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# A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF MAJUPHALLA INCORPORATED ZINC OXIDE WITH OZONE OIL AND ZINC OXIDE EUGENOL AS AN OBTURATING MATERIALS IN PRIMARY TEETH AGAINST ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS"- AN IN-VITRO STUDY.

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## ABSTRACT

### Introduction

Preservation of primary tooth with non-vital pulp is a major concern for a Pediatric dentist, for which the preferred treatment modality is to perform pulpectomy. The objective of pulpectomy is the complete removal of necrotic and irreversibly infected pulp of an affected tooth but, despite chemo mechanical preparation and copious irrigation of the canal, failure of pulp therapy occurs due to entrapped micro-organisms in canal space. Thus, for optimal success obturating material with antimicrobial properties is advocated for obturation.

### Aim

The study aimed to assess the antimicrobial efficacy of majuphalla incorporated zinc oxide with ozone oil and zinc oxide eugenol as an obturating material against *E. faecalis and Candida albicans*.

### Materials and Methods

The present study compared the antimicrobial efficacy of zinc oxide mixed with majuphalla powder and ozone oil as the study group and zinc oxide eugenol as a control group. Antimicrobial efficacy was evaluated against E. faecalis and Candida albicans. Statistical analysis was done using SPSS 21.0 version, IBM, Chicago, inter-group comparison of continuous variables was done using One-way ANOVA, and Intra-group comparison of continuous variables was done using repeated measures ANOVA.

### Results

Intergroup comparison at 24 hours ,15$^{th}$ day and 30$^{th}$ day revealed significant difference amongst both groups. Zinc oxide and majuphalla with ozone oil had significantly higher zone of inhibition as compared to ZOE group against E. faecalis and Candida albicans.

### Conclusion

Antimicrobial activity of Zinc oxide with majuphalla and ozone oil is more than zinc oxide eugenol for both *E. faecalis* and *Candida albicans.*

**Key words**

Pulpectomy, ZOE, Majuphalla, Ozone oil, antimicrobial efficacy

## INTRODUCTION

Dental caries is an infection that can cause the hard and soft tissues of a tooth to break down. In India, children have an average DMFT of 2, and caries prevalence increases with age, ranging from 51.9% to 63.1% in the 5-15 age group, according to the 2002-2003 National Oral Health survey. Despite technological advancements, dental caries remains a major public health issue, with intense pain being the most frequent complaint when the carious lesion reaches the pulp.[2].

When a child's baby teeth are affected by cavities or damage to the pulp tissue, it's important to perform pulp therapy to maintain the health and function of those teeth until they are replaced by permanent teeth. Pulpectomy is a treatment that involves using chemical and mechanical preparation, along with an intra-canal dressing that has antibacterial properties[3]. The success of this treatment depends on complete removal of infected bacteria. However, the complex anatomy of primary teeth root canals makes it difficult to clean properly using instrumentation and irrigations, due to the many accessory, curved, thin, and tortuous canals that are interconnected. This makes it challenging to eliminate microorganisms from inaccessible areas with intra-canal dressing[4].

The endodontic flora, which is the collection of microorganisms found in the root canal dentinal tubules and the apical portion of the root canal, is composed of a poly-microbial mixture of gram-positive and gram-negative bacteria[5,6]. In addition to this, bacterial biofilms have been identified in these areas.[7,8]

Endodontic failure is often associated with peri-radicular lesions. Research indicates that micro-organisms are present in the peri-radicular tissue in 35-100% of cases where teeth are filled with obturating materials. These micro-organisms are mostly facultative anaerobes and a few obligate anaerobes, which differ from the microbial flora found in primary infections that are mainly composed of gram-positive bacteria. Due to factors such as complex root morphology, physiological root resorption, close proximity of permanent successor teeth, and challenging diagnosis due to immature patients, pulp therapy of primary teeth remains a topic of controversy[9].

Therefore, this makes it important to use obturating and sealer materials which has high antibacterial property to act on microorganism of inaccessible areas in root canals of deciduous teeth.

Zinc oxide eugenol is a popular substance used to seal and fill root canals in primary teeth and can be applied topically to relieve pain caused by pulpitis and dental hypersensitivity[10]. Nonetheless, research has revealed that eugenol could lead to tooth irritation and be associated with various adverse effects, including deflection of the path of eruption of permanent successors, necrosis of bone and cementum, periapical tissue irritation, and tooth discoloration. Furthermore, it has limited antibacterial properties and a slower absorption rate than deciduous teeth's roots[11,12].

