A Comparative Evaluation of Anti-Inflammatory and Antiplaque Efficacy of Citrus Sinesis Mouthwash and Chlorhexidine Mouthwash

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ABSTRACT

Background: Citrus sinesis belongs to Rutaceae family is an enriched source of vitamin C, flavonoid compounds and antioxidants are helpful in reducing inflammation. Few in-vitro studies the ethanolic extract of orange peels has successfully reduced periodontal pathogens which has influenced us to prepare a mouth wash with ethanolic extract of orange peel.

Aim: To evaluate and compare the effect of indigenously prepared 4% ethanolic extract of Citrus sinseis (Orange peel) mouthwash to commercially available 0.2% Chlorhexidine mouthwash as an anti-plaque agent.

Materials and Methods: Twenty subjects in the age group of 18-60 years with moderate to severe gingivitis were divided into two equal groups. Clinical parameters like Plaque Index, Sulcus Bleeding Index and Gingival Index were recorded at baseline, 7th and 14th day respectively. Following oral prophylaxis Group-I (n= 10) subjects were instructed to rinse twice daily with 10ml of 0.2% chlorhexidine mouthwash and Group-II (n= 10) subjects were instructed to rinse twice daily with 10ml of 4% ethanolic extract of Citrus sinesis mouthwash for 14 days. All the subjects were recalled on the 7th and 14th day for follow up to record the clinical parameters.

Results: Citrus sinesis 4% mouthwash was seen to be as efficient as 0.2% Chlorhexidine in reducing Plaque Index and more effectively reducing gingival inflammation and gingival bleeding index.

Conclusion: Citrus sinesis 4% mouthwash can be used for short term purpose without any potential side effects as an alternative to 0.2% Chlorhexidine mouthwash in reducing plaque and gingival inflammation.

Keywords: Citrus sinesis; gingival inflammation; mouthwash.

INTRODUCTION

Dental plaque is a biofilm of the oral cavity that adheres to the tooth surface. Plaque contains a variety of bacteria that causes dental decay, contribute to calculus formation and initiates inflammatory responses associated with periodontal disease progression in terms of soft (gingiva, periodontal ligament, connective tissue, junctional epithelium) and hard tissues (cementum, alveolar bone).[1] Progression of periodontal disease results from the mutual interplay of bacteria and host defense reaction.

Citrus sinesis belongs to Rutaceae family and is also known as sweet orange. It is the most commonly grown tree fruit in the world.[2] Vitamin C in oranges is concentrated mainly in the peel and the white layer just under the peel. The peels are the rich source of flavonoid compounds and antioxidants which are helpful in reducing inflammation.[3] Moreover in few in-vitro studies the ethanolic extract of orange peels has successfully reduced oral microbes. Thus we have indegenously prepared a mouthwash made of aqueous solution ethanolic extract of Citrus sinesis peels and conducted a randomised clinical trial.[4]

The aim was to evaluate the effectiveness of indigenously prepared 4% ethanolic extract of *Citrus sinseis* (Orange) peel mouthwash to commercially available 0.2% Chlorhexidine mouthwash as an anti-plaque and anti-inflammatory agent.

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MATERIALS AND METHODS

Preparation of Citrus Sinesis mouthwash:[5] Fresh orange peels were collected and were washed thoroughly under running tap water to remove impurities and dirt from the surface of the peels. They were cut into small pieces and were dried under sunlight for 6 to 7 days in order to remove moisture from the peels. The peels were then crushed and pulverized with a grinder to prepare a fine powder.

A hundred gram of the orange peel powder was soaked in 1000 ml of hydrous concentrated ethanol (95% v/v and with a proof of 190) for 24 hours at room temperature. It was then filtered through a sterilized *Whatman* No.1 filter paper followed by filtering the extract and concentrating under vacuum below 40°C. The anhydrous extract of orange peel thus obtained was exposed to UV rays for 24 hours for sterilization. Subsequently, the orange peel extract was diluted with distilled water making a 4% solution.

