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A COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF ZINC OXIDE WITH OZONE OIL, ZINC OXIDE WITH MTAP, ZINC OXIDE WITH HYALURONIC ACID AND ZINC OXIDE EUGENOL AS AN OBTURATING MATERIAL IN PRIMARY TEETH AGAINST ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS"- AN IN-VITRO STUDY

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ABSTRACT

Introduction: Preserving non-vital pulp in primary teeth is a challenge. Pulpectomy is the preferred treatment. However, pulp therapy can fail due to trapped microorganisms. To ensure success, obturating material with antimicrobial properties is recommended. Aim: Aim of the present study was to assess antimicrobial efficacy of ZINC OXIDE WITH OZONE OIL, ZINC OXIDE WITH MTAP, ZINC OXIDE WITH HYALURONIC ACID AND ZINC OXIDE EUGENOL as an obturating material against E. Faecalis and Candida albicans. METHODOLOGY: In order to measure the diameter of zone of inhibition 40 agar plates were prepared, out of which 20 were nutrient agar and 20 were potato dextrose agar. The number of samples for all four groups was 10 each (n=10). A well of 6mm diameter was made by removal of agar with open end of micropipette and was filled with the test materials. The standard strains of E. faecalis and C albicans were used in this study. Both microorganisms were cultivated in MH Broth at 37 ° C for 24 hours and then was seeded on their respective agar plates. For uniform distribution of bacterial dilution, the lawn technique was used. The plates were maintained at room temperature for 2 hours for rediffusion of material and then were incubated at 37°C for 24 hours. After incubation, the diameters of zone of inhibition around the plates were measured in millimeters with the help of Hi Antibiotic zone scale (Hi Media). Results: Ozone oil, Mtap and hyaluronic acid showed more antimicrobial efficacy than eugenol when combined with zinc oxide.

Keywords: pulpectomy, zinc oxide, eugenol, ozone oil, Mtap, hyaluronic acid

INTRODUCTION

In cases where the pulp tissue of a primary tooth is damaged due to decay or injury, pulp therapy may be necessary to maintain the tooth health and function until permanent teeth replace them. The preferred therapy is pulpectomy[1], which involves using a combination of chemicals and mechanical tools to clean the root canals and fill them with resorbable antibacterial materials[2]. However, due to the intricate anatomy of primary teeth, it can be challenging to clean all root canal areas, including small and interconnected ones. This can pose a risk of bacterial infection if microorganisms are not thoroughly removed during the procedure.[3]

Enterococcus faecalis are gram-positive cocci facultative anaerobes often found in cases of root canal failures or persistent root canal infections[4]. They tend to bind to dentinal tubule collagen, leading to periapical disease and recurrent endodontic infections. This species is difficult to eliminate using root canal medications, and its presence substantially reduces the success rate of root canal therapy.[4]

The majority of the fungal microbiota is made up of Candida species. Candida albicans is commonly detected in endodontic infections and in the root canal. Its presence in the oral cavity of healthy humans ranges from 30-45%[5]. In cases of chronic apical periodontitis, Candida albicans is the most commonly detected fungus, with a prevalence ranging from 7-18%, as indicated by numerous studies. Its ability to form hyphae and exhibit thigmotropism allows it to penetrate dentin deeply, enabling it to thrive in persistent apical periodontitis due to its virulence.[5]

Zinc Oxide Eugenol (ZOE) has been used as a filling material in primary teeth. It was discovered in 1837 and introduced in dentistry in 1876.[6] ZOE is composed of zinc oxide, white resin, zinc acetate, zinc stearate, eugenol, and olive oil. Although eugenol has anti-inflammatory and analgesic properties, it can irritate periapical tissues and cause necrosis of bone and cementum.[7]

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

Ozone is being considered for dental use due to its antibacterial properties and minimal toxicity to oral cells. It is seen as a potential alternative antiseptic agent in dentistry because of its high antimicrobial power without leading to drug resistance. Ozone gas is used for root canal disinfection and has been found to be slightly less harmful to cells compared to NaOCl (2.5%). Ozone in its aqueous form has shown minimal toxicity to oral cells in laboratory studies, making it a viable alternative to eugenol[8].

The use of antibiotics as a treatment for root canal infections dates back to 1951 when Grossman used a polyantibiotic paste. Over time, other broad-spectrum antibiotics have been used alone or in combination to address the complex infections found in the root canal system[9]. One such addition is the Triple Antibiotic Paste (TAP) introduced in 1992 by Sato et al.[10], consisting of ciprofloxacin, metronidazole, and minocycline. While the efficacy of this paste has been supported by laboratory studies, clinically, the inclusion of minocycline has been associated with complications such as tooth discoloration, antiangiogenic activity, and weakening of the dental structure. Recent research by Doneria D et al[11]. has suggested that clindamycin could be a suitable alternative to minocycline in TAP, showing promise in terms of antimicrobial efficacy, absence of dentin discoloration, and reduced cytotoxicity. Based on this insight, a combination of ciprofloxacin, ornidazole (which has a longer shelf life than metronidazole), and clindamycin was chosen for the present study.

Hyaluronic acid has anti-inflammatory, anti-edematous, and antibacterial effects for periodontal disease treatment. It also aids in faster healing of laser-induced wounds and stimulates cell-rich rearrangement of pulp tissue. Its high molecular weight in water increases its viscoelasticity, making it easy to use as an injectable scaffold[12]. Limited studies have been done on its use for the pulpectomy of primary teeth, so more research is needed to evaluate its effectiveness against root canal pathogens and as a replacement for eugenol.