As a result of the need for materials with strong antimicrobial properties, a study was conducted using a mixture of zinc oxide powder, majuphalla powder, and ozone oil to determine its effectiveness against resistant microorganisms such as Enterococcus faecalis and Candida albicans.

Enterococcus faecalis, a type of Gram-positive cocci facultative anaerobes, is commonly found in cases of root canal failures or persistent root canal infections, especially in retreatment cases of apical periodontitis. This species has a tendency to attach to dentinal tubule collagen and survive within the tubules, which can cause periapical disease and recurrent endodontic infections. Unfortunately, eliminating E. faecalis through root canal medications is challenging once it has established itself in the dentinal tubules, resulting in a lower success rate of root canal therapy during obturation[13].

Research has revealed that Candida species account for the majority of fungal microbiota in endodontic infections, with Candida albicans being the most commonly identified. Interestingly, studies have found that 30-45% of healthy individuals have Candida albicans in their oral cavity, whereas its incidence in root

canals varies from 1-17%. In cases of chronic apical periodontitis, Candida albicans is the most frequently detected fungus, with a prevalence of 7-18%. Its capacity to create hyphae and deeply infiltrate dentin, combined with its thigmotrophic properties, render it a potent pathogen in persistent apical periodontitis[12].

It was necessary to search for new materials like zinc oxide powder with majuphalla powder and ozone oil to test their antimicrobial efficacy against Enterococcus Faecalis and Candida Albicans, due to the shortcomings of Zinc oxide eugenol.

## MATERIALS AND METHOD

In this research, 20 Muller Hinton (MH) agar plates were used and each group contained 10 samples (n=10). To create wells of 6mm diameter, agar was removed using the open end of a micropipette and then filled with test materials. For the test materials and ZOE paste, zinc oxide powder (0.2 mg) was mixed with majuphalla powder (0.2 mg) and 7 drops of ozone oil, and zinc oxide powder (0.2 mg) was mixed with 7 drops of eugenol on a sterile glass slab using a cement spatula. E Faecalis and C albicans, two standard strains of bacteria and fungi, were used. Both microorganisms were grown in MH Broth at 37°C for 24 hours and then seeded in MH agar. After injecting the test materials, the seeded agar was added directly over the plates to ensure uniform distribution of the bacterial dilution using the lawn technique. The plates were left at room temperature for 2 hours for prediffusion of material and then incubated at 37□ for 24 hours. After incubation, the diameters of the zone of inhibition around the plates were measured in millimetres on the 24th hour, 15th day and 30th day using an antibiotic zone scale.

## STATISTICAL ANALYSIS

Data were entered in Microsoft Excel spreadsheet and data were analyzed using statistical package of social sciences 25.0 software (SPSS Inc., Chicago, USA). The normality of data was checked using the Shapiro-Wilk test. Data reached normality. Thus, inferential statistics were performed using parametric tests of significance. Intra-group comparison of continuous variables was done using repeated measures of analysis of variance (ANOVA). The inter-group comparison of continuous variables was done using One-way ANOVA. For all statistical purposes, a p-value of $\leq 0.05$ was considered significant.

## RESULT

In first group, data demonstrated statistically significant difference ($p \leq 0.05$) in the mean zones of inhibition of the ZoE materials against the 2 micro-organisms (Candida albicans and E.faecalis). Zinc Oxide Eugenol showed the highest mean of zone of inhibition against E.faecalis compared to Candida albicans. Against E.faecalis the mean zone of inhibition at 24 hours - 11mm, after $15^{th}$ - day 8.2mm and after $30^{th}$ day - 4mm respectively. Against Candida albicans the mean zone of inhibition at 24 hours - 8.80mm, after $15^{th}$ day - 3.20mm and after $30^{th}$ day - 0.20mm respectively. (Table 1)

**Table 1- Comparison of the mean antimicrobial efficacy of Zinc oxide eugenol group against Candida albicans and E. faecalis (n-10)**

| Zinc oxide + Eugenol (Group 1) | n | ZONE OF INHIBITION (mm) | | | F value | p-value |
|---|---|---|---|---|---|---|
| | | 24 hours | 15th day | 30th day | | |
| | | Mean ± SD | Mean ± SD | Mean ± SD | | |
| E. faecalis | 5 | 11.00±0.70 | 8.20±1.48 | 4.00±2.00 | 21.402 | 0.007* |
| Candida albicans | 5 | 8.80±0.83 | 3.20±1.09 | 0.20±0.44 | 11.207 | 0.000* |
| p-value | | 0.002* | 0.000* | 0.003* | | |

