferred directly for microbiological analysis, and cultured on blood agar plates in aerobic and anaerobic conditions, and the bacterial growth was counted as colony forming units (CFUs) using manual counting technique. The antibacterial effectiveness of the tested materials was evaluated by the decrease in the CFUs from S1 to S2.

Results: NaOCl, CHX, and BA solutions showed significant reduction in the bacterial count from S1 to S2 ($P < 0.05$) with no significant difference between them P=0.136. Cleaning and shaping resulted in > 99.5% decrease in the count of bacteria from S1 to S2 samples.

Conclusions: BA could be considered as a promising root canal irrigant owing to its comparable antibacterial effect with the most commonly used root canal irrigants (NaOCl, CHX). BA gel reduced the possibility of post-operative pain compared to the other medicaments.

KEYWORDS: Boswellia Carterii, Root canal treatment, Endodontic microbiology.

INTRODUCTION

Apical periodontitis is an infectious disease that is caused by bacterial invasion from infected root canals. The outcome of the root canal treatment procedure depends on the how much the canal was disinfected and avoiding reinfection of the root canal.

The cleaning and shaping of the root canal process depends on a chemomechanical disinfection procedure, mechanically through canal shaping

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aiming to remove inflamed & necrotic pulps tissues and infected debris from the root canal system, and chemically by the use of chemical antibacterial irrigants to disinfect the root canals and cause aggressive decrease in the bacterial load inside the root canal system. [1]

Since complete eradication of microorganisms is impossible due to the complexities of the root canal system(2), decreasing the bacterial load using different irrigating solutions could be the solution. Obturation also provides entombing of any remaining resistant bacteria.

Sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are the most commonly used root canal irrigants for this purpose.

Therefore, recent trends were targeting natural alternatives to overcome the limitations of the well known popular irrigants. Recently, several natural alternatives with therapeutic properties as anti inflammatory and antifungal properties represent the main concern in endodontic irrigants. [3] So In this study, the performance of Boswellic acids in endodontics had been evaluated.

AIM OF THE STUDY

This study will be intended to evaluate the Antibacterial effect of Boswellic acids from Boswellia carterii in different pharmaceutical formulations in comparison to the most commonly used irrigants sodium hypochlorite and chlorhexidine.

MATERIALS AND METHODS

The sample size selection for the microbiological part of the study was determined to be based on a previous study by Peters et al.[14] where a difference of 48% in the culture of bacteria in the tested groups, with power =80% and alpha level =0.05. And the sample size was increased to 15 (n=15) to increase the statistical power.

And the sample size was increased to 15 (n=15) for more statistical power and to be the same as sample size of microbiological analysis.

Participants forty five patients came to the endodontic clinic at faculty of Oral and Dental Medicine, Future University in Egypt diagnosed clinically and radiographically as pulp necrosis with asymptomatic chronic apical periodontitis were included in this study. All patients were informed with the procedures with a written consent ensuring that the patient fully understands the research procedures and its risks.

The selection of the teeth to be involved in the study was based upon strict inclusion/exclusion criteria.

- **Inclusion criteria**

1. Maxillary incisors and canines with single root and single canal.
2. Teeth with necrotic pulps with asymptomatic apical periodontitis, confirmed clinically and radiographically.
3. Teeth with intact pulp chamber walls
4. Teeth with no previous root canal treatment.

- **Exclusion criteria**

1. Symptomatic teeth featuring pain, tenderness to percussion or swelling.
2. Teeth with crown root fracture.
3. Teeth with incompletely formed root.
4. Teeth with periodontal disease, having pocket depth more than 4 mm.
5. Involved teeth from patients that had taken antibiotics in the 3 weeks before the treatment.
6. Patients with chronic systemic disease.
7. Pregnant or lactating female patients

Intervention

Diagnosis After obtaining the medical and dental history from all patients, clinical and radiographic check was done. The chief complaint were taken from patients in their own words. Clinical evaluation included detection of carious lesions, discoloration, or large restorations. Percussion and palpation were done to indicate the presence or absence of tenderness or swelling. Periodontal probing

also done to detect any periodontal problem. The necrotic pulps of the teeth were confirmed with electric pulp tester and cold sensibility tests and by absence of bleeding after access cavity preparation. Pre-operative radiographs were taken to assess root condition and peri-apical status and measuring the size of apical radiolucency. The patients were divided into 3 groups according to the used irrigant solution; NaOCl, CHX and BC solution.

