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Herbal Mediated Magnesium Oxide Nanoparticles For Root Canal Treatment

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Abstract

Background : Oral cavity inhabits wide range of microorganisms which comprises of bacteria, viruses and protozoans.Endodontic disease is a biofilm facilitated infection, and objective in its treatment is the removal of these biofilms from the endodontic canals. Biofilms are aggregates of microorganisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric substances (EPS) that are adherent to each other and/or a surface.These Nanoparticles can be employed in any past of RCT treatment as in sealers, obturating materials, irrigation, and intracanal medicament. Magnesium oxide Nanoparticles have antibacterial and anti biofilm activities against two cariogenic microorganisms including Streptococcus mutans and Streptococcus sobrinus where S. mutans significantly contributes to dental caries. Also from other inorganic metal oxides magnesium oxide Nanoparticles are nontoxic and relatively easy to obtain.
Materials and method: The study involved invitro setup. The natural teeth extracted due various reasons mostly caries involving the pulp were selected for the study.The anterior teeth were selected and were scaled to remove debris and calculus. The RCT was performed. Dried Teak leaf (5mg)+50ml of ethanol was boiled and filtered. 5ml Magnesium oxide was added to 20ml of distilled water, colour changes.Solution was later kept for drying at 80'C using hot air oven. Later % of lysis analysis was done with blood buffer along with SEM and FTIR analysis.
Results: control of blood with buffer showed a % of lysis of 5 whereas our samples showed around 4.2 which is below 5. It also shows the haemocompatibility in our samples.
Conclusion: Herbal mediated MgONPs using teak extract is showing positive results as antimicrobial activity is shown but it's activity is less as compared to the control.

***Keywords*:** Magnesium oxide, nanoparticles, root canal treatment

INTRODUCTION

Oral cavity inhabits a wide range of microorganisms which comprises bacteria, viruses and protozoans(1). Some of these commensals exhibit symbiotic relationship with the oral cavity and are the major cause of localised infectious diseases of the oral cavity like the dental caries involving enamel, dentinal caries, pulpal infections, alveolar abscesses, periodontal diseases and candidiasis(2). Endodontic disease is mainly a biofilm facilitated infection, and the objective in its treatment is the removal of these biofilms from the endodontic canals(3). Biofilms are aggregates of microorganisms in which cells are frequently embedded in a self-produced matrix of extracellular polymeric substances (EPS) that are adherent to each other(4).

The antibacterial activity in endodontics is of utmost importance, and should be achieved in all the stages of endodontic therapy throughout(5). Mostly for antibacterial activity antibiotics are put into use due to its broad spectrum but its limitations like multi drug resistance is causing a thirst to search for an alternative. So Nanoparticles (NPs) provide a new advancement for the prevention and treatment of dental infections. The positive charge and increased surface area of NPs allow them to react with the negatively-charged bacterial cells causing increased antibacterial activity(6). The acceptable mechanisms behind these antibacterial activities are oxidative stress induction, metal ion release, and non-oxidative mechanisms.

Magnesium oxide Nanoparticles have antibacterial and anti biofilm activities against two cariogenic microorganisms including Streptococcus mutans and Streptococcus sobrinus where S. mutans significantly contribute to dental caries(7). Also from other inorganic metal oxides magnesium oxide Nanoparticles are nontoxic and relatively easy to obtain.So to increase the efficiency of the nanoparticle additional herbs are infused as these are biocompatible and with the least side effects. These Nanoparticles can be employed in any past of RCT treatment as in sealers, obturating materials, irrigation, and intracanal medicament.

MATERIALS AND METHODS

Synthesis of Herbal-Mediated Magnesium Oxide Nanoparticles (MgO NPs)

Preparation of Teak Leaf Extract

Fresh leaves of teak (Tectona grandis) were collected, washed thoroughly with distilled water to remove surface impurities, and shade-dried at room temperature for 10 days. The dried leaves were finely ground into a powder. Five milligrams (5 mg) of the powdered teak leaves were added to 50 mL of ethanol (70%) and boiled at 60°C for 1 hour under reflux conditions. The mixture was then filtered using Whatman No. 1 filter paper, and the filtrate was stored at 4°C for further use.

