Research Article

AHB Advances in Human Biology

Adv Hum Biol. 2015;5(2):78-87.

Evaluation of Antimicrobial Efficacy of Ozonated Sesame Oil, Calcium Hydroxide and their Combination as Intracanal Medicament against Candida Albicans: An in-vitro study

K Sashidhar Reddy[1] S Datta Prasad[2] P Durga Sirisha[3*] V Prashanth[4]

[1]Professor, Department of Conservative Dentistry and Endodontics, C.K.S.Theja Institute of Dental Sciences, Tirupati, Andhra Pradesh, India.
[2]Professor and Head, Department of Conservative Dentistry and Endodontics, C.K.S.Theja Institute of Dental Sciences, Tirupati, AP, India.
[3]Post Graduate Student, Department of Conservative Dentistry and Endodontics, C.K.S.Theja Institute of Dental Sciences, Tirupati, AP, India.
[4]Post Graduate Student, Department of Conservative Dentistry and Endodontics, C.K.S.Theja Institute of Dental Sciences, Tirupati, AP, India.

ABSTRACT

Aim: This in vitro study was done to evaluate the antimicrobial effect of intracanal medicament in root canals contaminated with Candida *albicans.*

Materials and Method: Twenty four extracted human single rooted teeth were selected. Access preparations and biomechanical preparations were done. Specimens were first sterilized and contaminated with Candida and incubated for 48hrs. Confirmation of Candida was done and then divided into 3 experimental groups and 2 control groups. The experimental groups were having 6 teeth each and they were treated with A) Ozonised oil, B) Calcium hydroxide, C) Ozonised oil + Calcium hydroxide respectively. The control groups were further subdivided in 1) Negative control and 2) Positive control with two teeth each. Intracanal medicament was placed into each root canal corresponding to the groups. First sampling was done after 48hrs and second or final sampling was done after one week of placement of intracanal medicament. Microbial growth was checked by counting CFU (Colony forming units).

Results: In the first sampling ozonised oil was 100% efficient followed by Calcium hydroxide group while combination of Ozonised oil + Calcium hydroxide showed similar results. In the second or final sampling after one week ozonised oil was highly efficient when compared to other groups. Calcium hydroxide was moderately efficient whereas combination of ozonised oil + calcium hydroxide was least effective as it showed highest CFU/ml.

Conclusion: Ozonised oil was most effective for longer duration when compared to other groups and can be used as an alternative intracanal medicament.

Keywords: Candida albicans, Ozone, Root canal.

INTRODUCTION

Microorganisms may survive upto 40-70% of their initial concentration even after Chemo mechanical preparation[1,2]. Among microbes Candida albicans & E.faecalis were found to be most resistant & seen most commonly in persistent or failing root canals. As these are facultative anaerobes they can survive under high pH. Such cases require inter appointment dressing with an intracanal medicament. Calcium Hydroxide has been most commonly used intracanal medicament.

Received: Aug. 11, 2014: **Accepted:** Jan. 4, 2015
*Correspondence Dr. P Durga Sirisha.
Department of Conservative Dentistry and Endodontics, C.K.S.Theja Institute of Dental Sciences, Tirupati, Andhra Pradesh, India.
Email: sirishaputchala@gmail.com

pISSN 2321-8568
eISSN 2348-4691

But even calcium hydroxide $Ca(OH)_2$ could not completely eradicate these species as it is based on high pH and needs direct contact. Moreover its low solubility & diffusibility makes it difficult to cause an increase in pH and gets neutralised by buffering system and acids present in deeper layers of dentin thus decreasing its bioavailability[3]. Moreover $Ca(OH)_2$ provides Ca ions necessary for growth of Candida, hence it becomes ineffective against resistant species[4].

Various forms of OZONE- Gaseous, water and oil based have been used as irrigant & intracanal medicament in root canal disinfection. Ozone therapy has been found to be more efficient on anaerobic bacteria which are the predominant species in the oral cavity[2]. Among these ozonated water is least cytotoxic than gaseous ozone and other irrigants like NaOCl, CHX and H_2O_2, but lacks residual effect (half-life 40min, 20ºc) and needs to be freshly prepared[5,6].

