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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THE ANTIMICROBIAL EFFICACY OF SODIUM HYPOCHLORITE WITH PROPOLIS AGAINST E-FAECALIS

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ABSTRACT

Introduction: Endodontic success relates to the 'endodontic triad' composed of instrumentation, disinfection and obturation, which are interwoven. Intricacies of the root canals in the primary teeth make it impossible for instrumentation alone to reach the finer, more tortuous aspects of the anatomy. Moreover, endodontic infections are polymicrobial in nature with a predominance of stubborn anaerobic organisms like E-Faecalis which further complicates the outcome. Irrigation thus permits efficient removal of the residual tissue and the microbial load. Although sodium hypochlorite remains a well accepted choice for irrigation, it possesses certain disadvantages like burning of surrounding tissues, damage to the permanent tooth follicles, tissue necrosis and chemical burns. Hence newer products with lesser disadvantages and potent antimicrobial activity like propolis are slowly gaining popularity in the pediatric population.
Aim: The present study was undertaken to determine and compare the efficacy of sodium hypoclorite and propolis against E-Faecalis present in the infected root canals of the primary teeth.
Materials and Methods: The selected teeth with dento-alveolar abscess were divided into two groups randomly. In Group A -11% Ethanolic extract of propolis and in Group B - 3% sodium hypochlorite were used as the irrigating solutions. The bacterial samples were collected both pre- and post-irrigation and were cultured.
Results: A mean reduction of 85.2% and 92.2% in the microbial load was observed with propolis and sodium hypochlorite, respectively. The difference was found to be statistically insignificant.
Conclusion: The present study puts forth the use of propolis as an effective root canal irrigant in pediatric patients with anti-microbial efficacy comparable to sodium hypochlorite. Additional research with propolis will further help in improving its properties and establishing it as a potent irrigant.

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INTRODUCTION

The aim of any pediatric endodontic treatment is to maintain the primary tooth in the arch so that it serves its function and facilitates eruption of permanent successor. Microbial infection of the pulp is considered to be the most common reason for the endodontic treatment (Vahdaty *et al*., 1993). Endodontic infections are polymicrobial in nature dominated by obligate anaerobic bacteria. The microbial invasion prompts the host to respond with a combination of non specific inflammatory processes and specific immunologic response (Poonam Shingare and Vishwas Chaugule, 2013). Moreover, the pulp which is infected, devitalized, and necrosed can lead to apical periodontitis, hampering the success of endodontic treatment (Moller *et al*., 1981). The prevalence of anaerobic microorganisms in the root canals of primary teeth with long acting chronic lesions was found to be around 96.8% (Luciana Cunha Pazelli *et al*., 2003). E Faecalis continues to be one of the most persistent organisms found in dentoalveolar abscess cases. The microbial load reduction or elimination from root canals of infected teeth leads to successful treatment. Endodontic instrumentation alone cannot successfully get rid of the microflora from the root canals of primary teeth mechanically due to the anatomical complexity of the root canals in primary teeth. Primary teeth have zones inaccessible to debridement, such as accessory canals, ramifications, and dentinal tubules. This is overcome by the use of irrigating solutions which permit bacterial neutralization and toxin inactivation without negative interference with the healing process (Thomas *et al*., 1994). Numerous solutions have been used in endodontics to achieve the desired chemical effect since sodium hypochlorite was introduced by Walker in 1936 (Walker, 1936). Sodium hypochlorite has remained a popular

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root canal irrigant because of its antimicrobial potential and its ability to dissolve organic material. However sodium hypochlorite is not only an irritant to the periapical tissues, but also inherently possesses certain disadvantages like burning of surrounding tissues, damage to the permanent tooth follicles and oral mucosa, tissue necrosis and chmical burns, staining of instruments and clothes, etc. Apitherapy is a branch of alternative medicine that uses honey bee products including honey, pollen, bee bread, propolis, royal jelly and bee venom. The presence of arginine, vitamin C, pro-vitamin A, vitamin B-complex, and trace minerals such as copper, iron, zinc as well as bioflavonoids cause stimulation of various enzyme systems, cell metabolism, circulation and collagen formation could contribute to the hard tissue bridge formation by Propolis (Park *et al*., 2002). The present study was thus carried out to determine the antimicrobial efficacy of Propolis as a root canal irrigant in primary molars and comparing it with that of sodium hypochlorite.

MATERIALS AND METHODS

The study was conducted in 30 patients between the age group of 4 - 8 years of both the sexes in the Department of Pedodontics and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Pune who fulfilled the following inclusion criteria.

