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Comparative evaluation of effectiveness of different concentration of sodium hypochlorite with conventional irrigation method versus ultrasonic irrigation in the elimination of *Enterococcus faecalis* from root canals: an in-vitro study

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ABSTRACT
Aim: *The aim of this study is to compare the decrease in bacterial population in the root canals with 3 different concentrations of NaOCl and with two different irrigation systems*
Methods and Material: *The study was conducted on 70 single rooted extracted teeth. Teeth were decoronated and the apices were sealed with auto polymerizing resin. The teeth were inoculated with a suspension comprising Enterococcus faecalis (E. faecalis). The canals were prepared were divided into three groups based on irrigation technique: Group 1; irrigation with 1% sodium hypochlorite, Group 2; irrigation with 3% sodium hypochlorite & Group 3; irrigation with 5% sodium hypochlorite. All three groups were divided into 2 subgroups. In subgroup A irrigation was done by conventional side vented needle and in subgroup B with passive ultrasonic agitation. A fourth control group was also added in which conventional needle irrigation was done with normal saline. The samples were inoculated on the growth media and the mean colonies units were calculated.*
Statistical analysis used*: The statistical analysis used was Student's unpaired t-test for intergroup comparison and one-way ANOVA and Tukey's post hoc test for intragroup comparison.*
Results: *In our study, groups with passive ultrasonic irrigation showed better antimicrobial effect compared to groups which use conventional needle irrigation.*
Conclusions: *Ultrasonic irrigation showed better antimicrobial activity compared to conventional needle irrigation.*
Key-words: *Conventional irrigation, Enterococcus faecalis, Passive ultrasonic irrigation, Sodium hypochlorite, Side vented needle.*

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I. INTRODUCTION

Whether mechanical debridement is carried out with hand files or rotating devices, it is insufficient to eradicate germs from the root canals during endodontic treatment. Firstly, the intricate structure of the root canal system is not accessible with tools. Second, difficult-to-disturb complex biofilms have the potential to form in these inaccessible areas.

Thirdly, the instrumentation produces a smear layer that hinders the obturation material's proper adaptation to the canal wall. An effective irrigation schedule can aid in the delivery of antimicrobials to these difficult-to-reach root canal system locations, as well as in penetrating the dentine and removing biofilm and smear layers.[1]

Since irrigation serves several crucial mechanical, chemical, and (micro) biological purposes, it is essential to the success of root canal therapy. Irrigation is also the only way to impact those areas of the root canal wall that are not touched by mechanical instrumentation.[2] Irrigation reduces friction between the instrument and dentine, improves the cutting effectiveness of the files, dissolves tissue, and cools the file and tooth, especially during the use of ultrasonic energy.[3]

There have been constant efforts throughout the history of endodontics to create more efficient methods for sending and agitating irrigant solutions within the canal system. These technologies fall into two main categories: agitation techniques that are mechanical and manual. Rotating brushes, simultaneous irrigation with rotating canal instrumentation, pressure alternation devices, and sonic and ultrasonic systems are examples of machine-assisted operations. When compared to traditional syringe and needle irrigation, all of them seem to enhance canal cleansing. Richman first introduced ultrasonic instrumentation to endodontics in 1957 for root canal therapy with Cavitron© as irrigation and obtained good results However, ultrasonically activated K files were not used for preparing canals before filling until the study by Martin et al.[4]

It was Weller et al. (1980) that originally described passive ultrasonic irrigation (PUI). Although the process is active, the phrase "passive" does not accurately characterize it; in its original context, it was used to refer to the "non-cutting" action of the ultrasonically activated file. PUI works by sending sound waves to an irrigant inside the root canal via a smooth wire or oscillating file. Ultrasonic waves are used to transfer the energy, which might cause the irrigant to cavitate and produce acoustic streaming.[5] Following the root canal's shaping to the master apical file (irrespective of the preparation technique used), a small file or smooth wire is introduced in the center of the root canal, as far as the apical region. The root canal is then filled with an irrigant solution and the ultrasonically oscillating file activates the irrigant.[6]