Search for newer materials with effective antimicrobial properties has motivated us to conduct this study with the use of ZINC OXIDE WITH OZONE OIL, ZINC OXIDE WITH MTAP, and ZINC OXIDE WITH HYALURONIC ACID to determine its antibacterial efficacy against the most resistant microorganisms like E. faecalis and C. albicans.

MATERIALS AMD METHOD

The present study was done in, CSRD (Centre for Scientific Research and Development), PEOPLE'S UNIVERSITY. ATCC strain of E. faecalis and Candida Albicans was procured from ATCC nonprofitable organization. The research was initiated after obtaining approval from the Institutional Ethical Committee (IEC/2022/700/02).

PREPARATION OF NUTRIENT AGAR PLATE: A total of 20 nutrient agar plates were prepared. Using a weighing dish and balance mass 23 g of nutrient agar was measured and then transferred to a 2-L beaker. Using a 1000-mL graduated cylinder, 1000 mL of distilled water was measured and transferred to the beaker of nutrient agar. Solution was stirred until the solid got evenly distributed and the solution was placed in an autoclave for 15 minutes at 121 °C at 15 lbs pressure. The solution was allowed to cool to 50–55 °C and poured into sterilized culture dishes. Plates were allowed to set for 24 hours before seeding of the microbe on the agar plate.

PREPARATION OF POTATO DEXTROSE AGAR PLATE: Potato dextrose agar (PDA) is a common microbiological growth media that is made from potato infusion and dextrose is used for the growth of fungi. To prepare PDA, 39 grams of PDA was mixed with 1000 ml of distilled water, which was measured using a graduated cylinder. The mixture was then boiled until the medium was completely dissolved and sterilized by autoclaving at 15 lbs pressure at 121°C for 15 minutes. After sterilization, the solution was allowed to cool to 50–55 °C, mixed well by stirring, and poured into sterilized culture dishes. The plates were allowed to set for 24 hours before seeding of the microbe on the agar plate.

CULTIVATION OF MICROBIAL CULTURE: The colonies of E. faecalis and candida albicans were touched using a sterile wore loop. The organisms were transferred separately into a sterile test tube prefilled with sterile brain heart infusion broth of 1.5 mL. The test tube was incubated at 37°C for 24 hours, to cultivate a moderately turbid microbial suspension. The microbial suspension density is standardized, comparing its density with 0.5 McFarland units of the barium sulfate standard. The standard is equivalent to 108 colony-forming units per millilitre. The agar culture medium was used for inoculation. A sterile cotton swab dipped in the microbial suspension was taken, removing the excess by rolling the swab to the test tube side. The entire surface of the culture medium plate was streaked with the cotton swab in three directions evenly.

INVESTIGATION OF ANTIBACTERIAL ACTIVITY : To investigate the antimicrobial activity of Zinc Oxide with Ozone Oil, Zinc Oxide with Mtap, Zinc Oxide with Hyaluronic Acid, And Zinc Oxide Eugenol, the agar diffusion method is used. Four wells 6 mm in diameter were made by the end of the micropipette at the same distance and filled with test materials. The mixing was done as per the manufacturer's instructions for zinc oxide eugenol, zinc oxide ozone oil was mixed in ratio of 2:1, zinc oxide with mtap and ozone oil mixed in a ratio of 1:1:1(ornidazole:ciprofloxacin:clindamycin::1:1:1), zinc oxide with hyaluronic acid in a ratio of 2:1 was mixed. For prediffusion, the plates were kept for 2 hours at room temperature. The incubation was done at 37°C for 24 hours under suitable gaseous conditions. The diameter of the zone of inhibition was measured in millimetres around the plate after incubation.

RESULT

Data was analysed using SPSS 25.0 software. Normality was confirmed with the Shapiro-Wilk test, and parametric tests were used for inferential statistics. Intra-group comparison used Repeated measures ANOVA,

while inter-group comparison used one-way ANOVA and t-tests. Analysis considered a p-value of ≤0.05 as significant.

In this study, we analysed the effectiveness of Zinc Oxide Eugenol (ZoE) materials against Candida albicans and E. faecalis. ZoE showed higher inhibition against E. faecalis compared to Candida albicans. The differences were statistically significant at 8 hours, 24 hours and 15 days, but not at 30 days.(Table 1, Graph 1)

The average zones of inhibition were measured for zinc oxide and ozone oil against Candida albicans and E.faecalis. Group II had the highest inhibition against E.faecalis compared to Candida albicans. The intra-group difference was statistically significant (p=0.000) for both microorganisms. When comparing inter-groups, highly significant differences were found at all time points (p<0.001).(Table 2 , Graph 2)

The mean zones of inhibition of zinc oxide and MTAP against Candida albicans and E. faecalis were calculated. Group III had the highest mean zone of inhibition against E. faecalis compared to Candida albicans. The difference between the two groups was statistically highly significant at 8 hours, 24 hours, and after 15 and 30 days. (Table 3, Graph 3)

When comparing the mean zones of inhibition of zinc oxide and hyaluronic acid against E. faecalis and Candida albicans, the group showed a higher mean zone of inhibition against E. faecalis. The differences within each group were statistically significant (p=0.000), and the difference between the two groups was highly significant at all time points (p=0.000).(Table 4, Graph 4)

On comparing the mean inhibition zone among all four groups against E. faecalis, zinc oxide and MTAP (group III) showed the highest mean zone of inhibition (27mm) after 8 hours, followed by group II (26.80mm), group III (25.80mm), and group I with the least mean inhibition zone (19.80mm). Group II consistently exhibited the highest mean zone of inhibition after 24 hours and 30 days, with statistically highly significant differences between the groups at each interval (p < 0.000).(Table 5, Graph 5)