Ozonised oil because of its viscosity remains in the root canal for prolonged periods, thus facilitating its use as an intracanal medicament[7]. The use of ozonated oil is still not widely used in dentistry and very few studies have been done[8] on its use. Sesame oil has been selected in this study because of its cost effectiveness, antimicrobial properties, prolonged half-life & healing effect[9]. Apart from sesame oil various plant extracts, ozonides of olive oil, castor oil, almond oil, carthame oil, peanut oil, jojoba oil, macadamia oil, theobroma oil, soybean oil, coconut oil, linseed oil, thistle oil, wheat germ oil, croton oil, safflower oil, avocado oil, Murmuru Butter, Cupuacu Butter, Caprylic/Capric Triglyceride, Shea Butter, Rice Bran Oil, Argan Oil, Camellia Oil, Rosehip Seed Oil, Dilo Nut Oil, Pomegranate Oil, Red Raspberry Seed Oil, evening primrose oil, Moluccana Oil, palmarosa oil, rosewood oil, sunflower seed oil, pumpkin seed oil, Grapeseed oil and soybean oil have also been used in other studies[10,11].

History

Ozone was first observed by a German chemist Christian Friedrich Schonbein[12] in 1840 when he detected an "Odorful Gas" on passing electrical discharge through water (Ozen = Odor).

AIM

The purpose of this study was to evaluate the antimicrobial effect of ozonated sesame oil, calcium hydroxide and their combination as intracanal medicament in root canals contaminated with Candida albicans.

MATERIALS AND METHODS

Sample preparation

Twenty four freshly extracted human single rooted teeth were selected. Access cavity preparations were done and working length 1mm short of apex was determined (Figures 1 and 2). Biomechanical preparation was done by step back technique upto size 50 k-file. During instrumentation 3ml of 3% NaOCl irrigant was used.

Then they were subjected to 17% EDTA for 3min to remove smear layer followed by irrigation with 5ml of 0.9% Normal Saline to wash out the residual irrigants, NaOCl & EDTA from the canal. Root apices were sealed with sticky wax and then root surfaces were coated with nail varnish with specific colour coding for group identification except the cervical openings. All the specimens were sterilized in an autoclave at 134ºC, 32 psi for 5 min.

Samples were divided into 5 groups - 3 Experimental Groups: A) Ozonised Oil, B) Calcium Hydroxide, C) Ozonised Oil + Calcium Hydroxide

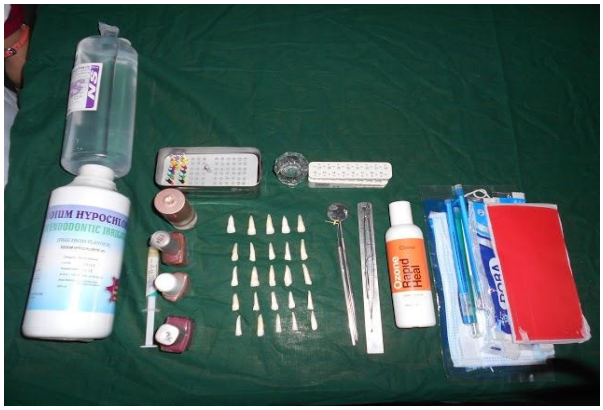

Fig 1: Materials required.

Fig 2: Samples.

Fig 3: Confirmation of candida albicans growth by CFU.

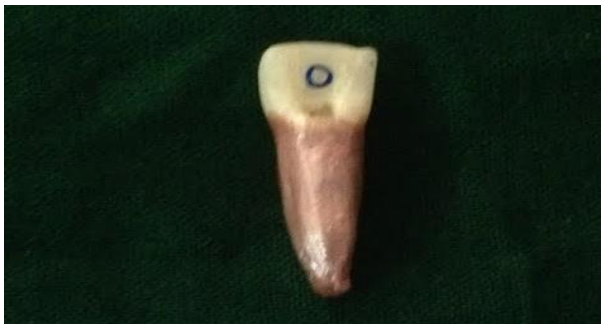

Fig 4: Ozonised oil group.

Fig 5: Calcium hydroxide group.

Fig 6: Ozonised oil + calcium hydroxide group.

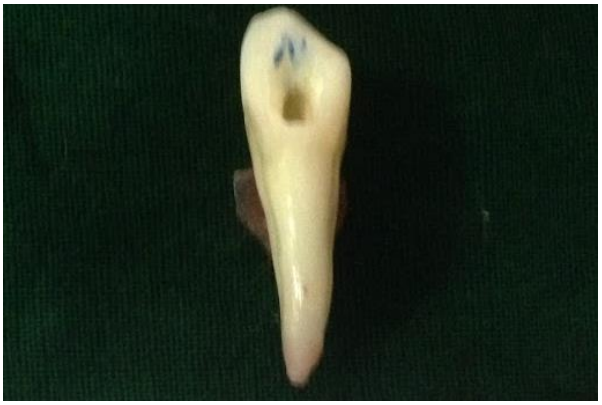

Fig 7: Negative control group.

