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Comparative Evaluation of Oral Rinsing with 01 Curcumin and 02 Chlorhexidine Mouthwash as an Adjunctive to Scaling and Root Planning in the Treatment of Moderate Periodontitis

Kishore Kumar Katuri[1], Juhee Keerthana Alluri[2], Susmitha Karyamsetti[3], Appaiah Chowdary Bollepalli[4]

[1]Professor, Department of Periodontics, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India

[2]Department of Periodontics, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India

[3]Department of Periodontics, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India

[4]Professor, Department of Periodontics, SIBAR Institute of Dental Sciences, Guntur, Andhra Pradesh, India

[1]Corresponding Author E-mail: *kishoreuga[at]yahoo.co.in*

Abstract: ***Aim: The present study was done to compare 0.1 % curcumin mouthwash with 0.2% chlorhexidine gluconate mouthwash as an adjunctive to scaling and root planing in the treatment of moderate periodontal pockets. Material & Methods: A total of 66 patients with generalized chronic periodontitis were divided into 3 groups. Group I= SRP+ 0.2% chlorhexidine mouthwash, Group II= SRP + 0.1% curcumin mouthwash, Group III= SRP + placebo 0.9% sodium chloride solution. Plaque Index (PI), pocket depth (PD) and clinical attachment level (CAL), and microbial load per sample is estimated by determining the Colony Forming Units (CFU/ml). All the parameters were recorded before (baseline) and after scaling and root planning (SRP), at 1 month and 3 months intervals. The obtained data were analyzed with ANOVA and Bonferroni post hoc tests. Results: PI and BOP scores showed significant (p<0.05) decrease from baseline to 1 month and 3 months in all the three groups except in Group III, where no significant difference was found from 1month to 3 months. Intergroup comparison at 3 months between Group I and Group II with Group III showed a statistically significant difference (P<0.001). PD and CAL showed a significant difference (P<0.001) in all three groups from baseline to three months with no significant difference between them at 3 months post-operative. The differences in CFU values from baseline to three months (P<0.001) were significant in all three groups with no significant difference was observed in Group III at 3 months post-operative. Conclusion: Results observed in this study suggest that 0.1% CMN can be used as an adjunctive antimicrobial agent along with SRP in the treatment of moderate periodontal pockets.***

Keywords: Chemical plaque control, Periodontal pockets, Randomized trial, Scaling and Rootplaning.

1. Introduction

Dental plaque is considered as the primary etiological factor for the cause of periodontal disease, resulting in the destruction of periodontal fiber apparatus and alveolar bone with subsequent pocket formation.[1]

Mechanical plaque removal by scaling and root planing using ultrasonic scaler and curettes have become effective procedure in the treatment of mild to moderate periodontal pockets[2], and regular effective removal of plaque using brushing, flossing and use of interdental cleansing aids depends on the individual time, motivation and manual dexterity. [3, 4] Chemical plaque control agents play an essential role as adjunctive agents along with scaling and root planing. Antimicrobial substances like metal salts, essential oils, phenols, fluorides, and oxygenating agents are used for plaque control; chlorhexidine digluconate is considered as gold standard and most effectively used an oral antimicrobial agent.[5,6] The long-term use of chlorhexidine is associated with some adverse effects.[7] Alternative mouth rinses like essential oils and herbal extracts have gained significant interest in the treatment of periodontal diseases mainly gingivitis, and Periodontitis.[8,9]

Curcumin (CMN) is a major component of turmeric, an ancient dietary spice, and food coloring agent used in the cooking of Southeast Asian countries. CMN is widely used in Ayurveda, Unani and Siddha medicine for cosmetic and medical preparations as well as in the treatment of various diseases. It is derived from the rhizome of Curcumin longa, belonging to the ginger family, Zingiberaceae. Curcumin is shown to possess anti-microbial, anti-inflammatory, anti-oxidant, anti-mutagenic properties, and photodynamic effects.[10]