Microbiological Root Canal Sampling (MRS) & Access cavity preparation was done under strict aseptic condition by doing the following:

1. Before access cavity preparation, supragingival plaque and calculus were removed from the tooth surface by scaling, root planning and polishing.
2. Defective restorations and caries were removed using sterile highspeed burs.
3. Isolation with rubber dam was done before exposure of the pulp chamber exposure and gingival barrier Opal Dam was used to gain tight seal around leaking dams.
4. After isolation, disinfection of the operative field was done using Hydrogen peroxide 3% and sodium hypochlorite 5.5%.
5. Penetration to the pulp space and complete deroofing was done by another sterile high speed burs.

Then the operative field was disinfected again.

Samples collection from group 1

- Pre-preparation sample (S1) was taken from the root canal Just after the tooth was accessed.

The access cavity was flooded with sterile saline without overfilling and a small H-file #15 or #20 was then introduced inside the canal to the extent of 1 mm from the arbitrary working length taken from the pre-operative radiograph, followed by gentle filling motion to loosen the debris and necrotic tissue to be loaded into the saline solution.

Then three sterile paper points were introduced and left inside the root canal for about 1 minute. Then immediately transferred with the used H-File to a sterile eppendorf tube containing the transfer media Brain heart infusion (BHI) broth. The samples were then directly placed in an ice box and transferred for culture.

Post preparation sample (S2) After taking the first sample (S1), apical patency and determination of working length was done with the aid of an electronic apex locator J Moritta Dentaport Root ZX. Confirmation of working length done using periapical radiograph.

Canal shaping was done using Protaper Gold system up to F3, (30/0.09) F4,(40/.06) or F5 (50/,05), with aid of about 10 mL the tested irrigants (NaOCl, CHX, BA) for lubrication, flushing of shaping debris and canal disinfection.

Plastic disposable syringe with a 30-gauge needle after each file use. It was used passively without forceful dispensing of the irrigant, placed 2mm short from the working length, which was verified by rubber stoppers.

At last rinse with 5 mL of irrigation was done after finishing canal preparation and EQS sonic activator used for 1 minute to aid in disinfection protocol. Then the canal was flushed with sterile saline and 5 mL sodium thiosulfate in NaOCl group to neutralize the effect of remnants of NaOCl. To take post-instrumentation sample S2 the canals were refilled with sterile saline once again and gentle filing motion was done using H-file and post-preparation sample (S2) was taken with the aid of sterile paper points as previously described.

Microbiological analysis In order to evaluate the antibacterial effect of the tested materials samples collected from root canals (S1 & S2) were subjected to microbiological analysis by aerobic and anaerobic bacterial culture to assess their bacterial load. The microbiological analysis was analyzed by manual counting the colony forming units (CFUs), and the percent of bacterial reduction after each step was calculated.

Aerobic culture method

The paper points were transferred immediately into sterile eppendorfs of 2 ml brain heart infusion (BHI) broth. Immediately, the collected specimens were delivered to the Microbiology Diagnostics and Infection Control Unit. BHI broth was cultured on blood agar plates (Oxoid) by using calibrated bacteriologic loop (10 ul) then incubated for 24 hours at 37°C. Then the bacterial growth was counted as CFUs using manual counting technique.

Anaerobic culture method

The lids of the test tubes were opened slightly, and paper points were inserted immediately in the BHI broth to maintain the anaerobic environment. The collected specimens were delivered to the microbiological lab. BHI broth was cultured on blood agar plates (Oxoid) by using calibrated bacteriologic loop (10 ul). The culture plates were placed in the anaerobic gas jar with its accessories (anaerobic gas bag and anaerobic indicator strip) and then incubated for 24 h at 37°C. [15]

After 24 h, the bacterial growth was counted as CFUs using the manual counting technique. Bacterial count was done for both aerobic and anaerobic culture in the form of CFUs, along with the inoculation and dilution factors to calculate the total CFU counts. CFU= Count on plate X inoculation factor X Dilution factor = CFU / 0.1 ml

Bliniding As the microbiological results depend on the detection whether there is bacterial growth or not, and manual counting the CFUs in the positive samples, the eppendorfs containing the samples taken from the root canal were marked by number codes so the laboratory staff would not know the samples belongs to which step of canal disinfections (S1, S2) and to which of the tested material the samples belong.(Single blind)

Statistical analysis:

