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Evaluation of Propolis as Intracanal Medicament against *Enterococcus faecalis*

Randa O. Elsayed[1], Sanaa O. Yagoub[2], Neamat Hassan Abubakr[3*]

[1]Dental Materials Department, Faculty of Dentistry, Al Neelain University, Khartoum, Sudan
[2]Microbiology Department, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan
[3]School of Dental Medicine, University of Nevada, Las Vegas, NV, USA
Email: Randaosman909@hotmail.com, Soyagoub08@hotmail.com, *neamat.hassan@unlv.edu

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Abstract

The present study aimed to comparatively evaluate Propolis, Calcium hydroxide $Ca(OH)_2$, Chlorhexidine gel 2% (CHX) intracanal medicaments, and a mixture of Propolis with $Ca(OH)_2$ and CHX against *Enterococcus faecalis*. **Material and methods:** One hundred and eight single-rooted extracted human teeth were used; all teeth were cut 14 mm away from the apex and prepared until size 25 master apical file. Samples were sterilized and dipped in Brain Heart Infusion Broth (BHI) contaminated with *E. faecalis* for 21 days at 37˚C. Samples removed, divided into six groups, injected with different medicaments as following: group A: Propolis; group B: $Ca(OH)_2$; group C: CHX; group D: Propolis + $Ca(OH)_2$; group E: Propolis + CHX, group F: control group with no medicament, teeth sealed with filling material and nail varnish for three times periods 2, 7 and 10 days. After each time period, samples were opened and irrigated with normal saline. H-file applied to the full length to obtain dentine chips, the files collected into test tubes containing sterile BHI and stored for 48 hours under anaerobic conditions. Samples were then removed and culture in Mueller-Hinton agar, then samples incubated for one week at 37˚C after which colony forming units counted using colony counter, statistical analysis was done with ANOVA and post hoc use LSD. **Results:** Propolis, 2% Chlorhexidine gel, and a mixture of Propolis with Calcium hydroxide were effective against *E. faecalis*, Calcium hydroxide, and a mixture of Propolis Chlorhexidine was not effective against it. **Conclusions:** Propolis showed a promising role as an intracanal medicament.

Keywords

Propolis, Chlorohexidine, Calcium Hydroxide, *Enterococcus faecalis*

1. Introduction

The endodontic treatment's success is most significantly dependent upon eliminating root canal infection and preventing contamination during treatment [1]. The microbial flora in canals after endodontic failure was limited to predominantly Gram-positive microbial species. Facultative anaerobes, especially *E. faecalis*, were the most commonly isolated microorganisms [2].

Intracanal medicaments have been thought of as an essential step in killing the bacteria in the root canal, and if multiple-visit endodontics is chosen, an intracanal medicament is strongly recommended [3]. The intracanal medicaments may be used for several purposes; it eliminates any remaining bacteria after canal instrumentation, reduces inflammation of periapical tissues and pulp remnants, renders canal contents inert and neutralizes tissue debris, acts as a barrier against leakage from the temporary filling, and help to dry persistently wet canals [4]. Currently, calcium hydroxide is the most used intracanal dressing [5]. However, its effectiveness against *E. faecalis* is controversial [6] [7]. Chlorhexidine having an advantage of bactericidal action, substantivity, biocompatibility, low toxicity, and lesser chances of developing resistance [8], as it possesses a wide range of antimicrobial activity; CHX has been used in Endodontics as an irrigating substance or intracanal medicament [9]. However, a recent study shows that Chlorhexidine reduced the success of root canal treatment [10].

Propolis is natural material synthesis by Bee; it has a complex composition such as esters, phenolic compounds, and flavonoids. Propolis has been revealed to possess antibacterial, anti-inflammatory, antiviral, antifungal, antioxidant, and immunomodulatory effects [11]. Propolis exhibits antimicrobial activity against *E. faecalis*, *S. aureus*, *C. albicans* and it could be used as intracanal medicament [12], it has been demonstrated in various *in vitro*, *in vivo* and *ex vivo* studies, as well as in human clinical trials [13]. Several studies revealed that Propolis had good antibacterial activity against *E. faecalis* in the root canals and suggested that it could be used as an alternative intracanal medicament [12] [14] [15] [16].

