



Research Article

EVALUATION OF NANOCURCUMIN MOUTHWASH AS AN ADJUNCTIVE PLAQUE CONTROL AGENT: A CLINICAL PILOT STUDY

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ABSTRACT

The use of adjunctive plaque control methods such as mouthwashes has shown to be effective in prevention of plaque accumulation. The gold standard for mouthwashes is 0.2% chlorhexidine, however, various side effects compels researchers to divert towards herbal alternatives. Curcumin has been enhanced with nanotechnology to enhance its efficacy and water solubility. The aim of study was to compare the efficacy of 0.1% nanocurcumin mouthwash to 0.2% chlorhexidine gluconate as an adjunct to scaling and root planning in localized chronic periodontitis patient. In this randomized controlled clinical study, a total of 30 patients having localized mild to moderate periodontitis were included. The study population was divided into 2 groups by randomization protocol; Group A (n=15) was given 0.1% nanocurcumin mouthwash whereas Group B (n=15) was given 0.2% CHX mouthwash. Clinical parameters including Plaque index (PI), Modified Gingival Index (mGI) being recorded at baseline, 30th day and 45th day and Sulcular Bleeding Index (SBI), Periodontal Probing Depths (PPD), Clinical attachment levels (CAL) were recorded at baseline and 45th day. Subjective criteria included taste acceptability, burning sensation and dryness whereas objective criteria including ulcer formation, tongue and teeth staining were analyzed. Statistically significant improvement was observed in all clinical parameters when compared to baseline in both groups and difference was statistically non-significant on intergroup comparison. No adverse reaction was observed in both groups in terms of subjective and objective criteria. Within the limitations, it can be concluded that nanocurcumin can be a viable alternative to chlorhexidine to formulate a mouthwash.

INTRODUCTION

Periodontal disease is one of the most common diseases caused by the complex interplay between pathogenic microorganisms and host immune systems. Plaque removal by mechanical methods such as tooth brushing is effective however; they are dependent on personal skills. The use of adjunctive methods such as mouthwashes has shown to be effective in prevention of plaque accumulation.

The existing gold standard for mouthwashes is chlorhexidine, a bisbiguanide with pronounced antibacterial property. But many important adverse effects like dental staining, taste alteration, unpleasant taste and mucosal erosion by chlorhexidine has motivated the development of other antimicrobial agents.^[1]

Herbal alternatives have gained renewed importance in this modern era, for their antimicrobial properties, fewer side effects, better patient tolerance, renewable nature, economic and healing potential. Curcumin, also known as diferuloylmethane, is a yellow polyphenol derived from the rhizome of the tropical Southeast Asian plant turmeric (*Curcuma longa*). For centuries, turmeric has been used as a spice and coloring agent in Indian food, as well as a therapeutic agent in traditional Indian medicine.^[2] It possess anti-

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inflammatory, antioxidant, anti-carcinogenic, antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, hypotensive and hypercholesteraemic activities.^[3] The acceptable dose of curcumin is 12grams/day, with no significant toxicity and adverse reactions.^[4] Despite the fact that it is a very promising molecule, its poor water solubility and rapid degradation profile make it compromise over its bioavailability way below the threshold level on administration. The development of nano-range formulations of curcumin, also known as "nanocurcumin", has received a lot of attention in recent years.^[5] Researchers are trying to deliver the drug to the targeted tissue, release the drug at a controlled rate, form a biodegradable drug delivery system with minimum side effects by utilizing nanotechnology^[6]. Nanocurcumin's improved therapeutic potential is a promising tool for combating microbes and it will lead to a new age in modern biomedical applications.

To the best of our knowledge, till date no study has been conducted to assess the efficacy of 0.1% nanocurcumin as a mouthwash. Therefore, the aim of the study was to compare the efficacy of 0.1% nanocurcumin mouthwash with 0.2% chlorhexidine gluconate as an adjunct to scaling and root planning in the management of localized chronic periodontitis.

