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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF ANTIMICROBIAL EFFICACY OF ZINC OXIDE EUGENOL ZINC OXIDE WITH THYME OIL AND ZINC OXIDE WITH BLACKSEED OIL AGAINST ENTEROCOCCUS FAECALIS AND CANDIDA ALBICANS AS OBTURATING MATERIAL IN PRIMARY TEETH: AN IN VITRO STUDY

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Abstract

There has been tremendous increase in failure of pulpectomies due to re-infection in past years due to many organisms especially E. Feacalis. This necessitates the need for finding an obturating material with effective antimicrobial potential against these causative microbes.

Aim: To compare antimicrobial efficacy of Zinc oxide eugenol, Zinc oxide plus thyme oil and Zinc oxide with black seed oil against Enterococcus Faecalis and Candida Albicans.

Materials and Methods: This study was conducted on 40 Muller Hinton agar plates which were divided into three groups (n=20). 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°C for 24hrs in MH Broth. 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 with the help of Hi Antibiotic zone scale.

Results: The mean zones of inhibition were highest in for ZoBfollowed by ZoE and least for ZoE against Enterococcus faecalis. Similarly, ZoB had highest mean zones of inhibition, followed by ZoE and the least was ZoT against Candida albicans.

Conclusion: The antimicrobial efficacy was highest in ZoB against E faecalis and Candida albicans followed by ZoE and then by ZoT. All the test materials had better antimicrobial efficacy against Candida albicans compared to E faecalis.

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Introduction:-

Dental caries is a global disease caused by microorganisms resulting in demineralisation of inorganic portion and destruction of organic substances of the tooth.[1] In children presence of higher pulp horns and thinner enamel results in rapid involvement of pulpal tissues due to caries progression. The complex oral microbiota can infect not only root canal spaces but also colonizes isthmus, accessory canals, ramifications, apical delta etc. bacteria in these areas are polymicrobial in nature and predominantly anaerobic type. These organisms are difficult to eliminate through cleaning and shaping as 35 -53 % of the canals remain uninstrumented during the process. Although chemo mechanical process decreases bacterial load from canals but is not enough to get rid of them completely.[2]

Most of endodontic failures occur due to persistence of these microorganisms especially E. Faecalis and C. Albicans which inhabit the inaccessible areas of the root canals. Literature has stated presence of streptococci, black pigmented bacilli and E. Faecalis as predominant organisms in reinfected primary teeth.[3]

E. Faecalis is a gram-positive facultative anaerobe which is considered notorious for root canal failure and persistent infections. It cannot be eliminated easily due to its ability to survive in aerobic and starving conditions of root canal.

C.Albicans, a fungal species has been found in root canals at an incidence rate of 1-17% and due to presence of their hyphal formation and thrgmotropism it penetrates deeper into dentinal tubules making its elimination difficult & thus found to be associated with failure cases.[4]

Zinc oxide eugenol has been widely and frequently used as root canal obturating material in primary teeth but over the time certain shortcomings like slower rate of resorption in comparison to primary roots, tooth discolouration, periapical tissue irritationand limited antibacterial activity have surfaced making it a compulsory need to find out a better alternative in this regard.

Thyme oil is derived from the flowers of various plants belonging to genus Thymus and is considered as a therapeutic agent since long. Various agents like thymol, P-cymene, terpinene, caryophyllene, etc have shown to have potent antimicrobial, antifungal and antioxidant properties. Thoshar et al tested different essential oils to evaluate minimum inhibitory concentration and found lower concentrations to be used for E. Coli and higher for E. Feacalis and S. Aureus.[5]

Blackseed oilknown as “prophetic medicine”is derived from Ranunculacea family that have been used since ancient times for various ailments specially in Arab civilizations. It contains many active components like thymoquinone, alkaloids (nigellicines, nigelledine), saponins (heredin), flavonoids etc have anti-inflammatory, anti-oxidant and immunomodulatory effects and can be effectively used against microbes producing inflammation.[6]

Thus, we need to find out a root canal filling material which has effective antibacterial properties, ability to neutralise toxic bacterial products and prevent re infection of the root canals. These materials should create a conducive and favourable environment for healing process to take off in peri radicular as well as furcal areas.

So the aim of this study is to find out antibacterial efficacy of zinc oxide mixed with thyme oil and zinc oxide mixed with blackseed oil in comparison to zinc oxide eugenol against E. Faecalis and C. Albicans in failed or persistent root canal infections in primary teeth.

