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A Comparative Evaluation of Antimicrobial Efficacy of Zinc Oxide with Thyme Oil, Zinc Oxide with Peppermint Oil and Zinc Oxide Eugenol Sealers and Obturating Materials Against Enterococcus Faecalis and Candida Albicans"- An in-Vitro Study

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Abstract:- The increase in failure of pulpectomies due to re-infection by most common micro-organisms like Enterococcus faecalis and Candida albicans has been noticed in recent years. Thus need for an obturating material or sealer material having a high efficacy against these causative microbes is the need of the hour which can eliminate these microbes even in an event of re-infection after the completion of the root canal treatment in deciduous teeth. AIM: To compare antimicrobial efficacy of Zinc oxide eugenol, Zinc oxide plus thyme oil and Zinc oxide with peppermint oil against Enterococcus Faecalis and Candida Albicans. MATERIALS AND METHODS:This study was conducted on 30 Muller Hinton agar plates which were divided into three groups (n=15). Three wells of 6mm diameter were made by removal of agar at equidistant points and then were filled with the test materials. Both microorganisms were grown at 37℃ for 24hrs in MH Broth. The lawn technique was used for uniform distribution of bacterial dilution. The plates were maintained at room temperature for 2 hours for prediffusion of material and then incubated at 37℃ 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 RESULTS: The mean zones of inhibition were highest in for ZoE followed by ZoT and least for ZoP against Enterococcus faecalis. Similarly, ZoE had highest mean zones of inhibition, followed by ZoT and the least was ZoT against Candida albicans. CONCLUSION: The antimicrobial efficacy was highest in ZoE against E faecalis and Candida albicans followed by ZoT but Candida albicans were almost resistant to ZoP and had the least efficacy against E faecalis. All the test materials had better antimicrobial efficacy against Candida albicans compared to E faecalis.

Keywords:- *ZoT, ZoP and ZoE.*

I. INTRODUCTION

Dental caries is a multifactorial microbial infectious disease characterized by demineralization of in-organic and destruction of the organic substances of the tooth. It is also one of the most commonly seen oral health problem globally. Indian children reported to have a DMFT of around 2 and caries prevalence was increasing with age from 51.9% and 63.1% in 5-15 years of age group respectively in the last National Oral Health survey conducted in 2002-2003 [1].Dental caries continues to pose a major public health problem despite the recent scientific advances. One of the main complaints of dental caries is due to the unbearable pain caused once the carious lesion reaches the pulp [2].

When the deciduous teeth pulp tissue gets affected either by caries or trauma pulp therapy has to be performed in such cases so as to maintain the primary teeth in its functioning and healthy state until replaced by their permanent successors. Pulpectomy in primary teeth includes chemo-mechanical preparation and use of intra canal dressing with anti-bacterial properties and the success depends on proper removal of infected bacteria. Complex anatomy of primary teeth root canals makes ittough for cleansing proper cleansing by instrumentation and irrigations[3].Presence of numerous accessary, curved, thin tortuous, ribbon like canals which are connected to each other with interconnected canals in primary teeth, the intra canals dressing may fail to remove microorganisms from in accessible areas[4].

Typically poly-microbial flora consisting of gram positive and gram negative bacteria consist of the main endodontic flora [5, 6]. Microorganisms are harboured by the root canal dentinal tubules, the apical portion of root canal and extra radicular tissue may also harbour bacterial biofilms[7,8].

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Endodontic failure corresponds with the persistence of peri-radicular lesion. To conserve the update in endodontic treatment and materials is necessary, otherwise persisting micro-organisms or secondary infections mainly cause by insufficient coronal restorations and absence of anti-bacterial properties of sealer and obturating material can lead to loss of the tooth. In cases of tooth filled with obturating materials, 35-100% cases micro-organism will be present in the peri-radicular tissue. Earlier studies using culture media revealed predominantly facultative anaerobes and few obligate anaerobes which is distinctly different microbial flora compared to primary infection, including mostly gram-positive bacteria. Complex root morphology, inherent physiological root resorption, close proximity of permanent successor tooth, complex diagnosis due to immature patient which are some of the reasons why pulp therapy of primary teeth still remains controversial despite high success rates pointed out in previous studies[9].

