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Investigating the Effects of Herbal Nanoparticle Endodontic Irrigants on *Candida albicans* and *Enterococcus faecalis*: An *In Vitro* Study

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ABSTRACT

Sodium hypochlorite irrigant (NaOCl) has many disadvantages although it possesses antibacterial activity and having the capability to disband the pulp tissue.. The aim of this study was to compare the effect of two nanoparticle herbal irrigants to that of Sodium hypochlorite (NaOCl) on *Enterococcus faecalis* and *Candida albicans*. Sixty single-rooted teeth were mechanically prepared. Thirty teeth were inoculated with *Enterococcus faecalis*, and the other 30 with *Candida albicans*. These teeth were divided into three groups: Group 1 contained 20 *Enterococcus faecalis*-inoculated teeth, which were further divided into 10 teeth irrigated with nanocurcumin and 10 teeth irrigated with nanoneem. Group 2 contained 20 *Candida albicans*-inoculated teeth which were irrigated like Group 1. Group 3 contained ten *Enterococcus faecalis*-inoculated teeth and ten *Candida albicans*-inoculated teeth which were irrigated with 2.5 % NaOCl. Microbial swabs were collected from the teeth before and after irrigation and cultured. Pre-operative and post-operative colony forming units (CFU) were compared in each sub-group using the Mann-Whitney test and the Kruskal-Wallis compared the difference between the groups. Significant post-operative reduction of the CFUs was shown for the incubated bacteria and fungi in both the nanoneem and nanocurcumin groups. However, the NaOCl showed a significantly greater reduction of the CFU than the nanoparticle herbal irrigants. The use of nanoneem and nanocurcumin in conjunction with sodium hypochlorite as root canal irrigants is advantageous due to their antibacterial and antifungal effect.

***Keywords*:** Root canal irrigants, antibacterial and antifungal effect, Nano-curcumin, Nano-neem

Introduction

The success of endodontic treatment depends on the ability to properly clean the root canals of pulp tissue and disinfect it. This can be achieved with proper instrumentation and irrigation of the root canal. However, instrumentation reaches about 65% of the canal surface leaving some pulp tissue behind. Furthermore, shaping the canals produces a smear layer of dentin debris and over-instrumentation may lead to iatrogenic errors such as apical transportation, and crack formation as well as weakening of root structure.[1,2]

Failure to disinfect the root canals with antimicrobial irrigants may lead to failure of the root canal treatment. Many studies have shown that *Enterococcus faecalis (E. faecalis*) and *Candida albicans* (*C. albicans*) found in endodontic infections can penetrate the root wall and reach its outer surface,[3] leading to periradicular inflammatory lesions. [4,5,6]

Irrigating solutions should have a wide antibacterial action, dissolve nonvital pulp tissue, disrupt endotoxins, prevent the production of the smear layer, or remove it once it has formed.[7] Sodium hypochlorite irrigant (NaOCl) has a powerful antimicrobial effect and the ability to disband pulp tissue, so it is considered the gold standard for irrigation.

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The concentrations of sodium hypochlorite play a major role in removing *E. faecalis* in both biofilm and planktonic form. Higher concentrations result in better outcomes.[8] Unfortunately, NaOCl irrigant has some drawbacks, such as hypersensitivity reactions, tissue sepsis, damage to the instruments, irritation to periodontium tissue, failure to remove the smear layer, and an unfavorable taste and smell.[9] NaOCl has a substantial impact on the mechanical characteristics of dentin, including flexural strength, microhardness, roughness, elastic modulus, inorganic content, and organic-inorganic ratio.[10] Furthermore, NaOCl cannot penetrate narrow areas of the root wall.[11]

Herbal medicine has gained popularity as a therapeutic agent due to its biocompatibility, safety, and cost-effectiveness. Therefore, there is a need to investigate their possible use in dentistry.[12] The main benefits of using herbal irrigants are their low microbiological resistance, safety, availability, and extended shelf-life.[13] Its greater patient acceptance compared to other irrigants such as NaOCl, along with its sustainable nature, renders it an efficient antibacterial irrigant for the eradication of E. faecalis.[14] Many herbal irrigants are available, including *Morinda citrifolia*, *Curcuma longa*, Tea tree oil, and *Azadirachta indica* (Neem). These herbal plants possess therapeutic properties and powerful effects on different microbes. When used as endodontic irrigants, they are antibacterial, antioxidant, as well as biocompatible.[9,15] These advantages make herbal irrigants a possible alternative to NaOCl.

