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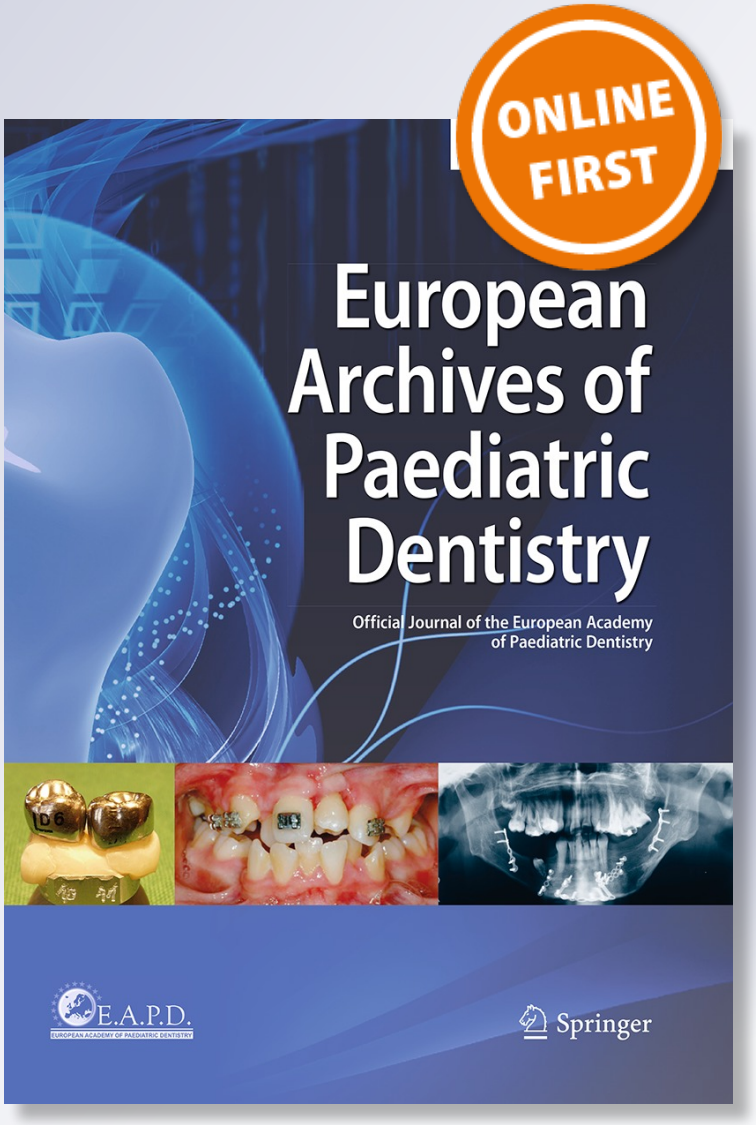

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ORIGINAL SCIENTIFIC ARTICLE

Clinical evaluation of sustained-release metronidazole gel versus metronidazole solution as an intracanal medicament in abscessed primary molars

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Abstract
Aim To evaluate the efficacy of metronidazole gel versus metronidazole solution against *Enterococcus faecalis* in abscessed primary molars.
Study design A clinical trial.
Method Twenty pulpally involved non-vital carious human primary mandibular second molars with furcal abscess were randomly allocated into two groups to evaluate the efficacy of metronidazole gel (3 % w/v) and metronidazole solution (0.5 % w/v) against *E. faecalis*. Subjects in the first experimental group were subjected to treatment with metronidazole gel (3 % w/v) and subjects in the second experimental group were treated with metronidazole solution (0.5 % w/v). Two microbial samples (pre-operative and post-operative samples) were obtained from the root canals of each subject from both the groups; sub cultured and efficacy of both the groups were evaluated.
Results Overall percentage reduction of the mean colony forming unit (CFU) count of metronidazole gel (3 % w/v) group was 96.39 % and metronidazole solution (0.5 % w/v) was 90.90 %. Results of intergroup *t* test of the percentage difference of mean CFU counts between both the groups revealed a statistically highly significant difference, i.e. *p* value—0.008 ($p < 0.01$).
Conclusions Metronidazole gel (3 % w/v) was more effective than metronidazole solution (0.5 % w/v) against *E. faecalis.*

Keywords Furcation · Non-vital · Metronidazole gel · Metronidazole solution · *Enterococcus faecalis*

Introduction

Microorganisms cause infection of the dental pulp and root canal as a sequel to dental caries, trauma, and operative dental procedures. The relationship between periapical inflammation and bacterial infection is well established (Kakehashi et al. 1966).