Data obtained from the microbiological analysis was evaluated by Paired sample t-test to assess the effectiveness of each material used in root canal disinfection. The comparison between different groups in the percent of bacterial reduction after each step of canals disinfection was statistically analyzed using a one way analysis of variance (ANOVA) at level of significance of 0.05. If significance was found, Mann-Whitney was used for pair wise comparisons. Statistical analysis was done using SPSS 22.0 (SPSS, Chicago, IL, US)

RESULTS

Subjects This study involved 45 teeth from patients diagnosed with necrotic pulps and chronic apical periodontitis, from those referred to the Endodontic Clinic at Faculty of Oral and Dental Medicine, Future University in Egypt.

Patients Data

1. Age: The selected patients age ranged from 18 to 57 years old with mean age of 32.4
2. Sex: From the 45 patients included in the study, 25 were Female , 20 Male.
3. Teeth Selected: Teeth included in the study were confined to maxillary incisors and canines having single root canal and single roots.

Results from microbiological analysis:

All samples (S1,S2) collected from the root canals were microbiologically analyzed after both aerobic and anaerobic culture to determine bacterial growth in the form of CFUs, and the antibacterial effectiveness of the tested materials was evaluated by the reduction of total bacterial counts and percent of bacterial reduction from initial samples S1, to post disinfection samples S2. From the 45 sampled teeth, anaerobic bacterial culture showed growth in all S1 samples(100%), while aerobic culture for S1 samples showed bacterial growth in only 12 cases (26.6%). The bacterial count in the form of colony forming units (CFUs) ranged from 1.5 × 103 to 2.9 × 106. Samples collected after chemomechanical preparation S2 showed no growth in all samples after aerobic culture while bacterial growth was evident in only 13 samples after anaerobic culture.

Evaluation of Antibacterial Effect of NaOCl

For NaOCl group, only 4 S1 samples showed bacterial growth after aerobic culture, while all of the S1 samples showed bacterial growth after anaerobic culture with bacterial count ranging from 1.1×104 to 1.2×106. After chemomechanical preparation, post instrumentation samples S2 showed no bacterial growth after aerobic culture in all samples S2 (100% bacterial reduction), while anaerobic culture revealed no growth in 10 S2 samples while bacterial growth was evident in only 5 S2 samples and CFUs in ranged from 1.5×103 to 4.2×103 and average bacterial reduction about 99.8%.

Evaluation of Antibacterial Effect of CHX irrigant

For cases treated with CHX irrigant, aerobic culture for S1 samples revealed bacterial growth in only 3 samples, while all of the S1 samples showed bacterial growth after anaerobic culture with total CFUs ranging from 3.8 × 104 to 1.09 × 106. After chemomechanical preparation using CHX irrigant, post instrumentation samples S2 showed no bacterial growth after aerobic culture in all samples S2 (100% bacterial reduction), while anaerobic culture revealed no growth in 10 S2 samples (100% bacterial reduction) while bacterial growth was evident in only 5 S2 samples with CFUs ranging from 3.2×103 to 6.1×103 and average percent bacterial reduction about 99.5%.

Evaluation of Antibacterial Effect of Boswellia irrigant

For Boswellia irrigant group, bacterial growth was evident in 5 S1 samples after aerobic culture and in all S1 samples after anaerobic culture. Aerobic culture for post instrumentation samples S2 showed no growth in all. Anaerobic culture for S2 showed 100% bacterial reduction in 11 cases, while 4 samples showed bacterial growth with CFUs ranging from 2.5×103 to 4.2×103 and average percent of bacterial reduction 99.9%.

Statistical analysis using paired sample t-test showed that each of the used irrigant during chemomechanical preparation of the root canal was significantly effective in reducing the bacterial count from S1 to S2 ($P < 0.05$). Regarding the comparison between groups, the statistical analysis after ANOVA showed that was no significant difference between the percent of bacterial reduction of the three tested irrigants (P value= 0.136).

TABLE (5) The number of total CFUs in S1 and S2 samples after anaerobic culture and percent of bacterial reduction according to the groups.

