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Full Length Research Paper

Iranian traditional medicine: Comparison of the antibacterial effect of ANNAS 0.2% and chlorhexidine 0.2%

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Anbarnesa smoke have a long history of antimicrobial effects in Iranian traditional medicine. The present study compared the effect of ANNAS (extract of Anbarnesara smoke) mouth wash 0.2% and Chlorhexidine 0.2% against some bacterial species. In this experimental study, the specific culture mediums were used for each species and the antibacterial efficacies of the mouth rinses were determined by means of agar diffusion test (for measurement of inhibitory zone diameter (IZD)) and dilution method (for measurement minimum inhibitory concentrations (MIC)). IZD and MIC were statistically analyzed by Kruskal-Wallis and Mann-whitney U tests. Both mouth rinses of ANNAS 0.2% and Chlorhexidine 0.2% had similar inhibitory growth zone for different bacterial species which was significantly better than control specimens. Chlorhexidine 0.2% induced higher MIC values than ANNAS 0.2% for the *streptococcus sanguis* and *Enterococcus faecalis* species, while no significant differences were found between two agents regarding MIC values against the other bacteria. Chlorhexidine 0.2% and ANNAS 0.2% showed higher growth inhibitory effect than control specimens against all bacteria except for *E. faecalis*. ANNAS mouth rinse 0.2% has some antibacterial properties, but it is not as efficacious as Chlorhexidine 0.2% on some selected species, with no significant effect on the *E. faecalis* species.

Key words: Antibacterial effect, mouth rinse, chlorhexidine, anbarnesa.

INTRODUCTION

Bacterial Plaque is a major causative factor for periodontal tissue destruction and plaque removal remains the cornerstone of periodontal disease prevention. Although mechanical methods such as brushing and flossing considered the basis of plaque control, some antibacterial mouthwashes (such as chlorhexidine, listesin etc) with topical or systemic effects can be prescribed as an alternative therapeutic aid (Shaju et al., 2011; Carranza and Newman, 2002).

Thanks to its ability to control microbial plaque, chlorhexidine can both prevent and reduce periodontal disease (Carranza and Newman, 2002). Because of some adverse effects of chlorhexidine such as tooth discoloration, allergic reactions and cytotoxic effects, a tendency to introduce a new mouthwash with the same antibacterial quality and less unwanted effects is an ongoing challenge (Bagis et al., 2011; Moshrefi, 2002; Hidalgo and Dominguez, 2001). In recent decade widespread development of alternative medicine

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attracted more attention toward traditional and herbal remedies in various fields of medicine and dentistry (Taheri et al., 2010; 2011; 2010). In Iranian Traditional Medicine smoke of Anbarnesa (yielded from burning of female donkey's droppings) has been used to treat ulcers and inflammatory conditions like stomatitis and ear infections (otitis).

According to our information no scientific research based on standards methodology accomplished on this field. The aim of this study was to compare the antibacterial effect of Anbarnesara mouthwash with conventional 0.2% chlorhexidine mouthwash on selected bacterial species.

METHODS

Anbarnesa (female donkey's droppings) was burned inside a closed container which walls coated with propylene glycol. After cooling the environment, substances in the smoke stick to the wall. This was repeated several times so that enough smoke stick to the wall. Then 10 ml propylene glycol solution was added to the container and mixed well with the extract on the wall. Then, the contents transferred into a falcon tube and suspended materials centrifuged for 30 min in 10 rpm (Hetich, Germany).To separate impurities using a Pasteur pipette, supernatant fluid was transferred to another tube. This solution named ANNAS. The concentration of the liquid was measured using liquid chromatography and diluted to the concentration of 0.2%. In this experimental (in vitro) study, the diameter of Inhibitory zone and minimum inhibitory concentration values were evaluated against different bacterial species. This evaluation was done with ANNAS and Cholhexidine 0.2% in comparison with control samples of propylene glycol. The bacteria species examined in the study include:

1. *Streptococcus mutans* (ATCC35668),
2. *Streptococcus sanguis* (ATCC10556),
3. *Streptococcus salivarious* (ATCC92220),
4. *Streptococcus Pyogenes* (ATCC8668)
5. *Enterococcus faecalis* (ATCC29212).