Achieving predictable long-term success of root canal treatment requires effective debridement and disinfection of the root canal system (Lee et al. 2008). Chemico-mechanical instrumentation removes the majority of infecting bacteria, together with necrotic pulp debris (Manzur et al. 2007). However, this is not always achieved completely because of anatomical complexity and the limitation in accessing the canal system by instruments and irrigants. The remaining bacteria may multiply during the period between appointments, often reaching the same level that it was at the start of the previous session, in cases where the canal is not dressed with a disinfectant between visits (Bystrom and Sundqvist 1981). Residual bacteria in obturated root canals may be denied access to nutrients and die (Peters et al. 1995), or they may survive and ultimately proliferate. Persistent endodontic infection may be

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attributed to the retention of microorganisms in dentinal tubules (Safavi et al. 1990).

Anaerobic bacteria, especially black pigmented gram-negative species, have been linked to the signs and symptoms of periapical disease (Gomes et al. 1994). Facultative bacteria such as *E. faecalis* have also been isolated from infected canals and may be related to failure of root canal treatment (Gomes et al. 1996). *E. faecalis* is a persistent organism that, despite making up a small proportion of the flora in untreated canals, plays a major role in the aetiology of persistent periradicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures and is able to survive in the root canal as a single organism or as a major component of the flora. Although *E. faecalis* possesses several virulence factors, its ability to cause periradicular disease stems from its ability to survive the effects of root canal treatment and persist as a pathogen in the root canals and dentinal tubules of teeth (Stuart et al. 2006).

This calls for the use of an effective intracanal medication that will assist disinfection of the root canal system. Such a medication should be effective throughout its period of application and penetrate the dentinal tubules, eliminating bacteria that may be present, with little toxicity to the periradicular tissues. However, it is impossible to achieve a sterile root canal space in all cases, even by thorough cleaning, shaping, and irrigating with disinfectants or antiseptics after one visit. Therefore, concern exists as to the fate and consequences of microorganisms left in the root canal. It has been shown that they may multiply rapidly in 2–4 days (to almost the original numbers) in cases where the canal is not filled or dressed with a disinfectant between visits.

Intracanal medicaments can help in eliminating the bacteria remaining even after chemico-mechanical instrumentation and can provide an environment conducive to periapical tissue repair (Lee et al. 2008).

Phenol and related volatile compounds were used for many years by endodontists and general practitioners for disinfection and caustic action. Camphorated monochlorophenol (CMCP) was one of the most commonly used medicaments for many years, but not anymore. Eugenol was once used for its obtundant action and mild antimicrobial action. Eugenol is now considered as a periradicular tissue toxin and its use is no longer recommended (Suresh Chandra and Gopi Krishna 1991).

Chlorhexidine gluconate has been widely used in periodontics due to its antibacterial activity (Gjermo 1974). Its use in endodontics has been proposed both as irrigant and intracanal medicament (Siqueira and Uzeda 1997). Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Known for its strong antibacterial activity against anaerobic cocci, as well as gram-negative and gram-positive bacilli, it has been used both systemically and topically in the treatment of periodontal disease. Metronidazole readily permeates bacterial cell membranes. It then binds to DNA, disrupting its helical structure and leads to very rapid cell death. It has been shown to have excellent activity against anaerobes isolated from odontogenic abscesses but has no activity against aerobes (Roche and Yoshimori 1997).

However, there are very few studies that have evaluated its efficacy in reducing facultative anaerobes in endodontic infections.