METHODOLOGY

The study was designed as a randomized controlled clinical study. It was approved by the Institutional ethical and review board and carried out in accordance with the Helsinki Declaration of 1975, revised in 2000. (BDC/Exam/165/2015-16). A total of 30 patients in the age range of 18-45 years were chosen from the outpatient department, 15 from each group, who were systemically healthy and had mild to moderate localized chronic periodontitis. Complete

medical and dental history was taken to ensure that the patients were in good general health and could maintain oral hygiene. The patients were divided into 2 groups by using coin toss randomization protocol; Group A received a nanocurcumin mouthwash while Group B received chlorhexidine mouthwash after completion of phase I therapy. Subjective parameters such as taste acceptability, burning sensation and dryness/soreness were assessed. Objective criteria such as ulcer formation tongue and teeth staining were analyzed.

Preparation of nanocurcumin mouthwash

A pilot study was performed to assess the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of various concentration of nanocurcumin against common periodontopathogens including *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans* *Streptococcus mutans* and *candida* species. At a concentration of 100µg/ml all the periodonto pathogens demonstrated sensitivity to nanocurcumin. Based on the values obtained, 0.1% concentration was taken for mouthwash preparation. Nanocurcumin of required amount was weighed and dissolved in alcohol. In a separate vessel, sodium saccharine, sodium benzoate, peppermint oil, menthol and glycerine were mixed. Once all the contents were mixed well, nanocurcumin dissolved in alcohol was added in the mixture. Distilled water was added to make the quantity sufficient. The whole solution was then kept under an agitator for 15 minutes for efficacious mixing of all the contents. The prepared nanocurcumin mouthwash was measured and required amount was dispensed into the bottles to be delivered to the patient. (Figure 1a &1b)

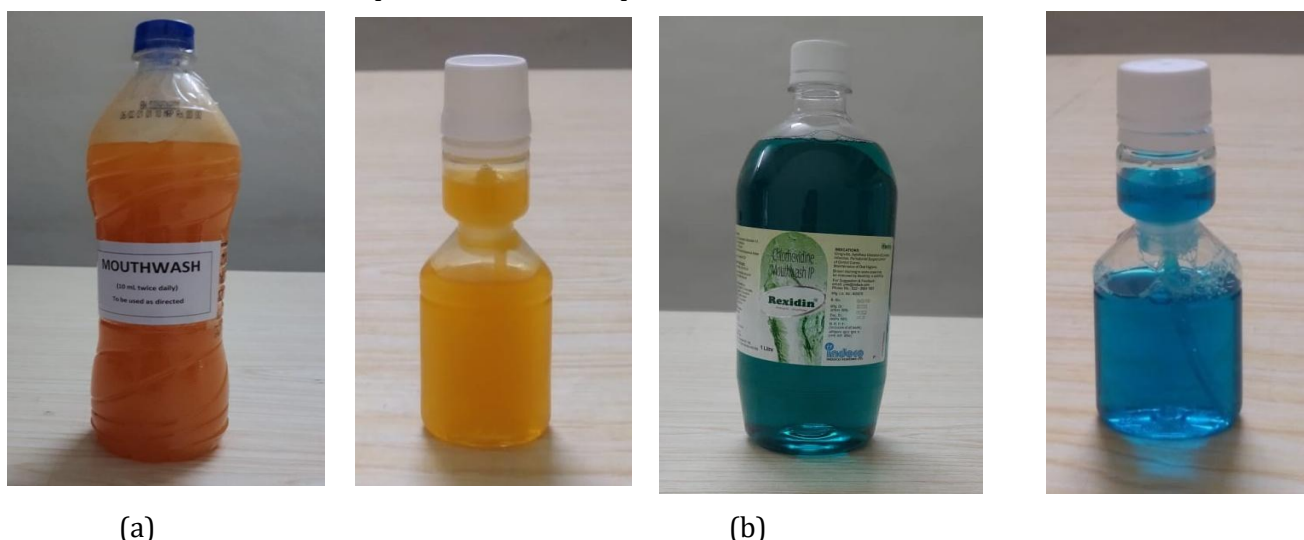


Figure 1: The required amount was measured
(a) Final packaging and dispensing of 0.1% nanocurcumin mouthwash
(b) Final packaging and dispensing of 0.2% chlorhexidine mouthwash

