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THE EFFECT OF PROPOLIS NANOPARTICLES IRRIGATION ON REDUCTION OF BACTERIAL ENDOTOXINS A RANDOMIZED CLINICAL TRIAL

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ABSTRACT

Aim: The aim of this study was to evaluate the effect of propolis nanoparticles irrigating solution versus sodium hypochlorite on reduction of bacterial endotoxins.

Materials and Methods: Forty-five single rooted non-vital permanent maxillary anterior teeth with tenderness to percussion and slight widening of lamina dura in medically free patients were included in the study. The patient's age ranged from 20-50 years, either males or females. Patients were randomly divided into three groups, 15 patients in each group, Group (1): 0.9% saline solution, Group (2): 2.5 % sodium hypochlorite (NaOCl) and Group (3): 20% Propolis nanoparticles. Two samples were taken for each group using paper points, one after access cavity preparation (S1) and the second sample after irrigation (S2). Samples were placed in a pyrogen free glass tube and stored at -20 degree Celsius then were assessed for quantification of bacterial endotoxins using ELISA kit, and subjected to statistical analysis.

Results: Results showed that the use of 20% propolis nanoparticles as a root canal irrigant resulted in significantly higher reduction of endotoxins level compared to 2.5% NaOCl root canal irrigation.

Conclusion: It could be concluded that the use of 20% propolis nanoparticles root canal irrigation allowed more reduction of endotoxins level significantly, compared to 2.5% NaOCl irrigation.

KEYWORDS: Sodium Hypochlorite; Propolis; Irrigation; Endotoxins; Enterococcus faecalis.

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INTRODUCTION

Several irrigating solutions have been used in endodontics for chemical disinfection of the root canal space, however, it has been known since the second half of the eighteenth century that sodium hypochlorite (NaOCl) (Household cleaning products of Egypt) remains the gold standard for irrigation in endodontics. This is due to its antibacterial action and tissue dissolving effect on organic and necrotic tissue.[1]

The most commonly found bacteria in the root canal space is the Enterococcus faecalis (E. faecalis). It is a gram positive bacteria which can be eliminated by NaOCl.[2] Also, it has been found in secondary infections following endodontic treatment.[3]

Endotoxins known as either lipoteichoic acid (LTA) for gram positive bacteria and lipopolysaccharide (LPS) for gram negative bacteria, were found on the outer membrane of bacteria.[4] Following the death of bacteria, such endotoxins can be emitted in contaminated root canal space leading to the development of a periapical lesion.[5] Therefore, it is necessary that such endotoxins be removed during endodontic treatment in-order to promote healing of the periapical tissues of the infected root canals. NaOCl can eliminate such endotoxins, but not entirely.[6]

Propolis is a natural product which can be used as an irrigant as it has been demonstrated to be efficient against E. faecalis owing to its chemical composition which renders it having an anti-microbial action.[7] Propolis in its raw state, is composed of aromatic oils and plant resins depending on its collection season.[8]

In addition, nano-materials are commonly used nowadays in clinical endodontics due to its efficient penetration power, drug steadiness and potency for treatment.[9]

Nano-materials can be bioactive, active or intelligent. They have small size and large surface area which helps them enter the bacteria and biofilm, hence, controlling infections.[10]

Therefore, the purpose of this study was to compare the ability of propolis nanoparticles irrigation versus NaOCl irrigation in reducing level of endotoxins in non-vital permanent teeth. The clinician at the end of this study will realize if the use of propolis nanoparticles irrigation will be more effective in reduction of endotoxins than NaOCl, or will this medicament has disadvantages and complains more than the traditional method if used.

Materials and Methods

This study was approved by the Ethical review Committee, Faculty of Dentistry, Cairo University. The researcher received a written consent from the patients accepting to participate in the trial before starting any treatment.

Forty-five single rooted non-vital permanent maxillary anterior teeth with tenderness to percussion and slight widening of lamina dura in medically free patients were included in the study. The patient's age ranged from 20-50 years, either males or females. Patients were randomly divided into three groups, 15 patients in each group, Group (1): {0.9% saline solution: sodium chloride 0.9%, Almotaheddon pharma, Egypt} Group (2): {2.5 % sodium hypochlorite (NaOCl): Household cleaning products of Egypt} and Group (3): {20% Propolis nanoparticles: Nanotech, Dreamland Egypt, for its preparation, polyglycolic acid solution (PGA) was used}. Participants represented Egyptian population which were recruited from Endodontic Clinic, Faculty of Dentistry, Cairo University.

