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COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF NEEM, MORINDA CITRIFOLIA AND GREEN TEA EXTRACT WITH SODIUM HYPOCHLORITE AGAINST E.FAECALIS AND C.ALBICANS. AN INVITRO STUDY

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ABSTRACT

AIM : The aim of this study is to evaluate the antimicrobial efficacy of morinda citrifolia, neem extracts green tea extracts, in comparison with 3% sodium hypochlorite against E.faecalis and C.albicans strains.

MATERIALS AND METHODS: Freshly prepared herbal extracts of morinda citrifolia (group1), neem extract (group 2), green tea extract(group 3) and 3% sodium hypochlorite were used against . Enterococcus faecalis and candida albicans using the agar diffusion test . Agar plates were incubated at 37 degree Centigrade for 24 hours in an incubator. The diameter of bacterial inhibition zones around each well was recorded to the nearest size in mm

RESULTS: Sodium hypochlorite was found to have maximum zones of inhibition followed by neem extracts, morinda citrifolia,green tea extracts. Significant difference between herbal extracts and sodium hypochlorite was obtained. No statistically significant differences of antimicrobial activity are obtained between three experimental groups.

CONCLUSION: There is no significant difference among the three herbal formulations as root canal irrigants. Sodium hypochlorite is still the gold standard for root canal irrigation.

KEYWORDS : *Azadirachta indica*, herbal extracts, *Morinda citrifolia*, green tea extracts, sodium hypochlorite

INTRODUCTION

The main aim of Root canal therapy is to eliminate all infected microbes from the pulp canals, hence more emphasis should be given on thorough cleaning and shaping of root canals in addition to proper protocol of chemical disinfection for successful eradication of endodontic biofilms. In this context, contemporary available endodontic irrigants have shown a promising potential to eradicate anaerobic microbes in root canals. However, the potential side effects of antibiotic resistance, cytotoxicity of irrigants[1], inability to effectively eradicate smear layer, concerns of safety are still debatable. Hence, alternative usage of plant based irrigant products has garnered enormous popularity in these recent years on account of their proven potential of excellent biocompatibility, antimicrobial and anti-inflammatory properties.

Morinda citrifolia juice was alternatively proposed to NaOCl as an endodontic irrigant as it exhibits broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, antihelmin, analgesic, anti-inflammatory and immune enhancing effects[2] Azadirachta indica the Indian neem is also known for its immune-modulatory, antibacterial, antiviral, antioxidant, anti carcinogenic property and has been under continuous research as a potential endodontic irrigant.[3] In addition, to this green tea polyphenols have showed a significant antimicrobial effect to reduce E.faecalis biofilms in invitro studies due to their significant antioxidant and anti-inflammatory properties.[4] These three root canal irrigants are used in this study for antimicrobial evaluation. Since the role of E.faecalis and C. albicans is well established in failed root canals and persistent endodontic infections, these strains are used in this study.

MATERIALS AND METHODS

In the present study, morinda citrifolia juice(Group1), neem extract(Group II), green tea extract(GroupIII) were selected as the experimental groups and 3% NaOCl as positive control group. The most pathogenic strains in infected root canal such as E.faecalis, and C.albians were used to evaluate anti microbial activity.

Preparation of neem extract- fresh mature neem leaves were collected from botanical garden in Hyderabad and 100 g of neem leaves were tied in muslin cloth and soaked in distilled water. The beaker was boiled till thick concentrate of extract was obtained and finally neem extract was cooled and filtered and stored. Morinda citrifolia extract was procured from basic ayurveda ,stall in Hyderabad.

Preparation of green tea extract- 100 mg of fresh green tea leaves are mixed with 5ml of dimethyl sulfoxide liquid and strained and stored for the experiment.

Agar –diffusion test

Test microbes *E. faecalis* and *C. albicans* suspensions obtained from cultures are inoculated in culture plates with Mueller- Hinton Agar and Sabouraud Dextrose agar respectively.

Using pipette, the herbal extracts of three experimental groups was added to the filter paper disk placed in Petri dishes, respectively, and then dried at room temperature for 3 hours. Disks were placed on agar and lightly pressed. These plates were incubated for 24 h at 37°C in an incubator. After incubation period, plates were removed and inhibition zones were measured in mm.

