Antimicrobial effects of analgesics, mefenamic acid and aceclofenac: an in vitro study.

Ashwini Anil Pokle[1], Adimulapu Hima Sandeep[2]
[1]Department of Conservative Dentistry and Endodontics, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu,India
Email: ashwini.pokle10@gmail.com
[2]Senior lecturer, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences Saveetha University, Chennai-600077, Tamilnadu,India
Email: himas.sdc@saveetha.com.
Corresponding author *
Adimulapu Hima Sandeep
Senior lecturer, Department of Conservative Dentistry and Endodontics, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences Saveetha University, Chennai-600077, Tamilnadu,India
Email: himas.sdc@saveetha.com.

Abstract
Non-Steroidal anti-inflammatory drugs (NSAIDS) are most frequently used analgesics. Mefenamic Acid (MA) which is a fenamate derivative of non-steroidal anti-inflammatory analgesic, is an over the counter (OTC) analgesic, which is used in cases of dysmenorrhea, menorrhagia. Aceclofenac, a phenylacetic acid derivative exhibiting anti-inflammatory and analgesic effects,often used to reduce joint pain and joint stiffness. Oral microflora such as Streptococcus Mutans, Staphylococcus Aureus, Enterococcus Faecalis, Candida Albicans are responsible for dental caries and infections. These resistant pathogens are responsible for root canal treatment failure as they adhere to the hard tissues thus causing biofilm induced infections. The aim of this in vitro study is to check for antimicrobial effects of analgesics used as intracanal instillation in root canals as they will give us both analgesic and antimicrobial effects. MA and Aceclofenac are prepared in the concentration of 100 µg,80µg, 60µg, 40µg, 20µg each. These analgesics are instilled in the wells made in the petri dishes containing the pathogens and observed for 24 hrs using a zone of inhibition test. MA showed better antimicrobial effects on S.mutans, S.aureus and E.faecalis than aceclofenac whereas the effect of the analgesics was negligible on C.albicans. MA and aceclofenac showed antimicrobial effects to some extent by damaging the cell membrane which can be used for root canal disinfection along with interappointment pain control.
Keywords: mefenamic acid, aceclofenac, S.mutans. S.aureus, E.faecalis, C.albicans

1. Introduction

'Fear of pain and what we do about it may be more disabling than pain itself', Waddell et al., 1993 with this quote, explained that chronic pain can reduce one's ability to complete daily chores.(Crombez et al., 1999) To reduce this pain and to accomplish the daily work patients with chronic pain usually tend to rely on painkillers. Non-Steroidal anti-inflammatory drugs (NSAIDS) are most frequently used analgesics and have likely been used for more than 2000 years.(Diener and Limmroth, 2001) In 1971 Vane (Vane, 1971) was the first to show that aspirin-like medicines work by blocking the enzymes that synthesize prostaglandins, implying the basic action of analgesics. At one or more stages in the endoperoxide biosynthesis pathway, all NSAIDs block the formation of prostaglandins. This one-of-a-kind quality is a common feature of such medications, and it is thought to be the source of their analgesic effect. (Cashman, 1996) Mefenamic Acid (MA) is a weak organic acid with a simple molecular structure, N-(2,3-xylyl), belongs to anthranilic acid group, is a fenamate derivative of non-steroidal anti-inflammatory analgesic. It is a mild over the counter (OTC) analgesic which shows both central and peripheral effects and is used in cases of dysmenorrhea, menorrhagia. It is also used to diminish pain after surgeries, muscular pain, arthritis, headache and post operative dental treatments. Its usage has become few and far between due to the gastrointestinal side effects it manifests.(Mavrikakis, Madkour and Buchanan, 1978) (Or and Bozkurt, 1988) The mechanism of action is that it reversibly inhibits cyclooxygenase, the enzyme which mediates the production of prostaglandins (PGs), since PGs play an important role in inflammatory reactions. (Moore, Derry and McQuay, 2009) Aceclofenac is a phenylacetic acid derivative which exhibits anti-inflammatory, it is an analgesic that is quickly and