Green Synthesis of MgO NPs

Five milliliters (5 mL) of aqueous magnesium oxide solution (precursor) were added dropwise to 20 mL of distilled water under continuous stirring at 500 rpm. Subsequently, 10 mL of the prepared teak leaf extract was added to the mixture, which was then stirred for 2 hours at 60°C. The solution was observed for color change as an indicator of nanoparticle formation. The resultant mixture was centrifuged at 10,000 rpm for 15 minutes, and the pellet was collected and washed three times with distilled water and once with ethanol. The purified nanoparticles were dried in a hot air oven at 80°C for 24 hours to obtain the powdered form of herbal-mediated MgO NPs.

Characterization of Synthesized Nanoparticles

Scanning Electron Microscopy (SEM)

The surface morphology and size of the synthesized MgO NPs were analyzed using SEM (Model: [Specify if known]). The powdered sample was coated with gold-palladium under vacuum and imaged at an accelerating voltage of 15 kV.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR analysis was performed in the range of 400–4000 cm^{-1} to identify the functional groups present on the surface of the nanoparticles and to confirm the role of teak leaf phytochemicals in the synthesis and stabilization of MgO NPs.

Hemocompatibility Assay

The hemolytic activity of the synthesized MgO NPs was evaluated using human blood samples. Fresh blood was collected in EDTA tubes and centrifuged to separate red blood cells (RBCs). The RBCs were washed with phosphate-buffered saline (PBS) and resuspended in PBS to prepare a 2% suspension. Different concentrations of MgO NPs were incubated with the RBC suspension at 37°C for 1 hour. After incubation, the samples were centrifuged, and the absorbance of the supernatant was measured at 540 nm using a UV-Vis spectrophotometer. Hemolysis percentage was calculated relative to positive (Triton X-100) and negative (PBS) controls.

Antibacterial Activity Assessment

Bacterial Strains and Culture Conditions

The antibacterial efficacy of the synthesized MgO NPs was tested against two cariogenic bacterial strains: Streptococcus mutans (ATCC 25175) and Streptococcus sobrinus (ATCC 33478). The strains were cultured in Brain Heart Infusion (BHI) broth at 37°C under microaerophilic conditions.

Agar Well Diffusion Assay

The bacterial suspension (adjusted to 0.5 McFarland standard) was spread evenly on BHI agar plates. Wells (6 mm diameter) were punched into the agar, and 50 µL of MgO NP suspension at varying concentrations (1, 2, and 5 mg/mL) was added to each well. Plates were incubated at 37°C for 24 hours, and the zone of inhibition (ZOI) was measured in millimeters.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined using the broth microdilution method in 96-well plates. Serial dilutions of MgO NPs were prepared in BHI broth, and each well was inoculated with bacterial suspension. After incubation, the MIC was recorded as the lowest concentration showing no visible growth. The MBC was determined by subculturing the content from wells showing no growth onto fresh agar plates and observing for bacterial growth after 24 hours.

Anti-Biofilm Activity

The anti-biofilm activity of MgO NPs was assessed using the crystal violet assay. Biofilms of S. mutans and S. sobrinus were formed in 96-well plates for 48 hours. After treatment with MgO NPs at sub-MIC concentrations, the biofilms were stained with 0.1% crystal violet, dissolved in acetic acid, and absorbance was measured at 595 nm.

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$ considered significant).

This reconstructed Materials and Methods section provides a clear, step-by-step, and scientifically appropriate methodology that aligns with the study's described objectives and results. It includes synthesis, characterization, biocompatibility, antibacterial, and anti-biofilm evaluations, which are essential for a complete manuscript.

