PULMONOLOGY

Evaluation of Essential Oil–Modified Zinc Oxide Pastes for Antifungal Activity against Candida albicans

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Abstract

Background: Endodontic treatment of primary teeth is required to eliminate microorganisms from the infected root canals having complex root canal anatomy.
Objectives: To assess the antifungal effect of zinc oxide paste with tea tree oil, thyme oil, peppermint oil on candida albicans and to compare it with zinc oxide eugenol paste.
Materials and methods: Zinc oxide pastes containing essential oils used in the present study were zinc oxide tea tree oil paste (ZOT); zinc oxide thyme oil paste (ZOTh); zinc oxide peppermint oil paste (ZOP) which were compared with zinc oxide eugenol paste (ZO). Antifungal effect in the form of zones of inhibition was evaluated by agar diffusion method. Sabauraud's dextrose agar medium was used for evaluation of antifungal effect of candida albicans. Statistical analysis was done using one way ANOVA and Tukey's post-hoc test with significance at the level of 5%.
Results: Antifungal effect in the form of zones of inhibition against candida albicans found in decreasing order was ZOTh paste (50.00±0.00)> ZOP paste (45.66±0.51)> ZOT paste (44.33±0.51)> ZOE paste (16.83±0.91) respectively with difference found to be statistically significant (0.0001, S,p<0. 05).
Conclusion: ZOTh paste can be successfully used for root canal filling of deciduous teeth against candida albicans.
Keywords:
ZOTh paste, ZOT paste, ZOP paste, ZOE paste, candida albicans.

Introduction

Primary goal of endodontic treatment is to eliminate the microorganisms from the infected root canal system[1]. Complexity of primary root canals prevent the normal instrumentation and irrigation difficult to remove these microorganisms including fungi i.e; candida albicans.

The most commonly found fungus in the cases of persistent apical periodontitis as concluded from various studies is candida albicans in the range of 7-18%[2]. It is considered that C. albicans is a part of normal flora of oral cavity. Therefore there are high chances that it can be present in the infected root canals[3-5]. Virulence factors of C. albicans makes it survive in persistent apical periodontitis. C. albicans has properties like hyphal formation and thigmotropism which allows it to penetrate deep into dentine[6].

Zinc oxide has been said to have limited antimicrobial effect[7]. Also it has certain disadvantages like slow resorption of material as compared to normal physiologic root resorption[8,9]. Medicinal plants have been successfully used as natural antimicrobial agents as reported its usage in many fields also including dental field[10]. Essential oils, called as volatile oils, are the aromatic oils obtained from different parts of the plants like buds, seeds, flowers, fruits , leaves, twigs, herbs, bark, wood and roots[11]. The antimicrobial effect of essential oils is due to a number of small terpenoids and phenol compounds[12]. Essential oils are reported to have its use in aromatherapy[13], fragrance industries[11] and food preservation[14]. Essential oils defend against pathogens[15] and they have been considered to be safe[16].

Considering the well documented benefits of tea tree oil, thyme oil and peppermint oil as available in the literature, present study was planned to find out the antifungal effect of zinc oxide based cement with tea tree oil, thyme oil, peppermint oil against Candida albicans and its comparison with zinc oxide eugenol.

Materials and methods

Institutional ethical committee approval was taken before starting the study. Essential oils used in the study were tea tree oil, thyme oil, peppermint oil (Aromatantra, Mumbai).

Zinc oxide powder and eugenol of the department of Pedodontics and Preventive Dentistry was used (Prime Dental Products Pvt. Ltd., Thane). For all the materials studied, 1 scoop (0.2gm) of powder was taken and mixed with 7 drops of oil (0.07cc) on the dry and sterile glass slab using cement spatula to get the desired soft creamy consistency[17].

Strain of Candida albicans used for the present study was obtained from the Department of Microbiology, Jawaharlal Nehru Medical College, Wardha, Maharashtra, India. Strain of Candida albicans used for the study was ATCC 90028.

