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COMPARISON OF THE ANTIBACTERIAL EFFICACY OF IBUPROFEN VERSUS TRIPLE ANTIBIOTIC PASTE AGAINST ENTEROCOCCUS FAECALIS IN SINGLE ROOTED TEETH [AN IN-VITRO STUDY]

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ABSTRACT

Aim: The aim of the present study was to compare the antibacterial efficiency of the anti-inflammatory non-antibiotic ibuprofen versus triple antibiotic paste as an intra-canal medication against *Enterococcus faecalis*.

Methodology: Forty four single rooted teeth were decoronated and instrumented up to F4 ProTaper rotary files. Apical foramen was sealed with acrylic resin, and all the external surfaces were made impermeable with nail varnish, except for the coronal access. Roots were autoclaved at 121°C for 20 minutes, placed in Eppendorf tubes and contaminated with *E. faecalis* for 14 days. Teeth were divided into two groups of 22 teeth; (Group1: ibuprofen, Group2: triple antibiotic paste (TAP). Colony forming unit (CFU) counts were taken before placing the intracanal medications by a paper point sampling (CFU1). The tested medicaments were mixed with distilled water (1:1 w/v), placed inside the root canals, then canals were temporarily sealed, and incubated at 37°C for 7 days. After intracanal medication removal, the second count (CFU2) was taken as (CFU1) and the antibacterial action of each medication was determined by calculating the percentage of bacterial reduction when comparing CFU2 to CFU1, and then the antibacterial action of the tested medications was compared with each other.

Results: There was no statistically significant difference between (T.A.P) and (Ibuprofen) groups in the percentage of bacterial reduction where (p=0.073). Both medications caused a reduction in the bacterial count from CFU1 to CFU2.

Conclusion: Ibuprofen proved to have an antibacterial effect comparable to that of TAP.

KEYWORDS: Intracanal medicaments, TAP, anti-inflammatory non-antibiotics, NSAIDs, *Enterococcus faecalis*.

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INTRODUCTION

Bacterial infection is considered as the primary cause of apical periodontitis, therefore the successful outcome of the endodontic treatment procedures relies on the effective disinfection of the root canal, which remains a crucial challenge in endodontics (Arruda *et al*. 2018).

In order to combat intracanal bacteria, an efficient debridement of the root canal system and removing the harmful irritants is mandatory; however, it is challenging due to different canal complexities. It has been shown that more than 50% of the dentinal canal walls were left untouched after mechanical instrumentation such as canal irregularities, isthmuses, lateral canals, ramifications... etc.(Vieira *et al*. 2012, Alves *et al*. 2016).

Intra-canal medications have been shown to contribute to canal disinfection through promoting significant bacterial elimination after chemo-mechanical procedures by reaching areas that instruments and irrigants could not reach (Arruda *et al*. 2018).

The combination of minocycline, metronidazole, and ciprofloxacin known as the triple antibiotic paste (TAP) was shown to be very effective in eliminating endodontic pathogens in vitro, in situ and in vivo especially *E. faecalis*. Concerns associated with intra-canal use of antibiotics included: fear of promoting antibiotic resistance, the possibility of tooth discoloration due to minocycline, risk of allergic reactions and biocompatibility of the medicament. Also excessive concentration of TAP might affect the host tissue and limit tissue regeneration (Kahler *et al*. 2016, Valverde *et al*. 2017, Zargar *et al*. 2019).

It was realized that the conventionally used class of analgesics non-steroidal anti-inflammatory drugs (NSAIDs) might possess additional therapeutic properties such as antibacterial efficacy through inhibition of bacterial DNA synthesis, impairment of membrane activity, alteration in genes encoding transport/binding proteins and cell envelope, down regulation of efflux pumps, prevention of bacterial colonization and biofilm formation by interfering with the quorum sensing of bacteria, and anti-plasmid activity. Therefore, the possibility of using non-antibiotics like ibuprofen as an intra-canal medication in root canal disinfection procedures could be a safe alternative to TAP (Chockattu *et al*. 2018).