Aspartame was added to the solution as sweetening agent for better acceptance amongst the subjects. Sodium benzoate 0.1 gm was added as a preservative. The prepared solution was then dispensed into small bottles of 280 ml capacity each.

Study design: Twenty subjects in the age group of 18-60 years were enrolled from the out-patient department of Periodontics, Pacific Dental College and Research Centre, Udaipur. The study design was submitted to the ethical committee and clearance was obtained subsequently. An informed consent from the subjects participating in the study was obtained before the commencement of the study.

Inclusion Criteria:

- Subjects with chronic generalized gingivitis
- Moderate to severe gingivitis (Gingival Index score of 1 to 2).
- No evidence of radiographic bone loss.
- No clinical attachment loss.
- No history of any systemic disorder.

Exclusion Criteria:

- Patients who have received antibiotics in the past 6 months.
- Inability to comply with the follow-up visit requirements.
- Current smokers were excluded.
- Pregnant and lactating females.
- Allergic to ingredients used in the study.
- Orthodontic treatment or bridge work that would interfere with evaluation.

Group Distribution

The subjects were then divided into two equal groups:

Group-I (n= 10) - Subjects using 0.2% Chlorhexidine gluconate mouthwash (Control Group)

Group-II (n= 10) - Subjects using 4% aqueous solution of ethanolic extract of *Citrus sinesis* peel mouthwash (Test Group).

Clinical parameters recorded

1. Quigley Hein Plaque Index,[6]
2. Gingival Index (Loe and Silness),[7]
3. Sulcus bleeding index (Muhleman and Sons).[8]

All the parameters were recorded at baseline, 7th and 14th day.

All the descriptive data that include mean and standard deviation were determined. The data derived for each group was analyzed by paired and unpaired Student's 't' test. For all tests, a p value of <0.05 was considered significant and p value of <0.001 was considered highly significant.

RESULTS

All 20 patients enrolled in this study reported for the recall schedule for post treatment evaluation. So the response rate to the study was 100%.

Plaque Index (PI):

The mean Plaque Index scores in control group at baseline was 2.97 ± 0.49 which reduced to 2.56 ± 0.46 and 2.26 ± 0.44 at 7 and 14 days postoperatively respectively; which was statistically highly significant (p ≤0.001) when compared to the baseline.

The mean Plaque Index scores in test group at baseline was 3.82 ± 1.32 which reduced to 3.31 ± 1.25 and 2.86 ± 1.19 at 7 and 14 days postoperatively respectively; which were statistically highly significant (p ≤0.001) when compared to the baseline.

When intergroup comparisons were made between the mean Plaque Index scores; there were no statistically significant differences at baseline (p = 0.072) or 7th day (p = 0.095) and 14th day (p = 0.095) postoperatively (Table-1), (Figure-1).

Table 1: Comparison of plaque index.

	Baseline			7 days			14 days		
	Group-I	Group-II	p- value	Group-I	Group-II	p- value	Group-I	Group-II	p- value
Mean	2.97	3.82	0.072 (NS)	2.56	3.31	0.095 (NS)	2.26	2.86	0.095 (NS)
Standard Deviation	0.49	1.32		0.46	1.25		0.44	1.19	
p-value from baseline				<0.001 (HS)	<0.001 (HS)		<0.001 (HS)	<0.001 (HS)	

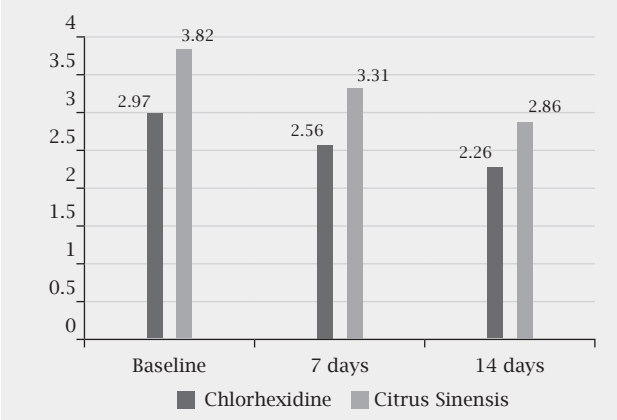

Figure 1: Plaque index.