	NAOCL	*CHX*	*BA*	*p VALUE*
S1 MEAN (SD)	6.4×10^5 ($\pm 5.9 \times 10^5$)	5.2×10^5 ($\pm 4 \times 10^5$)	7.8×10^5 ($\pm 8 \times 10^5$)	0.477
Min	1.1×10^4	7.3×10^3	6×10^4	
Max	1.4×10^6	1.3×10^6	2.9×10^6	
S2 Mean (SD)	1.3×10^3 ($\pm 3.4 \times 10^3$)	1.5×10^3 ($\pm 2.2 \times 10^3$)	9.7×10^2 ($\pm 1.6 \times 10^3$)	0.88
Min	0	0	0	
Max	4.2×10^3	6.1×10^3	4.2×10^3	
P Value	< 0.001*	< 0.001 *	0.002 *	
Percent of reduction in the total bacterial count				
S1-S2	99.8% (±0.34)	99.5% (±1.06)	99.9% (±0.18)	0.136

P < 0.05 is considered statistically significant

TABLE (2) The number of total CFUs in S1 and S2 samples after aerobic culture and percent of bacterial reduction according to the groups

	NAOCL	*CHX*	*BA*
S1 MEAN (SD)	1.5×10^3 ($\pm$ 3.3×10^3)	8.3×10^2 ($\pm 1.7 \times 10^3$)	4.3×10^3 ($\pm$ 1.2×10^3)
Min	0	0	0
Max	1.2×10^4	4.7×10^3	4.6×10^4
S2 Mean (SD)	0	0	0
Min	0	0	0
Max	0	0	0
P Value	0.018	0.085	0.02
Percent of reduction in the total bacterial count			
S1-S2	100%	100%	100%

DISCUSSION

The usage of herbal medicine is rising again in the last few decades for safety concerns and the increased antibiotic resistance. In endodontics, sodium hypochlorite is the gold standard in the process of root canal disinfection, concerns regarding biocompatibility and cytotoxic effects on the surrounding peri-apical tissues encouraged researchers to seek an alternative herbal disinfectant.

Several herbal extracts had been tested for endodontic use as root canal irrigants e.g. Morinda Citrofolia, Liquorice, Propolis.[10, 11, 16]

Boswellic acids is widely used in the treatment of several medical disorders and could be used in endodontics, owing to its safety and potent anti-inflammatory and antimicrobial properties. In vivo genotoxicity evaluation of Boswellic acids on animal showed that BA was totally safe and no genotoxicity was evident up to the dose of 1gm/kg. Besides, BA showed promising in vitro performance as root canal irrigant and intracanal medication. [13]

In this study, BA was tested for its effectiveness as root canal irrigant clinically against the most commonly used irrigants NaOCl solution & CHX. The BA solution was prepared with concentration 1 ug/mL, this was based upon the previous study testing the biocompatibility of BA in comparison with conventional and most commonly used irrigants (NaOCl, CHX).

The MTT assay revealed that BA with 1 ug/mL showed > 95% vitality and was significantly more biocompatible than the other tested materials used in the study. Beside the excellent biocompatibility of BC at concentration 1ug/mL, BA showed strong and comparable antibacterial properties.[13]

As a root canal irrigant, Boswellia extract solution was compared to NaOCl 2.5% and CHX solution. In this study, only maxillary incisors and canines with single root with single canal were included in our study. These teeth were chosen as they usually have wide straight canals which allows evaluation of disinfection on the root canal with decreased probability of procedural errors during instrumentation and irrigation. Also including these teeth in the study provides a nearly standard instrumentation conditions, as challenges faced during canal preparation such as curved canals, calcifications, isthmuses that may affect the quality of disinfection were avoided.

The whole clinical procedures of the root canal treatment was done under strict rubber dam isolation which was placed before the access cavity preparation. This ensures no probable contamination of the pulp chamber and root canals with saliva, blood, or gingival fluids loaded with bacterial microorganisms.[17]

Field decontamination was done using 5.25% NaOCl just after rubber dam placement and second application of NaOCl was done just before pulp penetration after removal of caries and defective restoration to avoid any false positive results from bacterial contaminants that may persist on the rubber dam sheet. According to Ng et al. 2003, they compared the effectiveness of iodine tincture to 5.25% NaOCl in field disinfection using culture and PCR tests, the results showed there were no significant difference between both solution in field disinfection using culture technique, while NaOCl was more effective than iodine tincture after evaluation the degree of field disinfection using PCR.[17]

In our study microbiological bacterial culture method was used to evaluate the antibacterial effect of the tested materials. Bacterial culture is considered to be the gold standard the most commonly used methods used for microbiological identification. It has the advantage of being broad range with the ability of detection of great variety of microbial species in one sample.

