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Evaluation of Jojoba Oil Efficacy in Endodontic Infection Control

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ABSTRACT

Aim: This study was designed to evaluate the antimicrobial effectiveness of Jojoba-Oil against endodontic pathogens involved in endodontic infection. **Materials and methods:** Regarding the in vitro part, sterile saline, crude Jojoba-Oil, mixtures of calcium hydroxide powder with sterile saline and calcium hydroxide powder with Jojoba-Oil were tested against selected strains ***(S.aureus, E.coli, E.faecalis and C.albicans)*** using agar diffusion method. Zones of inhibition were measured after 24, 48, 72 and 7 days incubation periods. Regarding the in vivo part, a total of forty patients were selected according to specific inclusion and exclusion criteria and randomly divided into four groups (10 each) according to the assigned irrigant and intracanal medicament. Three clinical samples were collected from each patient in each group under strict aseptic conditions for microbiological studies. **Results:** Ca(OH)2 + Jojoba-Oil group produced the statistically significant largest mean inhibition zone against the selected strains at 24, 48 and 72 hours incubation periods. The in vivo part showed that, chemomechanical preparation produced a significant reduction in both bacterial and fungal count in the four experimental groups. In groups using intracanal medication, there was further significant reduction in both bacterial and fungal count compared to non-medicated groups. **Conclusions:** The combination of calcium hydroxide with Jojoba-Oil as a vehicle has an antimicrobial activity comparable to calcium hydroxide mixed with saline.

KEYWORDS

Jojoba Oil, Calcium hydroxide, endodontic infection control.

INTRODUCTION

The success of the endodontic treatment depends on thorough debridement of the root canal system. In order to predictably eliminate as many bacteria as possible from the entire root canal system, a combination of mechanical instrumentation and irrigating solutions is used to remove or dissolve organic and inorganic debris, to destroy bacteria

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and to remove the smear layer. However, it has been reported that mechanical instrumentation with antibacterial irrigation will only render 50–70 per cent of infected canals free of microorganisms, depending on which irrigants are used.

Since there is no entirely predictable way, in one treatment session, to ensure complete elimination of root canal bacteria, an effective antimicrobial agent in the root canal is required for a predetermined time period to predictably eradicate or destroy any remaining bacteria. Therefore, antimicrobial agents used as inter-appointment medicaments must be able to penetrate through the dental tissues in the presence of microbes to reach a sufficiently high concentration in order to eliminate the disease-causing bacteria in a predictable manner [(1)].

Historically, different substances and medications have been used as intracanal medication between endodontic treatment sessions to assist in the control of microbial infection, including calcium hydroxide-based pastes, polyantibiotic pastes, formalin tricresol, camphorated paramonochlorophenol, and chlorhexidine alone or in combination with other substances. Despite conflicting claims, no medicament appears to be ideal, and there is controversy over their use [(2)].

Interest in the use of herbal remedies in endodontics has recently grown. Medicinal herbs are supposed to be effective in the treatment of infectious diseases, are biocompatible, and mitigate the side-effects of synthetic antimicrobials [(3)]. Jojoba oil is extracted from ground crushed seeds of simmondosia chinensis and was introduced in Egypt in 1984 by Food and Agriculture Organization (FAO) [(4, 5)]. It possesses anti-inflammatory, anti-microbial actions and does not interfere with biological processes due to its indigestibility and purity [(6, 7)]. Searching for a root canal irrigant and intracanal medicament prompted the interest of evaluating Jojoba-oil (natural material having a substantial role in medicine) as an irrigant and a vehicle for calcium hydroxide powder.

MATERIAL AND METHOD

In vitro part

The present study used Staphylococcus aureus strains cultured on blood agar (Fluka Company), Candida albicans strains cultured on Sabourad's Dextrose agar (SDA) (Scharlab Company, S.L., Barcelona, Spain), Eishrecia coli and Enterococcus faecalis strains cultured on Mueller Hinton agar (BBL 211438 Becton Dickinson, Sparks, MD, USA). Agar diffusion method was used to test the selected strains against: **sterile saline** (Al Mottahedoon Pharma, Egypt), **crude Jojoba-Oil** (Egyptian Company for Natural Oils), **Mixture of calcium hydroxide powder with Jojoba-Oil** and **mixture of calcium hydroxide powder with sterile saline**.