At baseline, all the patients underwent thorough ultrasonic scaling & polishing (Cavtron Bobcat® India). Root planing was accomplished with either 4R-4L, 2R-2L Columbia, universal curettes (Hu-Friedy). Sub gingival irrigation was done with normal saline to remove the debris/dislodged remnants of calculus from the sulcus. Thoroughness of the root planing was assessed with an explorer. After thorough scaling and root planning, patients were advised in both the groups to rinse with 10ml of mouth rinse twice daily; once in morning 30 minutes after the brushing and once at night before retiring for 30 seconds. Patients were advised to refrain from eating, drinking and rinsing 30 minutes after the use of mouthwash. Patients were evaluated at baseline 30th and 45th day in both group A and group B. Clinical parameters including Plaque Index (PI) and Modified Gingival Index (mGI) were evaluated at baseline, (1st visit), 30th day (2nd visit) and 45th day (3rd visit) while Sulcular bleeding Index (SBI), Periodontal Pocket depth (PPD) and Clinical attachment level (CAL) were evaluated only at baseline (1st visit) and 45th day (3rd visit). Subjective parameters such as taste acceptability, burning sensation and dryness/soreness were assessed. Objective criteria such as ulcer formation, tongue and teeth staining were also analyzed. (Figure 2a & 2b, 3a & 3b)

Figure 2a: Measurement of clinical parameters at baseline in Group A



Figure 2b: Measurement of clinical parameters at 45 days in Group A

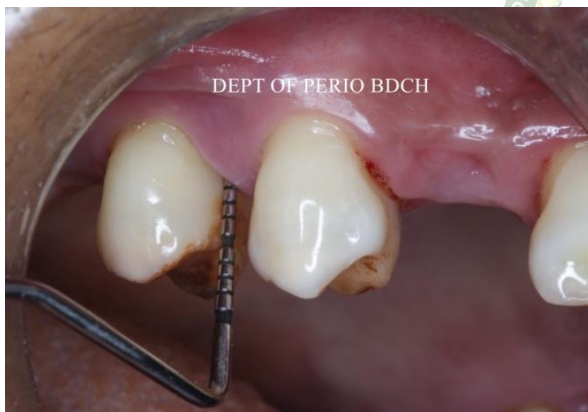


Figure 3a: Measurement of clinical parameters at baseline in Group B

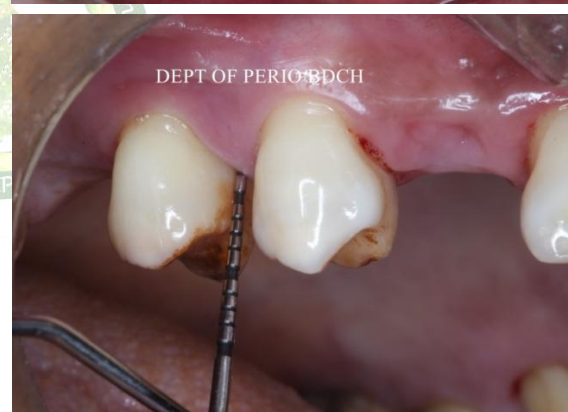


Figure 3b: Measurement of clinical parameters at 45 days in Group B

RESULTS

All the patients fulfilled the protocol and completed the follow-up period. The data obtained from clinical evaluation were presented as mean ± standard deviation and subjected to statistical evaluation.

The baseline values of Plaque index (PI) were comparable for group A and group B and no statistical significant difference was observed between the two groups ($p > 0.05$) whereas at 45 days follow-up, a statistically significant improvement in PI was observed in both group A and group B as compared to baseline. ($p < 0.05$) When the difference was compared between the 2 groups it was found to be statistically non-significant ($p > 0.05$).

Table 1: Inter and Intragroup Comparison of Plaque Index (PI) at Various Time Intervals

Parameter- Plaque Index (PI)	N	A	B
Baseline	15	1.42 (0.14)	1.39 (0.16)
30 days	15	0.81 (0.32)*	0.94 (0.16)*
45 days	15	0.54 (0.30)*	0.57 (0.22)*

*Statistically significant as compared to baseline on intragroup comparison

Similarly, the baseline values of the modified gingival index (mGI) for groups A and B were comparable, and at 30 and 45 days follow-up, both groups showed a statistically significant improvement over baseline ($p < 0.05$). However, when compared between the 2 groups this difference was statistically non-significant ($p > 0.05$) (Table 2)

Table 2: Inter and intragroup comparison of Modified gingival index (mGI) at various time intervals