In comparing the mean inhibition zone of all four groups against Candida albicans, zinc oxide and ozone oil (group II) showed the highest mean zone of inhibition (22.80mm) at 8 hours, 24 hours, and 30 days. The difference between the groups at each interval was statistically highly significant (p 0.000).(Table 6, Graph 6)

Table 1- Comparison of the mean antimicrobial efficacy of Zinc oxide + eugenol (Group I) against Candida albicans and E. faecalis (n=10)

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

Figure 1- Comparison of the mean antimicrobial efficacy of Zinc oxide + eugenol (Group I) against Candida albicans and E. faecalis

Zinc oxide + Eugenol (Group 1)	n	ZONE OF INHIBITION(mm)				F value	p-value
		8 hours	24 hours	15th day	30th day		
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
E. faecalis	5	19.80±0.83	18.00+1.22	14.20±2.04	10.80±0.83	41.690	0.001*
Candida albicans	5	16.40±0.89	13.20+1.64	11.40±0.89	10.40±0.54	25.876	0.003*
p-value	10	0.000*	0.001*	0.023*	0.397		

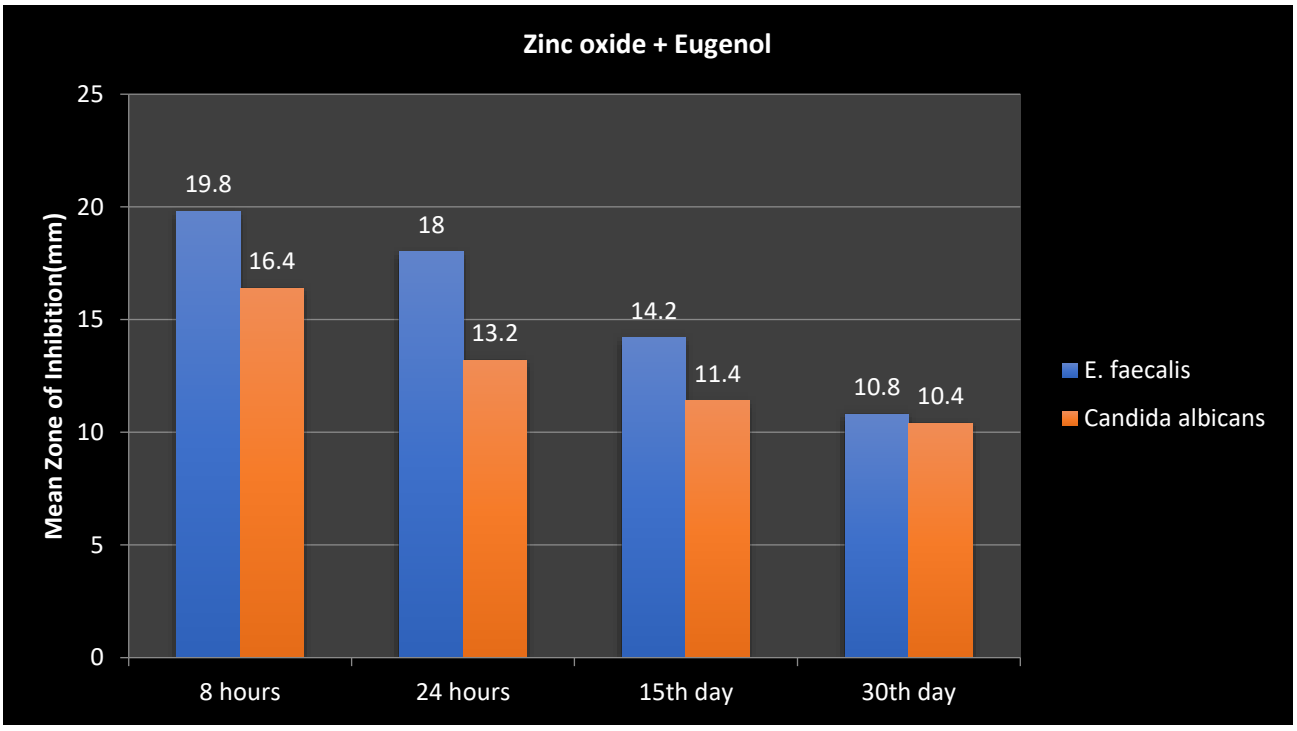


Table 2- Comparison of the mean antimicrobial efficacy of Zinc oxide + Ozone oil (Group II) against Candida albicans and E. faecalis (n=10)

	n	ZONE OF INHIBITION(mm)

Zinc oxide + OZONE OIL (Group II)		**8 hours**	**24 hours**	**15th day**	**30th day**	**F value**	**p-value**
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**		
E. faecalis	**5**	**26.80+0.44**	**25.60+0.89**	**20.40+0.54**	**18.20+1.30**	**119.41**	**0.000***
Candida albicans	**5**	**22.80+0.83**	**20.60+0.54**	**14.00+1.41**	**13.00+1.87**	**102.42**	**0.000***
		0.000*	**0.000***	**0.000***	**0.001***		

Figure 2- Comparison of the mean antimicrobial efficacy of Zinc oxide + Ozone oil (Group II) against Candida albicans and E. faecalis

