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An in vivo comparative evaluation of antibacterial efficacy of Sodium hypochlorite, Triphala, and Turmeric against microorganisms in root canal disinfection.

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ABSTRACT

Aims: The study aimed to individually evaluate and compare the aerobic and anaerobic antibacterial efficacy of Sodium hypochlorite, Triphala, and Turmeric as root canal irrigants.

Methods and Material: Sixty patients were selected and randomly allocated to one of the three groups (n = 20) The pre-irrigation and post-irrigation samples were collected through sterile paper points and microbial culturing was done on blood agar plates, followed by incubation for culturing of aerobic and anaerobic bacteria.

Statistical analysis used: Manual colony-forming unit counting was done, and statistical analysis was performed. Analysis of variance (one-way) followed by a post hoc test was performed as a parametric test to compare the difference between the groups for both aerobic and anaerobic bacteria.

Results: The comparison between the differences of mean CFUs for the three groups was statistically significant and maximum antimicrobial activity was shown by sodium hypochlorite followed by Triphala then Turmeric.

Conclusions: Within the limitations of this study, it can be concluded that NaOCl, Triphala, and Turmeric showed considerable antibacterial efficacy against anaerobic and aerobic microorganisms.

Key-words: Root canal irrigation, Sodium hypochlorite, Triphala, Turmeric

Key message: NaOCl, Triphala, and Turmeric showed considerable antibacterial efficacy against anaerobic and aerobic microorganisms.

I. INTRODUCTION:

Endodontic treatment 'success' has been defined as the prevention and elimination of periapical radiolucency and symptoms, with 'failure' being the development or persistence of Apical Periodontitis and/or symptoms (European Society of Endodontology 2006).[1] A favorable outcome of root canal treatment is nothing but the reduction of a radiographic lesion and the absence of clinical symptoms of the affected tooth after a minimal observation period of 1 year.[2]

Bacteria are the first etiologic factor in thegrowth of pulpal and periapical diseases. If the microorganisms that are remained in the dentinal tubules are not eliminated, they may reinfect the root canal.[3]

A significant percentage of the root canal surface always remains untouched, regardless of the instruments used for mechanical preparation.[4] Such unreached areas may protect micro-organisms from root canal disinfecting protocols. (Siqueira Jr et al. 2018)[5].

Irrigation plays an important role in such areas. An ideal irrigant with potent antimicrobial activity; the ability to dissolve organic and inorganic components; disinfect and flush out debris from the instrumented and untouched areas of canals; provide lubrication; and cause no adverse effects to the periradicular tissues or structural integrity of the tooth.[6]

The gold standard and the most effective irrigant is sodium hypochlorite (NaOCl). The widely used concentration is 0.5% to 6.0%. Hypochlorite preparations are sporicidal and virucidal and show far greater tissue dissolving effects on necrotic than on vital tissues. Its bactericidal activity and ability to dissolve vital and necrotic organic tissue make it the irrigating solution of choice. All these features prompted the use of aqueous sodium hypochlorite in endodontics as the main irrigant as early as 1920.[7]

Along with the merits of sodium hypochlorite, it has various undesirable characteristics. Very few complications in the use of NaOCl in endodontics have been recorded. The literature contains accidental injections of the solution beyond the root, severe pain, a rapidly developing edema, hematomas, necrosis, tissue toxicity, risk of emphysema when overfilling, allergic potential, a disagreeable smell and taste and abscesses are the commonly reported features. The complications are caused by the oxidizing effect of NaOCl on the vital tissues surrounding the endodontically treated tooth, followed by an inflammatory reaction of the body. Moreover, it

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might not completely cleanse the surfaces of the root canal walls.[8]

Therefore, the search for newer irrigants commenced. With the increasing popularity of traditional and holistic/alternative medicines due to their natural origin, easy availability, efficacy, safety, and fewer side effects, various herbal extracts have been used as effective endodontic irrigants.[9]

Triphala [three (tri) fruits (phala)] is a plant-derived composition developed in India; the powder is a combination of three dried plants named Terminaliabellerica, Terminaliachebula, and Emblicaafficinalia with tanic acid being its principal constituent. Compared to commonly used root canal irrigants, it is relatively safe. The most important advantages of Triphala include easy access, low cost, long-term substantivity, less toxicity, and absence of microbial resistance.[10]

Turmeric, also called Curcuma longa, is used as a medicinal herb, and dye in Asian countries. The main component in turmeric is curcumin, which has a wide range of properties, such as anti-inflammatory, antioxidant, antimutagenic, and antimicrobiological properties.[11]

My study aimed to comparatively evaluate the antibacterial efficacy of sodium hypochlorite, triphala, and turmeric against microbes in root canal disinfection. The null hypothesis was that there is no difference between the antibacterial effectiveness of sodium hypochlorite, triphala, and turmeric.

