Original Research Article

ANTIBACTERIAL EFFICACY OF DIFFERENT INTRACANAL IRRIGANTS ON ROOT CANAL TREATMENT: An In-Vitro study

ABSTRACT

Aims: Root canal treatment is a procedure employed to disinfect and leave the root canal system sterile of any bacterial microorganism. To be sure of whether the canal anatomy is sterile of any microorganism, disinfection is key. Many disinfecting agents have been employed for this purpose. Out of many properties that endodontic disinfecting agents 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 such agents on the bacterial microflora and to see how efficient they are against them

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 during February 2020 till 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 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 statistically significant difference in mean diameters of inhibition zone observed between the three groups for mean inhibition zone (F=12.28, P=0.001).

Conclusion: There was statistically significant difference between chlorohexidine and sodium hypochlorite (*P*=0.014) and chlorohexidine and neem extract (*P*=0.001). However, no significant difference was observed between sodium hypochlorite and neem extract.

Key words: *Root canal treatment, cholorohexidine, sodium hypochlorite, neem, intracanal irrigants, neem extract, intracanal medicament*

INTRODUCTION

Endodontic treatment is one of the main procedures employed to keep teeth that have undergone irreversible pulpitis into retaining their function as well as at times esthetic role with in the oral cavity. The success of an endodontic treatment depends on effective disinfection and complete sealing of the tooth. (7). Elimination of microorganisms from the root canal space is important in pulpal disease management. Isolated microorganisms from the infected root canals have serious implications on oral and systemic health (16). It has been observed from several studies that intracanal instrumentation does not remove all the microorganisms, but use of intracanal medicament helps in removal of remaining bacteria after cleaning and shaping. To achieve a more effective eradication of these microorganisms, endodontic instrumentation must always be implemented with abundant irrigation, which has to achieve chemical, mechanical and biological effects (4). The irrigators most used today are Sodium hypocholoite, Chlorohexidine and EDTA, released into the root canal system through different techniques. Irrigants are used to wash out canal debris, dissolve pulpal remnants and lubricate the canal, thus improving the efficiency of canal preparation. Irrigants may be delivered into the root canal using either a needle and syringe, or an ultrasonic device, and have been widely divided into chemical agents and natural agents. The objective of this study was to evaluate if a natural agent such as Neem has a similar antibacterial efficacy against more common irrigants used today, since it has been employed in herbal medicine and Ayurveda and possess many qualities including antibacterial, antifungal, antiviral, anti-inflammatory, as well as analgesic.

MATERIALS AND METHODS

The study was conducted in the Department of Endodontics at Fatima Jinnah Dental College and Hospital, Karachi, Pakistan during February 2020 till March 2020. Inclusion criteria were patients between 18 – 50 years both male and female, all teeth except those indicated for 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 blood agars. 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 or gram negative rods and cocci (Fig 1). 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% Cholohexidine, 3% NaOCl and Neem extract. Three antibacterial sensitivity discs were then added onto a specific distance from each other each of them containing the irrigants. Once placed onto the MHA agar the sample was incubated again at 37^0 C for 24 hours. Zones of inhibition (the zone in which there is antimicrobial activity seen was visible as a transparent areas over the agar plate) were checked after incubation of each plate against the bacterial colonies identified. Zones were measure using a transparent scale and then entered on to the proforma later to be analysed.