Materials And Methods:-

This study was conducted on 40 Muller Hinton (MH) agar plates. The no. of samples for all three groups were 20 in each. Three wells of 6mm diameter and 4mm deep were dug by the removal of the agar at equidistant points from each other with the help of an open-ended micropipette.

These wells were filled with three test materials on in each. The test materials were prepared by mixing one scoop of zinc oxide powder (0.2g) and 7 drops of oil on a dry sterile glass slab.

The standard strains of E. Faecalis (ATCC 29212) and C. Albicans (ATCC 14053) were used in this study. The strains were first revived and inoculated under laminar air flow to prevent any kind of contamination. Cultivation of these organisms was done in MH broth at 37 degree for 24 hours to produce fresh working cultures. After this, they

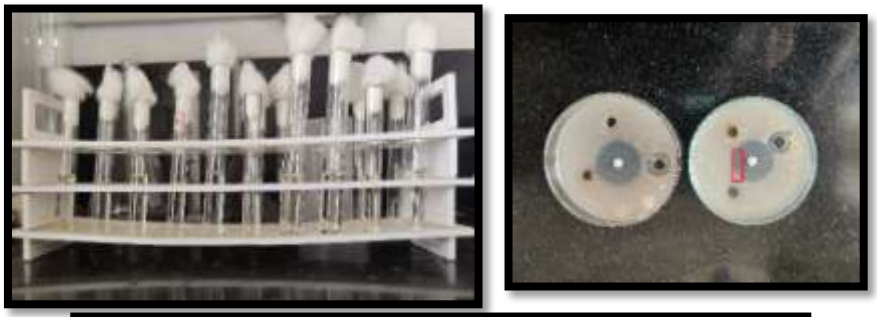
were seeded in MH agar to develop 0.5 turbidity on Mc Farland scale corresponding to 10^8 CFU/ml concentrations. This is called as standardization of the inoculum.

After the production of cultures, the wells were dug and test materials were injected into them directly. After this, the plates were maintained at room temperature for 2 hours for pre diffusion of test material into the culture plates and then incubated for 24 hours at 37 degrees temperature.

After the incubation period was over, the zone of inhibition surrounding the test material were measured using ZOI reader.

Strains of E. Feacalis&C. Albicans

Muller hinton broth and agar



Zone of inhibition of various test materials

Statistical Analysis: -

The recorded data was compiled and entered in a spreadsheet (Microsoft Excel) and then exported to data editor of SPSS Version 20.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as Mean±SD. Analysis of variance (ANOVA) was employed for comparing antimicrobial efficacy between the groups and Student's t-test was applied to compare antimicrobial efficacy within the groups. A P-value of less than 0.05 will be considered statistically significant.

Results:-

The data shows a statistically significant difference in the zones of inhibition of the different test materials against E. Faecalis and C. Albicans. Zinc oxide mixed with blackseed oil (ZnOB) showed highest mean of zone of inhibition (ZOI) of 44.2 mm against C. Albicans followed by zinc oxide eugenol (ZnOE) with a mean of ZOI 40.4 mm and zinc oxide thyme (ZnOT) showed mean ZOI as 26.8 mm.

Similarly, Zinc oxide mixed with blackseed oil (ZnOB) showed highest mean of zone of inhibition (ZOI) of 26.5 mm against E. Faecalis followed by zinc oxide eugenol (ZnOE) with a mean of ZOI 22.2 mm and zinc oxide thyme

(ZnOT) showed mean ZOI as 12.1 mm. ZnOB showed greater zone of inhibition against C. Albicans than E. Faecalis

Table 1: Comparison of the antimicrobial efficacy between the groups

Micro organism	Obturating Material	N	Mean	SD	Range	F-value	P-value
Enterococcus faecalis	ZNOE	20	22.2	2.94	18-28	151.06	<0.001*
	ZNOT	20	12.1	1.62	10-15		
	ZNOB	20	26.5	3.20	20-32		
Candida albicans	ZNOE	20	40.4	3.70	32-45	130.83	<0.001*
	ZNOT	20	26.8	3.48	22-32		
	ZNOB	20	44.2	3.58	38-50		

*Statistically significant (P-value<0.05)