Inclusion Criteria

1. Healthy children between 4 - 8 years of age.
2. Maxillary first deciduous molars with dento-alveolar abscess requiring pulpectomy treatment.
3. Indicated teeth with atleast 2/3rd of its root length intact.

Exclusion criteria

1. Children with a systemic disorder.
2. Teeth with un-restorable crowns.
3. Teeth with abnormal anatomy or calcified canals.
4. Teeth with evidence of extensive internal/external pathological root resorption.
5. Children undergoing antibiotic therapy.
6. Children allergic to Apitherapy/products containing honey.

A written informed consent was obtained from parents of the children to be included in the study. The design of this study and the consent forms were reviewed and approved by the ethical committee.

Procedure

The children with dento-alveolar abscess (Fig. 1), thus selected were instructed to rinse their oral cavity for 30 seconds with 5 ml of povidone iodine mouth rinse. This was followed by rubber dam placement for the primary teeth. A high speed hand-piece was used for coronal necrotic pulp removal and to gain access to the root canals of the teeth (Fig. 2). For the sampling procedure, the palatal canal of the maxillary first deciduous molars was selected. Followed by pulp extirpation, a sterile paper point was placed into the canal for 1 minute (Fig. 3). It was then removed and placed immediately into the transport medium. This pre- irrigation sample was sealed and taken for microbiological testing.

The children were then randomly divided into 2 groups:

A- Receiving 11% Ethanolic extract of Propolis
B- Receiving 3% Sodium hypochlorite

Following the pre-irrigation sample collection, canals were irrigated, after using each file size upto size 35 with 5 ml of the respective test solutions for about 5 minutes (Fig. 4). Sterile paper points were again placed in the canal and second samples of the root canal contents were collected (Fig. 5). This constituted the post-irrigation sample, which was also immediately taken for microbiological culturing. The pre and post irrigation readings were noted, tabulated and analyzed statistically.