Following local anesthesia, teeth disinfection and isolation with rubber dam, access cavity was carried out using a high speed round diamond bur size 2 (Dentsply, Tulsa Dental, Dentsply Maillefer, USA). First root canal sample (S1) was then taken from each group using sterilized paper point size 15 (Dentsply Maillefer, Switzerland) and immediately

placed in a pyrogen free glass tube and stored at -20 degree Celsius. Working length was checked with apex locator (Dent Port Zx J. Morita, Irvine, Japan) and confirmed with digital x-ray. Coronal preflaring was performed using Gates Glidden drills (Mani, Tochigi, Japan) sizes 2, 3 and 4. Root canal preparation was carried out using ProTaper Next rotary system (Dentsply, Tulsa Dental, Dentsply Maillefer, USA) till size X5 using endodontic motor (Dentsply Maillefer, USA) with torque and speed adjusted according to manufacturer's instructions.

In Group (1): root canals were prepared using rotary files with 3 ml of 0.9% saline solution (Sodium chloride 0.9%, Almotaheddon Pharma, Egypt) as an irrigant with Ethylene Diamide Tetra Acetic Acid (EDTA) gel (MD-Chelcream, Meta Biomed Co Ltd, Korea) applied to each rotary file before being introduced in the root canal space. A second sample (S2) was then taken using sterilized paper point size 50 and immediately placed in a pyrogen free glass tube and stored at -20 degree Celsius. Then the root canal was irrigated with 3 ml of 2.5% NaOCl solution and a final flush with 0.9% saline solution.

In Group (2): using a syringe with 27 gauge needle, irrigation was performed using 3 ml of 2.5% NaOCl solution. EDTA gel was applied to each rotary file before being introduced in the root canal space. A second sample (S2) was then taken using sterilized paper point size 50 and immediately placed in a pyrogen free glass tube and stored at -20 degree Celsius. The canal was then washed out with 0.9% saline solution.

In Group (3): the same procedures were exactly carried out as in Group (2) but using 20% Propolis nanoparticles irrigation instead of 2.5% NaOCl.

Endotoxins (ET) quantification was performed using Sandwich-ELISA method for both samples {after access (S1) and after irrigation (S2)} in all groups and subjected to statistical analysis.

Statistical analysis:

Categorical data were presented as frequency and percentage values and were analyzed using Fisher's exact test. Numerical data was represented as mean and standard deviation (SD) values. Shapiro-Wilk's test was used to test for normality. Homogeneity of variances was tested using Levene's test. Endotoxins data were normally distributed but the homogeneity assumption was violated so they were analyzed using Welch one-way ANOVA followed by Games-Howell post hoc test. Gender and age data didn't violate both assumptions and were analyzed using one-way ANOVA test. The significance level was set at $p<0.05$ within all tests. Statistical analysis was performed with R statistical analysis software version 4.3.2 for Windows*.

RESULTS

The study was conducted on 45 cases that were equally and randomly allocated to each of the tested groups (i.e. 15 cases each). There was 9 males and 6 females in the saline group while in other groups there were 8 males and 7 females. The mean age in 0.9% saline group was (30.27±7.28) years, in 2.5% NaOCl was (29.87±6.39) years, and in 20% Propolis nanoparticles group it was (29.60±5.49) years. There was no significant difference between all groups regarding gender and age ($p>0.05$). Demographic data are presented in table (1) and in figures (1) and (2).

Results of intergroup comparisons and summary statistics for endotoxins reduction are presented in table (2) and in figure (3). Results showed that there was a significant difference in reduction measured with different irrigants with 20% Propolis nanoparticles having the highest reduction followed by 2.5% NaOCl and with saline having the lowest reduction ($p<0.001$).

* R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

TABLE (1) Demographic data

Parameter			*0.9% saline*	*2.5% NaoCl*	*20% Propolis nanoparticles*	*Test statistic*	*p-value*
Gender	***Male***	**n**	9	8	8	**0.18**	**1**
		%	60.0%	53.3%	53.3%		
	Female	**n**	6	7	7		
		%	40.0%	46.7%	46.7%		
Age (years)	**Mean±SD**		30.27±7.28	29.87±6.39	29.60±5.49	**0.04**	**0.960**

TABLE (2) Intergroup comparison and summary statistics for endotoxins reduction.