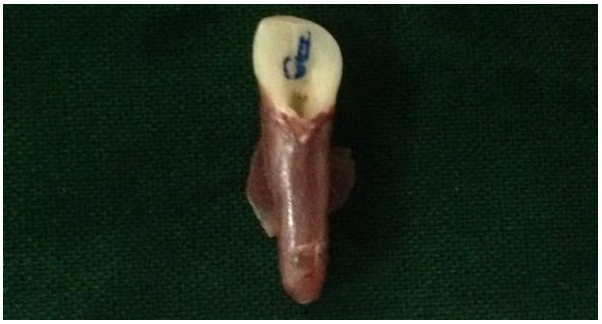

Fig 8: Positive control group.

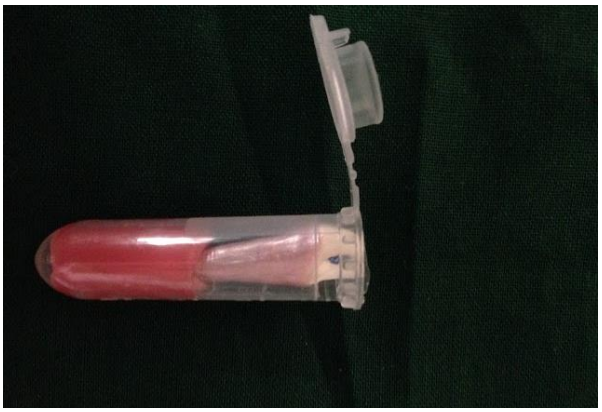

Fig 9: Tooth suspended in eppendorf tube.

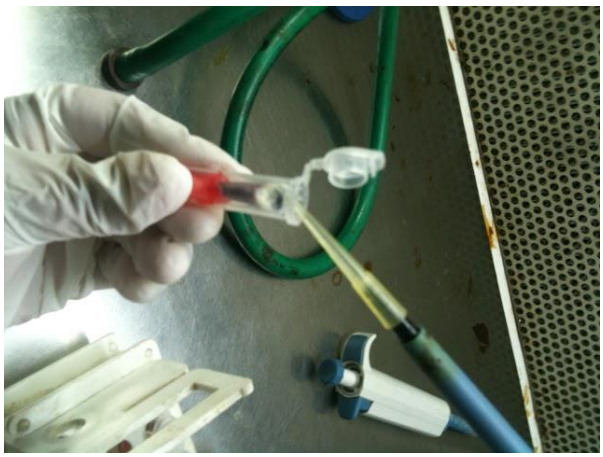

Fig 10: Intracanal medicament carried specimen using automated micropipette.

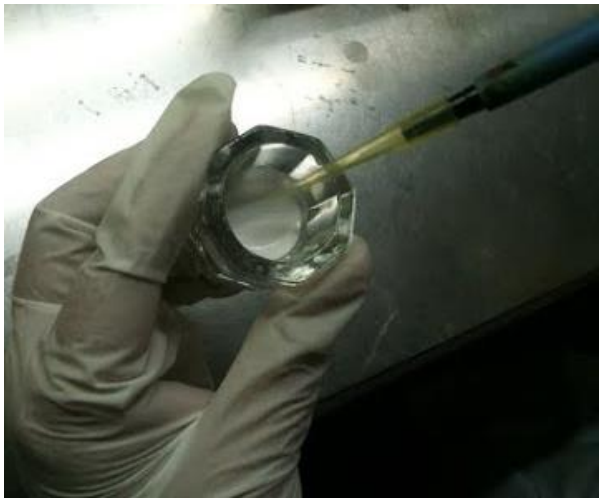

Fig 11: Preparation of Ca(OH)2 solution.

Fig 12: Intracanal medicament ozonated oil.

Fig 13: Intracanal medicament Ca(OH)2 powder.

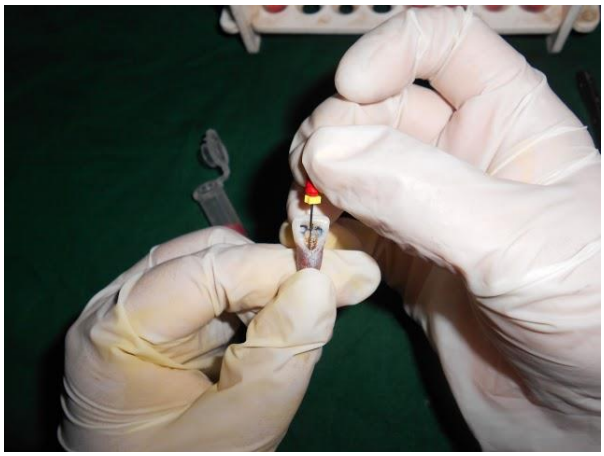

Fig 14: Dentinal scrapings collected from root canals using k files.

