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Evaluation of Antibacterial Efficacy of Calcium Hydroxide Combined With Magnetic Nanoparticles (MNP) and SilverNanoparticles (AgNP) Against E. Feacalis in Single-Rooted Mandibular Premolars – An Invitro Study

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ABSTRACT

The emergence of antibiotic-resistant bacterial strains has necessitated the exploration of alternative antimicrobial agents. This study investigates the antibacterial efficacy of magnetic nanoparticles (MNPs) and silver nanoparticles (AgNPs) against Enterococcus faecalis, a pathogenic bacterium known for its resistance to conventional antibiotics and its role in endodontic re-infections.

Materials and Methods: Forty extracted single rooted mandibular premolars were collected. After following appropriate cleaning and shaping upto F3, the samples were categorised into 4 groups (n=10) . group 1 – control group (without any medication), Group 2 – calcium hydroxide intracanal medicament, Group 3 – ca(OH)2 + silver nanoparticles (AgNP), Group 4 - ca(OH)2 + silver nanoparticles (AgNP) + Magnetic nanoparticles (MNP). Then the prepared intracanal medicaments were introduced into already incubated E faecalis tooth sample. The antimicrobial efficacy were evaluated at the interval of 24 hours and7 days.

Results: The antibacterial activity of the nanoparticles was evaluated using the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. The results demonstrated that both MNPs and AgNPs exhibit significant antibacterial properties against E. faecalis, with MNPs showing superior efficacy. The MIC values for AgNPs were found to be in the range of 2-4 µg/mL, while MNPs required slightly higher concentrations (5-10 µg/mL) to achieve similar inhibitory effects.

Conclusion: This study underscores the potential of MNPs and AgNPs as effective antimicrobial agents against E. faecalis, highlighting their promise for application in dentistry and therapeutic formulations. Future studies should focus on the in vivo efficacy and safety profiles of these nanoparticles to facilitate their translation into clinical settings.

Keywords: Magnetic nanoparticles; Silver nanoparticles; Antimicrobial efficacy; Targeted drug delivery; Intracanal medicaments

INTRODUCTION

The removal of the pathogenic microorganisms within root canal systems is closely linked to the long-term success of

endodontic therapy [1]. The creation of firmly sealed root canals and intracanal medicaments with ongoing antibacterial properties are critical tactics for preventing root canal therapy failure and persistent apical periodontitis (PAP) [2].

However, some teeth continue to harbour infection even after proper root canal treatment [3]. This is mainly due to biofilms and planktonic bacteria such as Enterococcus faecalis (E. faecalis) in the root canal were not entirely eliminated and the complicated nature of root canal system which includes C-shaped root canals, apical bifurcation, and supplementary root canals [4].

When the root canal is significantly infected and interappointment time periods are lengthy, employing an intracanal antibacterial medication as part of controlled asepsis has benefits [5]. Thus, it is crucial that we choose the appropriate intracanal medication on an individual basis, and it is wise to realize that local antibacterial dressings and irrigation in the root canals are components of a coordinated effort to treat endodontic infections.

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One common intracanal medication used to lower root canal bacteria in between appointments is calcium hydroxide (Ca(OH)2). It dissolves and promotes the development of hard tissue and encourages the removal of apical exudates [7]. Although calcium hydroxide has broad antibacterial activity, its effectiveness is restricted against certain bacterial species, including E. faecalis and Candida albicans [8].

Enterococcus faecalis is a Gram-positive, facultativeanaerobe that is closely linked to secondary endodontic infections. Because of its high alkaline pH, it can survive even in harsh environments . Even though it was effectively reduced by biomechanical preparation, still they resides in isthmuses, lateral canals, and apical deltas [6].

Introduction of nanotechnology in dentistry provides the pathway for excellent biomaterials with distinctive physical, chemical, and biological properties [9]. Silver nanoparticles (AgNPs), which ranges from 1 to 100 nm, are the most biocompatible and broad- spectrum of the different NPs . Because of itsminuscule particle size and larger volume-to surface area ratio, (NPs) have betterantibacterial effects [10].