*Statistically significant, SD- Standard Deviation, n-Number of subjects

Second group Zinc oxide eugenol, Majuphalla and ozone oil group also demonstrated a statistically significant difference ($p \leq 0.05$) in the mean zones of inhibition of against the 2 micro-organisms (Candida albicans and E.faecalis). This group showed the highest mean of zone of inhibition against E.faecalis compared to Candida albicans. Against E.faecalis the mean zone of inhibition at 24 hours -25.60mm, after 15th day -21.20 mm and after 30th day -17.80mm respectively. Against Candida albicans the mean zone of inhibition was 24 hours -18.20mm, after 15th day -15.80mm and after 30th day -10.40 mm respectively. (Table 2)

**Table 2- Comparison of the mean antimicrobial efficacy of Zinc oxide eugenol, Majuphalla, ozone oil group against Candida albicans and E. faecalis (n-10)**

| Zinc oxide + Majuphalla + Ozone oil (Group 2) | n | ZONE OF INHIBITION (mm) | | | F value | p-value |
|---|---|---|---|---|---|---|
| | | 24 hours | 15th day | 30th day | | |
| | | Mean ± SD | Mean ± SD | Mean ± SD | | |
| E. faecalis | 5 | 25.60±1.67 | 21.20±2.68 | 17.80±1.09 | 17.783 | 0.001* |
| Candida albicans | 5 | 18.20±0.83 | 15.80±1.48 | 10.40±0.89 | 70.412 | 0.000* |
| p-value | | 0.000* | 0.004* | 0.000* | | |

*Statistically significant, SD- Standard Deviation, n-Number of subjects

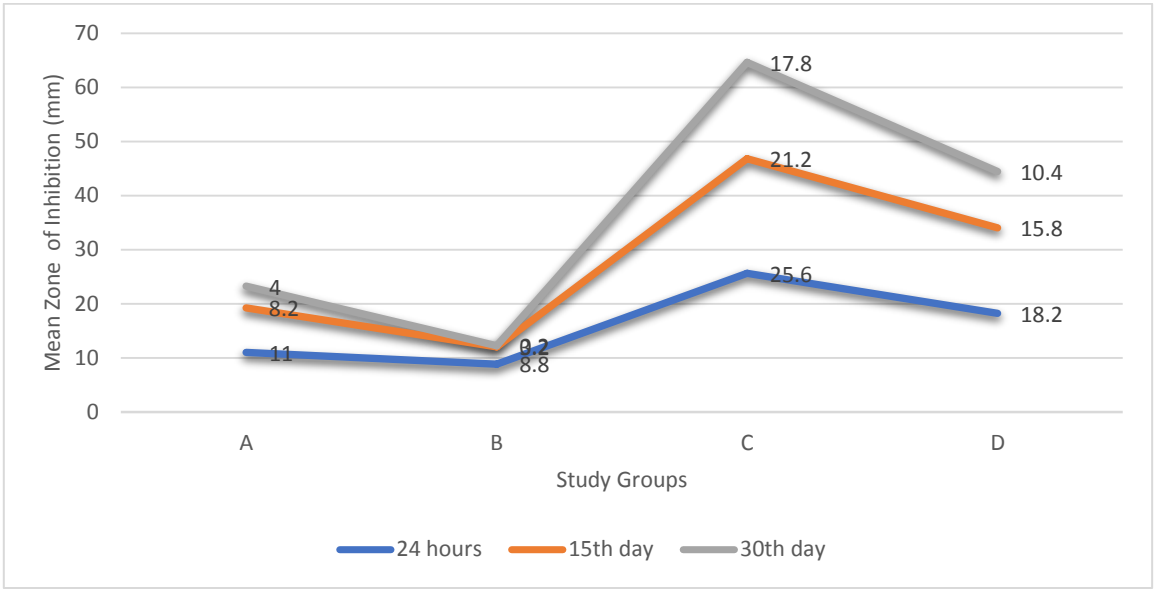
When we compared group 1 and group 2 against E.faecalis and Candida albicans, the difference was statistically significant. Group 2 showed the highest mean of zone of inhibition against E.faecalis and Candida albicans compared to group 1(ZoE). (Table 3, Figure 1)

