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COMPARISON BETWEEN THE EFFECT OF CALCIUM HYDROXIDE AND COLLOIDAL GOLD /CHARCOAL EXTRACT COMBINATION ON TWO COMMONLY ISOLATED BACTERIA FROM DENTAL CANAL ROOT – ENTEROCOCCUS FAECALIS AND STREPTOCOCCUS MUTANS

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ABSTRACT

Aim: The aim of this study was to compare the effect of two different materials which are colloidal gold/charcoal extract combination and calcium hydroxide on two commonly isolated bacteria from dental canal root which are *Enterococcus faecalis* and *Streptococcus mutans*.

Methodology: A total of ten *Enterococcus fecalis* and ten *Streptococcus mutans* strains were selectively isolated from infected human teeth on MacConckey's agar and Mitis-Salivarius agar respectively. For each bacterial strain, the antibacterial effects of colloidal gold/charcoal extract combination and calcium hydroxide were tested via in vitro disk diffusion susceptibility assay. Zones of growth inhibition were measured and compared between the two formulations.

Results: When comparing the mean range of bacterial zone of inhibition around both materials, the mean range was higher for colloidal gold/charcoal extract combination than that of calcium hydroxide in both bacteria. The results were significant as suggested by the P value (<0.05).

Conclusion: Bacterial inhibition effect of gold/charcoal combination was stronger than the effect of calcium hydroxide on both types of tested bacteria.

KEYWORDS: Colloidal gold solution, nanoparticles, charcoal extract, calcium hydroxide, root canal bacteria, *Enterococcus faecalis*, *Streptococcus mutans*.

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INTRODUCTION

A root canal infection, also known as endodontic infection, occurs when bacteria enter and infect the pulp of a tooth. Root canal infections are usually caused by untreated dental decay, deep cavities, cracked or fractured teeth, or dental trauma. Symptoms of a root canal infection may include severe toothache, sensitivity to temperature, swelling and tenderness, and abscess formation. Several types of bacteria can be involved in root canal infections. The most common bacteria implicated in root canal treatment including *Enterococcus faecalis, Streptococcus mutans, Streptococcus anginosus*, and various anaerobic bacteria such as *Prevotella spp., Porphyromonas spp., and Fusobacterium spp.*[1]

Recent advances have been introduced for the treatment of such infections. Gold nanoparticles (GNPs) have been tested in the treatment of gum disorders, dental caries, tissue engineering, dental implantology, and cancer diagnostics due to their nanostructures, high surface volume, and biocompatibility. [2]

One of the most important properties of GNPs is that they have antifungal and antibacterial properties, thus they are added to other biomaterials to boost up their effects. They also increase the mechanical qualities of materials, producing better results. They come in a variety of sizes and concentrations to demonstrate their therapeutic effects. These characteristics of GNPs make them a popular filler for biomaterials.[2]

According to a study by Pissuwan et al., GNPs improve the inhibitory power of dental substances against various root canal bacteria as *Streptococcus mutans*. GNP addition can aid to boost a material's antibacterial activity.[3]

Colloidal gold foam is now introduced to the market by Shiny Pharma containing nanoparticles with exceptional properties and improved effects which is tested in our in-vitro study to test its effect on two of the most common strains of bacteria which are *Enterococcus faecalis and Streptococcus mutans*.

In addition to GNPs, other materials are now tested for their beneficial effects in dentistry including the charcoal extract. Charcoal is known to have the ability to inhibit growth of pathogenic bacteria causing root canal infection thus improving the oral health. Charcoal has the ability to remove toxins from the mouth and tooth surfaces. During mouth washing, it binds with the poisons and exits the oral cavity. Because it has the power to alter the pH of the mouth, it prevents bacteria and germs that cause disease from thriving and procreating there, keeping the mouth healthy and safe.[4,5]

Calcium hydroxide is commonly used in dentistry for its antibacterial properties. It has been used for many years in various dental procedures due to its ability to eliminate or control bacteria in the oral cavity. Antimicrobial effect of calcium hydroxide has been proven in root canal treatment. After cleaning and shaping the root canal, calcium hydroxide is usually placed inside the canal to disinfect it and eliminate any remaining bacteria. It has a strong alkaline pH, which helps kill bacteria and disrupt their cellular processes, reducing the risk of reinfection.[6,7,8]

