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EVALUATION OF HERBAL MOUTHWASH AND CHLORHEXIDINE MOUTHWASH ON SALIVARY MICROBIAL COLONIES FOLLOWING EXPOSURE TO ACIDIC BEVERAGE: A COMPARATIVE STUDY

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ABSTRACT **Background:** Acidic beverages are known to alter the oral environment and promote bacterial growth by reducing salivary pH. Mouthwashes are commonly used as adjuncts for maintaining oral hygiene and reducing microbial load. Chlorhexidine mouthwash is considered the gold standard antimicrobial rinse; however, herbal mouthwashes are increasingly being explored as safer alternatives with fewer side effects. **Aim:** To comparatively evaluate the antimicrobial efficacy of herbal mouthwash and chlorhexidine mouthwash on salivary microbial colonies after exposure to an acidic beverage. **Materials and Methods:** A comparative study was conducted among 30 participants aged 18-25 years and divided into two groups Group1(Chlorhexidine mouthwash) and Group 2(Herbal mouthwash) consisting of 15 participants each. Baseline stimulated saliva sample was collected 30-45 minutes after exposure to an acidic beverage then, participants were asked to rinse using the assigned mouthwashes and again immediate saliva sample was collected and smeared on blood agar plates. The agar plates were then incubated and microbial growth was assessed. **Results:** Both mouthwashes demonstrated a reduction in salivary microbial growth following exposure to the acidic beverage. **Conclusion:** Chlorhexidine showed greater anti-microbial efficacy than herbal mouthwash.

KEYWORDS : Chlorhexidine, Herbal Mouthwash, Acidic Beverage, Saliva, Blood Agar, Antimicrobial Efficacy.

INTRODUCTION

The oral cavity contains a diverse microbial flora that plays an important role in maintaining oral health as well as in the initiation and progression of oral diseases.(1) Any alteration in the oral environment may favor the growth of pathogenic microorganisms and increase the risk of dental caries and periodontal diseases. Among various contributing factors, acidic beverages are known to reduce salivary pH and create conditions favorable for enamel demineralization and bacterial proliferation. Carbonated soft drinks such as Coca- Cola® are widely consumed, particularly among young adults, and their acidic nature may adversely affect oral health.(1)Mouthwashes are commonly used as adjuncts to mechanical plaque control for reducing microbial load and maintaining oral hygiene. Chlorhexidine gluconate (CHX) is considered the gold standard antiplaque and antimicrobial mouthwash because of its broadspectrum activity and substantivity.(2) However, long-term use of chlorhexidine has been associated with certain adverse effects including tooth staining, altered taste sensation, and mucosal irritation, limiting its prolonged use.(3)In recent years, herbal mouthwashes have gained popularity due to their natural composition, fewer side effects, costeffectiveness, and antimicrobial properties Herbal formulations containing plant extracts and essential oils have demonstrated inhibitory effects against various oral microorganisms and may serve as suitable alternatives to conventional chemical mouthwashes.(4)Several studies have reported the efficacy of herbal mouthwashes in reducing plaque accumulation and bacterial count.(5)Saliva plays a major role in maintaining oral homeostasis and reflects changes occurring within the oral cavity. Assessment of salivary microbial count using culture media such as blood agar provides a simple and effective method for evaluating the antimicrobial efficacy of oral rinses. Therefore, the present study was undertaken to comparatively evaluate the antimicrobial efficacy of chlorhexidine mouthwash and herbal mouthwash on salivary microbial colonies following exposure to an acidic beverage.

MATERIALS AND METHODS:

Study Population and Sample Size:

The study was conducted on 30 participants aged 18-25 years which included 15 male and 15 female participants. Informed consent of all the participants was taken beforehand.The study was carried out in the Microbiology Lab of the Dental College under sterilised conditions.The participants were divided equally into two groups: Group 1: Chlorhexidine mouthwash Commercially available nonalcoholic 0.12%(Hexidine® 60ml ICPA Health Products Ltd,Mumbai,India) group (n = 15) Group 2: Herbal mouthwash (HiOra[HiOra Mouthwash Regular Manufactured by the Himalaya Drug Company,Bengaluru,India] group (n = 15)

Sampling Method: Participants were selected using a non-probability convenience sampling method.

Inclusion Criteria: Participants aged between 18-25 years who are willing to participate in the study and individuals with apparently healthy oral cavity were included in the study

Exclusion Criteria: Individuals with systemic illness, undergoing antibiotic therapy, active oral infections or lesions and individuals who are allergic to any component of the mouthwash used were excluded from the study.

Data Collection Method:

Fig.1 Armamentarium used for data collection

All participants were exposed to 50ml Coca-Cola® then, stimulated morning saliva samples were collected from each participant after 30-45 minutes.The collected saliva samples were smeared on blood agar plates (Sheep Blood Agar) using sterile swab sticks and swabbing method.Each agar plate was divided into 4 sections.Participants in Group 1 were instructed to rinse with chlorhexidine mouthwash for 30 seconds.Post-rinse saliva samples were collected from Group 1 participants and smeared on blood agar plates.The same procedure was repeated for Group 2 participants after rinsing with herbal mouthwash for 30 seconds.All inoculated blood agar plates were subjected to incubation under appropriate laboratory conditions.Following incubation, microbial growth was assessed and compared between the two groups. Fig.1 shows the armamentarium used for data collection.