Parameter- Modified gingival index (mGI)	N	A	B
Baseline	15	1.70 (0.30)	1.64 (0.30)
30 days	15	0.96 (0.16)	0.95 (0.18)
45 days	15	0.75(0.20)	0.82 (0.17)

*Statistically significant as compared to baseline on intragroup comparison

When the baseline values of sulcular bleeding index (SBI) were compared between group A and group B, no statistically significant difference was found. ($p > 0.05$) At 30 days and 45 days follow-up, a statistically significant improvement in SBI was observed for both the groups when compared at baseline, however, the difference between the two groups was found to be statistically non-significant. ($p > 0.05$). (Table 3)

Table 3: Inter and Intragroup Comparison of Sulcular Bleeding Index (SBI) at Various Time Intervals

Parameter- Sulcular bleeding index (SBI)	N	Group A	Group B
Baseline	15	2.07(0.80)	2.00(0.76)
45 days	15	0.27(0.46)*	0.20(0.41)*

*Statistically significant as compared to baseline on intragroup comparison

For Group A, mean PPD at baseline was 6.00 ± 0.54 which was reduced to 4.40 ± 0.74 at 45th day follow-up and this difference was statistically significant ($p < 0.05$) (Figure 2a & 2b) whereas for Group B, mean PPD at baseline was 5.93 ± 0.46 which was reduced to 4.4 ± 0.83 at 45th day follow-up (Figure 3a & 3b) and this difference was statistically significant ($p < 0.05$). When the mean PPD of the two groups was compared at various time intervals, it was shown that there was no statistically significant difference between them. ($p > 0.05$). (Table 4)

Table 4: Inter and Intragroup Comparison of Periodontal Probing Depth (PPD) at Various Time Intervals

Parameter- Periodontal Probing Depth	N	Group A	Group B
Baseline	15	6.00 (0.54)	5.93 (0.46)
45 days	15	4.40 (0.74)*	4.40 (0.83)*

*Statistically significant as compared to baseline on intragroup comparison

The clinical attachment level for Group A, changed from 6.27 ± 0.59 at baseline to 4.60 ± 0.83 at 45th day and this value was statistically significant ($p < 0.05$). For Group B, the clinical attachment level improved from 6.07 ± 0.88 to 4.40 ± 0.74 at 45th day and this value was also statistically significant when compared to baseline ($p < 0.05$). However, when the mean PPD at various time intervals was compared between the two groups, it was observed that there was no statistically significant difference between group A and group B. ($p > 0.05$) (Table 5)

Table 5: Inter and Intragroup Comparison of Clinical Attachment Level (CAL) at Various Time Intervals

Parameter- Clinical attachment level	N	Group A	Group B
Baseline	15	6.27(0.59)	6.07(0.88)
45 days	15	4.60 (0.83)*	4.40(0.74)*

*Statistically significant as compared to baseline on intragroup comparison

Subjective Criteria

There were no complaints of burning or dry mouth among the patients in either group. Also, the taste was acceptable in both the groups except for 1 patient in each group A and B at 45 days who reported the taste to be tolerable; however the finding was statistically non significant. ($p > 0.05$) (Table 6)

Table 6: Intergroup comparison of subjective criteria at 45 days

Group	Burning sensation		Dryness/Soreness		Taste acceptability		
	0-Absent	1-Present	0-Absent	1-Present	0-Acceptable	1-Tolerable	2-Unacceptable
A(N=15)	15	0	15	0	14	1	0
B(N=15)	15	0	15	0	14	1	0

Objective Criteria

None of the patient reported any history of ulcer formation in both the groups. Also, no ulcers were seen at the 30th and 45th day follow-up visits, and no tongue or teeth discoloration was observed in either group. (Table 7)

Table 7: Intergroup comparison of objective criteria at 45 days

Group	Ulcer formation		Staining of teeth		Staining of tongue	
	0-Absent	1-present	0-Absent	1-Present	0-Absent	1-Present
A (N=15)	15	0	15	0	15	0
B (N=15)	15	0	15	0	15	0

DISCUSSION

Periodontitis is a multifactorial disease. Researches over the last few decades have established that most periodontal diseases are infections instigated by microorganisms, chiefly bacteria. The inflammatory responses which they provoke in the gingival tissue is responsible for progressive damage of collagen attachment of the tooth to the underlying alveolar bone, which, if unimpeded, can ultimately result in tooth mobility and culminate in the grim fate of tooth loss.^[8]

Meticulous mechanical and surgical treatment approaches can possibly arrest the progress of periodontal attachment loss in most individuals to a certain extent.^[9] The use of chemical products should be adjunctive to that of the mechanical devices as it reduces the amount of biofilm formation and also disrupt its structure which allows a more effective action of the chemical agent.