In addition, calcium hydroxide is used as a temporary intracanal dressing between root canal visits. It provides antibacterial activity and helps maintain a sterile environment within the root canal system during the treatment process. Moreover, calcium hydroxide is used in the treatment of periapical lesions, such as periapical abscesses or cysts. It helps eliminate bacteria and promote healing in the periapical tissues.[8]

It's important to note that while calcium hydroxide has antibacterial effects, it may not be effective against all types of bacteria. Dentists may choose alternative antibacterial agents or combinations of medications based on the specific

case and the targeted bacteria. All these innovations in dental materials help the dentist to improve the quality of treatment they offer to the patient and improve the oral and general health.[9]

MATERIALS AND METHODS

Bacterial isolates:

A total of ten *Enterococcus faecalis* and ten *Streptococcus mutans* strains were isolated from infected human teeth and included in this study.

Tested Substances:

- Colloidal gold/Charcoal extract combination.
- Calcium hydroxide.
- Normal Saline (Control).

Culturing media:

- **MacConckey's agar and Mitis-Salivarius agar:** MacConckey's agar and Mitis-Salivarius agar are used for selective isolation of *Enterococcus fecalis* and *Streptococcus mutans* respectively. Identification of isolated bacteria was performed by various microbiological techniques as described by ***Patricia, 2021***.[10] Isolation of the desired bacterial strains was performed inside the laboratory of Medical Microbiology and Immunology department at Faculty of Medicine, Ain Shams university. All instruments used during the whole procedure were sterile.
- **0.5 McFarland Standard**: This medium is prepared to adjust the proper bacterial density required for inoculation of Muller-Hinton agar for antibacterial sensitivity testing.
- **Muller Hinton Agar**: This medium is prepared for performance of disk diffusion susceptibility assay to test antibacterial effect of the tested substances on *Enterococcus faecalis*.
- **Mueller Hinton Agar with 5% Sheep Blood**: This medium is prepared for performance of disk diffusion susceptibility assay to test antibacterial effect of the tested substances on *Streptococcus mutans*.

Preparation of McFarland Standard: Following the instructions in CLSI, 2023, a 0.5 McFarland standard was made internally by adding a 0.5-ml aliquot of 0.048 mol/liter BaCl2 to 99.5 ml of 0.18 mol/liter H2SO4 and stirring continuously to maintain a suspended state. A spectrophotometer with a 1-cm light path and matched cuvette was used to measure absorbance to confirm that the density of the turbidity standard was accurate. Following that, 4- to 6-ml aliquots of the suspension of barium sulphate were put into screw-cap tubes that were the same dimensions as those used to standardize the bacterial inoculums. Followed by complete sealing of the tubes that were kept at room temperature in the dark.

Preparation of Muller Hinton agar: Muller Hinton agar plates were prepared following the manufacturer's instructions ***(HiMedia, India)***. 38.0 grams of the agar powder were suspended in 1000 ml purified/ distilled water then heated to boiling to dissolve the medium completely. Sterilization of prepared suspension was performed by autoclaving at 121°C for 15 minutes. The suspension was allowed to cool to 45-50°C then well mixed and poured into sterile Petri plates. As for Muller Hinton agar with 5% Sheep Blood, ready-prepared agar plates provided by HiMedia, India were used.

Bacterial inoculum preparation: For each bacterial isolate to be tested, fresh broth culture was made. Bacterial inocula were prepared through dilution of the broth culture to match a 0.5 McFarland turbidity standard ***(CLSI, 2023)[11].***

Disk Diffusion Susceptibility Assay: This assay was conducted following guidelines of ***CLSI, 2023***:

- **Implantation of bacterial isolates on Muller Hinton agar plates:** A sterile swab was inserted into the inoculum tube for each created bacterial

inoculum. The excess fluid was then removed by rotating the swab firmly against the tube's wall (above the fluid level). The Muller Hinton agar plate's dried surface was inoculated by streaking the swab across it three times, rotating the plate each time by around 60 degrees to achieve a uniform dispersion of the inoculum. The swab was then used to clean the plate's rim of any extra liquid. The surface of the agar plate was ultimately allowed to dry for at least 3 to 5 minutes, but no longer than 15 minutes, before moving on to the following stage.

- **Mixing and application of tested substances:** Using a metal punch, a total of three holes of 4 mm in diameter were punched on the agar surface, leaving around 10-15 mm from the petri dish's edge. To prevent zones of inhibition from overlapping, these holes were spaced apart by a distance of at least 20 mm. Each hole was labelled as follows: hole I for colloidal gold/ charcoal extract combination, hole II for calcium hydroxide, and hole number III for normal saline as a control. Each hole contained one of the tested substances. Prior to presenting the findings, all the agar plates were left to incubate for up to 24 hours in aerobic conditions at 37°C.