Fig.1. Dento-alveolar abscess seen with 64

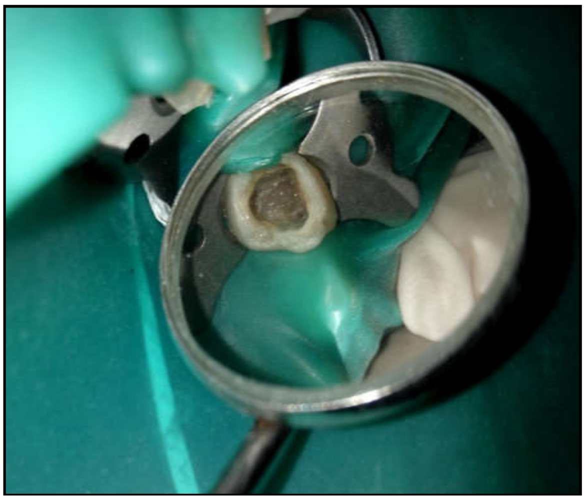


Fig.2. Access opening performed

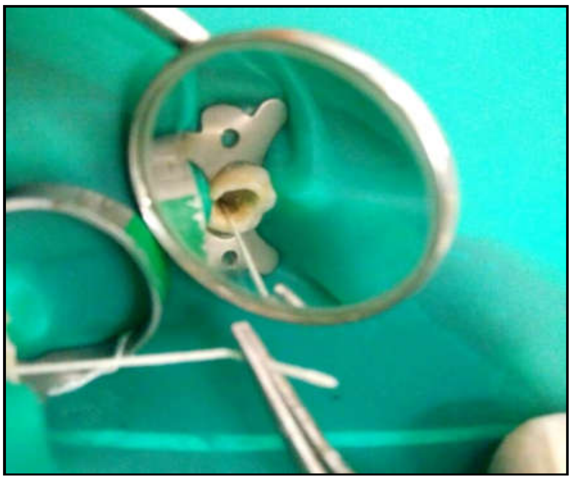


Fig.3. Collection of pre-irrigation sample using paper points

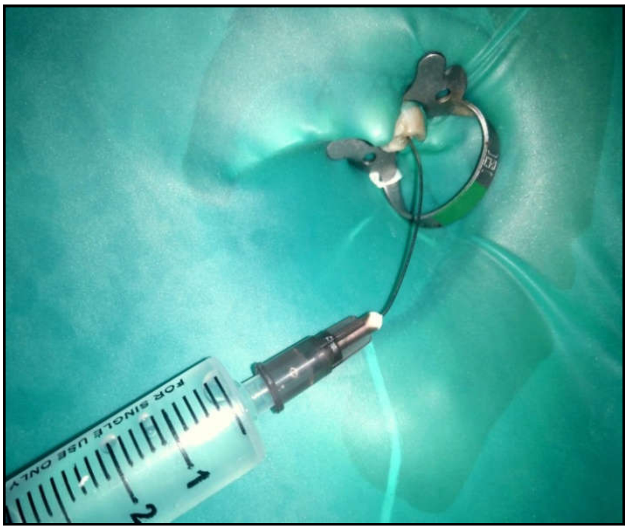


Fig.4. Intermittent irrigation using test irrigant

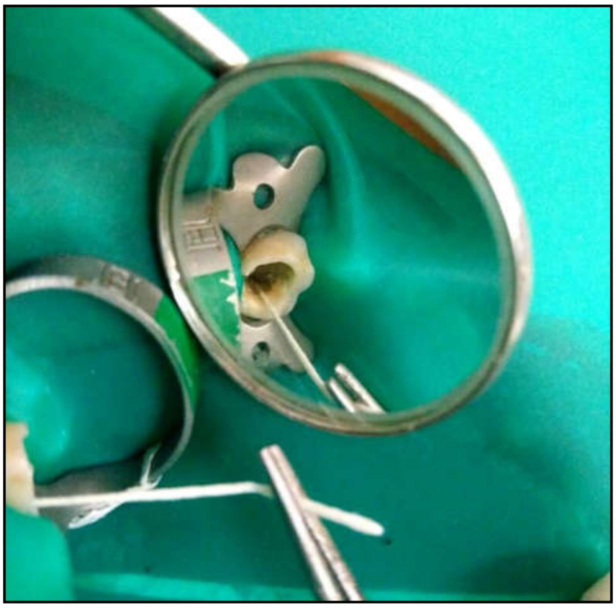


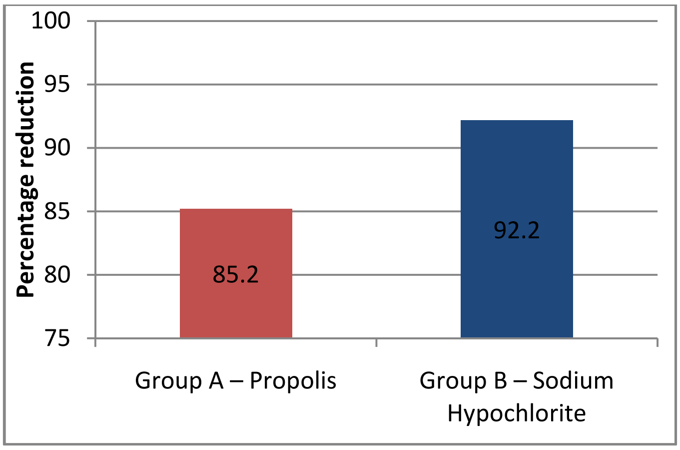
Fig.5. Collection of post irrigation sample using paper points

RESULTS

The total microbial count was estimated using a digital colony counter. Count per milliliter of diluted broth was calculated and multiplied by dilution factor. In both the groups, there was a statistically significant decrease seen in the microbial count in between the pre and post irrigation samples. (Table 1) In group A, a mean reduction of 85.2% was observed with the microbial load, while in Group B it was found to be 92.2% (Graph 1). Group statistics was done by using t test. When group A was compared with group B the difference was found to be statistically insignificant (t test – 0.222; p value – 0.826), there by proving comparable efficacy in both the groups as seen in Table 1.

Table 1. Comparison of the mean difference (pre - post irrigation values) {Mean (SD)} among both the groups using unpaired t test

Mean difference	No of samples	Mean (SD)
Group A – Propolis	15	199.13 (55.2)
Group B – Sodium Hypochlorite	15	202.86 (34.4)
t value	-	0.222
P value	-	0.826



