ISSN: 1812–1217

Evaluation of Antibacterial Efficacy Against *Enterococcus Faecalis* of Newly Prepared Endodontic Irrigant Solution (an In vitro study)

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الخلاصة

الاهداف: تهدف الدراسة الى تقييم فعالية محلول ارواء لبي جديد التحضير وهو مزيج من (٢٠) % منظف الخروع و (٤) % انزيم البابين (MCP) ضد بكتريا السبحيات البرازية . **المواد وطرائق العمل**: تالفت عينة البحث من (٦٠) سن بشري مقلوع تم تحضيرهم باستخدام نظام الدوار ,protaper NiTi)تم ختم الفتحة الذروية لكل عينة بواسطة الراتنج الاكريلي ثم غمر كل جذر بمادة طبعة سلكونية ثم تعقيمها ثم حقن مامقداره (١٠) ميكرولتر من معلق المزيج بلطف داخل كل قناة من المجموعات (A,B,C) ووضعها في الحاضنة لمدة (٢٤) ساعة عند درجة حرارة (٣٧م^{o}) اما المجموعة D فلم تحقن باعتبارها مجموعة شاهدة سالبة. العينات في المجموعات(A,B,C) تم تطهيرها كالاتي: المجموعة A باستخدام محلول (MCP) المجموعة ,B باستخدام محلول)NaOCl2.5% المحضر حديثا. المجموعة (D) فلم تعالج باي محلول باعتبارها مجموعة شاهدة موجبة. ثم تركت وتركت داخل القناة لمدة خمس دقائق ثم تم تجفيف القناة باستخدام اقماع التجفيف المعقمة. ثم اخذت العينات البكتيرية للمجموعات الاربعة باستخدام مبرد معقم حجم (٤٠) بادخاله على طول القناة وتدويره(٣٦٠)^{o} ثم وضعت المبارد المعقمة في علبة بلاستيكية معقمة تحتوي على(١) مللتر من السيروم الطبيعي واخذت (٢٠٠) مللتر ونشرت على وسط اجار السبحيات ووضعت في الحاضنة لمدة (١٨) ساعة عند (٣٧ م^{o})ثم تم حساب عدد المستعمرات البكتيرية. **النتائج**: كلا السائلين (MCP & NaOCl) يحدث محو تام لبكتريا السبحيات البرازية من قناة الجذر في خمس دقائق. **الاستنتاجات**: ان محلول منظف الخروع ٢٠% وانزيم البابين ٤% MCP له القدرة على المحو التام لبكتريا السبحيات البرازية من قناة الجذر المصابة مختبريا ولمدة خمس دقائق. ان تاثيره المضاد للبكتريا مشابه لتاثير محلول ٢.٥%NaOCl .

ABSTRACT

Aims: To evaluate the efficacy of a newly prepared endodontic irrigant solution, against *E. faecalis.* Materials and Methods: Sixty human extracted single rooted teeth samples were prepared by using protaper NiTi rotary system. The apical foramen of each sample was sealed by acrylic resin on the apical 3mm. Each root was embedded in silicon impression material block and autoclaved. A ten µl ($4X10^5$) of inoculated broth media (nutrient broth) with *E. faecalis* was injected gently inside the canals of group A,B&C was incubated for 24hours at 37C^{o}. Group D was left without inoculation to serve as a control negative. The samples of group A,B and C were disinfected as fallow: Group A was disinfected with MCP solution(mixture of 20% castor detergent and 4% papain). Group B was disinfected with freshly diluted 2.5% NaOCl solution. Group C not treated with any solution control positive. The disinfected solutions were left inside the canal for 5 min then each canal was dried with sterilized paper point, then bacterial samples were taken from the samples of 4 groups using size 40 sterilized file which was inserted to full canal length and rotated 360 degrees in clock wise direction for dentin engagement. The file was put inside the sterilized plastic tube containing 1ml of normal saline then 200 µl was inoculated and spread on *Enterococcus* agar media and incubated at 37C^{o} for 18hours, the numbers of bacterial colonies were counted. **Results**: Both solutions (MCP and NaOCl) produce complete eradication of *E Faecalis* within 5 minutes. **Conclusion**: Castor detergent 20% and papain enzyme 4% (MCP) has ability to completely eradicate *Enterococcus Faecalis* bacteria from the infected root canal in vitro in 5 min. It's antibacterial action is similar to the action of 2.5 % NaOCl **.**