II. SUBJECTS AND METHODS:

The study included all males and females from the age group of 18 to 55. The total sample size was 60. All three groups had 20 participants each. The teeth with carious exposure of pulp, teeth with mature apices, and teeth with single and straight root canals were included. Teeth with periapical pathology, multiple roots, dilacerated roots, immature apices, and calcified canals were excluded from the study. Patients with a history of allergy to any of the materials being used in the study were excluded. Patients who abstain from consuming triphala and turmeric were also excluded from the study. Consent in English, Marathi, and Hindi was obtained from every patient participating in the study. All the teeth included in the study were analyzed by preoperative radiographs followed by detailed medical and dental history recordings of the patient.

Randomization was done using the sealed envelope technique wherein different envelopes mentioning the groups to be allotted were prepared and randomly picked by a person other than the operator, just before beginning the treatment. Each patient was blinded to the disinfection protocol being followed and randomly divided into three groups corresponding to the protocol:

Group I – sodium hypochlorite (n =20)
Group II- triphala (n =20)
Group III – turmeric (n =20)

The tooth and surrounding area were disinfected by swabbing with an iodine tincture. Local anesthesia was administered, and isolation was done with a rubber dam followed by an access opening of the root canal through sterile round bur. The root canals were accessed with a size 10K file (Mani, Inc., Tochigi, Japan). Debridement was done using sterile water. Working length was determined radiographically which was kept at 0.5-mm short of the apex. This was confirmed through an electronic apex locator. Then, the initial pre-treatment root canal culture sample was taken with pre-sterilized paper points. Two paper points were placed in the canal for 60 seconds and then transferred into two separate pre-sterilized Eppendorf tubes of 2 ml brain–heart infusion broth (BHI broth) for aerobic culture and thioglycollate broth for anaerobic culture in each tube. Cleaning and shaping of each root canal were completed using Protaper gold rotary files (Dentsply Sirona, USA). Each patient was then randomly allotted to one of the three groups of irrigation solution.

Group 1: Irrigation was done with 20 ml of 3% NaOCl for 20 min. This was followed by flushing the canal with sterile water followed by sample collection through paper points.

Group 2: Ripe fruits of Terminalia chebula, Terminalia bellirica, and Embellica officinalis were collected dried, and powdered. 25 g of the powder of each of the three fruits was separately passed through an 80# sieve and then mixed in equal proportions to produce uniformly blended triphalachurna. This powder was then mixed with distilled water and allowed to stand for seven days in a glass chamber. Triphala preparation was 20 gms in 80 ml of distilled water. Irrigation was done with 20 ml of aqueous triphala solution for 20 min. This was followed by flushing the canal with sterile water followed by sample collection through paper points.

Group 3: The Curcuma longa rhizomes were washed with distilled water and patted dry. They were then cut into pieces and completely dried in an oven by a tray drying process at a temperature of 40±5°C for about 7-10 days till they were moisture-free. The pieces were ground to form a coarse powder which was then placed in a large glass chamber into which 80ml of sterile distilled water

was added to prepare the aqueous extract. Turmeric preparation was 6.4 gms in 80 ml of distilled water. Irrigation was done with 20 ml of an aqueous solution of turmeric for 20 min. This was followed by flushing the canal with sterile water followed by sample collection through paper points.

After the irrigation protocol in each group, the inoculation of each sample was done under a laminar air flow hood to avoid the contamination of the samples and the agar plates. Swabbing was done on blood agar plates followed by incubation. For the growth of aerobic bacteria, incubation was done at 37°C for 24 h, and for the anaerobic bacterial growth, incubation was done using gas packs inside a McIntosh and Fildes' anaerobic jar to create the optimal environment for the growth of anaerobic bacteria and incubation at 37°C for 7 days. The microbial analysis was done by counting the colony-forming units (CFUs) through a digital colony counter.