It enters the dentinal tubules and even at lower dosages, have a persistent antibacterial effect at the infection site They emit Ag ions, which result in strong bactericidal effects.In addition, they collect in the surface pits of the cells and denature the membrane, causing cell lysis and death [11].
Magnetic Nano Particles (MNP) were the most promising agents and they mainly focussed on targeted drug delivery. It have the potential to completely transform present clinical treatment approaches. This is because they allow a drugto be transported directly to the disease center in a variety of situations and treatedpurposefully with no negative side effects [12].

Applications involving "intelligent" particles with an a magnetic core (to direct the particles to the vicinity of the target and also for hyperthermia or temperature-enhanced drug release), a recognition layer (to which appropriate receptors are attached), and a therapeutic load (adsorbed inside the pores or hosted within internal cavities of the particles) are likely to have the greatest therapeutic potential [13].

On focussing the previous literature studies, Magnetic nanoparticles were used with root canal sealers mainly because of their targeted drug delivery. In light of these modern developments, as a novelty, we incorporated MNP with gold standard silver nanoparticles and conventional calcium hydroxide in the form of intracanal medicament so that it can easily invade and kill the harbored microbes in the dentinal tubules. Hence, this present study aimed to evaluate the antibacterial efficacy of calcium hydroxide incorporated with silver nanoparticles(AgNP) and magnetic nanoparticles against most resistant E faecalis.

MATERIALS AND METHODS

Sample Selection and Preparation:

The study followed guidelines from modified CONSORT for in vitro research and acquired ethical clearance from the Institutional Ethical Board before commencing the study. It was conducted in Adhiparasakthi dental college and hospital. The total sample was calculated based on G* Power software where the level of significance was found to be 0.005 and the statisticalpower of the study was found to be 95%.

In the present invitro study, 40 freshly extracted single-rooted mandibular premolars extracted for orthodontic reasons were included. The teeth samples were selected based on inclusion criteria such as fully formed apices, with a canalcurvature of less than 10 degrees, and teeth without dental caries and fractures. Whereas teeth with complicated root canal anatomy, calcifications, and internal or external root resorptions are to be excluded.

The extracted teeth samples were washed thoroughly with running water, curette with residual periodontal ligament, and scaled to remove calculus. After this, the samples were soaked with 0.5% chloramine T for 7 days to disinfect. Then they were stored in sterile saline until the samples were used[(14)].

Sample Preparation

With the goal to retain a standard length of 10 mm, the samples were decoronated 2 mm below the cemento enamel junction using a diamond disc (Dental Lab Mini, model no.: 88613, 19mm diameter). Endo access bur (DentsplyMaillefer, size # 2) was employed to open the access, and barbed broaches (MANI, INC. short broaches # 21mm) were used for eliminating the pulp tissues.

After determining the working length, the canal was negotiated using ISO size 10k and 15 k files (MANI, INC. stainless steel k files # 21mm). Dentsply Protaper Gold rotary files (Dentsply Maillefer, # 25mm, 6% taper) and Dentsply X Smartendomotor (Dentsply Maillefer) were utilized for biomechanical preparation in the following order of importance: SX (0.19/0.4%), S1 (0.18/0.2%), S2 (0.20/0.4%), F1 (0.20/0.7%), F2 (0.25/0.8%),and F3(0.30/0.9%).

In parallel, a side-vented 22 gauge needle (HMD UNILOK 2.5ml syringe) was used to inject 2 ml of sodium hypochlorite (PRIME dentistry -5.25%) for a duration of one minute. Then, 2% Chlorhexidine (CHX) and 17% EDTA solution(PREVEST Dent Pro) were utilized for 30 seconds respectively to remove organic and inorganic debris. Following the prescribed procedure, these solutions were ultrasonically agitated (using an Ultra X cordless ultrasonic activation equipment, Orikam) and then flushed with sterile saline solution (PROMEA therapies - 0.9% w/v) for a duration of one minute. Using absorbent paper points (META BIOMED), the prepared root canals were dried. Finally, the teethsamples were autoclaved with moist heat at 132°C (270°F) with a pressure 27 pounds for 30 minutes [15].

PREPARING BACTERIAL CULTURE

In a 5% CO2 incubator, E. faecalis strain (ATCC 29212) was cultivated on Petri dishes using Brain Heart Infusion agar as the nutritional medium for 24 hours at 37°C. After that, using a BD PhoenixSpec ™ nephelometer (BD Company), a bacterial suspension was created and adjusted to (4.5×10^8) colony forming units per mL (No. 1.5 McFarland Standard). Subsequently, a sterile micropipette was used to apply 50µl of bacterial suspension (4.5×108 [CFU/mL]) into the prepared root canals. For the same duration of one minute per sample, the suspension was initiated in the canal using a sterile file size of 20.

