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CLINICAL, RADIOGRAPHICAL, AND ANTIBACTERIAL EFFECT OF GOLD AND SILVER NANOPARTICLES AS IRRIGANT SOLUTIONS IN PRIMARY ANTERIOR TEETH

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ABSTRACT

Objectives: This study was directed to evaluate the clinical, radiographical, and antibacterial effects of gold and silver nanoparticles as irrigant solutions in primary anterior teeth. **Subjects and methods:** Thirty kids between the ages of 3 and 5 who had single-rooted primary anterior teeth that needed pulpectomy therapy were chosen. The Nano-Gate helped with the root canal irrigant solutions. A test for antimicrobial activity was assessed. **Results:** The results of this study revealed that chlorehexidine CHX and AgNPs irrigant solutions had a significantly higher antimicrobial effect against E. faecalis microorganisms when compared to the tested AuNPs solution. According to the study's clinical and radiographic findings, there was no noticeable difference between the group of teeth irrigated with CHX or AgNPs, but the clinical and radiographic results of this present study revealed that the AuNPs have lower success rates after different follow-up periods when compared with CHX and AgNPs. **Conclusion**: Based on the results of this study, The 100 ppm silver nanoparticles and The 100 ppm gold nanoparticles can be used as effective alternative to chlorehexidine irrigant solutions in primary teeth.

KEYWORDS: Pulpectomy, Primary Anterior teeth, Irrigation.

INTRODUCTION

The fundamental goal of pulp therapy is to treat any compromised pulp due to caries, trauma, or other factors while preserving the health and integrity of the tissues supporting teeth [(1)]. The goals of root canal therapy are to get rid of the germs and enhance the periapical condition [(2)].

The use of irrigation solutions in conjunction with mechanical instruments is more important when cleaning the root canal system because it will remove soft and hard tissue remnants from various root canal system locations that are inaccessible to instruments [(2)].

Enterococcus faecalis (E. faecalis) is the most common bacteria cultivated from unsuccessful root canals that need to be redone as well as from cases of first endodontic infection. It is a facultative, gram-positive, Facultative anaerobic organism that forms a biofilm and can tolerate root canal therapy and obturation while also being resistant to chemical and mechanical cleaning. It may result in the root canal operation failing if untreated [(3)].

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Some transition metals, such as silver (Ag) and gold (Au), have potent antibacterial effects when utilized as nanoparticles. Due to their small particle size, nanoparticles have a high surface-to-volume ratio that enables the insertion of several functional ligands that can improve interactions with target bacteria [4].

Clinicians can learn more and gain useful knowledge about the best endodontic irrigation strategy by analyzing the bacterial load reduction in patients who underwent endodontic treatments with various irrigation techniques. In addition, it's important to guarantee a high degree of predictability and adequate levels of disinfection during endodontic therapy. Therefore, this clinical study sought to evaluate the efficiency of irrigation solutions in clinical settings with Multidrug-resistant (MDR) bacteria in order to identify a standardized solution that can ensure comparability of the results of various solutions and identify the most effective solution to raise the success rates of endodontic treatments. Recent developments in nanotechnology provide up new opportunities for the creation of novel formulations based on various types of nanoparticles (NPs) with variable sizes, shapes, and antibacterial capabilities.

SUBJECTS AND METHODS

Materials:

1. 100 parts per million (ppm) of Ag-NPs solution (Nano Gate company).
2. 100ppm of Au-NPs solution (Nano Gate company).
3. 2% chlorhexidine gluconate solution (jk dental company, Cairo, Egypt).

Ethical Consideration

The ethics committee of the Faculty of Dental Medicine at Al-Azhar University in Cairo, boys, gave its approval before this study could be conducted. (NO:782/1943)

Before the research began, each parent received a thorough explanation of the process and was asked to sign a permission form.