To accomplish these ends, a wide variety of irrigants and irrigant combinations have been employed in endodontics. These include Sodium hypochlorite, Chlorhexidine, EDTA, Iodine potassium iodide, Hydrogen peroxide, saline and/or water, and Mixtures of irrigants, etc.[7] Sodium hypochlorite (NaOCl) was first described as an endodontic irrigant in 1919. It is reasonably priced, acts quickly, and has a wide range of effects. Proteins are denatured and optimal cell conditions are compromised by the high pH produced by hydroxyl ions, which also harm bacterial DNA and lipid membranes. Chloride ions break peptide bonds dissolving protein and releasing further chloramines that are antibacterial.[8] Sodium hypochlorite is both an oxidizing and hydrolyzing agent. it is bactericidal and proteolytic.[9]

Sodium hypochlorite solutions, with concentrations ranging from 1% to 5.25%, are now commonly used for root canal irrigation. As an endodontic irrigant, sodium hypochlorite solution is relatively cheap; is bactericidal and virucidal; it dissolves proteins, has a low viscosity, and it has a reasonable shelf life. It is not without disadvantages, principally due to its toxicity, it damages all living tissues except keratinized epithelia. sodium hypochlorite is extremely corrosive to metals; is strongly alkaline, hypertonic, and has a very unpleasant taste.[10,11] It is the only used solution that can dissolve organic matter in the canal presently. NaOCl ionizes in water into sodium (Na+) and the hypochlorite ions, OCl–, and establishes an equilibrium with hypochlorous acid (HOCl). At acidic and neutral pH, most of the chlorine exists as HOCl, whereas at pH of nine and above, OCl– is most abundant. Hypochlorous acid has the strongest antibacterial effect while the OCl– ion is less effective.[12]

Bacteria and their by-products play an essential role in the development and perpetuation of pulpal and periradicular diseases. E. faecalis is a predominant microorganism found in persistent intraradicular infections.[13]

II. MATERIALS AND METHODS

The study was conducted on 70 single-rooted extracted human teeth.

Inclusion criteria:

·None of the teeth should have visible root caries, fractures, or cracks.

·No signs of internal or external resorption or calcification, all should have completely formed apex.

Exclusion criteria:

·Teeth with more than one root canal.

·Teeth with root caries, fractures, or cracks.

·Teeth with internal or external resorption or calcification.

Procedure:

Selected teeth were decoronated with diamond discs. (Figure 1) The sample teeth were prepared to #40 size with saline as an irrigant. Canals were dried with paper points, and the apices were sealed with auto-polymerizing resin. The prepared roots were sterilized via autoclave for 15 min at 121°C. A Suspension of *Enterococcus faecalis (E. faecalis)* in blood agar was obtained (Figure 1). 0.1ml of the suspension was placed in each root canal, and sterile cotton was placed at the canal entrance. After that, the blocks were put inside stainless steel boxes and left for a whole day at 37°C incubation. The decoronated roots were then randomly divided into 3 groups and 1 control group based on irrigation protocols (Table 1. & Figure 1.)