MATERIALS AND METHODS

Sample preparation

- The teeth were inspected and cleaned from blood, tissue debris and disinfected with 3% sodium hypochlorite (Clorox, Household Cleaning Products, Egypt) for 5 min and scraped to remove the attached periodontal cells from the root surface, then were stored in a plastic container containing normal saline until use.
- All teeth were decoronated to standardize root length to 14 mm.
- Working length was determined by inserting #10 K file (MANI, Japan) into each root canal until it is visible at the apical foramen, then subtracting 1 mm from this point.
- The roots were instrumented using Protaper rotary system up to size F4 (Dentsply Maillefer, Switzerland) and irrigated with 2.5% NaOCl after each instrument, and were finally irrigated with 2 mL of 17% EDTA (METABIOMED CO.LTD,Korea) allowing it to remain for 1 min followed by rinsing with 2 mL of saline.
- After drying with size 40 Protaper absorbent paper points, the apical foramen was sealed with self-cure acrylic resin (Acrostone Dental Manufacture, Egypt). All external surfaces were covered with nail varnish (YOLO, YOLO cosmetics, France) to make it impermeable, except for coronal opening.
- Teeth were autoclaved (TOMY KOGYO CO., LTD.Tagara ,ner ima-ku ,Tokyo , Japan)

at (121°C for 20 min) and placed in 1.5 mL Eppendorf tubes then in racks.

- Triple antibiotic paste ciprofloxacin (Ciprobay, Hikma Pharma, Egypt), metronidazole (Flagyl, Sanofi-Aventis, Egypt), and minocycline (Minocin, TEOFARMA s.r.i., Italy) was crushed in the form of powder and mixed with proportions of equal weight of 1:1:1 with concentration of 1 mg/ml.
- Ibuprofen (Brufen,Kahira pharmaceuticals and industries company under license from Abbott Laboratories Limited- USA) powder was extracted from tablet with concentration (≥98%).
- The intra-canal medicaments powder was freshly prepared and mixed with distilled water (1:1 w/v) at time of insertion in the form of paste.

Contamination protocol

- The bacterial strain of *E. faecalis* (ATCC 29212) from the stock was revived by plating on blood agar medium.
- With the aid of a syringe 5 µL of *E. faecalis* microbial suspension adjusted to 0.5 McFarland standards was inoculated into the previously autoclaved teeth using sterile micropipette.
- This procedure was repeated every 72 h for 14 days.
- During this period, the teeth were kept in an incubator (FISHER ISOTEP* INCUBATOR. WTC Binder, TUTTLINGEN/ GERMANY) at 37°C.
- The first sample was taken before intracanal medication application by paper point sampling colony forming unit (CFU1) then it was transferred to Wasserman tube containing 1 ml of Phosphate Buffered Saline (PBS) to remove loosely attached bacteria, after that it was transferred to another Wasserman tube containing 1 ml of Brain Heart Infusion (BHI) and tubes were vortexed to re-suspend the remaining viable bacteria on the paper point.
- Serial 10-fold dilutions of the suspensions were made (10^1 to 10^5) form each sample.
- A volume of 20 microliter was taken from each dilution and plated on bile esculin plates. These plates were incubated for 24 hrs at 37 aerobically.

After 24 hrs, approximate number of colony forming unites (CFU) per ml was calculated.

The Protocol For application of ibuprofen and triple antibiotic paste as intracanal medication to measure the bacterial count

The teeth were randomly divided into two groups by simple randomization:

- **The intervention group [1]**

Ibuprofen paste was inserted in the canal using endodontic plugger, temporarily sealed, and incubated (37°C; 7 days).

- **The comparator group [2]**

Triple antibiotic paste (metronidazole, ciprofloxacin, and minocycline) was inserted in the canal using endodontic plugger, temporarily sealed, and incubated (37°C; 7 days).