Endotoxins reduction (Pg/ml) (Mean±SD)			*f-value*	*p-value*
0.9% Saline	**2.5% NaOCl**	***20% Propolis nanoparticles***		
0.002±0.001^{C}	0.025±0.007^{B}	0.039±0.011^{A}	**149.720**	**<0.001***

Values with different superscript letters within the same horizontal row are significantly different *Significant (p<0.05)

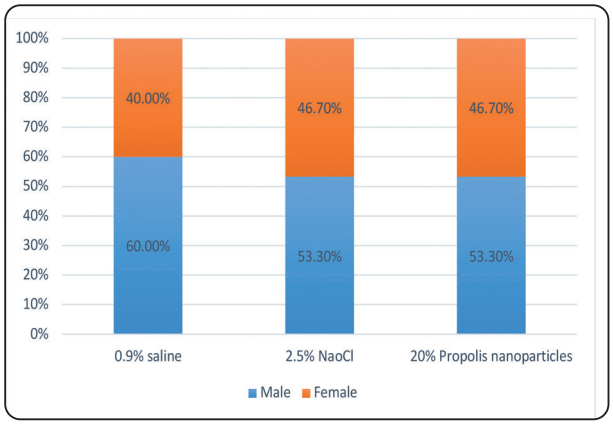


Fig. (1) Stacked bar chart showing gender distribution.

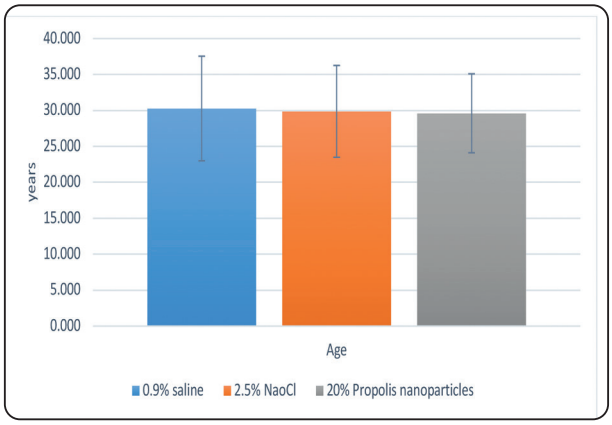
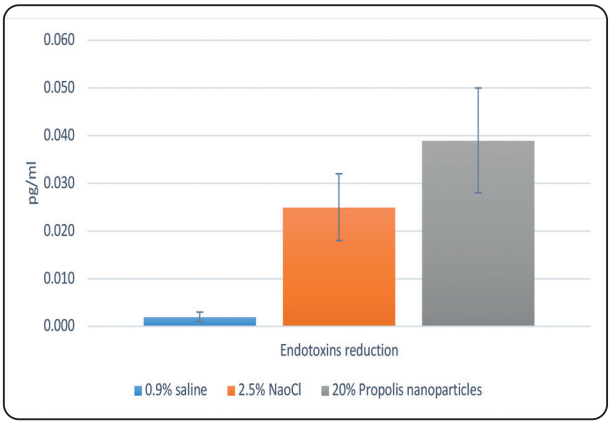


Fig. (2) Bar chart showing mean and standard deviation values of age (years)



Fig, (3) Bar chart showing mean and standard deviation values of endotoxins reduction (Pg/ml).

DISCUSSION

Successful root canal treatment can be achieved through intra-canal bacterial load control inside the root canal space before obturation.[11] Mechanical instrumentation resulted in 50% reduction in endotoxins level inside root canal space.[12] Therefore chemo-mechanical preparation is essential for better elimination of bacterial endotoxins.

Bacterial Endotoxins have been detected on the outer membrane layer of gram positive and gram negative bacteria.[4] These Endotoxins can be recognized as foreign by the immune system resulting in the release of cytokines which lead to pain and apical periodontitis.[13]

In the present study, an informed consent was signed by every patient who approved to participate in this clinical trial.

A wide age range (20-50 years) was selected to investigate its relation to the amount of endotoxins inside the root canal. However, the age didn't reveal any significant effect. Also, the gender didn't reveal any significant effect on endotoxins level.