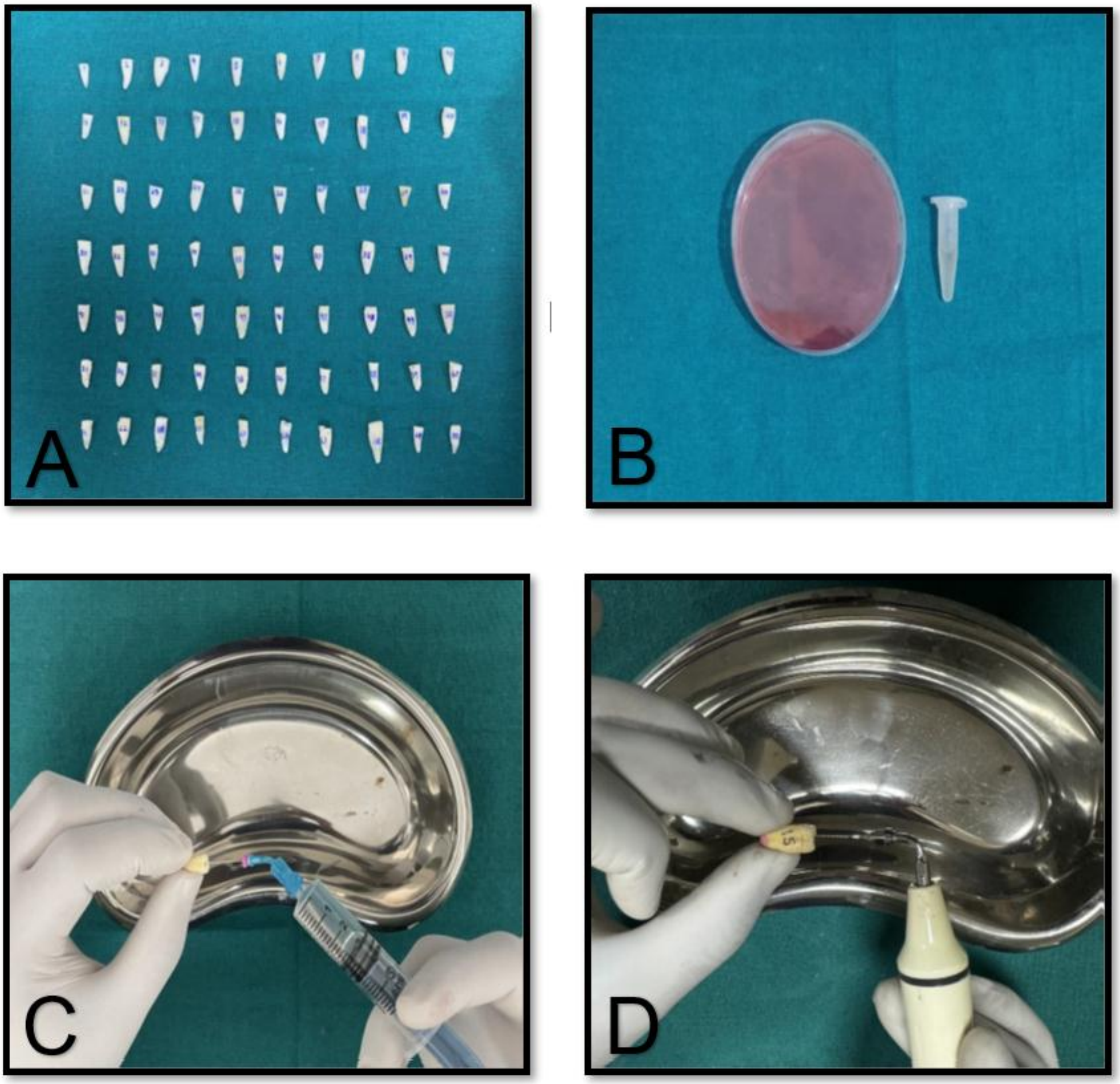