The number of colonies in 2 ml (2000 µl) of broth = No. of CFUs × 2000 µl/10 µl.

After the manual calculation of CFUs, statistical analysis was carried out.

III. DATA ANALYSIS METHOD

Analysis of variance (one-way) followed by post hoc test was performed as a parametric test to compare the difference between the groups for both aerobic and anaerobic bacteria ($P < 0.05$ considered as statistically significant at 95% confidence level).

IV. RESULTS:

The comparison between the differences in mean CFUs for the three groups was statistically significant. According to the results obtained Group 1 sodium hypochlorite showed maximum antimicrobial activity against aerobic and anaerobic bacteria followed by Group 2, and finally Group 3.

V. DISCUSSION:

Infections in the root canals are polymicrobial and a combination of aerobic and anaerobic bacteria are responsible for pulpal infections.12 Therefore, cleaning, disinfection, and preparation of the root canal and eradication of the bacteria are indispensable requirements for successful endodontic treatment.13

Although mechanical instrumentation eliminates the majority of intracanal microorganisms, it has been demonstrated that it is not possible to eliminate microorganisms. While cleaning and shaping reduce microorganisms, the use of irrigants is complementary to instrumentation in facilitating their removal.14

For a long time in endodontics, sodium hypochlorite has been used as a root canal irrigating solution at different concentrations, ranging from 0.5% to 5%. 15

The microbial reduction produced by sodium hypochlorite (98.07%) may be explained based on the observations of Rutala and Weber16, who suggested that, when combined with water, sodium hypochlorite produces hypochlorous acid, which contains active chlorine. Chlorine exerts its bactericidal action through the irreversible oxidation of sulfhydryl groups of essential bacterial enzymes, disrupting the metabolic function of bacterial cells.17

Triphala is a well-known ayurvedic herbal formulation consisting of the dried and powdered fruits of three medicinal plants, namely Terminalia bellerica, Terminalia chebula, and Emblica officinalis with tannic acid being its principal constituent. Other chemical constituents of triphala include Quinones, Flavones, Flavonoids, Gallic, and Vitamin C. The most important advantages of triphala include easy access, low cost, long-term stability, less toxicity, and absence of microbial resistance. It has anti-cariogenic and thermogenic effects and can act as a probiotic. The antibacterial property of triphala extract has been shown in the present study by the measurement of the zone of inhibition against E. 18

Curcuma longa, commonly called turmeric has been shown to have a wide spectrum of actions like anti-inflammatory, antioxidant, antibacterial, antifungal, antiprotozoal, and antiviral activities. The components of turmeric are polyphenols with a strong antioxidant function.19

Triphala and turmeric are proven to be safe, containing active constituents that have beneficial physiologic effects apart from their curative property such as antioxidant, anti-inflammatory, and radical scavenging activity, and may have an added advantage over the traditional root canal irrigants. 20

In our study, while all three groups significantly decreased the viable bacteria from the root canals, when comparisons were drawn between the groups, maximum antibacterial activity was exhibited by NaOCl followed by triphala and finally turmeric.

Similar results were found in a study by Jenny et al. who found triphala to be the most effective herbal product when used as a root canal irrigant.21 Dissimilar results were shown by Brar et al. The study found turmeric to be the most effective herbal product when used as a root canal irrigant. However, this was an in vitro investigation

which may not have demonstrated the full clinical potential of a tested material.18

Randomization was followed for the allocation of patients to each group along with blinding of the patient to the disinfection protocol being applied. Randomization helps prevent bias. Bias occurs when a trial's results are affected by human choices or other factors not related to the treatment being tested. Randomization eliminates the selection bias, balances the groups concerning many known and unknown confounding or prognostic variables, and forms the basis for statistical tests, a basis for an assumption of a free statistical test of the equality of treatments.22

Paper point sampling of the root canal contents provides a better indication of the bacteriologic status of the root canal system. This study explored the ability of absorbent paper points used for microbial sampling, to reflect spatial distributions of bacteria in a sample site.23

However, the study had a limited sample size because of which the results cannot be correlated with clinical success and more such in vivo studies are required to evaluate the effectiveness of triphala and turmeric as an endodontic irrigant.10

VI. CONCLUSION

Within the limitations of this randomized trial, it can be concluded that NaOCl, triphala, and turmeric show considerable antibacterial efficacy against anaerobic and aerobic microorganisms.