CMN has effective action of inhibiting the LPS induced NF-$_k$B cytokine gene expression at both the mRNA level and protein level in the gingival tissues. [11, 12] CMN also acts as a host modulatory agent in periodontal disease pathogenesis by decreasing alveolar bone loss by down-regulating expression of IL-17/IL-23 axis and retinoic acid receptor-related orphan receptor (ROR) γt.[13]

CMN mouthwash is an alcohol and sugar-free preparation containing curcumin at 95% with minor ingredients like thymol, eucalyptol, clove oil, mentha oil, and tea tree oil, which has the properties of anti-inflammatory and anti-bacterial agent.[14,15]

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The study aims to compare 0.1 % curcumin mouthwash and 0.2% chlorhexidine gluconate as an adjunctive to scaling and root planing in the treatment of moderate periodontal pockets.

2. Materials and Methods

Study population
The study was double-blinded randomized clinical controlled trial, and the study samples were randomized into three groups. Sixty-six patients with generalized moderate chronic periodontitis were recruited from out-patient Department of Periodontics,

Sample size determination was done by taking Probing pocket depth (PPD) and clinical attachment level (CAL) as primary outcome parameters. Total sample size was calculated using formula: $N=(r+1)(Z_{\alpha/2}) +(Z_{1-\beta})^2\ \sigma^2/rd^2$. A mean difference of 1mm in PPD and CAL between the groups at different intervals requires a sample size of ≥ 20 in each group to obtain a significant difference with 80% power. A total of 66 patients were taken in to the study and were divided into 3 groups with 22 patients in each group.

The requisite study-protocol was approved by the Ethical Committee of the Institute (pr.97/IEC/SIBAR/2017), and the study was done according to the Declaration of Helsinki of 1975, as revised in 2000, and registered with the Clinical Trials Registry – India; ref number: CTRI/2017/10/10257) and doi link is http://ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=20513 &EncHid=&userName.

This randomized clinical study was conducted according to CONSORT statement guidelines.[16] the details of the study design were shown in study flow chart (fig-1).

Inclusion and exclusion criteria
Patient inclusion criteria in this study were: 1) Patients diagnosed as generalized chronic moderate periodontitis with probing pocket depth of 4mm to 6mm[17], 2) having at least 20 permanent teeth, 3) Patients who have not undergone any form of periodontal therapy in the last 1 year, 3) Patients with no history of usage of antibiotics and analgesics within the preceding 6 months.

Exclusion criteria were as follows: 1) Patients with systemic diseases, 2) Patients allergic to the constituents of the formulations of the drug, 3) Patients with hematologic disorders, 4) Patients with any form of tobacco use and 5) Patients who were pregnant or lactating.

Based on the inclusion and exclusion criteria recruited patients were divided into 3 groups as Group I, SRP+ 0.2% chlorhexidine mouthwash (Hexidine®),* Group II= SRP + 0.1% curcumin mouthwash (Turmix®),† and Group III, SRP + placebo 0.9% sodium chloride solution ($Nacl_2$). ‡

Clinical parameters and plaque sample collection:
Patients who were willing to undergo the trials were fully informed of the study, and their written consent was obtained. At baseline before phase-1 therapy, clinical parameters like plaque Index (PI) (Quigley & Hein, as modified by Turesky, 1970),[18] bleeding on probing (BOP) was calculated by modified secular bleeding index (mSBI) (Mombelli 1987),[19] pocket depth (PD) and clinical attachment level(CAL) were recorded from the patients. Measurement of PPD and CAL were done using Michigan O probe with William's markings. §

Full mouth ultra-sonic scaling was performed to remove supra-gingival plaque & calculus and recalled after one week for subgingival plaque sample collection followed by scaling and root planning