Agar diffusion test methods (agar diffusion test: ADT) and the dilution method for determining the minimum inhibitory concentration (minimum inhibition concentration: MIC) were used to determine the antibacterial activity of two mouth washes.

For medium preparation, 15 ml specific culture medium poured equally in each plate and 3 wells with equal diameter and depth were created by the Pasteur pipette. Then, 0.5 ml of the prepared bacterial suspension was cultured. Each well was filled with 50 micro liters of each material (ANNAS or Chlorhexidine) and after 30 min incubated at temperature 37°C. After 48 h, inhibition zone diameter for each material was measured. At the same time, the plates were monitored not to be contaminated by other microorganisms.

Dilution method test

0.5 ml of TSB (Tryptic Soy Broth) medium was added to 8 tubes. Chlorhexidine and ANNAS were prepared with different concentrations (from 1 to 1 / 256). Equivalent of 0.5 ml of bacterial suspension with a concentration of 0.5 McFarland was added to each tube and incubated for 24 h. In the next phase, 0.5ml of the tube contents was calculated in appropriate media for bacterial species.

The three groups was compared in terms of inhibition zone diameter and minimum inhibitory concentrations of various species using Kruskal-Wallis non-parametric analysis meanwhile non parametric Mann-whitney U test was used to analyze different variables of mouth rinses in each bacterial species.

RESULTS

Mean diameter of inhibition zone of ANNAS 0.2 %, chlorhexidine 0.2% mouthwash and control group were shown in Table 1.

Results of Kruskal-Wallis test showed significant differences between the three groups in all bacterial species except for *E. faecalis* ($p < 0.374$). (*Streptococcus mutants* ($p<0.012$), *S. salivarious* ($p<0.017$), *S. sanguis* ($p<0.013$), *Streptococcus pyogenes* ($p<0.008$)).

The results of Mann-whitney U test showed no significant differences between ANNAS and Chlorhexidine in terms of the diameter of inhibition zone of studied bacterial species(*S. mutants* ($p=0.114$), *S. salivarious* ($p=0.846$), *S. sanguis* ($p=0.2$), *S. pyogenes* ($p=0.057$), *Enterococcus fecalis* ($p=0.2$)). On the other hand, ANNAS 0.2% and Chlorhexidine 0.2% mouthwashes had relatively equal antimicrobial potency. Results of minimum inhibition concentration test (MIC) were shown in Table 2.

There were significant differences between the three groups in all bacterial strains (*Streptococcus*. *mutants* ($p=0.009$), *S. salivarius* ($p=0.018$), *S. sanguis* ($p=0.007$), *S. Pyogenes* ($p=0.007$), *E. fecalis* ($p=0.014$)).

According to Kruskal-Wallis test, no significant differences were found between ANNAS 0.2% and chlorhexidine 0.2% in terms of minimum inhibitory concentrations of bacterial species (*Streptococcus mutans* ($p =0.686$), *S. Salvarius* ($p=0.1$) ,and *S. Pyogenes* ($p =0.114$)) whereas, differences regarding to *S. sanguis* ($p =0.029$) and *E. faecalis* ($p =0.029$) were significant. The average of minimum inhibitory concentration of ANNAS for these species was more than Chlorhexidine. The average of minimum inhibitory concentration of ANNAS and Chlorhexidine for all species were significantly lower than control group (except for *E. fecalis* in ANNAS ($p=1.0$))

DISCUSSION

The results of this study showed no significant difference in antibacterial effects of Chlorhexidine and ANNAS for selected strains, although IZD of Chlorhexidine mouthwash was slightly more. But mouthwashes were more potent than propylene glycol against bacterial species with the exception of *E. faecalis* in ANNAS. The growth inhibitory effects of Chlorhexidine and ANNAS were relatively the same in *S. mutans, Salivarius* and *S. pyogenes species.*

However, Chlorhexidine acted significantly better on *E. faecalis* than ANNAS. Dental caries in the presence of

Table 1. Antibacterial properties of ANNAS 0.2%, Chlorhexidine 0.2% and control group against some oral pathogens according to the diameter of zone of inhibition.