Keywords: Antibactrial, E Faecalis, New irrigant

Zakarea NA. Mohamad T.Taqa AA. Evaluation of Antibacterial Efficacy of Newly Prepared Endodontic Irrigant Solution Against *Enterococcus Faecalis* (an Invitro study). *Al–Rafidain Dent J*. 2014;14(1): 153-160.

Received: 6/8/2013 ***Sent to Referees:*** 12/8/2013 ***Accepted for Publication:*** 24/10/2013

INTRODUCTION

The treatment of apical periodontitis and root canal infection consists of removing the source of the microbial invasion and creating space in the root canal system for irrigation with an

antimicrobial solution .[1] Biomechanical preparation associated with antimicrobial irrigants is important for the elimination of the microorganisms from the root canal.[2] Therefore the irrigating solution must have adequate antimicrobial action on microorganisms present in the root canal, dentinal tubules, apical ramifications, cementum, and areas of root resorption. [3,4] *Enterococcus faecalis* is the most commonly isolated species from the canals of teeth presenting post-treatment diseases. *Enterococcus faecalis* account for up to 77% of therapeutic failures.[5] Sodium hypochlorite (NaOCl) has been widely used as an irrigant since its introduction in endodontics. [6] Sodium hypochlorite has been documented as the most effective irrigant in terms of antimicrobial activity [7] and has several disadvantages, such as: cytotoxicity to vital tissues, foul smell, taste, and inability to remove smear layer when used alone. [8] In addition, it may alter the dentin structure and may interfere with bonding of adhesive obturating materials to dentin. [9] Tartari *et al* (2013) founded that NaOCl is able to significantly decrease the dentin microhardness even when used alone.[10] The associated sequelae of NaOCl extrusion have been reported to include life threatening airway obstructions.[11] facial disfigurement requiring multiple corrective surgical procedures. [12] permanent paresthesia with loss of facial muscle control. [13] and the least significant consequence tooth loss. [14] Sodium hypochlorite is generally not utilized in its most active form in a clinical setting. For proper antimicrobial activity, it must be prepared freshly just before its use. [15,16]

Recently, there has been a growing trend to seek natural remedies as part of dental treatment. [17] Thus, some alternative irrigating solutions with antimicrobial action and biocompatibility have been proposed.[18] One of these substances is the castor oil. This phytotherapeutic polymer is obtained from the seeds of the Ricinus communis plant. It has been reported that 10% castor oil detergent has an acceptable biological and antibacterial properties.[19,20,21,22] Such studies evaluated the agent in comparison to other irrigating solutions(2% CHX and NaOCl) and concluded that gram positive microorganism was significantly affected by detergent.

Papain enzyme acts as a debris removing agent. It acts only on affected tissues, which lack the α1.antitrypsine plasmatic antiprotease that inhibits proteolysis in healthy tissues. [23]Papain has bacteriocidal, bacteriostatic and anti-inflammatory activity, and debriding agent. It does not damage healthy tissue, but accelerates the cicatricial process.[24] Bhardwaj find that papain enzyme has comparable antibacterial effect to calcium hydroxide when used in gel form as an intracanal medicament against *E. faecalis*. [25]

The purpose of this in vitro study was to evaluate the efficacy of a newly prepared endodontic irrigant solution prepared from a mixture of 20% castor detergent and 4% papain enzyme (MCP) against *E. faecalis*.

MATERIALS AND METHODS

Preparation of irrigant solution:

The (MCP) is an acronym of mixture of castor detergent and papain enzyme. The experimental solution was prepared by converting the castor oil (HEMANI Company, Pakistan) to sodium castorate powder by adding sufficient molar ratio of NaOH to the oil. A twenty grams of sodium castorate powder and 4 gm of papain enzyme powder (HIMEDIA Company India, molecular weight=23000) were dissolved in 100 ml deionized water to produce a solution of 20% castor detergent, and 4% papain enzyme.