Sabouraud's dextrose Agar was used for evaluating the susceptibility of Candida albicans.

Procedure: Standard inoculum of Candida albicans was obtained by passing single colony of Candida albicans in nutrient broth. It was then incubated at 37°C for 4-6 hrs. turbidity was of broth was adjusted with McFarland 0.5 turbidity standard. Colonies of Candida albicans were picked up with the help of sterile cotton swab. By using lawn technique, microbial colonies were spread uniformly on Sabouraud's dextrose Agar. Holes of 6 mm diameter were punched at two equidistant points. Freshly mixed material was then filled in the punched holes. To ensure prediffusion of material in agar, plates were kept for 2 hrs. at room temperature. Whole experiment was six times repeated for each material. All the plates were kept in incubator at 37ºC for 24 hrs. Diameter of zones of inhibition in millimeters around each test material was measured with the help of HiAntibiotic Zone Scale (HiMedia). 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.

Results

Table 1 shows antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans. Diameters of zones of inhibition in mm for ZOTh paste were largest against Candida albicans i.e; 50.00±0.00. Zones of inhibition of ZOP paste against Candida albicans was 45.66±0.51. With ZOT paste, it was 44.33±0.51 and with ZOE paste it was 16.83±0.98 (Figure 1).

Table 2 shows Antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans by using One way ANOVA. Difference between and within groups was found to be statistically significant (p-value: 0.0001, $p<0.05$).

Table 3 shows antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans by using Multiple Comparison: Tukey Test. When antifungal effect of ZOT was compared with ZOTh, ZOP and ZOP paste, it was observed that difference was statistically significant (p-value: 0.0001, $p<0.05$).
Antifungal effect of ZOTh when compared with ZOP and ZOE paste, the difference was found to be statistically significant (p-value: 0.0001, $p<0.05$).
When ZOP was compared with ZOE paste for antifungal effect, the difference was found to be statistically significant (p-value: 0.246, $p>0.05$).

Discussion

Root canal infections are polymicrobial in nature. Candida albicans, though are less in quantity of all the microorganisms, are found in infected root canals and are isolated from infected dental pulp[18]. Mechanical instrumentation and irrigation fail to remove this fungus from the complex structure of root canals of primary teeth.

It is widely known that the plant essential oils including tea tree oil, thyme oil, peppermint oil possess antimicrobial efiicacy. Therefore in the present study antifungal effect of ZOT, ZOTh and ZOP paste was evaluated against Candida albicans and compared with the routinely used zinc oxide eugenol paste.

In the study of Amorim et al, (2006)[19], the antimicrobial efficacy of root canal filling pastes used in pediatric dentistry i.e; Guedes-Pinto paste (GPP), calcium hydroxide paste (CHP), chloramphenicol + tetracycline + zinc oxide and eugenol paste (CTZP), zinc oxide-eugenol paste (OZEP) and Vitapex was evaluated against S. aureus, P. aeruginosa, E. faecalis, B. subtilis and C. albicans. It was concluded that all materials formed inhibition zones except Vitapex.

Cassanho et al, (2005)[20] had evaluated the antimicrobial activity of zinc oxide-eugenol cements and glass ionomer against Candida albicans. and concluded that ZOE cement was more effective in vitro against Candida albicans than GIC. But in the present study ZOE was least effective in showing antifungal effect against C. albicans.

Almeida et al, (2016)[21] had evaluated the anti-biofilm efficacy and anti-Candida effect of two essential oils from Cinnamon cassia (cinnamon) and Cymbopogon winterianus (citronella). It was concluded from the study that citronella and cinnamon essential oils have potential for daily anti-candidal denture cleansing. In the present study, plant essential oils used were tea tree oil, thyme oil and peppermint oil which showed good antifungal effect compared to zinc oxide eugenol paste against Candida albicans and can be used as root canal filling material for primary teeth effectively.