This is an *ex vivo* study aimed to evaluate Propolis, Calcium hydroxide paste, Chlorhexidine gel 2%, and their mixtures as intracanal medicaments against *E. faecalis*.

2. Materials and Methods

This study was conducted at the Faculty of Dentistry and Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al Neelain University, Khartoum, Sudan. Ethical consent was obtained from Al Neelain University Ethical and Research Committee. Verbal consent was also requested by the patient to volunteer in providing their extracted teeth for experimental purposes.

2.1. Study Population

The single rooted human teeth were collected after extraction due to different

dental indications in Khartoum state.

2.2. Sample Size

Sample size was 108 teeth, divided into six groups from (A to F); each group contains 18 teeth based on the calculation from previous study by Bolla *et al.* [17].

2.3. Preparation of the Extracts

100 gram of Propolis sample was extracted, extraction was carried out according to method described by Sukhdev *et al.* [18], and 1000 ml with 80% ethanol (S D Fine Chemicals, India) was used to dissolve the sample. The yield percentage was calculated as followed: Weight of extract obtained/weight of plant sample × 100.

The 100 gms of the Propolis was yield as 38.26 g giving a yield percentage of 38.26% Propolis ether extract.

2.4. Preparation of the Samples

The collected single-rooted teeth were stored at formalin solution until used. The methodology used was adapted from Gomes *et al.* [19]. Before starting root canal preparation, scaling was done with an ultrasonic scaler to remove tissue remnants and stains around the teeth. To standardized root canal preparation, all teeth crown was cut 14 mm away from the apex using a diamond disc in the micro motor (EMMI VI Sri—Badia Polesine RO, Italy). Then root canals were prepared until size 25 using K-file (Mani, Inc, Tochigi, Japan). After preparation, the apical region sealed by Glass ionomer cement (GIC) (Changshu Shang Chi Dental Materials Co. Ltd) to block bacterial microleakage. The smear layer removed using EDTA 17% (Prep Gel, MIXODENT, Delhi, India). Teeth were kept in sterilizing pouches, then autoclaved at 121˚C for 30 min.

2.5. Preparation of the *Enterococcus faecalis*

The *Enterococcus faecalis* was obtained from the Department of Microbiology, Faculty of Medical Laboratory, Al Neelain University. The isolate was reconfirmed by morphological appearance, cultural characteristic and biochemical reactions. The average number of viable organisms per ml of the stock suspension was determined by using surface viable counting technique (Miles and Misra technique) [20], the number of developed colonies (CFU) was counted. It was about 6000×10^{-6}. Brain Heart Infusion Broth (Himedia, Mumbai, India) was prepared with a 37 gm/Liter concentration. Seven bottles with 200 ml each of brain heart infusion broth were prepared. The sterilized teeth were divided into six bottles containing sterile media (18 teeth for every group) and autoclaved at 121˚C for 20 minutes. One bottle containing only sterile media was applied as Control for media sterility.

2.6. Inoculation of Samples

After sterilization, the six bottles that contained teeth were removed and wait until it reaches room temperature, then 2 ml of bacterial suspension prepared

before was added to them. All bottles were incubated at 37˚C for 21 days. The presence of viable bacteria was checked during the incubation period from 7 days to 21 days. The bottle prepared for the sterility following was clear, unlike the other six bottles, which showed turbidity.

2.7. Antimicrobial Activity of the Medicaments

After the incubation period (21 days), the teeth were removed divided into six groups the teeth canal were injected with the different intracanal medicament (Table 1). The distribution of the groups were as follows: group I [60% Propolis]; group II [($Ca(OH)_2$ paste (Well-paste, Vericom CO, LTD, Korea)]; group III [2% Chlorhexidine Gluconate gel (PPH Cerkamed, Poland)]; group IV [Propolis + $Ca(OH)_2$]; group V [Propolis + CHX 2%]; group VI [Control group with no medicament]. Teeth were then seal with temporary restorative material (Ora-fill-G temporary filling material, PREVEST, DenPro, limited, India) and nail varnish. The samples were applied and sealed in sterile Petri plates at 37˚C, each group was divided into three subgroups containing six teeth; one subgroup was examined after two days, the second subgroup was examined after seven; the last group was examined after ten days. At the target time, the samples were removed, opened, and irrigate with sterile normal saline. H-file size 25, applied to the full working length to obtain dentine chips. The H files were inserted into a test tube containing BHI, and stored for 48 hours in anaerobic condition at 37˚C. The number of bacteria in the broth was counted from the control group, it was more than 300×10^{-2} considered as un countable number of bacteria, then samples were removed and aseptically culture in Mueller-Hinton agar for one week at 37˚C, Colony-forming units were counted using colony counter. All readings were taken after seven days.