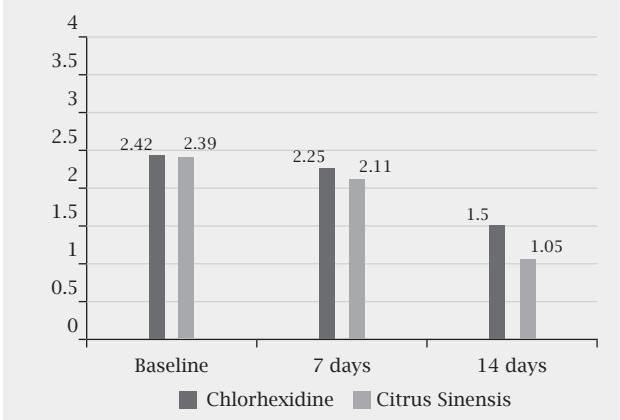

Figure 2: Gingival index.

Table 2: Comparison of gingival index.

	Baseline			7 days			14 days		
	Group-I	Group-II	p- value	Group-I	Group-II	p- value	Group-I	Group-II	p- value
Mean	2.42	2.39	0.072 (NS)	2.25	2.11	0.299 (NS)	1.75	1.05	0.04 (S)
Standard Deviation	0.39	0.22		0.31	0.27		0.21	0.23	
p-value from baseline				0.08 (NS)	<0.001 (HS)		<0.001 (HS)	<0.001 (HS)	

Gingival index (GI):

The mean Gingival Index scores in control group at baseline was 2.42 ± 0.39 which reduced to 2.25 ± 0.31 at 7 day revaluation which seemed to be statistically non-significant; however the score further reduced to 1.75 ± 0.21 at 14th days postoperatively; which seemed to be statistically highly significant (p ≤0.001) when compared to the baseline.

The mean Gingival Index scores in test group at baseline was 2.39 ± 0.22 which reduced to 2.11 ± 0.27 and 1.05 ± 0.23 at 7 and 14 days postoperatively; which were statistically highly significant (p ≤0.001) when compared to the baseline.

When intergroup comparisons were made between the mean Gingival Index scores; there were no statistically significant differences at baseline (p = 0.072) or 7th day (p = 0.299). However, there seemed to statistically significant difference at 14th day postoperatively in favour of test group (p = 0.04) (Table 2, Figure 2).

Sulcus bleeding index (SBI)

The mean Sulcus bleeding Index score of control group at baseline was 3.04 ± 0.43 which reduced to 2.64 ± 0.38 at 7th re-evaluation day, which was statistically non-significant (p = 0.095) and further reduced to 2.54 ± 0.30 at 14th day re-evaluation, which seemed to be statistically highly significant (p ≤0.001) when compared to the baseline.

The mean Gingival Index scores of the test group at baseline was 3.28 ± 0.38 which reduced to 2.71 ± 0.19 and 1.31 ± 0.16 at 7th and 14th days postoperatively which were statistically highly significant (p ≤0.001) when compared to the baseline.

When intergroup comparisons were made between the mean Sulcus Bleeding Index scores; there were no statistically significant differences seen at baseline (p = 0.16) or 7th day (p = 0.595) and at 14th day (p = 0.42) (Table 3, Figure 3).

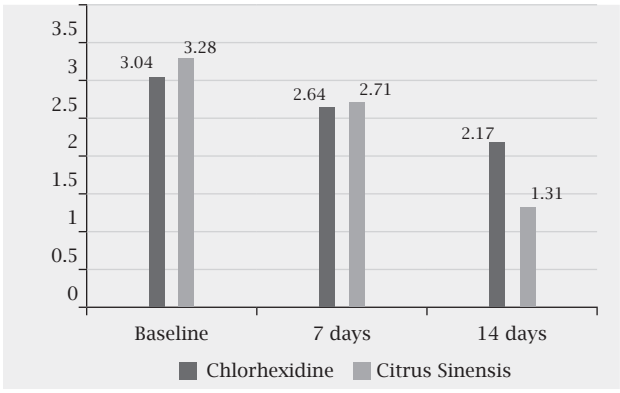

Figure 3: Sulcus bleeding index.

