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.

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ABSTRACT

Candida albicans and Enterococcus faecalis are the most predominant microorganisms recovered from root canals of teeth where, previous treatment has failed. Thorough debridement of an infected root canal and complete elimination of microorganisms are objectives of an effective endodontic therapy.

For thousands of years humans have sought to fortify their health and cure various illnesses with herbal remedies. While hundreds of substances have been tried and tested, few have withstood modern scientific scrutiny. Perhaps no other botanical herb better meets the true definition of a panacea or cure-all than neem, a tropical evergreen native to India.

Although different agents have been suggested as root canal irrigants, sodium hypochlorite is the most popular. The present study was aimed to evaluate alternative inexpensive, simple and effective method for the sanitization of the root canal system. The antimicrobial efficacy of neem leaf extract as irrigant is evaluated and compared with the standard irrigant sodium hypochlorite.

INTRODUCTION

Azadirachta indica A. Juss is a commonly seen medicinal tree in India, which is considered holy. Popularly known as “Indian neem/ Margosa tree” or “Indian lilac”, is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. In Sanskrit, it is called “arishtha” meaning “reliever of sickness” and is regarded as the village dispensary of India. Importance of neem tree has been recognized by US National Academy of Sciences where neem is entitled as ‘a tree for solving global problems’.

Each part of the neem tree has some medicinal property and is thus commercially exploitable. Biologic activities and pharmacologic actions of neem is very well established with crude extracts and their different fractions from its leaf, bark, flowers, roots, seed and oil.

Neem has a broad range of therapeutic effects. Antimicrobial effects of natural plant extracts on endodontic pathogens have generally not been evaluated and reported except for ‘articum lappa extract’ and ‘morinda citrifolia juice’, which were effective in disinfecting root canals.

Endodontic infections are polymicrobial. E. faecalis and yeasts in particular of candida genus are commonly isolated from recalcitrant endodontic
infections and root canals with pulp necrosis\textsuperscript{5,6}.

Sodium hypochlorite has been widely recommended as an irrigant for chemomechanical debridement of root canals because of its tissue dissolution and antimicrobial activity, thus making it an irrigating solution of choice irrespective of its several undesirable characteristics such as tissue toxicity, risk of emphysema, allergic potential and disagreeable smell and taste\textsuperscript{7,8}.

In dentistry, Azadirachta indica has been investigated, due to its antimicrobial potential against oral microorganisms especially those associated with gingivitis and periodontitis, and is concluded to be highly efficacious as an alternative to chlorhexidine in cases of periodontal disorders\textsuperscript{9,10}.

Currently none or very few natural products that might be used as an alternative to NaOCl or CHX as an effective root canal irrigating solution have been identified.

Literature has shown that neem has antimicrobial and therapeutic effects suggesting its potential to be used as an endodontic irrigant\textsuperscript{1}, but there is lack of any documentation or data regarding neem research in endodontics.

**AIMS & OBJECTIVES**

The purpose of this in vitro study was to compare the antimicrobial activity of 2\% sodium hypochlorite and Neem leaf extract against \textit{E. faecalis}, \textit{C. albicans} and mixed culture and to assess the antimicrobial property of neem leaf extract against endodontic pathogens, using the agar diffusion method.

**MATERIALS & METHODS**

Neem leaf extract, 2\% sodium hypochlorite, absolute ethanol, \textit{E. faecalis}, \textit{C. albicans} and mixed culture were the materials used in this study.

**Preparation of neem leaf extract**

Mature fresh \textit{Azadirachta indica} leaves were collected from the medicinal garden of YMT Dental College & Hospital and taxonomic identification of the plant was performed. Leaves were washed in sterilized distilled water and weighed in a sterile disposable cup. 25gms of fresh neem leaves were added to 50ml of absolute ethanol. Mixture was macerated for 1-2 mins and care was taken to prevent temperature rise beyond 45-50\textdegree{}C. Extract was filtered through muslin cloth for coarse residue. Extraction process was repeated again using coarse residue and 25ml ethanol. Both the extracts were pooled together and filtered through fast filter paper. Alcohol part was removed from the extract on water bath till the volume was about 25ml. Extract was ready (fig.1) and stored in airtight amber coloured container\textsuperscript{3}.

**Agar - diffusion test**

\textit{E. faecalis} isolated from root canal infected sample. \textit{Candida albicans BX} was included in the study. Cultures were maintained on brain heart infusion (BHI) broth and agar.

Cultures grown overnight at 37\textdegree{}C in BHI broth on a rotary shaker 150 rpm and bacterial growth checked by changes in turbidity at 24hrs\textsuperscript{5}.

