Effect of Eucalyptus and Chloroform on Mineral Content of Radicular Dentin: an in vitro Study

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Abstract

The aim of this study was to evaluate the mineral content of root canal dentin before and after treatment with eucalyptus and chloroform.

For this study were used 20 extracted human premolars. After pulp extirpation and teeth disinfection in an autoclave, dentin powder was collected from the root canal after preparation with endodontic files and stored in Eppendorf tubes. Before the application of eucalyptus and chloroform solvents, the levels of Ca, Fe, K, Mg, Mn, Na and Pb were measured with an Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) apparatus. After treatment with eucalyptus oil and chloroform solution for 10 and 20 minutes, the teeth were again instrumented with the endodontic files to obtain dentin powder from the root canal and levels of Ca, Fe, K, Mg, Mn, Na and Pb were measured again. The composition of minerals was measured as a percentage of mass.

The results were statistically analyzed by analysis of variance (ANOVA) and T-test.
Mean calcium (Ca) values increased significantly for $p < 0.05$ after 20 minutes of chloroform treatment. Mean iron (Fe) values decreased after 10 minutes of chloroform treatment and after 10 and 20 minutes of eucalyptus treatment. Mean potassium (K) values decreased significantly after 10 minutes of chloroform treatment and increased significantly after 10 minutes of eucalyptus treatment. Mean values of magnesium (Mg) increased significantly for $p < 0.05$ after 20 minutes of chloroform treatment and after 10 minutes of eucalyptus treatment..

The mean values of manganese (Mn) decreased after treatment with either chloroform or eucalyptus. Mean sodium (Na) values increased significantly for $p < 0.05$ after 10 minutes of chloroform treatment and after 10 minutes and 20 minutes of eucalyptus treatment.

Mean lead (Pb) values tended to decrease after treatment with either chloroform or eucalyptus. Eucalyptus and chloroform have effect on mineral content of radicular dentin.

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Introduction

Gutta-percha is the most commonly used solid core filling material for canal obturation. It exhibits minimal toxicity, causes minimal tissue irritability, and is the least allergenic material available when retained within the root canal system.[1] Gutta-percha has been used in endodontic therapy as a filling material for over 100 years and remains the material of choice today.[2] One of the disadvantages of gutta percha as an obturating material is the lack of an effective seal,[3] which can result in endodontic failure. Retreatment is a nonsurgical therapeutic option for an endodontic failure. [2]

Nonsurgical endodontic retreatment involves the removal of material from the root canal space to correct deficiencies or to repair pathological or iatrogenic defects, followed by cleaning, shaping and three-dimensional obturation.[4,5] Techniques for gutta-percha removal include the use of rotary instruments and heated instruments.[6,7] Retreatment requires the complete removal from the root canal space of the filling material, which usually comprises

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gutta-percha and endodontic cement.[8] Gutta-percha cones are composed of a vegetable resin and can be softened by chemical solvents.[9]

Chloroform is the solvent most often used for the removal of root canal filling material.[10] Chloroform is an excellent solvent, but it is hepatotoxic and nephrotoxic and is classified as a potential carcinogen.[11,12-14] Additionally, chloroform and xylene may change the physical and chemical properties of dentin, and this issue is clinically important.[15]

Eucalyptus oil and peppermint oil also possess solvent properties, and are currently used as a viable alternative to chloroform and xylene. Halothane, which is slower acting than chloroform, is also used as a solvent for gutta-percha. Even methyl chloroform is a good alternative to chloroform for dissolving gutta-percha, as it is less toxic, non-carcinogenic and is more effective than xylene and eucalyptol.
Other solvents that have been tested for softening gutta-percha include orange oil, carbon disulfide and benzene.[16-18]

Studies testing the performance of some alternatives to chloroform concluded that at room temperature, eucalyptol dissolves very slowly in comparison to other solvents, although its dissolution effect increases when it is heated.[9,13]
The aim of this study was to evaluate the mineral content of root canal dentin before and after treatment with eucalyptus and chloroform.

Materials and methods

This study included 20 human premolars that were extracted for various orthodontic reasons. The teeth were stored in distilled water at room temperature until use. Soft and hard deposits were removed from the teeth, and the crown and the apical third of the root was removed with a diamond disc under continuous water flow. The dental pulp was extirpated and the teeth were disinfected in an autoclave (Vacuklav, Melag, Germany).

The teeth were then irrigated with physiological solution and instrumented with root canal instruments Endostar E5 (Poldent Co., Warsaw, Poland) using endodontic handpiece (Entran WH, Dental Werk-Bumoos, GmbH, Austria). The dentin powder produced was collected from the root canal.