RESULTS

The results were analysed with ANOVA analysis of variance. ANOVA shows that there is significant difference between the zone diameters of experimental groups and the sodium hypochlorite group against E.faecalis and candida strains.($p<0.05$.) No significant difference is seen in between the experimental groups against both strains.(p.0.05) Over all, neem extract (Gp 2) has found to show higher zones of inhibition when compared to MCJ and green tea extract experimental groups. The zones of inhibition diameters for sodium hypochlorite are high when compared to herbal extracts.

Zones of inhibition- table 1

Zone diameter	E.faecalis	Candida albicans
Morinda citrifolia Gp1	8mm	7mm
Neem extract Gp2	12mm	9mm
Green tea Gp3	7mm	5mm
NaOCl 3%	15mm	12mm
Statistical analysis p value	P<0.05 statistically significant	p>0.05 not statistically significant

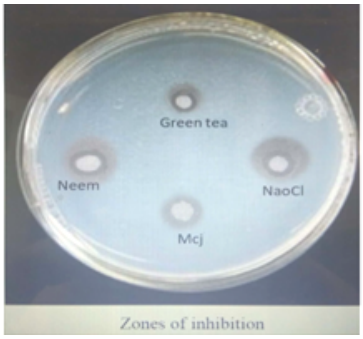

FIGURE 1

DISCUSSION

The golden standard of endodontic irrigation is sodium hypochlorite. But characteristics of NaOCl such as tissue toxicity, inability to remove smear layer, staining of clothes, disagreeable smell and taste has provoked many researchers to look for alternative irrigants.[4] Among the alternatives published are herbal formulations or natural plant extracts that have become popular in the recent era. They claim to have superior antimicrobial activity, excellent biocompatibility with less cytotoxic potential and better anti inflammatory and anti-oxidant property.[5] In this study readily available herbal products were used as they can have ease of availability and preparation which is acceptable for clinical practice.

Azadirachta indica is considered as age old medication for medical and dental problems. Neem and its active metabolities have been considered as treatment of dental plaque and gingivitis and several researchers have also proven their efficacy as root canal irrigant.[6] Azadirachta indica additionally has found to be possessing anti-adherence property of bacterial cells and this antimicrobial property is important for decreasing bacterial load. A study done by Aarti Bohora concluded that neem extract has significant antimicrobial effect against E.faecalis and candida albicans and mixed strains.[7]The results of this study were consistent with the above study findings.

On the other hand, Noni the Indian Mulberry has believed to express anti microbial activity due to presence of acubin, L-asperuloside, alizarin, and some anthraquinones. In 2008 Murray etal concluded MCJ effectively removed smear layer when used in conjunction with NaOCl and 17% EDTA.[8] Due to its higher biocompatibility MCJ was recommended as root canal irrigant as it does not cause serious injuries when extruded out.[8] MCJ appears to be the first juice identified as alternative to NaOCl. Similarly, study done by Rajesh Podar etal in 2015 have showed that the antimicrobial efficacy of MCJ, A.Indica and 3% NaOCl is similar.[9] Similarly, antimicrobial efficacy of propolis, Morinda citrifolia, neem and 5% sodium hypochlorite on candida albicans biofilms was studied by Tyagi etal in 2013. This study revealed that antifungal activity of neem and MCJ is limited when compared to propolis and 5% NaOCl.[10] In our study, same findings were obtained probably owing to usage of directly obtained morinda citrifolia extract. 3% NaOCl is used in this study as it is found to have satisfactory antimicrobial activity in many studies conducted.

