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Comparative Evaluation of Antimicrobial Activity of 3% Sodium Hypochlorite And Propolis Against Gram Negative Bacteria Porphyromonas Gingivalis: In-Vitro Study

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Abstract
Background: *Successful endodontic treatment requires complete elimination of micro-organisms from the root canal system. Porphyromonas gingivalis are the most predominant microorganisms recovered from teeth requiring retreatment. Sodium hypochlorite has been the most widely used irrigant. The constant increase in antibiotic resistant strains and side effects caused by synthetic drugs has prompted researchers to look for herbal alternatives , like Propolis in endodontic irrigation*
Aim To check the comparative evaluation of antimicrobial activity Of 3% Sodium Hypochlorite And Propolis Against Gram Negative bacteria Porphyromonas gingivalis.
Materials & Methods: *Propolis, 3% sodium hypochlorite(NaOCl), normal saline (control) & pure cultures of Porphyromonas gingivalis. Bacteria were grown in a 5 ml Brain heart infusion broth (HIMEDIA) for 7 days at 37°C.After confirming the growth of bacteria in culture , the broth was mixed with Brucella agar plates supplemented with Hemin and Vitamin K1 (HIMEDIA) which was kept for 24 hours at 37 °C. Culture was adjusted to 1 Mc Farland scale (3 x108CFU/ml).The experiment was performed 3 times, mean of the readings was recorded in mm & statistical analysis was performed.*
Results:*Propolis showed a continuous antimicrobial effect over 1st , 2nd and 4th week .*
Conclusion:*With Propolis the sustained zone of inhibition demonstrated over 4 weekscan be considered as an alternative irrigant.NaOCl,the most commonly used root canal irrigating solution showed significant activity.*

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I. Introduction

Success of endodontic treatment mainly depends on elimination of infecting microorganism which is achieved through chemo-mechanical preparation of root canals.(1) Endodontic infections are polymicrobial in nature , with obligate anaerobes dominating the micro flora of primary endodontic infection.Porphyromonas Gingivalis , obligate gram negative anaerobic rods is the most frequently isolated bacteria from the infected root canals.(2) Elimination of microorganism from the infected root canal system is a complicated task , if microorganism persist during obturation there is a high risk of treatment failure.(1) Sodium hypochlorite has been used as root canal irrigant because of its tissue dissolution and antimicrobial activity, however it has some undesirable characteristics like tissue toxicity, allergic potential and disagreeable smell and taste. (3)In an attempt to avoid the side effects of the synthetic irrigating solutions, recently focus has shifted towards herbal extracts which are known since ages for their antimicrobial effect in traditional medicine.Among these natural products, Propolis shows promising biological propertiesIt has antibacterial, antifungal and anti-inflammatory action against a wide range of Gram-negative pathogens. (4)The aim was to compare the antimicrobial efficacy of a Conventional Root Canal Irrigant 3% Sodium Hypochlorite and Propolis against gram negative anaerobe bacteria Porphyromonas gingivalis, and objective was to evaluate the antimicrobial effect of Propolis as anRoot canal Irrigant.

II. Materials & Methods

Propolis [Figure 1] ,3% sodium hypochlorite[Figure 2], normal saline & pure culture of Porphyromonas gingivalis (ATCC 33277) [Figure 3] were the materials used.

Microbial Culture

Bacteria were grown in a 5 ml Brain heart infusion broth (HIMEDIA) for 7 days at 37°C.After confirming the growth of bacteria in culture , the broth was mixed with Brucella agar plates supplemented with Hemin and Vitamin K1 (HIMEDIA) which was kept for 24 hours at 37 oC. Culture was adjusted to 1 Mc Farland scale (3 x108CFU/ml).

Agar Diffusion Method

After confirming the growth of microorganism , bacterial suspension was spread with sterile swabs over the entire Brucella Agar plates. After inoculation of bacteria , 5 saturated paper disks 6 mm in diameter, were placed on each agar plate. Four of paper disk were saturated with one of the two test solutions, and one was saturated with sterile distilled water (control group). Brucella Agar plates with bacterial suspension were incubated for three days in sealed anaerobic jar with anaerobe tab to create an anaerobic atmosphere prior to measuring the inhibition zones for Propolis. The plates were incubated for 24hrs in an incubator at 37ºC & examined for zones of inhibition. Similarly eight agar plates were taken for sodium hypochlorite and were incubated. All assays were repeated three times to ensure reproducibility and the microbial zones of inhibition were measured in millimetres.

III. Results

The results were tabulated and statistically analyzed using SPSS software version 17. Unpaired‘ t test’ was applied to test the significance between the two solutions[Table1 - 3]. Also descriptive Statistics were expressed as mean ± standard deviation (SD) for each group. Treatment with 3% Sodium Hypochlorite induced larger zone of microbial inhibition for Porphyromonasgingivalis than Propolis.[Figure 4 Figure 5][Graph 1-3] In the above test, p value less than or equal to 0.001 ($p \leq 0.05$) was taken to be statistically significant.