Therefore this makes it important to use obturating and sealer materials which has high antibacterial property to act onmicroorganism of inaccessible areas in root canals of deciduous teeth[10].

Zinc oxide eugenol is widely used for root canal sealing and obturation in primary teeth[3].In dental practice, eugenol has topical uses such as relieving pain from different sources like pulpitis and dental hypersensitivity [3,11]. Certain studies have reported that eugenol has irritating action on the tooth [11]. It may have some side effects as its presence per-apically may deflect the path of eruption of permanent successors and may cause necrosis of bone and cementum, periapical tissue irritation and may cause tooth discoloration[4]. It has a disadvantage that includes slower rate of resorption than the root of deciduous teeth. It is also believed to have limited antibacterial activity [4,12].

Thus the search for newer materials with effective antimicrobial properties has led to conduct this study with use ofzinc oxide powder with thyme oil and zinc oxide powder with peppermint oilto determine its antibacterial efficacy against most resistant microorganisms like Enterococcus Faecalis and Candida albicans.

Enterococcus Faecalis is gram positive cocci facultative anaerobes which are seen in cases with root canal failures or persistent root canal infections,[3,6] isolated from retreatment cases of apical periodontitis,[5,6,13,14] enterococcus Faecalis' tendency to bind to dentinal tubule collagen and stay viable within tubules, which can induce periapical disease and recurrent endodontic tooth care deficiencies. [5,6], it is tough to eliminate this species through root canal medications when established in the dentinal tubules [5,6,13The success rate of root canal therapy at the time of obturation is substantially decreased in the presence of Enterococcus Faecalis[13].

The largest proportion of fungal microbiota is formed by candida species. Candida albicans has been the most commonly detected fungal species in endodontic infections.The oral cavity occurrence of Candida Albicans has been reported to be 30-45% in healthy humans. Their incidence in the root canal ranges from 1% to 17%.In infected canals, there are also strong risks that candida albicans may be identified. In chronic apical periodontitis cases, as inferred from numerous studies of candida albicans in the range of 7-18%, it is the most commonly detected fungus. Properties such as hyphal formation and thrgmotropism that enable it to penetrate dentin deeply and make it thrive in persistent apical periodontitis with its virulence[12].

Therefore because of the shortcomings of Zinc oxide eugenol search for newer materials like zinc oxide powder with thyme oil and zinc oxide powder with peppermint to test their antimicrobial efficacy against Enterococcus Faecalis and Candida Albicans which are most commonly seen in secondary or failed root canal infections**.**

II. MATERIALS AND METHODS

This study was conducted on 30 Muller Hinton (MH) agar plates. The number of samples for all three groups were 15 each (n=15). Three wells of 6mm diameter were made by removal of agar at equidistant points with open end of micropipette and then were filled with the test materials. For all the test materials and ZOE paste, using a cement spatula, 1 scoop (0.2 mg) of zinc oxide powder was mixed with 7 drops of oil (0.07) on a dry, sterile glass slab.

The standard strains of bacteria and fungi, 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 seeded in MH agar to develop 0.5 turbidity on the McFarland scale, corresponding to 108 CFU/mL concentrations. The 2nd layer of this MH broth was included. Following the injection of test materials, the seeded agar was added directly over the plates. For uniform distribution of bacterial dilution the lawn technique was used.

The plates were maintained at room temperature for 2 hours for prediffusion of material and then incubated at 37°C for 24 hours. After incubation, the diameters of zone of inhibition around the plates were measured in millimetres with the help of Hi Antibiotic zone scale (HiMedia).

III. STATISTICAL ANALYSIS

A power analysis was established by G*power, version 3.0.1(Franz Fauluniversitat, Kiel, Germany). A sample size of 36 subjects (12 in each group which can be rounded off to 15 in each group comprising the total sample to be 45) would yield 95% power to detect significant differences, with effect size of 0.7 and significance level at 0.05.

Antimicrobial behavior data was statistically evaluated at a significance level of 5 percent using one way ANOVA and Tukey's post-hoc test.Data was entered in the excel spread sheet. Descriptive statistics like mean, standard deviation and percentages was calculated. Inferential

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statistics like ANOVA with post-hoc Bonferroni was used to compute the significant difference among the groups using SPSS version 22. Any other necessary tests will be dealt at the time of analysis.