1.Sample selection and preparation

Sixty extracted human single rooted teeth free of root resorption, root fracture, sever curvature, were selected teeth were decoroneted at the cement-enamel junction. The patency of each root canal was confirmed by inserting size 15 K file, the working length of each canal was determined by inserting size 15 K type file inside the canal until the tip of the file was just become visible at the apical foramen under stereomicroscope X20 magnification. The file was reduced 1mm. from the measured working length, each canal length was adjusted to 14mm.

working length by cutting from the cervical part of the root.The apex of samples were closed from the apical area by sticky wax and all root canal samples were prepared by using protaper NiTi rotary system Protaper (Dentsply Maillefer Switzerland) starting from SX, S1, S2, F1, F2& F3 at 3000 rpm speed and 2.5 Ncm torque using micro motor (NSK Comp. Mekanushi, japan) hand piece. Preparation of each canal was done with 1% of NaOCl (Clorox, Kingdom of Saudi Arabia) 3ml between each file size, the time was standardized to 10 mints for each canal.[26]

The irrigation was performed by endodontic syringe with flexible silicon tip inserted up to 2mm from the apex. The speed of irrigation was 1ml/5 seconds.[27] Finally each canal was irrigated with 5 ml of distilled water and immersed in 1liter of distilled water for 24h to eliminate the residual effect of NaOCl. Samples were removed from distilled water and dried with sterile endodontic paper point.

2.Sterilization of teeth: The apical foramen of each root sample was sealed by applying acrylic resin on the apical 3mm of root to prevent fluid leakage during microbiological work. Each root was embedded in silicon impression material block up to 2 mm of cervical margin Figure (1**)**

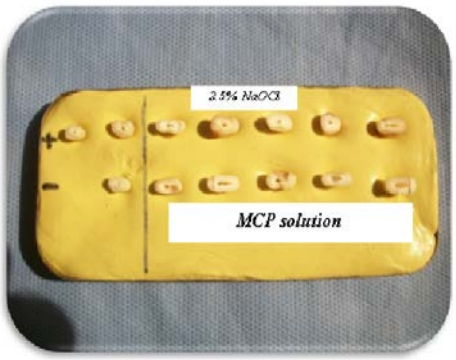

Figure (1): Samples of extracted teeth after decoronation fixed in heavy body impression material.

To facilitate the grasping and holding of root samples. The block of tooth samples was covered with aluminum foil and autoclaved at 121C^{o} for 15 min at 15 pound/inch^{2}.The microbiological part was performed in Dental Basic Science Department University of Mosul.

The samples were divided randomly as follow:

Group A n=20 were irrigated with MCP solution.

Group B n=20 were irrigated wit 2.5% NaOCl.

Group C n=10 control +ve the samples just inoculated without treatment

Group D n=10 control -ve the samples just autoclaved without bacterial inoculation.

3.Culturing of bacteria:

A single colony of *Enterococcus Faecalis* was inoculated on Enterococcus agar and incubated aerobically for 18 hours.[28] Single colony of fresh bacteria was inoculated in screw capped vial containing 5 ml of nutrient broth (HiMedia India) and incubated for 18hours, at 37C^{o} then 0.5 ml of bacterial suspension was added to 0.5 ml of nutrient broth in screw capped vial. The vials were shake well manually in vertical direction, the final dilution of inoculated broth become 4X10^{7} cfu/ml .[29]

4.Inoculation of Root Canal with *Enterococcus Faecalis* A ten µL (4X10^{5}) of mixed suspension was taken by micropipette and injected gently inside the each canals of group A,B&C under aseptic condition using micropipette and incubated for 24 hours at 37^{o}C.[30] group D was not inoculated and consider as a control positive Figure (2).