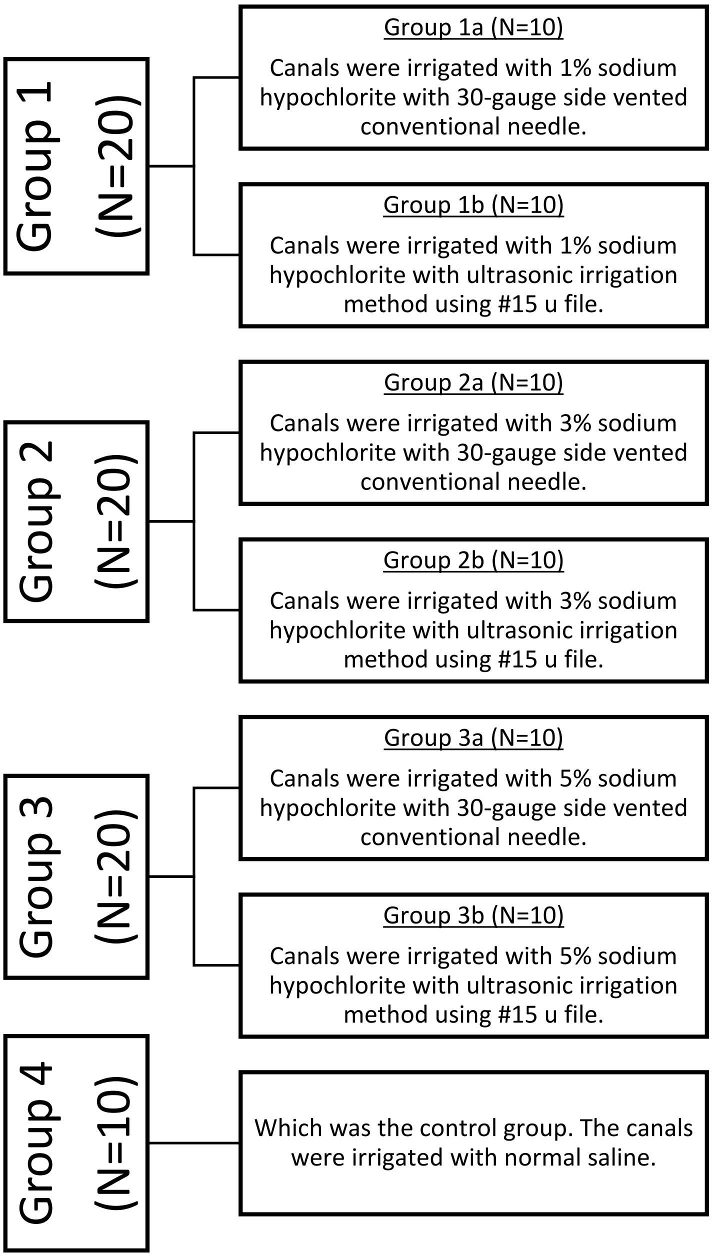


