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Research Paper

In Vitro Antimicrobial Efficacy of Zinc Oxide with Peppermint Oil in Comparison to Zinc Oxide Eugenol against Four Root Canal Microorganisms

Dr.Nilima Thosar[1], Dr.Manoj Chandak[2], Dr.Manohar Bhat[3], Dr.Silpi Basak[4]
[1]*Professor, Dept. Of Pedodontics & Preventive Dentistry, Sharad Pawar Dental College, Datta Meghe Institute Of Medical Sciences (Deemed University), Wardha-442004, Maharashtra, India.*
[2]*Professor and Head, Dept. Of Conservative Dentistry And Endodontics, Sharad Pawar Dental College, Datta Meghe Institute Of Medical Sciences (Deemed University), Wardha-442004, Maharashtra, India.*
[3]*Professor and Head, Dept. Of Pedodontics & Preventive Dentistry, Principal, Jaipur Dental College, Jaipur.*
[4]*Professor, Dept. Of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute Of Medical Sciences (Deemed University), Wardha-442004, Maharashtra, India.*

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ABSTRACT: *Antimicrobial efficacy of zinc oxide with peppermint oil and zinc oxide with eugenol oil was assessed by using agar diffusion method. Muller Hincton agar was punched at equidistant points into which test materials were filled with six times repetitions for each microorganism and kept in incubator at 37°C for 24 hours. After 24 hours, zone of inhibition in millimeter was measured with Antibiotic sensitivity scale. Statistical analysis was done using ANOVA and Tukey's post-hoc comparison test. P-value < 0.05 was considered for level of significance. Results: For zinc oxide mixed with peppermint oil (ZOP), zones of inhibition were highest against E.Coli, followed by Staph. Aureus and equal zones of inhibition against E.Faecalis and P.Aeruginosa. Zones of bacterial growth inhibition in decreasing order for ZOE paste were against E.Coli, followed by Staph. Aureus, E.Faecalis and P.Aeruginosa respectively. Difference was significant statistically in both the pastes i.e; ZOP paste and ZOE paste (p-value: 0.0001, p<0.05).Conclusion: Antibacterial activity shown by ZOP paste was highest against E.coli and Staph.aureus as compared to that of ZOE paste; while antibacterial activity of ZOE paste against E.faecalis and P.aeruginosa was highest as compared to ZOP paste.*

Keywords: *Antimicrobial efficacy, Zinc oxide peppermint oil, Zinc oxide eugenol*

I. INTRODUCTION

Pulp therapy in deciduous teeth is challenging as the root canals have complex anatomy due to presence of numerous accessory and lateral canals which makes it difficult to remove infecting bacteria completely with instrumentation and irrigation. Various materials have been tried till date in dentistry as intracanal antimicrobials [1].

Zinc oxide eugenol is the widely used root canal filling material in the field of Pediatric Dentistry. Its antimicrobial effect is due to its eugenol content. But the side effects associated with zinc oxide eugenol paste as mentioned in literature includes its slow resorption as compared to normal physiologic root resorption of deciduous tooth; due to its presence periapically, it may deflect the path of eruption of permanent successor; it may cause necrosis of bone and cementum [2]; periapical tissue irritation; it may cause tooth discoloration also.

Peppermint oil is derived from the leaves of the peppermint plant or *Mentha pipertita*, a hybrid of the water mint and spearmint plants, and *M. arvensis* var. *piperascensa*, a plant from the Labiatae family [3]. It is a colorless, pale yellow or pale greenish-yellow liquid which has a characteristic odor and taste followed by a sensation of cold [4]. Menthol is the main constituent of peppermint oil which is used in the toothpastes, lozenges, pain balms, cold balms, Dabur Pudin Hara, cough drops and Vicks Vaporub etc [3]. Other constituents found in peppermint oil are menthyl acetate, 1,8-cineole, limonene, beta-pinene, and beta-caryophyllene [5]. It is an important medicinal plant which is widely used in several indigenous systems of medicine for various therapeutic benefits viz. analgesic, anesthetic, antiseptic, astringent, carminative, decongestant, expectorant,

*Corresponding Author: Dr.Nilima Thosar[1]

[1]*Professor, Dept. Of Pedodontics & Preventive Dentistry, Sharad Pawar Dental College, Datta Meghe Institute Of Medical Sciences (Deemed University), Wardha-442004, Maharashtra, India.*

nervine, stimulant, stomachic, inflammatory diseases, ulcer and stomach problems [3]. Studies have shown that peppermint oil exhibits antiviral, antimicrobial, antifungal, antioxidant, analgesic, radioprotective, and antiedema properties [6].

Uses of peppermint oil in medical field are wide as found in literature. But in dentistry, it is only used to prevent bad breath by using it in the form of mouthwash. In the present study antimicrobial efficacy of zinc oxide powder mixed peppermint oil was carried out which was compared with the routinely used zinc oxide eugenol cement.