Curcuma longa (C.longa) is an herbal plant that belongs to the Zingiberaceae family commonly called turmeric. It is characterized by its anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal, and antiviral properties.[16] However it is less soluble in water with subsequent poor absorption, and fast metabolism.[17] Curcumin is only soluble in organic solvents, which are toxic making it of limited biological use.[18]

Neem is another botanical extract with antibacterial, anti-inflammatory, and anti-cariogenic activities.[19] Because neem contains a variety of

active phytoconstituents, including flavonoids, isoprenoids, and acid metabolites, it is a preferred material for root canal irrigation and a safer substitute for chemical irrigants like sodium hypochlorite.[20] Neem is highly biocompatible.[21] Thus, it can serve as a potent herbal substitute for sodium hypochlorite irrigant. Adding sweets and other flavors can counteract the bitter taste of neem, which is the main downside that decreases the patient's capacity to use the medicine.[14] While many studies concluded that neem has an efficient antimicrobial effect compared to endodontic irrigants, specifically NaOCl, others showed that neem has limited action against the main microbes in root canal infections like, *E. faecalis* and *C. albicans*.[22, 23] The use of nanotechnology to modify the particle size may improve the properties and efficacy of herbal irrigants. The process of synthesizing curcumin nanoparticles improves the drug's cellular penetration, water solubility, and bioavailability. It also enables targeted distribution to specific target locations and increases the drug's systematic delivery of tiny molecules such as proteins and nucleic acids.[24] Some studies showed that nanocurcumin showed stronger antifungal properties than native curcumin.[25] Also downsizing particles of the neem extract may improve its antimicrobial action against microorganisms causing endodontic infection. Few studies have assessed nanocurcumin as endodontic irrigants and to our knowledge, this is the first study to introduce nanoneem as an irrigant. This study evaluated the effect of nanoparticle herbal irrigants on *E. faecalis* bacteria and *C. albicans*.

Materials and Methods

Materials and reagents

Preparation of irrigants utilizes high-power ultra-sonication probe (Sonics Vibra Cell, Ningbo Haishu Kesheng Ultrasonic Equipment Co., Ltd., China), homogenizer (Unidrive X1000 model, CAT, Germany), Transmission Electron Microscopy (TEM) (Spectra 300, Thermo Fisher Scientific Co., Waltham, MA, USA).

While inoculation of pathogenic microorganisms includes pathogenic yeast (*Candida albicans* ATCC 10231) and gram-positive bacteria (*Enterococcus faecalis* ATCC 29212), Erlenmeryer flasks (Taizhou Sun Trine Biotechnology Co., Ltd., Taizhou, Jiangsu, China), cryovial (YD-2.0ML, Foshan Yuyang Medical Instrument Co., Ltd., China).

Extracted teeth from the surgical department, at Cairo University were collected, diamond disc (Mani, Inc., Tochigi, Japan), light-cure composite (3M ESPE Z250, USA), No. 4 round carbide bur (DENTSPLY Maillefer, OK, USA), Gates Glidden drills size 1:4 (Mani, Inc., Tochigi, Japan), Pro-Taper (DENTSPLY Maillefer, OK, USA) were used for preparing the teeth.

Methods

Preparation of nanoneem and nanocurcumin irrigants

The preparation of solid lipid nanoparticles (SLN), is accomplished in several studies using an ultrasonic-solvent emulsification technique.[26-29]. A high-power ultra-sonication probe was used to treat the coarse emulsion to 55 W for 5 min. with a water bath (0 ^{0}C). A homogenizer was used to scatter the cold nanoemulsion into cold water. Traces of organic solvents were removed by magnetic stirring. Impurities were removed from the oil-loaded SLN suspension and then stored at 4 ^{0}C for further bioassays. Transmission Electron Microscopy (TEM) analysis was used to characterize the nanoneem and nanocurcumin particles, their sizes were below 100 nm. The effective electric charge on the surface of nanoparticles for both liquids showed a negative zeta potential.