Graph 1. Comparison of the mean percentage reduction among both the groups

DISCUSSION

Preservation of primary teeth is essential for the harmonious development of occlusion, maintenance of arch length, optimum function of chewing and speech and preservation of healthy oral environment. When dental caries are left untreated, it inadvertently leads to a dento-alveolar abscess. The fate of the infection depends on the virulence of the bacteria, host resistance factors and regional anatomy. After entering the periapical tissues via the apical foramen, these bacteria are capable of inducing acute inflammation leading to pus formation. As per graham DB, abscesses account for 47 % of all dental-related attendances at paediatric emergency rooms in the United States (Graham *et al*., 2000). Thus; in the present study salvageable maxillary first deciduous molars with dento-alveolar abscess requiring pulpectomy treatment were included. According to Nair PN, the pathogenesis of dentoalveolar abscess is polymicrobial in nature, comprising of various facultative anaerobes like E-Faecalis, viridans group streptococci and the Streptococcus anginosus group, strict anaerobes, especially anaerobic cocci, Prevotella and Fusobacterium species (Nair, 2004). According to Sundqvist G *et al*., E. faecalis is found in 4 to 40% of primary endodontic infections (Sundqvist *et al*., 1998). Studies investigating its occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (Molander *et al*., 1998). In some cases, E. faecalis has been found as the only organism (pure culture) present in rootfilled teeth with periradicular lesions (Sundqvist *et al*., 1998). Enterococci are well adapted for survival and persistence in a variety of adverse environments. It may explain its survival in root canal infections, where nutrients are scarce and there are limited means of escape from root canal medicaments. In in vitro studies, Enterococcus faecalis has been shown to invade dentinal tubules. According to Guven Kayaoglu *et al*., it can colonize root canal and survive without the support of other bacteria (Guven Kayaoglu and Dag Orstavik, 2004). As per Abo Al-Samh D *et al*., the simple act of irrigation allows flushing away of loose, necrotic, contaminated materials before that they are inadvertently pushed deeper into the canal and apical tissues, compromising the periapical tissue and permanent bud. Thus the use of irrigants is essential for ensuring bacterial elimination and eradication of organic tissue remnants (Abo Al-Samh and Al-Bagieh, 1996). Moreover, according to Thomas AM, primary teeth have zones inaccessible to debridement, such as accessory canals, ramifications, and dentinal tubules. The complex morphology and the irregularity of the root canals of primary teeth negatively affect the success of chemo-mechanical endodontic treatment (Thomas *et al*., 1994).

Several root canal irrigants such as Sodium Hypochlorite, Saline, Chlorhexidine, etc. have been used over centuries for the purpose of disinfection. Several ideal requirements of such irrigants are given by Zehnder (2006). These include broad antimicrobial spectrum, high efficacy against anaerobic and facultative microorganisms organized in biofilms, ability to dissolve necrotic pulp tissue remnants, ability to inactivate endotoxin, ability to prevent the formation of a smear layer during instrumentation or to dissolve the latter once it has formed and being systemically nontoxic when they come in contact with vital tissues with ablsolutely no potential to cause an anaphylactic reaction. Sodium hypochlorite (NaOCl) has widely been accepted as a root canal irrigant since its first reported use by Walker in 1936 (Sassone *et al*., 2008). Sodium hypochlorite (NaOCl) is a strong base (pH>11) that acts as an organic and fat solvent, degrading fatty acids and transforming them into fatty acid salts (soap) and glycerol (alcohol) that reduce the surface tension of the remaining solution. The high pH of sodium hypochlorite interferes in the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism, and phospholipid degradation observed in lipidic peroxidation (Estrela *et al*., 2002). NaOCl is used in concentrations varying from 0.5% to 5.25%. Siqueira *et al*. evaluated the effectiveness of 4% NaOCl against Enterococcus faecalis in vitro reporting that it was significantly more effective than saline solution (control group) in disinfecting the root canal (Siqueira *et al*., 1997). In this study, a 3% concentration of sodium hypochlorite has been used as a root canal irrigant and its anti-microbial efficacy has been tested. NaOCl was highly effective in bringing down the E-Faecalis count. The contact time of 5 minutes was chosen in this study, as a lesser contact time has been found to be ineffective for Sodium Hypochlorite (Radcliffe *et al*., 2004).

NaOCl, in both in vitro and In vivo conditions, exhibits excellent antibacterial activity. Despite its fulfillment of other desirable properties including availability and low cost, it has a few drawbacks. As per Hülsmann M, apart from its unpleasant taste and smell, tendency to bleach clothes and it is potentially corrosive, there is a concern regarding its noxious effects if concentrated solutions were inadvertently forced into the periapical tissues during irrigation or leaked through the rubber dam (Hülsmann *et al*., 2009). Besides, some controversies as highlighted by Dakin HD do exist with regard to its antimicrobial activity at lower concentrations, which are advocated as an attempt to reduce its toxic reactions (Dakin, 1915; McDonnell and Russell, 1999). Owing to such drawbacks, several herbal and api-products which have been introduced long back for curing diseases are again being looked upon as an alternative to commercially available chemical formulations. In dentistry, propolis has been used for surgical wound repair, root canal irrigation, direct and indirect pulp capping, for reduction of dentin hypersensitivity, in caries prevention and as a storage media for avulsed teeth (Elaine *et al*., 2014). Considering the immense potential of propolis as an antimicrobial agent, as evidenced in literature, the present study was undertaken to assess its usage as an intracanal irrigant in endodontic therapy of primary teeth. According to Talas *et al*., the main chemical classes present in propolis are flavonoids, phenolics and other various aromatic compounds. Flavonoids are well known plant compounds that have antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties (Talas *et al*., 2009). Bankova V has also put forth the fact that propolis possesses distinguishing and pleasing aromatic smell and varies in color depending on its source and age (Bankova *et al*., 2000). Moreover Jain S *et al*. observed that propolis does not lose much of its antibiotic activity even when stored for 12 months or longer (Jain *et al*., 2014).