Green tea extract has proven activity against a wide spectrum of microbes due to its antioxidant, anti-inflammatory properties as well as its ability to inactivate free radicals. The comparative evaluation of antimicrobial efficacy of green tea , triphala , MTAD, 5% sodium hypochlorite by Prabhakar etal showed that the herbal alternatives exert significant antimicrobial activity.[4] In this study E. faecalis and candida albicans were used. The microbiological status of root filled teeth has showed that enterococci was the most frequently recovered bacteria.(Molander A,Reit C 1998). The association of E.faecalis with different forms of radicular diseases is quite evident from a study done by Rocas IN, Siqueira etal, who deduced that E.faecalis was strongly associated with persistent infections. [11]Role of candida albicans in teeth with apical periodontitis and failed root canals was also well understood and so root canal irrigants with the antifungal properties are recommended by researchers.[12]

In the present study, sodium hypochlorite had obtained a mean diameter of 15mm and 12mm against E.faecalis and C.albicans respectively. The high antimicrobial activity of NaOCl is due high ph of NaOCl which interferes with cytoplamic membrane integrity and causes alterations in cellular metabolism.[13]The lower antimicrobial efficacy of herbal alternatives in this present study could be attributed to the herbs chosen and preparatory method used. Studies showing the significant antimicrobial effects of herbal extracts and the clinical acceptability of taste and odour, concentration of irrigants by addition of appropriate vehicles should be given priority for further use and recommendations of these herbal formulations in clinical practice.

CONCLUSION

Within the limitations of this study, sodium hypochlorite has shown significant antimicrobial activity against E. faecalis and C. albicans when compared to morinda citrifolia, neem extract and green tea extract. Neem extract showed better antimicrobial activity when compared to MCJ and green tea extracts. However, the concentration of herbal extracts, method of preparation of extracts, shelf life of herbal formulations, taste and smell as well as acceptability to patient should be emphasized in further studies.

Conflicts of interests –none

REFERENCES

1. Ok E, Adanir N, Hakki S. Comparison of cytotoxicity of various concentrations origanum extract solution with 2% chlorhexidine gluconate and 5.25% sodium hypochlorite. Eur J Dent. 2015;9:6–10.
2. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, et al. Morinda citrifolia (Noni): A literature review and recent advances in Noni research. Acta Pharmacol Sin. 2002;23:1127–41.
3. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. Curr Med Chem Anticancer Agents. 2005;5:149–6.
4. Prabhakar J, Senthilkumar M, Priya MS, Mahalakshmi K, Sehgal PK, Sukumaran VGEvaluation of antimicrobial efficacy of herbal alternatives (Triphala and green teapolyphenols), MTAD, and 5% sodium hypochlorite against Enterococcus faecalis biofilm formed on tooth substrate: an in vitro study. J Endod. 2010;36(1):83-6.
5. Madhu Pujar, Saleem Makandar .Herbal Usage In Endodontics- A Review.Int. Journal Of Contemporary Dentistry. 2011:2(1).
6. Behl H, Sidhu O, Kumar V, Singh D, Saimbi C, Efficacy of neem active metabolites for prevention of dental plaque and gingivitis, Neem Foundation 2002
7. Aarti Bohora , Vibha Hegde .Sharad Kokate .Comparison of the antibacterial efficiency of neem leaf extract and 2% sodium hypochlorite against E. faecalis, C. albicans and mixed culture - An in vitro study. Journal of endodontology.
8. Murray PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F.Evaluation of Morinda citrifolia as an endodontic irrigant. J Endod. 2008 Jan;34(1):66-70.
9. Rajesh Podar,Gaurav P. Kulkarni,1 Shifali S. Dadu,1 Shraddha Singh,1 and Shishir H. Singh In vivo antimicrobial efficacy of 6% Morinda citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants. Eur J Dent. 2015 Oct-Dec;9(4):529-34
10. TyagiSP, SinhaDJ, GargP, SinghUP, MishraCC, NagpalR.Comparison of antimicrobial efficacy of propolis, Morinda citrifolia, Azadirachta indica (Neem)and 5% sodium hypochlorite on Candida albicans biofilm formed on tooth substrate: An in-vitrostudy. Journal Of conservative dentistry. 2013:16(6);532-535
11. Roças IN, Siqueira JF Jr, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod. 2004 May;30(5):315-20
12. Jay Kumar, Rohit Sharma, Madhurima Sharma, V Prabhavathi, John Paul, and Chava Deepak Chowdar.Presence of Candida albicans in Root Canals of Teeth with Apical Periodontitis and Evaluation of their Possible Role in Failure of Endodontic Treatment. J Int Oral Health. 2015 Feb;7(2):42-5
13. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod. 1992;18:605–12