II. MATRIALS AND METHODS

Present *in vitro* study was approved by institutional ethical committee. In the present study, zinc oxide powder was mixed with peppermint oil (Aromatantra, Mumbai). It was compared with zinc oxide eugenol paste (Prime Dental Products Pvt. Ltd., Thane).

The powder liquid ratio which was used for both the materials was as per the formula given by Tchaou et.al, 1995. One scoop of zinc oxide powder equivalent to 0.2 g was taken into which 7 drops of oil equivalent to 0.07 cc was used. Powder was mixed with oil on a dry sterile glass slab using cement spatula [7].

Microorganisms used for the present study were obtained from Microbiologics, USA, Hi Media, Pvt., Ltd.and was procured by the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. Microbial strains studied were Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Enterococcus faecalis (ATCC 29212) and Psudomonas Aeruginosa (ATCC 27853).

The agar medium used in the present study was Mueller Hinton Agar because it is the most commonly used growth medium for checking the susceptibility of Staphylococcus aureus, Escherichia coli, Enterococcus faecalis and Psudomonas Aeruginosa [8].

Test *microorganism* from the available stock culture of microbiology department was added to 5ml BHI broth. It was incubated at 37°C for 24 hrs. and sub-cultured onto a blood agar plate. It was again incubated at 37°C for 24 hrs. After 24 hrs. microbial colonies were inoculated in nutrient broth for 6 hours. Density of nutrient broth was adjusted equivalent to 0.5 standard of McFarland opacity standard scale [9]. Procedure was carried out in laminar flow chamber. Microbial colonies were picked from the nutrient broth with the help of swab and lawn technique was used for uniform distribution of bacterial dilutions. Open end of 6 mm diameter micropipette was used to punch the holes of 6 mm diameter at equidistant points. Fresh mix of test material and control material was then immediately filled in the hole. Plates were kept for 2 hrs. at room temperature so as to allow for pre-diffusion of the material in MH-agar. Six repetitions were done for each microorganism. All the plates were kept in incubator at 37ºC for 24 hrs. After 24 hrs., zones of inhibition around each material was measured in millimeters with the help of HiAntibiotic Zone Scale (HiMedia) [10]. Zones having wider diameters were considered to have greater antibacterial activity against that specific microorganism.

Data of antibacterial activity was analyzed statistically using ANOVA and Tukey's post-hoc test at a significance level of 5% using the Graph Pad Prism 4 software.

III. RESULTS

TABLE 1 shows zones of bacterial growth inhibition in mm of ZO+P oil against four bacterial strains. Zone of inhibition in mm for E.coli was 20.66±2.06 (Fig.2); for Staph.Aureus was 18±0.00 (Fig.1) and for E.faecalis and P.Aeruginosa was 8.00±0.00 (Fig.3and Fig.4) respectively with statistically significant difference (p-value: 0.0001, $p<0.05$).

TABLE 1b shows multiple comparison Tukey Test of zones of bacterial growth inhibition of ZO+P oil against four bacterial strains. Difference was statistically significant between Staph.aureus and E.faecalis; between Staph.aureus and E.coli; between Staph.aureus and P.aeruginosa (p-value: 0.0001, $p<0.05$). Difference between E.coli and E.faecalis; between E.coli and P.aeruginosa was statistically significant (p-value: 0.0001, $p<0.05$). There was no statistically significant difference between E.faecalis and P.aeruginosa (p-value: 1.000, $p>0.05$).

TABLE 2a shows zones of bacterial growth inhibition in mm of ZOE against four bacterial strains. Zones of inhibition in mm of ZOE in decreasing order were for E.coli (19.00±1.09) (Fig.2), for Staph.aureus (16.00±0.00) (Fig.1), for E.faecalis (10.83±1.47) (Fig.3) and P.aeruginosa (10.33±0.51) (Fig.4) respectively. Difference was found to be significant statistically (p-value: 0.0001, $p<0.05$).

TABLE 2b shows multiple comparison Tukey Test of zones of bacterial growth inhibition of ZOE against four bacterial strains. Statistically significant difference was observed between Staph.aureus and E.coli (p-value: 0.0001, $p<0.05$), between Staph.aureus and E.faecalis (p-value: 0.0001, $p<0.05$), between Staph. aureus and P.aeruginosa (p-value: 0.0001, $p<0.05$), between E.coli and E.faecalis (p-value: 0.0001, $p<0.05$) and between E.coli and E.faecalis (p-value: 0.002, $p<0.05$). Difference between E.faecalis and P.Aeruginosa (p-value: 0.893, $p>0.05$) was not significant statistically.