Mouth rinse	Bacterial species	Mean (mm)	SD	Minimum (mm)	Maximum (mm)
ANNAS	*S. mutans*	12.5	1.29	11	14
	S. salivaris	12.5	2.06	10	15
	S. sanguinis	10.25	0.95	9	11
	S. pyogenes	9.25	0.95	8	10
	E. faecalis	3	0.81	2	4
Chlorhexidine	*S. mutans*	14.5	1.29	13	16
	S. salivaris	13	0.81	12	14
	S. sanguinis	11.5	1.29	10	13
	S. pyogenes	11.25	0.95	10	12
	E. faecalis	1.75	1.89	2	6
Propylene glycol	*S. mutans*	2.25	0.95	1	3
	S. salivaris	0	0	0	0
	S. sanguinis	0	0	0	0
	S. pyogenes	0	0	0	0
	E. faecalis	3	2.16	1	6

Table 2. Minimum inhibitory concentrations (MIC) of ANNAS 0.2%, Chlorhexidine 0.2% and control group.

Mouth rinse	Bacterial species	Mean (mm)	SD	Minimum (mm)	Maximum (mm)	MIC
ANNAS	*S. mutans*	0.004	0.002	0.003	0.007	9/7648
	S. salivaris	0.005	0.002	0.003	0.007	11/7188
	S. sanguinis	0.017	0.009	0.007	0.031	35/1562
	S. pyogenes	0.006	0.001	0.003	0.007	13/6718
	E. faecalis	0.625	0.25	0.5	1	1250
Chlorhexidine	*S. mutans*	0.004	0	0.004	0.004	7/8125
	S. salivaris	0.005	0.002	0.004	0.007	11/7188
	S. sanguinis	0.004	0.001	0.004	0.007	9/7656
	S. pyogenes	0.004	0	0.004	0.004	7/8126
	E. faecalis	0.234	0.009	0.015	0.031	46/875
Propylene glycol	*S. mutans*	0.187	0.072	0.125	0.25	
	S. salivaris	0.375	0.144	0.25	0.5	
	S. sanguinis	0.312	0.125	0.25	0.5	
	S. pyogenes	0.5	0	0.5	0.5	
	E. faecalis	0.625	0.25	0.5	1	

acidogenic bacteria such as Streptococcus mutans rose sharply and this species is the first and most important microorganism present in dental plaque (Sullivan et al., 2011; Slot et al., 2011; Theondor et al., 2006).

Numerous experiments have shown that Chlorhexidine mouthwash, in therapeutic and even lower concentrations is far more efficient than the most common mouthwashs (Jones, 1997).

Rubernes et al. (2004) reported that Chlorhexidine can inhibit bacterial growth. Their results showed that the growth of *S. aureus*, *S. mutans* and *S. sobrinus* was reduced up to 66, 71 and 88%, respectively (Ruberns et al., 2004). The bacteriostatic and bactericidal effect of chlorhexidine results from cationic structure that penetrates the cell membrane and lead to cytoplasmic coagulation (Bebek et al., 2009). Antimicrobial effects of chlorhexidine mouthwash, is largely due to the destruction of cell integrity (Shah et al., 1993; Löe et al.,

1976).

It should be noted that the antibacterial effects of mouthwashes is affected by affinity of salivary proteins to the cationic part of mouth rinses, continuous cleansing action of saliva and short contact of mouthwash with microorganisms. (Schuster, 2002; Babich et al., 2009).In previous studies the minimum inhibitory concentration of Chlorhexidine for streptococci and lactobacilli was reported 0.25 – 8 µgr/ml and the minimum concentration to eliminate actinomysis and *S. surbinus* was l 0.125 – 8 µgr/ml and 0.008 – 0.0002, respectively (Botelho, 2000), (Steinberg and Rothman, 1996).

Low concentrations of Chlorhexidine compared to ANNAS are required to inhibit bacterial growth. Because of some antibacterial properties of ANNAS 0.2% it might be considered as an antimicrobial agent. However, its probable side effects and range of potency on other bacterial species are matters to be elucidated.

Conclusion

ANNAS 0.2% mouthrinse has some antibacterial properties, although its antibacterial efficacy was lower than Chlorhexidine 0.2% with no significant effect on *E. faecalis* species.

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