IV. Discussion

Porphyromonas gingivalis is one of the obligate gram-negative anaerobes found in 90% primary endodontic infections. It is predominant endodontic pathogen. Sundqvist reported the presences of 90% of P gingivalis in necrotic teeth. Bystrom reported that in teeth with periapical infection, 80% of anaerobes were P. gingivalis.(5) Gomes et al reported a study in which cases with Endodontic pain werecaused by Porphyromonas gingivalis.(6) Siqueira et al also reported a study in which 32% cases of acute periradicular abscess which were infected with P.gingivalis.(7) Hence proper irrigation of the root canal system during endodontic treatment is essential for successful treatment.

The most widely used endodontic irrigant is sodium hypochlorite because of its bactericidal activity and ability to dissolve vital and necrotic organic tissue. On the other hand , the usage of sodium hypochorite in high concentration is undersirable because it is irritating to the periapical tissues. (8)Propolis evaluated for antibacterial activity against endodontic pathogens is believed to have been coined by Aristole who identified how propolis was used to protect and define the hive. Propolis (from the greek) means ‘Before the city or Defender of the city’.Propolis is resinous material/sap that is collected after it oozes out from tree bark and bud. After bees have collected propolis they mix it with wax flakes and their saliva in the hive. This mixture is what they use to cover the interior of the hive. The bees not only use propolis as a building material and structural defence mechanism but their health is maintained as a result of its immune system enhancing properties. Propolis forms the bees external immune defence system, making the beehive one of the most sterile environments known to nature. (9)

It is a hard resinous material derived by bees with large amounts of flavinoids as it main active ingredient with phenol derivatives.(4) Literature does not have sufficient evidence on the use of natural products in elimination of obligate anaerobic bacteria, especially as root canal irrigants. In dentistry, propolis has been used for the treatment of aphthous ulcers, candidiasis, gingivitis, periodontitis and pulpitis. Propolis being a good antimicrobial and anti inflammatory agent , can serve as a better intracanal irrigant and intracanal medicament.(10) So far no study has been done to test the antimicrobial effect of propolis on obligate anaerobe P. gingivalis.In our study results showed that, the mean zone of inhibition at 1st week, 2nd week and 4th week for Sodium Hypochlorite was significantly higher in comparison to Propolis($.p \leq 0.05$).It is confirmed that Sodium Hypochlorite is effective against Porphyromonas gingivalis. While assessing the zone of inhibition of propolis, it has shown a continuous antimicrobial effect over 1st , 2nd and 4th week [Graph 1,2, 3] .This demonstrated that there wasa sustained effect of propolis over 4 weeks.. Propolis is non toxic , biocompatible and does not alter the structural integrity of the dentin and with a prolonged effect over 4 weeks , it can be considered as an alternative irrigantTo check the antibacterial activity of two solutions agar diffusion test is generally accepted procedure, but has some limitations like pH of the substrate, incubation period, and diffusion capacity of the drug having effect on activity of test materials. However evidence also suggests agar diffusion tests shows good correlation with other antimicrobial susceptibility tests.

V. Conclusion

Theantimicrobialefficacy of Sodium Hypochlorite was superior to Propolis but potential for microbial Inhibition of Propolis opens perspectives for its use as an Irrigant, an “In Vitro” observation of Propolis appears PROMISING !!!! It may help in our effort to practise “Green Dentistry”.