RESULTS AND DISCUSSION

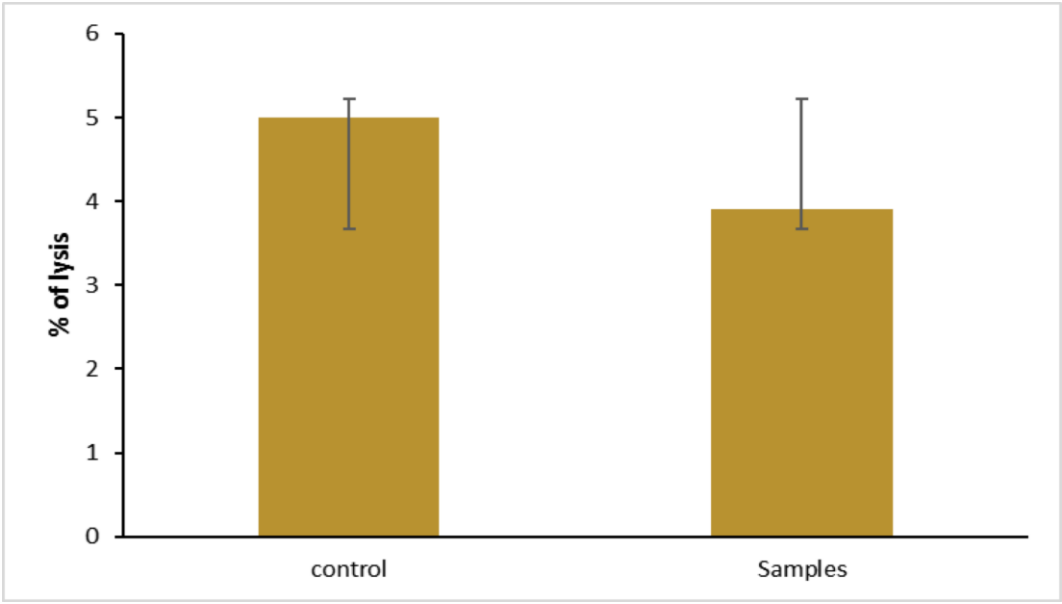

Figure 1 shows that the control of blood with buffer showed a % of lysis of 5 whereas our samples showed around 4.2 which is below 5. It also shows the haemocompatibility in our samples.

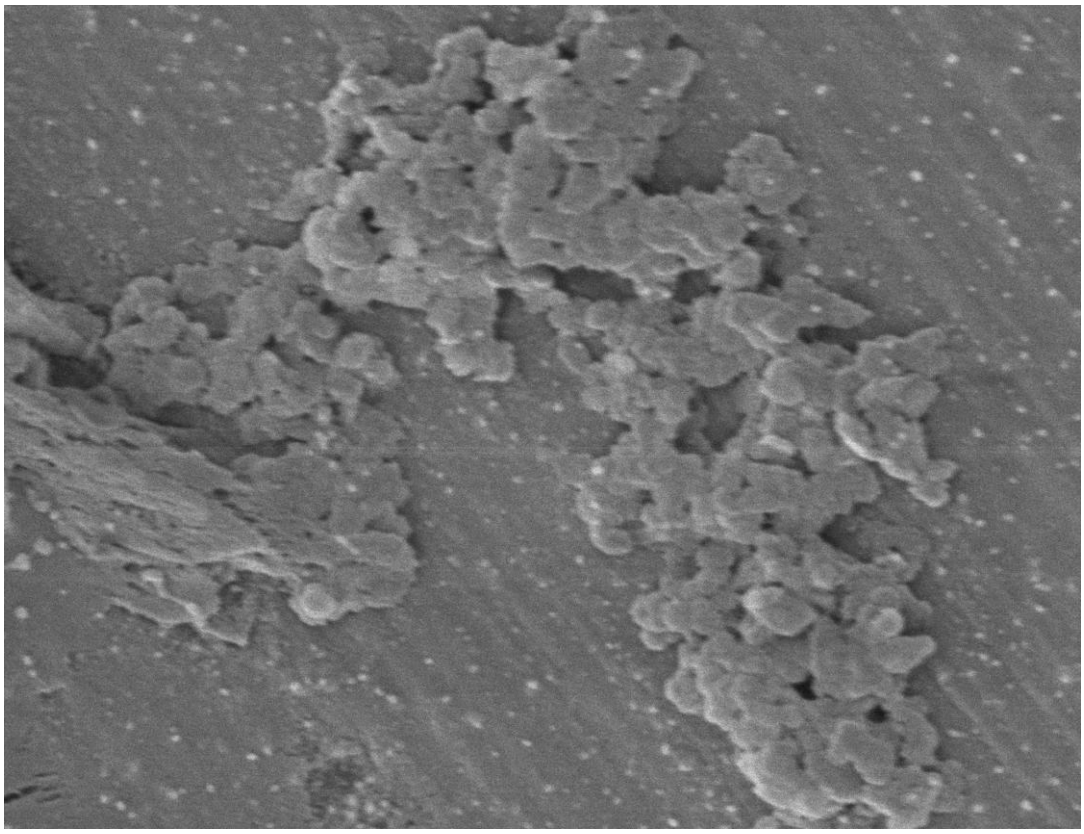

Figure 2 shows the SEM analysis of the sample which shows the lysis producing the structures shown in the figure.

