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A study on determination of chelation value & antibacterial property of different dental irrigants

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Abstract: The purpose of this study is to analyse the chelating power and antibacterial property of different dental irrigants taken for the studies. The chelation power and antibacterial activity achieved through wet lab and microbial analysis in a GLP certified laboratory in Pune.

Keywords: Chelating agent, Ethylenediaminetetraacetic acid, 17% EDTA solution, Suspension test (EN 1040:2005), Staphylococcus aureus.

Introduction: M. Hulsmann et.al described in the international endodontic journal that chelates are stable complexes of metal ions with organic substances as a result of ring-shaped bonds. This stability is a result of the bond between the chelator which has more than one pair of free electrons, and the central metal ion.

In 1957, Nygaard Ostby introduced chelating agents into endodontics as an aid for the preparation of narrow and calcified root canals. One of the most critical parameter for an ideal dental irrigant is chelating property. Chelating agents such as ethylenediaminetetraacetic acid (EDTA), citric acid and tetracycline are used for removal of the inorganic portion of the smear layer. NaOCl is an adjunct solution for removal of the remaining organic components. Irrigation with 17% EDTA for one minute followed by a final rinse with NaOCl is the most commonly recommended method to remove the smear layer. Longer exposures can cause excessive removal of both peritubular and intratubular dentin.

Chelating agents are claimed to remove the inorganic component of the smear layer from root canal dentin, being commonly used for final irrigation during endodontic treatment. The most common chelating agent is ethylenediaminetetraacetic acid (EDTA), which, at neutral pH, reacts with the calcium ions in dentin and forms soluble calcium chelates. However, if extruded into the periapical tissues, it can cause apoptosis, necrosis, inflammation, and cytotoxic effects. Acetic acid (AA) can remove dentin calcium ions, favoring smear layer removal, but it can also reach the inorganic structure of dentin, causing subsequent erosion and changes in dentin microhardness.

Although around 500 species of bacteria have been identified in the oral environment, only a limited number have been found to colonize the root canal system. Based on this, few dental irrigants studied against Staphylococcus aureus by standard EN 1040:2005 suspension test. In a phase 1 suspension test, 8 parts of the test product is added to 1-part test microorganism and 1-part water. The mixture is allowed to interact for the duration of the contact time. One part of the mixture is added to 8 parts of neutralizer and 1-part water for 5 minutes to halt bactericidal activity. The final mixture is then acquired and incubated for 2 days to allow surviving bacteria (if any) to proliferate. The bacterial colony is counted and compared against the original culture size.

Materials: 17% disodium EDTA (prepared in Ross Lifescience Limited R&D Centre), Dent wash (aqueous 17% EDTA) from prime dental products private limited, EDTA liquid (17% EDTA solution) from Waldent innovations

private limited, Canalarge (17% Disodium EDTA solution) by Ambient, NEOEDTA Liquid (17% EDTA solution) from Orikam Healthcare India private limited, DebFree (0.2% Chitosan solution) from Swakit Biotech Bengaluru, sodium hypochlorite solution, chlorhexidine solution.

Laboratory materials: Disodium EDTA, Sodium Hydroxide pellets, 0.5M NaOH solution, 0.1M Calcium Carbonate, Ammonium buffer solution, EBT Indicator, burette.

Method: The marketed samples were purchased and subjected to basic physical parameters and chemical analysis. Basic physical parameters includes appearance, color, odor and pH. Basic chemical parameter include chelation power by titration method.

Weighed 17g of disodium EDTA in a 100ml of distilled water. To this add quantity sufficient sodium hydroxide pellets to dissolve until solution gets clear, colorless, transparent flowable liquid. This prepared 17% disodium EDTA was analysed. Appearance of this solution was clear, colorless, transparent flowable liquid. Odor and color observed odorless and colorless. pH observed was 6.5-7.50. Prepared 17% disodium EDTA solution used as a test material for comparing the market samples.

Antibacterial activity by Standard Suspension Method 1040:2005.

Test Procedure

- 1 ml of test suspension (bacteria) was added to 1 ml of sterile distilled water.
- 8 ml of undiluted sample (dental irrigant) was transferred to the mixture.
- The test mixture was mixed well and placed in 20°C±1°C water bath.
- After 5 minutes of contact time for bacteria, 1 ml of the test mixture was transferred into a tube containing 8 ml of neutralizing medium and 1 ml of distilled water.
- After neutralization, 1 ml of the mixture was tested immediately using pour plate method with Tryptone Soya Agar (TSA) for bacterial strains.
- The final mixture is then acquired and incubated for 36.8°C for 48 hours for bacteria to allow surviving microorganism (if any) to proliferate.