IV. RESULTS

The datashows a statistically significant difference in the zones of inhibition of the different materials against the 2 test micro-organisms(Candida albicans and E.faecalis). Zinc Oxide Eugenol (ZoE) showed the highest median of zone of inhibition of 45mm against Candida albicans followed by Zinc Oxide Thymol (ZoT) with median of 38mm of zone of inhibition and ZoP showed no zones of inhibition when made to react with Candida Albicans species. Similar results were seen when the study materials were made to react against E Faecalis species, ZoE showed the highest zone of inhibition with median score of 21mm followed by ZoT 12mm and ZoP showing the least median score with 8mm forthe zone of inhibition (table 1)

ZoE showed greater zones of inhibition against Candida albicans compared to E Faecalis and the difference was found to be statistically significant. The two zones of inhibition for E Faecalis & C albicans with ZoP showed no statistically significant difference among them. ZoT showed greater zones of inhibition against C albicans compared to E Faecalis and this difference was found to be statistically significant (table 2)

TABLE 1: COMPARISON OF THE ANTIMICROBIAL EFFICACY AMONG THE GROUPS USING KRUSKAL-WALL

		Minimum	Maximum	Median	IQR	Kruskal-wallis	P value
Candida albicans	ZoE	40	48	45	5	34.86	0.00*
	ZoP	0	35	0	8		
	ZoT	0	45	38	5		
E faecalis	ZoE	18	25	21	3	37.29	0.00*
	ZoP	0	12	8	12		
	ZoT	12	13	12	1		

*significant

TABLE 2: COMPARISON OF THE ANTIMICROBIAL EFFICACY WITHIN THE GROUP BETWEEN CANDIDA ALBICANS AND E FAECALIS USING MANN-WHITNEY TEST

		Minimum	Maximum	Median	IQR	U value	P value
ZoE	C.albicans	40	48	45	5	0.00	0.00*
	E.faecalis	18	25	21	3		
ZoP	C.albicans	0	35	0	8	80.00	0.126
	E.faecalis	0	12	8	12		
ZoT	C.albicans	0	45	38	5	15.00	0.00*
	E.faecalis	12	13	12	1		

*significant

V. DISCUSSION

Antibacterial activity is one of the most important and desired properties for an ideal obturating material and sealer to be used on primary teeth. Incorporation of antibacterial components in an endodontic obturating material has become essential to prevent the re-growth of bacteria leading to secondary infection. This is usually caused by the residual microorganisms left behind due to the nature of the root canals in deciduous teeth. It is also essential to control the re-entry of bacteria into the root canal system. However, these components should not cause any specific toxicity toward the innate microorganisms found in the root canal system because of which it may exert toxic effect on the host cells causing irritation and inflammation. Therefore, biocompatibility of the antibacterial components is very important factor when selecting it as an obturating and sealer material [15]

Enterococcus faecalis is a gram positive, facultative anaerobic microorganism found in persistent per-apical infections and failed root canal treatments[16]. It has been isolated in cases of persistent root canal infections. Rocas IN et al concluded that the prevalence of E Faecalis is 4-40% in primary endodontic infections and 67-77% in secondary infections, consistently higher.E Faecalis forms a defensive biofilm capable of blocking lymphocytic function, rendering the bacteria 1000 times more resistant to phagocytosis, antibodies and antimicrobials. [17]. E Faecalis can also endure prolonged periods of nutritional deprivation[14].With the involvement of E. faecalis in the pulp canal system at the time of obturation, the effective rate

of root canal therapy is substantially decreased. [18]. Above all, relative to other species, E.faecalis rapidly colonizes the dentinal tubules and is therefore especially difficult to eradicate. [19]. Due to all these reasons, it represents a standard against which the antimicrobial action of an intra-canal medicament is tested[19,20]

Candida albicans is the most frequently isolated fungal species from contaminated root canals, and because of its invasive affinity to dentin, this species is considered a dentinophilic microorganism·[21].Candida albicans though are found in reduced quantities when compared to other microorganisms which are isolated from infected dental pulp[22]. The study conducted by Baumgartner et al assessed the presence of Candida albicans from abscess and cellulite aspirates of endodontic origin and contaminated root canals using the PCR method and concluded the study by documenting the presence of Candida albicans in 5 of 24 root canal samples. [23].