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completely absorbed when taken orally and it is highly protein bound in the plasma. It reduces joint pain, joint stiffness and is used in the treatment of various painful disorders (e.g, dental and gynecological). It is usually well tolerated; however, it often leads to gastrointestinal disturbances. (Dooley, Spencer and Dunn, 2001) The Oral cavity harbors diverse yet distinctive microflora, which is present on both the hard (tooth surface) and soft tissue (oral mucosa).(Marsh, 2000) The oral pathogens present on the hard tissue of the oral cavity; mainly in the root canal includes bacterias like Streptococcus Mutans(D'Arcangelo, Varvara and De Fazio, 1999), Staphylococcus Aureus(Fidalgo et al., 2010), Enterococcus Faecalis(Kishen et al., 2008); Fungi like Candida Albicans(Kumar et al., 2015) which are resistant to traditional root canal irrigants and are responsible for root canal treatment failure as they adhere to the hard tissues thus causing biofilm induced infections. In order to counteract these resistant bacterias and prevent the failure of endodontic treatment, thorough use of irrigants should be done along with biomechanical preparation of the canals. Incidence of interappointment pain during root canal treatment are plenty and several strategies are being adopted for its management, one of it being intracanal instillation of the analgesic agent. (Pai and Nivedhitha, 2020) Apart from its analgesic activity, analgesics are also believed to possess antimicrobial effects which are yet to be studied in detail. Our team has extensive knowledge and research experience that has translated into high quality publications (Choudhari and Thenmozhi, 2016; Govindaraju, Jeevanandan and Subramanian, 2017; Ravi et al., 2017; Vikram et al., 2017; Gupta, Ariga and Deogade, 2018; Hannah et al., 2018; Kavarthapu and Thamaraiselvan, 2018; Pandian, Krishnan and Kumar, 2018; Ramamurthy and Mg, 2018; Ashok and Ganapathy, 2019; Ramesh et al., 2019; Sharma et al., 2019; Venu, Raju and Subramani, 2019; Wu et al., 2019; Samuel, Acharya and Rao, 2020)

Hence the aim of this study is to determine the antimicrobial effects of analgesics like mefenamic acid and aceclofenac at different concentrations on S. mutans, S. aureus, E.faecalis and C. albicans using the zone of inhibition test.

2. Methods and materials

2.1 Preparation of the Analgesics

2.1.A Mefenamic Acid was obtained in the powder form and since it was soluble in basic hydroxides, sodium hydroxide solution was prepared by dissolving 0.1g of sodium hydroxide pellet in 10ml of distilled water. This solution was then used to dissolve 0.1g of mefenamic acid which resulted into 100µg/ml mefenamic acid solution.

2.1.B Aceclofenac powder is soluble in phosphate buffer at pH 6.8 and so 1.38g of potassium dihydrogen sulfate and 3.5g of disodium hydrate were mixed in 100ml distilled water resulting in Phosphate Buffer saline, in which 0.1g of aceclofenac is mixed giving 100 µg/ml, aceclofenac solution. Both Mefenemic acid and Aceclofenac were diluted to 5 different concentrations 100µg/ml, 80µg/ml, 60µg/ml, 40µg/ml and 20µg/ml.

2.1.C Saline and Calcium Hydroxide (Apexical) was used for control.

2.2 Preparation of strains

The most popular test for determining the antimicrobial activity of dental materials is the agar diffusion test. (Orstavik and Haapasalo, 1990) (Milosevic, 1993)

Streptococcus mutans (S.mutans) which is a gram positive, non-motile streptococci (Hamada and Slade, 1980), Enterococcus faecalis (E.faecalis) which is a nonmotile, gram positive, spherical bacterium (Jhamb, Nikhil and Singh, 2010), Candida albicans a dimorphic, commensal as well as an opportunistic fungus (Ramage et al., 2001) and Staphylococcus aureus (S.aureus) a gram positive round shaped bacterium were grown in Mueller-Hinton Agar. 6 wells each were made in every Petri dish.