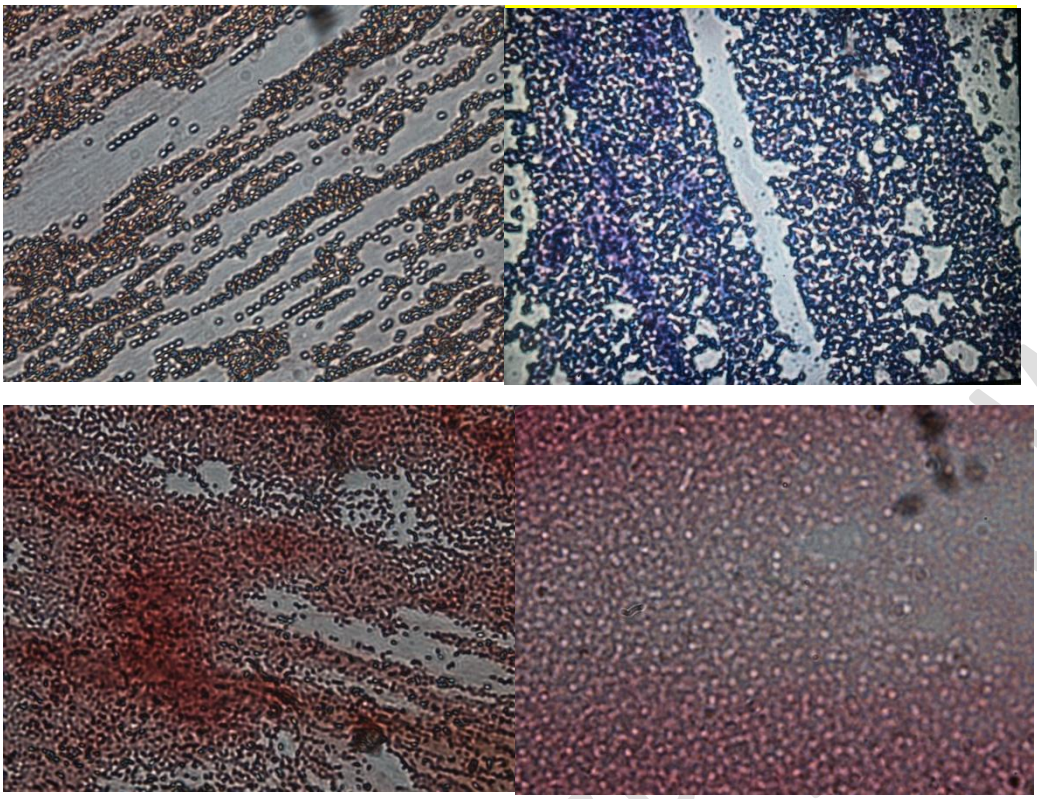

Fig 1 : Different gram stained bacterial colonies under 100x magnification

RESULTS AND DISCUSSION

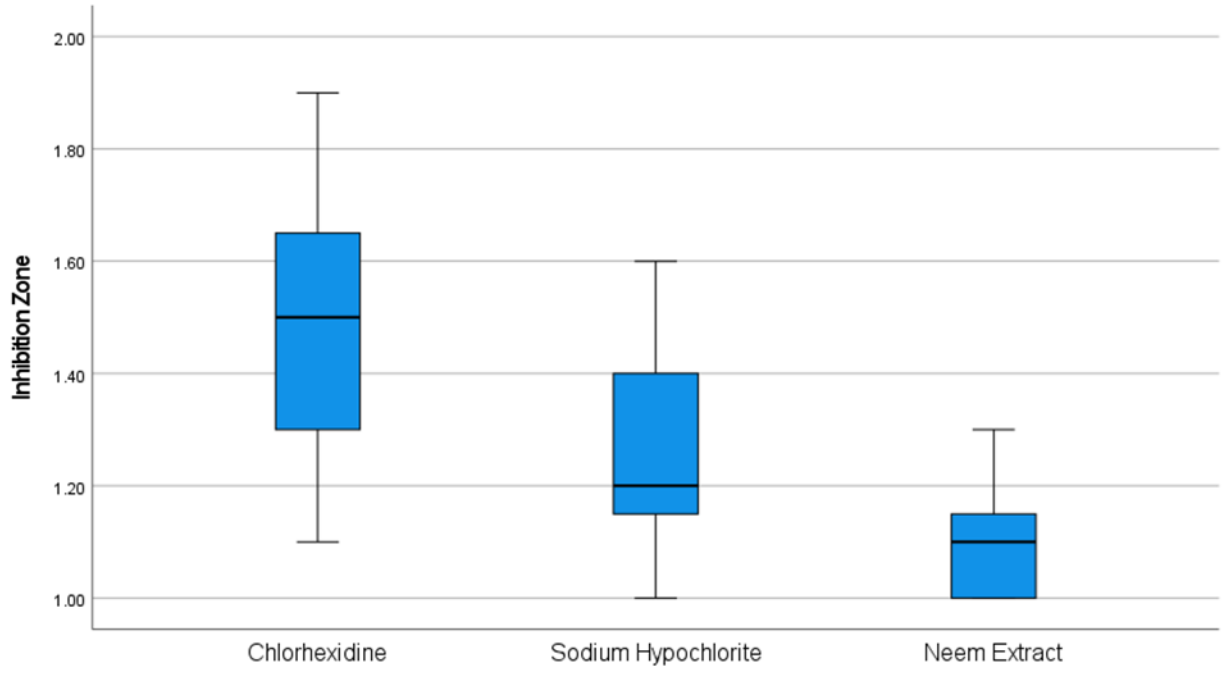

Fig 2: Difference between mean diameters of zones of inhibition

Total 36 infected samples were included in the study, wherein 12 samples were irrigated with 2 % chlorohexidine, 12 with 3 % sodium hypochlorite and 12 with neem extract. Chlorohexidine had significantly higher mean inhibition zone (1.49±0.25mm) as compared to sodium hypochlorite (1.25±0.19mm) and neem extract (1.10±0.11mm) respectively. Hence, there was statistically significant difference in mean diameters of inhibition zone observed between the three groups for 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

Irrigants have been widely used for the elimination of root canal bacteria over past few years. Irrigation provides effective cleaning of the root canal system along with elimination of microbial colonies housed within the root canal system The ideal requirements for any endodontic irrigants is not only to have a broad spectrum of antimicrobial activity but should include high efficacy against anaerobic, aerobic and facultative bacteria, ability to dissolve necrotic pulp tissue remnants, ability to inactivate endodotoxins.

Chlorohexidine is known to be effective against both gram-positive as well as gram negative microorganisms due it being a broad-spectrum antimicrobial agent(2) Chlorohexidine gluconate 2% is used as the gold standard antimicrobial agent with the most potent chemotherapeutic activity against many microbes(4) (5).It is said to be bacteriostatic in low concentration and bactericidal in high concentration(6).Chlorhexidine gluconate has been used for the past years for caries prevention,in periodontal treatment and as an oral antiseptic mouthwash. The major advantages of chlorohexidine over