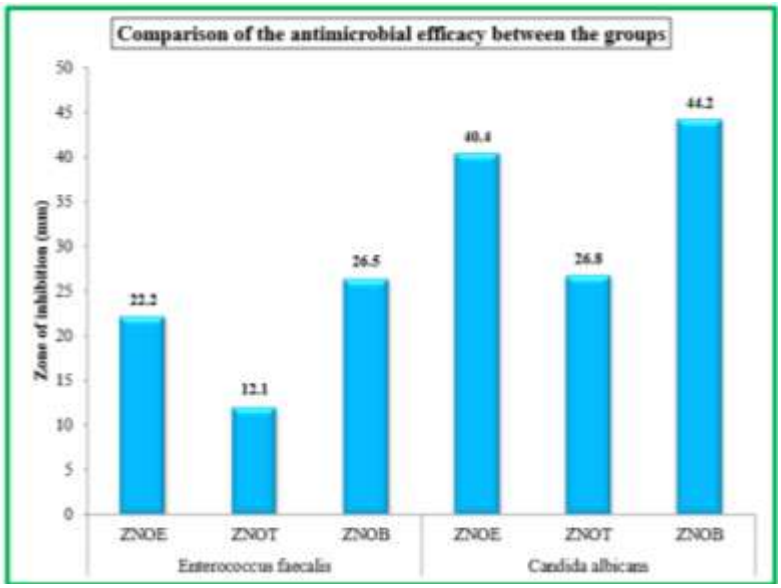
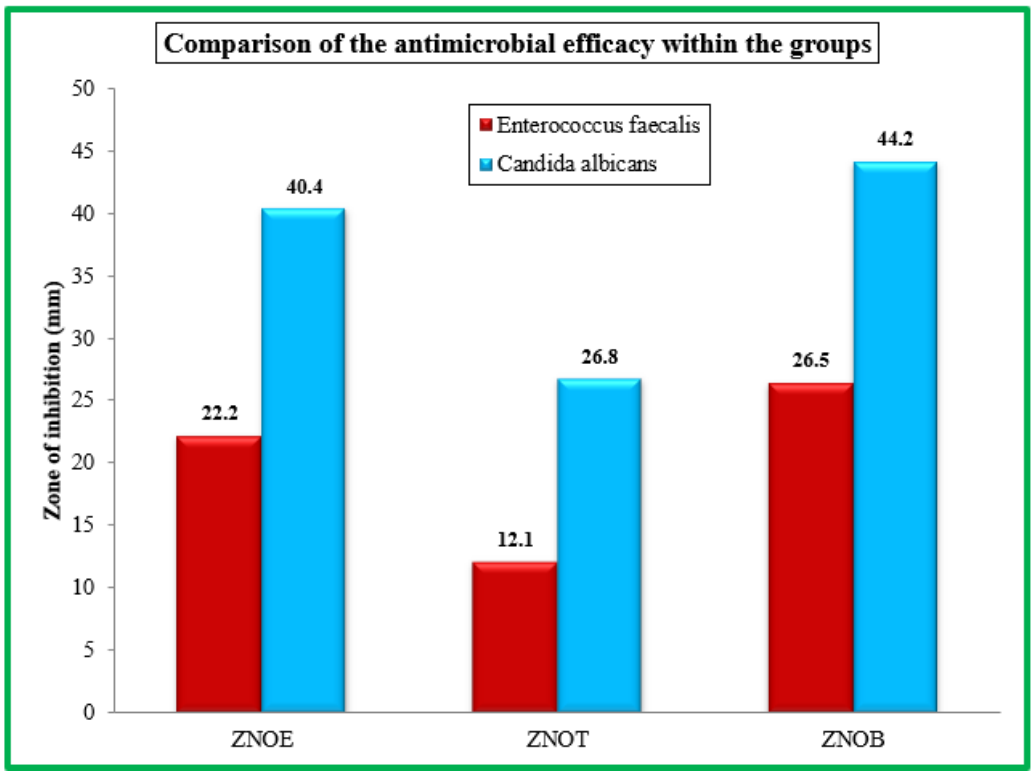


Table 2: Comparison of the antimicrobial efficacy within the groups

Obturating Material	Enterococcus faecalis		Candida albicans		t-value	P-value
	Mean	SD	Mean	SD		
ZNOE	22.2	2.94	40.4	3.70	17.25	<0.001*
ZNOT	12.1	1.62	26.8	3.48	17.08	<0.001*
ZNOB	26.5	3.20	44.2	3.58	16.53	<0.001*

*Statistically significant (P-value<0.05)



