ANTIBACTERIAL EFFICACY OF DIFFERENT INTRACANAL IRRIGANTS ON ROOT CANAL TREATMENT

An In-Vitro study

ABSTRACT

Aims: Out of many properties that an endodontic disinfecting agent should possess, the most important is that of having a wide range of antibacterial efficacy. This study has been performed to see the effect of different agents on the bacterial microflora and to see how efficient they are against them. Our study has used 3 different agents (Chlorohexidine, Sodium Hypochlorite, and Neem extract) and compared their efficacy against bacterial microflora.
Study Design: Experimental study design
Place and Duration: The study was conducted in the Department of Endodontics at Fatima Jinnah Dental College and Hospital, Karachi, Pakistan from February 2020 to March 2020.
Methodology: Infected samples from individuals were collected through paper points and then allowed to be cultured and incubated on blood agar plates at 37 degrees in an incubator for 24 hours. The colonies were then identified through the gram staining procedure and grown on MHA agar to conduct the disk diffusion test for sensitivity.Individual zones of inhibition for irrigants were measured and compared against each other.
Results: A total of 36 infected samples were included in the study out of which 12 samples were irrigated with chlorohexidine, 12 with sodium hypochlorite, and 12 with neem extract. there was a statistically significant difference in mean diameters of the inhibition zone observed between the three groups for the mean inhibition zone (F=12.28, P=0.001).
Conclusion: Chlorohexidine showed greater efficacy against bacterial microflora, compared to both sodium hypochlorite and neem extract.

Keywords: *Root canal treatment, chlorohexidine, sodium hypochlorite, neem, intracanalirrigants, neem extract, intracanal medicament*

INTRODUCTION

The goal of endodontic treatment is to cleanse the root canal system and periapical tissues completely and avoid reinfection. The current procedures, equipment, and irrigants for root canal sterilization are limited ..(Ruksakiet, K., Hanák, L., Farkas, N., Hegyi, P., Sadaeng, W., Czumbel, L.M.et al 2020).

The main goals of endodontic treatment are to shape and clean a root canal system while also preserving the surrounding periodontal tissues. While the mechanical parts of a root canal treatment receive the most of the emphasis, irrigation is a critical component.as well.(Dioguardi, M., Di Gioia, G., Illuzzi, G., Laneve, E., Cocco, A.Troiano, G., 2018.)

Irrigants in endodontics have been associated for a long time now. Instrumentation, in combination with irrigation, helps to reduce microbial excess in the root canals. Irrigants can help with mechanical debridement by washing debris out of the root canal system, disintegrating tissue, and disinfecting it. Chemical debridement is especially important for teeth with intricate internal anatomy, such as fins or other anomalies that instrumentation may overlook.. (Kandaswamy D. and Venkateshbabu N, 2010)

Chemical irrigation's main goal is to destroy germs and disintegrate pulpal tissue. Sodium hypochlorite and chlorohexidine, for example, have been shown to be efficient antimicrobials in vitro and are commonly utilised during root canal therapy around the world. However, according to a systematic review, there is a paucity of high-quality evidence to support the use of one irrigant over another in terms of both short- and long-term therapeutic outcome.(Fedorowicz, Z., Nasser, M., Sequeira-Byron, P., de Souza, R.F., Carter, B., and Heft, M., 2012.)

An irrigant should be able to disinfect and penetrate dentin and tubules, provide a long-term antibacterial effect, remove the smear layer, and be non-antigenic, nontoxic, and non-carcinogenic in order to properly clean and disinfect the root canal system. It should also have no negative effects on dentin or the capacity of filling materials to seal. Furthermore, it should be reasonably priced, simple to use, and free of tooth discolouration. The ability to disintegrate pulp tissue and inactivate endotoxins are two other desirable qualities of an ideal irrigant.. (Borzini, L., Condò, R., De Dominicis, P., Casaglia, A. and Cerroni, L., 2016).