Fig. 1: Zone of inhibition of ZOP paste and ZOE paste against Staph.aureus

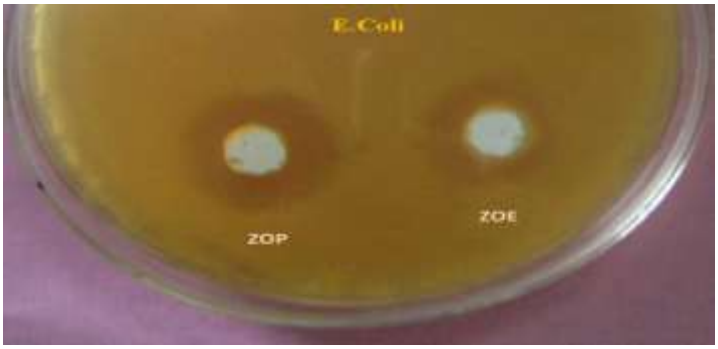

Fig. 2: Zone of inhibition of ZOP paste and ZOE paste against E.Coli

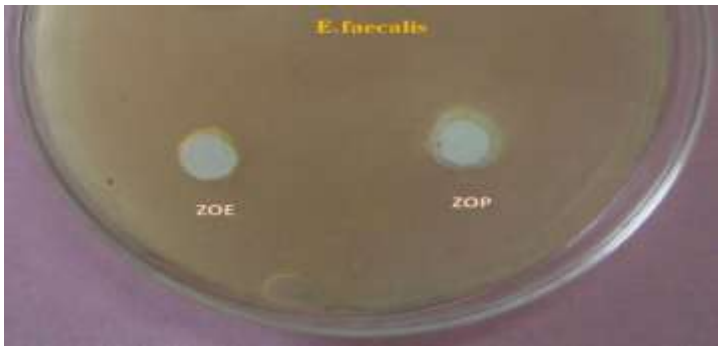

Fig. 3: Zone of inhibition of ZOP paste and ZOE paste against E.faecalis

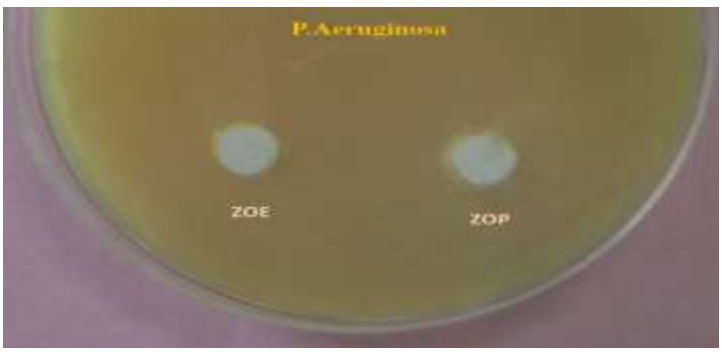

Fig. 3: Zone of inhibition of ZOP paste and ZOE paste against P.aeruginosa

Table 1a: Zones of bacterial growth inhibition in mm of ZO+P oil against four bacterial strains

Microorganisms	N	Mean	Std. Deviation	p-value
Staph Aureus	6	18.00	0.00	0.0001

E-Coli	6	20.66	2.06	S,p<0.05
E.Faecalis	6	8.00	0.00	
P.Auruginosa	6	8.00	0.00	

Table 1b: Multiple Comparison: Tukey Test of zones of bacterial growth inhibition of ZO+P oil against four bacterial strains

Microorganisms		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Staph Aureus	*E.Coli*	-2.66	0.54	0.0001,**S**	-4.28	-1.05
	E.Faecalis	10.00	0.54	0.0001,**S**	8.38	11.61
	P.Aeruginosa	10.000	0.54	0.0001,**S**	8.38	11.61
E.Coli	*E.Faecalis*	12.66	0.54	0.0001,**S**	11.05	14.28
	P.Aeruginosa	12.66	0.54	0.0001,**S**	11.05	14.28
E.Faecalis	*P.Aeruginosa*	0.00	0.54	1.000,**NS**	-1.61	1.61

Table 2a: Zones of bacterial growth inhibition in mm of ZOE against four bacterial strains

Microorganisms	N	Mean	Std. Deviation	p-value
Staph.aureus	6	16.00	0.00	0.0001 S,p<0.05
E.coli	6	19.00	1.09	
E.faecalis	6	10.83	1.47	
P.aeruginosa	6	10.33	0.51	

Table 2b: Multiple Comparison: Tukey Test of zones of bacterial growth inhibition of ZOE against four bacterial strains

Microorganisms		Mean Difference	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Staph.aureus	*E.coli*	-3.00000*	0.55377	0.0001,**S**	-4.62	-1.37
	E.faecalis	5.16667*	0.55377	0.0001,**S**	3.54	6.79
	P.aeruginosa	5.66667*	0.55377	0.0001,**S**	4.04	7.29
E.coli	*E.faecalis*	8.16667*	0.55377	0.0001,**S**	6.54	9.79
	P.aeruginosa	8.66667*	0.55377	0.0001,**S**	7.04	10.29
E.faecalis	*P.aeruginosa*	0.50000	0.55377	0.893,**NS**	-1.12	2.12