Subsequently, all of the samples were incubated for 24 hours at 37°C in an aerobic environment. Following that, five random samples were used to obtain bacterial swabs and were cultivated to verify the growth of bacterial colonies within the root canal before the application of the prepared intracanal medicaments [16]. Thetotal teeth samples (n=40) were categorized into 4 groups (n=10), based on prepared intracanal medicaments.

Grouping the Specimens Based on Materials Used

Group A – Control group without any intracanal medicament

Group B – Calcium hydroxide

Group C – Calcium hydroxide + silver nanoparticles (AgNP) (1:1) by weight Group D - Calcium hydroxide + silver nanoparticles (AgNP) + Magnetic Nanoparticles (MNP) (1:1:1) by weight.

The samples were measured in the previously mentioned ratio using a digital measuring scale and a measuring scoop. They were then combined with a sonic activator (Flasher Scientific, 550 Sonic Dismembrator) that had a total dimensionof 15 * 18 * 9 inches and a 20 kHz ultrasonic frequency. For two hours, they were configured in pulse mode (every 45 seconds). After achieving the necessary homogeneous combination, the medicaments were inserted using lentulospiral into the prepared aseptic root canals.

The standardization of silver nanoparticles and magnetic nanoparticles were performed as per manufacturer instructions.

Testing Procedures

The wells were then filled with the appropriate medications from each group and were aseptically repeated five times. Incubating culture plate at 37°C for 7 days and then the diameter of bacterial growth inhibitory zones were evaluated at the end of 14 days, whereas colony forming units (CFU) were analysed at the time interval of 24 hours and 7 days.

Evaluation Of Antibacterial Effects

The antibacterial activity of Enterrococcus faecalis were evaluated in this present study. The minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) were acquired using logarithmic phases of pathogenic growth. It was derived from the culture plate based on the amount of colony forming units. Another method of verifying bacterial growth was zone of inhibition and this was achieved by measuring the diameter inhibitory areas [20] and it was performed using VITEK® 2 SYSTEM (bioMerieux), microdilution method.

Statistical Analysis

The statistical analysis were performed using SPSS software (17 version) (IBM) with significant p – value (<.05) and

confidence level of 95%. Data were obtained after 24 hours and 7 days and Kruskal wallis test followed by Mann Whitney Post hoc test was employed to compare the antibacterial efficacybetween 4 study groups at apical 1/3rd portion of root canal system.

RESULTS

The antibacterial activity of all groups of intracanal medicaments has been presented in the following tables. The descriptive statistics of mean, standard deviation (SD) and percentages of decimal logarithm (log of 10) of bacterial cell number after antibacterial effect (both in MIC and Zone of inhibition) were found to be reduced with the incorporation of silver nanoparticles and conventional calcium hydroxide. In contrast, incorporating Magneticnanoparticles into the afore-mentioned group produced the lowest count ofbacteria at the result of 7 and 14 days. The synchronized impact of calcium hydroxide, silver nanoparticles, and magnetic nanoparticles demonstrated more antibacterial activity than their actions when the overall activity of test groups was compared.

Table 1: Represents The Intergroup Comparision Of AntimicrobialEfficacy Of Various Intracanal Medicaments

GROUPS	CONTENTS	MEAN	S.D	95% CONFIDENCE INTERVAL		MEAN SQUARE	F	SIG
				LOWER	UPPER			
A	Control group	62.70	3.83	59.95	65.41	201.16	142.15	.000
B	Ca(OH)2	81.52	2.77	79.53	83.47			
C	Ca(OH)2 + AgNP	294.31	17.95	281.46	307.12			
D	Ca(OH)2 + AgNP + MNP	129.50	16.09	117.98	140.98			