Keeping this in mind, the present study was aimed to assess the efficacy of metronidazole as an intracanal medicament against *E. faecalis* in the dentinal tubules of necrotic human mandibular second primary molars.

Materials and methods

Ethical approval

The study aims were fully explained to the parents/guardians, who signed a written consent form authorising children's enrolment in the study. The study protocol was reviewed and approved by the institutional research ethics committee.

A total of 40 subjects aged 6–9 years reporting to the outpatient section of the department of paedodontics and preventive dentistry, JSS dental college and hospital, Mysore were selected for the study. Forty subjects were assigned randomly into two groups namely—metronidazole gel (3 % w/v) group (20) and metronidazole solution (0.5 % w/v) group (20). Subjects aged 6–9 years fulfilling following inclusion criteria based on clinical and radiographic examination were included in the study: (1) teeth with furcal abscess (confirmed pulp necrosis) due to caries but with sufficient coronal structure to permit isolation of the operative field with rubber dam and further restoration, (2) less than two-third of root resorption, (3) presence of *E. faecalis* as a monobacterial organism in the infected root canals, (4) Mobility degree 0 or 1, (5) no periodontal pockets (periodontal probing depth <3 mm), and (6) no previous root canal intervention.

Preparation of metronidazole solution (0.5 % w/v)

Metronidazole solution was obtained commercially under the trade name of metrogyl injection (5 mg/ml) manufactured in India by J.B. Chemicals and Pharmaceuticals LTD., batch no. RIW 0026.

Preparation of metronidazole dispersed gel (3 % w/v)

Fifty millilitres of de-ionised water was dispensed in a clean calibrated beaker and 1.66 g of pure metronidazole

powder was added and dissolved in water using a magnetic stirrer (manufactured by Aarti Drugs Limited, India; batch no. MTZ/8030143). This was stirred for 5–10 min and then HPMC E-15 (5 %) powder was added gradually to the above mixture increasing the viscosity to the desired gel consistency. It was again stirred and kept aside for the developed foam to disappear gradually (4–5 h) and then checked for syringeability of the preparation. The preparation was covered with a lid or tin foil and stored in a cool dry place to avoid exposure to sunlight. Before using, it was sterilised to prevent any contamination in the operating field.

Measurement of gel viscosity

Dispersion viscosity was measured using Brookfield Viscometer spindle = SC4-18 small volume adaptor and was found to be of appropriate thickness for injectability.

Methodology of clinical procedure and microbial sample collection

All the clinical procedures and microbial sample collection were carried out by a single operator (experienced paedodontist from the department of paedodontics and preventive dentistry, JSS dental college and hospital, Mysore, Karnataka).

Local analgesia (0.2 % xylocaine with 1: 200,000 adrenaline) was given followed by rubber dam application to gain isolation. The tooth was cleaned with pumice, and the surrounding field was cleaned with 3 % hydrogen peroxide and decontaminated with 2.5 % NaOCl for 30 s. The solution was inactivated with 5 % sodium thiosulphate. After this, pulp chambers were opened under aseptic conditions with sterile water-cooled, high-speed diamond burs. K-file no. 15 was introduced to a level approximately 1 mm short of the tooth apex based on diagnostic radiographs and working length was determined. Afterwards, two sequential paper points were placed to the level of working length and used to soak up the fluid in the canal. Each paper point was retained in position for 60 s. Paper points were then transferred to 2 ml sterile Eppendorf tubes containing brain heart infusion (BHI) broth and samples were processed within 2 h. Aseptic techniques were used for instrumentation during access to and removal of the contents from the pulp space, and sample collection (Figs. 1–5). In each case, a single root canal was sampled to confine the microbial evaluation to a single ecologic environment. The criterion used to choose the canal to be microbiologically investigated in the multirooted teeth was the root canal with largest diameter, i.e. the distal canal of mandibular primary second molar in this case. If the root canal was dry, a small amount of sterile saline solution was introduced into the canal to ensure viable sample acquisition. Chemical active ingredients were never used before sampling. After collection of pre-operative samples, necrotic or infected pulp tissue was removed using a barbed broach. Filing was carried out to the determined working length and each canal was enlarged three instrument sizes greater than the first file. Irrigation with normal saline (1 ml) was carried out between the uses of each instrument to aid in removing debris and no other chemical agent was used to prevent false positive results. After mechanical instrumentation, canals were dried and 0.33 ml of 3 % metronidazole gel was used in first experimental group as an intracanal medicament to deliver a total of 10 mg of metronidazole.

