In vitro antimicrobial efficacy of photoactivated cow urine against enterococcus faecalis

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Abstract

Introduction: The success of the endodontic treatment depends on the complete debridement of the root canal to prevent infection. E. faecalis is the predominant bacteria responsible for endodontic treatment failure. Various irrigants are used to disinfect the root canals and eradicate the microorganisms. Sodium hypochlorite (NaOCl) is the irrigant of choice in endodontic practice. However, there is a need for alternative irrigant to overcome the shortcomings caused by the use of sodium hypochlorite.

Materials and Methods: A total of 75 uniradicular teeth without fractures and caries were collected, decoronated and irrigated with 2.5% NaOCl to remove any remnants. Canals were prepared using protaper universal rotary files upto F3 size and the teeth were inoculated with E.faecalis. The contaminated teeth were randomly divided into group 1 (n=15) Control, group 2 (n=15) 2.5% NaOCl (Positive control), Group 3 (n=15) photo-activated cow urine and incubated for 60, 90 and 120 min at 37°c. Colony forming units and percentage reduction was determined at various tested time intervals. The obtained data is statistically analyzed using student t test.

Results: Both the groups treated with 2.5% sodium hypochlorite and photoactivated cow urine, exhibited effective inhibitory potential E.faecalis when compared to control group. The mean number of colonies formed was 0 and the percentage reduction was 100% in 2.5% NaOCl group. The photoactivated cow urine group exhibited inhibitory potential with increase in exposure time showing 85% reduction at 120 min.

Conclusion: From the study, it can be concluded that photoactivated cow urine can be used as an alternate irrigant to eradicate the E.faecalis bacteria from the root canal systems.

Keyword: Antibacterial Activity, Enterococcus faecalis, Photoactivated cow urine, Sodium hypochlorite.

Introduction

With relevant increase in the incidence of antibiotics misuse and overuse, researchers are focusing towards the naturally available products to combat infections in human beings.[1] Since ancient times, the use of natural products has been in practice in both eastern and western traditional medications. Nature is considered as the primary drug house with a very wide range of animals, plants and innumerable microorganisms acting as an infinite source for discovery, development and supply of various drugs to treat a large variety of clinical conditions.[2]

In endodontics, the use of biological medication with naturally available products is gaining importance because of the cytotoxic effects caused by most of the intracanal medicaments used during the treatment procedures and their inability to eliminate the infection from the dentinal tubules.[3] The success of an endodontic treatment depends on many factors with the reduction or elimination of bacterial infection being the most important one.[4] However, complete sterilization of the root canal system cannot be achieved even with advanced endodontic instrumentation because of the extremely complex anatomy of the root canal.[5] The residual pulpal tissue acts as the nutritional source for the bacteria, thus resulting in infection of root canal system. Hence, the role of irrigants holds a major place in successful endodontic treatment[6].

The endodontic infections are polymicrobial in nature with *Enterococcus faecalis* as the most predominant bacteria showing a prevalence of 24% to 77% in endodontically failure teeth. [7,8,9,10] Sodium hypochlorite (NaOCl) is considered as the best irrigant to eliminate *Enterococcus faecalis* from the root canal space and the dentinal tubules.[11] However, few studies reported the resistance of *Enterococcus faecalis* to NaOCl.[12] Because of its unpleasant taste and cytotoxic reactions on the structural integrity of dentin, there is a need for an alternative agent.[13]

In India, the Cow (Kamadhenu), scientifically known as Bos indicus is considered as the sacred and most veneered animal. This is because of the therapeutic benefits obtained from its products. All the products like Urine, Milk, Dung, Curd and Ghee, collectively known as "Panchagavya" are the chief ingredient in ayurvedic medicine. They are used to treat a wide range of health conditions since ancient times.[14] Ancient literatures like Bhav Prakash Nighantu, Sushruta Samhita and Astanga Sangrah described cow urine as the most effective secretion with various therapeutic uses with antimicrobial, antifungal properties.[15] Cow urine acts as an effective agent against a wide range of gram negative and gram

positive bacteria.[16] Therefore, the present study aims at evaluating the antimicrobial efficacy of photo activated cow urine against *Enterococcus faecalis* infections.