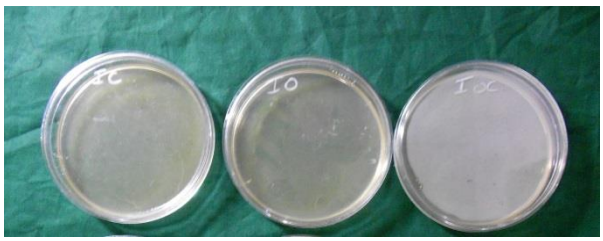

Fig 15: First sampling result- calcium hydroxide of ozonised oil + calcium hydroxide.

2. Control Groups: D) Negative Control- No contamination and no treatment was done. E) Positive Control- Contamination was done but no treatment.

Samples of corresponding groups with specific colour coding were suspended in eppendorf tubes.

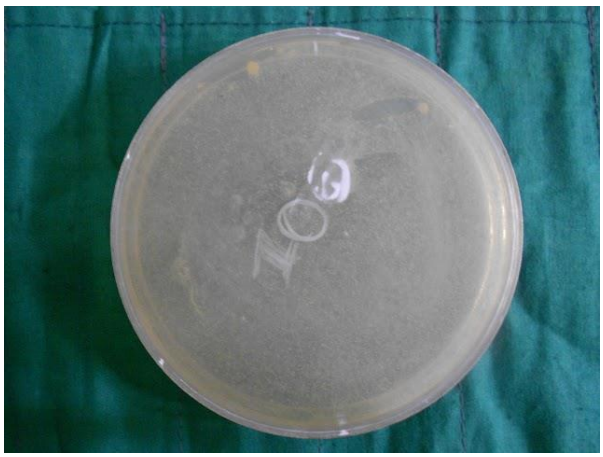

Fig 16: Second sampling result-Ozonised oil.

Fig 17: Second sampling result-calium hydroxide.

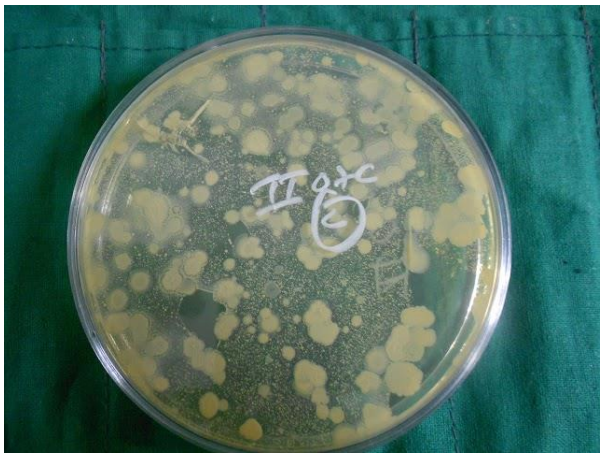

Fig 18: Second sampling result-Combination of Ozonised oil+ Calcium hydroxide.

Microbial preparation

All microbial procedures were performed under aseptic conditions, in laminar flow chamber. Candida albicans was previously cultivated on sabouraud's dextrose agar medium. Microbial suspension was prepared to match turbidity of $1.5x10^8$ cfu/ml (equivalent to 0.5Mc Farland standard). 10µl of microbial suspension was inoculated into each root canal with automated micropipette and cervical openings were sealed with temporary cement. Microplates containing specimens were incubated at 37ºc for 48hrs.

After this period of incubation contamination confirmation of Candida was done as follows 1) direct visualization which appeared as creamy/white colored, smooth pasty colonies. 2) Counting of colonies. Except negative control all the other groups showed similar counts. Negative control was zero (Figure 3). 3) Germ tube test – rapid quantitative production of chlamydospore was assessed. Two different culture media (corn milk broth+5% milk) and serum milk was inoculated into it &placed in a water bath at 45ºc & results were read after 8 & 16 hrs. Chlamydospores formation was observed under wet conditions when stained with LPCB (Lactophenol cotton blue stain), which is a confirmatory test for Candida albicans. Grouping was done & intracanal medicament was placed into 3 experimental groups (n=6).