In the second experimental group after mechanical instrumentation, the canals were dried and 2 ml of 0.5 % w/v metronidazole solution was injected and used as an intracanal medicament to deliver a total of 10 mg of metronidazole. After delivering the agent, a wet cotton pellet was placed in the chamber and closed with a ZOE cement

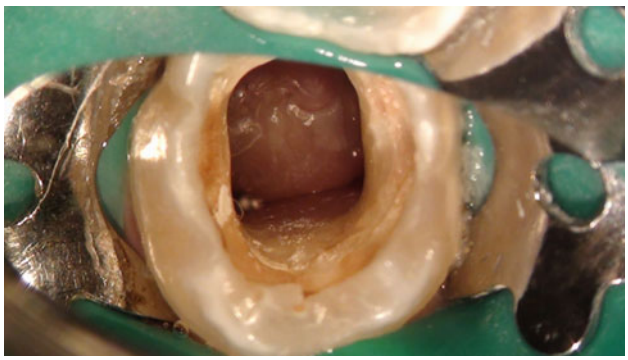

Fig. 1 Surgical microscope image of access opening, ×13

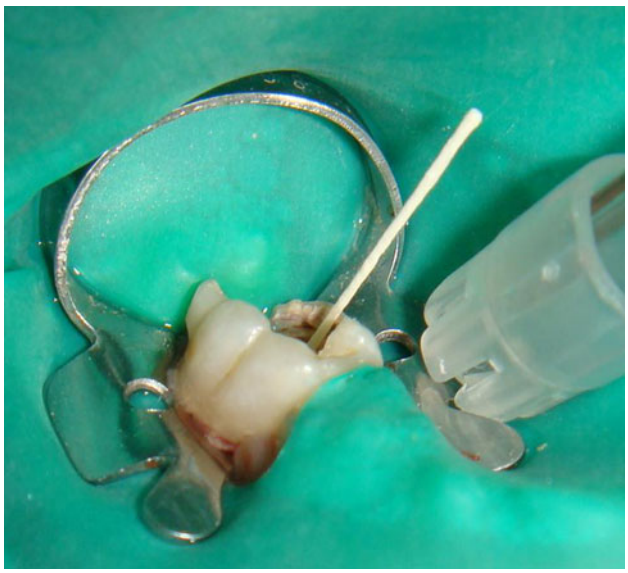

Fig. 2 Sample collection with sterile paper point

Fig. 3 Paper point in BHI broth

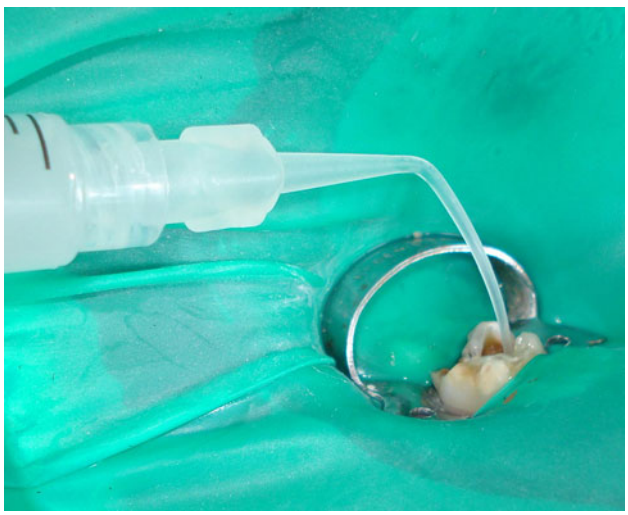

Fig. 4 Injecting 3 % metronidazole gel

dressing was given. Post-operative instructions were given to the patient and no other antibiotics were prescribed to prevent false positive results. Patients were recalled after 7 days and the condition was evaluated clinically as well as radiographically. Under aseptic conditions, the closed dressing was removed using slow-speed diamond burs, saline moistened cotton rolls on K-files were used to remove the residual intracanal medicament. Post-operative samples were taken using sterile #20 paper points in the same way as described earlier. After obtaining post-operative samples, the teeth were obturated and permanent