Table 3: Comparison of sulcus bleeding index.

	Baseline			7 days			14 days		
	Group-I	Group-II	p- value	Group-I	Group-II	p- value	Group-I	Group-II	p- value
Mean	3.04	3.28	0.16 (NS)	2.64	2.71	0.595 (NS)	2.54	1.31	0.042 (S)
Standard Deviation	0.43	0.29		0.38	0.19		0.30	0.16	
p-value from baseline				<0.095 (NS)	<0.001 (HS)		<0.001 (HS)	<0.001 (HS)	

DISCUSSION

The aim of the present study was to evaluate the effectiveness of indigenously prepared 4% aqueous solution of ethanolic extract of orange peel mouthwash for dental plaque reduction and anti-inflammatory activity and compare it with the 0.2% Chlorhexidine mouthwash which has been considered as gold standard.

Fruits are known to be an integral part of diet. Besides their delicious taste and flavor, they are known to reduce the risk of several chronic disease including cancer. The protective nature of orange is due to presence of phytoconstituents such as poly phenolic compounds, antioxidants, vitamin-C, essential oils etc. Flavonoids are polyphenolic plant secondary metabolites ubiquitous in foods of plant origin (Havsteen, 1983). They occur naturally as glycosides and consist of flavones, flavonols, flavanones and isoflavones.[9] Hesperidin and naringin are the main flavanone glycosides naturally occurring in citrus fruits. They exert interesting pharmacological properties such as antioxidant and anti-inflammatory action in humans.[10] Microbiological study has revealed that ethanolic extract of citrus sinesis has good potential in reducing oral microbiota.[11] Miyake and Hiramitsu had extracted four antimicrobial compounds from orange peel and those were subjected to Porphyromonas gingivalis and other microbes. The key compounds have been found to be active against oral pathogens.[12] Based on these facts, this clinical trial has been attempted. As there is no previous study to measure the clinical efficacy of this newly introduced product, it was found important to measure the efficacy of this product and compare it with the gold standard.

The present study has demonstrated almost similar anti-plaque activity in both the groups as statistically significant difference has been found from baseline, 7th and 14th day re-evaluation period. 8-geranyloxypsolaren, 5-geranyloxypsolaren, 5-geranyloxy-7-methoxycoumarin and phlorin may be responsible for anti-bacterial efficacy. This was in accordance with an in-vitro study where the authors had stated that *Prevotella intermedia* and *Porphyromonas gingivalis* were resistant to aqueous extracts while *Aggregatibacter actinomycetemcomitans* was inhibited at very high concentrations. Ethanolic extracts showed significantly higher zone of inhibition than cold ethanolic extract.[13]

Considering gingival index and Sulcus bleeding index at baseline the scores were non-significant when inter-group comparison was done. Both the Group-I and Group-II has shown statistically significant reduction of scores up to 7th day re-evaluation period. But at 14th day Group-II has showed more reduction of both scores than group-I indicating greater anti-inflammatory activity than 0.2% chlorhexidine mouthwash. The flavonoid compounds and phenolic group compounds may be responsible for anti-inflammatory activity. Moreover, citrus sinesis also contains vitamin-C which can be effective to prevent scurvy.

Lang stated that the substantivity of an antimicrobial agent needs sufficient gingivitis and hence forth reduces the gingival inflammation.[14] Chlorhexidine with a substantivity of 8-12 hours is considered highly effective, whereas the substantivity of orange peel mouth-wash is not yet known, which has been found to be a drawback of this study.