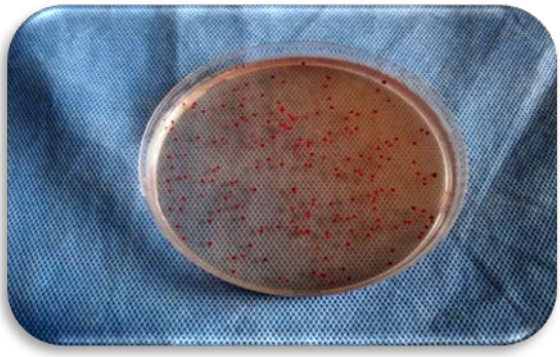

Figure (2): Petri dishe with Enterococcus faecalis growth of control positive.

5. Canal irrigation: After 24hours incubation of the samples, each experimental canal was treated by injection 10 µL of freshly prepared corresponding solution and left for 5 minutes and then each canal was dried with sterilized paper point, group D was not treated with any solution to consider as control negative then bacterial samples were taken from the samples of 4 groups using size 40 sterilized file which was inserted to full canal length, then the file is rotated 360 degrees in clock wise direction for dentin engagement. The file was inserted inside the sterilized plastic tube immediately under aseptic condition to tube containing 1ml of normal saline, and shake in vertical direction for 3 seconds, and then 200 µl was inoculated and spread on Enterococcus agar media and incubated at 37C^{o} for 18hours. After 24hours the numbers of bacterial colonies were counted under good illumination and manual lens for magnification, then multiplied by the dilution factor and the mean values were computed Figure(3).

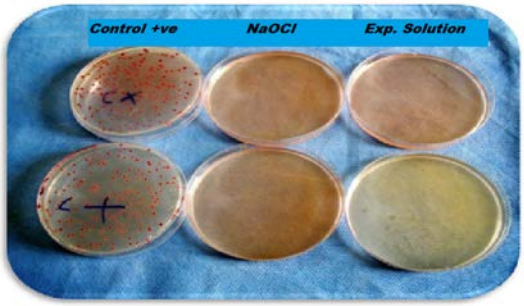

Figure (3): Petri dishes with Enterococcus faecalis growth of control positive, (left side) 2.5%NaOCl (intermediate), and MCP solution (right side).

RESULTS

The results of bacterial count showed clearly that group A (MCP solution) and group B (2.5% NaOCl) were complete eradication of the bacteria from the root canal, all the samples was negative culture Figure (4).The mean score for both solutions are zero Table (1). The mean score value of control positive was equal to 523.9 (Figure 3), while the control negative the mean score value was zero. (Table 1).

Table (1): the mean of bacterial count for four groups

Group	NaOCl 2.5%	MCP solution	Control +ive	Control -ive
mean	0	0	523.9	o

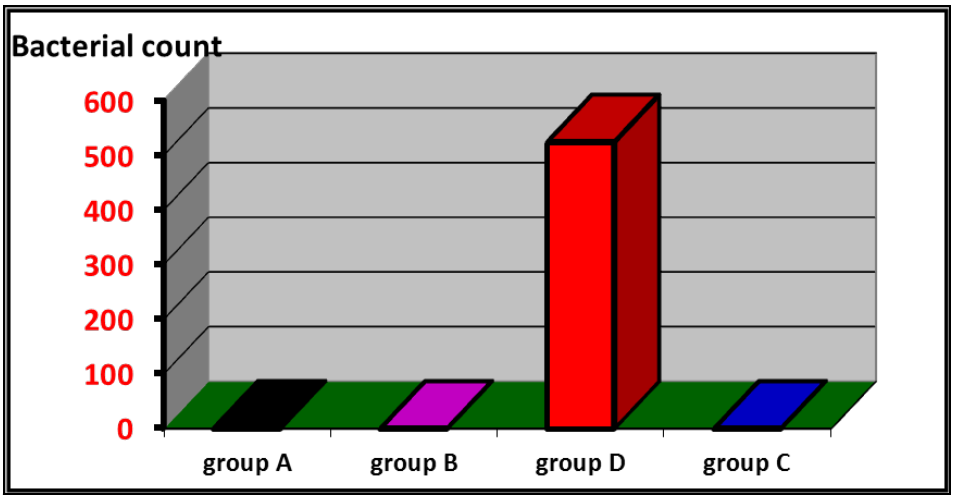

Figure (4): Histogram of bacterial count of four groups

DISCUSSION

Control of bacterial infection of the root canal and prevent its recurrence is the main objective of endodontic therapy, *Enterococcus faecalis* was chosen as the primary test organism because it is the most resistant bacteria found in the root canal system and has been associated with treated root canal failures. [31] inherent antimicrobial resistance, the ability to invade into the dentinal tubules where they are protected from endodontic medicaments and are therefore difficult to eliminate. [32,33] The results of control negative conform complete sterilization of the samples by autoclaving, while the results of control positive confirm the root canal inoculation by bacteria after its sterilization.