In our study, the samples were marked by number codes so the laboratory staff would not know the samples belongs to which tested material which is considered as an essential precaution, as our results depend on the detection whether there is bacterial growth or not, and on manual counting the CFUs in the positive samples.

The operator was not blind and no effort was made for this purpose which may show come concerns, but it was difficult to make the operator unaware with the applied materials, as the tested materials had different color, consistency and odour.

In accordance to our results, bacterial growth was evident in all initial samples S1 and this confirmed the correlation between apical periodontitis and bacterial colonies invading necrotic root canal system. The initial samples were dominated with anaerobes as all S1 samples showed anaerobic growth with high bacterial population number, while aerobic culture showed bacterial growth in only 18% of the S1 samples with lower bacterial count compared to the anaerobic population, which could be attributed to the hard living conditions inside the closed root canal system in the form of low oxygen and availability of nutrients, This coincide with the previous studies. (18,19)

In our study, there was a substantial statistically significant reduction in the bacterial load after chemomechanical preparation of the root canal. The results showed that percent of bacterial reduction from S1 to S2 samples was above 98% in all cases and coincided with a previous study by Siquera et al 2007 [3] , in their study the percent of bacterial reduction was more than 95% in all cases after chemomechanical preparation after using NaOCl as root canal irrigant, which reflects the importance of the cleaning and shaping step in the process of canals disinfection It is clear that antibacterial effectiveness is one of the most important properties of any root canal irrigant. The statistical analysis showed that the three tested irrigants significantly reduced the total CFUs from S1 to S2 samples, with no significant difference between them. This showed that BA solution was as effective as NaOCl and CHX solution in bacterial elimination. The antibacterial activity of BA could attributed to its ability to disrupt the permeability of the microbial outer membrane structure as stated by Raja et al. 2011 [12] found that the ability of AKBA to destroy microbes results from its ability to interrupt the permeability barrier of microbial membrane components. Al-Saidi et al. 2012 [20] tested the antibacterial effectiveness of four esstential oils of oleogum resins of Boswellia, they found that the

four oils were effective against gram positive and gram negative bacteria, and they stated that the possible antibacterial effect of BA could rise from its ability to inhibit enzymes responsible for energy production, and destruction of the bacterial genetic material, beside the disruption of the cytoplasmic membrane.

Sodium hypochlorite and chlorhexidine are the most commonly used root canal irrigants, the results of our study showed that both irrigants were equally effective in the reduction of the bacterial population with no significant difference between them, and this came in agreement with an in vivo study made by Rocas et al. 2016 [8] who found no significant difference between NaOCl and CHX in their antibacterial effectiveness during root canal treatment.

A recent systematic review and meta-analysis of randomized clinical trials made by Ruksakiet et al. 2020 [21] comparing the antibacterial effect of both NaOCl and CHX in their use during root canal treatment of permanent teeth. From 8 studies included in this systemic review, the authors stated that both irrigants were effective in reducing the bacterial load with no significant difference between both them. Owing to the limitations of the sampling procedure, and the reduced sensitivity of the culturing technique, A negative results after bacterial culture does not mean sterility of the root canal, sometimes survived bacteria microorganisms may be present in the inaccessible areas of the root canal such as ramifications, lateral canals and dentinal tubules.[9] Also absence of bacterial growth after canal disinfection may indicates reduced bacterial population below a level that cannot be detected by bacterial culture methods, these levels usually allow peri-radicular healing in most of cases.[7] However, the great reduction in the percent of bacterial population >98% reduction gives an indication for the effectiveness of the disinfection protocol, even if there were survived bacterial microorganisms. From the above results, it is clear that BA solution could be an alternative to NaOCl as a root canal irrigant. But the high antimicrobial effectiveness is not the only major requirement of the used root canal irrigants, the tissue dissolving ability is also of extreme importance to dissolve the remaining necrotic tissues that could be found in the ramifications of the root canal. A property that is not found in any of the used irrigants except NaOCl. This means that using NaOCl is of a must during cleaning and shaping procedure and the other irrigants could be used to aid NaOCl in the disinfection of the canals to get advantage of their properties, or in patients with allergy to NaOCl. [5]

CONCLUSIONS

Upon the results of our study we concluded that BA could be considered as a promising root canal irrigant owing to its comparable antibacterial effect with the most commonly used root canal irrigants (NaOCl, CHX)

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