Sodium hypochlorite is the most commonly used irrigant with a broad spectrum of antibacterial action and a high potential for disintegrating pulpal tissue. However, because of the pH of 11-12, it has a toxic action that induces protein oxidation, resulting in hemolysis and necrosis. Sodium hypochlorite has a number of drawbacks, including clothing damage, injury to the patient's or operator's eye, and air emphysema while

injecting in the canal..(Zehnder M, 2006, Hulsmann M, Hahn W, 2000). Due to the following disadvantages, there is a need for a new biocompatible and effective root canal irrigant.

Chlorohexidine on the other is another intracanalirrigant, possessing a wide range of antimicrobial activity. It is effective against both gram-positive as well as gram-negative bacteria.(Bhardwaj A, Ballal S, Velmurugan N, 2012). Chlorohexidinegluconate is used as the gold standard antimicrobial agent with the most potent chemotherapeutic activity against many microbes(Agarwal P, Nagesh L, Murlikrishnan, 2012) (Prabhakar AR, Basavraj P, Basappa N, 2013).It is bacteriostatic in low concentration and bactericidal in high concentration(. Bazvand L, Aminozarbian MG, Farhad A, Noormohmmadi H, Hasheminia SM, Mobasherizadeh S, 2014).

Chlorohexidine produces staining of teeth, altered taste, and development of microbial resistance(Agarwal P, Nagesh L, Murlikrishnan, 2010). Sodium hypochlorite has unwanted side effects such as tissue toxicity, allergy, and disagreeable smell and taste(Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D, 2013). Side effects of non-herbal medicines, herbal medicines are gaining importance.

MATERIALS AND METHODS

The study was conducted in the Department of Endodontics at Fatima Jinnah Dental College and Hospital, Karachi, Pakistan fromFebruary 2020 to March 2020. Inclusion criteria were patients between 18 – 50 years both male and female, all teeth except those indicated for the extraction or had undergone previous endodontic treatment were included in the study.

Canals that were shaped till Rotary shaper files were used to collect the specimen. Once the canals had gone through initial filing and prepared till the last shaper file of rotary.A paper point of size 35 was introduced into the canal. The paper point was then carried into a sterile vial containing 1mm of saline. The paper point was then streaked onto blood agar plates and incubated at 37^0C for 24 hours in an incubator. After 24 hours bacterial growth was observed on the bloodagar (Fig 1) .The colonies were then inoculated onto slides for gram staining and identification. The slides were then viewed under a 100x magnification oil immersion lens of a compound microscope. Different bacteria were identified as either gram-positive orgram-negative rods andcocci (Fig 2). The bacteria were then transferred using a sterile culture swab again onto the MHA agar to perform the disk/agar diffusion method to identify bacterial sensitivity against 2% Chlorohexidine, 3% NaOCl, and Neem extract. Three antibacterial sensitivity discs were then addedat a specific distance from each other each of them containing the irritants. Once placed onto the MHA agar the sample was incubated again at 37^0 C for 24 hours. Zones of inhibition (Fig 3) (the zone in which there is an antimicrobial activity seen as visible as a transparent area over the agar plate) were checked after incubation of each plate against the bacterial colonies identified. Zones were measured using a transparent scale and then entered onto the proforma later to be analyzed.