Microorganisms and culture conditions

Qualitative evaluations were carried out in nutrient broth according to Elboraey et al. & Sultan et al.[30,31] The study's adopted inoculation of pathogenic microorganisms consisted of pathogenic yeast (*Candida albicans*) and gram-positive bacteria (*Enterococcus faecalis*), which were obtained from fresh overnight broth cultures in nutritional broth medium. The microbial stoke inoculum was prepared by inoculating 100.0 µL from each test strain separately into 100.0 mL-volume of Erlenmeryer flasks incorporating 25.0 mL of nutrient broth medium, and it was incubated for 24 hours at 37 °C.[31, 32] 20.0 µL of the above-prepared inoculum of each test strain were separately inoculated into each 2.0 mL-volume cryovial containing one tooth with one central hole filled with 1.0 mL of the sterile nutrient broth medium (NB). The cryovials containing the inoculated teeth were incubated at 37 °C for 21 days for more test strain attachment. After the proper incubation period, the first inoculum was taken from each cryovial as a control sample.

Teeth selection and preparation

Sixty extracted teeth, including incisors, canines, and premolars were selected. Once the intraoral periapical radiograph was thoroughly examined, the inclusion criteria comprised a single canal and a completely developed apex. Exclusion criteria were teeth with caries, fracture, resorption, or malformation. De-coronation of all teeth was done using a diamond disc. As shown in Figure 1. All tooth samples had their apical foramina sealed with light-cure composite (3M ESPE Z250, USA) and then coated with two layers of varnish to prevent any leakage. A No. 4 round carbide bur was used to refine the access. Using radiography as a guide, the working length was established and maintained at 0.5 mm below the radiographic apex. Preparation was done using the crown-down technique. Coronal preparation was done using Gates Glidden drills size 1:4. Following initial instrumentation up to #30 stainless steel hand files, Pro-Taper was used for rotary instrumentation up to F3. To get rid of all the bacteria, all of the teeth were autoclaved twice at 121 °C. Sixty teeth samples are divided into three groups and undergo different interventions.

Group 1: contains 20 teeth inoculated with *E.feacalis* strains divided into two subgroups; each subgroup contains 10 teeth irrigated with 2 mL nanocurcumin and 10 teeth irrigated with 2 mL nanoneem using a 25-gauge needle at a distance of 1 mm from the apex. Left for 5 minutes in contact with the root surface, then returned to the cryovials.

Group 2: contains 20 teeth inoculated with *C. albicans* strains divided into two subgroups; each subgroup contains 10 teeth irrigated with 2 mL of nanocurcumin and 10 teeth irrigated with 2 mL of nanoneem in the same way as the first group.

Group 3: contains 20 teeth: teeth samples inoculated with *E. faecalis* (n = 10) and teeth samples inoculated with *Candida albicans* (n=10), both of them were irrigated with 5.25 % NaOCl and left for 5 minutes.

From the dilution 10^{-4}, 100.0 µL from each sample, either (pre-inoculum or treated sample) was inoculated separately into 9.0 cm sterile plastic Petri-dishes containing 20.0 mL of solidified nutrient agar medium (NA). These plates were incubated at 37 ^{0}C for 24 hours. The microbial inhibition was determined (total viable count technique) by counting the colony-forming units (CFU).

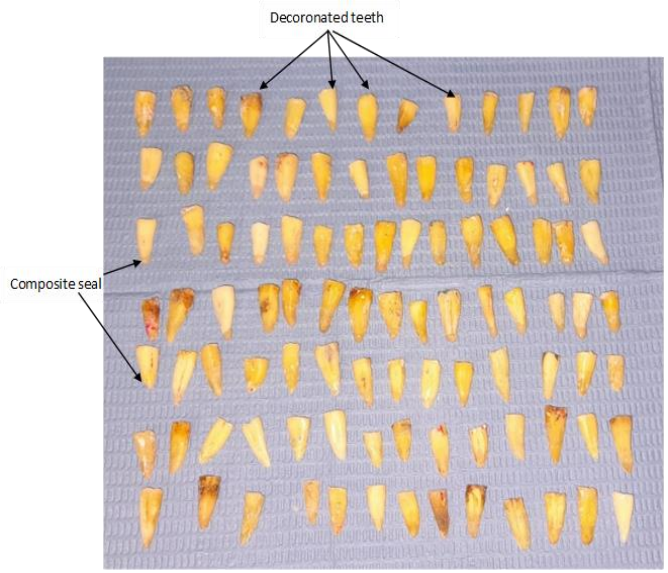

Figure 1: Teeth after decoronation and sealing apically with composite

Statistical methods

The SPSS software application, version 25.0, was used to convert and alter all test data. The mean, standard deviation, standard error of the mean (SE), median, interquartile range (IQR), and range with minimum