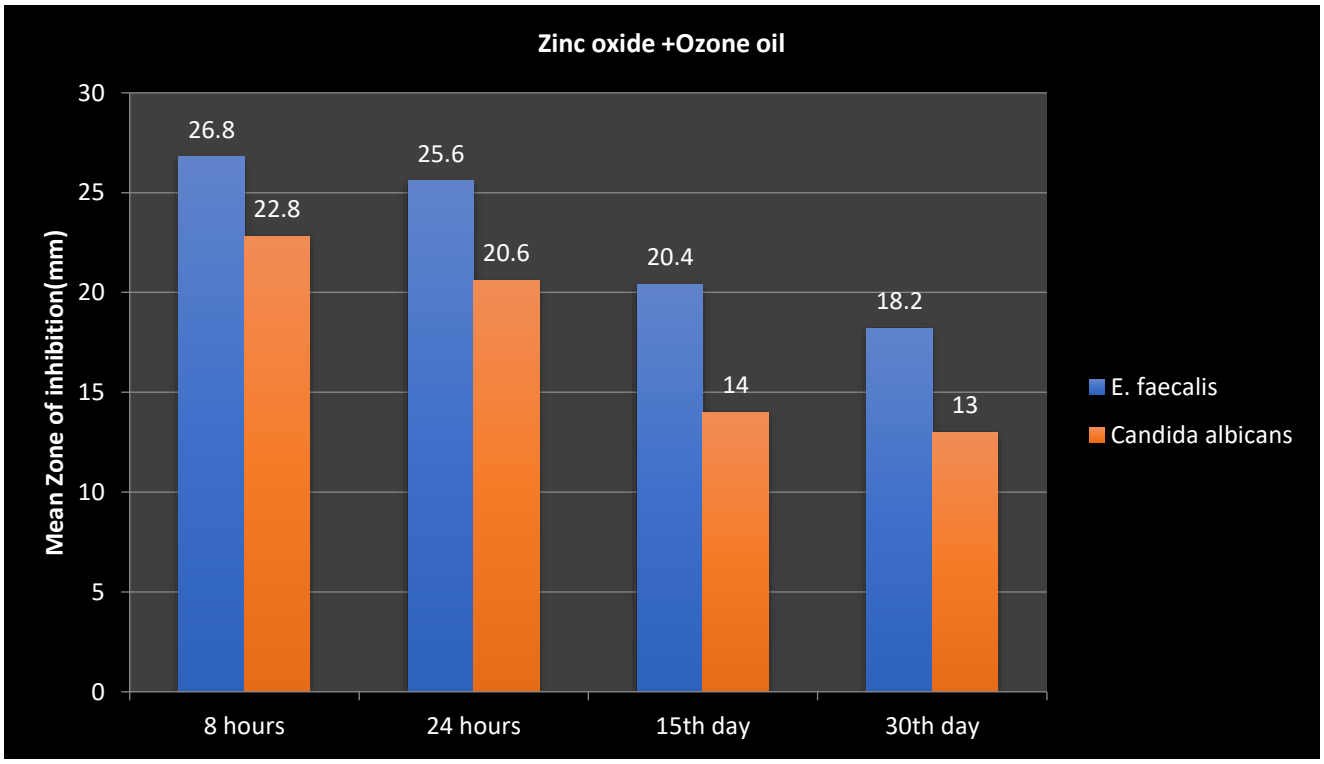


Table 3- Comparison of the mean antimicrobial efficacy of Zinc oxide + MTAP (Group III) against Candida albicans and E. faecalis (n=10)

Zinc oxide + MTAP (Group III)	n	ZONE OF INHIBITION(mm)				F value	p-value
		8 hours	24 hours	15th day	30th day		
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
E. faecalis	5	27.00+1.41	25.80+0.83	20.40+0.54	14.60+0.89	138.21	0.000*
Candida albicans	5	21.40+0.54	19.60+0.54	14.80+0.44	10.40+0.54	398.43	0.000*
		0.000*	0.000*	0.000*	0.000*		

Figure 3- Comparison of the mean antimicrobial efficacy of Zinc oxide + MTAP (Group III) against Candida albicans and E. faecalis (n=10)