Study Setting and Population

This investigation was a prospective, three-arm, parallel group, randomized controlled clinical trial from patients utilizing the outpatient clinic of the Pedodontics Department at the Faculty of Dental Medicine (Boys, Cairo), Al-Azhar University, 30 healthy children between the ages of 3 and 5 were chosen.

Eligibility criteria for the selected teeth

Inclusion criteria: [5-7]

Primary teeth with deep carious lesion with uncontrolled bleeding after pulp amputation.

Apical root resorption is less than 1/3 of roots. With History of spontaneous, unprovoked toothache and Sufficient coronal tooth structure to support a rubber dam and to be restored with Zirconia Crown (ZC).

Exclusion criteria: [5-7]

Children with parents refused to participate in the study or Uncooperative child. And Childs with systemic diseases can affect their immunity or cooperation.

Children have mobile tooth that was indicted for extraction. Or with resorption of more than 2/3 of Root [5,6]. Root canal obliteration or anatomic anomalies. And Children who had taken antibiotics up to two weeks prior to the sampling, as well as those who had any systemic impairment, non-restorable teeth, perforated pulpal floors, excessive mobility, or pathological root resorption, would be disqualified[6].

Sample Size Calculation

According to Hassan et al.[8]. findings, the minimum estimated sample size was 24 participants (8 in each group), which was then expanded to 30 participants (10 in each group) to account for follow-up loss. G power Statistical Power Analyses Version 3.1.9.2 was used to calculate the sample size. Using a two-sided hypothesis test and a significance level

(error) of 0.05 for the data, the sample size was computed at an effect size of (0.92), a power of (0.8), and an effect size of (0.92).

Patient Consent

Consent was obtained from the children's parents.

Preoperative Assessment:

A. History of the Patient:

We took a complete medical and drug history as well as patient's data (name, gender and age) were collected. As regarding the medical history, all patients were free from any systemic diseases.

B. Clinical Examination:

- ***Extraoral examination:***

Include examination of face and general appearance [9].

- ***Intraoral examination***

- Soft tissue includes examination of Gingiva, tongue and floor of mouth
- Hard tissue examination includes Teeth

Grouping

The involved children were divided randomly into three groups (n=10) according to the type of the used irrigant solution as the following:

* Group A: pulpectomized primary anterior teeth irrigated with 100 ppm of Ag-NPs solution.
* Group B: pulpectomized primary anterior teeth irrigated with 100 ppm of Au-NPs solution.
* Group C: pulpectomized primary anterior teeth irrigated with 2% chlorhexidine gluconate solution.

Preparetion of the solution

Silver nanoparticles Solution

The synthesis of silver nanoparticles in an aqueous solution was successfully carried out via the chemical reduction of silver nitrate (AgNO3) with sodium borohydride (NaBH4) and chitosan biopolymer as a stabilizing agent, as described by Wei et al.[10].

Gold nanoparticles Solution

Synthesis of gold nanoparticles with different size were prepared according to Bastús et. al [11]. A solution of 2.2 mM sodium citrate in Milli-Q water [Double Distilled Water DDS] (150 mL) was heated with a heating mantle in a 250 mL three-necked round-bottomed flask for 15 min under vigorous stirring. A condenser was utilized to prevent the evaporation of the solvent. After boiling had commenced, 1 mL of HAuCl4 (25 mM) was injected. The color of the solution changed from yellow to bluish gray and then to soft pink in 10 min. The resulting particles (~12 nm) [Particle size performed by transmission electron microscope] are coated with negatively charged citrate ions and hence are well suspended in H2O figure (1).

