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Dentistry Section

A Comparative Analysis of Antimicrobial Property of Wine and Ozone with Calcium Hydroxide and Chlorhexidine

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ABSTRACT

Background: The antibacterial properties of wine and ozone have been established but their antibacterial efficacies against endodontic pathogens are yet to be ascertained.

Aim: The purpose of this study is to comparatively evaluate the antibacterial property of ozonated water, white wine (14%) and de-alcoholised white wine.

Materials and Methods: *S.mutans* and *E.faecalis* were subcultured and inoculated in a nutrient broth for 24 hours. The following groups were formulated: Group 1A:2% Chlorhexidine (Control group); Group 1B:White wine; Group 1C:Dealcoholised white wine; Group 1D:Ozonated water; Group 2A: $Ca(OH)_2$ + Chlorhexidine (Control group); Group 2B: White wine + $Ca(OH)_2$; Group 2C:De-alcoholised White wine + $Ca(OH)_2$ + chlorhexidine; Group 2D:White wine + $Ca(OH)_2$ + chlorhexidine and group 2E: Dealcoholised white wine + $Ca(OH)_2$ + chlorhexidine. The samples were allowed to diffuse into the culture medium for two hours, later the *S. mutans* were streaked on to the blood agar medium and the *E. faecalis* were streaked on to the Muller Hilton agar medium and incubated for 48 hours at 37^{0}C the zone of inhibition was measured after 48 hours.

Results: There was no growth of microorganisms seen with ozonated water. Chlorhexidine showed large zone of inhibition compared to the other groups. White wine has better antimicrobial property than de-alcoholised white wine, but when mixed with calcium hydroxide the dealcoholised white wine has better action against the microorganisms.

Conclusion: Ozonated water has the best antibacterial property and the antibacterial action of Calcium hydroxide is enhanced when it is mixed with de-alcoholised white wine.

Keywords: Antimicrobial efficacy, *Enterococococcus feacalis*, Reactive oxygen species, *Streptococcus mutans*

INTRODUCTION

Moderate wine consumption has beneficial effects on human health. Wine is said to have antibacterial property, this could be because of the presence of the anti oxidant properties of grapes or because of the acidic content of the wine. The organic acids commonly present in the wine such as acetic acid, citric acid, lactic acid, succinic acid, malic acid and tartaric acid is said to be responsible for the antibacterial property of wine. The antioxidant and antiradical properties, particularly of red wine, attributed mainly to high polyphenols content, appear to protect against the risk of coronary heart disease and cancer [1].

Several strains of oral streptococci are capable of initiating the formation of dental plaque, which plays an important role in the development of caries and periodontal disease in humans. *Streptococcus mutans*, a potent cariogenic, can colonize tooth surfaces and initiate plaque formation by its ability to synthesize extracellular polysaccharides from sucrose, mainly water insoluble glucan, using glucosyltransferase. Streptococcal growth inhibition by antibacterial agents has extensively been investigated. A small number of recent studies have reported antimicrobial activity of natural agents against selected oral pathogens. Naturally effective antimicrobial agents against oral pathogens could play an important role in preventing dental caries. The activity of wine against oral bacteria has not yet been investigated [1].

Calcium hydroxide ($Ca(OH)_2$) is one of the most versatile medicaments in dentistry, especially for its use as an intracanal dressing in endodontic therapy. $Ca(OH)_2$ has been successfully employed in the treatment of perforation repair arising from internal resorption and in apexification procedures. The main mechanism of action of $Ca(OH)_2$ is due to its high alkaline pH which has a lethal effect on protein strands in the bacterial cell membrane. As an intracanal dressing it is highly biocompatible and even when extruded periapically it is well tolerated. It cannot be however considered a universal intracanal medicament due to its ineffectiveness against all bacterial species seen in the root canal space [2,3].

Chlorhexidine gluconate (CHX) has a remarkable substantial activity against a number of gram-positive and gram-negative bacteria. For this reason it is widely employed in endodontics as an irrigant and intracanal medicament. The mechanism of action of CHX is based on the interaction between the positive charge of the molecule and the negatively charged phosphate groups on the bacterial cell wall. This enables the CHX molecule to infiltrate into the bacteria with toxic effects [4]. The idea of combining $Ca(OH)_2$ and 2% CHX is to augment the antimicrobial effectiveness especially against resistant microorganisms, such as *Enterococcus Feacalis* frequently isolated in failed endodontic cases [5].