Using cotton rolls, the site of interest presenting with deepest periodontal pocket in each patient was isolated an sterile paper-point of ISO 40 standard ‖ was inserted to the depth of periodontal pocket and allowed to remain in the site for a period of 20 seconds. After that, the paper points were transferred to a screw-capped 2ml poly vial having reduced transport fluid (RTF), as a transport medium. Samples were allowed to incubate in room-temperature for 3 to 4 hours permitting self-adjustment of bacteria to the culture environment and then stored at a temperature below 15^0C.[20]

Randomization and procedure
Under local anesthesia with 2% lignocaine hydrochloride, scaling and root planing was performed using piezoelectric ultrasonic scalar and handheld instruments like area specific curettes (Hu-Friedy®) by a single clinician (JKA). It was followed by computerized randomization using 9.0 statistical software ensuring that each patient in all the groups got the unlabeled concealed mouthwash bottles equally, which was monitored by a nonclinical investigator (TP) who kept assignment hidden from the participant and observers till the study was completed.

Oral hygiene instructions were given to all the patients in each group, followed by directing them individually to rinse undiluted form of 10mL twice daily times (morning and night) for ten days. At one month and three months postoperatively, clinical parameters were recorded and subgingival plaque sample was collected. Clinical parameters recording and plaque sample collection at all the intervals was carriedout by a single examiner (KKK).
The flowchart marked as figure 1 shows the study-schedule.

Microbial Load:
Estimating microbial load of every sample is done by determination of the sample's Colony Forming Units (CFU/ml). After a series of tests, 10^{-4} dilution was selected the most suitable one for determination for CFU. Dilutions were conducted sequentially using the medium of sterile RTF on appropriate media-plates, and it was allowed to incubate at 37°C for seven days in an anaerobic chamber (80/10/10, N2 /H2 /CO2). The count of CFU/ml on the growth plate was recorded.[21]

For strictly isolating anaerobes, samples were kept on non-selective ATMB (Anaerobic Thioglycolate Medium Base + serum, bacitracin 75 ug/ml, vancomycin 5 ug/ml) agar plates for selectively recovering anaerobic Gram-negative obligate rods, which was cultured at 37°C in micro-aerophilic environment (5% CO2, 95% N2).[22] These steps of

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procedures were conducted at baseline, 1 month, and 3 months postoperatively.

Statistical analysis:
The collected data at each interval from all the three groups were stored in Microsoft Excel and analyzed using SPSS version statistical software¶ CAL was taken as the primary outcome and used to estimate sample size. A mean difference of 1mm in CAL and 1mm difference in standard deviation between the groups at different intervals required ≥20 patients in each group to get the significant difference with 80% power.** An intragroup comparison was made by using repeated measures of ANOVA. Multiple pairwise comparisons in each group were carried out by Bonferroni post hoc tests. Mean values comparison in between the groups is made by using ANOVA. Results were considered statistically significant at P< 0.05, at 95% confidence interval.

3. Results

A total of 66 patients were recruited into the study (37 males and 29 females) and were divided into three groups with 22 patients in each group. Among them, five patients (2 patients in group I and group III and one patient in group II) failed to complete the study due to no-availability for follow-up and personal reasons. Mean age of all the patients in three groups is found to be 49±2.5. PI showed significant (p<0.001) decrease from baseline to 1 month in all the groups (Table 1), whereas an increase in the PI values was observed at three months, with higher values were noticed in Group III followed by Group II and Group I respectively (Table 2). In between the groups, comparison showed a statistically significant increase (p<0.001) in PI scores was observed in Group III (placebo) when compared with group I and group II (Table 3).

BOP scores were also reduced in all the three groups from baseline at one month and three months post-operatively, which were statistically significant (p<0.001)(Table1). When compared with baseline, BOP scores reduced significantly (p<0.001) in both group I and group II than group III at one month and three months interval, which were similar (Table 2). Intergroup comparison of BOP scores at baseline and 1month showed no statistically significant difference among the three groups, whereas at three months post-operatively showed a significant (p<0.05) difference in BOP scores in group 1 and group 2 in comparison with group 3(Table 3).