and maximum of the CFU*10^{-4} of all pre-and sampled groups were reported. The independent t-test for normally distributed data and the Mann-Whitney for nonparametric data were used to compare the quantitative data. Kruskal Wallis test was used for comparing more than two nonparametric groups with a pairwise test between each group among multiple comparisons using the Bonferroni correction for multiple tests.

Results and Discussion:

Table 1 showed that all irrigants had an effective antibacterial effect against *Enterococcus faecalis*, with significantly better results than the pre-treatment count. Comparison between nanoneem (median 132.5, IQR 10.0-361.5), nanocurcumin (median 169.5, IQR 0.8-298.3) and NaOCl showed no growth of bacterial *Enterococcus faecalis*. The results revealed that there was a statistically significant difference among the groups ($p<0.01$) Comparison between each group and another showed no statistically significant difference between nanoneem and nanocurcumin ($p>0.05$).

On the other hand, the difference was statistically significant between nanoneem and NaOCl ($p<0.001$) and nanocurcumin and NaOCl ($p=0.004$). The comparison of nanoneem, nanocurcumin, and NaOCl CFU*10^{-4} on the bacterial *Enterococcus faecalis* is shown in Figure 2. The mean values reveal statistically significant differences between nanoneem and NaOCl post-treatment samples ($p<0.001$) and nanocurcumin and NaOCl post treatment samples ($p=0.004$). While the distinction between the two nanoherbal irrigants is statistically insignificant ($p>0.05$).

Table 2 shows the efficient antimicrobial effect of nanoneem and nanocurcumin irrigants against *Candida albicans* with significantly better results compared to pre-treatment counts. It showed mean values of nanoneem (median 1006.0, IQR 886.0-1140.0), nanocurcumin (median 1007.5, IQR 810.0-1228.5) and NaOCl (median 5.0, IQR 0-37.5) on *Candida albicans*. The results revealed that there was a statistically highly significant difference among groups ($p<0.01$) while a comparison between each group and another showed no statistically significant difference between nanoneem and nanocurcumin ($p>0.05$). On the other hand, the difference was statistically highly significant between nanoneem and NaOCl ($p=0.001$) and nanocurcumin and NaOCl ($p<0.001$). Figure 3 shows a comparison between nanoneem, nanocurcumin and NaOCl CFU*10^{-4} on *Candida albicans*. Mean values show high statistically significant difference between nanoneem and NaOCl ($p=0.001$) and nanocurcumin and NaOCl ($p<0.001$) where CFU*10^{-4} of *Candida albicans* growth is much lower than other groups. The difference between the two nanoparticles herbal irrigants was statistically insignificant ($p>0.05$).

The goal of the current *in vitro* study was to assess and compare the efficacy of the herbal irrigations with nanoparticles for eliminating common root canal bacteria, particularly *E. faecalis*, which have been shown to penetrate deeper into the dentinal tubules.[3] *Enterococcus faecalis* infections in root canal systems are more resilient than those caused by planktonic forms because of the activation of the virulence factors, the production of biofilms, or the invasion of dentinal tubules.[33] The incubation period in the current investigation was 3 weeks, which has been found to result in a dense infection that extends 200–400 μm into the dentinal tubules.[34] Using a rotary Protaper system to provide more instrumented areas than hand instruments for canal preparation.[35] The apical third of the root canal was instrumented up to the F3 file, which opens the dentinal tubules to facilitate the more effective penetration of antimicrobials and increases the effectiveness of the elimination of intracanal bacteria by accessing inaccessible locations.[36] A 25-gauge side-vented needle could be placed up to 1 mm short of working length without binding, this lessened the applied apical pressure and avoided the vapor lock effect, it likewise rendered it easier for back-and-forth movement of the irrigant to occur in the canal. NaOCl was left for 5 minutes, in contrary to other studies that demonstrated that a contact time of at least 40 minutes with 5.25% NaOCl is more effective.[11]