Intracanal medicament Preparation and Placement-

A) Ozonated oil - ozonated oil was carried into canals with automated micropipette in 1:2 ratio of microbial suspension i.e, 10µl of candida : 20µl intracanal medicament ozonated oil. (Figure 4)

B) Calcium hydroxide- $Ca(OH)_2$ solution preparation- 48gm % of $Ca(OH)_2$ is present in commercially available $Ca(OH)_2$ powder. 1ml of $CaOH_2$ solution with sterile water was prepared. From this 20µl was taken & inoculated into each canal with micropipette. (Figure 5). The prepared $Ca(OH)_2$ solution was carried into canals with automated micropipette in 1:2 ratio of Microbial suspension i.e, 10µl of Candida : 20µl $Ca(OH)_2$ intracanal medicament.

C) Ozonated oil + Calciumhyroxide-10µl Candida Microbial suspension : 10µl of Ozonated oil + 10µl of $Ca(OH)_2$ solution of intracanal medicament [Figure 6] was inoculated in canals.

D) Negative control-no contamination and no treatment was done (Figure 7).

E) Positive control-contamination was done but no treatment (Figure 8).

Intracanal medicament was placed into each root canal of corresponding group and sealed with temporary cement (ZnO) (Figures 9-13).

Sampling

First sampling was done after 48hrs of placement of intracanal medicament. Dentinal scrapings were collected from all root canals using k-files, H-files and paper points & transferred into eppendorf tubes containing 1ml of peptone water which is the nutrient medium for growth of microbes (Figure 14). Then the tubes were subjected to agitation for 1 min. Aliquots of 0.1ml were seeded into petridishes containing sabouraud's dextrose agar medium & incubated at 37ºC for 48hrs (Figure 15). After this period microbial growth was measured by CFU/ml. After sampling the root canals were sealed with temporary cement.

Second sampling (Figures 16-18) was taken after 1week of placement of intracanal medicament. Samples were collected from root canals similar to first sampling. Results were submitted to logarithmic transformation. Kruskal-Wallis and Dunn's tests were used for comparison among the groups. Friedman's test was used for comparison among the samples within each group. The significant level was set at 5% for all analyses.

Table 1: After inoculation growth of candida into root canals of specimens of all the groups

Groups	1	2	3	4	5	6	Mean	Std dev
A	610	615	613	608	606	611	3663	3.271085
B	620	612	624	610	621	618	3705	5.43139
C	625	621	623	624	625	620	3738	2.097618
D	0	0	0	0	0	0	0	0
E	630	628	626	629	625	623	3761	2.639444

[A=Ozonised oil group, B=Calcium hydroxide, C=combination of Ozonised oil + calcium hydroxide, D=Negative control,E=positive control].

Table 2: Growth of candida after 48hrs of placement of intracanal medicament of specimens of all the groups

	1	2	3	4	5	6	Mean	Std dev
A	0	0	0	0	0	0	0	0
B	0	1	0	2	0	0	3	0.83666
C	1	0	3	0	1	0	5	1.169045
D	0	0	1	2	0	0	3	0.83666
E	632	628	628	629	626	622	3765	3.331666

Table 3: Candidal growth after 1 week of placement of intracanal medicament of specimens of all the groups.

	1	2	3	4	5	6	Mean	Std dev
A	2	21	11	1	0	2	37	8.280499
B	47	0	62	0	42	23	174	25.69047
C	122	74	91	108	33	39	467	36.2404
D	0	3	4	5	1	2	15	1.870829
E	632	630	629	633	624	623	3771	4.135215

Table 4: Amount of candidial growth in all groups at different time intervals.

Groups	Initial	After 48hrs	After 1week
A) Ozonated Oil	3663 ± 3.2	0 ± 0	37 ± 8.2
B) Calcium Hydroxide	3705 ± 5.4	3 ± 0.83	174 ± 25.6
C) Ozonated Oil + Calcium Hydroxide	3738 ± 2.0	5 ± 1.16	467 ± 36.2
D) Negative Control	0 ± 0	3 ± 0.8	15 ± 1.87
E) Positive Control	3761 ± 2.63	3765 ± 3.33	3771 ± 4.13

RESULTS

Microbial colony count (10^5) was carried out in initial, post medication (after 48hrs) & final sample (after 1week) phase.

The data was statistically analyzed with Kruskal-Wallis test & Dunn's Post-Hoc test to assess the differences in antimicrobial efficacy between groups. Friedman's test was used for comparison among the samples within each group ($p< 0.05$). The initial sample revealed similar CFU/ml for all groups except negative control. The highest microbial count was observed in positive control.