A significant difference (P<0.001) in PD was observed in all the three groups from baseline to 3 months (Table 1). Intragroup comparison from baseline to 1 month and baseline to 3 months showed a significant difference (P<0.001) in PD in all the three groups (Table 2). Intergroup comparison shows no significant difference at baseline in between the groups but a significant reduction (P<0.002) in PD was observed in group I and group II when compared with group III at 1 month and 3 months (Table 3).

Mean scores for CAL at baseline was similar in all the three groups. A statistically significant (P<0.001) gain in CAL was observed in all the three groups from baseline to 1 month and 3 months respectively (Table 1). Intragroup comparison from baseline to 1 month, baseline to 3 months and 1 month to 3 months showed a significant increase in CAL gain was achieved at baseline to 1 month in all the three groups than 1 month to 3 months (Table 2). Intergroup comparison showed no significant difference between yhe groups at all the intervals (Table 3).

A significant difference (P<0.001) in CFU values was observed in all the three groups from baseline to 3 months (table 4). Comparison of each interval among the groups showed statistically significant (P<0.001) reduction in CFU in both group I and group II, whereas in group III, no statistical difference was observed compared to the baseline (Table 5).

Intergroup comparison showed highly significant (P<0.001) decrease in group I and group II at one month and three months compared to baseline. At one month and three months, no difference was found between group I and group II, whereas a significant difference (P<0.001) was found between group I and group III and group II and group III (Table 6).

4. Discussion

To the best of our knowledge, this is the first randomized study done to investigate the effect of 0.1% CMN as a mouth rinsing agent both clinically and microbiologically in the treatment of periodontitis along with SRP. A follow up period of 3 months has been taken and observed

To reduce subgingival micro-organisms and also to improve the clinical parameters, control agents of chemical plaque are most often advised, out of which CHX has proved most effective against plaque-formation and gingivitis.[23,24] As seen in the previous studies, 0.2% CHX digluconate mouthrinse has been accepted as an active control for evaluation of anti-microbial characteristics of herb-based 0.1% CMN mouthrinse.[25] Similar to the previous studies, 0.2% CHX digluconate mouthrinse has been taken as a positive control to evaluate the antimicrobial property of herbal based 0.1% CMN mouthrinse.

Even though plaque scores improved after three months interval in both the groups, between CMN and CHX, the plaque scores remained the same which indicated the inhibitory performance of CMN against plaque forming periodontal pathogens. According to Izui S et al., CMN is shown to be inhibiting the formation of biofilm by periodontal pathogens like Porphyromonas gingivalis either with streptococcus gordonii or without it, analyzed with the aid of confocal laser scanner microscopy. The study concluded that CMN, in a dose-specific manner inhibits the growth of Prevotella intermedia, Porphyromonas gingivalis, Treponema denticola, and Fusobacterium nucleatum as also Porphyromonas gingivalis associated proteinases.[26]

CMN was thoroughly studied in sync with periodontal diseases for its anti-inflammatory characteristics. Chen D et al., established that CMN inhibited the stimulated macrophage activity by lipopolysaccharides from Porphyromonas gingivalis in a dose-specific approach and

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also TNF-α and IL-1β gene expression and protein synthesis was found to be suppressed.[27]

BOP was checked to evaluate the anti-inflammatory property of CMN. Effect of CMN as a mouth rinse in the present study has shown similar values to that of CHX postoperatively at one month and three months, indicating that CMN does possess anti-inflammatory property.