The pre-irrigation bacterial count provides a benchmark, while the post-irrigation bacterial count indicates antimicrobial activity for different irrigants. Because neem extracts include multiple active ingredients, including nimbidin, nimbin, nimbolide, and azadirachtin, it has been observed that these extracts exhibit a variety of pharmacological properties, responsible for their antibacterial action.[37] Neem irrigant in its nanoform has been investigated as a new endodontic irrigant to explore its effect on common types of microorganisms. It showed a significant reduction in *E. faecalis* compared to pretreatment samples. This was confirmed by additional research that found that irrigation with *Azadirachta indica (A. indica*) extract demonstrated a notable resistance to *E. faecalis* colonization.[38-44]

Table 1: Comparison between pre and postoperative treatment of first and third-group samples against *Enterococcus faecalis* CFU*10-4

		Nanoneem	Nanocurcumin	Sodium hypochlorite
Post Sample	Mean ± SD	203.4 ± 215.8	162.3 ± 156.4	0
	SE	68.3	49.5	0
	Median	132.5	169.5	0
	IQR	10.0-361.5	0.8-298.3	
	Range (min-max)	5.0-616.0	0-410.0	0
Pre Sample	Mean ± SD	705.2 ± 105.2	578.4 ± 67.2	616.0 ± 107.3
	SE	48.4	30.0	48.0
	Median	720.0	559.0	612.0
	IQR	615.0-788.0	522.0-644.5	510.0-724.0
	Range (min-max)	540.0-840.0	492.0-664.0	504.0-732.0
	P value	<0.001*	<0.001*	<0.001*
		P value	Adjusted significance	
Group 1 nanoneem vs. Group 3		<0.001	0.001*	
Group 1 nanocurcumin vs. Group 3		<0.001	0.005*	

Mann-Whitney test: *Statistically highly significant difference $p<0.01$

Table 2: Comparison between pre and postoperative treatment of second and third groups samples against *Candida albicans* CFU*10^{-4}

		Nanoneem	Nanocurcumin	Sodium hypochlorite
Post sample	Mean ± SD	989.8 ± 203.6	1089.3 ± 414.0	25.1 ± 42.3
	SE	64.4	130.9	13.4
	Median (IQR)	1006.0	1007.5	5.0
		886.0-1140.0	810.0-1228.5	0-37.5
	Range (min-max)	616.0-1254.0	645.0-2091.0	0-112.0
Pre sample	Mean ± SD	1591.2 ± 419.1	1950.4 ± 236.3	564.0 ± 144.0
	SE	187.4	105.7	64.4
	Median (IQR)	1592.0	1936.0	488.0
		1248.0-1934.0	1746.0-2162.0	452.0-714.0
	Range (min-max)	908.0-1952.0	1740.0-2320.0	420.0-764.0
	P value	0.002*	0.001*	0.001*
		P value	Adjusted significance	
Group 2 nanoneem vs. Group 3		<0.001	<0.001*	
Group 2 nanocurcumin vs. Group3		<0.001	<0.001*	

Kruskal-Wallis test: *Statistically significant p<0.05

Additionally, the antibacterial activity of neem extract, tulsi extract, and chlorhexidine—herbal irrigants—was evaluated by Chandrappa et al. They discovered that against E. faecalis, all of the irrigants listed were statistically efficacious.[45] This could be because nanoneem contains a variety of active phytoconstituents, including alkaloids, flavonoids, isoprenoids, and acid metabolites.