Ozone is currently being discussed as a possible alternative antiseptic agent in dentistry because of its reported high antimicrobial power without the development of drug resistance. Ozone gas in a concentration of 4 g/m^3 (Heal Ozone; KaVo, Biberach, Germany) is already being used clinically for endodontic treatment. Regarding the demand on relative non-toxicity toward periapical and oral mucosal tissue for the endodontic irrigants, the ozone gas concentration currently used in endodontics (4 g/m^3) has been shown to be slightly less cytotoxic than NaOCl (2.5%) and aqueous ozone (up to 20 µg/mL) showed essentially no toxicity to oral cells in vitro [6,7].

Though there are various irrigants and intra canal medicaments in today's endodontic therapy the need for a better antimicrobial agent is always prevalent. The aim of this study is to compare the antimicrobial property of chlorhexidine and calcium hydroxide with wine and ozonated water against the caries causing *Streptococcus mutans* and against the *Enterococcus faecalis* which causes root canal failure.

MATERIALS AND METHODS

Chlorhexidine, calcium hydroxide, wine and ozonated water were studied for their antibacterial effect. The in-vitro study was conducted in Anand's multispecialty dental hospital, Chennai, India for duration of one week in a sterile environment. The materials to be tested for antimicrobial property was divided into two major groups in this study which are as follows.

Group I: It has the following 4 subgroups

- Sub Group A - 2 % chlorhexidine (control group)
- Sub Group B - White wine
- Sub Group C - De – alcoholised white wine
- Sub Group D – Ozonated water

Group II: It has the following 5 subgroups

- Sub Group A - $Ca(OH)_2$ + Chlorhexidine
- Sub Group B - White wine + $Ca(OH)_2$
- Sub Group C - De-alcoholised White wine + $Ca(OH)_2$
- Sub Group D - White wine + $Ca(OH)_2$ + chlorhexidine
- Sub Group E - Dealcoholised white wine + $Ca(OH)_2$ + chlorhexidine

The white wine used in this study contained 17% ethanol, the dealcoholised white contained 0.5% ethanol.

Preparation of Ozonated water: In this study aqueous ozone was used, it was prepared by passing gaseous ozone through water in a glass beaker. The ozone gas was generated by a table top ozone generator. One end of the silicon tube was connected to the outlet of the ozone generator and the other end was placed inside the beaker containing water. Ozone gas was passed through the water for 5, 10 and 15 min and the antibacterial property was checked.

Pure strain of *E. faecalis* (ATCC #24212) and *S. mutans* (ATCC # 25175) was obtained from Institute of Basic Medical Sciences, Chennai, India. It is then subcultured in Brain Heart Infusion (BHI) culture media also obtained from Institute of Basic Medical Sciences, Chennai, India and passed under gaseous condition to confirm their purity. Mueller-Hinton agar was prepared on sterile Petri dishes and kept for sterility check at 37°C for 24 hour. After sterility check, the inoculae of *E. faecalis* and *S. mutans* strains were used to make lawn culture on Meuller-Hinton agar plates.

Wells of 4 mm diameter and 4mm depth was prepared in the sterile agar plates. 200 µl of the experimental solutions were taken using a micropipette and was placed into the punched out wells in the agar plates. The agar plates were then placed inside the UV chamber for one hour for the solutions to diffuse through the culture medium. Later the organisms were taken from the culture medium and were streaked on the agar plates which were previously diffused by the solutions. The medium was left undisturbed for 48 hours, after which the zones of inhibition was measured and the values were noted. The antimicrobial property of ozonated water was checked by streaking the cultured organisms on to the agar plates immediately after the ozonated water was micropipetted into the punched wells. This was because the ozone gas dissipates within 10 to 15 minutes. The zone of inhibition was measured after 48 hours from the agar plates and was tabulated.

RESULTS

Ozonated water shows the total inhibition against *S. mutans* and *E feacalis*. Combination of dealcoholised wine with calcium hydroxide and chlorhexidine has better antibacterial efficacy against *E. feacalis* and *S. mutans* when compared with the other groups in group II.