Results: Freshly prepared 17% disodium EDTA batch number RLS/NPD/DCI/07 shown 360mg/g of calcium carbonate & this was also subjected for antibacterial activity by suspension test method (study number 896) shown 99.9949% log reduction in 5 minutes against Staphylococcus aureus. The marketed Canalarge batch number CNL105 sample having 17% EDTA solution along with other quaternary ammonium salt showed maximum chelation value around 1640mg/g of calcium carbonate & this was also subjected for antibacterial activity by suspension test method (study number 894) shown 100% log reduction in 5 minutes against Staphylococcus aureus. DebFree (0.2% Chitosan solution) from Swakit Biotech Bengaluru batch number SBT/A21/02 shown 177.66mg/g of calcium carbonate also which was subjected for suspension test method (study number 896) and shown 99.9928% log reduction in 5 minutes against Staphylococcus aureus. An attempt was also made with only EDTA solution and it shown 4550mg/g of calcium carbonate. Dent wash (aqueous 17% EDTA) from prime dental products private limited shown no results for chelation value also which was subjected for suspension test and shown 100% log reduction in 5 minutes against Staphylococcus aureus.

Discussion: Laboratory grade disodium ethylenediaminetetraacetic acid (EDTA) was selected to this studies. Marketed dental irrigants were subjected to few parameters including appearance, color, odor and pH. Mandate was to study the chelation power of marketed dental irrigants. Principle of the total chelation value is determined by titrating with a solution of calcium salt in the presence of oxalate ion at a pH of 11.0 or slightly higher. The chelating agent will complex the calcium until an excess of calcium is present; this end point is indicated by the appearance of the white calcium oxalate precipitate.

Preparation of Calcium Carbonate 0.1M is followed by adding dissolving 10 g of in 1000 ml of distilled water**,** If not dissolved, then add 1:1 HCL, Then it will dissolved. Preparation of Ammonia Buffer Solution (To make basic p H

required >10. And adjusted the p H) is followed by dissolving 2.7 g ammonium chloride (NH4Cl) in 17.8 mL concentrated ammonia (NH3, 16 M) and diluting to 100 mL with distilled water. Preparation of EBT Indicator 1% (This indicator is required to know any changes in the p H Value of the solution) is followed by Dissolve 1.0 g of Eriochrome Black T in 80 mL 95% ethanol. Make up to 100 mL with 95% ethanol.

By the completion of preparing all the above required chemical solutions, the chelation power analysis carried out in Ross Lifescience limited GLP certified laboratory in Bhosari site. This was followed by this procedure;

Step1: 0.2g 2NA EDTA in Conical Flask, add sufficient amount of water to dissolve it

Step2: Add 15ml of Ammonia Buffer and few drops of EBT Indicator

Step3: Titrate it with 0.1M Calcium Carbonate

Step4: End Point-Blue to Wine Red appears.

Step5: Note down the burette reading and calculate the chelation power and purity of sample

Purity of EDTA $NA_{2.}2H_2O$ (Disodium EDTA Dihydrate) = $\frac{372.24 \text{ X Burette Reading}}{\text{Weight of EDTA taken (Weight of test sample)}}$

These marketed dental irrigants were also subjected to antibacterial analysis as EDTA itself having some antibacterial property. Staphylococcus aureus ATCC 6538 was taken to use in this S. aureus is a facultative aerobic, Gram-positive coccal (round) bacterium also known as "golden staph" and "oro staphira". S. aureus is nonmotile and does not form spores. In medical literature, the bacterium is often referred to as S. aureus, Staph aureus. S. aureus appears as staphylococci (grape-like clusters) when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates.

As per the literature, in the direct exposure test, sodium hypochlorite NaOCl had better antimicrobial effectiveness for all microorganisms at all times. Chlorhexidine CHX was effective for S. aureus, E. faecalis and C. albicans at all times, and ineffective for P. aeruginosa, B. subtilis and the mixed culture. Hence we wanted to analyse the dental irrigants prepared with 17% EDTA solutions against Staphylococcus aureus.

A detailed method of antibacterial analysis followed by standard suspension test 1040:2005 was given as above.

Conclusion: Hence the chelation power of freshly prepared 17% disodium EDTA solution exhibit 360mg/g of calcium carbonate as compared to marketed product Canalarge which contain 17% EDTA solution 1640mg/g of calcium carbonate. It has been concluded that freshly prepared 17% EDTA solution will be effective for chelating of metal ion as well as antibacterial agent against Staph aureus.

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