Bonastre (1837) found zinc oxide eugenol, which was later used by Chisholm (1876)[24] in dentistry. The first root canal filling material to be recommended for primary teeth was Zinc oxide eugenol paste, as defined by Sweet in (1930)[25] and has since been the most widely used root canal filling material in dentistry[4].ZOE has historically been the material of choice for filling deciduous teeth in the root canals[26], and was the only material specifically prescribed in the clinical recommendations established by the American Academy of Pediatric Dentistry until 2008. (AAPD) [27]. The antimicrobial effectiveness of zinc oxide eugenol may be due to the eugenol content. Owing to its capacity to induce hydrophobicity, Eugenol is bactericidal in nature, allowing them to separate the lipids of the bacterial cell membrane and mitochondria, spreading the cell structures and making them more permeable. Extensive bacterial cell leakage or the exit of essential molecules and ions will lead to cell death[28].

Oyedemi et al 2009 investigated the bactericidal action mechanism of eugenol against gram-positive and gram-negative bacteria and concluded that eugenol caused damage to the cell wall resulting in leakage of protein from the bacterial cell wall. [29].Hashieh conducted a study which show that the level of eugenol released from a zinc oxide-eugenol-based sealer beyond the apex is very low and decreases over timebut still at these concentrations, eugenol is said to have anti-inflammatory and analgesic properties that are very useful after a pulpectomy procedure[30]. However, 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 [4].

In the present study eugenol has been compared to peppermint and thyme oil because of the drawbacks seen from the use of eugenol. These are plant essential oils which can exhibit antimicrobial activity against the microorganism which are commonly found in secondary infection like E faecalis and Candida albicans.

Studies done by Markowitz K et al. and Saggar V et al. concluded that Zinc oxide eugenol containing sealers were more superior in inhibiting the micro-organisms found in primary root canal system[31,32]. A study done by Rahul et al in 2015, showed that ZoE based sealer Endoflas FS had the highest zones of inhibition against E Faecalis and C albicans which is in agreement with the results of the present study[6] . Zones of inhibition were seen highest with ZoE obturating material compared to the herbal extracts in the study done by Shipra et al[33] which was similar to the result seen in study done by Navit et al& Markowitz et al (1992)[33,34]. A study was done to assess the antimicrobial activity of endodontic sealers on Enterococcus Faecalis by Anjali et al 2012 concluded that Eugenol based endodontic sealers had greater zones of inhibition compared to that of non eugenol sealers which was same as the results seen in experiments conducted by Andre Mickel et al[13,35].

Verma et al 2015 in a study concluded that Zinc oxide eugenol (ZoE) had comparable antimicrobial effect with other obturating materials used against E Faecalis in the primary root canal dentine [36]. Similarly, Queiroz AM et al 2009 concluded that ZoE had the highest antibacterial effect when compared with other materials used against E Faecalis[37].

In a clinical study, 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[38], 82.5% by Gould[39] and 86.1% by Coll et al[40]. A more recent retrospective evaluation by Bahrololoomi and Zamaninejad 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[41].

Thymol is the primary ingredient of thyme oil. In dental practice, thyme oil demonstrates antibacterial activity and has proven useful[42]. Thymol tends to prevent the development of oral pathogens in the mouth and can decrease tooth decay in conjunction with other essential oils[43,44]. One of the essential oils present in Listerine with antibacterial effects is Thymol·[45].

In a study done by Nilima et al in 2016, the zones of inhibition were highest for ZoT, followed by ZoE and least for ZoP against E Faecalis which contradicts the results of the present study where ZoE had highest zones of inhibition followed by ZoT[3].

NilimaThosar et al 2013 conducted a study to assess the antimicrobial efficacy of 5 essential oils against oral pathogens which concluded that Thyme oil had the highest antifungal effect on candida albicans followed by peppermint oil while the least effect was seen with eugenol which refutes the results of the present study[11]. Studies done by Gislene et al (2000), Hili et al (1997)and Nzeako et al (2006) showed identical conclusions[46,47,48] .Similar results were also seen in studies done by Kovac et al (2013)[49], Amorim et al (2006) [50]and Baumgartner et al

(2000)[23].ZoP showed better antimicrobial efficacy compared to ZoE in study done by Nilima et al 2016 [4].