CHX is the most widely evaluated and the most efficacious agent against oral biofilms. CHX is most often formulated in mouth rinses with a concentration of 0.1–0.2%. These concentrations achieve the ideal CHX dosage of 18–20mg/application. CHX has shown to be active against gram-positive and gram-negative bacteria, yeast, and viruses. In addition to its antimicrobial effect, CHX molecules adhere to the tooth surface and interfere with bacterial adhesion. CHX also interacts with salivary glycoproteins, thus leading to reduced salivary pellicle formation. Its molecules bind reversibly to oral tissues, with a slow release, a property known as substantivity. Although CHX considered to be the gold standard, certain side effects on prolonged use such as staining, hypersensitivity reactions, delayed wound healing etc compels the researchers to look for more natural and herbal alternatives.^[10]

Among the herbal alternatives available and tested, curcumin drew researchers attention which possess high medicinal value and has been identified to boost immunity, metabolic functions and abridging inflammation in several formulations. Its efficacy has been tested against number of microorganisms namely *P. gingivalis*, *Fusobacterium nucleatum*, and *Treponema denticola* and the bacterial growth was suppressed almost completely at very low concentrations of curcumin.^[11]

Despite showing such promising medicinal properties, curcumin's application as an effective therapeutic agent is still limited. This can be attributed

to its poor pharmacodynamic action *in vivo* because of poor aqueous solubility, poor absorption in gastrointestinal tract and rapid metabolism along with rapid systemic elimination. Also, curcumin, being lipophilic in nature, is highly vulnerable to reticuloendothelial system (RES) uptake and thus does not reach up to the therapeutic threshold causing a low systemic bioavailability. A high dose of curcumin with repeated administration is needed to maintain the required therapeutic concentration.^[3]

More recently, implementation of nanotherapeutics led to the formulation of curcumin loaded nanoparticles or “nanocurcumin” to increase its biodistribution. This conversion significantly strengthens its pharmacological effects such as better solubilization, superior biocompatibility, enhanced pharmacokinetic profile and controlled drug release.^[11] Nanoencapsulation of curcumin may thus allow improved drug circulation and retention in the body. This not only causes the reduction in the dosage but also maintains the threshold level of curcumin and thus transforms its image from a nutritional spice to a clinical medicine. It has been previously used for many applications including cancers, leukemia's, various tumors, HIV, atherosclerosis and thrombosis etc to name a few.^{[12],[13]}

Hence, the impetus for the current study was the lack of scientific literature regarding the antimicrobial efficacy of nanocurcumin as a mouthwash in the management of localized periodontitis patients and its comparison to the current gold standard i.e., chlorhexidine mouthwash.

Plaque is considered to be the prime etiologic agent in the causation of periodontal disease. Baseline values for plaque index in both the groups were comparable and no statistically significant difference was found. The improvement in the plaque indices can be attributed to its antimicrobial effect. This was shown in an in-vitro study by Gera & colleagues where nanocurcumin showed >99% antimicrobial activity and higher zone of inhibition against pathogens.^[14] Nanocurcumin being water soluble has greater ability to penetrate the bacterial cell wall and causes bacterial cell lysis as demonstrated by Gopal & colleagues.^[15] Our findings are also in accordance with Negahdari & colleagues where they evaluated the anti bacterial efficacy of nanocurcumin on implant fixtures and showed that the inhibitory rate of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and

Enterococcus faecalis in nanocurcumin group was above 99%.^[16] The reduction in PI in both the groups at the end of 30 days could also be attributed to the reinforcement and motivation of oral hygiene measures by the clinician and maintenance by the patient or it could also be due to the Hawthorne effect. A similar improvement in modified gingival index was also observed at 30th and 45th day follow up in both Group A and Group B. In group A, this could be attributed to the anti inflammatory and anti oxidant properties of nanocurcumin in resolving inflammation at an early stage. It acts similarly to aspirin and aspirin like agents in diminishing anti-inflammatory mediators of arachidonic acid metabolism. Our findings are in accordance with the study by Naganuri and colleagues where they compared the effects of 0.1% turmeric mouthwash to that of 0.2% CHX mouthwash.^[17]