Method of evaluation: After observation period of 24 hours, a poly gauge millimeter ruler was used to quantify the zone of microbial growth inhibition (lack of bacterial colonization) around the holes at its biggest diameter.

Statistical analysis: SPSS software (version 16.0, SPSS, Chicago, IL, USA) was used to analyse the data. The ANOVA and Kruskal-Wallis tests were used to compare the data in each group. The significance level was set at 0.05.

RESULTS

After 24 hour incubation period, the zones of bacterial inhibition around the two tested materials (colloidal gold/charcoal extract combination and calcium hydroxide) were measured and the mean values for the inhibition zones for Enterococcus faecalis and Streptococcus mutants were recorded.

This table compares between the relation between the effect of colloidal gold/charcoal extract combination and calcium hydroxide on both *E. faecalis* and *S. mutans*. When comparing the mean range of bacterial zone of inhibition around both materials, the mean range was higher for colloidal gold/charcoal extract combination than that of calcium hydroxide in both bacteria. The results were significant as suggested by the P value (<0.05).

TABLE (1)

	Enterococcus faecalis Growth Inhibition			***Streptococcus mutans*** Growth Inhibition		
	Range in mm	Mean (±SD)	P Value	Range in mm	Mean (±SD)	P Value
Colloidal Gold/Charcoal Extract	21-2	23.9 (±2.46)	0.002	14-23	19 (±3.26)	0.0002
Calcium Hydroxide	15-23	18.4 (±2.98)		9-15	11.7 (±1.88)	
Normal saline	0	0		0	0	

DISCUSSION

The combination of charcoal and gold nanoparticles has been explored for its potential antibacterial effects in dentistry. Both charcoal and gold nanoparticles have individually shown antimicrobial properties, and researchers have investigated whether their combination could enhance these effects. Charcoal, specifically activated charcoal, is known for its adsorptive properties and has been used in various medical applications. It can bind to toxins and bacteria, effectively removing them from the system. In dentistry, activated charcoal has been used in toothpaste formulations and oral care products due to its potential to adsorb bacteria and toxins from the oral cavity.[12]

Gold nanoparticles, on the other hand, have unique properties that make them attractive for biomedical applications. They possess a large surface area-to-volume ratio, which enhances their reactivity. Additionally, gold nanoparticles can be functionalized with various molecules, such as antibiotics or antimicrobial agents, to enhance their antibacterial properties.[2]

Studies have investigated the combination of charcoal and gold nanoparticles to explore their synergistic antibacterial effects. For example, researchers have coated gold nanoparticles with charcoal and tested their antibacterial activity against oral pathogens. The results have shown promising antimicrobial effects, indicating that the combination of charcoal and gold nanoparticles can inhibit the growth of bacteria commonly associated with dental infections. The addition of gold nanoparticles to dental materials can help prevent bacterial adhesion and biofilm formation, reducing the risk of infections.[13,14]

Our study revealed that colloidal gold/charcoal extract combination had a more potent in-vitro antibacterial effect based on range of zones of growth inhibition of both *Enterococcus faecalis and Streptococcus mutans* around the two tested formulations. The mean range around the colloidal gold/charcoal extract combination was 23.9 and 19 for *Enterococcus faecalis* and *Streptococcus mutans* respectively. In comparison, the mean range around calcium hydroxide was 18.4 and 11.7 for *Enterococcus faecalis* and *Streptococcus mutans* respectively. This can be explained by several studies that revealed drawbacks of calcium hydroxide treatment as its inability to deeply penetrate through the microscopic channels of dentinal tubules. These channels can harbor bacteria and their byproducts. Because calcium hydroxide's antimicrobial effect is primarily surface-bound, this can result in persistent infection or reinfection of the root canal system.[15,16]

CONCLUSION

Within the limitations of the present study, it was concluded that colloidal gold/charcoal extract combination exhibited a stronger antimicrobial activity than calcium hydroxide against bacteria that are commonly implicated in root canal infections. However, it's important to note that while this combination has shown potential antibacterial effects, further research is still needed to fully understand their mechanism of action, optimize their formulation, and evaluate their long-term safety and efficacy in dental applications.

RECOMMENDATIONS

More in-vivo studies on the colloidal gold/charcoal extract combinations are required to confirm these revolutionary results and get better understanding about this combination.

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