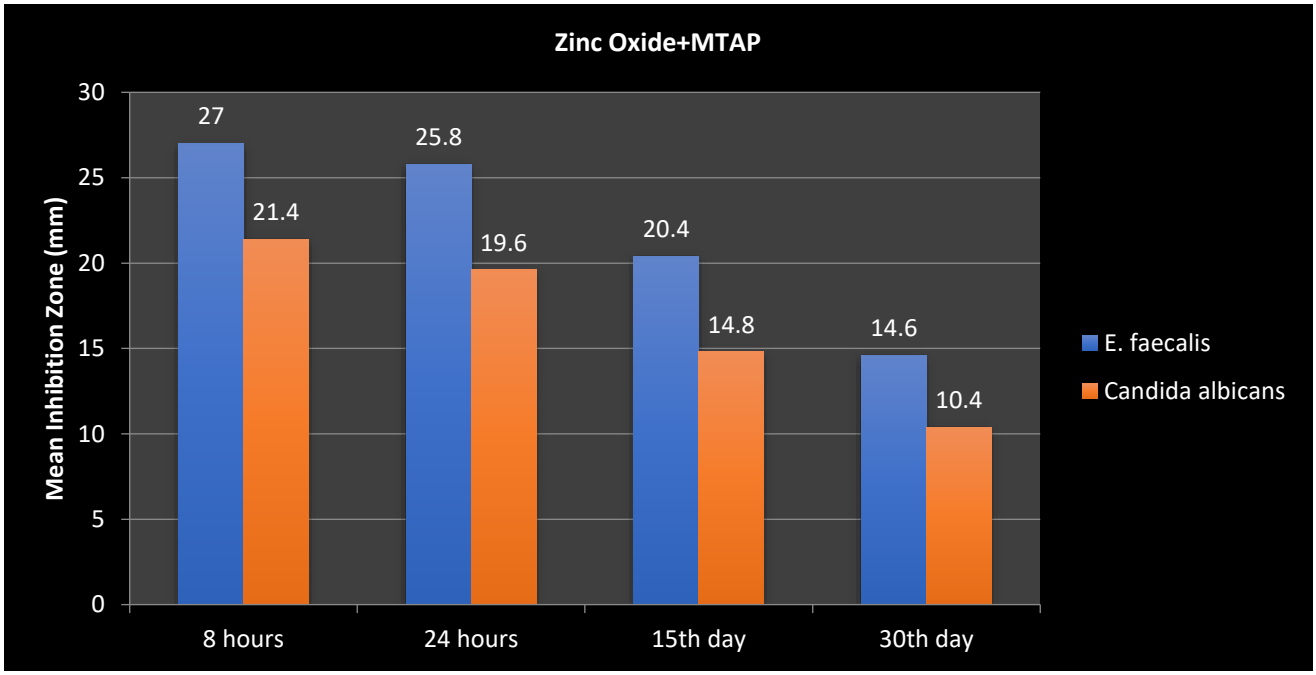


Table 4- Comparison of the mean antimicrobial efficacy of Zinc oxide +Hayluronic Acid (Group IV) against Candida albicans and E. faecalis (n=10)

Zinc oxide + n HAYLURONIC ACID (Group IV)		ZONE OF INHIBITION(mm)				F value	p-value
		8 hours	24 hours	15th day	30th day		
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
E. faecalis	5	25.80+0.44	24.80+0.44	18.20+0.44	13.00+0.70	514.81	0.000*
Candida albicans	5	22.60+0.54	20.40+0.54	13.40+0.54	10.40+0.54	639.22	0.000*
		0.000*	0.000*	0.000*	0.000*		

Figure 4- Comparison of the mean antimicrobial efficacy of Zinc oxide +Hayluronic Acid (Group IV) against Candida albicans and E. faecalis

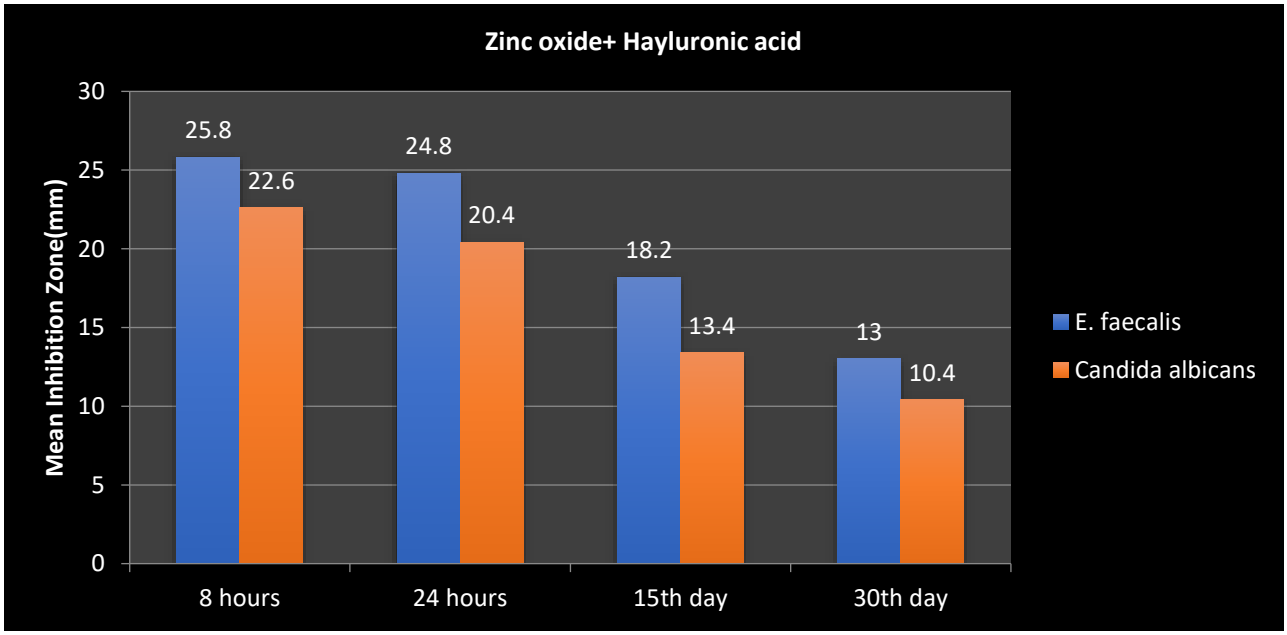


Table 5- Comparison of the mean antimicrobial efficacy of four groups at different interval against E. faecalis (n-20)

	Groups	n	ZONE OF INHIBITION(mm)			
			8 hours	24 hours	15th day	30th day
E. faecalis	Zincoxide + Eugenol	5	19.80±0.83	18.00+1.22	14.20±2.04	10.80±0.83
	Zinc oxide + Ozone oil	5	26.80+0.44	25.60+0.89	20.40+0.54	18.20+1.30
	Zinc oxide + MTAP	5	27.00+1.41	25.80+0.83	20.40+0.54	14.60+0.89
	Zinc oxide + Hayluronic acid	5	25.80+0.44	24.80+0.44	18.20+0.44	13.00+0.70
	F-value		74.903	86.729	34.187	52.523
	p-value		0.000*	0.000*	0.000*	0.000*

Figure 5- Comparison of the mean antimicrobial efficacy of four groups at different interval against E. faecalis (n-20)