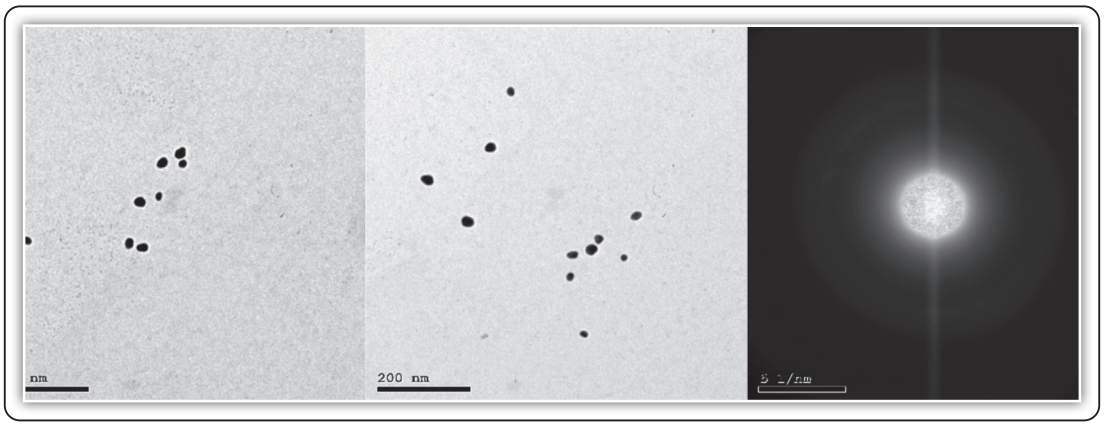

FIG (1) Shows the TEM images of the prepared AuNPs

Pulpectomy procedure:

After injection of local anesthetic solution, the involved tooth was isolated with a rubber dam before the beginning of any operative procedure**.** Then, the caries removal and access cavity opening was performed in necrotic primary anterior teeth with a sterile round carbide bur , Barbed broaches were used to remove the pulp from the pulp chamber and root canals, Next, the root canal was mechanically cleaned and shaped using endodontic files(Main INC., Co, Japan) till file size #35,The root canal preparation was shorter than the radiographic apex by 1mm[5,6].

Immediately after gaining the access cavity; the first microbial swab (S1) sample was taken from each root canal by a sterile paper point of a size compatible with the root canal diameter and was placed for 30 seconds. The retrieved paper points were placed into a sterile tube with thioglycolate) Sigma Aldrich Chemical, USA)[5,6]**.** Then, mechanical cleaning and shaping of canals with endodontic files were performed [6,7]**.**

After that, the root canals of each involved tooth were cleaned with one of the three tested irrigant solutions according to the allocated group in sample grouping, The removal of organic debris and root sterilization was conducted with periodic irrigation for 30 seconds with a syringe containing either 100ppm of AgNPs, 100 ppm of AuNPs or 2% CHX solutions,for all irrigation solutions, a 5mL syringe was employed,the irrigation was performed by plastic disposable syringe had a needle with soft tip and side-vented to the working length by gentle pressure,after that, sterile absorbent paper points of a size compatible with the root canal diameter was placed for 30 seconds in the root canals for the collection of the second microbial swab (S2) sample. The retrieved paper points were placed into a sterile tube with thioglycolate[5,6]. then, the root canals of each involved tooth were cleaned with 2 ml of 0.9% sterile saline solution used as a Neutralizer.

After microbial sample collection, the placement of zinc oxide and eugenol (ZO/E) as pulpectomy material was performed[7]. Then, a suitable base material of glass ionomer cement (GIC) [Medicam,Promedica Dental Material GmbH, Germany] was used over the pulpectomy material[5,6].

After that, the final restoration of composite was placed in each cavity and the tooth was restored with Zircona Crown. [3M, ESPE, USA]. All treatments were performed in a single visit [6].

Observation

1- Clinical and radiographic evaluation: [7]

Clinical and radiographic evaluations were performed at different intervals (after 1-month, 3-month,6-month)

Clinical criteria of success:

Absence of pain, swelling, fistula and sensitivity to percussion.

Radiographic criteria of success:

Absence of deviated eruption of succedaneous teeth with No external/internal root resorption is more than 1/3 of its length, and absence of preapical radiolucency.