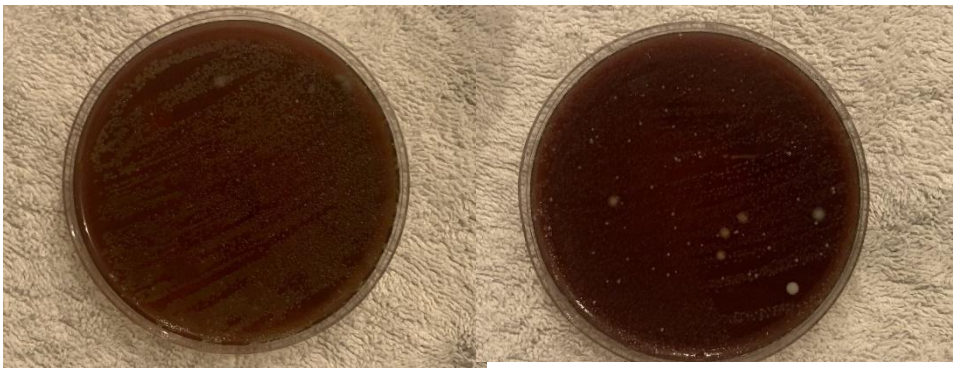

Fig 1: Blood agar plates with bacterial colonies

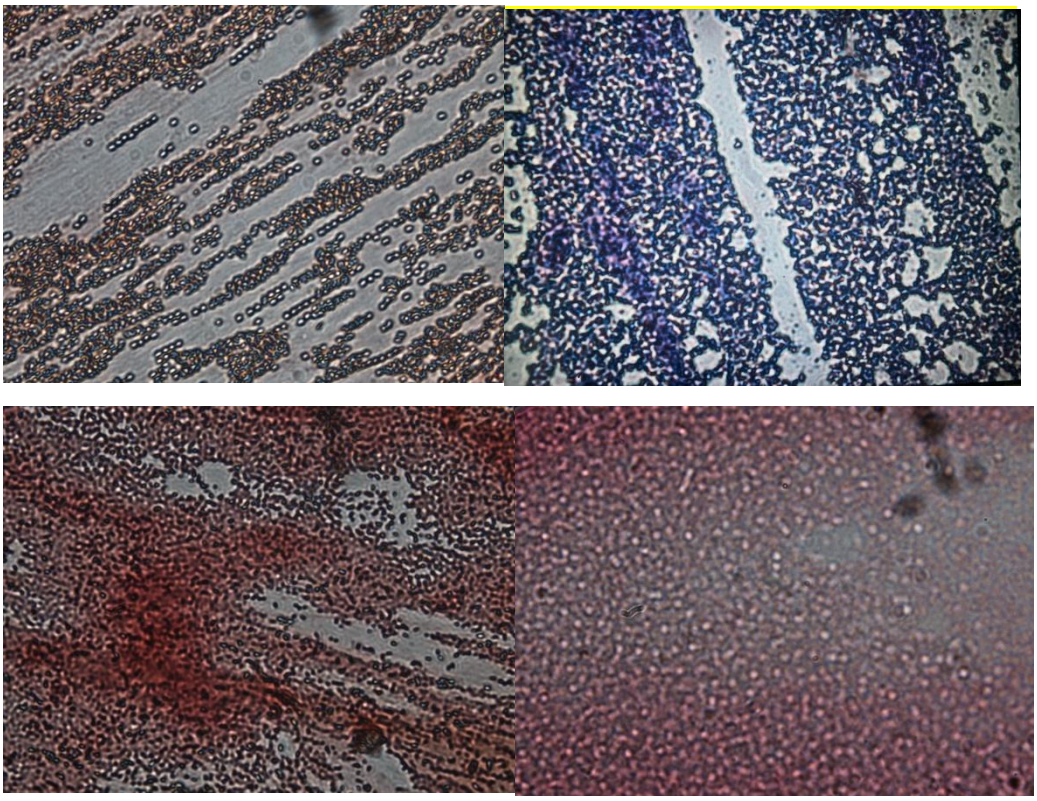

Fig 2: Different gram stained bacterial colonies under 100x magnification

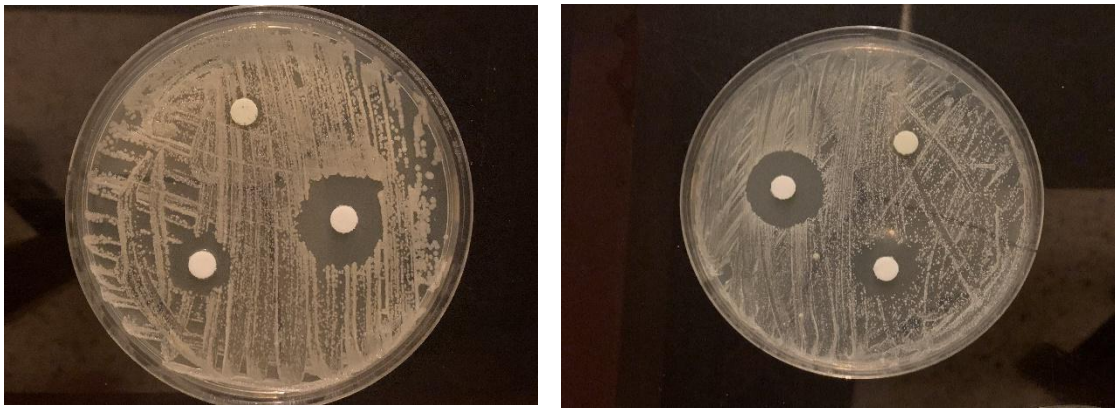

Fig 3: Zones of inhibition on MHA plates

RESULTS AND DISCUSSION

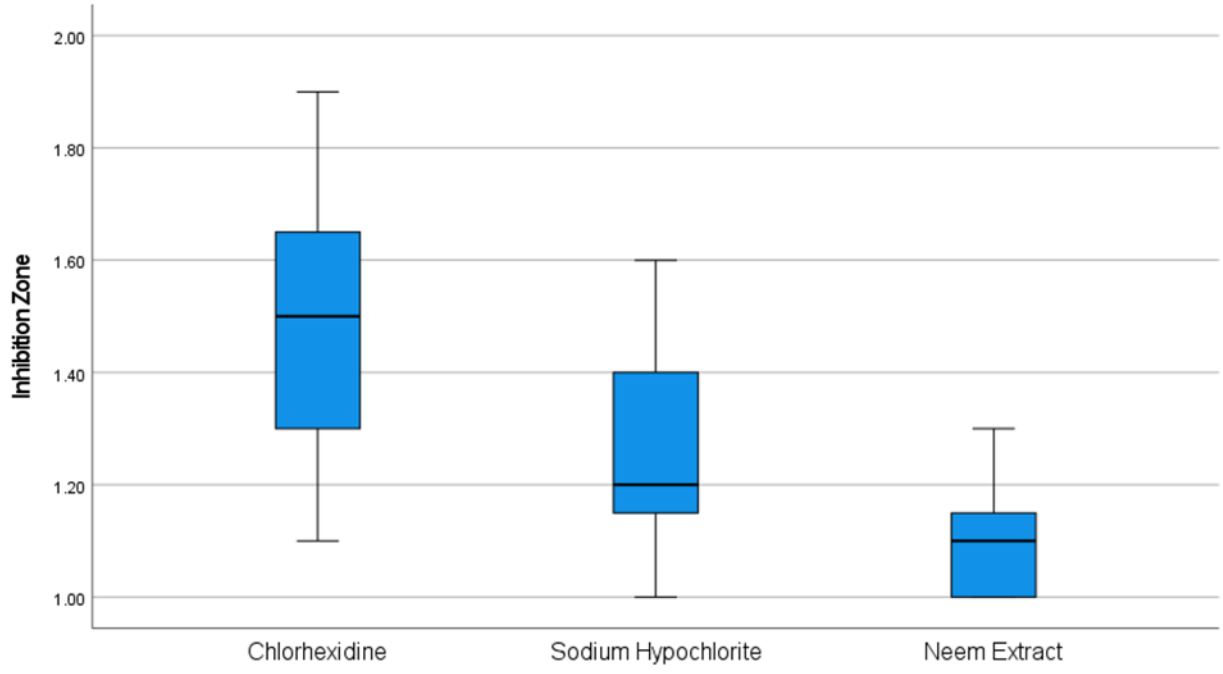

Fig 2: Difference between mean diameters of zones of inhibition

A total of36 infected sampleswere included in the study, wherein 12 samples were irrigated with 2 % chlorhexidine, 12 with 3 % sodium hypochlorite, and 12 with neem extract. Chlorohexidine had a significantly higher mean inhibition zone (1.49±0.25mm) as compared to sodium hypochlorite (1.25±0.19mm) and neemextract (1.10±0.11mm) respectively. Hence, there was a statistically significant difference in mean diameters of the inhibition zone observed between the three groups for the mean inhibition zone (F=12.28, *P*=0.001). (Fig 2)