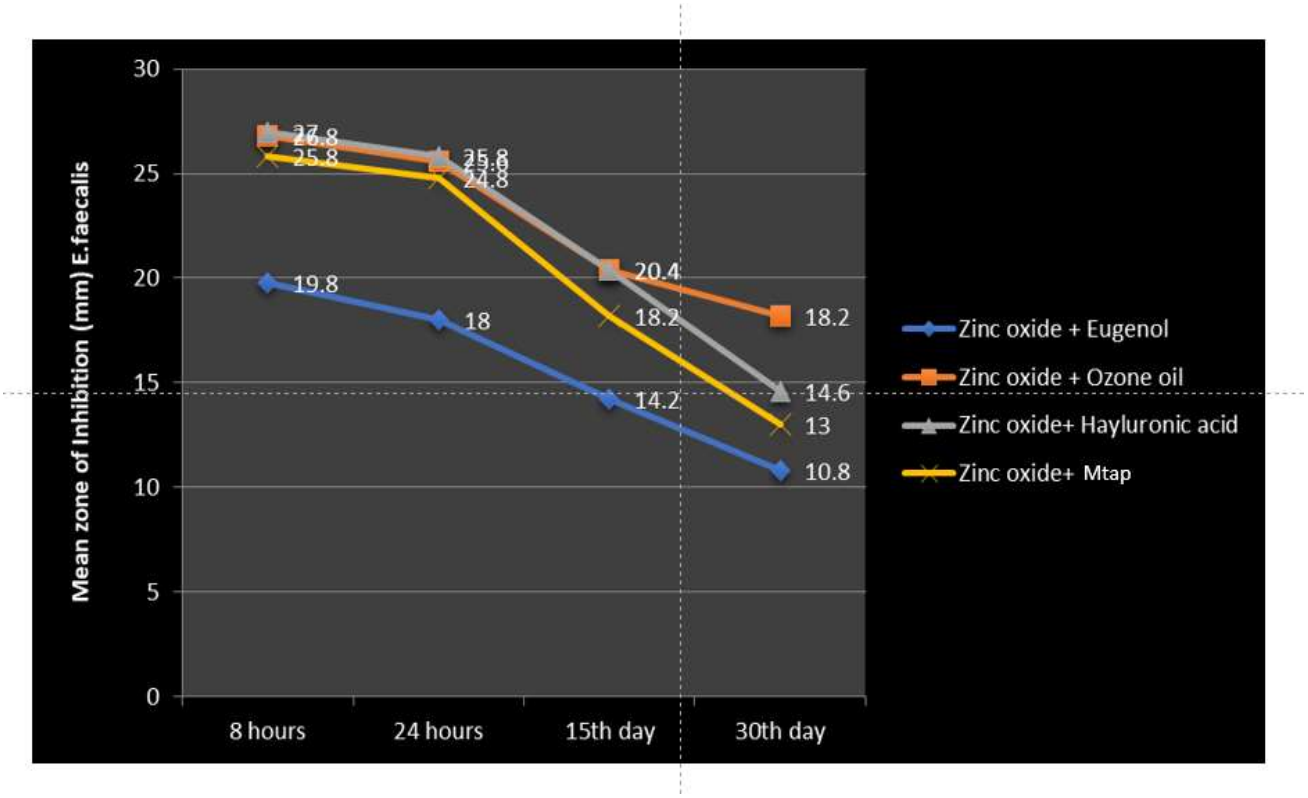
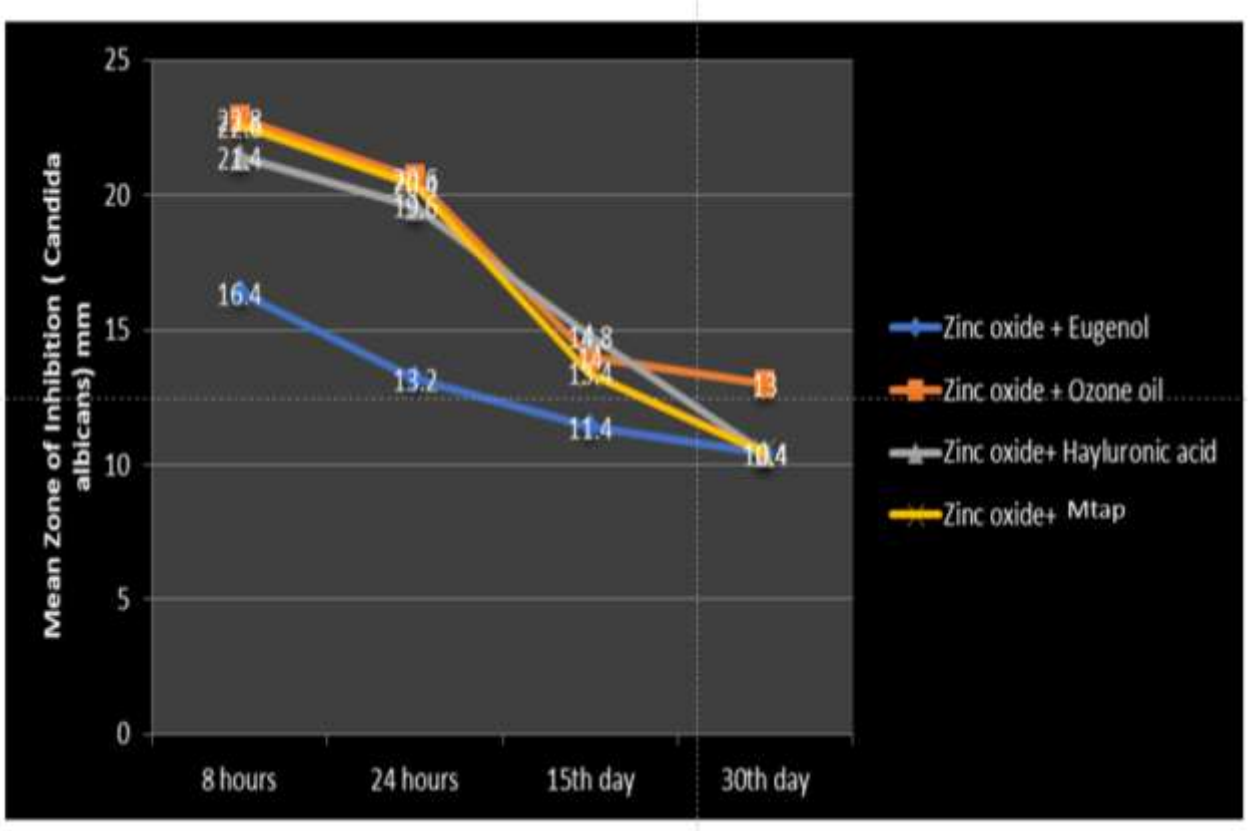