In this study, NaOCl was much more effective against *E. faecalis* compared to nanoneem irrigant. In accordance, a study found that the most effective antibacterial agent was found in 3% NaOCl, which was followed by neem leaf extract using polymerase chain reaction (PCR),[44,22] due to the high NaOCl concentration, the same as in the present study. Recent studies illustrate that, when compared to neem, sodium hypochlorite showed the biggest inhibitory zone, which could be explained by neem's high diffusiveness and instability on the agar plate. Therefore, the size of the inhibition zones does not indicate the entire antibacterial activity of the substance.[46] Numerous factors, including the unexpected nature of medications, the loading dose on agar plates, the agar solubility and diffusion, and the procedure of extracting herbs, are crucial.[47] Saxena D. et al. analyzed the antimicrobial activity of five herbal extracts, i.e., *Azadiracta indica* (AI), *Propolis, , Triphala, and C. Longa*, They concluded that of all the herbal extracts, propolis exhibited the biggest zone of inhibition, comparable to that of sodium hypochlorite, followed by the other irrigants.[48, 49] Babaji et al. found that A. indica had a smaller inhibitory zone than sodium hypochlorite; this could be explained by the increased incidence of *E. faecalis* in secondary endodontic infections.[50,23] In contrast to the current study, in an in vivo investigation, samples were assessed for colony-forming units following irrigation with neem extract and NaOCl by Dutta et al., who concluded that neem had similar antibacterial efficacy as sodium hypochlorite irrigant, so could be considered for endodontic use. Furthermore, the combination of NaOCl and ethanolic A. indica leaf extract has an anti-microbial effect,[51] This difference in results may be attributed to the difference in nanoneem concentration, which was 5 mg/ml. In the same way, a study stated that neem yielded the same antibacterial activity in comparison to 5% NaOCl.[52] Furthermore, neem showed a significantly higher zone of inhibition compared to 3% sodium hypochlorite against *E. faecalis*.[53-55] This could be because *A. indica* is a strong alternative to sodium hypochlorite because of its antioxidant and antibacterial qualities, which are attributed to its active components such as Nimbinin, Azadirachtin, and Nimbidin.[56] Nanoneem was effective against candida compared to the pre-treatment count, while NaOCl was the best significantly. Likewise,

Neem has been shown by Bohora et al. and Tyagi et al. to be an efficient root canal medication against *Candida albicans* and *E. faecalis*,[57,58] besides being biocompatible with oral and periapical tissues.[59] This may support the antimicrobial effect of nanoneem. A study reported that although sodium hypochlorite is a much more effective irrigant; increasing its concentrations did not significantly affect the zone of inhibition against *C. albicans*. 5%, 3%, and 0.5% NaOCl concentrations showed similar zones of inhibition.[60]

In comparison to the present study, no significant difference was observed between the neem extract and the 2% NaOCl against *C. albicans*. The biggest zone of inhibition was displayed by the neem leaf extract.[57,54] Another study reported a comparable zone of inhibition with 3% NaOCl against *Candida albicans*,[61] supporting nanoneem results in this study compared to 5.25% NaOCl.

Jose *et al*.[23] reported that *A. indica* leaf extract was less effective against *Candida*, while Dedhia J. showed that neem exhibited no activity against *C. albicans*. This may be due to the major disadvantage of herbal extracts; a lower concentration of active ingredients and the requirement for fresh preparation could be the cause of the decreased efficacy.[62]

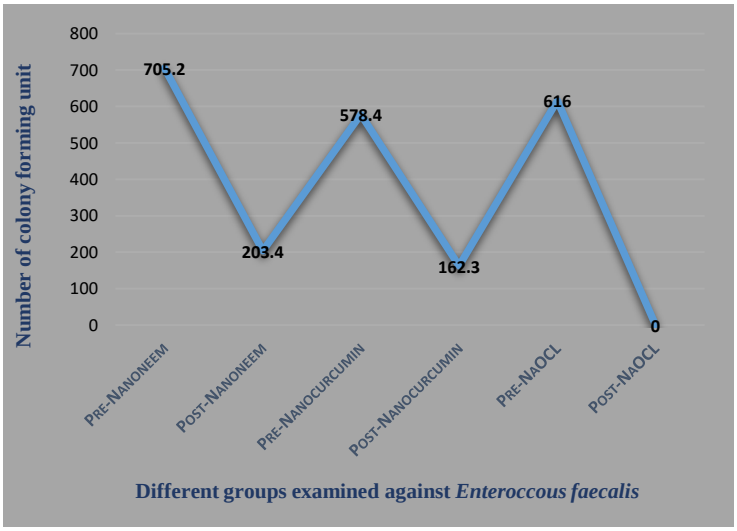

Figure 2: Comparison between mean values of pre-Nanoneem and pre-Nanocurcumin samples on *Enterococcus faecalis* using CFU*10^{-4} (n=10 samples in each group).