NaOCl are its lower cytotoxicity and lack of foul smell and bad taste. However, unlike NaOCl, it cannot dissolve organic substances and necrotic tissues present in the root canal system. In addition, like NaOCl, it is unable to kill all bacteria and cannot remove the smear layer.Chlorhexidine produces staining of teeth, altered taste, and development of microbial resistance.(4)

Sodium hypochlorite (household bleach) is a commonly used root canal irrigant. An antiseptic and inexpensive lubricant that has been used in different dilutions ranging from 0.5% to 5.25%. Free chlorine in NaOCl dissolves vital and necrotic tissue by breaking down proteins into amino acids. Decreasing the concentration of the solution reduces its toxicity, antibacterial effect and ability to dissolve tissues.Increasing its volume or warming it increases its effectiveness as a root canal irrigant. NaOCl advantages include its ability to dissolve organic substances present in the root canal system and its affordability. The major disadvantages of this irrigant are its cytotoxicity when injected into periradicular tissues, foul smell and taste, ability to bleach clothes and ability to cause corrosion of metal objects.In addition, it does not kill all bacteria, nor does it remove all of the smear layer. It also alters the properties of dentin.The results of a recent in vitro study show that the most effective irrigation regimen is 5.25% at 40 minutes, whereas irrigation with 1.3% and 2.5% NaOCl for this same time interval is ineffective in removing E. faecalis from infected dentin cylinders. Based on the findings of this study, the authors recommend the use of other irrigants to increase the antibacterial effects during cleaning and shaping of root canals. Sodium hypochlorite is generally not recommended in its most active form in a clinical setting.It is prepared freshly just before its use.