Chlorhexidine is considered as a gold standard in mouthwashes. There are a number of advantages inherent with the molecule. Jenkin et al have stated that chlorhexidine has immediate bactericidal action on plaque bacteria and plaque fungi and is among the most effective active agents to reduce and inhibit plaque accumulation. It is able to kill both gram-positive and gram-negative microbes. This could be due to the mechanism of action of chlorhexidine on bacteria, which involves the disruption of bacterial cell membrane.[15]

The alcohol-based mouthwashes has shown better anti-inflammatory activity compared non-ethanol based mouthwashes concluding an anti-gingivitis effect of alcohol.[16] Thus it is suggestive of further studies required to compare between the anti-inflammatory effects of Citrus sinesis and alcohol.

CONCLUSION

From the present study, it can be concluded that 0.2% chlorhexidine is better than Citrus sinesis mouthwash in terms of antibacterial and antiplaque activity, but *Citrus sinesis* mouthwash has shown better anti-inflammatory activity and better acceptability among the subjects owing to its natural herbal ingredient use and better taste. As far as substantivity is concerned, further randomized double blinded clinical trials with long term follow-up and more in-vitro studies are required to investigate. However, the subjects who are allergic to chlorhexidine, can be prescribed this new product as an adjunct to scaling and root-planing.

REFERENCES

1. Ximénez-Fyvie LA, Haffajee AD, Socransky SS. Comparison of microbiota of supra- and subgingival plaque in subjects in health and periodontitis. J Clin Periodontol. 2000;27:648-57.
2. Favela-Hernández JM, González-Santiago O, Ramírez-Cabrera MA, Esquivel-Ferriño PC, Camacho-Corona Mdel R. Chemistry and Pharmacology of Citrus sinensis. Molecules. 2016;21(2):247.
3. Anastasssiadis S, Morgunov IG, Kamzolova SV, Finogenova TV. Citric acid production patent review Recent Pat Biotechnol. 2008;2(2):107-23.
4. Monforte MT, Trovato A, Kirjavainen S, Forestieri AM, Galati EM, Lo Curto RB. Biological effects of hesperidin, a citrus flavonoid. (note II): hypolipidemic activity on experimental hypercholesterolemia in rat. Farmaco. 1995;50:595-9.
5. Mehmood B, Dar KK, Ali S, Awan UA, Nayyer AQ, Ghous T. Short communication: in vitro assessment of antioxidant, antibacterial and phytochemical analysis of peel of Citrus sinensis. Pak J Pharm Sci. 2015;28:231-9.
6. Quigley GA, Hein JW. Comparative cleansing efficiency of manual and power brushing. J Am Dent Assoc. 1962;65:26–9.
7. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand. 1963 Dec;21:533-1.
8. Muhleman HR, Son S. Gingival sulcus bleeding- a leading symptom in initial gingivitis. Helv Odontol Acta. 1971;33:107-13.
9. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20(7):933-56.
10. Pietta PG. Flavonoids as antioxidants. J Nat Prod. 2000;63(7):1035-42.
11. Khushwaha A, Singh RP, Gupta V, Singh M. Antimicrobial properties of peels of citrus fruits. Int J Univ Pharma Life Sc. 2012;2(2):24-48
12. Miyake Y, Hiramitsu M. Isolation and extraction of antimicrobial substances against oral bacteria from lemon peel. J Food Sci Technol. 2011;48(5):635-9
13. Hussain KA et al. Antimicrobial effects of citrus sinensis peel extracts against periodontopathic bacteria: an in vitro study. Rocz Panstw Zakl Hig 2015,66(2):173-8.
14. Lang NP. Chlorhexidine digluconate–an agent for chemical plaque control and prevention of gingival inflammation. J Periodontal Res. 1986;21(S16):74-89.
15. Jenkin S, Addy M, Wade W. The mechanism of action of chlorhexidine. A study of plaque growth on enamel inserts in vivo. J Clin Periodontol. 1988;15(7):415-24.
16. Eaton KA et al. The effects of a 0.12% chlorhexidine-digluconate-containing mouthrinse versus a placebo on plaque and gingival inflammation over a 3-month period. A multicentre study carried out in general dental practices. J Clin Periodontol. 1997;24(3):189-97.