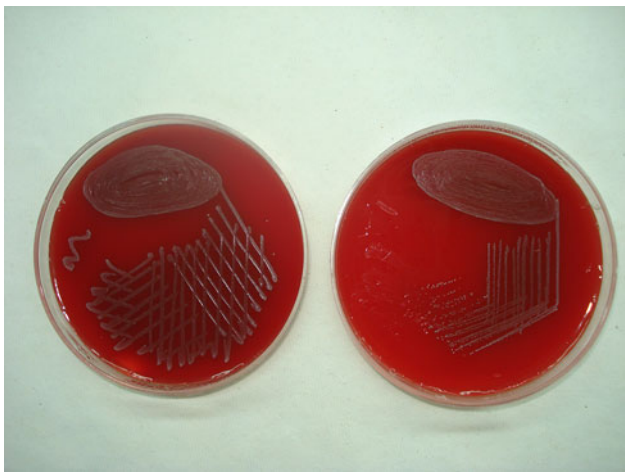

Fig. 5 Pre- and post-operative *E. faecalis* growth

restorations placed. On receiving the microbiological reports, the pre- and post-operative readings of colony forming unit (CFU) of *E. faecalis* were compared and the results were obtained.

Antimicrobial assessment

For microbiological procedures, the sterile screw cap vials containing the samples in BHI broth were preincubated for 30 min at 37 °C and shaken vigorously in a vortex mixer for 60 s. Serial tenfold dilutions were made up to $1{:}10^6$ in 1 % sterile peptone water. From the serial dilutions, 0.1 ml was transferred and plated on Hi Veg blood agar to confirm the presence of *E. faecalis*. The plates were incubated in an anaerobic chamber for 48 h and the *E. faecalis* counts were determined as CFU/ml using a colony counter.

The purity of the cultures was confirmed by gram staining, catalase production, colony morphology on blood agar and using a biochemical identification kit. The plates were assigned to the following groups ($n = 40$ plates).

- Metronidazole gel 3 % w/v group (pre- and post-operative)
- Metronidazole solution 0.5 % w/v group (pre- and post-operative)

The results thus obtained were evaluated with descriptive statistics and paired and independent *t* tests for comparison within the groups and between both the groups.

Results

A total of 40 patients were examined in the department of paedodontics and preventive dentistry, JSS dental college and hospital, Mysore, Karnataka. Twice the number of subjects were included bearing in mind the possible flare-

ups and dropouts. These 40 patients were allocated equally to both the groups namely: metronidazole gel 3 % w/v group (20) and metronidazole solution 0.5 %w/v group (20). On procuring an initial pre-operative microbiological sample and culturing, it was found that of the 40 patients, 10 cases yielded polymicrobial colonies like *E. faecalis*, *F. nucleatum*, *P. gingivalis* and *P. intermedia*, respectively. Five cases could not make up to the scheduled treatment and the remaining five patients returned with dislodged closed dressings resulting in loss of intracanal medicament with contamination. Coronal seal loss must have been attributed due to the dietary habits. As a result, only 20 patients could meet the strict treatment guidelines and yielded *E. faecalis* as a monobacterial organism.

Descriptive statistics of CFU counts for the metronidazole gel 3 % w/v group during the pre-operative evaluation were 5.311×10^4 and during the post-operative evaluation were 0.0541×10^4. Similarly, CFU counts for metronidazole solution 0.5 % w/v group during pre-operative evaluation were 5.5×10^4 and during post-operative evaluation were 0.46×10^4 (Table 1; Fig. 6).