**Table 3- Comparison of the mean antimicrobial efficacy of group 1 and group 2 against Candida albicans and E. faecalis (n-20)**

| | Groups | n | ZONE OF INHIBITION (mm) | | |
|---|---|---|---|---|---|
| | | | 24 hours | 15th day | 30th day |
| E. faecalis | Zincoxide + Eugenol | 5 | 11.00±0.70 | 8.20±1.48 | 4.00±2.00 |
| | Zinc oxide + Majuphalla + Ozone oil | 5 | 25.60±1.67 | 21.20±2.68 | 17.8±1.09 |
| | p-value | | 0.000* | 0.000* | 0.000* |
| Candida albicans | Zincoxide + Eugenol | 5 | 8.80±0.83 | 3.20±1.09 | 0.20±0.44 |
| | Zinc oxide + Majuphalla + Ozone oil | 5 | 18.20±0.83 | 15.80±1.48 | 10.40±0.89 |
| | p-value | | 0.000* | 0.000* | 0.000* |

*Statistically significant, SD- Standard Deviation, n-Number of subjects

**Figure 1- Comparison of the mean antimicrobial efficacy of group 1 and group 2 against Candida albicans and E. faecalis (n-20)**




(Group A- Zinc oxide eugenol against *E. faecalis*,Group B- Zinc oxide eugenol against *Candida albicans*, Group C- Zinc oxide + Majuphalla + Ozone oil against *E. faecalis*,Group D- Zinc oxide + Majuphalla + Ozone oil against *Candida albicans*)

## DISCUSSION

The key objective of pulp therapy for primary teeth is to preserve their function until they naturally fall out. This is crucial because primary teeth play a vital role in maintaining adequate space for permanent teeth to develop properly[15,16]. In cases where the pulp of a primary tooth is irreversibly infected or has died due to decay or trauma, a pulpectomy may be necessary. However, cleaning the intricate and winding root canal system can pose a challenge due to changes in morphology caused by root resorption. To ensure the success of pulp therapy, it is recommended to use root filling materials that have antimicrobial properties. By incorporating antimicrobial components into the obturating materials, the risk of recurrent infections can be reduced.[17,18].

Enterococcus faecalis is a microorganism that is commonly found in cases of persistent periapical infections and failed root canal treatments. It is a gram-positive, facultative anaerobic bacterium. Studies have shown that the prevalence of E. faecalis in primary endodontic infections ranges from 4-40%, and 67-77% in secondary infections.[19] This bacterium can form a biofilm that makes it up to 1000 times more resistant to phagocytosis, antibodies, and antimicrobials[20]. Furthermore, it can survive for extended periods without proper nutrition. E. faecalis is especially problematic because it can rapidly colonize dentinal tubules. As a result, it is used as a reference to assess the efficacy of intracanal medicaments' antimicrobial properties.[21,22]

It is typical to discover Candida albicans as the most predominant fungal species in polluted root canals due to its strong affinity for dentin, making it a dentinophilic microorganism[23]. However, in comparison to other microorganisms detected in infected dental pulp, Candida albicans is commonly found in smaller quantities[24]. Baumgartner et al conducted a study using the PCR method to determine the presence of Candida albicans in abscess and cellulite aspirates of endodontic origin, as well as polluted root canals. The results of the study revealed that Candida albicans was detected in only 5 out of 24 root canal samples.[25].

Zinc oxide eugenol was first discovered by Bonastre in 1837 and later utilized in dentistry by Chisholm in 1876[26]. In 1930, Sweet recommended the use of zinc oxide eugenol paste as a root canal filling material for primary teeth and since then, it has become the most commonly used material in dentistry for this purpose.[27] ZOE is the preferred material for filling deciduous teeth in the root canals and was the only material recommended by the American Academy of Pediatric Dentistry until 2008.[28] The antimicrobial properties of zinc oxide eugenol stem from its eugenol content, which induces a bactericidal hydrophobicity. Eugenol causes the lipids of the bacterial cell membrane and mitochondria to separate, dispersing the cell structures and making them more permeable[29]. This ultimately leads to extensive bacterial cell leakage and the escape of essential molecules and ions, resulting in cell death.[30].