2- Microbial evaluation: [5,6]

Microbial evaluation for counting the number of *E. faecalis* was performed after access cavity (S1), and immediately after irrigation with different irrigant solutions (S2). Using cell spreaders, the samples were streaked on Enterococci agar media (selected media for E. faecalis). The pre-and post-irrigation samples obtained were incubated for 24 hours at 37°C in an anaerobic chamber (85% N2, 10% H2, 5% CO2). Later, the bacteria present in these samples was counted by the turbidimetry method.

Statistical analysis of the data:

A commercially available software application was used for statistical analysis (SPSS Chicago, IL, USA). In order to reflect the normalcy of the

data, numerical values were either reported using the mean and standard deviation or the median and range. Based on the degree of normalcy, either the Wilcoxon signed Rank test or the ANOVA test was used to compare the data. P≤0.05 was used as the significance threshold. All testing used a two-tail design.

RESULTS

I. Demographic Data:

1. Age distribution:

The statistical analysis of age for the researched groups revealed that there was no statistically significant difference between the recorded mean age values among the studied groups, as evidenced by the One-way ANOVA test, the p-value equals 0.917.

The group of children treated with 100 ppm AgNPs irrigant solution (group A) had the lowest recorded age means and standard deviation (5.1±0.7), followed by the group of children who received 2% CHX (group C) (5.1±1.7) and the group of children treated with 100 ppm AuNPs irrigant solution (group B) (5.3±0.5).

2. Gender distribution:

The statistical analysis of gender revealed that there were no statistically significant differences between the analyzed groups, as shown by the chi-square test, which recorded a p-value of 0.873.

The male and female percentages are the similar (60 and 40%) in groups B and C, which both group of children who received 100 ppm AuNPs and 2% CHX irrigation. However, groups of children who get irrigation with 100 ppm AgNPs (group A) have a 50% male to female ratio.

II. Bacterial count results:

1. E. faecalis baseline count (CFU/ml) along the study:

One-way ANOVA was used to statistically compare the affected root canals between the examined groups based on the E. faecalis baseline count and reported as CFU/mL. The E. faecalis baseline count (CFU/mL) statistical analysis results, which reveal the mean and standard deviation of the included root canals across the analyzed groups, were compiled in Table 2 and visually shown in Fig. (2). The One-way ANOVA test showed that there was no statistically significant difference in E. faecalis baseline count (CFU/ml) between the included root canals among the analyzed groups, with a p-value of 0.353. A higher (mean SD) E. faecalis count was seen in the root canals that included to receive 100 ppm AgNPs irrigant solution (2418.3±178.45), followed by those that included to receive 2% CHX solution (2342.7±125.9). The E. faecalis count was lower (2312.6±185.73) in the affected root canals that included to receive 100 ppm AuNPs irrigant solution.

TABLE (1) Comparison of E. faecalis baseline count (CFU/ml) throughout the study:

Variable	Mean	SD	f-ratio	p-value
Group A	2418.3	178.5		
Group B	2312.6	185.7	1.082	0.353 ns
Group C	2342.7	126		

**; Significance level at p≤0.05.*

; ns= Non-significant level at p>0.05.

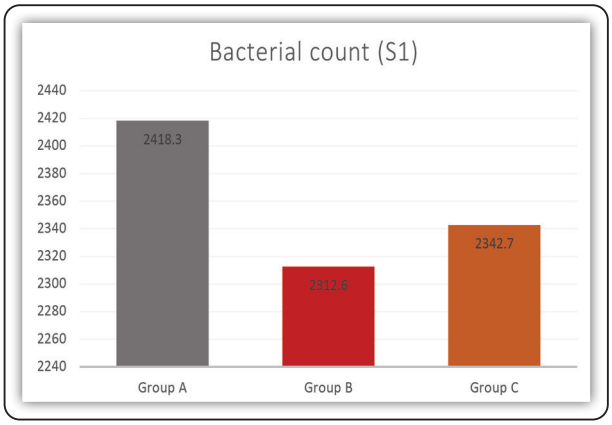

FIG (2) Comparison of E. faecalis baseline count (CFU/ml) throughout the study.