In Group one Mefenemic acid with concentration 100µg/ml,80µg/ml,60µg/ml,40µg/ml,20 µg/ml was added in 5 wells respectively in all the strains. Saline was added in the 6th well.In Group two Aceclofenac with concentration 100µg/ml,80µg/ml,60µg/ml,40µg/ml,20 µg/ml was added in 5 wells respectively in all the strains. Saline was added in the 6th well.

The Petri dishes were kept under observation for 24 hrs and checked for zones of inhibition around the wells.

3. Results

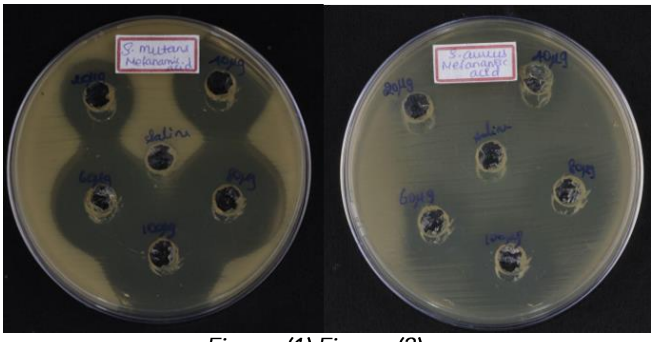
The Petri dishes were divided into 2 groups, Group 1 was MA and Group 2 was Aceclofenac. After keeping the petri dishes under observation for 24 hrs we got the following results.

S.mutans showed the zone of inhibition between 22mm-24mm in 20µg, 30mm-32mm in 40µg, 32mm-34mm in 60µg, 34mm-36mm in 80µg, 38mm-40mm in 100µg in group1 and 10mm-12mm in 20µg, 14mm-15mm in 40µg, 14mm-16mm in 60µg, 19mm-21mm in 80µg, 20mm-22mm in 100µg of group 2. S.mutans showed a zone of inhibition of 9mm around saline and 30mm-32mm in calcium hydroxide.

S.aureus showed the zone of inhibition between 12mm-14mm in 20µg, 19mm-21mm in 40µg, 20mm-22mm in 60µg, 26mm-28mm in 80µg, 30mm-32mm in 100µg in group1 and 9mm-10mm in 20µg, 9mm-12mm in 40µg, 9mm-11mm in 60µg, 14mm-18mm in 80µg, 19mm-20mm in 100µg in group 2. S.aureus showed a zone of inhibition of 9mm-10mm around saline and 25mm-27mm around calcium hydroxide.

E.faecalis showed the zone of inhibition between 10mm-12mm in 20µg, 23mm-25mm in 40µg, 28mm-30mm in 60µg, 30mm-33mm in 80µg, 35mm-37mm in 100µg ingroup1 and 9mm-10mm in 20µg, 9mm-10mm in 40µg, 9mm-10mm in 60µg, 10mm-11mm in

80µg, 11mm-13mm in 100µg in group 2. E. faecalis showed a zone of inhibition of 9mm around saline and 12mm-15mm around calcium hydroxide.
C.albicans showed the zone of inhibition between 9mm in 20µg, 9mm in 40µg, 9mm in 60µg, 9mm in 80µg, 9mm in 100µg in group1 and 9mm in 20µg, 9mm in 40µg, 9mm in 60µg, 9mm in 80µg, 9mm in 100µg in group 2. E. faecalis showed a zone of inhibition of 9mm around saline and 14mm-15mm around calcium hydroxide.