This collected powder was then weighed for each tooth individually (Sartorius, Analytic, Gottingen, Germany), (Figure 1) and stored in Eppendorf tubes marked with numbers 1–20 as a control group. Before the application of eucalyptus and chloroform solvents, the levels of Ca, Fe, K, Mg, Mn, Na and Pb were measured with an Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) apparatus (Optima 2100 DV, Perkin Elmer).

Figure 1. Analytical scale.

Eucalyptus oil (eucalyptus oil, Heman, 100% natural, Karachi, Pakistan) and chloroform (Chloroform GR stabilizied with amylene, Lach-Ner, Neratovice, Czech Republic) were used in this research. These solvents were incubated at 37 °C and 100% humidity for 15 minutes. All samples were treated with the same solution at 37 °C.

The ICP-OES was calibrated with a standard solution. The number of ions released from each dentin extract was verified and the mean value of the three readings obtained from this apparatus was obtained.

After treatment with eucalyptus oil and chloroform solution, the teeth were again instrumented with the Endodontic handpiece to obtain dentin powder from the root canal. The teeth were then divided into four experimental groups of five teeth each according to the type of solvent used and the duration of exposure: group 1 (exposed to chloroform for 10 minutes); group 2 (exposed to chloroform for 20 minutes); group 3 (exposed to eucalyptus oil for 10 minutes); and group 4 (exposed to eucalyptus oil for 20 minutes).

Levels of Ca, Fe, K, Mg, Mn, Na and Pb were measured again after exposure of the teeth to eucalyptus oil and chloroform solvent. The composition of minerals was measured as a percentage of mass.

The results were statistically analyzed by analysis of variance (ANOVA) and T-test.

Results

In table 1 are shown mean values and standard deviation of each tested mineral before and after treatment.

Minerals	Ca	Fe	K	Mg	Mn	Na	Pb
Pretreatment	171902.6±66523.1	7850.3±15303.2	2042.6±1556.7	6240.6±2311.8	282.8±508.7	7574.1±5728.5	787.5±1091.7
Treatment with chloroform after 10 min	135496.4±75217.5	845.6±1104.7	302.8±217.7	5209.9±3127.8	6.7±27.4	21412.4±30880.2	21.3±10.0
Treatment with chloroform after 20 min	226236.8±40591.8	7492.7±8808.7	1066.3±593.9	9530.6±2322.2	62.5±67.0	11174.2±4568.3	17.7±11.3
Treatment with eucaliptus, after 10 min	232759.7±62685.4	5938.3±6712.4	3924.1±1226.1	9296.1±2603.2	68.1±73.4	13093.9±5294.9	114.3±16.3
Treatment with eucaliptus, after 20 min	209828.1±44027.7	2191.7±1499.1	2423.7±2224.3	6404.8±3735.2	13.9±24.4	13669.7±6830.5	57.4±21.9

Table 1. Mean mineral values and standard deviations before and after treatment with chloroform and eucalyptus.

Mean calcium (Ca) values increased significantly for $p < 0.05$ after 20 minutes of chloroform treatment ($t = -1.73$; $p = 0.048$) and after 10 minutes of eucalyptus treatment ($t = 1.1.84$; $p = 0.038$), (Figure 2).

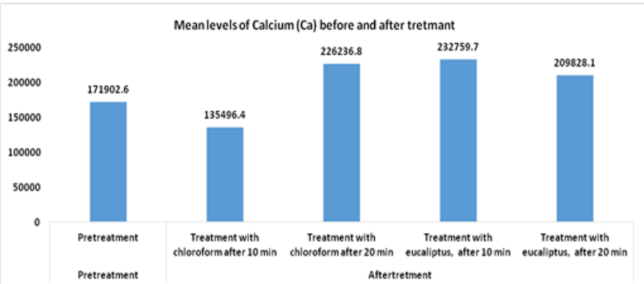

Figure 2. Mean calcium values before and after treatment.

Although the mean iron (Fe) values decreased after 10 minutes of chloroform treatment and after 10 and 20 minutes of eucalyptus treatment, these differences were not significant (Figure 3).

Mean potassium (K) values decreased significantly for $p < 0.05$ after 10 minutes of chloroform treatment ($t = 2.45$; $p = 0.011$), but increased significantly for $p < 0.001$after 10 minutes of eucalyptus treatment ($t = -2.50$; $p = 0.0099$), (Figure 4).