Similarly, studies undertaken by Guimaraes MR et al. inferred that CMN efficiently causes inhibition of stimulation of nuclear factor-kB cytokine gene expression in periodontitis induced experimentally in rats, at both mRNA and protein level in gingival tissues.[28]

Reduction of PD and CAL scores on similar lines was seen between CMN and CHX groups which indicate that CMN has similar therapeutic advantages in managing moderate periodontal pockets, which can be ascribed to the anti-bacterial and anti-inflammation propensities of wound healing potential. According to Elburki MS et al., deduced the inhibitory effect of CMN, the chemically modified strain, (CMC2.24) on NFκB and MAPK signaled production of an inflammatory cytokine in experimental periodontal disease models in rats. It stated that CMC2.24 substantially reduced in inflammatory cytokines and MMPs in gingival tissues, besides decreasing bone loss in locally induced as well as in systemically associated periodontitis.[29]

In a similar study by Elburki MS et al., it was inferred that CMC2.24 works not just as a potential anti-inflammatory mediator through suppression of inflammatory cytokine production (IL-1β, IL-6, TNF-α) and matrix metalloproteinase secretion (MMP-2, MMP-9, MMP-8), but it also prevents hyperglycemia and bacteria-propelled destruction of connective tissues of skin and bone.[30] Again, a gain in clinical attachment level as well as decrease in probing depth seen in this study could be owed to CMN's immunomodulatory characteristics, and this would have caused enhanced healing of wound as well. According to studies conducted by Smith PC et al., CMN raises the turnover rate of proteins in the extra-cellular matrix by activating the epidermal growth factor directly, and this in return, enables the expression of urokinase plasminogen reflex in the gingival fibroblasts.[31] Correa MG et al., in their study on Wistar rats, found that CMN and resveratrol possess immunomodulatory property in bringing down inflammatory cytokines like IL-1β, IL-4, IFN-ɣ, and TNF-α, causing reduced alveolar bone loss in periodontitis that was experimentally induced.[32]

Reduction of PD and gain in CAL scores was seen in both CMN and CHX groups, indicating that CMN has similar therapeutic benefits like CHX in the managing moderate periodontal pockets, which can be ascribed to the anti-bacterial and anti-inflammation propensities of wound healing potential. Similar to the earlier studies, present study results re-establish that 0.1% of CMN mouthwash can effectively work against plaque development and thus causes the diminution of PD and CAL, as could see in CHX at one month and three months in post-operative scenarios.[14,33] Again, gain in clinical attachment level as well as decrease in probing depth seen in this study could be owed to CMN's immunomodulatory characteristics, and this would have caused enhanced healing of wound as well.

Anaerobic gram-negative bacteria (aggregatibacter actinomyces, porphyromonasgingivalis, prevotellaintermedia, tannerellaforsythus, Treponema denticola,etc.) are inclusive factors primarily connected with periodontal problems [34]. To manage the continuity of periodontal diseases, early identification of the above commonly associated periodontal pathogens and controlling their growth in subgingival clusters are of high importance.

Boutaga K et al, compared CFU with Real time PCR for detection of subgingival bacteria mainly presence of Porphyromonas gingivalis and found that CFU can be used as quantification method for identification of periodontal pathogens [35]. The present study observations are in accordance with previous study that, CFU in both CMN and CHX at each time interval has been low, even though a little increase was seen in CFU formation with CMN group at three-month duration, a comparison with CHX not to be considered significant. This decrease is suggestive of potential inhibition of CMN against plaque formation for a longer period similar to that of CHX [36].

Limitations of the present study was only moderate periodontal pockets with 4 to 6mm depth were included but observation in deep periodontal defects can explain the anti-bacterial effect of the two mouth rinses as deep periodontal pockets often associated with significant subgingival plaque formation. Professional irrigation of the agents will have more effective action of the drug subgingivally. Qualitative assessment of the plaque by PCR analysis will help to give a definitive identification of presence of putative pathogens causing periodontitis. Longitudinal trials with larger sample size are required to evaluate the benefits of CMN as mouth rinse in the management of chronic periodontitis.

