



COMPARISON OF ANTIMICROBIAL EFFICACY OF PROPOLIS AND 5.2% SODIUM HYPO CHLORITE, 0.2% CHLOROHEXINATE GLUCONATE ON CANDIDA ALBICANS BIOFILM FORMED ON TOOTH SUBSTRATE: AN IN-VITRO COMPARATIVE STUDY

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Abstract

Candida albicans is the most common fungus isolated from failed endodontic cases. Due to the inherent disadvantages of sodium hypochlorite, researchers have looked for herbal alternatives. Thus, the present study is aimed to explore newer irritants which will probably be as effective or more and at the same time would be able to overcome the drawbacks of NaOCl.

Methodology- 0.2% chlorohexinate Gluconate showed a moderate zone of inhibition of all microbial inhibition stains. Extracted human mandibular premolar were biomechanically prepared, vertically sectioned, placed in tissue culture wells exposing the root canal surface to Candida albicans grown on Saboraud Dextrose Agar to form a biofilm. At the end of 2nd week, all groups were treated with test solution and control for 10 minutes and evaluated for Candida growth and number of colony forming units.

Results & Conclusion- Propolis performed equally well as that of NaOCl and while chlorohexidine showed moderate results.

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Introduction

Endodontic infections are usually polymicrobial in nature with obligate anaerobic bacteria as the commonest causative organisms involved. To accomplish successful long term root canal treatment needs effective debridement and disinfection of root canal system. It is difficult to achieve owing to the complex anatomy involved and the difficulty in accessing the canal system with instruments and irrigants. *Candida albicans* is the commonest fungus implicated in infections of the root canal, 21 % and 18% in primary infections and retreatment cases respectively^{1,2}. *Candida albicans* has a tendency to thrive in harsh conditions because of its capacity to form biofilms as well as its physicochemical properties that help it to modify and survive in accordance to the existing environmental and nutritional surroundings. Biofilm empowers the organism to withstand its own destruction by enabling it to resist phagocytosis, antibody destruction and killing by antimicrobial agents³. Biofilm forms a protective barrier around the organism, composed of extracellular matrix. *C. albicans* is also resistant to Calciumhydroxide, which is the most commonly used intra canal medicament.

A popular root canal irrigant with good antimicrobial potential and propensity to dissolve organic matter which is used frequently is 5.2% Sodium hypochlorite. However, it not only causes irritation to the periapical tissues but also has certain other inherent disadvantages including staining of instruments, causing burns to the surrounding tissues, an unpleasant taste, high toxicity, instrument corrosion, inability to remove smear layer, reduction in elastic modulus and flexural strength of dentin^{7,8}. Propolis is a new herbal agent with potent antimicrobial, antioxidant and anti-inflammatory properties owing to the presence of flavonoids, phenolics and other aromatic compounds. It is a brownish resinous substance collected by bees mainly from plants, and is used by them to reinforce their hives and keep the environment aseptic. It is. The present study is aimed to explore newer herbal irrigants which have equivalent or higher antimicrobial potential as that of sodium hypochlorite, chlorohexinate with minimal side effects and disadvantages^{2,9,10,11}.

Materials & Methods

The samples were further inoculated on Sabouraud Dextrose Agar (SDA) medias and incubated at 37 degree celcius and 22 degree celcius and adjusted to an optical density of 1 with sterile BHI broth...

cultures were examined for growth daily for the first week and twice a week for the subsequent 3 weeks. Fungal isolates so observed were finally identified with lactophenol cotton blue (LPCB) staining technique and cornmeal agar^{20,21}.

Propolis was prepared by diluting a 33 % commercially available alcoholic extract using warm saline in a ratio 2: 1, to form a 11 % alcoholic extract².

2. pre- molar extracted in department of oral surgery were collected. All premolars were collected and blocks were made on acrylic. All the Single rooted human pre-molar teeth were sectioned below the cemento-enamel junction to obtain a standardised tooth length of 8 mm. The teeth were cleaned of superficial debris, calculus, tissue tags and stored in normal saline¹¹.

The root canals were then instrumented using the crown down technique and rotary instruments to an apical size of Pro Taper F of 5.2 % Naocl was used between each instrument during the cleaning and shaping procedure.

All teeth were vertically sectioned along the mid-sagittal plane into two halves. The concave tooth surface was minimally grounded to achieve flat surface to enable placement in tissue culture wells exposing the root canal surface to *C. Albicans* to form a biofilm^{20,21}.

The samples were divided into 5 experimental groups with 10 samples each, namely -

Group 1 -Propolis

Group 2-5% Sodium Hypochlorite

Group 3- Sterile Saline

Group 4- chlorohexidine gluconate 0.2%

The samples were then sterilised by ultraviolet radiation and placed in the wells of tissue culture plates. The cultured yeast was inoculated in the wells containing tooth samples at 37°C for 48 hours. Each sample was then irrigated with 5ml of their respective irrigant for 10 minutes.