IV. DISCUSSION

Endodontic treatment can be said successful depending on the reduction or elimination of the infecting bacteria [11]. Bacteria can be reduced or eliminated by adequate root canal debridement through proper instrumentation, by antibacterial irrigations, and antibacterial filling materials [7]. Bonastre (1837) discovered zinc oxide eugenol and it was subsequently used in dentistry by Chisholm (1876) [2]. It is the most widely used root canal filling material in dentistry. It has disadvantages like slow resorption, deflection of permanent tooth bud, tooth discoloration, irritation to periapical tissues. All these disadvantages led to the search of numerous materials for root canal filling in primary teeth.

Two major components of peppermint oil as found in various studies are menthol and menthone [6,12,14] which indicates its wide use in lozenges, toothpastes, balms, and rubs. The basic raw material used for preparation of for mint oil is leaves of a plant *Mentha arvensis*[14]. India is world's largest producer and exporter of mint oil. Mint oil and its constituents and derivatives are used in food, pharmaceutical and perfumery and flavouring industry. Peppermint oil is commonly used as flavoring in foods and beverages and as a fragrance in soaps and cosmetics. Peppermint oil also is used for a variety of health conditions and can be taken orally in dietary supplements or topically as a skin cream or ointment and also may help relieve symptoms of irritable bowel syndrome and indigestion [15]. Peppermint oil is used for treating stomach disorders like indigestion, gas problem, acidity, etc. The aroma of peppermint oil has been shown to improve memory and raise alertness.

There are very few studies available in the literature in the field of dentistry where peppermint oil has been used. Thosar et al, 2013 [16] carried out study to find out the minimum inhibitory concentration (MIC) and

the minimum bactericidal concentration (MBC) of peppermint essential oil against oral pathogens namely Staphylococcus aureus ATCC 25923, Enterococcus fecalis ATCC 29212, Escherichia coli ATCC 25922. Results showed that peppermint oil exhibited inhibitory effect with mean MIC of 0.62 ± 0.45 and mean MBC of 9.75 ± 14.88. It was concluded from the study that peppermint oil can be effectively used as an intracanal antiseptic solution against oral pathogens.

Studies have shown that the mouthwash containing peppermint oil is helpful in reducing bad breath and gum infections [17]. Peppermint oil extract has been shown to be more effective than the chlorhexidine mouthwash in preventing the development of biofilm that contribute to cavities [18]. Other studies available where peppermint oil was used was the study carried out by Shahdad et al, 2007 [19] to find out the effect of various food-simulating solvents on the hardness of denture teeth after varying storage times, using a Martens hardness test. Martens hardness (HM) was assessed at baseline and during storage up to 1 month in distilled water (DW), peppermint oil (PO), heptane (HT) and 75% ethanol (ET) for four commercially-available denture teeth; Vivodent (VIV), Double-cross-linked Postaris (DCL), Orthosit (ORT), Candulor porcelain (POR) and two polymer based experimental denture teeth: Experimental 1 (EXP1); a hybrid nanocomposite with two different sized silanated filler particles and Experimental 2 (EXP2); containing an organic copolymer based upon urethanedimethacrylate and polymethyl methacrylate. Results showed that specimens stored in water, heptane and peppermint oil showed minor fluctuations in hardness which was not statistically significant.

Results of the present study showed that the diameters of zones of bacterial growth inhibition in mm of ZO+P oil against four bacterial strains in decreasing order were for *E.coli* (20.66±2.06) followed by for *Staph.Aureus* (18±0.00) and equal zones of inhibition for *E.faecalis* and *P.Aeruginosa* i.e; 8.00±0.00 respectively with statistically significant difference (p-value: 0.0001, $p<0.05$). Zones of inhibition obtained by using ZOE paste also followed the same pattern in decreasing order for microorganisms as that of ZOP paste. But the highest values of diameters of zones of inhibition in mm were obtained in case of ZOP paste when compared with ZOE paste against all the microorganisms studied in the present study.

In the present study, it was surprising to observe that ZOP paste showed good results with respect to antimicrobial efficacy against the four most commonly found microbial strains in root canals of primary teeth as compared to ZOE paste. So ZOP paste can be successfully used as root canal filling material in primary teeth.

V. CONCLUSION

Present study concludes that zinc oxide peppermint oil paste is the new material which can be used in the field of Pediatric Dentistry as a root canal filling material in primary teeth as it has shown good results against the root canal pathogens when compared with the routinely used zinc oxide eugenol paste. Further elaborative studies are required to prove it successful.

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