A total of 16 wells were prepared in four plates (4 wells in each plate).One gram of each tested material was placed in each well. In each plate, three wells were filled with crude Jojoba-Oil, a mixture of calcium hydroxide powder with crude Jojoba-Oil and a mixture of calcium hydroxide powder with sterile saline, while the fourth well contained sterile saline which served as negative control. The agar plates were incubated at 37°C for 24, 48, 72 hours and 7 days incubation periods, after which the zones of inhibition were measured using a plastic ruler and was recorded for each material. The results were statistically analyzed using (ANOVA) test.

In vivo part

1. Patients selection:

Forty single-rooted teeth (32 mandibular second premolars and 8 maxillary central incisors) from forty male patients (aged 18-22 years old) were selected for this study based on inclusion/exclusion criteria. Inclusion criteria were: patients has a non-contributory medical history. The selected teeth should have necrotic pulps confirmed by negative response to sensitivity pulp tests, radiographic evidence of asymptomatic apical periodontitis and suf-

ficient coronal structure for adequate isolation with a rubber dam and were free of periodontal pockets deeper than 4 mm. All patients were informed on the objective and design of the study, expected outcomes or alternative management, then the informed consent was signed by the patients.

2. Patients grouping:

The selected patients were randomly divided into four groups (10 patients each) according to the assigned irrigant and intracanal medicament as following, **group (I):** teeth were irrigated with crude Jojoba-Oil and received a mixture of calcium hydroxide powder with Jojoba-Oil as an intracanal medicament, **group (II):** teeth were irrigated with sodium hypochlorite 2.6%NaOCl (Egyptian company for house hold bleach, Egypt) and received a mixture of calcium hydroxide powder with sterile saline as an intracanal medicament, **group (III):** teeth were irrigated with crude Jojoba-Oil with no intracanal medicament, **group (IV):** teeth were irrigated with sodium hypochlorite (2.6%NaOCl) with no intracanal medicament.

3. Samples collection:

Three clinical samples were collected from each patient under strict aseptic conditions for microbiological studies as follows: **S1** (initial sample) before any root canal treatment (after access cavity preparation), **S2** (post-instrumentation & irrigation sample) after root canal instrumentation and irrigation, **S3** (post-medication sample) after application of intracanal medication or just sterile cotton pellet and temporary filling for seven days.

4. Clinical procedure:

4.1 First session

After rubber dam application, tooth disinfection was performed followed by access cavity preparation using sterile round bur. For collection of the first microbiologic sample **(S1)**, sterile saline solution was placed in the pulp chamber and the root canal walls were gently filed to suspend the canal contents in saline. Three successive size 25 sterile paper points were consecutively placed in the canal to soak up the fluid in the canal. The canal patency and the working length were established, then cleaning and shaping was carried out using ProTaper Universal rotary system (Dentsply, Maillefer, USA) in a crown-down manner with a brushing motion.

Between consecutive files, irrigation with 2 mL crude Jojoba-Oil for **groups (I, III)** and 2.6% NaOCl for **groups (II, IV)**. At completion of chemomechanical preparation, root canals were rinsed with 2 mL of 17% EDTA (Dentsply, Latin America, Brazil) followed by 2mL of 2.5% sodium hydroxide (NaOH) (Al-Gomhoreya Company, Cairo, Egypt) for **groups (I, III)** and 2mL of 1%NaOCl & 5% sodium thiosulfate (Al Gomhoreya Company, Cairo, Egypt)for **groups (II, IV)** for inactivation of crude Jojoba-Oil and NaOCl respectively. The root canals were finally flush with 2 mL of sterile saline. Subsequently, the post-instrumentation & irrigation sample **(S2)** was taken with three paper points matching the apical enlargement size, as described previously. The canals medicated with either a mixture of calcium hydroxide powder with Jojoba-Oil for **group (I)** or a mixture of calcium hydroxide powder with sterile saline for **group (II)**.No intracanal medicament was applied in **groups (III, IV)**, just a sterile cotton pellet and Coltosol for 7 days.

4.2 Second session:

After rubber dam application, operative field disinfection and temporary filling removal, the medicament was removed from the canal with a Hedstorem file of the same size as the master apical file and rinsed away with 5 mL of 2.5% sodium hydroxide (NaOH) then flushed with 5 mL of sterile saline solution for **group (I)** or 5mL of sterile saline solution only for **group (II)**. A third microbiological sample **(S3)** was acquired from **all groups (I, II, III and IV)** with three paper points matched to the apical enlargement size.