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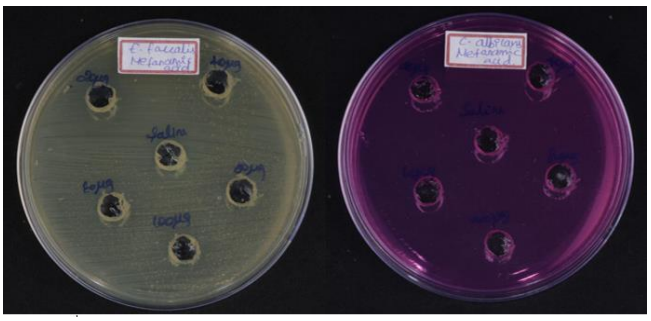

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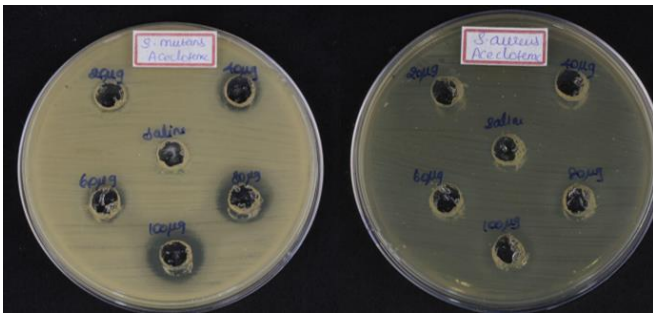

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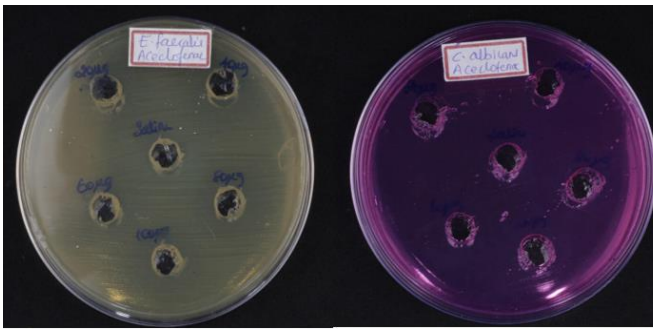
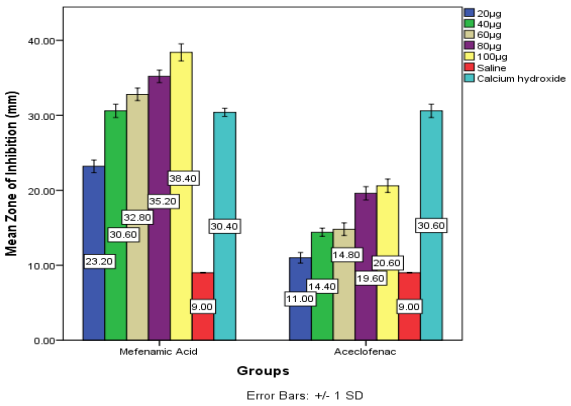


Figure (7) Figure (8)

100µg showed the maximum antimicrobial activity followed by 80µg, 40µg, 60µg and 20µg in both MA and aceclofenac in the particular strain. Saline showed a zone of inhibition of 9mm in all the strains. Calcium hydroxide showed a zone of inhibition of 30-32mm in *S. mutans*, 25-27mm in *S.aureus*, 12-15mm in *E.faecalis* and 14-15mm in *C.albicans*.

There was a significant amount of antimicrobial activity of MA and Aceclofenac in *S.mutans*, *S.aureus* and *E.faecalis* whereas the zone of inhibition was negligible for *C.albicans* in both MA and Aceclofenac. MA and Aceclofenac in the concentration of 100µg showed the maximum activity in every petri dish. The pilot study showed significant results.



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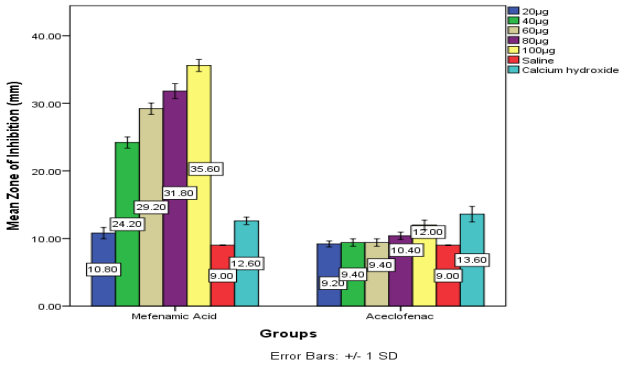