Intragroup comparison of mean CFU counts for 3 % w/v metronidazole gel group during pre- to post-operative evaluations revealed statistically significant reduction of the colonies ($t = 3.376$, $p = 0.008$). Similarly for 0.5 % w/v metronidazole solution group, statistically significant reduction of the colonies was observed ($t = 3.645$, $p = 0.005$) (Tables 2, 3; Figs. 7, 8).

When inter-group values were compared and calculated through percentages, a significant difference was observed between the two groups, where the obtained t value of 3.303 was found to be significant ($p = 0.004$). From the mean values, it was clear that the gel group had a higher percentage reduction (mean 96.39 %) compared to the solution group (mean 90.90 %) (Tables 4, 5; Fig. 9).

Discussion

The significance of this study derives from its being conducted under in vivo conditions where the use of an intracanal medication could disrupt the established nutritional interrelationships, eliminating some bacteria that might have been essential to the growth of others or leaving some bacteria whose presence would inhibit the growth of others (Oncag et al. 2006).

Few studies have evaluated the microbiota of root canals of primary teeth with necrotic pulp and the available ones were limited to detect microorganisms in teeth with chronic periapical lesions.

Metronidazole is known to be more effective against obligate anaerobic bacteria than on aerobic and facultative anaerobic bacteria. But there is evidence of its antibacterial activity on *E. faecalis*, a facultative anaerobic bacterium. Anaerobic bacteria contain electron transport components such as ferrodoxin that have a sufficiently negative redox potential to donate electrons to metronidazole. This single electron transfer forms a highly reactive nitro radical action that targets DNA and other biomolecules (Gao et al. 2004).

Side-effects to systemic administration of metronidazole are relatively frequent, but mostly non-serious: anorexia, nausea, metallic taste and abdominal cramps are the most common. Looseness of stools is occasional. Less frequent side-effects are headache, glossitis, dryness of mouth, dizziness, rashes and transient neutropenia. Prolonged administration may cause peripheral neuropathy and CNS effects. Seizures also occur following very high doses. Thrombophlebitis of injected veins occur if the solution is not well diluted. However, there is no documentation of side-effects of local application of metronidazole in dentistry.

The drug was prepared in the gel form to allow a longstanding action (Siqueira and Uzeda 1997) and also to have the following advantages: (1) it releases the drug gradually helping the medicament to stay in the root canal for a longer period of time, making it a slow drug delivery system. (2) During root canal preparation, the antimicrobial medicament used should also act as a lubricant, remove smear layer, be water soluble, be biocompatible with periapical tissues, and have contact with the microorganisms (Siqueira et al. 1996).

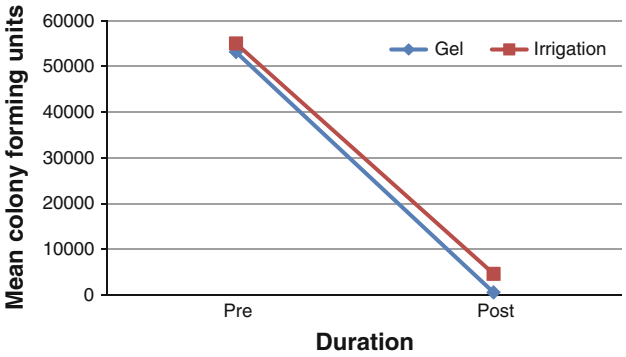

Fig. 6 Mean CFU counts for gel and solution groups during pre-operative and post-operative evaluations

Table 1 Comparison of mean CFU counts for 3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution groups during pre- and post-operative evaluations

Evaluation	Group	Mean CFU ($\times 10^4$)	SD ($\times 10^4$)
Pre-operative	Gel	5.31	4.96
	Solution	5.50	4.74
Post-operative	Gel	0.05	0.05
	Solution	0.46	0.47

Table 2 Intra-group comparison by paired sample statistics for the mean values of CFU counts for 3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution groups from pre- to post-operative evaluations

Group CFU		Mean CFU ($\times 10^4$)	SD ($\times 10^4$)
3 % w/v metronidazole gel	Pre-operative	5.31	4.96
	Post-operative	0.05	0.05
0.5 % w/v metronidazole solution	Pre-operative	5.50	4.74
	Post-operative	0.46	0.47