After this period each tooth was irrigated with 5 mL saline to remove intracanal medication from the canal. Second sample was taken after the second incubation period by paper point sampling for both groups (CFU2) as the technique used to collect the first set of CFU counts

RESULTS

Antibacterial effect of each intracanal medication at both observation points:

Intervention (Ibuprofen):

The mean and standard deviation (SD) values of the *E.faecalis* counts (CFU/ml) in Group A (Ibuprofen). The mean and standard deviation (SD) values of the *E.faecalis* count were 6.84±0.11 in the pre-medication sample (CFU1), and 2.26±2.79 in

the post- medication sample (CFU2). There was a statistically significant difference between (CFU1) and (CFU2) groups (p<0.001), where the highest bacterial count was found in the pre-medication (CFU1) group, while the least bacterial count was found in the post medication (CFU2) group.

Control (T.A.P):

The mean and standard deviation (SD) values of log 10 of the *E.faecalis* counts (CFU/ml) in Group A (Ibuprofen). The mean and standard deviation (SD) values of the *E.faecalis* count were 6.80±0.13 in the pre-medication sample (S1), and 3.90±2.62 in the post- medication sample (S2). There was a statistically significant difference between (CFU1) and (CFU2) groups (p<0.001), where the highest bacterial count was found in the pre-medication (CFU1) group, while the least bacterial count was found in the post medication (CFU2) group.

Relation between the intervention (Ibuprofen) and the control (TAP) groups in the percentage of bacterial reduction:

The mean and standard deviation (SD) values of the percentage of bacterial reduction of the *E.faecalis* counts (CFU/ml) in Group A (Ibuprofen) and Group B (TAP). The mean and standard deviation (SD) values of the percentage of bacterial reduction were 97.82% ±3.62 for intervention (Ibuprofen) group and 95.77% ±3.60 for the control (TAP) group.

There was no statistically significant difference between the (T.A.P) and the (Ibuprofen) groups in the percentage of bacterial reduction (p=0.073), where the highest mean of bacterial reduction was found in the (Ibuprofen) group, while the least mean of bacterial reduction was found in the (T.A.P) group.

DISCUSSION

Successful endodontic treatment depends on proper cleaning, disinfection, shaping and the filling material used during obturation. Biomechanical preparation is considered the key element to establish successful root canal treatment through proper disinfection of the root canal and its ramifications (Tanomaru Filho *et al*. 2002). Since, irrigants alone cannot achieve complete eradication of bacteria, the use of an antibacterial intracanal medication is considered an important adjunct to reduce and eliminate different bacterial strains (Kontakiotis *et al*. 2008).

The teeth were decoronated at the cementoenamel junction by using a water cooled double sided disc rotating in a low speed hand piece to standardize the length of the used roots at a length of 14 mm to reduce variability and ensure that the specimens were cut at the same root level (Nagas *et al*. 2014, Chockattu *et al*. 2018).

The apices of all roots were covered with auto polymerizing resin to simulate in-vivo conditions where the root is closed in a bony socket and also to prevent bacterial leakage within the root canals (Berber *et al*. 2006). A coronal seal at the coronal opening of the root canal was placed to restrict the nutrient supply to the bacteria under study (Sponchiado *et al*. 2014).

In the present study, *E. faecalis* strain (ATCC 29212) was used to infect the teeth, since it is considered the most resistant intracanal bacteria and one of the main causes for endodontic failures and flare ups, it can persist within the tubules for at least 10 days without nutrient supply, it plays a role in the pathogenesis of persistent apical periodontitis and it has the ability to adhere, aggregate and grow to form a biofilm, thus improving its survival potential and rendering it more resistance to the antimicrobial agents (Haapasalo and Orstavik, Portenier *et al*. 2003).

Measuring the outcome using colony forming units counting (CFUs) was chosen to evaluate the antibacterial efficacy of intracanal medicaments as they would signify the quantity of the live residual bacteria present in the root canals to define a percentage of reduction in CFUs in infected dentine before and after the application of

intracanal medicaments. Microbial root culturing is commonly used to assess the effectiveness of endodontic treatment measures (Zerella *et al*. 2005, Chua *et al*. 2014a). A logarithmic transformation (log 10 transformation) of each CFU/ml count was performed to normalize the data before statistical evaluation (Best 1970, Ge *et al*. 2008).

