

Evaluation of Antimicrobial Efficiency of Herbal Irrigants of Pulpectomized Primary Teeth

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Abstract:

Objective: This study aimed to evaluate the antimicrobial efficiency of 2 herbal extracts versus 2.5% sodium hypochlorite and saline in eliminating *Enterococcus faecalis* (*E. faecalis*) bacteria within infected primary root canals. **Materials and Methods:** Forty roots of extracted lower primary molars were obtained and divided into 4 groups according to the type of irrigant: Group 1 (control) NaOCl 2.5%, Group 2 (12.5% turmeric irrigant), and Group 3 (25% propolis irrigant) and Group 4 (saline). Root canals were contaminated with *E. faecalis* bacteria. Bacterial counts were performed from samples that were taken from each canal pre-irrigation and post-irrigation. Data was analyzed using t-test and ANOVA. **Results:** There were statistical differences between all groups; NaOCl and turmeric extract showed a high effective reduction of a bacterial number followed by propolis. A slight reduction of *E. faecalis* was demonstrated following saline irrigation. **Conclusions:** All herbal irrigation solutions showed effective eradication of *E. faecalis* and can be used as an alternative to NaOCl in the irrigation of infected root canals of primary teeth.

Introduction:

Bacteria is the main etiologic factor for pulpal diseases. Successful endodontic treatment in primary teeth relies on several factors as the complete removal of microorganisms from the root canal and periapical tissues, proper mechanical preparation, and irrigation, and adequate obturation of root canals to allow tissue healing.^(1,2)

Medicament and intracanal irrigants are essential in endodontic therapy of primary teeth for their chemical disinfection, lubrication, and removal of necrotic tissues. Mechanical instrumentation alone can not provide proper disinfection after cleaning and shaping the canals. Various irrigants are available for the removal of bacteria and disinfecting the root canals.⁽³⁾

The most common pathogenic micro-organism in primary root canals is *Enterococcus faecalis*. *E. faecalis* can survive in a highly alkaline conditions.⁽⁴⁾ Various intracanal irrigants are used for pulpectomy of primary teeth as sodium hypochlorite which is universally used because of its antimicrobial effect and ability to dissolve organic material. However, it possesses some disadvantages such as unaccepted taste and odor, burning to surroundings, the possibility of damaging permanent tooth follicles, and the inability to fully eradicate microbes. In addition, chlorhexidine gluconate, tetracycline isomer, and hydrogen peroxide were used as root canal irrigants.^(5,6) Chlorhexidine is a broad-spectrum antimicrobial agent as in low concentration has bacteriostatic action. At 2% CHX may have a toxic effect and impair tissue healing. When it is mixed with sodium hypochlorite produces a harmful product.⁽⁷⁾

Due to the worldwide trend of 'Back to Nature', nowadays-herbal alternatives are widely used in

dentistry because of their safety and beneficial effects. Natural phytochemicals extracted from medicinal plants are potential sources of bioactive components. Studies suggested their biocompatibility, antimicrobial ability, non-toxic effect, better acceptance by the patient, and anti-inflammatory properties. Considering the Side effects and inefficient results of synthetic irrigants, many studies were performed to search for herbal alternatives that are effective and less irritating to the tissues than synthetic ones.^(8,9)

The therapeutic effects of Various herbal extracts, such as neem, curcumin, aloe vera, and propolis have been evaluated to be used as root canal irrigants.⁽¹⁰⁾

Propolis is a natural product, which has several uses in dentistry. It is used as pulp capping agent, storage media for avulsed teeth, and reduction of caries and dentine hypersensitivity. Propolis is effective against *E. faecalis*, *S. aureus*, and *Candida albicans*. The antimicrobial effect may be attributed to the flavonoid content which breaks down bacterial cell walls and prevents the division of bacterial cells. It is also effective against some Gram -ve bacteria.⁽¹¹⁾ A previous study suggested the effectiveness of Propolis against *E. faecalis*, it reported that 300µg/ml Propolis irrigant was as effective as 6% NaOCl and 2% CHX in eliminating *E. faecalis* biofilms.⁽¹²⁾

Turmeric is a flavoring and medicinal herb. The main constituent of turmeric is mainly curcumin. For centuries, Curcumin is widely used in dentistry and medicine, due to its therapeutic properties as it is antimicrobial, anti-inflammatory, nontoxic, anticarcinogenic, and analgesic effect.⁽¹³⁾

In dentistry, curcumin can be used for the treatment of periodontitis, mouthwash, to relieve pain and swelling, and in pit and fissure sealant. Few studies showed the efficacy of turmeric extract as a root canal irrigant

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solution in primary teeth.⁽¹⁴⁾

The anti-inflammatory effect of 10% Turmeric mouthwash was as effective as chlorhexidine mouthwash in the prevention of gingivitis.⁽¹⁵⁾ Significant reduction in plaque and gingival index was observed when using 2% turmeric gel therefore, it can be used as an adjunct for treating periodontal disease.⁽¹⁶⁾

Few studies were performed to evaluate the efficacy of herbal extract as a root canal irrigant in primary teeth. Therefore, this study aimed to comparatively evaluate the antimicrobial efficacy of commercial herbal extract for the irrigation of pulpectomized primary teeth

Materials and methods:

This study was performed in the Department of Pediatric Dentistry, Faculty of Dentistry, Mansoura University. The ethical approval was taken (M24030123) from the Ethical Committee of Mansoura University.