2.8. Statistical Analysis

Data was manipulated through the statistical software: Statistical package for social science (SPSS) version 20. The data were statistically analyzed with a one-way analysis of variance using the ANOVA and post hoc test with least significant difference (LSD) multiple comparison tests to check the difference between the different medicaments. P-value ≤ 0.05 was considered statistically significant.

Table 1. Description of the examined groups.

Group Number	Component of the Intracanal Medicament
Group I	60% Propolis
Group II	Calcium hydroxide [$Ca(OH)_2$] paste
Group III	2% Chlorhexidine [CHX] gel 2%
Group IV	60% Propolis + $Ca(OH)_2$
Group V	60% Propolis+ 2% CHX gel
Group VI	Control group with no medicament

3. Results

1) Number of the bacteria counting from teeth treated with different medicaments after two days' (group 1).

In this study, CHX showed the best activity for inhibition of the *E. faecalis*, the number of the inoculated bacteria was dramatically reduced from 6000×10^{-6} to 1.5×10^{-2}. Similarly both Propolis and the mixture of Propolis and calcium hydroxide showed apparent reduction of the number of the bacteria to 18.5×10^{-2}, and 46×10^{-2}, respectively. While, the teeth that received calcium hydroxide alone, the mixture of Propolis and Chlorhexidine, and control (teeth receiving no treatment), all showed uncountable (more than 300×10^{-2}) number of the *E. faecalis* (Table 2).

2) Number of the bacteria counting from teeth treated with different medicaments for the seven days' (group 2).

This group was evaluated after 2 days, 4 days and 7 days. Chlorhexidine showed the best activity against *E. faecalis* the number of the inoculated bacteria was reduced from 6000×10^{-6} to 100×10^{-2}, 90×10^{-2} and 26×10^{-2} after 2, 4 and 7 days respectively. Propolis showed an apparent reduction of bacterial colonies from uncountable after 2 days to 116×10^{-2} after 4 days and 72×10^{-2} after 7 days. The mixture of Propolis and calcium hydroxide also showed a reduction of the number of the bacteria to 3×10^{-2}, and 7×10^{-2} and 16×10^{-2} respectively. While, calcium hydroxide alone, the mixture of Propolis and Chlorhexidine and teeth that inoculated with the bacteria and not receive any treatment (control) were all showed an uncountable number of the *E. faecalis* during these examination, (Table 3). Showed number of the bacteria counting from teeth treated with different medicaments during seven days (CFU 10^{-2}).

Table 4, showed the statistical analysis of these groups, the table showed that there is highly significant difference between medicaments.

3) Number of the bacteria counting from teeth treated with different medicaments for the ten days' (group 3).

This group was evaluated after 2 days, 4 days and 7 days. In this group, Chlorhexidine showed the highest activity of inhibition of the *E. faecalis* after two, four and seven days. The number of the inoculated bacteria was

Table 2. Number of the bacteria count (CFU 10^{-2}) from teeth treated with different medicaments for group 1.

Medicaments	Number of bacteria CFU 10^{-2}
Calcium hydroxide	Uncountable
Chlorhexidine	1.5×10^{-2}
Propolis	18.5×10^{-2}
Propolis + Calcium hydroxide	46×10^{-2}
Propolis + Chlorhexidine	$>300 \times 10^{-2}$
Control group (without medicament)	$>300 \times 10^{-2}$

Table 3. Number of the bacteria counting from teeth treated with different medicaments for seven days (CFU 10-2).