5. Conclusion

Based on the merits of non-alcoholic herbal formulation and without any untoward complications like burning sensation, varied taste, tooth staining associated with CHX mouth-rinse, CMN can be considered an alternative herbal mouth-rinse for patients under a periodontal treatment protocol CMN's plaque controlling efficacy is co-related to CHX gluconate mouth-rinse when used for a period of three months. Results of the current study indicate that 0.1% CMN can be administered as an adjunctive anti-microbial agent together with SRP in treating periodontal pockets of moderate intensity.

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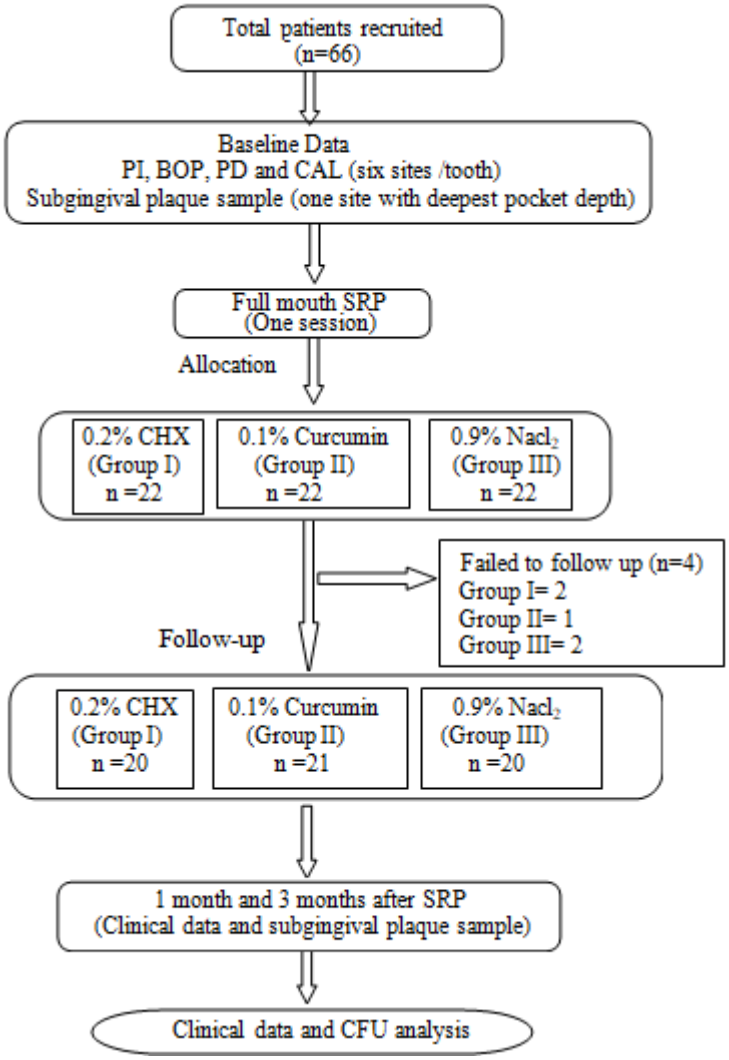

Figure 1: Flowchart of the study schedule

Table 1: Intragroup comparison of mean PI and PD, CAL at Baseline, 1 month and 3 months by using repeated measures ANOVA

Groups	Time	Mean	F-value	P-value
PI Group I	Baseline	1.682	431.63	0.001**
	1 Month	0.909		
	3 Months	0.955		
PI Group II	Baseline	1.727	107.69	0.001**
	1 Month	0.864		
	3 Months	1		
PI Group III	Baseline	1.955	319.924	0.000**
	1 Month	0.955		
	3 Months	1.364		
BOP Group I	Baseline	1.621	204.18	0.001**
	1 Month	0.500		
	3 Months	0.483		
BOP GroupII	Baseline	1.592	146.23	0.001**
	1 Month	0.471		
	3 Months	0.554		
BOP Group III	Baseline	1.614	81.40	0.082
	1 Month	1.240		