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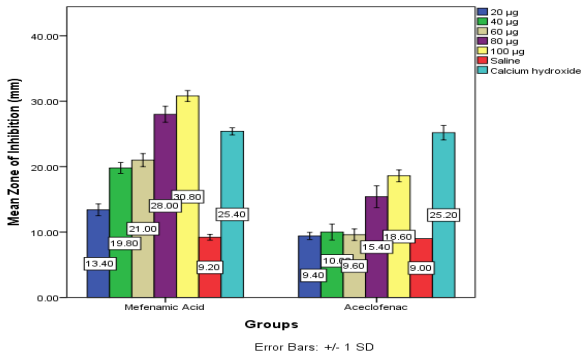
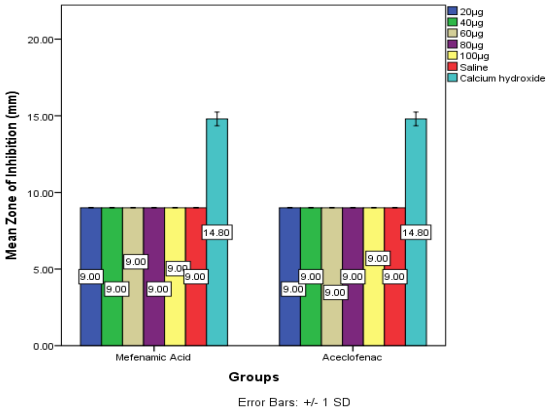


Graph (3)



Graph (4)

4. Discussion

The thought of root canal treatment brings about fear and anxiety thus delaying the treatment as for the fear of pain is the most likely reason why patients usually avoid dental treatments. A study shows patients who have undergone root canal treatment are less scared than the ones who have not undergone the treatment(SAGE Journals: Your gateway to world-class research journals, no date) thus becoming very important to give a positive root canal treatment experience. This can be done by prescribing different kinds of analgesics like opioids, Nsaids or their combination to give a better result in severe cases of inflamed pulp, necrosed pulp, postoperative flare up, acute periapical abscess etc. (Website, no date) Recently the use of Nsaids has increased not only as an anti-inflammatory but also as an analgesic and can be used for all kinds of dental pain. The current Nsaids are selective Cox 2 inhibitors, thus the damage to the gastric mucosa is less.(Haas, 2002)

Intracanal instillation is used after canal instrumentation to kill microbes present in the canal, it functions as a barrier against leaks from the temporary filling; it also minimizes inflammation of periapical tissues and pulp remnants. (Chong and Pitt Ford, 1992).

Group 1, 100µg of MA showed maximum antimicrobial effect on S.mutans followed by E.faecalis. 80µg of MA on E.faecalis showed similar antimicrobial activity as 100µg of MA on S.aureus. Group 2, 100µg of Aceclofenac showed similar antimicrobial activity on both S.mutans and S.aureus followed by E.faecalis. C. albicans showed resistance to all the concentrations of Aceclofenac and MA

When the two groups were compared, group 1 i.e. MA showed bigger areas of zone of inhibition than in group 2.

Overall aceclofenac showed lesser antimicrobial activity than mefenamic acid in all concentrations. These Analgesics work by suppressing prostaglandin production via the cyclooxygenase enzyme in the human body. Due to the absence of cyclooxygenase in microbes this mechanism does not take place (Al-Janabi, 2010). The synthesis of DNA is essential for the growth of all bacterial cells, whereas RNA is required for transcription and the provision of information for the synthesis of proteins and different enzymes. As a result, any disruption of DNA or RNA production can stop a bacterium from growing. Inhibitory chemicals are those that attach to DNA or RNA in such a way that their message cannot be read. (Dastidar et al., 2000) The bacterial growth inhibition around the analgesic wells is mainly due to inhibition of DNA synthesis and damage of the bacterial cell membrane. (Salem-Milani et al., 2013) The exact mechanism is unknown and it needs further research on how these analgesics show antimicrobial effects. This study has limitations since the zone of inhibition can be diffuse and have irregular borders,also it depends on the agar and the diffusion rate (Salem-Milani et al., 2013). Overall, our findings show that these analgesics have antimicrobial properties that should be researched further, mainly in clinical settings.