2. Comparison of E. faecalis count (CFU/ml) after irrigation along the study:

One-way ANOVA was used to statistically assess the included root canals across the study groups based on the E. faecalis count following irrigation and reported as CFU/mL.

The statistical analysis findings of the E. faecalis count (CFU/mL) following irrigation, which displays mean± SD of the included root canals among the investigated groups, were compiled in Table 2 and visually depicted in Fig. (3).

TABLE (2) Comparison of E. faecalis count (CFU/ml) after irrigation throughout the study.

Variable	Mean	SD	f-ratio	P-value
Group A	1168.5^{A}	126.9		
Group B	1780.5^{B}	109.4	97.79	<0.001*
Group C	1177.7^{A}	98.3		

**; Significance level at P≤0.05.*

; ns= Non-significant level at P>0.05.

; Different uppercase litters mean statistically significant.

- *Between AgNPs and AuNPs (P =0.000*)*
- *Between AgNPs and CHX (P =0.981 ns)*
- *Between AuNPs and CHX (P =0.000*)*

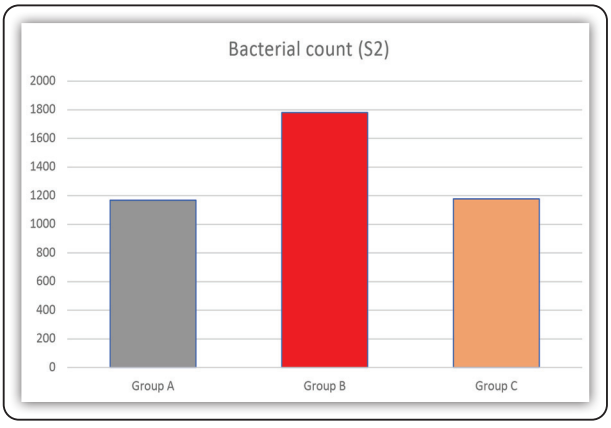

FIG (3) Comparison of E. faecalis count (CFU/ml) after irrigation throughout the study.

The One-way ANOVA test revealed a statistically significant difference in the E. faecalis count (CFU/ml) between the implicated root canals among the tested groups following irrigation, with a p-value <0.001.

A greater (mean± SD) E. faecalis count was seen in the included root canals that got 100 ppm AuNPs irrigant solution (1780.5±109.4) than in the root canals that received 2% CHX (1177.7±98.3). However, the affected root canals that received irrigant solution containing 100 ppm AgNPs displayed a reduced E. faecalis count (1168.5±126.9).

It has been found that E. faecalis count among group (A) and group (C) has no statically difference (P =0.981 ns).

While E. faecalis count among group (A) and group (B) has statically difference (P =0.000*).

Meanwhile E. faecalis count among group (B) and group (C) has statically difference (P =0.000*).

III. Clinical and radiographic evaluation:

1. Clinical evaluation:

After 1, 3, and 6 months of follow-up, the clinical evaluation of the implicated teeth in the study groups was compared for success/failure and expressed as a number and percentages. This data was then statistically evaluated using the Chi-square test.

The clinical evaluation's statistical analysis findings, which showed the number and percentages of cases involved in each study group following irrigation at 1, 3, and 6 months of follow-up, were compiled in **Table 3** and graphically displayed in **Fig. (4)**

The results of the Chi-square test at 1, 3, and 6 months of follow-up revealed a statistically significant difference in the success and failure rate among the various examined irrigants, with p-values of (p = 0.000, <0.001, and <0.001), respectively.