Medicaments	At 2 days	At 4 days	At 7 days
$Ca(OH)_2$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$
CHX	100×10^{-2}	90×10^{-2}	26×10^{-2}
Propolis	$>300 \times 10^{-2}$	116×10^{-2}	72×10^{-2}
Propolis + $Ca(OH)_2$	3×10^{-2}	7×10^{-2}	16×10^{-2}
Propolis + CHX	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$
Control group	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$

Table 4. Statistic analysis using ANOVA for the different medication groups.

Medicament	Mean	Std. Deviation	Std. Error	F	P-Value
$Ca(OH)_2$	300.00	0.00	0.00		
CHX	41.50	43.24	17.65		
Propolis	103.50	100.35	40.97		
Propolis + $Ca(OH)_2$	17.83	13.82	5.64	16.932	**<0.001***
Propolis + CHX	169.67	142.99	58.38		
Control group	300.00	0.00	0.00		
Total	133.38	134.79	20.80		

reduced from 6000×10^{-6} to 1.5×10^{-2} after 7 days the count increased to 30×10^{-2}. Propolis showed an apparent reduction of bacterial colonies to 52×10^{-2} after 2 days and to 39×10^{-2} after 4 days, then the count increased to 42×10^{-2} after seven days. The mixture of Propolis and calcium hydroxide reduced the number of bacteria to 12×10^{-2} after 2 days and to 31×10^{-2} after 4 days and the count increased to 38×10^{-2}. While the mixture of Propolis and Chlorhexidine gel 2% show pattern of activity against the *E. faecalis* and the number of colonies was reduced to 25×10^{-2} after 2 days, then count increased 47×10^{-2} after 4 days and then decreased 46×10^{-2} after 7 days. Both calcium hydroxide and control groups all showed uncountable number of the *E. faecalis* (Table 5).

Results showed a high significant difference between medicaments, so there is a need for multiple comparisons. Multiple comparison was done use post hock test least square different (LSD), shown in Table 6.

The value of the coefficient of determination (R2) indicates that group and medicament explain 99.5% of CFU variation. There is a highly significant effect of group on CFU, also highly significant effect of medicament on CFU. The interaction between the two independent variables and medicaments is highly significant; each medicament's effect on CFU is not the same for all groups. The final comparison between tested medicaments in different groups (CFU) through different periods is shown in Figure 1.

Table 5. Number of the bacteria counting from teeth treated with different medicaments for ten days (CFU 10-2).

Medicaments	After 2 days	After 4 days	After 7 days
$Ca(OH)_2$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$
CHX	1.5×10^{-2}	1.5×10^{-2}	30×10^{-2}
Propolis	52×10^{-2}	39×10^{-2}	42×10^{-2}
Propolis + $Ca(OH)_2$	12×10^{-2}	31×10^{-2}	38×10^{-2}
Propolis + CHX	25×10^{-2}	47×10^{-2}	46×10^{-2}
Control group	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$	$>300 \times 10^{-2}$

Table 6. Multiple comparisons for CFU by medicament.

(I) Medicament		Mean Difference (I-J)	Std. Error	P-Value
($Ca(OH)_2$)	2% CHX gel	258.5000	42.5424	0.000
	Propolis	196.5000	42.5424	0.000
	Propolis + $Ca(OH)_2$	282.1667	42.5424	0.000
	Propolis + 2% CHX gel	130.3333	42.5424	0.005
	Control group	0.0000	42.5424	0.000
2% CHX gel	Propolis	−62.0000	42.5424	0.155
	Propolis + $Ca(OH)_2$	23.6667	42.5424	0.582
	Propolis + 2% CHX gel	−128.1667	42.5424	0.005
	Control group	−258.5000	42.5424	0.000
60% Propolis	Propolis + $Ca(OH)_2$	85.6667	42.5424	0.053
	Propolis + CHX	−66.1667	42.5424	0.130
	Control group	−196.5000	42.5424	0.000
60% Propolis + $Ca(OH)_2$	Propolis + CHX	−151.8333	42.5424	0.001
	Control group	−282.1667	42.5424	0.000
60% Propolis + 2% CHX gel	Control group	−130.3333	42.5424	0.005