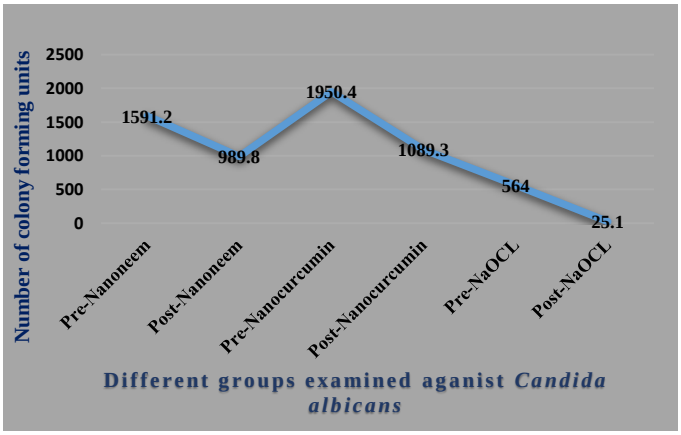

Figure 3: Comparison between the second group and NaOCl group samples against *Candida albicans* using CFU*10^{-4} (n=10 samples in each group).

5.25% NaOCl was better and statistically significant compared to nanocurcumin irrigant against *E. faecalis*. In the same way, the highest level of inhibition was attained with 5% NaOCl, and then with *C. longa*, where NaOCl achieved 100% killing of *Enterococcus faecalis*.[63] The present study showed efficient antibacterial activity compared to pretreatment count, by reporting that Turmeric's hydro-alcoholic and aqueous extracts had antibacterial action against *E. faecalis*, but not to the same extent as the NaOCl group, due to the high concentration of NaOCl used.[64, 65] However, on the other hand, *Turmeric's* ethanolic extract did not exhibit any antibacterial action at any of the tested dosages against either *E. faecalis* [66] or *C. albicans*.[62] Possible causes of the variation of antimicrobial action include differences in extract concentrations and extraction procurement procedures.[62, 65] Similarly, Hegde discovered that there is no zone of inhibition against *E. faecalis* in the hydroalcoholic extract of turmeric.[67]

Nanocurcumin irrigant was statistically significant for antibacterial activity compared to the pretreatment count against *Enterococcus faecalis*. This outcome suggested that curcumin's antibacterial activity was enhanced by reducing the particle size to nanoforms. Numerous investigations have demonstrated *C. longa's* strong antibacterial activity against *E. faecalis*.[68,69] One possible reason for this could be the disruption of bacterial cells.[70] In the same words, comparing curcumin with *Aloe vera* and calcium hydroxide, the antibacterial efficacy of curcumin is maximal against *Escherichia coli, Pseudomonas aeruginosa, E. faecalis, and Staphylococcus aureus*. This effect increases with time and dose, ultimately leading to 100% bacterial eradication.[72] Ninety-five percent of curcumin's antibacterial, antifungal, antiplatelet, and strong antioxidant properties come from *C. longa*.[73,74,75] Rai et al. report that curcumin directly interacts with filamenting temperature-sensitive mutant Z (FtsZ) to impede bacterial cell division and enhance FtsZ's guanosine triphosphatase activity, which is fatal to the bacteria.[76] On the contrary, According to Swapnil SM. et al. and Damre PG. et al., curcumin had less antibacterial effect on *E. faecalis* in its planktonic condition when compared to *Triphala* and calcium hydroxide.[55,77]

Curcumin inhibits fungal growth and shows good inhibitory activity against *C. albicans*.[54,55,78] Per our study, nanocurcumin showed efficient results against *Candida albicans* compared to pretreatment samples. Although NaOCl was better and gave statistically significant results, this may be due to the high concentration of NaOCl used.

Conclusion

The use of herbal irrigants in nanoform might prove to be advantageous because of their antimicrobial activity. When considering the several undesirable characteristics of NaOCl, herbal irrigants could be as adjunctive in cleaning process of endodontic treatment. Alternate use of herbal nanoparticle irrigants would cover gaps in preclinical investigations and, eventually, clinical use. It is necessary to do in-depth in vivo studies to ascertain their toxicity, capability, and susceptibility. The study can be further improved by assessing the antibacterial efficacy using more standardized techniques, such as PCR.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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