TABLE (3) Clinical follow-up results among the studied groups:

Variables		Group A	Group B	Group C	p-value
1 month	Success; n (%)	9 (90%)	9 (90%)	7 (70%)	**0.000***
	Failure; n (%)	1 (10%)	1 (10%)	3 (30%)	
3 months	Success; n (%)	9 (90%)	9 (90%)	6 (60%)	**<0.001***
	Failure; n (%)	1 (10%)	1 (10%)	4 (40%)	
6 months	Success; n (%)	8 (80%)	8 (80%)	5 (50%)	**<0.001***
	Failure; n (%)	2 (20%)	2 (20%)	5 (50%)	
p-value		**0.055 ns**	**0.055 ns**	**0.015***	

**; Significance level at P≤0.05.* *; ns= Non-significant level at P>0.05.*

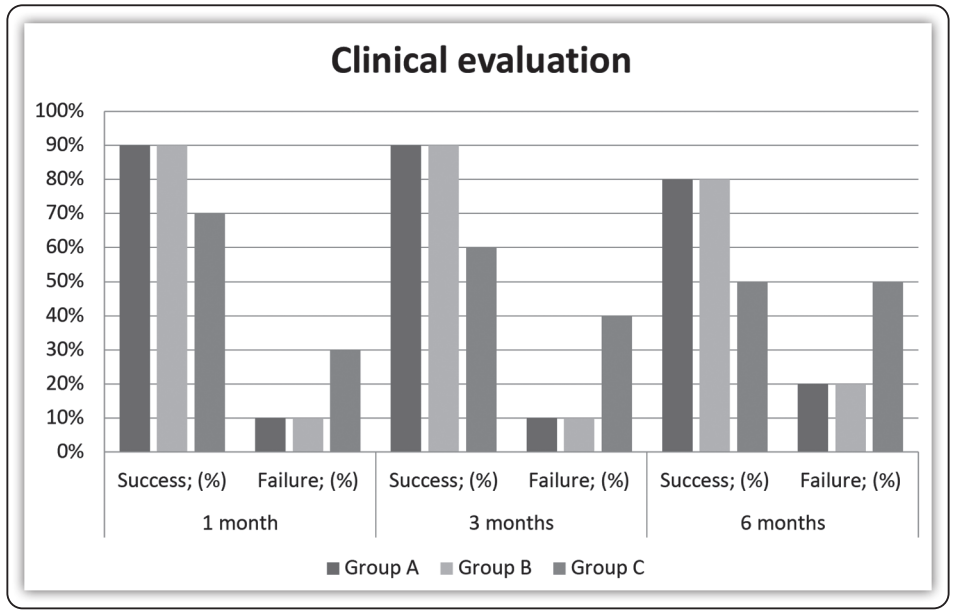

FIG (4) Clinical follow-up results among the studied groups.

The results of the Chi-square test of 100 ppm Group (A) AgNPs andGroup (C) 2% CHX irrigant solutions revealed a non-statistically significant difference in the success and failure rate among the various examined follow-up periods (1, 3, and 6 months), with p-values of (p = 0.055). However, the Chi-square test of 100 ppm Group (B) AuNPs irrigant solution revealed a statistically significant difference in the success and failure rate among the various examined follow-up periods (1, 3, and 6 months), with p-values of (p = 0.015).

In groups of children those received 100 ppm AgNPs and100 ppm AuNPs irrigant solutions (group A and group B) after 1,3 months , only one tooth was scored failed clinically in each group with success rate of (90%). While, after 1 month in group of children those received 2% CHX irrigation (group C), 3 teeth were scored failed clinically with success rate of (70%).

Meanwhile, in groups of children those received 100 ppm AgNPs and 100 ppm AuNPs irrigant solutions (group A and group B) after 6 months, only two teeth were scored failed clinically in each group with success rate of (80%). However, in group of children those received 2% CHX (group C), 4 teeth were scored failed clinically after 3 months and 5 teeth were scored failed clinically after 6 months with success rate of (60% and 50) respectively.

2. Radiographic evaluation:

After 1, 3, and 6 months of follow-up, the radiographic evaluation of the implicated teeth in the study groups was compared for success/failure and expressed as a number and percentages. This data was then statistically evaluated using the Chi-square test.