The result of this study concluded that ZoE was found to be superior to Zinc oxide with thyme oil and Zinc oxide with peppermint oil in inhibiting E faecalis and Candida albicans but study done in 2016, Nilima et al observed that ZoT had a more effective anti-fungal effect on the antifungal effect of zinc oxide-based pastes containing various essential oils against Candida albicans, accompanied by ZoP and ZoE, this may be attributed to different culture media used in the research, which is sabourauds dextrose agar. [12]. In another study done by Nilima et al in 2016, the zones of inhibition were highest for ZoT, followed by ZoE and least for ZoP against E Faecalis which contradicts the results of the present study where ZoE had highest zones of inhibition followed by ZoT[3]

The rationale for performing this in vitro study was to provide information that zinc oxide eugenol has the highest antimicrobial efficacy followed by zinc oxide with thyme oil and then zinc oxide with peppermint oil 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 might modify the antimicrobial efficacy of the obturating material. Therefore, further in vivo studies are needed to evaluate the antimicrobial properties of the obturating materials used inthe present study.

VI. CONCLUSION

All materials developed zones of microbial growth inhibition against Enterococcus faecalis and candida albicans within the limitations of the sample.On intergroup comparisons of mean zones of inhibition in the different group, maximum zone of inhibition was seen in Zinc oxide eugenol followed by zinc oxide with thyme oil and least with zinc oxide with peppermint oil which was the least against Enterococcus faecalis and candida albicans. Candida albicans were almost resistant to zinc oxide with peppermint oil in most of the cases. It could also be concluded that ZoE, ZoT and ZoP has better antimicrobial efficacy against Candida albicans as compared to Enterococcus faecalis.

REFERENCES

[1]. Mehta A. Trends in Dental Caries in Indian Children for the past 25 Years. Indian J Dent Res 2018; 29:323-8.
[2]. Goenka P, Dutta S, Marwah N, Sarawgi A, Nirwan M, Mishra P. Prevalence of Dental Caries in Children of Age 5 to 13 Years in District of Vaishali, Bihar, India. Int J ClinPediatr Dent 2018; 11(5):359-64.
[3]. Thosar N, Chandak M, Bhat M, Basak S. Antibacterial efficacy of zinc oxide paste with various essential oils against enterococcus Faecalis and comparison with zinc oxide eugenol paste. Int J Dev Res. 2016; 6(12):10663-6
[4]. Thosar N, Chandak M, Bhat M, Basak S. In vitro antimicrobial efficacy of zinc oxide with peppermint oil in comparison to zinc oxide eugenol against four root canal microorganisms. J Adv Med Dent Scie Res.2016;3(9):53-8.
[5]. Arora R, Raiwat P, Bhayya D P.A comparative evaluation of antimicrobial efficacy of three endodontic sealers: Endoflas FS, AH Plus and sealapex against enterococcus Faecalis Int J Med Dent Sci.2014;13(3):90-3
[6]. Bhargava R, Shashikala K, Bhargava R. Antibacterial efficacy of three root canal sealers against enterococcus Faecalis ans candida albicans-an in vitro study. SRM J Res Dent Sci.2018;6(3):170-4
[7]. Navit S, Jaiswal N, Khan S A, Malhotra S et al. Antimicrobial efficacy of contemporary obturating material used in primary teeth: An in vitro study. J ClinDiagn Res.2016;10(9):9-12
[8]. Hammer K A, Carson C F, Riley T V. Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol.1999;86:985-90.
[9]. Anderson AC, Hellwig E, Vespermann R, Wittmer A, Schmid M, et al. Comprehensive Analysis of Secondary Dental Root Canal Infections: A Combination of Culture and Culture-Independent Approaches Reveals New Insights. PLoS ONE 2012;7(11): e49576
[10]. Thosar N, Chandak M, Bhat M, Basak S. Evaluation of antimicrobial activity of two endodontic sealers:- zinc oxide with thyme oil and zinc oxide eugenol against root canal microorganisms. Int J ClinPediatr Dent 2018;11(2):79-82
[11]. Thosar N, Basak M, Bahadure R N, Rajkumar M .Antimicrobial efficacy of five essential oils against oral pathogens: an in vitro study. Eur J Dent.2013;7:71-7
[12]. Thosar N, Chandak M, Bhat M, Basak S. Antifungal effect of zinc oxide based pastes containing various essential oils against candida albicans and comparison of its effects with zinc oxide eugenol. Indian J Med Res Pharm.2016;3(11):60-5.
[13]. Kaiwar A, Nadig G, Hegde J, Lekha S. Assessment of antimicrobial activity of endodontic sealers on enterococcus Faecalis: an in vitro study. J Dent(Shiraz).2012;3(1):26-31
[14]. Punathil S, Bhat S S, Bhat S V, Hegde S K. Microbiological analysis of root canal flora of failed pulpectomies in primary teeth. Int J CurrMicroboil App Sci.2014;3(9):241-6.
[15]. Smadi L, Mahafzah A, Khraisat A. An In vitro Evaluation of the Antimicrobial Activity of Nine Root o Canal Sealers. J Contemp Dent Pract 2008 July; (9)5:060-067.
[16]. Stuart, C.H., Schwartz, S.A., Beeson, T.J., Owatz, C.B. 2006. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J.Endod.2006; 32:93–8.
[17]. Jaidka S, Somani R, Singh J W, Sheikh T, Chaudhary N. Herbal combat against E Faecalis- an in-vitro study. J Oral BiolCraniofac Res 2017; 7: 178-81.