6. Laboratory procedures

All the samples were transferred immediately to the regional center for mycology and biotechnology (Al-Azhar University). Serial dilutions, plating and counting of live microbial cells were performed to determine the number of bacteria and yeast in each given sample. After preparing serial dilutions of a solution containing unknown number of bacterial and yeast, samples were plated on sheep blood agar media to count bacteria and on Sabourad's Dextrose agar media to count yeast. The numbers of colony forming units (CFU's) are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria or yeast per m.L. that present in the original sample according to the following equation:

$$\frac{\textit{Number of colony forming units (CFU's)}}{\textit{Volume plated (mL)} \times \textit{Total dilution}}$$

RESULTS

(I) In vitro part:

Comparison of the antibacterial activity among tested groups regarding incubation period: (Table 1) (Figure 1)

The negative control group (saline) didn't show any antibacterial activity during the four incubation periods (24, 48, 72 hours and 7 days). Tukey's post hoc test revealed that, $Ca(OH)_2$ + Jojoba-Oil group had the statistically significant largest mean inhibition zone compared to $Ca(OH)_2$ + Saline group and Crude Jojoba-Oil at 24, 48 and 72 hours incubation periods ($P \leq 0.05$). On the other hand, at 7-days incubation period, ANOVA showed that, there was no statistically significant difference among Crude Jojoba-Oil group, $Ca(OH)_2$ + Saline group and $Ca(OH)_2$ + Jojoba-Oil group in the mean inhibition zone ($P \geq 0.05$).

Table (1): *The mean and standard deviation (SD) values of inhibition zone (mm) of the tested groups in each incubation period.*

Groups \ Time	Inhibition zone (mm)							
	24 hours		48 hours		72 hours		7 days	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Crude Jojoba-Oil	9.00[a]	0.82	9.50[a]	1.29	9.75[a]	0.96	10.25	1.50
Ca(OH)2 + Saline	10.75[ab]	0.96	11.50[ab]	1.29	11.50[ab]	1.29	12.25	1.71
Ca(OH)2 + Jojoba-Oil	12.25[b]	0.96	13.00[b]	1.41	13.00[b]	1.41	13.25	1.50
Saline	0.00[c]	0.00	0.00[c]	0.00	0.00[c]	0.00	0.00	0.00
P-value	**0.002***		**0.012***		**0.012***		**0.064**	

Mean with different letters in the same column indicate statistically significance difference.

**: significant, ns: non-significant.*

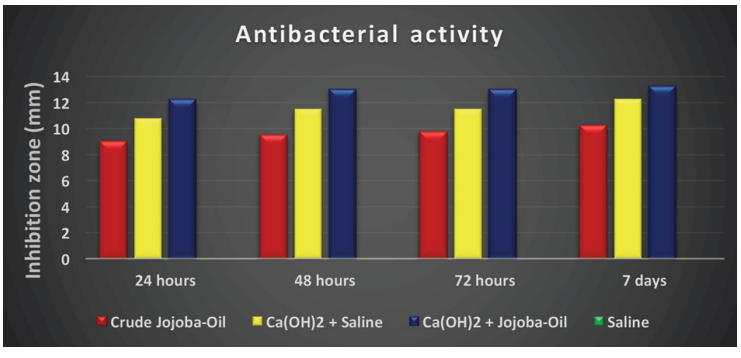

Fig. (1) Bar chart representing mean of inhibition zone (mm) of tested groups in the four incubation period.

(II) In vivo part:

a. Effect of irrigation and medication on the total bacterial and fungal count in each tested group: (Table 2,3) (Figure 2,3)

The results of the in vivo part showed that chemomechanical preparation produced a significant reduction in both bacterial and fungal count in the post-instrumentation and irrigation samples **(S2)** of the four experimental groups. Samples after 7-days of intracanal medication **(S3)** in groups **(I, II)** showed a further significant reduction in both bacterial and fungal count in comparison to **(S2)**, while samples after 7- days without intracanal medication in groups **(III, IV)** showed a significant increase in total bacterial and fungal count in comparison to post instrumentation and irrigation samples **(S2)**.

Table (2): *The mean and standard deviation (SD) values of total bacterial count of the tested groups after initial, post-instrumentation & irrigation and post-medication samples.*

Groups / Samples	Total bacterial count							
	Group I		Group II		Group III		Group IV	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S1(Initial samples)	$3.98x10^{7a}$	$0.80x10^{7}$	$3.28x10^{7\,a}$	$1.05x10^{7}$	$3.28x10^{7\,a}$	$0.77x10^{7}$	$2.76x10^{7\,a}$	$1.16x10^{7}$
S2(Post instrumentation & irrigation samples)	$8.61x10^{3\,b}$	$0.70x10^{3}$	$1.24x10^{3\,b}$	$0.74x10^{3}$	$6.77x10^{3\,b}$	$3.07x10^{3}$	$1.58x10^{3\,b}$	$1.19x10^{3}$
S3(Post-medication samples)	$2.70x10^{3\,c}$	$0.51x10^{3}$	$0.19x10^{3\,c}$	$0.07x10^{3}$	$3.37x10^{5\,c}$	$1.38x10^{5}$	$1.98x10^{4\,c}$	$2.01x10^{4}$
P-value	≤0.001*		≤0.001*		≤0.001*		≤0.001*	