The radiographic evaluation's statistical analysis findings, which showed the number and percentages of cases involved in each study group following irrigation at 1, 3, and 6 months of follow-up, were compiled in **Table 4** and graphically displayed in **Fig. (5)**.

The results of the Chi-square test at 1, 3, and 6 months of follow-up revealed a statistically significant difference in the radiographic success and failure rate among the various examined irrigants, with p-values of (p = 0.001, 0.008, and 0.003), respectively.

The results of the Chi-square test of the all tested irrigant solutions revealed a non-statistically significant difference in the success and failure rate among the various examined follow-up periods (1, 3, and 6 months), with p-values of (p>0.05).

In groups of children those received 100 ppm AgNPs and 100 ppm AuNPs irrigation solutions (group A and group B) after 1 month, 2 teeth were scored failed clinically in each group with success rate of (80%). While, after 1 month in group of children those received 2% CHX irrigant (group C), 4 teeth were scored failed clinically with success rate of (60%).

Meanwhile, in groups of children those received 100 ppm AgNPs irrigant solutions (group A) after 3 months , no difference occur .However, children those received 100 ppm AuNPs irrigant solutions (group B) after 3 months, 3 teeth were scored failed clinically with success rate of (70%).

Meanwhile, in groups of children those received 100 ppm AgNPs and 100 ppm AuNPs irrigation solutions (group A and group B) after 6 months, 3 teeth were scored failed clinically in each group with success rate of (70%). However, in group of children those received 2% CHX irrigant (group C), 4 teeth were scored failed clinically after 3 months and 5 teeth were **scored** failed clinically after 6 months with success rate of (60% and 50) respectively.

TABLE (4) Radiographic follow-up results among the studied groups:

Variables		Group A	Group B	Group C	p-value
1 month	Success; n (%)	8 (80%)	8 (80%)	6 (60%)	**0.001***
	Failure; n (%)	2 (20%)	2 (20%)	4 (40%)	
3 months	Success; n (%)	8 (80%)	7 (70%)	6 (60%)	**0.008***
	Failure; n (%)	2 (20%)	3 (30%)	4 (40%)	
6 months	Success; n (%)	7 (70%)	7 (70%)	5 (50%)	**0.003***
	Failure; n (%)	3 (30%)	3 (30%)	5 (50%)	
p-value		**0.155 ns**	**0.181 ns**	**0.257 ns**	

**; Significance level at P≤0.05. ; ns= Non-significant level at P>0.05.*

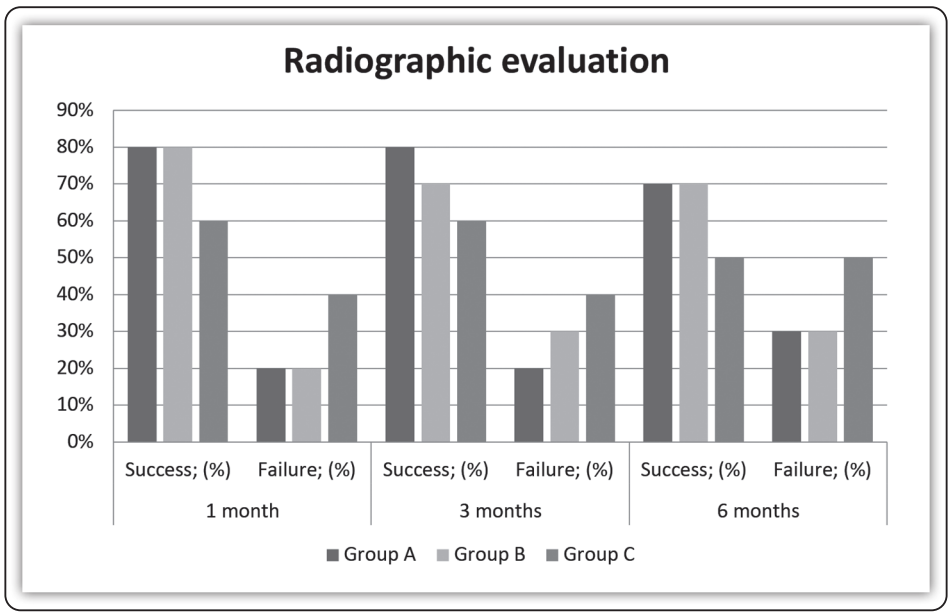

FIG (5) Radiographic follow-up results among the studied groups.