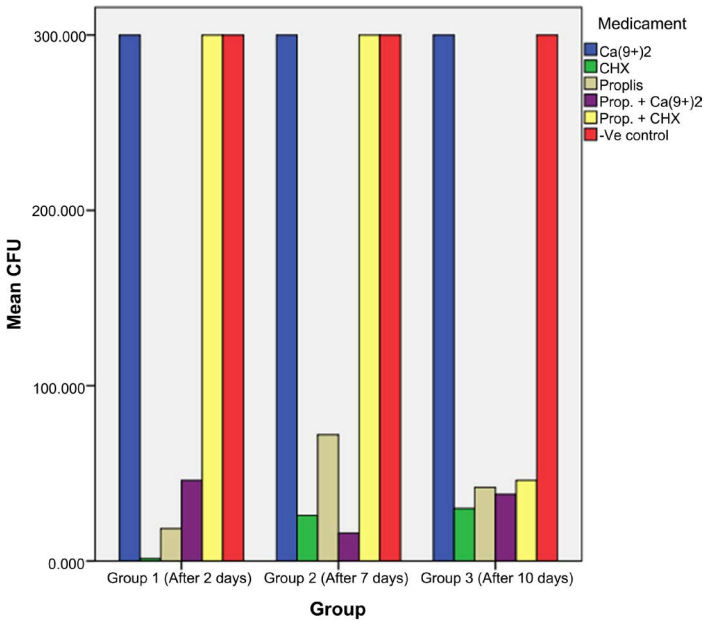

Figure 1. Comparison between different medicaments in different groups.

4. Discussion

The causative factors of pulpal and peri-radicular inflammation are bacteria and their byproducts. The cleaning and shaping of the root canal significantly reduce the microbial content; however, the root canal anatomy provides areas in which bacteria can be hidden. Consequently, there is an essential need for the use of an intracanal medicament in addition to the irrigants [21]. The present study focuses on comparing the antibacterial properties of different intracanal medicaments against *E. faecalis*, which could be considered a limitation of this study as it is better to measure effectiveness against bacterial biofilm. *Enterococcus faecalis* is the main species that cause endodontic failure [21]. It is known that most of the herbs are safe, readily available, increased shelf life, cost-effective, and lack microbial resistance [22]. Propolis also has these advantages. This study showed that ethanolic extracted Propolis (EEP) was effective against *Enterococcus faecalis*, it had an apparent effect against *E. faecalis* in all periods of the study, follow up of bacterial growth show a considerable reduction in the bacterial count, this indicates that Propolis will be useful intracanal medicament especially in the treatment of root canal failure. The present findings agree with others, who concluded that Propolis had potent antibacterial activity against *E. faecalis* in human teeth [12] [23]. AL-Beitawi *et al.* also evaluated the efficacy of Propolis and calcium hydroxide in *ex vivo* as a short-term intracanal medicament. They were found that Propolis is very useful in rapidly eliminating *E. faecalis* [24]. Other investigations also revealed that Propolis is effective against *E. faecalis* bacteria [14] [15] [16] [25]. It was indicated that the EEP is effective as an intracanal medicament *ex vivo* [26] and also in extracted teeth [27]. Another investigation indicated the effectiveness of Propolis in controlling dental infections [28].

CHX has been used in endodontics as an intracanal medicament; present findings showed that 2% CHX gel is patent material against *E. faecalis*; it reduces bacterial growth after 2 days 7 days after 10 days, the bacterial colonies were increased in number. Zohreh Ahangari *et al.* found that 2% Chlorhexidine Gluconate (CHX) was influential on *Enterococcus faecalis* contaminated root canals of human extracted teeth [29]. It was shown that 2% CHX gel was the most effective agent against *E. faecalis* inside dentinal tubules, while $Ca(OH)_2$ alone was ineffective [30]. Sodium hypochlorite (NaOCl) is the most commonly used root canal irrigant; some studies reported that when NaOCl and CHX have combined, the occurrence of dark-brown color changes and precipitation is noticeable [31] [32], this is due to the chemical reaction of both materials. This might indicate that practitioners should be careful when using CHX and sodium hypochlorite as an irrigant. The use of ethanol extracted Propolis was suggested to be safe and effective as CHX on oral microorganisms [33]. The present findings agree with previous studies' results, which indicated that both CHX and Propolis were the most effective against *E. faecalis* [34] [35]. Based on the present findings, it is recommended to use Propolis as the first alternative for CHX, especially when using sodium hypochlorite as an irrigant, and more investigations

are recommended to evaluate the interaction between Propolis and different used irrigants.