	Mean difference	p-value	Significance
1 vs 2	0.242	0.014*	Significant
2 vs 3	0.150	0.206	Insignificant
1 vs 3	0.392	0.001*	Significant
1=Chlorohexidine, 2= Sodium hypochlorite, 3=Neem extract			

Table 1: Comparison of mean between the irrigants

For many years, herbal medicine has been associated with medical uses. Neem in particular, due to its vast variety of qualities, which include antibacterial, anti-inflammatory, and antifungal capabilities. Since it was utilised in medicine, it was felt that it would be wise to employ it in dentistry as well, combining its qualities into usage as an intracanalirrigant, to use not only as a pain reliever but also to eliminate a wide range of bacteria residing within the root canal system. Previous research has revealed that neems have antibacterial as well as anti-adherent properties, affecting bacterial adherence and colonisation capacity. (Kishen A, Sum CP, Mathew S, Lim CT 2008) (Rosaline H, Kandaswamy D, Gogulnath D, Rubin Ml 2016)

About three differentstudies at a point (Prabhakar AR, Basavraj P, Basappa N 2013), Arora T, Kang RS, Mann JS, Khurana NS, Aggarwal R, Walia G 2015),(Rani A, Thakhu S, Gupta S, Gauniyal P, Bhandari M, Gupta H 2015) founda highest antimicrobial effect with 0.2% chlorhexidine compared to herbal medicament (*Morindacitrifolia*, garlic, and turmeric), whereas a different study showed that neem extract is more effective than sodium hypochlorite 5.25% against *E. faecalis*.(Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D 2013)

In a recent study though no significant difference was found in antibacterial efficacy between Cholorohexidine and NaOCl treatments, which showed that intracanal endotoxin levels decreased compared with the initial levels after applying Cholorohexidine and NaOCl. However, they found that NaOCl was more effective in the reduction of gram-negative bacterial endotoxin than Cholorohexidine, but none of the gram-positive bacterial parameters were investigated. (Ruksakiet, K., Hanák, L., Farkas, N., Hegyi, P., Sadaeng, W., Czumbel, L.M.et al 2020).

Bacteriologic samples were taken before, during, immediately after, and 24 hours after instrumentation, irrigation, and treatment with Chlorhexidinegluconate and NaOCl, respectively. Following the instrumentation and irrigation procedures, there was an extremely significant reduction in microorganisms in the Chlorhexidine-treated specimens. Another study (Jeansonne M.J., White R.R. 1994) compared 2% Chlorhexidine to 5.25% NaOCl in vitro and found that Chlorhexidine was more successful in reducing the number of positive cultures, despite the fact that the difference was not statistically significant.

However, in another study conducted (Mustafa M 2016), the antimicrobial efficacy of neem was compared with that of the chlorhexidinegluconate and NaOCl, and it was found that neem efficacy was comparable to that of other commonly used gold standard compounds. In this study, it was shown that the zone of inhibitionin the agar diffusion test showing the antimicrobial efficiency of the neem extract was comparable to that of 2% chlorhexidine and 3% NaOCl.

The antimicrobial efficacy of CHX and NaOClirrigants was compared in root canal therapy of permanent teeth. No significant differences in their antimicrobial efficacy were found.In conclusion, the obtained evidence suggested that both CHX and NaOCl significantly, but not completely, reduced endodontic infections during root canal therapy. They were found to be equally effective despite their different molecular mechanisms. (Ruksakiet, K., Hanák, L., Farkas, N., Hegyi, P., Sadaeng, W., Czumbel, L.M., et al 2020)

Antimicrobial drug resistance is a major problem in the medical and dental fields (Reddy RR, Kumari K, Lokanatha O, Mamatha S, Reddy D 2012) which is why dental professionals are looking for alternatives, such as herbal products, which possess significant antibacterial properties. Of all these natural medications, neem is drawing significant attention since the plant possesses excellent antibacterial and antifungal properties (Raghavendra SS 2014)