Single rooted non-vital permanent maxillary anterior teeth with tenderness to percussion and slight widening of lamina dura were included in the study to confine the microbial evaluation to a single ecological environment.[14]

ProTaper Next Rotary system were used in this study for mechanical preparation as NiTi instruments allow minimal debris extrusion compared to hand instruments.[15]

In this study, a human endotoxin ELISA kit was used to measure the endotoxins found inside the root canal. ELISA (Enzyme-Linked Immuno-Sorbent Assay) is a plate-based assay technique designed for detecting and quantifying substances such as peptides, antibodies, proteins and others. Its main advantage is its high sensitivity, as it is 2-5 times more sensitive than direct or indirect ELISAs. In addition, it delivers high specificity as two antibodies were used to detect antigen.

Several studies have concluded that using hand files with 2.5% sodium hypochlorite (NaOCl) allowed reduction of bacterial lipopolysaccharides by 59% [16] whereas using rotary instruments with 2.5% sodium hypochlorite (NaOCl) and 17% EDTA allowed reduction of bacterial lipopolysaccharides by 98.06%.[16] This might be due to the uniform round root canal space preparation, better disinfection and centering ability.[17] Hence, ProTaper Next Rotary system was used in this study. In addition, apical preparation size was enlarged to size X5 which is equivalent to 50/0.06 as bacterial endotoxins is better removed by large sized files compared to smaller ones.[18]

In this study, 0.9% saline solution was used as an irrigant in group (1) to isolate the effect of preparation alone and then this group received the standard irrigation protocol after the second sample (S2) was taken.

Sodium hypochlorite (NaOCl) is the gold standard irrigation, normally 2.5%. This is due to its strong antibacterial action and organic tissue dissolving capacity. Higher concentration was found to be more effective, however, it was proved to have toxic effect on periapical tissues.[19] Therefore, sodium hypochlorite (NaOCl) with a concentration of 2.5% was used in this study.

Due to the fact that lower concentration of sodium hypochlorite might be less effective on bacterial counts inside the root canal, and higher concentration is toxic, finding an alternative irrigant would be beneficial. The use of nanoparticles as antimicrobial agents has recently attracted considerable attention in the medical field as a result of their superior antibacterial properties compared with those of other antimicrobial agents, together with a low potential to produce microbial resistance.[20]

In this study, 20% Propolis nanoparticles irrigant was used. Propolis is a natural product which is obtained by the combination of alcohols, amino and fatty acids, minerals, vitamins and flavonoids being the main component, where Pinto et al,[21] reported that propolis has low toxicity, as its main component, has low toxicity. Propolis with 20% concentration was used, as it was reported to have the highest antimicrobial effect.[22]

In this study, the results of statistical analysis revealed that in group (1) (mechanical preparation and irrigation using 0.9% saline solution) presented the lowest reduction in bacterial endotoxins compared to group (2) (2.5% NaOCl) and group (3) (20% propolis nanoparticles). This result coinsides with the finding of Martinho & Gomes[12] who reported that mechanical instrumentation resulted in 50% reduction in endotoxins level inside root canal space. Hence, the mechanical preparation with 0.9% saline solution alone isn't sufficient to eradicate the endotoxins completely supporting the significant use of chemical disinfectant to get rid of bacterial endotoxins from the root canal space. This is proved from the results of this study through the use of 20% propolis nanopartciles when compared to 2.5% NaOCl irrigation.

In the present study, the results of statistical analysis showed that the use of 20% propolis nanoparticles intra-canal irrigant resulted in significantly higher reduction of bacterial endotoxins compared to 2.5% sodium hypochlorite (NaOCl) irrigation. This is in agreement with the findings of Parolia et al,[23] who concluded that propolis nanoparticles were more effective against Enterococcus faecalis bacteria compared to NaOCl.[23] This could be attributed to the fact that propolis nanoparticles can penetrate dentinal tubules without being deactivated with organic tissues.[22] In addition, nanoparticles have large surface area to mass ratio for contact with bacteria.[10, 24] Moreover, the presence of flavonoids in propolis has shown to have a high antibacterial property, as it acts on bacterial membrane causing structural damage.[23]

CONCLUSION

From this study, it could be concluded that the use of 20% propolis nanoparticles in root canal irrigation significantly resulted in reduction of endotoxins level compared to 2.5% sodium hypochlorite (NaOCl), regardless the gender or the age.

Further studies are needed to assess the effect of different concentrations of propolis nanoparticles and sodium hypochlorite (NaOCl) on bacterial endotoxins level inside the root canal.

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