In this study we don't use any other method with the irrigation which increase the effectiveness of root canal irrigant (sonic, ultrasonic and mechanical instrumentation) this was done by slowly injection of the solution in to the canal by micropipette to ensure that any reduction in the bacterial count is due to antibacterial action of the tested solution.

(MCP) solution is a promising solution because in clinical endodontic we use larger amount of irrigant solution (3-5 ml for each irrigation) this will give more irrigant fluid volume and velocity inside the canal which will give more potent disinfecting action than in vitro study due to increases both volume and velocity of the fluid. The concentration of NaOCl 2.5% was selected according to modern reduction of NaOCl because the range of concentration is between 0.1 to 6%, so 2.5% concentration is less toxic, more biocompatible and had a good solving and antibacterial actions similar to 5.25%.[34] The concentration 2.5 % NaOCl was used because NaOCl is highly toxic to vital tissues at undiluted high concentrations. At higher concentration, for example, 1:10 (vol/vol) dilution, the tissue irritation may be substantial [35] and displacing highly concentrated NaOCl into periapical tissues can cause severe tissue damage.[36] Also there is some dispute regarding the most effective concentration of NaOCl, although theoretically the concentration should be kept to the lowest level that is effective. When used in contaminated canals of extracted teeth, Siqueira *et al.* (2000). found no difference in the antibacterial effect of 1%, 2.5% and 5.2% NaOCl. [7]

Both concentrations of 4% papain and 20% castor detergent were selected according to study of different concentrations for both materials and measurement the inhibition zone to determined the optimum concentration for the antibacterial action of the solution. The results showed that the optimum concentration was 4% papain and 20% castor detergent which give similar inhibition zone to the 2.5% NaOCl. Also the concentrations of MCP solution

constituent were chosen according to the ability to remove smear layer by using scanning electron microscope (SEM), other part of Study.

Also the root canal sampling was done using endodontic file with engagement in the apical part to ensure good sampling from dentin particles from the apical area. The concentration of papain enzyme 4% and castor detergent 20% in MCP solution was selected according to pilot studies to get maximal anti-bacterial action, the ability to remove smear layer, and surface tension of solution (other part of the study). Table (1) showed that MCP solution has antimicrobial activity identical to that of 2.5 % NaOCl in which both of them completely eradicate the microbial inoculation of all samples of both MCP and NaOCl solutions, so MCP solution is a promising endodontic irrigant solution which could overcome many disadvantages of NaOCl. The two component of MCP solution was selected to remove both organic and inorganic (papain enzyme for organic part and castor detergent for inorganic part) component of smear layer which is produced during root canal instrumentation.

The castor oil detergent acts by breaking sugar leakage of the cellular wall of pathogenic microorganisms, consequently the loss of cytoplasmic material leads to cell destruction. [37,38] Also the papain enzyme has a well-known bacteriocidal, bacteriostatic and antinflammatory activity and it is possibly make a synergistic effect to kill bacteria by affecting the protein of its cell membrane.[24] These results coincide with the results of Ferreira *et al* (1999). [21], Leonardo *et al* (2001) [22] who found that 3.3% castor oil solution and 0.5% sodium hypochlorite showed similar antimicrobial action and Papaine gel (0.4%) presented less antimicrobial action on all the microorganisms evaluated. [26]

CONCLUSIONS

Within the limitation of this invitro study, it is concluded that mixture of 20% castor detergent and 4% papain enzyme (MCP) as endodontic irrigant solution has a strong antibacterial action that completely eradicate *Enterococcus faecalis* from the infected root canals when applied for 5 mints, it's antibacterial action is similar to that 2.5% NaOCl.

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