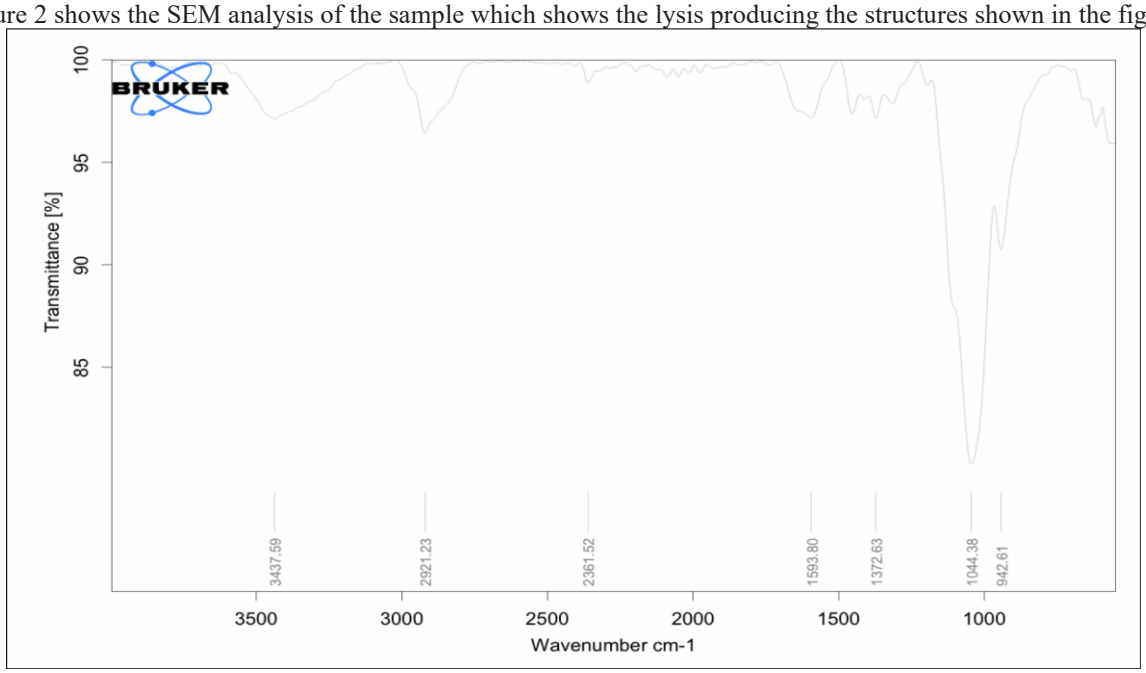

Figure3 explains the FTIR curve showing the elemental analysis of the groups present in the study sample.

The results of the antibacterial tests of synthesized magnesium oxide nanoparticles indicated a significant reduction in the number of Gram-positive and Gram-negative (8)bacteria. Consistent with the results of this study, previous studies reported the antibacterial activity of magnesium oxide nanoparticles alone or in combination with other antimicrobials agents](9). In line with the results of this study, the researchers suggested that magnesium oxide nanoparticles have a better antibacterial activity against Gram-positive bacteria compared to that against Gram-negative bacteria.

The precise mechanism of bactericidal action of magnesium oxide nanoparticles is still unclear; however, various mechanisms have been proposed(10). The mechanism of action of nanoparticles depends on their binding with bacterial surface as well as metabolism in the organism . Leung et al. reported that oxidative stress and lipid peroxidation do not play any role in cell death in Gram-negative bacteria (*Escherichia coli*) in the presence of magnesium oxide nanoparticles,

and cell membrane damage is the main cause of death in these cells(11). This difference can be due to different membrane structures of Gram-positive and Gram-negative bacteria. Gram-negative bacteria possess a complex outer membrane structure that act as a major barrier to the penetration of ROS into the cell. It has also been reported that increase in concentration and size of magnesium oxide nanoparticles improves antibacterial activity of these nanoparticles

CONCLUSION

Herbal mediated MgONPs using teak extract is showing positive results as antimicrobial activity is shown but it's activity is less as compared to the control.

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