In a Similar Study done with diclofenac on E. faecalis, it was concluded that diclofenac showed better antimicrobial activity than calcium hydroxide, but it was lesser than antibiotics. The exact mechanism is unclear, but it can be an impairment of cell membrane. (Salem-Milani et al., 2013) Frequent use of antibiotics both systemically and locally can cause resistance to various bacteria . An in vitro study was done to see the effect of non-antibiotics on S. aureus and P.aeruginosa(Hendricks, Butterworth and Kristiansen, 2003), thus use of these analgesics instead of antibiotics in the root canal after canal instrumentation can lower the resistance .

For an effective and successful endodontic treatment elimination of the pathogens from the root canal is necessary, without proper disinfection it results in failure. (Alghamdi and Shakir, 2020) (Lin, Skribner and Gaengler, 1992) Calcium hydroxide is usually used as an intracanal medicament and it shows great results with cases having peri apical infections.(Vianna et al., 2007) (Law and Messer, 2004) Chlorhexidine gluconate in the concentration of 0.2% kills the intra canal microflora effectively, sometimes better than calcium hydroxide (Jhamb, Nikhil and Singh, 2010). Use of corticosteroids and antibiotics together as an intracanal dressing reduces the inflammation and ceases the growth of bacteria in the canal respectively.(Ehrmann, Messer and Adams, 2003) Along with the disinfection, the interappointment and postoperative pain caused during the treatment should be minimum for patient compliance and a positive experience. Earlier a study showed use of analgesic as an intracanal instillation to reduce interappointment pain.(Pai and Nivedhitha, 2020) . If this analgesic instillation can provide both antimicrobial activity and reduce the pain in between the appointments, then it gives a positive reinforcement to the patient and a successful endodontic treatment

5. Conclusion

The effect of mefenamic acid was more than aceclofenac on S. mutans, S. aureus and E.faecalis. The effect of mefenamic acid and aceclofenac on C. albicans was insignificant. Overall MA showed better antimicrobial effects than aceclofenac.

Further research with larger sample size is required to find out the antimicrobial effects of these analgesics.

A study on the synergistic effect of these analgesics with antibiotics can be carried out. (Chan et al., 2017)

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7. Conflict Of Interest

The authors would like to declare no conflict of interest in the present study.

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Legends

Figure 1: Petri dish showing zone of inhibition of *S.mutans* around mefenamic acid wells.
Figure 2: Petri dish showing zone of inhibition of *S.aureus* around mefenamic acid wells.
Figure 3: Petri dish showing zone of inhibition of *E.faecalis* around mefenamic acid wells.
Figure 4: Petri dish showing zone of inhibition of *C.albicans* around mefenamic acid wells.
Figure 5: Petri dish showing zone of inhibition of *S.mutans* around aceclofenac wells.
Figure 6: Petri dish showing zone of inhibition of *S.aureus* around aceclofenac wells.
Figure 7: Petri dish showing zone of inhibition of *E. faecalis* around aceclofenac wells.
Figure 8: Petri dish showing zone of inhibition of *C.albicans* around aceclofenac well

Graph 1: Graph depicting comparison of antimicrobial effects between Group 1 (MA) and Group 2 (Aceclofenac) in S.mutans in various concentrations.

Graph 2: Graph depicting comparison of antimicrobial effect between Group 1 (MA) and Group 2 (Aceclofenac) in *S.aureus* in various concentrations.

Graph 3: Graph depicting comparison of antimicrobial effect between Group 1 (MA) and Group 2 (Aceclofenac) in *E.faecalis.*

Graph 4: Graph depicting comparison of antimicrobial effect between Group 1 (MA) and Group 2 (Aceclofenac) in *C.albicans* in various concentrations.