Figure 1. (a) 70 decoronated samples **(b)** *Enterococcus faecalis* suspension **(c)** Irrigation with side vented needle **(d)** Irrigation with Passive Ultrasonic irrigation

TABLE. 1



<u>Bacterial sample collection for each irrigated group</u>:
After the irrigation paper point was placed inside the root canal space of each sample for 1 min and then kept on petri plates containing blood agar, which were incubated at 37ºC for 24 hours. Colonies were counted, and the mean number of colonies was calculated.
<u>Statistical analysis</u>:
Comparison of microbial load in the root canal after irrigation among the four study groups was done with Student’s unpaired t-test for intergroup comparison and one-way ANOVA and Tukey’s post hoc test for intragroup comparison.

III. RESULTS

Figure 2. Shows no. of bacterial colonies formed in petri dishes after 24 hours of incubation.
Table 2. shows the comparison of microbial load in the root canal after irrigation among the four study groups having sample size n=70. The specimens were initially divided into 3 groups (each having 2 subgroups) based on the irrigation method used. The remaining 10 samples (n=10) were taken as a control group.
The mean number of colonies was obtained for all the groups.

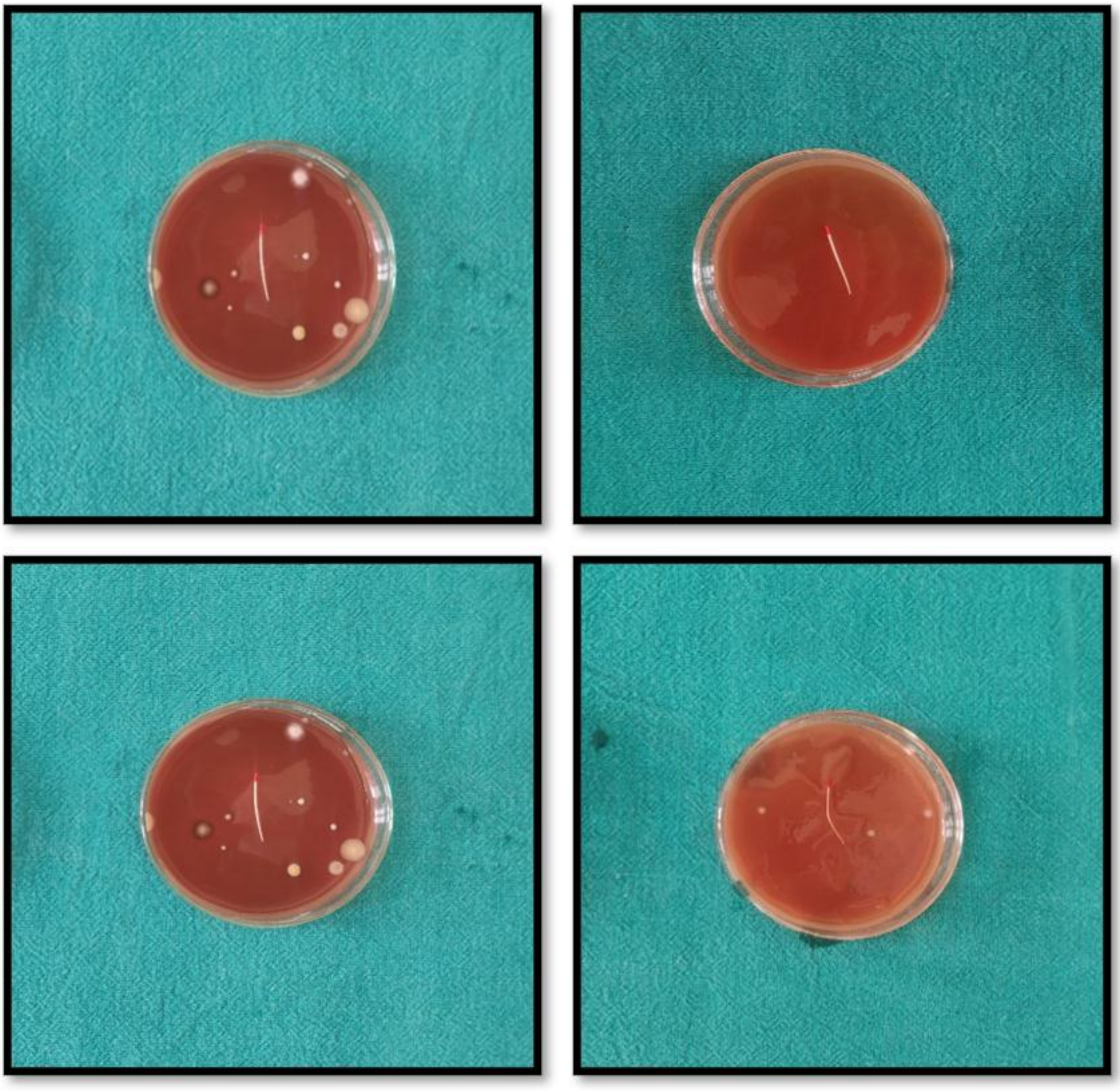


Figure 2. Bacterial colonies formed in petri dish after 24 hours of incubation

Table 2. Comparison of microbial load in the root canal after irrigation.

Mean, SD of colonies formed in irrigation groups and 1 control group														
Irrigants	1% sodium hypochlorite with side vented needle		1% sodium hypochlorite with ultrasonic irrigation		3% sodium hypochlorite with side vented needle		3% sodium hypochlorite with ultrasonic irrigation		5% sodium hypochlorite with side vented needle		5% sodium hypochlorite with side vented needle		control group - the canals were irrigated with normal saline.	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Mean colony count	**4.25**	**2.22**	**1**	**2.71**	**3.25**	**0.96**	**0.75**	**0.5**	**2**	**0.96**	**0.5**	**0.93**	**5**	**2.5**
	2.125				2				1.5				5	

Table 2 shows that Group 3 (Mean: 1.50) in which we used 5% sodium hypochlorite as an irrigating agent showed a better antimicrobial effect compared to Group 2 (Mean:2.0) in which we used 3% sodium hypochlorite as an irrigating agent and Group 1 (Mean:2.125) in which we used 1% sodium hypochlorite as an irrigating agent. Among the groups, Group 3 employed the greatest concentration of sodium hypochlorite, at 5%.