Teeth selection: Extracted lower primary molars that fulfill the following criteria were collected: no radiographic root resorption, presence of at least 2/3 of root length, and no root fractures or cracks. The teeth were cleaned by using a scaler for removing calculus and tissue tags and then soaked in 5% NaOCl for 24 h to remove any remaining debris. Teeth were stored in saline to prevent dehydration.

Specimens' preparation: All lower primary molars were decoronated below the cemento-enamel junction using a rotary double-sided diamond disc on a high-speed contra-angle with water coolant. Teeth were sectioned perpendicular to the long axis with a long cylindrical carbide bur from the cemento-enamel junction to separate the roots. Forty distal root canals were selected for the study. All canals were instrumented using hand files (K-type) and enlarged to size #35 for cleaning and shaping. Canals were flushed with saline after each instrument size to prevent blockage of the canal. After the determination of working length, root apices were sealed with α -cyanoacrylate adhesive (Amir Alpha co.) to avoid bacterial leakage. All specimens were sterilized at 121 °C and 15 PSI pressure for 15 minutes in a steam autoclave.⁽¹⁷⁾

Biofilm formation: The bacterial culturing was carried out in the Department of Microbiology Faculty of Medicine, Mansoura University. *E. faecalis* -a known resistant strain- was used in this study. The inoculating *E. faecalis* was cultured in the brain heart infusion broth after incubation at 37°C for 24 h. The prepared canals of the experimental teeth and the positive control group were inoculated for 72-hour with the prepared suspension of *E. Faecalis* using an insulin syringe and incubated at 37 for 4 weeks.⁽¹⁸⁾

Root canals grouping and irrigation: Following the contamination procedure, specimens were assigned into four groups (each = 10) according to the irrigant used. group (I): irrigated with 2.5% NaOCl solution

group (II): irrigated with 12.5% turmeric solution., group (III): irrigated with 25% water-soluble Propolis

solution, group (IV): irrigated with sterile saline. pre-irrigation samples were taken using sterile paper points which were placed in root canals to the apex and left for 1 minute. Then, they were removed with tweezers and placed in a tight sterile vial containing nutrient broth for culturing in a microbiological lab. 2 ml of irrigant was delivered into the canal with (24G) sterile plastic syringe for 5 min, and then the specimens were flushed with distilled water. Post-irrigation samples were taken in the same manner as pre-irrigation samples and transported immediately to the microbiological lab.

Preparation of 12.5% turmeric irrigant: Two hundred and fifty grams of the powdered *Curcuma longa* rhizomes were added to 1000 ml of absolute Ethanol in a flask. The flask was left overnight after wrapping it with aluminum foil. The extract was filtered by using filter paper then it is separated from ethanol by evaporation. About 112.5 gm was yielded which is diluted with distilled water to obtain a 12.5% turmeric solution.⁽¹⁸⁾

Preparation of 25% of propolis irrigant: Twenty-five grams of Propolis powder was triturated with 5 ml of 98% methanol until obtaining a homogenous emulsion. A few drops of Tween-80 were added until a clear solution was obtained. Then 50 ml of distilled water was added slowly with continuous stirring until complete dissolution followed by adding 50 ml of distilled water to get 25% water-soluble extract of Propolis then stored in a refrigerator until use.⁽¹⁹⁾

Bacterial count: Paper points of pre and post-irrigation were placed in Eppendorf containing 1 ml saline and agitated by vortex for 1 min. After 10-fold of serial dilution, aliquots of 0.1 ml were spread plated onto BHI agar plates and incubated at 37°C for 48 hours. The colonies were determined based on their morphology and counted as CFU/ml of sample.

Statistical analysis: Data was collected and analyzed using a t-test at a level of significance of 0.05 and the ANOVA analysis of variance

Results:

The antibacterial activity of NaOCl (group 1) was found to be greater than other groups. As the bacterial count was (7.3±4.95) followed by turmeric irrigant where the bacterial count post-irrigation was (15.5±13.07) followed by propolis and saline respectively (76.5±38.36, 132.2±74.78). Comparison between Turmeric (group 2) and propolis (group 3) showed better activity of Turmeric than propolis. Table 1. The antimicrobial effect of propolis (group 3) was observed higher than saline (group 4). No statistically significant difference in the post-irrigation bacterial count was found between Group 1 & Group II. Bacterial count reduction was statistically higher when