The clinical assessment of gingival colour, form and texture is subjective in nature whereas gingival bleeding is objective diagnostic sign of gingival inflammation and this may be observed and detected before change in colour, form or texture are manifested. A statistically significant improvement in mSBI was observed in both the groups which can be attributed to the reduction in inflammatory component and ulceration of the gingiva which in turn decrease its tendency to bleed. Nanocurcumin has been demonstrated to block prostaglandin E2 generation by inhibiting the microsomal prostaglandin E2 synthase-1 enzyme, lowering vascular permeability and improving clinical signs such as gingival bleeding. Similar results were obtained in a study by Zambarano et al., who found that the local administration of curcumin loaded nanoparticles in experimentally induced periodontitis in rats showed anti inflammatory and anti resorptive properties.^[18]

On intra group comparison, a highly significant reduction in PD and CAL was observed in both the group A & B ($p < 0.05$). However, on inter group comparison, the differences in mean PD and CAL were found to be statistically non-significant. This might be attributed to anti inflammatory and anti bacterial properties of nanocurcumin by promoting migration of fibroblast and an increase in fibronectin and transforming growth factor β transcription.^[19] Also, the anti-bacterial and anti inflammatory nature of nanocurcumin halts the further progression of periodontal pocket and also helps the pocket lining to convert into normal epithelial lining and thus reducing the depth of the pocket. Similar findings were found in the study by Zambrano & colleagues where significant improvement in periodontal probing was observed when chlorhexidine and curcumin gel applications were compared.^[20] The results of this study were also in accordance with the observations made in a similar

study by Singh et al where they have compared turmeric chip with chlorhexidine chip as a local drug delivery agent for the improvement of clinical indices such as PI, GI, PPD in the treatment of chronic periodontitis and found a significant deterioration in all the clinical parameters at 1 month follow-up in both the groups.^[21]

When the subjective criteria were assessed using taste acceptability, burning sensation and presence of dryness/soreness, nanocurcumin mouthwash was found to be generally acceptable by all the patients. However, 1 patient in naocurcumin group and 1 patient in chlorhexidine group found the taste tolerable. The alteration in taste perception might be attributed to the presence of alcohol which was added in the nanocurcumin mouthwash. Objective criteria were assessed by the presence of ulcer formation and the staining of teeth and tongue. None of the patient in either group reported any objective symptom. However, our findings are in contradiction with the study by Naganuri and colleagues where they reported transient staining of tongue in turmeric mouthwash group.^[17] This could be attributed to the enhanced water solubility and nano-sized particles of nanocurcumin form as compared to curcumin which increases its clearance from the intraoral hard and soft tissues and thus reduces the chances of staining.

In the present study the overall improvement in clinical parameters in nanocucumin and chlorhexidine group was significant individually and on comparison between both the groups the difference was not statistically significant. These can be attributed to the anti inflammatory, anti bacterial and improved wound healing property of nanocurcumin. The medicinal property of curcumin was known since long but incorporation of nanotechnology to obtain an improved version of curcumin particles enhanced its therapeutic efficacy. Due to the nano sized particle, it is easier to penetrate deeper into the tissues and overcome the major drawback of curcumin which is its lower water solubility. In the present study when 0.1% nanocurcumin mouthwash was compared with gold standard 0.2% chlorhexidine mouthwash as an adjunct to SRP in periodontitis cases, the results were comparable at 45 days follow-up. Also no staining of teeth and tongue was reported which was seen in previous studies when curcumin was used. These findings indicate that nanocurcumin mouthrinse could be an effective and viable alternative to that of chlorhexidine as an adjunct to non surgical management of localized chronic periodontitis.

CONCLUSION

Within the limitations of the study, it can be concluded that nanocurcumin can be used to formulate a mouthwash with efficacy similar to current gold standard to chlorhexidine mouthwash as an adjunct to

SRP in the management of localized chronic periodontitis.

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