Mean with different letters in the same column indicate statistically significance difference.: significant, ns: non-significant.*

Table (3): *The mean and standard deviation (SD) values of total fungal count of the tested groups after initial, post-instrumentation & irrigation and post-medication samples.*

Groups / Samples	Total fungal count							
	Group I		Group II		Group III		Group IV	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
S1(Initial samples)	$6.99x10^{5a}$	$0.49x10^{5}$	$8.60x10^{5\,a}$	$0.61x10^{5}$	$7.05x10^{5\,a}$	$0.50x10^{5}$	$6.62x10^{5\,a}$	$0.62x10^{5}$
S2(Post instrumentation & irrigation samples)	$6.25x10^{2\,b}$	$0.52x10^{2}$	$3.74x10^{4\,b}$	$0.62x10^{4}$	$8.31x10^{2\,b}$	$0.52x10^{2}$	$7.33x10^{4\,b}$	$0.80x10^{4}$
S3(Post-medication samples)	$8.57x10^{c}$	0.46x10	$6.08x10^{3\,c}$	$0.54x10^{3}$	$3.96x10^{2\,c}$	$0.64x10^{2}$	$6.81x10^{5\,a}$	$0.71x10^{5}$
P-value	≤0.001*		≤0.001*		≤0.001*		≤0.001*	

*Different letters in the same column indicate statistically significant difference *; significant ns; non-significant*

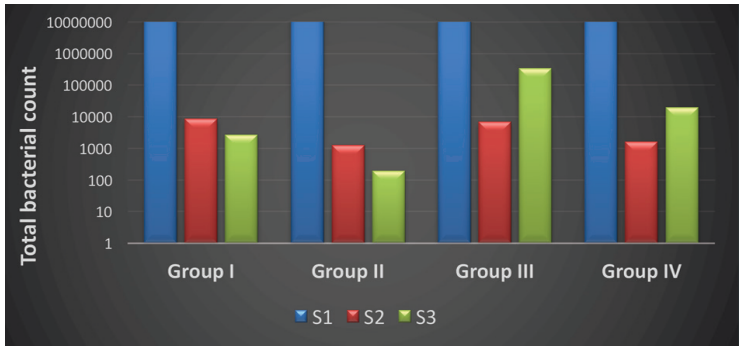

Fig. (2) Bar chart representing mean of the total bacterial count of tested groups after initial samples (S1), post-instrumentation & irrigation samples (S2) and post-medication samples (S3).

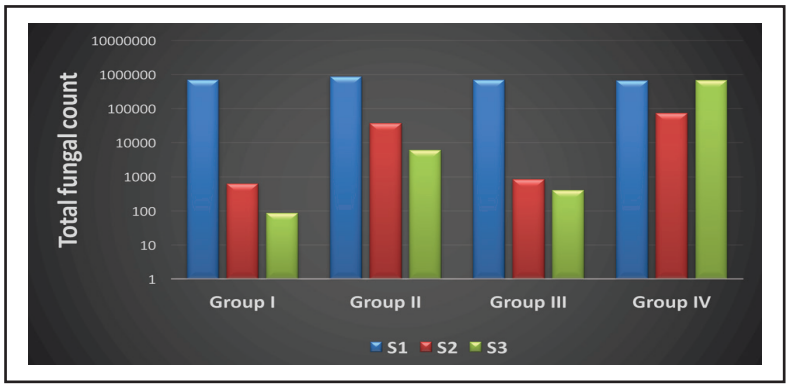

Fig. (3) Bar chart representing mean of the total fungal count of tested groups after initial samples (S1), post-instrumentation & irrigation samples (S2) and post-medication samples (S3).

b. Evaluating the antimicrobial and antifunal effectiveness of Jojoba-Oil: (Table 4)

There was a statistical significant difference between Jojoba-Oil groups and other groups in the bacterial and fungal count ($P \leq 0.05$). The results showed that, using Jojoba-Oil as an irrigant and intracanal medicament showed statistical lower bacterial and fungal count than other groups.

Table (4): Mean and standard deviation values of bacterial and fungal count of Jojoba-Oil groups and for other groups.