DISCUSSION

Weisse et al., [8] reported that red and white wines are as potent as bismuth salicylate against several bacteria responsible for traveller's diarrhoea and that diluted ethanol induced no significant reduction in colony counts. Sugita-Konishi et al., [9] stated that the antibacterial activity of red and white wines are due to acetic acid present in the red and white wines in his study. He also stated that acetic acid is present in the polyphenol free fractions of red and white wines. Similarly, Dolara et al., also suggested that the antibacterial activity against Gram-positive and Gram-negative pathogenic bacteria by two industrial and a homemade wine is due to acetic acid [10], a common wine component added to red and white wines.

This study was carried out to check for a better and efficient irrigant and intracanal medicament to eliminate the microorganisms during endodontic therapy. Though CHX is being considered as one of the good intracanal irrigants available today, it has its own drawbacks and effects. Tissue irritation and the reactive oxygen species (ROS) formation are few of the drawbacks of CHX. A systematic electronic literature search revealed that no antibacterial study was involved with wine and ozonated water for its potential use as an irrigant in endodontics. In order to overcome these drawbacks and to obtain an irrigant with better antibacterial property this study was carried out.

The results obtained shows that ozonated water has the best antibacterial property as there was no growth seen even after 24 hours of incubation [Table/Fig-1]. The properties of ozone, in both aqueous and in gaseous phases, make it a useful disinfectant. The oxidant potential of ozone induces the destruction of cell walls and cytoplasmic membranes of the micro organisms. As this process goes on ozone acts on the glycoprotein, glycolipids and other amino acids and inhibits and blocks the enzymatic control system of the cell resulting in an increased membrane permeability which is the key element of cell viability thereby causing immediate functional cessation. Thus, ozone molecules in both aqueous and in gaseous phases can readily enter the cell and cause the microorganisms to die [6,7].

Group I	*S. mutans*	*E. feacalis*
Sub group A: Chlorhexidine (control group)	30 mm	30 mm
Sub group B: White wine	25 mm	20 mm
Sub group C: Dealcoholised White wine	20 mm	18 mm
Sub group D: Ozonated water	No growth	No growth
Group II	***S. mutans***	***E. feacalis***
Sub group A: $Ca(OH)_2$ + Chlorhexidine	20mm	20mm
Sub group B: White wine + $Ca(OH)_2$	16mm	25mm
Sub group C: Dealcoholised white wine + $Ca(OH)_2$ + chlorhexidine	20mm	30mm
Sub group D: White wine + $Ca(OH)_2$ + chlorhexidine	18mm	23mm
Sub group E : Dealcoholised white wine + $Ca(OH)_2$ + chlorhexidine (Control)	20mm	20mm

[Table/Fig-1]: Antibacterial efficacy of wine and ozonated water against *S. mutans* and *E. feacalis* bacterial strains with and without chlorhexidine and calcium-hydroxide

Two types of wine were used in this study, they were, alcoholised white wine in which the alcohol content was 12 to 15% and de-alcoholised white wine in this the alcohol content was very minimal, it was about 0.5%. These two types of wine were tested for antibacterial property both individually against the two organisms, *E. faecalis* and *S. mutans* and the zone of inhibition were measured. Later these two wines were mixed with $Ca(OH)_2$ and the zone of inhibition was measured. When used alone alcoholised white wine has better antibacterial property than the dealcoholised wine. But the effect of the alcoholised wine against the control group chlorhexidine was

lesser. When mixed with $Ca(OH)_2$ the dealcoholised wine has better action against the microorganisms. The anti bacterial property was better than that of the mixture of chlorhexidine and $Ca(OH)_2$. This makes it a better intracanal medicament with better antibacterial property, but the presence of any harmful by products formed in the end product has to examined and the tissue response to these combinations has to be checked prior to its clinical usage.

LIMITATIONS OF OZONE THERAPY

Inhalation of ozone gas for long periods causes damage to the lungs and other organs and it should not be intravenously injected due to the risk of pulmonary air embolism. Ozone therapy should not be employed in pregnancy, severe anaemia, hyperthyroidism, thrombocytopenia, severe myasthenia, acute alcohol intoxication, recent myocardial infarction, hemorrhage from any organ, glucose-6-phosphatedehyrogenase deficiency and ozone allergy.

CONCLUSION

Within the limitations of this study it can be concluded that ozonated water has the best antibacterial property among the study groups and the antibacterial action of Calcium hydroxide is enhanced when it is mixed with de-alcoholised white wine. Further research has to be done to check for the biocompatibility of the resulting product and its clinical implications.

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