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	3 Months	1.422		
PD Group I	Baseline	4.955	5341.67	0.000**
	1 Month	3.909		
	3 Months	3.345		
PD Group II	Baseline	4.952	6134.16	0.000**
	1 Month	3.773		
	3 Months	3.424		
PD Group III	Baseline	5.045	7131.46	0.000**
	1 Month	4.045		
	3 Months	4.168		
CAL Group I	Baseline	5.091	3324.092	0.000**
	1 Month	3.909		
	3 Months	4.091		
CAL Group II	Baseline	5.001	15225.00	0.000**
	1 Month	4.045		
	3 Months	4.136		
CAL Group III	Baseline	5.000	9184.13	0.000**
	1 Month	4.136		
	3 Months	4.251		

$p<0.01$** denotes highly significant at 1% level.

Table 2: Multiple pair wise comparisons of mean PI, PD and CAL using Bonferroni post hoc tests

Groups	Time	Mean difference	P- value
PI Group I	Baseline – 1 month	0.773	0.000**
	Baseline - 3 months	0.727	0.000**
	1 month - 3 Months	-0.045	0.986
PI Group II	Baseline – 1 month	0.864	0.000**
	Baseline - 3 months	0.727	0.000**
	1 month - 3 Months	-0.136	0.249
PI Group III	Baseline – 1 month	1	0.000**
	Baseline - 3 months	0.591	0.000**
	1 month - 3 Months	-0.409	0.011
BOP Group I	Baseline – 1 month	1.122	0.001**
	Baseline - 3 months	1.143	0.001**
	1 month - 3 Months	0.020	0.210
BOP Group II	Baseline – 1 month	1.123	0.001**
	Baseline - 3 months	1.042	0.001**
	1 month - 3 Months	-0.080	0.344
BOP Group III	Baseline – 1 month	0.138	0.053
	Baseline - 3 months	0.135	0.060
	1 month - 3 Months	1	$P\leq0.001$**
PD Group I	Baseline – 1 month	1.182	0.000**
	Baseline - 3 months	1	0.000**
	1 month - 3 Months	-0.182	0.05*
PD Group II	Baseline – 1 month	0.995	0.000**
	Baseline - 3 months	0.864	0.000**
	1 month - 3 Months	-0.091	1.000
PD Group III	Baseline – 1 month	0.864	0.000**
	Baseline - 3 months	0.864	0.000**
	1 month - 3 Months	0.001	1.000
CAL Group I	Baseline – 1 month	1	0.000**
	Baseline - 3 months	-0.182	0.127
	1 month - 3 Months	0.995	0.000**
CAL Group II	Baseline – 1 month	0.864	0.000**
	Baseline - 3 months	-0.091	0.900
	1 month - 3 Months	0.864	0.000**
CAL Group III	Baseline – 1 month	0.864	0.000**
	Baseline - 3 months	0.001	1.000
	1 month - 3 Months	1	0.000**

$p<0.001$** denotes highly significant at 1% level.
$p<0.05$* denotes significant at 5% level.

Table 3: Comparison of mean PI, PD, and CAL among groups at Baseline, 1 month and 3 month by using ANOVA

Time	Groups	Mean	F-value	P-value
PI Baseline	Group I	1.68	2.447	0.095
	Group II	1.73		

