



AN IN-VITRO COMPARISON OF ANTIBACTERIAL EFFICACY OF NISIN, PROPOLIS, NEEM, PLATELET RICH PLASMA AS AN INTRACANAL MEDICAMENTS USED IN PRIMARY TEETH AGAINST ENTEROCOCCUS FAECALIS

Dental Science

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ABSTRACT

Context: Success of endodontic treatment complies on the debridement of root canal from microbes. However, due to complexities of root canal system, need of an adjunct to instrumentation is required.

Aim: To evaluate the antimicrobial efficacy of Nisin, Propolis, Neem extract, Platelet rich plasma and calcium hydroxide as an intra-canal medicament against *E faecalis*.

Settings & Design: 60 extracted human primary anterior teeth were collected, washed and kept in ultrasonic cleaner. The teeth were de-coronated and bio-mechanical preparation were done using 4% K3 file and irrigated with normal saline.

Methods and Materials: Then the root canals were inoculated with *Enterococcus faecalis* and incubated. After 21 days the canals were rinsed with saline and teeth were randomly divided into 5 treatment groups (n=10). In respective treatment groups teeth were inoculated with Nissin, PRP, Propolis, Neem extract, metapex and saline and incubated for 7 days. On the 8th day, to measure the degree of infection, dentinal chips from radicular dentin were harvested using a sterile rotary file (6%) and CFUs were checked.

Statistical Analysis Used: Kruskal Wallis test and Mann Whitney U test were applied. P value <0.05 was considered statistically significant.

Results: Nisin was more efficient in eradicating *E faecalis* from the root canal followed by Propolis and Neem when compared with Platelet rich plasma

Conclusions: The present study concluded that between all the medicaments used Nissin was found to be most effective against *E faecalis* followed by Propolis then Neem whereas minimal or no inhibitory effect was observed when PRP was used.

KEYWORDS

intra-canal medicaments, antibacterial efficacy, *E faecalis*.

INTRODUCTION:

Persistent root canal infections are generally attributed to intra-radicular infections, foreign body reactions and cholesterol containing cyst. Moreover, major cause of failure of endodontic treatment is due to the survival of microorganism at the apical one third of the root end.¹ In deciduous tooth, presence of tortuous canal, shorter root trunk and altered morphology due to root resorption restrict complete debridement of root canals. When left untreated, empty root canal provides foci for colonization of microbes which leads to secondary endodontic infections.² It is well documented that *Enterococcus faecalis* (*E.faecalis*) is a predominant organism found in about 4-40% cases of primary endodontic infections and nine times more likely seen in secondary endodontic infection. So despite of making up a small number of microflora in untreated canals, it is the major contributing factor in persistent peri-radicular infections.¹

The biomechanical preparation does not completely eradicate microorganism present inside radicular dentin. Therefore inter-approintment intracanal medicaments are more effective against pathogens especially that get lodged into dentinal tubules and canal ramifications as seen in persistent endodontic infections.^{3,4,5} Suitable medicaments prevents proliferation, inhibits the ingress and nutrients supply of pathogens during treatment intervals.⁶ Calcium hydroxide had been widely used as an intracanal medicaments with various combinations since it was introduced in 1920. Among various combinations, Metapex, a silicone oil-base calcium hydroxide with 38% idoform is commonly used medicament.⁷ The alkaline pH and strong capacity of inactivating bacterial endotoxins and lipopolysaccharides made calcium hydroxide as choice of medicament in necrotic pulp, however not found much effective against some

strains like *E.faecalis*.³

A novel intracanal medicament, Nisin which is a class I bacteriocin, natural antimicrobial peptide isolated from *Lactococcus lactis* by Mattick and Hirsch in 1947 was found effective against *E.faecalis*. It is used as preservative in dairy industry.⁸ Its antibacterial activity is due to interaction with anionic lipids leading to perturbation on bacterial cells, causing efflux of adenosine triphosphate (ATP), amino acids, pre accumulated rubidium and other vital ion gradients resulting in cell death of the microbes.⁹

Recently, herbal products have been studied for their antimicrobial properties since there is a relevant increase in the overuse of antibiotics.¹⁰ India has plethora of medicinal flora widely distributed throughout the country. *Azadirachta indica* commonly known as "Neem" is an age old medicinal plant for various human ailments.¹¹ It leads to alteration in bacterial adhesion resulting in anti-adherence activity and inhibits colonization of micro-organism.¹⁰ Neem contains active phytometabolites like alkaloids, glycosides, terpenoids, steroids and tannins which are biocompatible to human PDL fibroblast and highly effective against anaerobic infections.¹¹ Another biologically derived product is Propolis (commonly known as "Bee glue"), a resinous substance extracted from plant resin and collected by honeybees.¹² Its antibacterial activity is due to flavonoids which alter the cell membrane permeability in turn decreasing resistance of cells to other antibacterial agent.¹³

Autologous platelet concentrates are being widely investigated for its antibacterial effect. The platelet rich plasma (PRP) generate potent antimicrobial peptides like oxygen metabolites, including superoxide,

hydrogen peroxide and hydroxyl free radicals, which internalize micro-organism and enhances its clearance.¹⁴

Thus the aim of our study is to evaluate the antibacterial efficacy of calcium hydroxide, Nisin, Neem, Propolis and PRP against *E. faecalis* when used as an intracanal medicaments in human primary teeth.