Table 6- Comparison of the mean antimicrobial efficacy of four groups at different interval against Candida albicans (n=20)

	Groups	n	ZONE OF INHIBITION(mm)			
			8 hours	24 hours	15th day	30th day
Candida albicans	Zincoxide + Eugenol	5	16.40±0.89	13.20+1.64	11.40±0.89	10.40±0.54
	Zinc oxide + Ozone oil	5	22.80+0.83	20.60+0.54	14.00+1.41	13.00+1.87
	Zinc oxide + MTAP	5	21.40+0.54	19.60+0.54	14.80+0.44	10.40+0.54
	Zinc oxide + Hayluronic acid	5	22.60+0.54	20.40+0.54	13.40+0.54	10.40+0.54
	F-value		85.587	69.093	12.768	7.682
	p-value		0.000	0.000	0.000	0.002

Figure 6- Comparison of the mean antimicrobial efficacy of four groups at different interval against Candida albicans



DISCUSSION

Success rates of zinc oxide eugenol cement after obturation in primary teeth were reported by various authors as follows; 82.3% by Barr et al[13], 82.5% by Chandra S P [14] and 86.1% by Coll et al[15]. A more recent retrospective evaluation by Bahrololoomi and Zamaninejad[16] was done in 2015 to find out the success rate of ZOE as an obturating material for a mean follow-up period of 24 months. They reported that two-visit pulpectomy of primary molars using ZOE for root filling is one of the most reliable and successful treatments for necrotic primary teeth.

Despite ZOE being the most accepted and efficient obturating material, because of the shortcomings of eugenol, the search for its replacement was the rationale of the present study. Eugenol was replaced with ozone oil, modified triple antibiotic paste and hyaluronic acid to and their antimicrobial properties were checked against E.faecalis and C. albicans

Ozone has been considered as promising alternative because of its interesting biological characteristics viz: bactericidal action, debriding effect, angiogenesis stimulation capacity and high oxidising power (Guinesi et al. 2011)[17]. It is cur- rently being discussed as a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power without the development of drug resistance. Ozone gas is already being used for root canal disinfection and proved to be slightly less cytotoxic than NaOCI (2.5%). Ozone in aqueous form showed essentially no toxicity to oral cells in vitro (Huth et al. 2006)[18].

When the mean zones of inhibition for zinc oxide and ozone oil materials were calculated against the two micro-organisms, Candida albicans and E.faecalis, the highest mean zone of inhibition was found against E.faecalis compared to C.albicans. The mean zone of inhibition against E.faecalis was 26.80 mm at 8 hours, 25.60 mm at 24 hours, 20.40 mm at 15 days, and 18.20 mm at 30 days and against Candida albicans, the mean zone of inhibition was 22.80mm at 8 hours, 20.60 mm at 24 hours, 14 mm at 15 days and 13 mm at 30 days, respectively. Intra-group difference was also statistically significant (p-0.000) for both E.fecalis and Candidda albicans. Inter-group difference, was also statistically significant (p-0.00) at 8 hours, 24 hours, and after 15 days and 30 days. The antimicrobial effect of of ozone oil can be justified by the study done by Estrela et al.[19] in which they used ozonated oils to disinfect the root canal systems and to remove the necrotic debris by virtue of ozone's bactericidal and effervescent properties. Ozone oil when used as an irrigant, the canal sterilization is quicker and more efficient than conventional irrigation by the combination of sodium hypochlorite and sodium peroxide. Similarly Chandra S P [14] conducted a study to assess the success rate of the ozonated sesame oil and zinc oxide mixture as the primary teeth root-filling material. They concluded that after 12 months of follow-up, ozonated sesame oil–ZnO demonstrated good clinical and radiographic success, and can be considered as an alternative obturating material in infected primary teeth The results of present study are also in accordance with the study done by Pratyusha & Selvan[20] in 2017 who compared the antibacterial efficacy of ozonated olive oil, cold pressed neem oil, 2% chlorohexidine and calcium hydroxide against Enterococcus faecalis and Candida albicans. The results of their study showed maximum inhibitory effect against Enterococcus faecalis by ozonated olive oil. Also Kishore et al[21]. in 2016 investigated the antimicrobial efficacy of calcium hydroxide, ozonated sesame oil and their combination as intra canal medicament, they concluded that ozonized sesame oil was most effective for longer duration when compared to other groups.