Discussion:-

Antibacterial activity is considered as one of the most important and desired properties for an ideal obturating materialto be used in 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 torturous nature of the root canals in deciduous teeth. Also, these components should not cause any specific toxicity on the host cells resulting in irritation and inflammation. Therefore, biocompatibility of the antibacterial components is very important factor when selecting it as an obturating and sealer material.[7]

Enterococcus faecalis is a gram positive, facultative anaerobic microorganism found persistently in peri-apical infections and failed root canal treatment. Rocas et al concluded that the prevalence of E Faecalis is 4-40% in primary endodontic infections and 67-77% in secondary infections. It is seen consistently because E. Faecalis forms a defensive biofilm capable of blocking lymphocytic function, making it 1000 times more resistant to phagocytosis and antimicrobialsalongwith its ability to endure prolonged periods of nutritional deprivation.[8,9] Above all, relative to other species, E. faecalis rapidly colonizes the dentinal tubules and is therefore especially difficult to eradicate. Due to all these reasons, it represents a standard against which the antimicrobial action of an intra-canal medicament is tested.[10]

Candida albicans has invasive affinity to dentin making it one of the most commonly found organism in the root canals. The study conducted by Baumgartner et al assessed the presence of Candida albicans from abscess of endodontic origin and contaminated root canals using the PCR method and found presence of Candida albicans.[11]

Bonastre (1837) found zinc oxide eugenol, which was later pioneered by Chisholm (1876) in dentistry. Sweet recommended Zinc oxide eugenol paste as the first root canal filling material forthe primary teeth and since then has gained tremendous popularity in dentistry. ZOE has historically been the material of choice for rootcanal fillings in primary teethand was clinically recommended by the American Academy of Pediatric Dentistry until 2008.[12,13] The antimicrobial effectiveness of zinc oxide eugenol is due to its eugenol content acting on the lipids of the bacterial cell membrane and mitochondria, rendering them more permeable which is in accordance withOyedemi et al 2009.[14] Hashieh conducted a study which show that the level of eugenol released from a zinc oxide-eugenol paste 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. However, it has disadvantages like slow resorption, deflection of permanent tooth bud, tooth discoloration, irritation to periapical tissues which led to the quest of some biocompatible and equally effective material for root canal filling in primary teeth.[15]

In the present study eugenol has been compared to blackseed oil and thyme oil as these plant essential oils have been found to exhibit antimicrobial activity against E faecalis and Candida albicans commonly found in endodontic infections.

Studies done by Markowitz K et al. and Saggar V et al. concluded that Zinc oxide eugenol were more superior in inhibiting the micro-organisms found in primary root canal system.[16,17] In 2015, Rahul et al stated that ZoE had the highest zones of inhibition against E Faecalis and C albicans which is contradictory with the results of the present study. Zones of inhibition were seen highest with ZoEwhen compared to other obturating materials in the study done by Shipra et al, Navit et al which are not in accordance with present study.[18] The reason accorded to this fact is that zinc oxide eugenol was compared to traditional obturating materials and not with essential oils that are known to have better antibacterial properties.

Verma et al stated that Zinc oxide eugenol (ZoE) had comparable antimicrobial effect with other obturating materials when used against E Faecalis in the primary root canal treatment. Similarly, Queiroz AM et al concluded that ZoE had the highest antibacterial effect when compared with other materials used against E Faecalis.[19,20]

A more recent retrospective evaluation by Bahrololoomi and Zamaninejad was done in to find out the success rate of ZOE as an obturating material for a mean follow-up period of 24 months and found it to be reliable material.

In a study done by Nilima et al and Thosar et al, the antimicrobial efficacy of eesential oils had the highest antifungal and antibacterial effect than seen with eugenol which affirms the results of the present study in which also eugenol had less antibacterial effect than essential oils.[22,23]

The result of this study concluded that ZoBwas found to be superior to Zinc oxide with thyme oil and Zinc oxide with eugenol 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. [22,23]

However, blackseed oil has been used an irrigant against E. faecalis and has proved better antibacterial efficacy against E. Feacalisthan sodium hypochlorite which is considered as a gold standardas an irrigant reported by Jain et al (2018)[24] and it was due this that blackseed oil was used in our study to evaluate its effectiveness as a root canal filling material in primary teeth.

Conclusion:-

The rationale for performing this in vitro study was to provide information that zinc oxide with blackseed oil has the highest antimicrobial efficacy followed by zinc oxide with eugenol and then zinc oxide with thyme 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.

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