Regarding Calcium Hydroxide material in this study, it was clear that it was not effective against *E. faecalis* bacteria; it did not reduce bacterial growth. These results reported by many researchers, since 1987, Haapasalo and Ørstavik reported that a $Ca(OH)_2$ paste failed to eliminate, even superficially *E. faecalis* in dentinal tubules [36].

The mixture between Propolis and calcium hydroxide was done to take the benefits of calcium hydroxide, mainly the high pH, and observe a possible improvement of the calcium hydroxide paste against *E. faecalis*. Although $Ca(OH)_2$ alone did not showed activity against *E. faecalis*, it was surprising that mixing of 1:1 Propolis and $Ca(OH)_2$ showed evident antibacterial activity in eliminating the tested organism, mostly if left for one week inside the root canal. Attempts to improve the effectiveness of calcium hydroxide pastes have been proposed using chemicals such as p-chlorophenol and Chlorhexidine [37]. Dausage *et al.* also supported this finding in 2017, who reported that the diffusion ability of calcium hydroxide ions through dentinal tubules is facilitated by the addition of herbs [38]. It has been recognized that long-term exposure to calcium hydroxide alone decreases the strength of root dentin and changes its physical properties, while a mixture of calcium hydroxide with other materials decreases it is an adverse effect on root dentine [39].

This study is in accordance with others who revealed that the bacterial growth of *E. faecalis* was inhibited by brown Propolis extract alone and when mixed with calcium hydroxide [40]. Previously Calcium hydroxide-Propolis paste was able to diffuse through the dentinal tubules [41] [42].

Regarding the mixture of Propolis with 2% CHX gel, it was not effective against the bacteria after 2 days and 7 days of the insertion inside the root canal, but after insertion of the mixture for 10 days, it reduced the bacterial growth, this may indicate that mixture of Propolis and Chlorhexidine need to stay for a long time in the root canal to be effective. There are no many studies regarding the effect of the mixture between Propolis and CHX against *E. faecalis*. However, other studies evaluate the combination of CHX and $Ca(OH)_2$, and they found that $Ca(OH)_2$ decreases the antibacterial activity of CHX, possibly to loss of its ability to adhere to the bacterial cell wall [43]. Another study showed an additive antibacterial effect on mixing $Ca(OH)_2$ powder with 0.5% CHX, where the CHX had a reduced antibacterial action [25]. Still, the usefulness of mixing $Ca(OH)_2$ with CHX remains unclear. This may also occur when mixing Propolis with Chlorhexidine, the mixture may negatively affect the action of Chlorhexidine and affect its antimicrobial ability, or this mixture may cause difficulty in the diffusion ability of CHX. More researches are needed in this area and to study the effect of Propolis in *E. faecalis* biofilm.

5. Conclusion

Within the study's limitations, results showed that both Propolis and Chlorhex-

idine gel 2%, in addition to a mixture of Propolis with Calcium hydroxide, were effective against *Enterococcus faecalis*. Propolis showed a promising role as intracanal medicaments. It can be used alone or in a mixture with $Ca(OH)_2$ as an alternative for CHX, mainly when sodium hypochlorite is used as a root canal irrigant. However, further clinical trials and researches are required to be considered effective alternatives to synthetic intracanal medicaments.

Ethical Statement

Ethical consent was obtained from Al Neelain University Ethical and Research Committee on December 2016. Verbal consent was also requested by the patient to volunteer in providing their extracted teeth for experimental purposes.

Authors' Contribution

Concept—ROE, NHA; Design—ROE, NHA, SOY; Supervision—NHA, SOY; Funding—ROE; Materials—ROE; Data collection and/or processing—ROE; Analysis and/or interpretation—ROE; Literature search—ROE, NHA Writing—ROE, NHA, SOY; Critical Review—ROE, NHA, SOY.

Conflicts of Interest

The authors deny any conflicts of interest. We have no financial affiliation, or involvement with any commercial organization with direct financial interest in the subject or materials discussed.

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