To check the antimicrobial efficacy of neem leaf extract and 2\% NaOCl, agar well diffusion method was performed. BHI agar plates were prepared and cultures (200\textmu{}l) were spread onto agar plates. For mixed culture inhibition, 2 cultures were mixed in 1:1 and 200\textmu{}l of the mixed culture
was spread onto agar plates.

Wells of 6mm diameter were made in the agar surfaces. Neem leaf extract, sodium hypochlorite and control 50µl each was added to the respective wells and the plates were incubated for 24hrs at 37°C in an incubator (fig.2). Control used in the study is the solvent used for extraction of neem – ethanol. Activity of the solvent was checked earlier and didn’t give any zone of inhibition against cultures. After incubation period, plates were removed and zones of inhibition were recorded.

Experiment was performed 3 times and mean of the zone of inhibition was recorded in mm.

RESULTS

The results were tabulated and statistically analyzed using analysis of variance (ANOVA). (Table 1).

ANOVA shows that there is significant difference between the zone diameters of neem extract and 2% NaOCl against E. faecalis and mixed culture (p< 0.05, ANOVA; Fig. 3, Fig. 4). No significant difference between zone of inhibition for neem and NaOCl (p> 0.05, ANOVA; Fig 5) against C. albicans. The difference was also significant with respect to the solvent control which showed no inhibition zone.

| TABLE |
| Zone diameter (mm) | E. faecalis | C. albicans | Mixed |
| Neem Extract | 20.0 | 7.1 | 15.0 |
| NaOCl (2% v/v) | 17.0 | 6.0 | 10.0 |
| Control | 0.0 | 0.0 | 0.0 |
| Statistical Analysis (P value) | P< 0.05, Significant | P> 0.05, Not Significant | P< 0.05, Significant |

![Fig.1. Neem Leaf Extract](image1)
![Fig.2. Agar plates incubated for 24hrs at 37 deg Celsius](image2)
![Fig.3 Zone of inhibition of Neem extract and NaOCl against mixed culture](image3)
![Fig.4 Zone of inhibition of Neem extract and NaOCl against C. albicans](image4)
![Fig.5 Zone of inhibition of Neem extract and NaOCl against C. albicans](image5)

Table 1. Zone of Inhibition
DISCUSSION

Several studies on the antimicrobial activity of irrigation solutions in endodontics, such as 0.5%, 1%, 2.5%, 5% NaOCl and 2% CHX are found in literature5, 6.

On the other hand, lack of studies on phytotherapeutic substances such as chlorophyll, propolis, morinda citrifolia juice and neem do not permit more objective conclusions about their use.

Inclusion of C. albicans and E. faecalis in this study was based on the literature that relates these micro-organisms to pulp infections, mainly in recalcitrant infections after endodontic treatment4, 6, 11.

Methodology of this study followed the standard established for agar dilution tests. Study design presented in this paper is more consistent with other studies testing ability of antimicrobial action5.

The use of best possible irrigant during chemomechanical preparation is of great importance. Ideal irrigant should combine antimicrobial action and a capacity to dissolve organic and inorganic remnants. NaOCl in full concentration is well known for its bactericidal action and cytotoxicity6, 12.

Several pharmacological activities and medicinal applications of various parts of neem are well known. Interest on this substance is based on its properties like antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, analgesic, immunostimulant.1, 3, 13 Moreover, its anti-adherence activity by altering bacterial adhesion and ability of organism to colonize, also stimulated the study of this substance14.

Also Botelho et al (2008) and Behl et al in their experiments and trials concluded that Azadirachta indica is highly efficacious in the treatment of periodontal disease thus exhibiting its biocompatibility with human PDL fibroblasts9, 10. Use of neem as an endodontic irrigant might be advantageous because it is a biocompatible antioxidant and thus not likely to cause the severe injuries to patients that might occur via NaOCl accidents.

Bitter taste associated with this plant can be altered by different formulations due to addition of sweeteners and flavors to increase the patient compliance and acceptability10.

The results obtained in this in vitro study showed that neem leaf extract is a viable medicament against C. albicans, E. faecalis and even mixed state. Although 2% NaOCl showed comparatively less antimicrobial effect than neem, it still had an observable effectiveness against test organisms.

CONCLUSION

Under the limitations of this study, it was concluded that neem leaf extract has a significant antimicrobial effect against E. faecalis and C. albicans and mixed state. Microbial inhibition potential of neem leaf extract observed in this study opens perspectives for its use as an intracanal medication. However, preclinical and clinical trials are needed to evaluate biocompatibility & safety before neem can conclusively be recommended as an intracanal irrigating solution, but in vitro observation of neem effectiveness appears promising.

As the global scenario is now changing
towards the use of non toxic plant products that have traditional medicinal use, extensive research and developmental work therefore should be undertaken on neem and its products for their better economic and therapeutic utilization.

REFERENCES