After 48hrs in post medication samples Ozonised oil showed lowest CFU/ml. $Ca(OH)_2$ and Ozonised oil+Ca(OH)2 combination also showed similar results. After one week ozonised oil group revealed lowest counts, Calcium hydroxide group showed moderate CFU/ml and combination of ozonised oil+calcium hydroxide group showed highest CFU/ml. Ozonised oil was proven most efficient intracanal medicament. Calcium hydroxide second best, whereas combination of ozonised oil + calcium hydroxide was proven least effective as it showed highest colonies.

DISCUSSION

Candida albicans a dimorphic fungi can be found in secondary or persistent infections in root canals. This species has the ability to colonize and invade the dentin and seems to be resistant to calcium hydroxide dressing[13].

Irrigating solutions such as NaOCl and CHX have a wide spectrum of action on the microorganisms present in endodontic infections. However, during the treatment they act for a short time and often cannot penetrate inside some parts of the root canal system. Therefore, the use of intracanal dressings is necessary to allow a longer duration of action against microorganisms in root canals and prevent the proliferation of microorganisms, acting as a mechanical barrier to reinfection[14].

$Ca(OH)_2$ intracanal medicament is most appropriate for teeth with apical lesions and healing rates improving to about 10%. Histological periapical repair after obturation of infected root canals in dogs revealed better healing with $Ca(OH)_2$ in two appointments than one appointment[15].

Calcium hydroxide alone is less effective against C. albicans[16]. Hasselgren et al found $Ca(OH)_2$ to improve debridement efficacy of NaOCl when root canals were pretreated with $Ca(OH)_2$. It enhanced the tissue-dissolving capability of sodium hypochlorite, and so conferred an advantage to multiple-visit root canal treatment where NaOCl would be used following a period of $Ca(OH)_2$ medication[17].

Waltimo et al and Mohammadi et al stated that Calcium hydroxide has been found to be ineffective against C. albicans[16]. They demonstrated that *C.* albicans is highly resistant to $Ca(OH)_2$. Because *C.* albicans survives at a wide range of pH values, the alkalinity of saturated $Ca(OH)_2$ solution may not have any effect on *C.* albicans. In addition, $Ca(OH)_2$ pastes may provide the Ca ions necessary for the growth and morphogenesis of Candida. These mechanisms may explain why $Ca(OH)_2$ has been found to be ineffective against *C.* albicans[18].

Combination of $Ca(OH)_2$ with CMCP has previously been shown to be more capable of inhibiting the growth of bacteria than CHX and $Ca(OH)_2$ combined with sterile saline[19]. However CMCP was found to be cytotoxic to the target periodontal ligament cells by inhibiting cell viability

and proliferation[20]. $Ca(OH)_2$ accelerates the setting time of eugenol sealers. The interaction between calcium hydroxide and zinc oxide eugenol sealer, at the time of root-canal filling can affect the retention of calcium hydroxide on the canal wall compromising the quality of the seal and influences the prognosis of treatment[21,22]. In the present study Ca(OH)2 was found to be moderately efficient against Candida albicans. This is in agreement with other studies done by Silveira et al and Roberta Vieira Farac et al.

Ozone is chemical compound consisting of 3 oxygen atoms (triatomic oxygen). It is one of the most important gases in stratosphere due to its ability to filter UV rays which is critical for the maintenance of biological balance in the biosphere. Ozone is produced naturally from electrical discharges following thunderstorms when oxygen molecule receives an electrical discharge breaking into two O_2 atoms. The individual atoms combine with other O_2 molecule to form O_3[23]. Medical grade ozone is a mixture of pure oxygen and pure ozone in the ratio of 0.05% to 5% of O_3 with 95% to 99.95% of O_2[24].

Use of Ozone as a disinfectant started from 1881. During World War 1, Ozone was used medically to treat wounds and other infection. There is evidence in literature since 1990 of use of O_3 in dentistry. In dentistry, it is used as gaseous form, ozonated water and as ozonated oils. Artificially there are 3 different systems of generating O_3 gas-1) Ultra violet system-produce low concentrations of O_3. 2) Corona discharge system-produces high concentrations of O_3. 3) Cold plasma system. Nagayoshi et al in 2004 have shown that O_3 water has almost the same antimicrobial activity as 2.5% NaOCl, especially in combination with ultrasonic canal treatment, with low cellular toxicity. Ozone water can be considered to be a potential root canal disinfectant and is less cytotoxic than NaOCl which can cause necrosis[25]. It can be used can be used as an irrigant and intracanal medicament in root canals also.