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	Group III	1.95		
PI 1 month	Group I	0.91	0.186	0.830
	Group II	0.86		
	Group III	0.95		
PI 3 months	Group I	0.95	3.861	0.02*
	Group II	1.00		
	Group III	1.36		
BOP baseline	Group I	1.62	2.423	0.098
	Group II	1.59		
	Group III	1.61		
BOP 1 month	Group I	0.50	8.160	0.04*
	Group II	0.47		
	Group III	1.24		
BOP 3months	Group I	0.48	14.28	0.02*
	Group II	0.55		
	Group III	1.42		
PD Baseline	Group I	4.95	0.257	0.774
	Group II	4.95		
	Group III	5.05		
PD 1 month	Group I	3.91	3.884	0.026*
	Group II	3.77		
	Group III	4.05		
PD 3 months	Group I	3.34	4.341	0.002*
	Group II	3.42		
	Group III	4.16		
CAL Baseline	Group I	5.09	2.100	0.131
	Group II	5.00		
	Group III	5.00		
CAL 1 month	Group I	3.91	1.357	0.265
	Group II	4.05		
	Group III	4.14		
CAL 3 months	Group I	4.09	0.834	0.938
	Group II	4.14		
	Group III	4.25		

$p<0.05^{*}$denotes significant at 5% level.

Table 4: Intragroup comparison of mean CFU at Baseline, 1 month and 3 months by using repeated measures ANOVA

Groups	Time	Mean	F-value	P-value
CFU Group I	Baseline	176.091	2828.59	0.000**
	1 Month	14.45		
	3 Months	33.864		
CFU Group II	Baseline	173.591	3962.713	0.000**
	1 Month	19.227		
	3 Months	45.909		
CFU Group III	Baseline	176.13	2071.96	0.000**
	1 Month	61.545		
	3 Months	151.773		

$p<0.001^{**}$denotes highly significant at 1% level.

Table 5: Multiple pair wise comparisons of mean CFU using Bonferroni post hoc tests

Groups	Time	Mean difference	P- value
CFU Group I	Baseline – 1 month	161.63	0.000**
	Baseline - 3 months	142.22	0.000**
	1 month - 3 Months	-19.409	0.000**
CFU Group II	Baseline – 1 month	154.364	0.000**
	Baseline - 3 months	127.682	0.000**
	1 month - 3 Months	-26.082	0.000**
CFU Group III	Baseline – 1 month	114.591	0.000**
	Baseline - 3 months	24.364	0.061
	1 month - 3 Months	-88.227	0.044*

$p<0.001^{**}$denotes highly significant at 1% level.
$p<0.05^{*}$denotes significant at 5% level.

Table 6: Intergroup Comparison of mean CFU at Baseline, 1 month and 3 month by using ANOVA

Time	Groups	Mean	F-value	P-value
CFU Baseline	Group I	176.09	0.186	0.095

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	Group II	173.59		
	Group III	176.14		
CFU 1 month	Group I	14.45	32.700	0.000**
	Group II	19.23		
	Group III	61.55		
CFU 3 months	Group I	33.86	1004.758	0.000**
	Group II	45.91		
	Group III	149.77		

$p<0.001$** denotes highly significant at 1% level.

Legends

Figure 1: Flowchart of the study schedule
Table 1: Intragroup comparison of mean PI and PD, CAL at Baseline, 1 month and 3 months by using repeated measures ANOVA
Table 2: Multiple pair wise comparisons of mean PI, PD and CAL using Bonferroni post hoc tests
Table 3: Comparison of mean PI, PD, and CAL among groups at Baseline, 1 month and 3 month by using ANOVA
Table 4: Intragroup comparison of mean CFU at Baseline, 1 month and 3 months by using repeated measures ANOVA
Table 5: Multiple pair wise comparisons of mean CFU using Bonferroni post hoc tests
Table 6: Intergroup Comparison of mean CFU at Baseline, 1 month and 3 month by using ANOVA

Foot notes: * ICPA Health Products, Mumbai, India
† Sanat Products Ltd, Delhi,
‡ Otsuka Pharmaceutical India Pvt. Ltd. Ahmedabad, India
§ Hu-Friedy Mfg.co, Chicago, IL. USA
|| ISO 40, Pearl Dent co, Korea
¶ IBM SPSS statistics for windows version 20, Armonk, NY, USA
** G-star Power 3.1.9.2 software, Dusseldorf, Germany

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