According to a 2009 study conducted by Oyedemi et al., eugenol is a highly effective solution for combatting both gram-positive and gram-negative bacteria[31]. This is achieved by damaging the bacterial cell wall and causing protein leakage. Additionally, Hashieh's research has shown that eugenol found in zinc oxide-eugenol-based sealers possesses both anti-inflammatory and analgesic properties[32]. However, it's worth noting that the release of eugenol beyond the apex is limited and decreases over time. Despite its many benefits, eugenol does come with a few drawbacks. These include slow resorption, potential deflection of permanent tooth buds, tooth discoloration, and irritation to periapical tissues. Due to these issues, there is a need to explore alternative materials for root canal filling in primary teeth.

This study aimed to assess the efficacy of a combination of zinc oxide with majuphalla powder and ozone oil in comparison to zinc oxide eugenol. Given the potential adverse effects of eugenol, researchers sought to explore alternative antimicrobial agents. Notably, majuphalla powder and ozone oil have been shown to possess antimicrobial properties against microorganisms frequently linked to secondary infections, including E. faecalis and Candida albicans.

In 2014, SPChandra et al conducted a study on the effectiveness of a mixture of ozonated oil and zinc oxide as a primary teeth root filling material. Their findings showed that teeth filled with ozonated oil-zinc oxide had a success rate of 93.3%, which was better than zinc oxide eugenol's success rate of 63.3%. However, there was no significant statistical difference ($p = 0.408$) between the two groups. The study concluded that ozonated oil-ZnO was a viable alternative filling material for infected primary teeth, as it demonstrated good clinical and radiographic success at the 12-month follow-up.[33]

In 2020, Chandramohan Ravivarman and his colleagues conducted a study titled "Comparative Evaluation of pH and In Vitro Cytotoxicity of Zinc Oxide-Ozonated Eugenol and Conventional Zinc Oxide Eugenol as Endodontic Sealers". The study found that ozonating eugenol increased the pH level and cell survival rate compared to conventional ZOE..[34]

The galls of the Quercus infectoria (QI) are believed to have therapeutic benefits. They are said to have astringent, antitremorine, local anesthetic, antipyretic, anti-inflammatory, antibacterial, and antiviral properties. These extracts are commonly used in toothpaste and powder to treat oral diseases.[35]

This study aims to examine the antimicrobial effectiveness of combining majuphalla powder, zinc oxide, and ozone oil against root canal pathogens, which has not been previously researched. While the effectiveness of majuphalla alone against oral pathogens has been established.

In 2012, Basri et al. conducted a study to determine the efficacy of Quercus infectoria Olivier galls in combatting oral bacteria responsible for dental decay and periodontitis. The study evaluated methanol and acetone extracts against two Gram-positive bacteria (Streptococcus mutans ATCC 25175 and Streptococcus salivarius ATCC 13419) as well as two Gram-negative bacteria (Porphyromonas gingivalis ATCC 33277 and Fusobacterium nucleatum ATCC 25586). The results indicated that both extracts of Q. infectoria galls displayed comparable antibacterial activity against oral pathogens, suggesting that the galls could be an effective natural remedy for preventing oral pathogens.[36]

In 2012, Nagesh et al. conducted a study to evaluate the antibacterial effects and minimum inhibitory concentration (MIC) of gall extract derived from Quercus infectoria against Enterococcus faecalis. The results showed that an increase in the volume of gall extract led to a larger zone of inhibition, and the MIC of the extract against Enterococcus faecalis was determined to be 16.6 µl/ml. These findings suggest that the methanolic extract of galls from Quercus infectoria possesses antibacterial properties against Enterococcus faecalis.[37]

A 2020 study by Dsouza et al. tested the potential of Quercus infectoria Olivier galls in fighting oral bacteria that cause dental caries. The study found that S. aureus was the most susceptible among all tested bacteria, and the galls may be effective phototherapeutic agents for preventing biofilm formation by common oral pathogens.[38]

Quercus infectoria galls have been used in Unani medicine for centuries. They contain many bioactive compounds with potential pharmacological applications, such as anti-inflammatory, anti-tumor, and anti-microbial effects. Recent studies have shown their effectiveness in treating various conditions.[39]

## CONCLUSION

Based on the study findings, Zinc oxide and Majuphalla combined with ozone oil demonstrated the highest zone of inhibition against both Enterococcus faecalis and Candida albicans. Upon comparing the mean zones of inhibition between the two groups, it was noted that Zinc oxide and Majuphalla with ozone oil were more effective against Enterococcus faecalis than Candida albicans. Therefore, it can be concluded that Zinc oxide and Majuphalla with ozone oil exhibit superior antimicrobial efficacy against Enterococcus faecalis in comparison to Candida albicans..

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