Table 2 also shows that the antimicrobial effect of Group 3b (Mean: 0.50) in which we used 5% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 3a (Mean:2.0) in which we used 5% sodium hypochlorite with conventional irrigation, Group 2b (Mean:0.75) in which we used 3% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 2a (Mean:3.25) in which we used 3% sodium hypochlorite with conventional irrigation and similarly Group 1b (Mean:1.0) in which we used 1% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 1a (Mean:4.25) in which we used 1% sodium hypochlorite with conventional irrigation. Table 2. shows that the control group in which irrigation was done with saline shows the least amount of antimicrobial effect against disinfection of *E. faecalis.*

IV. Discussion:

A good irrigation schedule can assist in getting antimicrobials to these hard-to-reach root canal system locations, as well as into the dentine and smear layer to eradicate biofilm.[14]

In the present study, *E. faecalis* was the choice of pathogen to study the antimicrobial effect of the irrigant. It may be attributed to the factors that it has a frequent presence in persistent endodontic infections and its wide use in the testing of the effectiveness of disinfecting agents in endodontics. Additionally, enterococci are simpler to push into dentinal tubules due to their round shape and relatively small cell diameter.[15]

In this present study, the canals were prepared till the #40 K file. This was corroborated by the study done by **Ahmad et al**. They reported root canals had to be enlarged to the size of a #40 file, which is approximately the minimum size that would permit clearance and free vibration of the # 15 ultrasonic file.[16]

Results from this study show that Group 3 (Mean: 1.50) in which we used 5% sodium hypochlorite as irrigating agent showed a better antimicrobial effect compared to Group 2 (Mean:2.0) in which we used 3% sodium hypochlorite as irrigating agent and Group 1 (Mean:2.125) in which we used 1% sodium hypochlorite as irrigating agent. Group 3 used a 5% Concentration of Sodium hypochlorite which was the highest amongst other groups. Higher doses of sodium hypochlorite have consistently shown a more pronounced antibacterial impact against the disinfection of E. faecalis, even if the results were not statistically significant.

The results obtained in our study showed that the antimicrobial effect of Group 3b (Mean: 0.50) in which we used 5% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 3a (Mean:2.0) in which we used 5% sodium hypochlorite with conventional irrigation, Group 2b (Mean:0.75) in which we used 3% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 2a (Mean:3.25) in which we used 3% sodium hypochlorite with conventional irrigation and similarly, Group 1b (Mean:1.0) in which we used 1% sodium hypochlorite with ultrasonic irrigation was significantly better than Group 1a (Mean:4.25) in which we used 1% sodium hypochlorite with conventional irrigation. This suggests that using ultrasonic irrigation enhanced sodium hypochlorite's antibacterial activity. This could be explained by the fact that the files in ultrasonic irrigation vibrate transversely, forming a distinctive pattern of nodes and anti-nodes along their length. PUI works by sending sound waves to an irrigant inside the root canal via a smooth wire or oscillating file. The energy can cause the irrigant to cavitate and produce acoustic streaming when it is transferred through ultrasonic frequencies.[17]

The observations of the present study show that Group 4 i.e., the Control group in which the saline was used as the irrigating solution without the use of ultrasonic activation shows the least antimicrobial efficacy amongst all other groups. This suggests that saline alone should not be used as an intracanal irrigant.

A study performed by **Zhejun Wang et al.** corroborated our findings by contrasting the antibacterial activities of several disinfection agents on immature and mature E. faecalis biofilms in dentin canals. They also concluded that High-concentration NaOCl (6%) showed the strongest antibacterial effect among the solutions tested for both young and old E. faecalis biofilms.[18]

In contrast to the results of our study, **Richard K. Howard et al.** compared the effectiveness of debris removal between EndoVac, PiezoFlow (ultrasonic), or needle irrigation in mandibular molars. They concluded that, both before and after final irrigation, there were no statistically significant variations in the cleanliness of the canal and isthmus among the three groups.[19]

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