SUBJECTS & METHODS:

Preparation Of Sample:

Sixty individual samples were prepared from extracted human primary anterior teeth, washed from debris and kept in ultrasonic cleaner (Runyes, China) for 15 minutes and stored in distilled water (Bio Lab, India). The samples were de-coronated using a rotary diamond saw (Dentorium, Germany) under copious water irrigation and all were type I root canal configuration with round orifice. The working length was determined as 1 mm short of apex with average root length of all sample as 10mm. The bio-mechanical preparation was done using 4% K3 rotary files (Sybron Endo USA) in crown down technique with X smart (Densply, UK). Copious irrigation was done with 2.5% sodium hypochlorite (Photon, India) using 30 gauge sterile endodontic needle (Ultradent South Jordan) followed by rinsing with distilled water. Samples were then rinsed with 17% ethylenediaminetetraacetic acid (EDTA) (Vishal biotech, Mumbai, India) for 5 minutes in an ultrasonic cleaner to remove smear layer and finally rinsed with distilled water. The samples were dried and three layers of nail varnish were applied all over the root surface except the root canal and were allowed to dry. Then, samples were autoclaved at 121°C, 15psi for 30 minutes.^{6&8}

Preparation Of The Inoculums & Infection Of Root Canals:

Standard strains of *E. faecalis* (MTCC- 439) (Institute of Microbial Technology (IMTECH), Chandigarh, India) were used in the study. 10 µl culture broth was inoculated inside autoclaves root canals and were sealed with the paraffin wax, incubated at 37°C for 21 days. Re-inoculation of the microorganism was done at every 3 days of interval throughout the 21 days of incubation period.

Preparation Of Neem Extract:

Shade dried Neem leaves were subjected to soxhlet extraction with 200 ml of solvent. The extract obtained was subjected to evaporation of solvent to get extract in slurry form which was suitably diluted for its use.

Preparation of PRP:

20 ml blood was withdrawn by venipuncture in test tube and centrifugation was performed using a 'soff' spin. The supernatant was transferred in another test tube and again centrifugation was done at higher speed to obtain platelet concentrate. After centrifugation, lower one-third of the test tube was platelet rich plasma (PRP) and upper two-third was platelet-poor plasma (PPP). The PPP was removed and the platelet pellets were suspended in a minimum quantity of plasma (2-4 ml) by gently shaking the tube.¹⁵

Intracanal Medicaments:

After 21 days, canals were aspirated and copiously irrigated with 0.9% Normal saline solution (Marck Biosciences, India) using endodontic needle. After drying with paper points the teeth were randomly divided into six treatment groups including a positive & negative control (n=10). In Group I, 5 µl Nisin (Bimal Pharma, Mumbai); Group II, 5 µl Neem Extract (Lab preparation); Group III, 5 µl Propolis (Superbee, Singapore); Group IV, 5 µl Platelet Rich Plasma (Lab preparation); Group V, 5 µl Metapex (Meta Biomed, Korea); Group IV, 5 µl 0.9%w/v Saline (Marck Biosciences, India), were placed using Tuberculin syringe (Nanjing, China). In our study calcium hydroxide was taken as positive control and saline was taken as negative control. The samples were sealed again by paraffin wax and incubated for 7 days at 37°C. On the eighth day, dentin chips were taken from full length of root canals using 6% K3 rotary files (Sybron Endo USA) for harvesting in nutrient agar to know the degree of infection. After incubation for 24 hours, the developed colonies were counted by digital colony counter (EI products, India). Mean, standard deviation, minimum and maximum values of CFUs for *E. faecalis* were calculated. Shapiro-Wilk test showed that CFU counts did not follow normal distribution. Hence, non-parametric tests namely Kruskal Wallis test and Mann Whitney U test were applied for further data analysis. P value <0.05 was considered statistically significant. All analyses were performed using version 21.0 of the Statistical Package for Social Sciences (IBM Corporation, Armonk, New York, USA).

RESULTS:

Our study showed no colony forming units (CFUs) in Group I (Nisin) and maximum CFUs in Group IV (Platelet Rich Plasma) with 67 (10¹/ml) as shown in table 1. Positive control (Group V Metapex) showed 5(10¹/ml) CFUS while negative control (Group VI Saline) showed 120 (10¹/ml) CFUs as shown in Table I. Kruskal Wallis test showed significant difference for *E. faecalis* CFUs in different medicaments in primary teeth. When Mann Whitney U test was applied, it showed that in PRP group CFU was higher than any other group, followed by Metapex, Neem Extract, Propolis and Nisin in that order. Significantly least count was observed with Nisin. There was no significant difference between Neem Extract and Propolis. Neem Extract and Propolis were same for *E. faecalis* CFUs in primary teeth. Nisin was more efficient in eradicating *E. faecalis* from the root canal followed by Propolis and Neem when compared with Platelet rich plasma as shown in Graph I.