Table 3 Intra-group comparison by paired sample '*t*' test for mean values of CFU counts for 3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution groups from pre- to post-operative evaluations

Group	CFU ($\times 10^4$)	Mean difference	*t* value	*df*	*P* value
3 % w/v metronidazole gel	Pre-operative	5.25	3.376	99	0.008** ($P < 0.01$)
0.5 % w/v metronidazole Solution	Post-operative	5.04	3.645		0.005** ($P < 0.01$)

df degree of freedom

** Highly significant

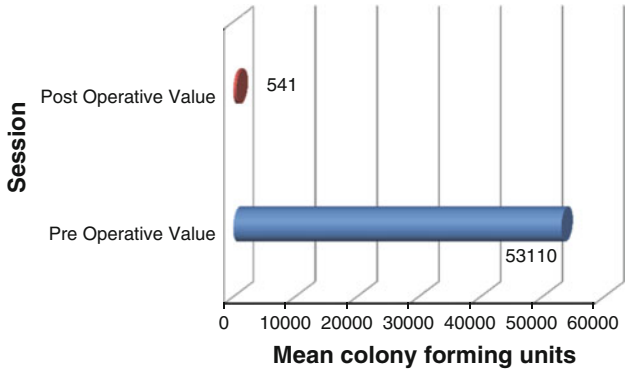

Fig. 7 Mean values of CFU counts of 3 % w/v metronidazole gel group during pre-operative and post-operative evaluations

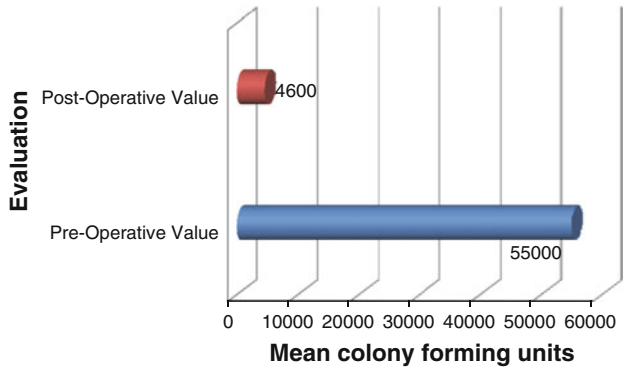

Fig. 8 Mean values of CFU counts for 0.5 % w/v metronidazole solution group during pre-operative and post-operative evaluations

Enterococcus faecalis was chosen as a test organism because it is a facultative organism that is non-fastidious, easy to grow, and efficiently and rapidly colonises tubules. *E. faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections. Its prevalence

Table 4 Inter-group comparison of percentage reduction from pre- to post-operative evaluations of mean CFU counts of 3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution groups

Group	Mean	SD	SE mean
% Difference			
Gel	96.39	4.41	1.39
Solution	90.90	2.84	0.90

Table 5 Inter-group comparison by independent sample '*t*' test for percentage difference from pre- to post-operative evaluations of mean CFU counts of 3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution groups

t	*df*	Sig.	Mean difference
% Difference			
3.303	18	0.004** ($P < 0.01$)	5.49 %

df Degree of freedom

** Highly significant

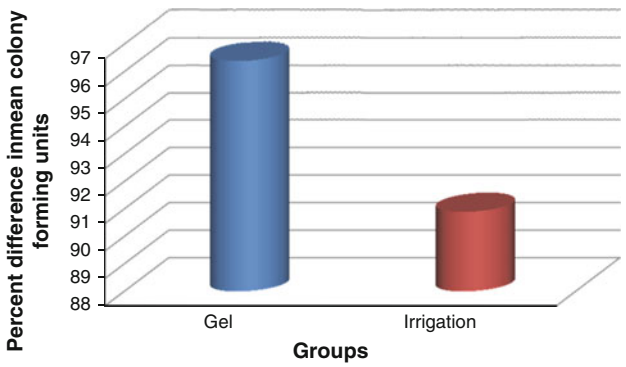

Fig. 9 Percentage difference from pre-operative to post-operative evaluations in CFU counts of 3 % w/v metronidazole gel group and 0.5 % w/v metronidazole solution group