Regarding effective drug dose for the intervention group (ibuprofen), its concentration was ≥98% pure powder and for the control group (TAP), studies reported that a concentration of 1 mg/1ml of TAP was effective in eradicating more than 99.99% of *E.faecalis* and various endodontic pathogens (Salem-Milani *et al*. 2013, Sabrah *et al*. 2015, Arruda *et al*. 2018, Chockattu *et al*. 2018),

The results of the present study showed that there was a statistically significant difference for the control (TAP) group between (CFU1) and (CFU2) groups ($p<0.001$), where the highest bacterial count was found in the pre-medication (CFU1) group, while the least bacterial count was found in the post medication (CFU2) group, with 95.77% as a percentage of bacterial reduction of the *E.faecalis* counts. These results were in agreement with (Prabhakar *et al*. 2008), who reported that a combination of 3 antibiotics was able to completely eradicate all intracanal bacteria present inside infected primary teeth after 1 day of application. This may be attributed to the action of the triple antibiotic paste which was able to penetrate into dentinal tubules and its minocycline component which inhibits protein synthesis on the surfaces of ribosomes which might be the reason for the antimicrobial property, while metronidazole and ciprofloxacin help fibroblasts in the synthesis of the extracellular matrix and collagen and contribute to the development of structural framework. Moreover, the combination of ciprofloxacin, metronidazole, and minocycline at a concentration of 25 μg/ml each per milliliter at a paste form was able to sterilize infected root dentin in vitro (Hoshino *et al*. 1996), it was also reported that a 50 μg/ml of each antibiotic per milliliter was sufficient to sterilize infected root dentin in situ. The results of the present study was also in agreement with (Chua *et al*. 2014b, Devaraj *et al*. 2016, Ravi 2017, Valverde *et al*. 2017, Zancan *et al*. 2019) who revealed that triple antibiotic paste had a great antibacterial activity against *E. faecalis* biofilms.

While the results of this study were in disagreement with (Ghabraei *et al*. 2018a) who found that a paste of $Ca(OH)_2$ mixed with 2% CHX was able to eradicate the E. *faecalis* biofilm in three days, while TAP was able to eradicate the biofilm of E. *faecalis* in seven days and were in disagreement with the results of (Lakhani *et al*. 2017) who found that the 2% CHX gel was the most effective medicament against *E. faecalis,* among the tested medicaments which were normal saline, calcium hydroxide, moxifloxacin or triple antibiotic paste at the end of first, 7th and 10th day.

The current study showed a statistically significant difference for the Intervention (Ibuprofen) group between (CFU1) and (CFU2) groups ($p<0.001$), where the highest bacterial count was found in the pre-medication (CFU1) group, while the least bacterial count was found in the post medication (CFU2) group, with 97.82% as a percentage of bacterial reduction of the *E.faecalis* counts. These results were in agreement with (Shirin *et al*. 2006) who demonstrated the efficacy of ibuprofen against *Helicobacter pylori*. The results were also in agreement with (Annadurai *et al*. 1998, Dastidar *et al*. 2000, Dutta *et al*. 2007b) who revealed that diclofenac had an anti-bacterial efficacy against *Salmonella typhimurium*, *Mycobacterium tuberculosis* and *Listeria monocytogens*. This may be attributed to its ability to inhibit bacterial DNA synthesis and/or impair its membrane activity, down regulate the efflux pumps, alter the genes encoding of the transport/binding proteins, DNA synthesis and cell envelope and the anti-plasmid activity. In addition, the results were in accordance with (Dutta *et al*. 2007a) who reported that the anti-inflammatory drug diclofenac sodium (Dc) showed a significant activity against most strains

of *Bacillus* spp, *Salmonella* spp., *Shigella* spp. and 17 species of *E. coli*.

The results of the present study were in accordance with (Salem-Milani *et al*. 2013) who was the first to comparatively assess and prove the anti-bacterial efficiency of ibuprofen, diclofenac and $Ca(OH)_2$ against *E.faecalis*, where they recommended the use of NSAIDs as intracanal medicaments.

CONCLUSIONS

It could be concluded that Ibuprofen had an antibacterial action against *E.faecalis* and could be used as an intracanal medicament. Ibuprofen was as effective as TAP in eliminating *E.faecalis* from infected root canals.

Conflict of Interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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