Having said both these common irrigants to serve a few disadvantages, and with that it is easy to feel the need for a newer, better and more biocompatible irrigant to be introduced into the field of endodontics. Herbal medicine has also been associated with medicinal uses for many years. Particularly Neem due its wide range of properties starting from being antibacterial, to having anti-inflammatory and a wide range of antifungal properties as well. Since its cohert use in medicine it was thought to be wise to be used in the dental arena of medicine as well, there by incorporating its properties into use as a intracanal irrigant, to utilize in being not only a medince to relive pain but also in eliminating a wide range of bacteria harbouring within the root canal system. Previous studies have shown neems ability of not only being antibacterial but also having antiadherent activity by altering bacterial adhesion and their ability to colonize (7) (8)

Neem has been in discussion for many of its uses over the past few years especially in the field of Ayurveda, but new researches have shown it to have considerable affect towards the field of endodontics, especially against endodontic bacteria. This is the first time in many years that neem has been considered against materials like cholorohexdine and sodium hypochlorite as an irrigant against endodontic bacterial microflora. However certain studies have shown Neem to be a more effective against Enteroccous species most commonly found in the root canals. In a study (9) it was discussed about neem being used as an endodontic irrigant for its antibacterial efficacy on endodontic microbes and

its potential role as an irrigant.The agar diffusion or disk diffusion method used in this research to compare the efficacy of these irrigants have been employed as the most commonly used method in evaluation of antibacterial activity and as an adequate method of comparing the efficacy of different irrigants against each other. We have used the same method for our research study and it has shown results that compare similar effcicay between NaOCl and Neem in an in vitro setting. Antimicrobial drug resistance is a major problem in the medical and dental fields (10) 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 (11)

The isoprenoid group (nimbin, nimbinin, nimbidinin, 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 (12) (13) (14) .The use of neem as an endodontic irrigant may be advantageous because neem is an excellent antioxidant with a 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.(15) About three different studies at a point (5), (16),(17) found highest antimicrobial effect with 0.2% chlorhexidine compared to herbal medicament (Morinda citrifolia, garlic and turmeric), whereas a different study showed that neem extract is more effective than sodium hypochlorite 5.25% against E. faecalis.(18).Studies also evaluated the use of 0.2% Chlorhexidine gluconate on infected root canals of extracted necrotic teeth (19) Bacteriologic samples were obtained before, during, immediately after and 24 hours after instrumentation, irrigation, and medication either with Chlorhexidine gluconate or with sterile saline. There was a highly significant reduction in microorganisms in the Chlorhexidine-treated specimens after the instrumentation and irrigation procedures (20). Another study (21) compared 2% Chlorhexidine and 5.25% NaOCl in vitro, showed that Chlorhexidine was more effective in reducing the number of positive culture, even if the difference was not statistically significant. However another study conducted (20) , the antimicrobial efficacy of neem was compared with that of the chlorhexidine gluconate 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. Therefore, it can be concluded that neem leaf extract could be used as alternative agent in root canal disinfection. However, further in vitro studies on its toxicological effects and optimal concentration against a wider spectrum of microorganisms have to be established. Keeping in mind the results of the present study that has been conducted by us Chlorohexdine has been shown to be most effective against the bacterial microbes compared to both sodium hypochlorite and neem. Despite its several disadvantages, it's advantages outweigh and still bring it to the most efficient position as an intracanal irrigant, 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 limitations of this study it was concluded that Cholorohexdine had the maximum amount of antibacterial efficacy as an intracanal irrigant against endodontic bacteria, while sodium hypochlorite and neem gave similar readings against the microbes making them both equally effective against the endodontic microbes. Having said that it has also been supported by the literature that neem extract has significant antibacterial properties and can be used as a subsititute for intracanal irrignats. It should also be taken into account that these studies have been in vitro studies and more studies need to be conducted for further results and conclusion regarding use of neem as an intracanal medicament or irrigant.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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