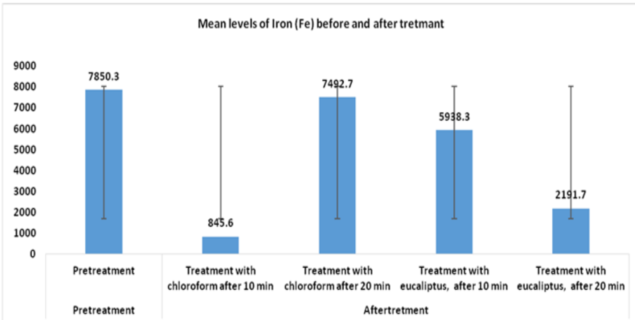

Figure 3. Mean iron values before and after treatment.

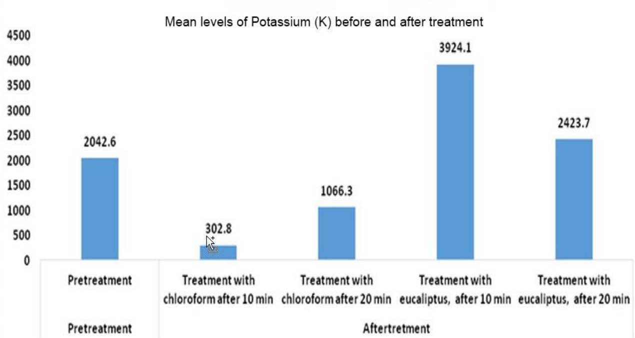

Figure 4. Mean potassium values before and after treatment.

Mean values of magnesium (Mg) increased significantly for $p < 0.05$ after 20 minutes of chloroform treatment and after 10 minutes of eucalyptus treatment (Figure 5).

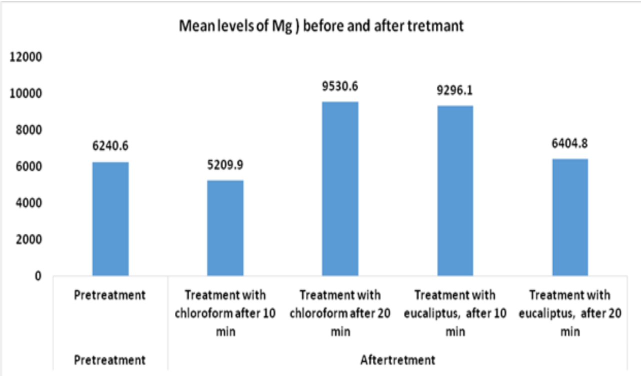

Figure 5. Mean values of magnesium before and after treatment.

The mean values of manganese (Mn) decreased after treatment with either chloroform or eucalyptus, but these differences were not significant (Figure 6).

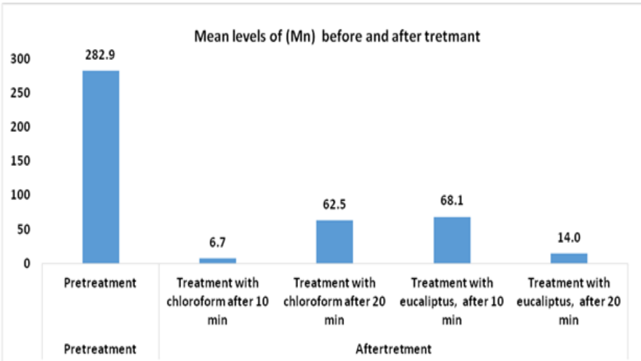

Figure 6. Mean manganese values before and after treatment.

Mean sodium (Na) values increased significantly for $p < 0.05$ after 10 minutes of chloroform treatment ($t = -1.99$; $p = 0.029$) and after 10 minutes and 20 minutes of eucalyptus treatment (Figure 7).

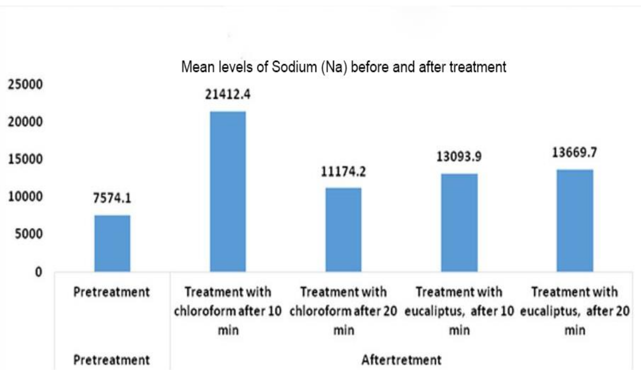

Figure 7. Mean sodium values before and after treatment.