Groups	Bacterial count		Fungal count	
	Mean	SD	Mean	SD
Jojoba-Oil groups	$1.01x10^{7a}$	$1.57x10^{7}$	$2.34x10^{5a}$	$3.33x10^{5}$
Other groups	$1.22x10^{7b}$	$1.78x10^{7}$	$3.87x10^{5b}$	$3.58x10^{5}$
P-value	≤0.001*		≤0.001*	

DISCUSSION

Achieving predictable long-term success of the root canal treatment requires effective debridement and disinfection of the root canal system. Chemomechanical instrumentation removes the majority of infecting bacteria, together with necrotic pulp debris. The need for an irrigating solution during biomechanical preparation is not questionable. Moreover, nature has bestowed a very rich botanical wealth, and a large number of diverse types of plants grow in different parts of world. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment for infectious diseases, and simultaneously they also mitigate many of the side effects that are often associated with synthetic antimicrobials [1,3,4]. Hence, the purpose of the present study was to evaluate the antimicrobial effectiveness of Jojoba-Oil against endodontic pathogens involved in endodontic infection.

Regarding the in vitro part of the present study, combination of calcium hydroxide with Jojoba-Oil showed the statistically largest mean inhibition zone compared to $Ca(OH)_2$ + saline, crude Jojoba-Oil and saline groups at 24, 48 and 72 hours against *S.aureus*,*E. Coli*,*E. faecalis* and *C. albicans*. This might be attributed to the role of Jojoba-Oil as a vehicle for calcium hydroxide, which improved its antimicrobial effect. Several studies have reported that gram-positive and gram-negative microorganisms were affected by the phenolic extracts of jojoba hulls [8,9].

At 7-days incubation period, $Ca(OH)_2$ + Jojoba-Oil group had the largest mean inhibition zone. However, ANOVA test showed that, there was no statistically significant difference among $Ca(OH)_2$ + Jojoba-Oil group, $Ca(OH)_2$ + Saline group and Crude Jojoba-Oil group in the mean inhibition zone ($P > 0.05$). This might be explained by the fact that bacterial cells may communicate and exchange genetic material to acquire new traits that lead to restricted entry and action of antimicrobial agents [10].

Regarding the in vivo part of the present study, chemecomechanical preparation using either Jojoba-Oil or NaOCl as an irrigant resulted in a substantial bacterial and fungal reductionfrom 10^7 to 10^3 in the post instrumentation and irrigation samples **(S2)**. For groups **(I, III)**, in which Jojoba-Oil was used as an irrigating solution, the significant reduction in the total bacterial and fungal count may be attributed to the phenolic constituents of the oil [11]. The inhibitory effect of phenols could be explained by interactions with the cell membrane of microorganisms and is often correlated with the hydrophobicity of the compounds. Phenolic compounds could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [12].

For groups **(II, IV)** where NaOCl was used as an irrigating solution, the significant reduction in the total bacterial and fungal count was attributed to the antimicrobial effectiveness of NaOCl which based on its high pH (hydroxyl ion action). The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degradation observed in lipidic peroxidation [13].Samples after 7-days intracanal medication **(S3)** in groups **(I, II)**, showed further significant reduction of CFU counts. This may be attributed to the antimicrobial activity of calcium hydroxide where the release and diffusion of hydroxyl ions (OH-) leads to a highly alkaline environment which is not conducive to the survival of micro-organisms.

In groups **(III, IV)**, after 7-days of chemomechanical preparation without intracanal medicament application, there was a statistical significant increase in the mean bacterial count. This might be explained that rotary instrumentation techniques tend to produce round preparations (especially in oval canals) leaving some areas uninstrumented and hence possibly containing infected debris. The remaining necrotic tissue may provide a source of nutrition for any surviving bacteria [14, 15]. Moreover, the irrigation may not reach to bacteria in biofilms on untouched canal walls, dentinal tubules, irregularities, and other anatomic variations.

CONCLUSIONS

Under the conditions of the present study, the following could be concluded:

1. In teeth with necrotic pulp, a thorough chemomechanical preparation is able to significantly reduce the bacterial and fungal load of root canals.
2. Combination of calcium hydroxide with Jojoba-Oil as a vehicle has an antimicrobial activity comparable to calcium hydroxide mixed with saline.
3. The use of intracanal medicament is necessary to overcome the regrowth of microorganisms.

RECOMMENDATION

As calcium hydroxide mixed with saline is not the ideal intracanal medicament dealing with E. faecalis, further studies are suggested to compare the effect of combination of calcium hydroxide with Jojoba-Oil versus calcium hydroxide mixed with chlorhexidine.

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