Materials and Methodology

Collection of Cow urine: Fresh cow urine was collected in a sterile container and is photo-activated by keeping it in sunlight for about 72 hours. Whatmann filter paper is used to filter the urine so as to remove any debris and is then stored at 4^0C. Evaluation of cow urine is done for microbial contamination prior to introduction to the samples.

Preparation of Samples

A total of 75 uniradicular teeth without fractures and caries were collected and decoronated at cementoenamel junction. The length of roots were standardized to 15mm. Preparation of canals was done with instrumentation using protaper universal rotary files upto F3 size. The canals were irrigated with 28 guage needle placed 1-2mm short of working length using 2.5% NaOCl followed by 17% EDTA. Finally, distilled water was used to flush the canals so as to remove the precipitates. Splitting of the teeth was done by making shallow grooves on the medial and distal sides using a diamond disk. Care was taken not to perforate the canal. The apical foramen of all the samples is sealed by coating with nail varnish. The samples were stored in 0.2% sodium azide at 4^0C after sterilizing with Ethylene oxide until further use.

Microbial Strain and preparation of sample

Two to three well isolated colonies of *E. faecalis* (ATCC 29212) were transferred to 3ml of Muller Hinton Broth tubes and incubated for 3-4 hrs at 37^0C with intermittent shaking at 150 rpm in water bath to ensure the logarithmic phasic cultures. The optical density of the inoculum was measured spectrophotometrically to obtain the density of 5 X 10^6 CFU/ml. Further, each sample was treated with 5µl of *E. faecalis* inoculum by pipette and incubated at 37^0C for 10 days. After incubation the samples containing *E. faecalis* were randomly divided into group 1 (n=15) Control, group 2 (n=15) 2.5% NaOCl (Positive control), Group 3(n=15) photo-activated cow urine. After the various treatments, all the groups were incubated at 60, 90 and 120 min at 37^0C.

After incubation, each sample was flushed with 2ml of sterile water and dried with gauze and paper points. To test for bacterial survival, dentin shavings from within the canal were collected using round burs at a depth of 0.5mm. The collected dentin material was transferred to 1ml brain heart infusion (BHI) broth and incubated for 6 hrs to enrich the bacterial cells in an orbital incubator at 120 rpm. The enriched samples were diluted, placed in triplicates on the BHI agar plates, which are then incubated for 24 hrs to determine the colony forming units.

The percentage kill of each test agent on the *Enterococcus faecalis* was determined by

$$\text{Percent kill} = \frac{\text{Initial colony} - \text{Final colony count}}{\text{Initial colony count}} \times 100$$

Statistical Analysis

The results obtained were statistically analyzed and compared among the groups using student t test. All the data were expressed as Mean ± SEM and the difference of $p<0.05$ or more is considered as significant.

Results

All the irrigating solutions tested showed antimicrobial activity against Enterococcus faecalis. Table 1 summarizes the colony forming units of the bacteria among different groups at all the tested time intervals. Maximum numbers of colonies were observed in Group I or the control group, at all the time intervals. The Group II or the group inoculated with bacteria treated with 2.5% NaOCl showed no bacterial colonies, showcasing the higher inhibitory potential of 2.5% NaOCl against the *Enterococcus faecalis* bacilli at all the time intervals. Whereas, photoactivated cow urine group showed decrease in the number of colonies. The number of colonies at 60, 90 and 120 minutes were 162, 138 and 64 respectively.

There was no percentage reduction of *Enterococcus faecalis* colonies in the control group at all the tested time intervals. Whereas, 2.5% NaOCl showed maximum reduction in the bacterial colonies (100%) indicating antibacterial efficacy. The percentage reduction of bacteria in photoactivated cow urine treated group was time dependent with 42%, 67% and 85% at 60, 90 and 120 minutes respectively (Table 2).

Table 1: **Reduction of average CFU mL^{-1} of Enterococcus faecalis within root canals treated with cow urine. Mean number of viable colonies of bacteria in the different groups**

Group	Number colonies CFU mL^{-1} ± SEM		
	60min	90min	120min
Group I (Control)	432.72±1.24	446.15±1.75	435.08±1.35
Group II (Positive control)	0.00±0.00	0.00±0.00	0.00±0.00
Group III (Test)	162.00±1.64***	138.00±1.92***	64.00±1.73***

All results are shown as Mean ± SEM from the data of a minimum of three separate experiments. ***p< 0.001 (when compared with respective control group).