Microbiological Assessment:

Blood agar plates were used for microbial culture and evaluation of the growth of the colonies.The antimicrobial efficacy of the mouthwashes was assessed by comparing the microbial colonies observed before and after rinsing with the mouthwash.(Fig.2 and Fig.3)

Statistical Analysis:

The obtained data were tabulated and analyzed using appropriate statistical methods.

Results:

Chlorhexidine showed higher levels of antimicrobial action than herbal mouthwash by reducing the colony forming units(CFU) from 105 to 102 while herbal mouthwash reduced the colony forming units from105 to 104 (Fig.4).Fig.5 shows the comparison between the mouthwashes before and after rinse.A p-value of <0.5 was considered non-significant.

Fig.3 Blood agar plate showing bacterial colonies before and after rinsing with herbal mouthwash.

Fig.2Blood agar plate showing bacterial colonies before and after rinsing with chlorhexidine mouthwash.

Mouthwash	Before rinse	After rinse	p - value
CHX mouthwash	10^5	10^2	0.38
Herbal mouthwash	10^5	10^4	0.41

Fig.4 Comparison of Microbial Colonies Observed Before and After Rinsing with CHX Mouthwash and Herbal Mouthwash.

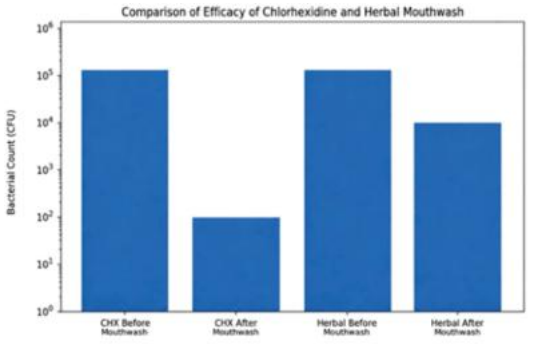

Fig.5 Bacterial count (CFU) before and after rinsing with chlorhexidine and herbal mouthwash.

DISCUSSION

The oral cavity harbors a complex microbial ecosystem that plays a crucial role in both oral and systemic health.(8) Acidic beverages are known to reduce salivary pH and create a favorable environment for the growth of cariogenic microorganisms.Coca-Cola® is one of the world's most highly consumed commercial carbonated beverages(9). It also possesses a high erosive potential.(10,11,12) A number of studies have shown that Coca-Cola® decreased the salivary pH immediately after consumption.(10,13,14,15,16) The pH value of 5.5 was found to be critical for enamel dissolution and caries development in vitro(25) but Coca-Cola® did not produce a decrease in salivary pH below 5.5.(10,13,14) The erosive potential of soft drinks may be reduced by increasing the salivary flow rate,(17,18,19) modification of the ingredients of the beverage(20,21,22), or using various oral care products immediately after the intake of soft drinks.(23,24,25) It has been shown that the use of various oral care products may result in rehardening of the enamel(24) and increase the salivary pH.(23) Maintenance of oral microbial balance after acidic exposure is therefore important in preventing dental caries and periodontal diseases. Mouthwash has been recommended being a regular adjunct along with mechanical therapy to maintain oral health.(6) Among the mouthwashes, CHX is considered to be the gold standard and is part of the periodontal treatment regimen.(7) In this study, both chlorhexidine and herbal mouthwash demonstrated a reduction in salivary microbial colonies after rinsing. However, chlorhexidine mouthwash showed comparatively greater antimicrobial efficacy than herbal mouthwash. The results of the study were similar to the findings of the study conducted by Jones CG et al., who reported that chlorhexidine remains the gold standard antiplaque and antimicrobial mouthwash because of its broad-spectrum antimicrobial activity and prolonged substantivity.(2) The results of the present study are also consistent with the findings of Gupta et al., who who observed a significant reduction in oral microbial count and gingival inflammation following the use of chlorhexidine mouthwash.(3) The study supports the observations made by George et al., who reported that herbal mouthwashes containing natural plant extracts possess antibacterial and anti-inflammatory properties beneficial in maintaining oral hygiene.(4) Similarly, Pathan et al. compared herbal mouthwash and chlorhexidine against periodontal pathogens and concluded that while chlorhexidine showed superior antimicrobial action, herbal mouthwash could serve as a safer alternative for long-term use.(1) An important aspect of the present study was the use of blood agar culture for microbiological assessment. Blood agar provides a simple, economical and reliable method for evaluating salivary microbial colonies and comparing antimicrobial efficacy between oral rinses. The study design also simulated a clinically relevant condition by assessing the effect of mouthwashes following exposure to an acidic beverage, thereby reflecting common dietary habits among young adults. However, the study has certain limitations, only salivary microbial colonies were assessed; specific identification of individual microbial species was not performed, the sample size was relatively small and only immediate antimicrobial effects were assessed. Long-term clinical trials with larger sample sizes and advanced microbial assessment methods are recommended for further validation of results.

CONCLUSION

In the present study, Chlorhexidine mouthwash showed higher levels of antimicrobial action.However, the herbal mouthwash too was effective thereby confirming with the other studies that herbal mouthwash can also be used as an adjunct to oral hygiene practices.

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