DISCUSSION

According to the findings of this study, using 2% CHX as an irrigant in infected root canals significantly decreased the number of bacteria present. Because CHX has a wide antibacterial spectrum and is a cationic bis-biguanide, this is the case. In order to interact with phospholipids and lipopolysaccharides on the bacterial cell membrane, CHX, a positively charged hydrophobic and lipophilic molecule, uses an active or passive transport mechanism. It functions by reacting with negatively charged phosphate groups on microbe cell walls, which alters the osmotic equilibrium inside the cell [12].

Moreover, Kushwaha et al. (2018) [13] reported that the most effective medication against E. faecalis has been found to be silver Nano particles and laser then 2% Chlorhexidine (CHX), which interacts with the negatively charged bacterial surface and promotes cellular breakdown. Moreover, in this study, the antimicrobial results revealed that AgNPs resulted in a significant reduction in the bacterial count when used as an irrigant in the infected root canals against the E. faecalis gram-positive bacteria. This is because laser and AgNPs are more effective against gram-negative bacteria. Gram-negative bacteria are surrounded by a thin peptidoglycan cell wall, which itself is surrounded by an outer membrane containing lipopolysaccharide. Gram-positive bacteria lack an outer membrane but are surrounded by layers of peptidoglycan many times thicker that may prevent nanoparticles from entering cells [14]. The distinct antibacterial responses of gram-negative and gram-positive bacteria to silver nanoparticles show that absorption of silver nanoparticles is crucial to the antibacterial action[15,16].

Additionally, this study's findings showed that when compared to the AuNPs solution under test, CHX and AgNPs irrigant solutions had a considerably greater antibacterial activity against E. faecalis microorganisms. These results agree with the results of the previous study by Kushwaha et al. (2018) [13] who stated that gold nanoparticles have poor antibacterial activity against E. faecalis microorganisms when compared with 2% CHX and AgNPs.

The clinical and radiographic results of this present study revealed that there was no significant

difference between the group of teeth irrigated with CHX or AgNPs this could be attributed to the similar ability of both tested irrigant solutions to debride the root canals and decrease the load of the E. faecalis microorganisms as proved by the antimicrobial results in this present study.

Moreover, various studies have shown that CHX is most biocompatible when used in therapeutic settings at levels between 0.2% and 2% [17,18]. This could also explain the higher clinical and radiographic success rates of 2% CHX irrigant solution in this present study. but the clinical and radiographic results of this present study revealed that the AuNPs have lower success rates after different follow-up periods when compared with CHX and AgNPs [4]. This could be attributed to its lower antimicrobial activity when compared with CHX and AgNPs as reported by previous investigations [13,19].

CONCLUSION

According to the study's findings, it was established that:

1. Silver and gold nanoparticles presented significant antimicrobial potential against Enterococcus Faecalis microorganisms.
2. The 100 ppm silver nanoparticles can be used as effective alternative to chlorhexidine irrigant solutions in primary teeth.
3. Chlorhexidine irrigant solution exhibited significant potential against Enterococcus Faecalis microorganisms.
4. Chlorhexidine irrigant solution is more effective against Enterococcus Faecalis microorganisms when compared to 100 ppm gold nanoparticles as irrigant solutions in primary teeth.
5. The 100 ppm silver nanoparticles are more effective against Enterococcus Faecalis microorganisms when compared to 100 ppm gold nanoparticles as irrigant solutions in primary teeth.

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