Mean lead (Pb) values tended to decrease after treatment with either chloroform or eucalyptus, but there was no significant difference ($p > 0.05$) (Figure 8).

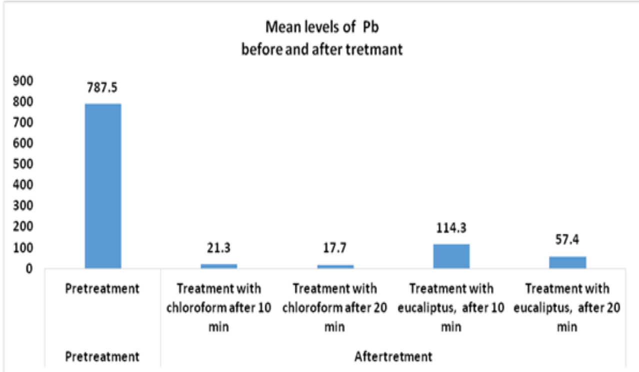

Figure 8. Mean lead values before and after treatment.

Discussion

The use of organic solvents during endodontic nonsurgical retreatment are suitable to decrease the resistance of filling material, but clinician should be aware that strong solvent due to dentine softening may cause root canal transportation and pericementitis.[19-21]

Numerous studies have investigated the dissolving efficacy of organic solvents for root canal filling materials.[21-23]

Bueno and Valdrigh (1998) measured gutta-percha before and after immersion in chloroform, xylol, halothane, eucalyptus, turpentine, and orange oil. Chloroform, xylol, and halothane yielded the same results after immersion for 5 minutes, although chloroform was more effective after immersion for 10 minutes.[24]

Wourms et al. (1990) tested the effects of solvents on gutta-percha for 15 minutes and concluded that the solvent becomes ineffective if it stays in the cavity for more than 15 minutes, and that chloroform, trichlorethylene and tetrachlorethylene are effective in dissolving gutta-percha at a temperature of 22 °C.[7]

Mushtaq et al. (2011) investigated the solvent effect of xylene, tetrachlorethylene, refined orange oil, and distilled water for 2 and 5 minutes. Their results showed that xylene performed better in reducing gutta-percha with Rezilion with no significant difference between the two durations.[2]

Oyama's (2002) study showed that xylol and orange oil are more effective than eucalyptus and halothane in dissolving gutta-percha. However, processed turpentine oil and chloroform are capable of completely dissolving gutta-percha.[25]

Filho et al. (2010) found that xylol was more effective than eucalyptus and orange oil in softening gutta-percha with Rezilion.[23]

Related to chloroform, Gomez et al. (2017) study showed that this solvent gave better results in the first 5 minutes than eucalyptus, orange oil or xylene.[6]

However, a few studies have shown that these solvents are also capable of softening the coronal enamel and dentin.[26, 27]

According to Panighi (1992) there is a positive correlation between the microhardness values and the mineral content of teeth.[28]

In a study by Rotstein et al.(1999) their results indicate that chloroform, xylene, and halothane may cause a significant softening effect on both enamel and dentin. The softening was shown after 5 minutes of treatment. Also, they reported that after 15 minutes treated with chloroform, microhardness decreased by 29%.[21]

But, by comparison of some other organic solvents, Khedmat et al. (2015) found no significant difference in dentin microhardness before and after treatment with orange oil, eucalyptus and chloroform.[27]

According to mineral content of radicular dentin, Erdemir et al. (2004) reported that halothane treatment resulted in a significant decrease in Ca and a significant increase in Mg in the teeth when compared with other tested elements such as P, K and S. There was a significant increase in Mg level after treatment with chloroform.[26] Our findings also revealed an increase in Mg levels after treatment with chloroform for 20 minutes and an increase in Ca levels after treatment with eucalyptus for 10 minutes.

In contrast with our findings, Dogan et al. (2001) reported that there was no significant differences in the levels of Ca, P and Mg in the radicular dentin after treatment with chloroform, xylene, eucalyptus, orange oil, halothane or physiological solution.[29]

Our findings suggest that eucalyptus and chloroform since they have effect on mineral radicular dentin content, they may effect on microhardness and fracture resistance, too.

According to Yavuz et al. (2008), mineral content of the teeth should be important to pediatric dentists.[30]

Knowing that minerals are important elements as material for the remineralization process,[31] further in vitro studies are required to confirm the effect of gutta-percha solvents on mineral content of radicular dentin.

Conclusions

Based on our results we can conclude that:

Gutta-percha solvents: chloroform and eucalyptus have effect on mineral content of root dentin.

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Declaration of Interest

The authors report no conflict of interest.

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