In this present study, a combination of ciprofloxacin, ornidazole and clindamycin was mixed with zinc oxide powder along with propylene glycol as a replacement of eugenol. The drug combination was chosen based on their spectrum of action against endodontic pathogens and when the mean zones of inhibition of Zinc oxide and MTAP was calculated, this group exhibited the highest mean zone of inhibition against E.faecalis in comparison to Candida albicans. Against E.faecalis, the mean zone of inhibition was 27 mm at 8 hours, 25.80 mm after 24 hours, 20.40 mm after 15 days, and 14.60 mm after 30 days. The intra-group difference was also statistically significant (p-0.000). Against Candida albicans, the mean zone of inhibition was 21.40 mm at 8 hours, 19.60 mm after 24 hours, 14.80 mm after 15 days and 10.40 mm after 30 days. The intra-group difference was also statistically significant (p-0.000). on comparing groups, the difference between the two

groups was statistically highly significant (p 0.00) at 8 hours, 24 hours, and not significant after 15th and 30th days, which is in accordance to study done by Hoshino et al[22] , agar diffusion test was used to evaluate the antimicrobial activities of the medicaments, the TAP was more effective than zinc oxide eugenol in a seven-day period and it eliminated EF from the root canal system and Sato et al[22] examined the antibacterial effects of a TAP on candida albicans within the root canal system and concluded that it can remove all target bacteria from the root canal within 48 hours. Similar results were found in the study done by Shaik J et al [23] in 2020 where they assessed the in vitro antimicrobial efficacy of different medicaments with two different vehicles against E. faecalis and Candida albicans (C. albicans), and concluded that the endodontic infections are polymicrobial in origin, the combined local drug delivery of antimicrobial agents such as ciprofloxacin, ornidazole, doxycycline with an efficient drug carrier (propylene glycol) is efficient to combat the microbial load. Healing properties of tap can be justified by the study done by Raison Bose who compared TAP, calcium hydroxide, and formocresol as intracanal medicaments in non-vital young permanent tooth. The triple antibiotic group showed the highest percentage increase in the dentin wall thickness compared with the other two groups. TAP can help promote functional development of the pulp–dentin complex. Reynolds et al.[24] achieved revascularization of a necrotic bicuspid using TAP. TAP contains both bactericidal (oetronidazole, ciprofloxacin) and bacteriostatic (clindamycin) agents to allow for successful revascularization.

The use of a hyaluronic acid to cover a dentin defect, stimulated a cell-rich rearrangement of pulp tissue with few inflammatory cells, as well as being an acceptable and biocompatible scaffold for regenerating the dental pulp.[25] Because of its high molecular weight when dissolved in water, hyaluronic acid's viscoelasticity increases, making it simpler to use as an injectable scaffold [26] Moreover, advantageous characteristics are related to the hyaluronic acid scaffolds as bioactivity, biocompatibility, and biodegradability, in addition to serving as a reservoir for growth factors[27]. As for zinc oxide and hyaluronic acid combination there is no literature data available yet. Keeping all the merits of hyaluronic acid in mind, zinc oxide powder was mixed with hyaluronic acid gel in the present study to check for its antimicrobial efficacy against E. faecalis and candida albicans. When the mean zones of inhibition against two microorganisms (Candida albicans and E. faecalis) were calculated, 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 was 25.80 mm after 8 hours, 24.80 mm after 24 hours, 18.20 mm after 15 days, and 13 mm after 30 days. The intragroup difference was statistically significant (p-0.000). Against Candida albicans, the mean zone of inhibition was 22.60 mm after 8 hours, 20.40 mm after 24 hours, 13.40 mm after 15 days, and 10.40 mm after 30 days. The intra-group difference was also statistically significant (p-0.000). On comparing the two groups, the difference between them was statistically highly significant (p-0.000) at 8 hours, 24 hours, 15 days, and 30 days.

On comparing the mean inhibition of all four groups against E. faecalis, it was observed that Zinc oxide and MTAP (group III) showed the highest mean zone of inhibition (27mm) after 8 hours followed by group II (26.80mm). Group III (25.80mm) and group I showed the least mean inhibition zone (19.80mm). After 24 hours, group II showed the highest mean zone of inhibition and group I showed the least mean zone of inhibition. After 30 days, group II showed the highest mean zone of inhibition, and the difference between the groups at each interval was statistically highly significant (p 0.000). On comparing the mean inhibition zone of all four groups against Candida albicans, Zinc oxide and ozone oil (group II) showed the highest mean zone of inhibition (22.80mm) at 8 hours, followed by group III (22.60mm), group II (21.40mm), and group I showing the least mean inhibition zone (16.40mm). After 24 hours, group II showed the highest mean zone of inhibition, and group I showed the least mean zone of inhibition. After 30 days, group II showed the highest mean zone of inhibition, and the difference between the groups at each interval was statistically highly significant (p-0.000).

The rationale for performing this in vitro study was to provide information that zinc oxide with ozone oil and zinc oxide with Mtap has the highest antimicrobial efficacy followed by zinc oxide with hyaluronic acid and

zinc oxide with eugenol against Enterococcus faecalis and Candida albicans. However, it should be taken into consideration that data presented here is related to an in-vitro condition and in-vivo conditions such as presence of saliva, presence of other micro-organisms, presence of dentine and the serum which might modify the antimicrobial efficacy of the obturating material. Therefore, further long-term in vivo studies are needed to evaluate the antimicrobial properties of the obturating materials used in the present study.

CONCLUSION

All materials developed zones of microbial growth inhibition against Enterococcus faecalis and candida albicans. However, against E. faecalis, it was observed that (zinc oxide and MTAP) group III showed the highest mean zone of inhibition followed by (zinc oxide with ozone oil) group II, (zinc oxide with hyaluronic acid) group III and (zinc oxide eugenol) group I showed the least mean inhibition zone and the difference between the groups at each interval was statistically significant. On comparing the mean inhibition zone of all four groups against Candida albicans, (zinc oxide and ozone oil) group II showed the highest mean zone of inhibition, followed by (zinc oxide with Mtap) group III, (zinc oxide with hyaluronic acid) group IV, and (zinc oxide with eugenol) group I showed the least mean inhibition zone. The difference between the groups at each interval was statistically significant. Thus, it can be concluded that zone oil, Mtap and hyaluronic acid have more antimicrobial efficacy than eugenol when combined with zinc oxide and can be used in place or as a substitute for eugenol to overcome its limitation.

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