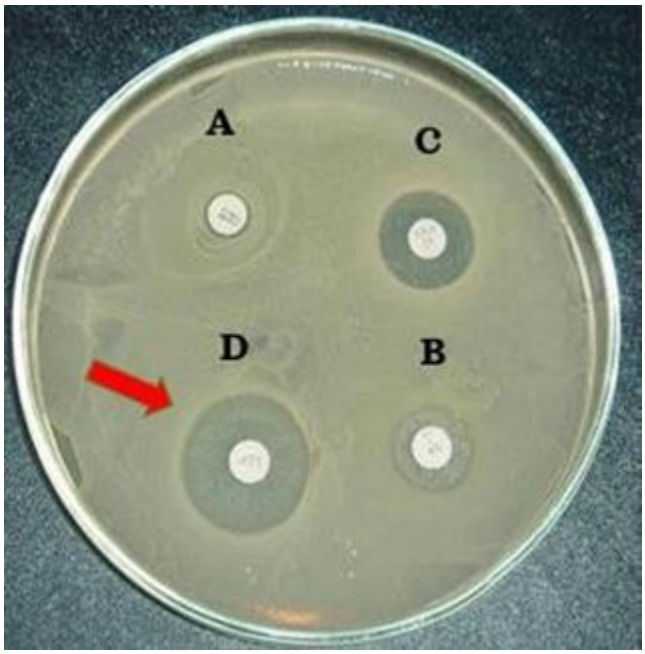
Table 2: Represents The Intergroup Comparision Of Antimicrobial Efficacy Of Various Intracanal Medicaments(In Colony Forming Units)

GROUPS	After 24 hours No of colonies	After 7 days No of colonies
Control group	Too numerous to count >300 colonies	Too numerous to count >380 colonies
Ca(OH)2	263(NG)	168 (NG)
Ca(OH)2 + AgNP	145(NG)	87(NG)
Ca(OH)2 + AgNP + MNP	86(NG)	21 (NG)

*** NG – no growth**

Table 3: Represents The Intergroup Comparision Of Antimicrobial Efficacy Of Various Intracanal Medicaments (Zone Of Inhibition - After 14 Days)

GROUPS	ZONE OF MEASUREMENTS AFTER 24 HOURS
Control group	7 mm
Ca(OH)2	11 mm
Ca(OH)2 + AgNP	16 mm
Ca(OH)2 + AgNP + MNP	19 mm



DISCUSSION

Several factors, including microbiological factors, alterations in periapical tissue pressure, chemical mediators, cyclic mediator changes, and other physiological parameters can produce pain during root canal treatment. The most frequent reason for pain is improper canal disinfection. Therefore, the main goal of endodontic therapy is to eradicate these bacteria as much as possible.

Originally, the three key components of a successful endodontic treatment were debridement, thorough cleaning, and root canal system obturation, with each component bearing equal importance. This compromised by complex anatomy and morphology knowledge, diagnosis and treatment planning [21].

Endodontic failures are polymicrobial but Enterococcus faecalis, residual facultative anaerobe in the root canal system accounting about 37% [23]. Edward et al indicates that these organisms can survive in harsh alkaline pH (>12) and can last for 30 minutes at 60 degrees Celsius. It can adhere collagen, primarily in the dentin, which serves as a nidus for the regrowth of microorganisms and eventually causes root canal failure. According to previous research, retreatment cases have nine times more E. faecalis than initial infections. This is mostly because of its inhibitory effect on lymphocytes which results in secondary infections [24]. According to Faisal Alghamdi et al., the prevalence rate of E. faecalis that was elevated between 77% and 79.5%. in endodontically treated teeth. The selection of Enterococcus faecalis for the current investigation was primarily motivated by this.

An antimicrobial drug called an intracanal medicament is inserted into the root canal in between appointments to eradicate any bacteria that may still be present and stop re-infection. They are employed to destroy microorganisms, lessen discomfort and inflammation, get rid of apical exudate, and regulate inflammatory root resorption [22].

Historically, intracanal medications were essential in multi-visit endodontics for root canal space disinfection. However, medicated sealers can be used in single-visit endodontics to boost antimicrobial activity, lower periapical microbial bioflora,

and thwart the growth of microorganisms that try to enter dentinal tubules [25].

Calcium hydroxide was one of the gold-standard intracanal medicament introduced to endodontics by Herman in 1920 to reduce microbial load inbetween appointments. It is a strong base, insoluble in alcohol, and a high pH of12.5–12.8 [26]. The method of action is caused by production of hydroxyl ions, which raises the medium's pH and encourages bacterial inactivation It mainly produces the antibacterial action and the induction of hard tissue deposition [27].