Table 1: Bacterial count (CFU/ml 10⁵) pre and post-irrigation in all groups

Sample no.	Group 1 (NaOCL)		Group 2 (Turmeric)		Group 3 (propolis)		Group 4 (Saline)	
	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation	Pre-irrigation	Post-irrigation
1	78	6	55	3	80	25	310	270
2	260	5	120	22	120	63	244	189
3	205	10	204	31	210	140	289	196
4	156	6	27	2	265	32	71	53
5	167	7	67	8	115	77	248	177
6	118	3	33	7	168	50	74	36
7	165	14	145	12	99	56	175	104
8	227	17	266	43	264	101	203	129
9	207	2	115	11	137	97	118	61
10	170	3	150	16	234	124	146	107
Mean ±SD	175.3±52.77	7.3±4.95 a d	118.2±76.84	15.5±13.07 b e	169.2±69.37	76.5±38.36 c d e	187.8±85.35	132.2±74.78 a b c
P value	T=10.24& P=0.000*		T=4.17&P=0.001*		T=3.7&P=0.002*		T=1.35& P=0.135	

* P is significant and ≤ 0.05 , Groups with the same letter means insignificant difference between them

comparing turmeric irrigant group with propolis irrigant group and saline group.

Discussion:

The use of anti-bacterial irrigants is recommended in endodontic therapy to eliminate and eradicate microorganisms and organic tissue remnants. Root canals of primary teeth are tortuous and characterized by their narrowness, and complex anatomy which negatively affect the success of endodontic treatment. ⁽²¹⁾ *E. faecalis* is a Gram-positive anaerobic and it was selected in this study as it is the most predominant species in infected primary root canals. Also, these bacteria can survive in highly acidic conditions with little nutrients. Specimens' apices in the present study were sealed with cyanoacrylate to avoid contamination from the outer circumstances that may affect the bacterial count. ⁽²²⁾

Chemical irrigants are used in endodontic treatment for the eradication of microorganisms. Ideal irrigants should be biocompatible, have a good antimicrobial effect, and be nontoxic to periapical tissue or injurious to underlying permanent successor teeth, especially in pediatric dentistry. ⁽²³⁾

The most widely known irrigant is NaOCl which is used in different concentrations ranging from 0.5% to 5.25 for its antibacterial effect and ability to dissolve organic tissue. The higher concentration is not advocated to avoid irritating and cytotoxic effects on periapical tissues. Therefore, a concentration of 2.5% NaOCl was used in this study to avoid possible damage to the underlying permanent tooth. ⁽²⁴⁾ Because of the adverse effect of NaOCl, many alternative and herbal irrigants were introduced to be used as root canal irrigants. In the present study,

2.5% NaOCl showed the highest significant reduction of mean bacterial count followed by the turmeric group followed by the propolis group, and the least reduction was seen with the saline group. The result is consistent with another study which reported that 2.5% NaOCl as root canal irrigant is extremely effective in removing vital pulp tissue from dentinal walls. ⁽²⁵⁾ The result of the present study showed a significant reduction in group 1 > group 3 > group 4 while the bacterial reduction was not significant between group 1 and group 2.

Turmeric is an available and economical herbal material that has antimicrobial, anti-inflammatory, and immune-stimulant properties, therefore, it is used for medicinal purposes. Also, it has an antibacterial effect against *Staphylococcus aureus* and *Enterococcus faecalis* and subsequently reduces rates of dental caries and gingival inflammation. The main component of turmeric is curcumin demethoxycurcumin 5 methoxycurcumin and dihydrocurcumin, which are natural antioxidants. A study conducted by Chandra stated that turmeric has antimicrobial properties therefore, it is incorporated into a mouthwash to treat inflammation, pain, and ulcerative area in the oral cavity. ⁽²⁶⁾

Our study agreed with a previous study that assessed the antibacterial activity of 12.5% turmeric solution and concluded that turmeric can be used as a safe natural alternative to NaOCl and CHX. ⁽²⁷⁾

Propolis has therapeutic and biological properties due to the presence of phenolic compounds, flavonoids, and aromatic acids. In the present study, propolis showed less bacterial reduction effect than NaOCl, which is in accordance with Asha Nara study. ⁽²⁸⁾ On the other hand, Al-Qathmi and Al-Madi ⁽²⁹⁾ suggested that propolis root canal irrigant was as effective as NaOCl

In the present study, a saline group showed a non-significant reduction of bacterial count post-irrigation when compared with pre-irrigation and this may be explained by the ability of saline to wash out debris from canals rather than having an antimicrobial effect.⁽³⁰⁾ This supports the result of Serafino et al.⁽³¹⁾ who found that irrigation with saline has a less antibacterial effect.

Conclusion:

Within the limitation of this study, it can be concluded that the use of 12.5% turmeric solution as a root canal irrigant can be promising. It can be considered a safe natural herbal alternative substitute for NaOCL. In the irrigation of infected primary root canals. The use of a 25% propolis solution in the elimination of *E. faecalis* is still debatable as complete elimination did not take place. 2.5% % NaOCL is still the irrigation solution of choice, due to its highly effective antibacterial activity.

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