DISCUSSION:

The penetration of *E. faecalis* inside the dentinal tubules were found to be 400 µm in vitro.¹⁶ This bacterial invasion occurs within 2-3 weeks when observed under light and scanning electron microscopy (SEM), which in turn play a significant role in failure of the endodontic treatment.¹⁷ The ability of *E. faecalis* to suppress IL-2 and IL-4 production by lymphocytes is one of the reason for commonly derived species in cases of persistent endodontic infections.¹⁸ Another reason being the ability of *E. faecalis* to survive in high alkaline pH is due to the presence of proton pump that lowers the internal pH of bacterial cell. Also, at a pH of 11.5 or greater *E. faecalis* is unable to survive, but the buffering capacity of dentin lowers down the pH instead. Thus rendering *E. faecalis* as a culprit in secondary endodontic infections.¹⁹ Nisin completely eradicate the *E. faecalis* from the root canal in our study, when used as an intracanal medicament in primary teeth. This was in accordance to Turner et al and Chinni et al who concluded that no growth of bacteria occur after use of Nisin in root canals.^{20&21} According to Hemadri et al, when compared, Nisin reduced 49% and calcium hydroxide reduced 31% of *E. faecalis* inside root canal.²² This was in contrast to our study where almost complete eradication was seen with Nisin while calcium hydroxide showed 5(10¹/ml) colonies of *E. faecalis*. The ability of Nisin to kill bacteria does not rely upon the alkalinity. Also, Nisin has been reported to be effective against a broad range of gram positive bacteria especially Streptococci and Enterococci.⁸

Neem showed maximum antibacterial activity at 50% concentration according to Prashant et al.²³ However lower concentration of Neem was ineffective against *E. faecalis*.¹¹ In our study 100% concentration of Neem extract was used which showed 2(10¹/ml) colonies of *E. faecalis*. This was confirmed by Ibrahim et al and Hedge et al who concluded that Neem had a strong inhibitory effect against *E. faecalis*.¹⁰ Propolis showed 1(10¹/ml) colonies forming unit in our study which was in contrast to Ramani et al who showed 10³ CFU/ml when inoculated for 7 days.¹³ The difference is due to the geological variation from where Propolis has been derived like location, season and area of vegetation which in turn affect its biological activity.²⁴ Oncag et al concluded that Propolis was more effective than calcium hydroxide when used as intra-canal medicaments which was akin to our study result.²⁵ In our study, highest CFUs were detected in PRP group up to 67(10¹/ml). However, until now no studies in literature have been done on antibacterial efficacy of Platelet rich plasma when used as an intracanal medicaments by measuring CFUs. Although Drago et al concluded that minimal inhibitory concentration of PRP against *E. faecalis* ranged up to 34.475/µl.¹⁴ Calcium hydroxide was used as a positive control in our study showed 5(10¹/ml) CFUs which was similar to study done by Jahromi et al and Rezende et al.^{2&26} Comparison of antibacterial efficacy between all the used medicaments in our study has not been addressed in literature until now.

CONCLUSION:

Our study concluded that between all the medicaments used Nisin was found to be most effective against *E. faecalis* followed by Propolis and Neem in that order, whereas minimal or no inhibitory effect was observed when PRP was used. So under the limitation of our in vitro study, it can be concluded that Nisin had greater antibacterial effect on *E. faecalis* but further in vivo or clinical trials are required to confirm the results.

Table I: CFU count in recovered medicaments from deciduous

tooth samples treated with *E.faecalis*.

S.N	Recovered Medicaments	Dilution Size	Inoculum Size	<i>E. faecalis</i> CFU Count	
				Mean $\pm$ SD	Min-Max
1.	PRP	1 ml	100 μ l	67.00 $\pm$ 1.76	64-69
2.	Neem Extract	1 ml	100 μ l	2.00 $\pm$ 1.56	00-05
3.	Propolis	1 ml	100 μ l	1.00 $\pm$ 0.82	00-02
4.	Nisin	1 ml	100 μ l	0.00 $\pm$ 0.00	00-00
5.	Metapex	1ml	100 μ l	5.00 $\pm$ 1.05	03-07
6.	Saline	1ml	100 μ l	120.00 $\pm$ 2.87	116-124
Kruskal Wallis test	$\chi^2 = 42.688$, P = 0.000 (<0.001), Sig. diff.				
Mann Whitney U test	PRP > Metapex > Neem Extract = Propolis > Nisin				

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