The isoprenoid group (Nimbin, nimbolide, and nimbidic acid) of constituents of neem has a broad range of therapeutic and antimicrobial effects suggesting its potential as an endodontic irrigant as suggested by these studies (Bohora A, Heghe V, Kokate S.2010) (Dutta A, Kundabala M 2014) (Ravishankar P, Laksmi T, Kumar SA 2011).The use of neem as an endodontic irrigant may be advantageous because neem is an excellent antioxidant with very high biocompatibility, and thus there is no risk of tissue toxicity with its use. Biocompatibility of neem to the human periodontal ligament fibroblasts has already been proved, and this is an important factor favoring its clinical application in endodontics.(Botelho MA, Santos RAD, Martins JG, Carvalho CO, Paz MC, Azenha CR et al, 2008)

Nimbina product of the seed kernel of *A. indica* demonstrates anti-inflammatory, antibacterial, antifungal, and antipyretic properties. Furthermore, neem exhibited substantial efficacy against periodontal pathogens and is biocompatible with PDL fibroblasts. Hence, its use as a biocompatible irrigant might be beneficial in endodontic therapy. (Dedhia, J., Mukharjee, E., Luke, A.M., Mathew, S., Pawar, A.M., 2018)Mistry *et al*. concluded in their study that neem extract showed significant activity against *S. aureus*. (Mistry KS, Sanghvi Z, Parmar G, Shah S.2014)

Whereas, Bohora *et al*. and Tyagi *et al*. reported neem to be an effective root canal medicament against E. faecalis and *C. Albican*(Bohora A, Hegde V, Kokate S. 2010) (Tyagi SP, Sinha DJ, Garg P, Singh UP, Mishra CC, Nagpal R, et al. 2013). However, the results of the study were not by them and neem exhibited less effectiveness against bacterial microflora.A present study (Panchal, V., Gurunathan, D. and Muralidharan, N.P., 2020) showed cinnamon extract irrigant to have better antibacterial effectiveness followed by sodium hypochlorite. Neem showed to have the least antibacterial effectiveness.This present study correlates to the result we see in our findingsofthe comparison of neem and sodium hypochlorite, showing less effectiveness.

Another recent study published in 2021 showed that Neem was associated with lower pain intensity. Neem and 2.5 % sodium hypocholoritesignificantly reduced endotoxin levels but were not effective in eliminating endotoxins from root canals of mandibular molars with necrotic pulps. (N. S. Hosny, S. A. El Khodary, R. M. El Boghdadi, O. G. Shaker, 2021).

Based on the above givenin thestudy, it can be concluded that neem leaf extract could be used as an alternative agent in root canal disinfection. However, further in vitrostudies on its toxicological effects and optimal concentration against a wider spectrum of microorganisms have to be established (Mustafa M 2016)

Keeping in mind the results of the present study that has been conducted Chlorohexdineis most effective against the bacterial microbes compared to both sodium hypochlorite and neem. Despite its several

disadvantages, its advantages outweigh and still bring it to the most efficient position as an intracanalirrigant, but further studies still need to be performed to come to a proper conclusion for the use of neem as an intracanal irrigant

CONCLUSION

Within the constraints of this investigation, it was determined that chlorohexdine had the highest antibacterial activity as an intracanalirrigant against endodontic germs, whereas sodium hypochlorite and neem had equivalent antibacterial efficacy against the microbes, making them both equally beneficial. Having said that, the literature supports the idea that neem extract has antibacterial qualities and can be utilised as an alternative for intracanalirrigants, however in light of our findings, more research on Neem is needed before it can be considered an appropriate endodontic irrigant.

COMPETING INTERESTS

There are no competing interests stated by the authors. The products employed in this study are widely and often used in our field of study and in our country. There is no conflict of interest between the writers and makers of the products because we do not plan to use them as a means of pursuing legal action, but rather to further knowledge. Furthermore, the research was not supported by the production firm, but rather by the writers' own efforts.

AUTHORS' CONTRIBUTIONS

Author 1 designed and wrote the final draft for the study
Author 2 wrote the first draft
Author 3 helped collect the sample and helped in writing the draft
Author 4 helped write the final draft
Author 5 read and approved the final draft
Author 6 managed the analysis of the study
All authors read and approved the final manuscript

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