In a study conducted by Chandra et al Ozonated oil with ZnO combination demonstrated good clinical & radiographic success at 12 months follow up & so can be considered an alternative obturating material in infected primary teeth[26].

Since O_3 is unstable in gas form, O_3 oil was used in this study. The O_3 present in oily vehicle could have advantages over gaseous or aqueous media[27,28]. Since the oil remains in contact with the surface of root canal for prolonged period of time, it exercises its functions for a longer period[29].

Preparation of Ozonated oil:

Bubbling of O_3 gas through plant, vegetable extracts which are rich in omega-3, 6 and 9 unsaturated fatty acids is done. They contain double bonds between Carbon atoms and contain 3 double bonds in omega-3 fatty acid (Leinolenic acid), 2 double bonds in omega 6 fatty acid (Leinoleic acid), and 1 double bond in omega -9 fatty acid (Oleic acid). They react with O_3– to form ozonoids– aldehydes, ketones, peroxides i.e Reactive Oxygen Species (ROS) and Lipid Oxidation Products (LOP)[30]. O_3 is strong oxidising agent which is responsible for antimicrobial property.

Mechanism of action- Ozonoids can induce disruption of microbial cell wall (Lipopolysaccharide moiety) & cell membrane, unsaturated fatty acids in the oil may also have antimicrobial effects, which can be due to their incorporation in the cytoplasmic membrane, inducing lethal structural perturbations, disruption of the membrane integrity and release of intracellular constituents[31]. Therefore, the antimicrobial activity of the ozonized oil may be result of action of aldehydes, unsaturated fatty acids, and hydrogen peroxide. Indeed, the oxidant effects of hydrogen peroxide may help to explain the excellent antibacterial effects of ozonized oil on anaerobic bacterial species commonly found in endodontic infections[32].

Efficiency Grading

Ozonized oils are available in three cncentrations i.e. Strong- >3000 meq, medium – 1500 to 1700 meq and light - <1000meq.

Middle concentration Peroxide Value (PV) – 1500 to 1700 meq has the most beneficial effect in accelerating the wound closure ratio. (The doses are given by Ozone Forum of India providers of ozonated sesame oil). Ozonated sesame oil used in the present study had PV-1500-700meq (Ozorie, Ozone Forum of India). Ozonated oils can augment

the wound healing process and is being used in various fields of medicine.

CONCLUSION

Ozonised Oil was proven effective & can be used as an alternative intracanal medicament because of its prolonged activity, antimicrobial & wound healing properties. It also saves time of the clinician as it can be used alone rather than using calcium hydroxide in combination with other medicament.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Silveira AMV, Lopes HP, Siqueira JF Jr, Macedo SB, Consolaro A. Periradicular Repair after Two-Visit Endodontic Treatment Using Two Different Intracanal Medications Compared to Single-Visit Endodontic Treatment. Braz Dent J. 2007;18(4):299-304..

2. Halbauer K, Prskalo K, Janković B, Tarle Z, Pandurić V, Kalenić S. Efficacy of ozone on microorganisms in the tooth root canal. Coll Antropol. 2013;37(1):101-7.

3. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: a systematic review and meta-analysis. Int Endod J. 2007;40(1):2–10.

4. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endo J. 2011;44(8):697–730.

5. Chirag M. Raiyani, Ruchi Arora, Deepak P. Bhayya. Ozone (Dentistry towards the greenery) – A Review. International Journal of Biomedical Research 2015;6(03):155-9.

6. Mohammadi Z, Shalavi S, Soltani MK, Asgary S.. A Review of the Properties and Applications of Ozone in Endodontics: An Update IEJ Iran Endod J. 2013;8(2):40-43.

7. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Med Res Rev. 2009;29(4):646–82.

8. Farac RV, Pizzolitto AC, Tanomaru JM, Morgental RD, Lima RK, Bonetti-Filho I. Ex-vivo effect of intracanal medications based on ozone and calcium hydroxide in root canals contaminated with Enterococcus faecalis. Braz Dent J. 2013;24(2):103-6.

9. Pai SA, Gagangras SA, Kulkarni SS, Majumdar AS. Potential of ozonated sesame oil to augment wound healing in rats. Indian J Pharm Sci. 2014;76(1):87-92.

10. E Mohamed Ali. Ozone application for preventing fungal infection in diabetic foot ulcer. Diabetologia Croatica 2013;42(1)3-22.