[18]. Kriplani R, Thosar N, Baliga MS, Kulkarni P, Shah N, Yeluri R. Comparative [11] evaluation of antimicrobial efficacy of various root canal filling materials along with aloevera used in primary teeth: A microbiological study. J ClinPediatr Dent. 2013;37(3):257-62.
[19]. Marsh SJ, Largent MD. A bacteriologic study of the pulp canals of infected [20] primary molars. ASDC J Dent Child. 1967;34:460-70.
[20]. Cohen MM, Joress SM, Calisti LP. Bacteriologic study of infected deciduous [21] molars. Oral Surg Oral Med Oral Pathol. 1960;13:1382-86.
[21]. Waltimo TMT, Haapsalo M, Zehnder M, Meyer J. Clinical aspects related toendodontic yeast infections. Endod Topics 2004;9:66-78.
[22]. Sabyasachi S, Sonali S, Firoza S. An In Vitro Evaluation of Antimicrobial Activity of Different Endodontic Sealers. J I D M R 2010;3(3):108-15.
[23]. Baumgartner JC, Watts CM, Xia T. Occurrence of Candida albicans in infections of endodontic origin. J Endod. 2000 Dec;26(12):695–8.
[24]. Praveen P, Ananthraj A, Venkataraghavan K, Pratibha Rani S, Sudhir R, Jaya AR. A review of obturating materials for primary teeth. A streamdent, 1(3), 2011.
[25]. Khairwa A, Bhat M, Sharma R, Satish V, Maganur P, Goyal AK. Clinical and radiographic evaluation of zinc oxide with aloe vera as an obturating material in pulpectomy: An in vivo study. J Indian SocPedodPrev Dent 2014; 32:33-8.
[26]. Rodd H. D, Waterhouse P. J, Fuks A. B, Fayle S. A, Moffat M. A. "Pulp therapy for primary molars," Int J Paediatr Dent.2006;16(1):15– 23.
[27]. American Academy of Pediatric Dentistry, "Guideline on pulp therapy for primary and young permanent teeth," Pediatric Dentistry.2005; 27(7):130–4.
[28]. Tantry B A, Kumar A, Rahima S, Tantry M N. Antibacterial evaluation and phytochemical screening of methanolic extract of ocimum sanctum against some common microbial pathogens. Glob Adv Res J Microbiol.2016;5(1): 1-6.
[29]. Oyedemi S O, Okoh A L, Mabinya L V, Pirochenva G, Afolayam A J. The proposed mechanism of bactericidal action of eugenol, -terpinol and g-terpinene against Listeria monocytogenes, streptococcus pyogens, proteus vulgaris and Escherichia coli. Afr J Biotechnol;8(7):1280-6.
[30]. Hashieh I A, Ponnmel L, Camps J. concentration of Eugenol apically released from ZnOE based sealers. J Endocrinol 1999;22:713-5.
[31]. Markowitz K, Moynihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide eugenol and zinc oxide eugenol. A clinically oriented review. Oral Surg Oral Med Oral Pathol. 1992; 73:729-37.
[32]. Saggar V, Chandra S, Jaiswal JN, Singh M. Antimicrobial efficacy of iodoformized zinc oxide eugenol sealer on microorganisms of root canal. J IndSocPedo and Prev Dent.1996; 14 (1):1-3.
[33]. Navit S, Jaiswal N, Khan SA et al. Antimicrobial efficacy of contemporary obturating materials in primary teeth-an in-vitro study. J ClinDiagn Res 2016 ; 10(9) : ZC09-12.