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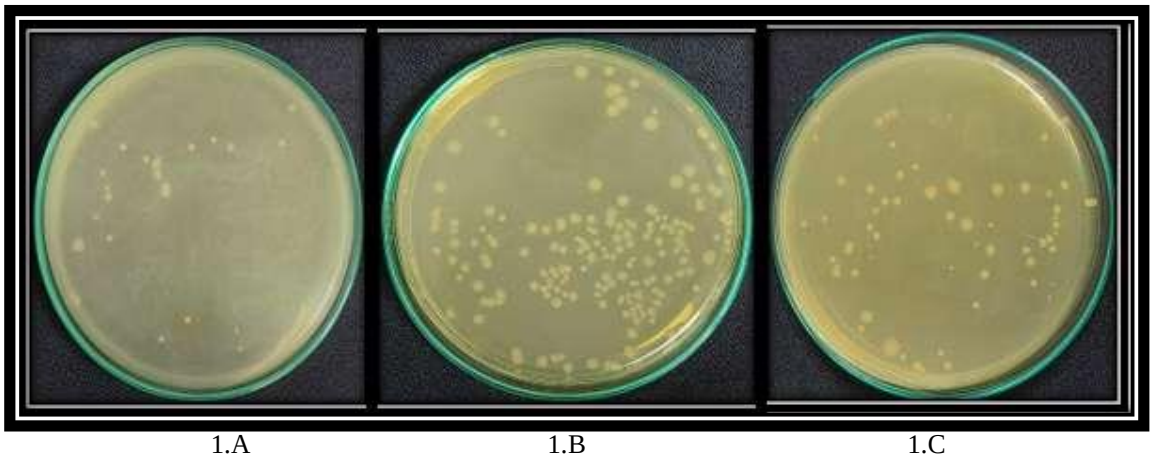

1.A 1.B 1.C

FIG 1.A POST-IRRIGATION WITH SODIUM HYPOCHLORITE (Anaerobic)
FIG 1. B POST-IRRIGATION WITH TRIPHALA (Anaerobic)
FIG 1. C POST-IRRIGATION WITH TURMERIC (Anaerobic)

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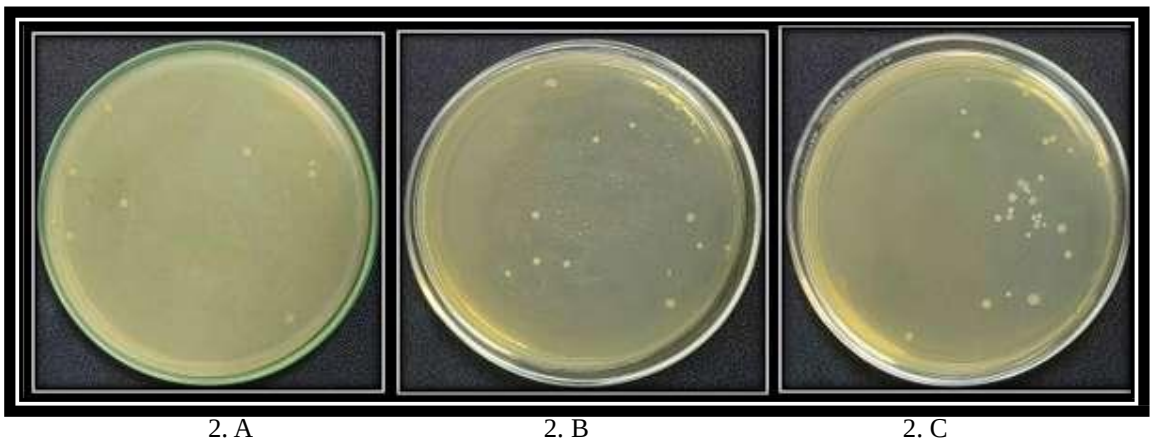

2. A 2. B 2. C

FIG 2.A POST-IRRIGATION WITH SODIUM HYPOCHLORITE (Aerobic)
FIG 2. B POST-IRRIGATION WITH TRIPHALA (Aerobic)
FIG 2. C POST-IRRIGATION WITH TURMERIC (Aerobic)