Each sample was then scraped with a paper point and inoculated in sabouraud dextrose agar and incubated at 37 C for 2 days in a petri dish, which was then analysed by the digital colony counter and the readings were subjected to statistical analysis using SPSS soft version 26 derived ANOVA and Post-Hoc Tukey tests.

Results

Readings of microbial count obtained from digital colony counter after irrigation with respective irrigant were as follows:

The Microbial Count Readings Obtained From The Digital Colony Counter After Irrigation 10x3

Saline	Propolis	Naocl	Chlorohexinate
104	0	0	2
102	1	0	5
113	0	1	7
117	0	0	2
114	1	0	2
103	0	0	4
104	1	1	2
115	0	0	1
117	0	0	11
119	0	0	14

The mean and standard deviations obtained were as follows (Table 2).

	N	Mean	SD	Min	Max
SALINE	10	110	6.24	102	119
PROPOLIS	10	.30	0	0	1
NAOCL	10	.20	0	0	1
Chlorohexidine	10	5	4.40	1	14
Total	40	34.7	4.24	103	135

Microbial count was maximum in Saline group and minimum in Propolis and Naocl groups While it was moderate in chlohexinate group. Absolute washout of microbes was observed in 6 out of 10 samples of Propolis and 7 Naocl groups.

The order of efficacy of different groups was as follows: Propolis=Naocl > chlorohedinate > Saline

Discussion

Candida albicans is amongst the commonest micro-organism encountered in recurrent endodontic infections and has a stron predilection to form biofilms. After an initial period of its adherence to metals for 0-2hours, there is microcolony formation after 2-4hours. After a period of 4-6 hrs, there is dimorphic switching from budding-yeast forms to filamentous pseudo and true-hyphal forms. Micro-colonies are produced which later on get inter linked as a result of the hyphal extensions that are formed, resulting in a confluent monolayer within the next 6-8 hours. Eventually the biofilm takes on a three-dimensional architecture with spatial heterogeneity.

The biofilm after 24 and 48 hours consists of a mixture of yeast cells, extracellular material and true hyphae. Filamentous forms account for the most crucial factor in this three-dimensional architecture, with yeast cells forming the the basal layer^{13,14}. There are some mutant strains of *C.albicans* mutants that lack the ability to produce hyphae have demonstrated an inability to form

three dimensional biofilms. Therefore, the dimorphic switching observed in this species is a pivotal factor for biofilm formation and the pathogenic potential of *C. albicans*, which is why the 48 hours biofilm model was used. Sodium Hypochlorite is the most commonly used irrigating solution in clinical practice and has the most established anti-microbial activity. Ayhan et¹⁵ aldemonstrated that Naocl lowered CFU below the limit of detection after only 10s in the case of *C.albicans*. In this study, Propolis has shown equal efficacy to that of Naocl and chlorohexinate . Propolis exhibits anti-microbial, anti-inflammatory, healing, anaesthetic and cariostatic properties. According to Tckaisi-Kikuni and Schilcher¹⁶, it prevents fungal cell division and also breaks down fungal cell wall and cytoplasm similar to the action of some antibiotics. Kujungiev et al¹⁷. reported the antimicrobial action of propolis is due to flavonoids and esters of phenolic acids. This is in support of our study aspropolis effectively inhibited Candidal biofilm. The pH chosen for propolis was six based on the results of the study conducted by Ivaneajiaet al¹⁸,since the inhibitory effect of propolis was the strongest in a slightly acidic environment(pH = 6). Inhibitory action of propolis is also solvent dependent. Acetone and ethanolic extracts were found to be more active towards most microorganisms, so we have use dan ethanolic extract of propolis.¹⁸ Kousedghi et al¹⁹ compared the antibacterial activity of propolis and calcium hydroxide against *C.albicans*, but the drawback of the study was that the biofilm model was hot used thereby it did not simulate a clinical condition. Until date, no study has been conducted comparing propolis with sodium hypochlorite and chlorohexinate in a biofilm model against *C.albicans* and therefore, this study holds ground for future research. According to the results of the study, we see a promising herbal irrigant in propolis against *C. Albicans* inroot canal infections as like sodium hypochloride and chlorohexinate Saline was taken as negative control which as expectedhad the least antimicrobial activity.

Conclusion:

Under the limitations of this study, it can be conauded that Propolis performed equally well as Naocl and while less effect of chlohexinate 0.2% against *C.albicans* has been observed .Thus, from the results of study, it can be suggested thatfor endodontic infections, proplis could beused as anadjuvant to Naocl but, further in vivo and long term studies needed.

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