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Figure 1 Figure 2 Figure 3

FIGURE 4

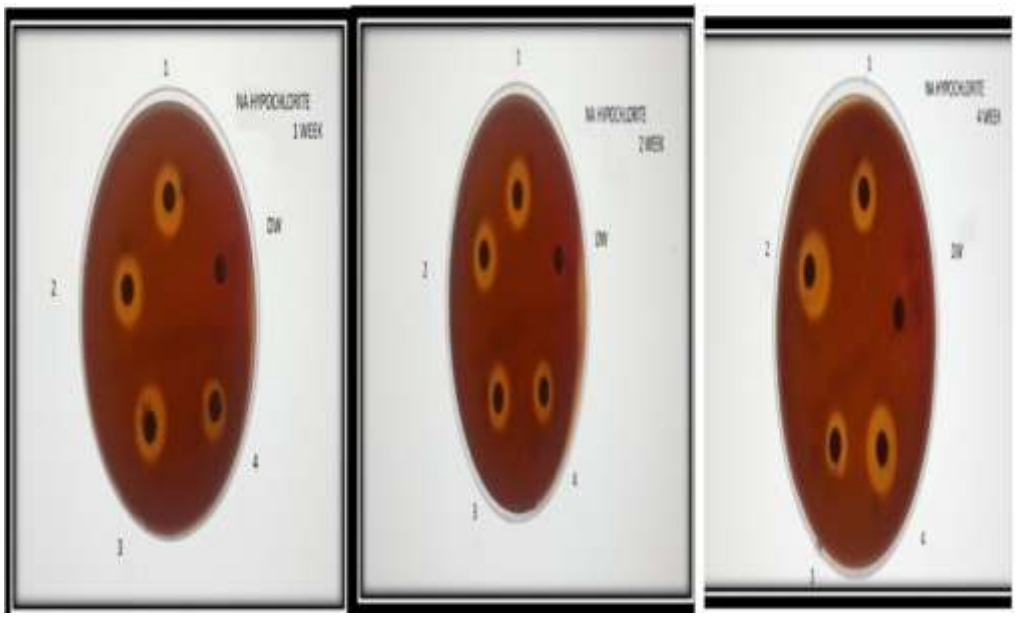


Figure 5

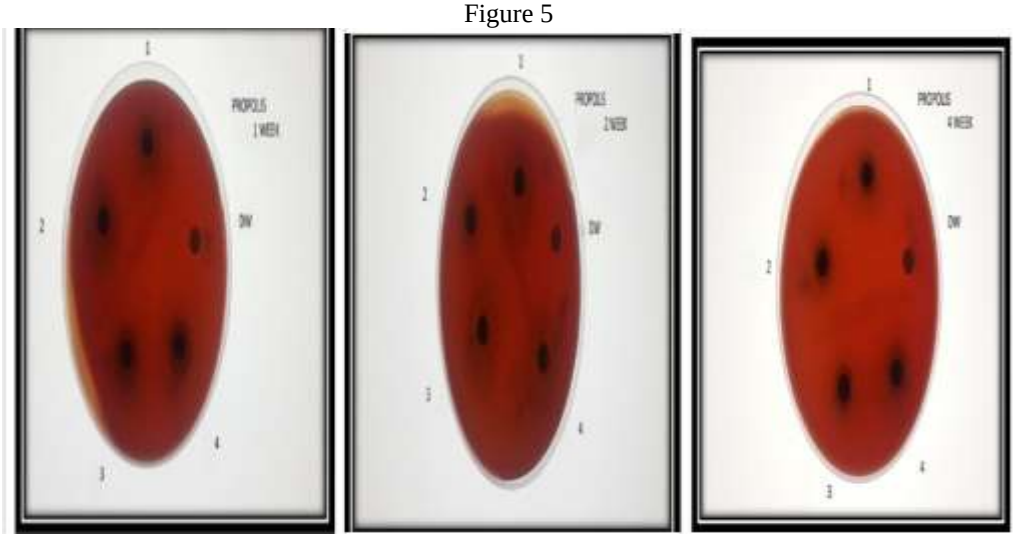


Table 1comparison of antibacterial activity of 3% Sodium hypochlorite and Propolis against Gram negative bacteria *P.Gingivalis*(Agar well diffusion method) at 1st week.

Agents	Mean (zone of inhibition)	Standard deviation	P value (Unpaired t test)
3% Sodium hypochlorite	**16.36**	0.41	<0.001*
Propolis	**11.99**	0.82	

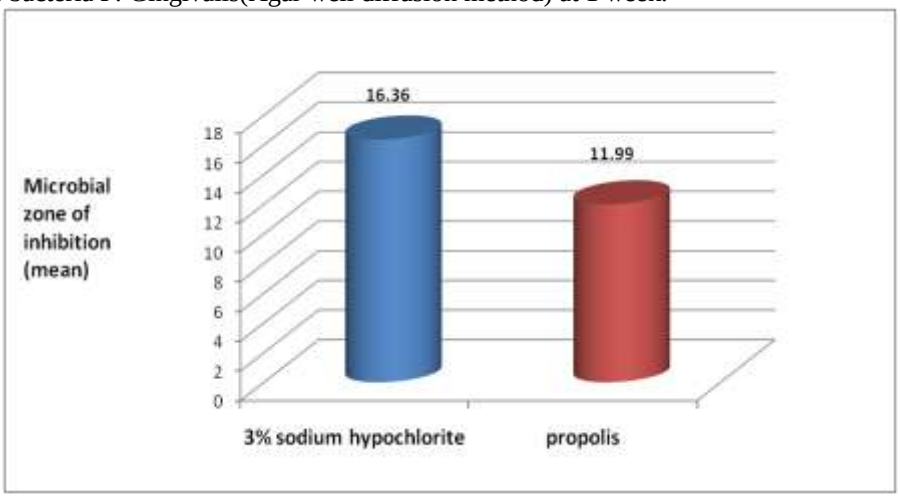
Table 2 Comparison of antibacterial activity of 3% Sodium hypochlorite and Propolis against Gram negative bacteria *P.Gingivalis*(Agar well diffusion method) at 2nd week.