Koo found that propolis had an antibacterial effect on several microorganisms and the antibacterial characteristic of propolis could be explained in a number of ways. These included propolis containing high concentrations of pinocembrin, chrysin, acacetin and galangin (Koo *et al*., 1998). One such study by Takaisi-Kikuni NB *et al*., reported that it prevented bacterial cell division and also broke down bacterial walls and cytoplasm similar to the action of some antibiotics (Takaisi-Kikuni and Schilcher, 1994). In another study, Kujumgiev reported that the antibacterial, antifungal and the antiviral activities of propolis to be due to flavonoids and esters of phenolic acids (Kujumgiev *et al*., 1999). Use of different concentrations of propolis extract ranging from 11% to 33% by weight to volume ratio has been tested. In this study, 11% alcoholic extract of propolis was used, as suggested by GP de Rezende *et al*. (2008). A significant reduction in microbial load was observed with the use of 11% ethanoloic extract of Propolis. The anti-microbial effects of propolis in this study are similar to those obtained by others who have evaluated the inhibitory effect of propolis solution on bacterial growth. Manjesh Kumar Verma *et al*. carried out a study to to assess the potential of water-soluble 25% propolis extract against microorganisms present in root canals of primary teeth during endodontic procedures. Considering the low toxicity concerns and antibacterial effectiveness of water-soluble extract of 25% propolis, they confirmed its use in the root canals of primary teeth in vivo (Verma Manjesh Kumar *et al*., 2014). Grang and Davey used a propolis dilution of 1:20 in nutrient agar and they found that it had an inhibitory effect on grampositive cocci and rods (Grange and Davey, 1990). The present study was conducted to determine and compare the anti-microbial efficacy of 3% Sodium hypoclorite and 11% Ethanolic Extract of Propolis against E-Faecalis present in the infected root canals of the primary teeth. Both the groups showed statistically significant decrease in the E-Faecalis count post irrigation. On comparing the anti-microbial efficacy between the 2 groups, NaOCl proved to be slightly better than propolis. However, the difference was statistically insignificant, leading to the conclusion that propolis proved to be equally efficacious as a root canal irrigant in primary teeth as sodium hypochlorite. Moreover since propolis possessed a sweeter and taste and smell, it seemed to be more accepted than sodium hypochlorite by the children.

A study by Hind Al-Qathami *et al* also showed similar results and concluded that the difference in the antimicrobial activity of Propolis and NaOCL was not significant (Hind Al-Qathami and Ebtissam Al-Madi, 2003). In contrast to this however, Poonam Shingare el al. in their study stated that propolis was less efficacious than Sodium hypochlorite (Poonam Shingare and Vishwas Chaugule, 2013). This was in accordance with the study carried out by Asha Nara *et al*. (2010). The study done by Shveta Gupta *et al* 1 showed that 30% propolis extract was not effective against E. faecalis owing to the discrepancy in the vehicle used and the pH obtained (Gupta *et al*., 2007). Several changes can be formulated for the various propolis preparations in order to make it more efficacious. These mainly include determination of a suitable vehicle which helps in retaining it inside the canals, thereby increasing the contact time. Moreover, preparations with higher pH values show greater anti-microbial properties. Addition of synergistic compounds to Propolis formulations or using it in combination with other agents may further help in improving its characteristics as a potent irrigating solution.

Conclusion

Thus, In pediatric dentistry, the role of natural products cannot be overemphasized as nearly all problems related to oro-dental region requires either direct contact of material and medicaments with oral mucosa in cases of a mouth rinse, intracanal irrigation, IPC, etc. or indirect contact with the hard and soft tissues as intracanal medicament and accidental extrusion of irrigant. Hence, the child is always at a risk of toxic reactions from man-made formulations either by direct contact or by systemic absorption. The present study puts forth the use of Propolis as an effective root canal irrigant in pediatric patients with anti-microbial efficacy comparable to Sodium Hypochlorite. However need for more research work in this field is a must in order to establish the efficacy of Propolis against the conventional root canal irrigants.

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