in such infections ranges from 24 to 77 %. Moreover, *E. faecalis* is found in 40 % of primary endodontic infections. The mechanisms by which *E. faecalis* enters and survives in the root canal for extended periods despite endodontic treatment are not well understood. However, it is well established that *E. faecalis* has the capacity to survive under various stressful environmental conditions, including intracellular survival in macrophages. It has been suggested that *Enterococci* may be selected in root canals undergoing standard endodontic treatment because of their low sensitivity to antimicrobial agents including the ability to resist the high pH of antimicrobial agents commonly used, such as calcium hydroxide paste. This finding can be explained by various survival and virulence factors possessed by *E. faecalis*, including its ability to endure prolonged periods of nutritional deprivation, bind to dentine and proficiently invade dentinal tubules, alter host responses, suppress the action of lymphocytes, utilise serum as a nutritional source, resist intracanal medicaments, compete with other cells and form a biofilm. Moreover, it possesses lytic enzymes, cytolysin, aggregation substance, pheromones, and lipoteichoic acid (Stuart et al. 2006).

A significant decrease in the mean CFUs was observed from pre- to post-operative test session. This proves that metronidazole in the 3 and 0.5 % w/v concentrations are highly effective against *E. faecalis* in primary endodontic infections. It has been mentioned in many studies that *E. faecalis* is highly resistant to metronidazole. An in vitro study conducted by Siqueira et al. (1996) where 10 % metronidazole gel was found to be effective against all obligate anaerobes tested and not at all effective against all facultative anaerobes including *E. faecalis*. However, in this study lower concentrations were used, i.e. 3 and 0.5 % w/v with excellent results. This result is totally in contrast to the results reported in a previous study (Siqueira et al. 1996). Reason for such a great variation in the results might be because of different methodological procedures used in this study and different vehicles used to prepare the gel. Also metronidazole was made as a slow drug delivery system (i.e. in gel form) which made it possible to stay within the root canal for a longer period of time (i.e. for 7 days in this study). These results are also very much superior to the results obtained and reported (Krithikadatta et al. 2007), where moderate inhibition of *E. faecalis* was seen with 2 % metronidazole gel at 200 and 400 μm depth in an in vitro study (Gao et al. 2004). It is also mentioned that by increasing the concentrations of metronidazole, complete elimination of *E. faecalis* might be achieved.

An in vitro study was conducted (Krithikadatta et al. 2007) where the reported results revealed overall percentage reduction of *E. faecalis* growth (at 200 and 400 μm depth) of about 86.5 % with 2 % metronidazole gel. In the present study, metronidazole was used at a concentration of 3 % w/v with overall percentage reduction of *E. faecalis* of 96.39 % which is far more superior to the results obtained by Krithikadatta et al. (2007). However, in that study, metronidazole was used at a concentration of 2 %, whereas the present study used a concentration of 3 %, the reason being an attempt to get the desired complete reduction of *E. faecalis* from the dentinal tubules which the above-mentioned study did not achieve. Also the discrepancy in the results could be possibly attributed to the use of a different vehicle to formulate the gel in the present study.

Conclusion

There was a significant percentage difference in the mean CFU counts during pre- to post-operative evaluation between both the experimental groups (3 % w/v metronidazole gel and 0.5 % w/v metronidazole solution).

Metronidazole gel 3 % w/v showed superior efficacy than 0.5 % w/v metronidazole solution in *E. faecalis* in eliminating non-vital carious human primary second mandibular molars with furcal abscess due to its sustained-release property.

Therefore, within the limitations of the present study, it can be said that 3 % w/v metronidazole gel can be used as an alternative intracanal medicament to other routine medicaments in treatment of non-vital carious human primary second molars with furcal abscess. Thus, clinical evaluation of these intracanal medicaments is the true judgemental test of their efficacy.

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