Agents	Mean (zone of inhibition)	Standard deviation	P value (Unpaired t test)
3% Sodium hypochlorite	**15.93**	1.60	<0.001*
Propolis	**12.02**	0.75	

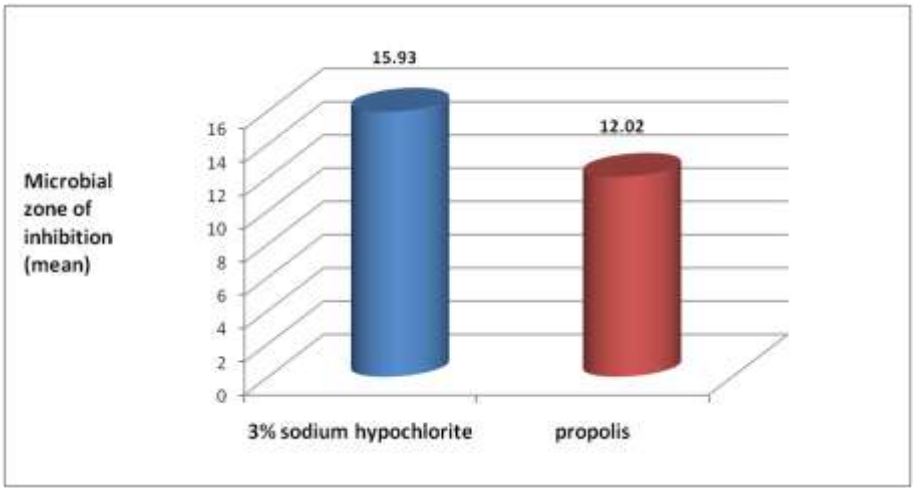
Table 3 Comparison of antibacterial activity of 3% Sodium hypochlorite and Propolis against Gram negative bacteria *P.Gingivalis*(Agar well diffusion method) at 4th week.

Agents	Mean (zone of inhibition)	Standard deviation	P value (Unpaired t test)
3% Sodium hypochlorite	**16.51**	0.52	<0.001*
Propolis	**12.11**	0.94	

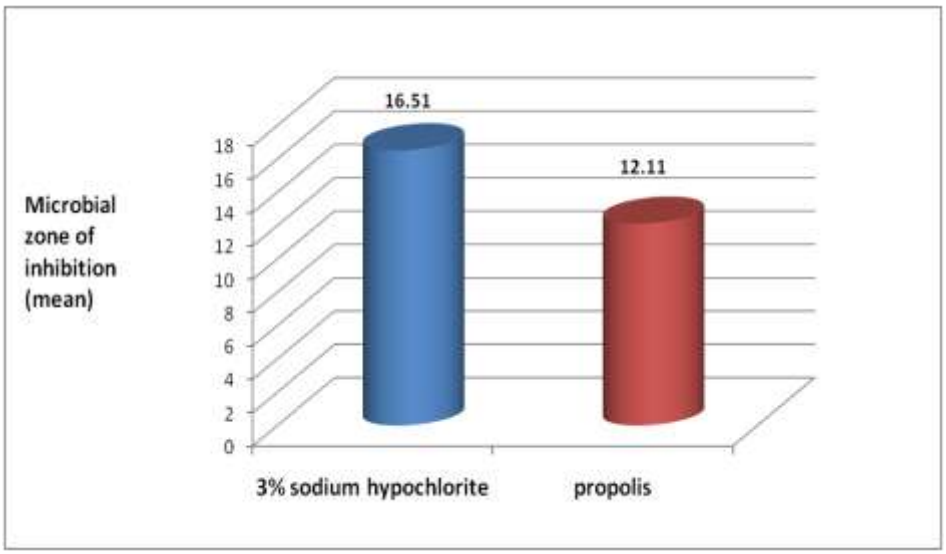
Graph 1 **:** Comparison of antibacterial activity of 3% Sodium hypochlorite and propolis against Gram negative bacteria P. Gingivalis(Agar well diffusion method) at 1 week.



Graph .2: Comparison of antibacterial activity of 3% Sodium hypochlorite and propolis against Gram negative bacteria P. Gingivalis(Agar well diffusion method) at 2 weeks.



Graph .3: Comparison of antibacterial activity of 3% Sodium hypochlorite and propolis against Gram negative bacteria P. Gingivalis(Agar well diffusion method) at 4 weeks.



*Priyanka Sawants. "Comparative Evaluation of Antimicrobial Activity of 3% Sodium Hypochlorite And Propolis Against Gram Negative Bacteria Porphyromonas Gingivalis :in-Vitro Study." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.8 (2017): 17-21