Table 2: **Percentage reduction of *Enterococcus faecalis* for different irrigants**

Group	Percentage kill ± SEM		
	60min	90min	120min
Group I (Control)	0.00±0.00	0.00±0.00	0.00±0.00
Group II (Positive control)	100±1.52	100.00±1.04	100.00±1.25
Group III (Test)	42.00±1.41	67.00±1.63	85.00±1.15

Discussion

Elimination of infection and obturation of canals to prevent reinfection is the major factor contributing to the success of endodontic treatment procedures.[4] Despite various chemomechanical preparations, complete eradication of microorganisms is still a challenge, because of the complex anatomical structure of the root canal system.[5] *Enterococcus faecalis* is termed as the star survivor in the root canal because of its high resistance to various antimicrobial agents[8,9]. Several microorganisms play a key role in endodontic treatment failures of which *Enterococcus faecalis* is the one of the most common bacteria[17].

Among all the irrigants, NaOCl is considered as the irrigant of choice because of its high tissue dissolving capacity and antimicrobial activity.[6] However, its unpleasant taste and smell, cytotoxic reactions, allergic potential results in a need for better compound with minimal toxicity.[13] As NaOCl targets tissue toxicity for antimicrobial activity, there is a raise in demand among the scientific community to explore new antimicrobial agents. Cow urine contains sodium, nitrogen, sulphur, Vitamin A, B, C, D, E, minerals, manganese, iron, silicon, chlorine, magnesium, citric, succinic, calcium salts, phosphate, lactose, carbolic acid, enzymes, creatinine and hormones[15]. Various studies described the antimicrobial activity of cow urine against a broad spectrum of bacteria by enhancing the phagocytic activity of macrophages and thus helpful against bacterial infections.[18,19,20,21] Therefore, the current *invitro* study was designed to evaluate the antibacterial activity of photo activated cow urine against *Enterococcus faecalis.*

It is reported that fresh cow urine shows very low antimicrobial properties, but effective control of proliferation of microorganisms can be observed with photoactivation.[22] With the photoactivation, the cow urine becomes slightly acidic because of decrease in its pH, thus increasing the bactericidal action.[23] Formation of biogenic compounds like Carbon dioxide, Methane, Ammonia, Propanol, Acetone, Methanol and other secondary metabolic nitrogenous products results in increasing the inhibitory potential of photoactivated cow urine against several bacterial strains when compared to fresh cow urine.[24,25] Therefore the present study was conducted by using photoactivated cow urine.

In the present *in vitro* study, the samples treated with NaOCl and photoactivated cow urine exhibited antimicrobial activity when compared to control at all the tested time intervals. However, photoactivated cow urine is found to be less effective when compared to 2.5% NaOCl. The results of our study demonstrated that the antimicrobial efficacy of the photoactivated cow urine depends on the exposure time. Photoactivated cow urine was less effective at 60 and 90 min but 85% reduction was observed at 120 min, indicating its antimicrobial efficacy against *Enterococcus faecalis* increases over time.

The antimicrobial effects of the tested cow urine in the study may be attributed to certain chemical compounds present in it. Earlier studies demonstrated that antimicrobial activity of the photo activated cow urine is due to the presence of certain volatile and non volatile components.[26] Linton and Dick states that the phenols present in the cow urine are bactericidal to both gram positive and gram negative bacteria.[27] Because of the presence of amino acids and urinary peptides, there is an increase in bacterial cell surface hydrophobicity, thus enriching the bactericidal effect.[28] Besides this, the presence of inhibitory compounds like aldehydes, ketones, sulfinol, amines, chlorides makes the photoactivated cow urine more effective than the fresh cow urine.[29]

Conclusion

Based on the results obtained in our study, we can conclude that both the 2.5% NaOCl and photoactivated cow urine are effective in eliminating the *Enterococcus faecalis* at all the tested exposure times. Further studies are needed before recommending it as an intracanal irrigant.

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