11. Travagli V, Zanardi I, Valacchi G, Bocci V. Ozone and ozonated oils in skin diseases: a review. Mediators Inflamm. 2010;2010:610418. doi: 10.1155/2010/610418. Epub 2010 Jul 4.

12. A SR, Reddy N, Dinapadu S, Reddy M, Pasari S. Role of ozone therapy in minimal intervention dentistry and endodontics - a review. J Int Oral Health. 2013;5(3):102-8.

13. Sen BH, Safavi KE, Spångberg LS. Colonization of Candida albicans on cleaned human dental hard tissues. Arch Oral Biol. 1997;42(7):513-20.

14. Siqueira JF Jr, de Uzeda M. Influence of different vehicles on the antibacterial effects of calcium hydroxide. J Endod. 1998;24(10):663-5.

15. Katebzadeh N, Hupp J, Trope M. Histological periapical repair after obturation of infected root canals in dogs. J Endod. 1999;25(5):364-8.

16. Mohammadi Z, Shalavi S, Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: a review. Chonnam Med J. 2012;48(3):133-40.

17. Hasselgren, G.; Olsson, B. & Cvek, M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endodon 1988;14:125-7.

18. Waltimo TM, Sirén EK, Orstavik D, Haapasalo MP. Susceptibility of oral Candida species to calcium hydroxide in vitro. Int Endod J. 1999;32(2):94-8.

19. Penesis VA, Fitzgerald PI, Fayad MI, Wenckus CS, BeGole EA, Johnson BR. Outcome of one-visit and two-visit endodontictment of necrotic teeth with apical periodontitis: a randomized controlled trial with one-year evaluation. J Endod 2008;34(3):251-7

20. Chang YC, Tai KW, Chou LS, Chou MY. Effects of camphorated parachlorophenol on human periodontal ligament cells in vitro. J Endod 1999;25(12):779-81.

21. Margelos J, Eliades G, Verdelis C, Palaghias G. Interaction of calcium hydroxide with zinc oxide eugenol type sealers: a potential clinical problem. J Endod. 1997;23(1):43-8.

22. Kim SK, Kim YO. Influence of calcium hydroxide intracanal medication on apical seal. Int Endod J, 2002;35(7):623-8.

23. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. J Contemp Dent Pract. 2008;9(4):75-84.

24. Ahmed J, Binnal A, Rajan B, Denny C, Shenoy N. Ozone applications in dentistry: an overview J Exp Integr Med. 2013;3(3):171-6.

25. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004;30(11):778-81..

26. Chandra SP, Chandrasekhar R, Uloopi KS, Vinay C, Kumar NM. Success of root fillings with zinc oxide-ozonated oil in primary molars: preliminary results. Eur Arch Paediatr Dent. 2014;15(3):191-5.

27. Oizumi M, Suzuki T, Uchida M, Furuya J, Okamoto Y. In vitro testing of a denture cleaning method using ozone. J Med Dent Sci. 1998;45(2):135-9.

28. Noetzel J, Nonhoff J, Bitter K, Wagner J, Neumann K, Kielbassa AM. Efficacy of calcium hydroxide, Er:YAG laser or gaseous ozone against Enterococcus faecalis in root canals. Am J Dent. 2009;22(1):14-8..

29. Guinesi AS, Andolfatto C, Bonetti Filho I, Cardoso AA, Passaretti Filho J, Farac RV. Ozonized oils: a qualitative and quantitative analysis. Braz Dent J. 2011;22(1):37-40.

30. Velio Bocci, Iacopo Zanardi, Valter Travagli. Oxygen/ozone as a medical gas mixture. A critical evaluation of the various methods clarifies positive and negative aspects. Med Gas Res. 2011;1(6):1-9.

31. Shapiro S. The inhibitory action of fatty acids on oral bacteria. Oral Microbiol Immunol. 1996;11(5):350-5.

32. Siqueira Junior JF, Rôças IN, Cardoso CC, Macedo SB, Lopes H.P. Efeitos antibacterianos de um novo medicamento—óleo ozonizado- comparados às pastas de hidróxido de cálcio. Rev. Bras. Odont. 2000;57(4):252–256.

How to cite this article:

Reddy KS, Prasad SD, Sirisha PD, Prashanth V. Evaluation of Antimicrobial Efficacy of Ozonated Sesame Oil, Calcium Hydroxide and their Combination as Intracanal Medicament against Candida Albicans: An in-vitro study. Adv Hum Biol. 2015;5(2):78-87.