[34]. Markowitz K, Monihan M, Lui M, Kim S. Biological properties of Eugenol and zinc oxide eugenol. Oral Surg Oral Med Oral Path 1992; 73: 729-37.
[35]. Mickel A K, Nguyen T H, Chogle S. Antimicrobial activity of endodontic sealer on Enterococcus Faecalis. J.Endod.2003;29:4.
[36]. Verma R., Sharma, D.S., Pathak, A.K. 2015. Antibacterial Efficacy of Pastes Against E Faecalis in Primary Root Dentin: A Confocal Microscope Study. Journal of Clinical Pediatric Dentistry 39, 247–54.
[37]. Queiroz, A.M. de, Nelson-Filho, P., Silva, L.A.B. da, Assed, S., Silva, R.A.B. da, Ito, I.Y. 2009. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex and EndoREZ. Brazilian Dental Journal 20, 290–6.
[38]. Barr E S, Flaitz C M,Hicks M J. A retrospective radiographic evaluation of primary molar pulpectomies. Pediatr Dent 1991;13:4-9.
[39]. Gould J M. Root canal therapy for infected primary molar teeth: Preliminary report. ASDC J Dent child 1972;39:269-73.
[40]. Coll J A, Josell S, Casper J S. Evaluation of a one appointment formocresol Pulpectomy technique for primary molars. Pediatr. Dent 1985; 7: 123-9.
[41]. Bahrololoomi Z, Zamaninejad S H. Success rate of zinc oxide eugenol in Pulpectomy of necrotic primary molars: A retrospective study. J Dent Mater Tech.2015; 4:89-94.
[42]. Meeker HG, Linke H A. The antibacterial action of eugenol, thyme oil, and related essential oils used in dentistry. Compendium 1988; 9(32): 34-5.
[43]. Shapiro S, Guggenheim B. The action of Thymol on oral bacteria. Oral MicrobiolImmunol 1995;10: 241-6.
[44]. Yu D, Pearson S K, Bowen W H, Luo D, KohutB E, Harper D S. Caries inhibition efficacy of an antiplaque/antigingivitis dentifrice. Am J Dent 2000;13:14C-7.
[45]. Kato T, IijimaH, Ishihara K, Kaneko T, Hirai K, Naito Y. Antibacterial effects of Listerine on oral bacteria. Bull Tokyo Dent Coll 1990;31:301-7.
[46]. Gislene GF, Paulo C,Giuliana L. Antibacterial activity ofplant extracts and phyto chemicals on antibiotic resistant bacteria. Braz J Microbiol 2000;31:314-25.
[47]. Hili P, Evans CS, VenessRG. Antimicrobial action of essential oils: The effect of dimethylsulphoxide on the activity of cinnamon oil. LettApplMicrobiol 1997;24:269-75.
[48]. Nzeako BC, Al-Kharousi ZS, Al-Mahrooqui Z. Antimicrobial activitiesof clove and thymeextracts. Sultan QaboosUnivMed J 2006;6:33-9.
[49]. Kovac J, Kovac D, Slobodnikova L, Kotulova D. Enterococcus faecalis and Candida albicans in the dental root canal and periapical infections. BratislLekárskeListy. 2013;114(12):716–20.
[50]. Amorim L de FG de, Toledo OA de, Estrela CR de A, Decurcio D de A, Estrela C. Antimicrobial analysis of different root canal filling pastes used in pediatric dentistry by two experimental methods. Braz Dent J. 2006;17(4):317–22.