In the study of Egan et al, (2002)[18], frequency of occurrence of yeasts in root canals was 10%. It was assumed in the same study that the presence of yeasts in root canals can be associated with its presence in saliva and role of yeasts in the initiation of periapical infection needs to be determined.

Study done by Baumgartner et al, (2000)[22] had evaluated the presence of Candida albicans from the aspirates of abscesses and cellulitis of endodontic origin and infected root canals by using PCR method concluded the study by finding the presence of Candida albicans in 5 of 24 samples which were taken from root canals i.e; 21%. They could not find the presence of Candida albicans in periradicular aspirates. Reason for this, given was that PCR being an extremely sensitive molecular method, may be used to identify Candida albicans in samples directly taken from the infections of endodontic origin.

Study of Kovac et al, (2013)[5] concluded that Enterococcus faecalis and Candida albicans can participate in root canal and periapical infections. Irrigating solutions and intracanal medicaments which effectively prevent endodontic therapy failures should be used. Unexpected finding observed in their study was the isolation of Candida albicans in the sample of apical periodontitis taken from a 9 year old child. So possibility of the presence of Candida albicans as a potential etiological agent in endodontic infections at young age can also be suspected.

In the present study, all the materials have shown antifungal effect, but the zones of inhibition obtained with ZOTh paste were highest i.e 50.00±0.00. Zones of inhibition obtained with ZOP paste were also higher i.e, 45.66±0.51 which were nearly comparable with the zones obtained with ZOT paste i.e; 44.33±0.51. Zinc oxide eugenol which is routinely used for root canal filling of primary teeth had shown minimal antifungal effect with zones of inhibition of 16.83±0.98. Hence zinc oxide based paste by using tea tree oil, thyme oil and peppermint oil can be the best option for evaluation of antifungal effect in pediatric root canal infections.

Conclusion

Present study concludes that ZOTh paste was the best material which had shown larger zones of inhibition against Candida albicans. Other materials like ZOP paste and ZOTT paste also had shown better zones of inhibition against Candida albicans. Zinc oxide eugenol could not show antifungal effect against Candida albicans effectively. Antifungal effect against Candida albicans in decreasing order was ZOTh> ZOP> ZOT> ZOE. All these materials except zinc oxide eugenol can be successfully used as root canal filling material for primary teeth.

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Table 1

Antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans

Zinc oxide based based pastes with essential oils	N	Mean	Std. Deviation	Std.Error	95% Confidence Interval for Mean	
					Lower bound	Upper bound
ZOT	6	44.33	0.51	0.21	43.79	44.875
ZOTh	6	50.00	0.00	0.00	50.00	50.00
ZOP	6	45.66	0.51	0.21	45.12	46.20
ZOE	6	16.83	0.98	0.40	15.80	17.86

Table 2

Antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans by using One way ANOVA

Source of variation	Sum of Squares	Df	Mean Square	F	p-value
Between Groups	4110.45	3	1370.15	3653.74	0.0001 **S**,p<0.05
Within Groups	7.50	20	0.37		
Total	4117.95	23			

S: Significant

Table 3

Antifungal effect of ZOT, ZOTh, ZOP and ZOE in the form of zone of inhibition on Candida albicans by using Multiple Comparison: Tukey Test

Zinc oxide based based pastes with essential oils		Mean Difference (I-J)	Std.Error	p-value	95% Confidence Interval	
					Lower bound	Upper bound
ZOT	ZOTh	-5.66	0.35	0.0001,**S**	-6.65	-4.67
	ZOP	-1.33	0.35	0.006,**S**	-2.32	-0.34
	ZOE	27.50	0.35	0.0001,**S**	26.51	28.48
ZOTh	ZOP	4.33	0.35	0.0001,**S**	3.34	5.32
	ZOE	33.16	0.35	0.0001,**S**	32.17	34.15
ZOP	ZOE	28.83	0.35	0.0001,**S**	27.84	29.82

S: Significant

Figure 1: Zones of inhibition with ZOT, ZOTh, ZOP and ZOE pastes against Candida albicans