The presence of hydroxyl ions creates an alkaline environment and produces highly oxidant free radicals that have antibacterial and early healing effects. To conclude, the damage to the bacterial cytoplasmic membrane, protein denaturation and DNA damage, Co2 adsorption, mineralization, and acid neutralization after an inflammatory processare the mechanisms that generate calcium hydroxide's fatal effect [28].

The long-standing era of nanotechnology left a lasting impression in the fieldof dentistry. Inorder to overcome the limitations of conventional antibacterial substances, they offers larger surface area to volume ratio, extremely small sizes and exhibits superior chemical and physical properties [27,28].

Comparing the combination effect of AgNPs and Ca(OH)2 , the colony-forming units (CFUs) of E. faecalis were decreased within a week of treatment. It was proposed that incorporating AgNPs into Ca(OH)2 lowered the drug's minimum inhibitory concentration (MIC) against E. faecalis and enhanced its antimicrobialefficacy(29) While AgNPs are less likely to cause bacterial resistance, recent research has shown that some endodontic germs may become resistant to them. They include extrinsic adaptation mechanisms like plasmids containing resistance genes and point and adaptive mutations, as well as internal ones like effux pumps, porins' downregulation, and chromosomal resistance genes [29,30].

Moreover, oxidative stress and the emergence of persistent bacteria like E. faecalis are caused by AgNP concentrations below the MIC. They enter a state of dormancy in which the antibiotic attaches itself to the bacteria but is unable to destroy them because of a subsequent inactivation of pathways [29,30].

The main drawbacks of higher AgNP consumption in bacteria may promote coregulation and core resistance to metals and antibiotics. They would so becomeresistant to antibiotics. The sublethal exposure to AgNPs may improve the development of biofilms, upregulate genes for gene transfer as well as to increase the protein and sugar in the biofilms, which are resistant against antimicrobial effect of AgNPs [31]. Hencein order to overcome these limitations and to improve antibacterial efficacy, magnetic nanoparticles (MNP), a novel material were incorporated in this presentstudy.

Inorganic metal-based core-shell nanomaterials have become popular drug delivery vehicles in recent years, particularly those consisting of an iron oxide core. It includes enhanced biocompatibility, simpler production, drug- loading capability, and controlled cargo release. Furthermore, pathogen membranes can be broken down by iron oxide magnetic nanoparticles, and thesematerials can produce reactive oxygen species that harm mitochondria. These advantageous characteristics encourage the use of magnetic nanoparticles in contemporary treatments as medication carriers [32].

Targeted magnetic nanoparticles decreases the adverse effects of systemic treatments by accumulating themedication at the site of requirement. Specifically, magnetic nanoparticles (MNPs) arenow being used in unique ways in nanodentistry for a wide

range of purposes, including imaging techniques, regulates targeted medicament delivery, minimizing side effects and multiple drug resistance [33].

The distinct electromagnetic and magneto-mechanical properties of magnetic nanoparticles (MNPs) enable communication and control their vibration, rotation, and translational movement, as well as the absorption of electromagnetic energy and the provision of remote heating and actuation. One excellent feature is the ability of magnetic fields to penetrate deeply through biological tissues without harming healthy cells. In general, MNPs exhibit biocompatibility and relative stability, particularly when shielded by a thick layer of self-assembling monolayer or coated in a layer of carbon, silica, or gold. The ability to link MNPs to various objects is a significant benefit [34].

Furthermore, several other extensively researched uses for MNPs exist, such as magnetically enhanced transfection, magnetically assisted gene therapy, magnetically induced hyperthermia, and magnetic force-based tissue. More than thirty years have been spent intensively researching MNPs as the next generation of targeted medication delivery because of their distinct physical characteristics and capacity to operate at the cellular and molecular level of biological interactions [35].

CONCLUSION

Within the constraints of the current investigation, it can be said that, in comparison to all other groups, Gorup 4's combination of calcium hydroxide, silver nanoparticles, and magnetic nanoparticles was thought to be the best intracanal medication. Two antibacterial assessments, including zone of inhibition and minimum inhibitory concentration, supported this. However, morein vivo research is required to evaluate the effectiveness of magnetic nanoparticles added to intracanal medicament.

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