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A Prospective, Open Label, Double Arm, Multi-center, Comparative Clinical Study on Mouth Rinse Containing Chlorhexidine and Hyaluronic Acid as a Potential Aid in Improving Overall Gum Health in Patients, Post Oral Surgical Procedures

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ABSTRACT:

Purpose: For fast wound healing in oral surgical procedures, support of adjuvant therapeutic agents is pivotal.

Methods: Prospective, open label, randomized, double arm, multi-center, comparative clinical study was conducted to ascertain safety and efficacy of the mouth rinse containing Chlorhexidine and Hyaluronic Acid. Wound healing using Healing index scale and Percentage healing index (PHI), along with overall gum health and subjective feedback was also recorded.

Results: Total 50 otherwise healthy male and female participants (25 in each arm) who had planned for oral surgical procedures viz. tooth extraction, implant surgeries or periodontal surgeries were enrolled. All subjects had completed the study with no dropout.Wound healing by Healing Index exhibited significant improvement on Day 03, 07 and 15 (P<0.0001), where the score was improved from 1 at baseline to 2.2, 3.4 and 4.2 respectively in subsequent visits. Healing in the test group was significantly more than the placebo group on Day 07 (P=0.0011) and Day 15 (P<0.0001). PHI demonstrated significant improvement by 50.7% on Day 07 and 68.4% on Day 15 (P<0.0001), as compared to baseline. PHI was significantly higher in the test group than the placebo on Day 07 (P=0.0032) and Day 15 (P=0.0005). Significant difference between two groups was recorded for overall gum health by investigator assessment wherein the test product was found effective. Subjective assessment revealed significant reduction in post-operative pain, faster wound healing, reduction in teeth staining, and overall gum health in test group. No adverse event was recorded during the study.

Conclusions: The test product was found significantly more effective than the placebo in aiding wound healing of oral surgical procedures. It also aided in improving overall gum health and reduced the post-operative pain, inflammation and teeth staining and can be recommended as an adjuvant to the patients of oral post-surgical procedures.

KEYWORDS: Chlorhexidine, Gum Health, Hyaluronic Acid, Oral Wound Healing, Periodontitis, Tooth Extraction.

I. INTRODUCTION

Oral diseases affect nearly 3.5 billion people worldwide which is close to 50% world's population, estimates the Global oral health status report of World Health Organization (2022). The prevalence is relatively higher among the people living in middle-income countries. The report highlights that over 95% Indian adults have dental caries and over 50% have periodontal disease. Over the past 30 years, there is rise of 1 billion cases

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globally due to deficiency of appropriate oral health care facilities such as prevention, risk protection, and restorative and rehabilitative services [1]. Lack of awareness among general population, limited access to dental health services and poor oral hygiene etc. are few causes for the increasing prevalence.

Dental caries (tooth decay) and periodontal (gum) diseases are most common oral disorders that leads to edentulism (total teeth loss). Extraction of tooth due to plaque is very common procedure performed to get permanent relief from toothache and also to save the other teeth from decay. However, the procedure may cause wound that needs to be healed to protect from pain, prolonged bleeding, swelling, developing infections and dry socket, which are most common postextraction complications [2]. Unlike the open dermal wounds, oral wounds need longer time to heal completely due to a dynamic environment of the mouth in which healing occurs in warm oral fluid containing a high microorganism load and continued physical activity[3, 4]. Infection is considered the key factor behind delayed healing process. In postextraction procedure and other periodontal surgeries, the wound site may serve as a niche for bacterial plaque formation if good oral hygiene is not maintained[5].

The healing progression for oral injuries comprises a sequence of complex biological processes that involves hemostasis, inflammation, proliferation, and remodeling. Often the traditional dressings are not able to fully meet the needs of wound treatment since the oral cavity contains millions of microorganisms in a remarkable environment [4]. Use of topical antimicrobial preparations in wound therapy is gaining attention of medical practitioners. Mouthwashes, sprays, gels etc. are most common topical medications, which are usually prescribed to prevent the wound sites from developing infections and chronicityof the wound. For wound stability and wound closure in a timely manner, the first postoperative week appears to be the most critical for the maintenance of wound stability [6].Topical medicated agents are often prescribed in order to reduce postoperative pain, promote healing, prevent from infections in oral cavity and to reduce detrimental effects on the oral tissues.

The efficacy of Chlorhexidine (CHX) in the field of periodontology has been extensively studied and well established. CHX possesses broad spectrum antibacterial action and is one of the most effective antimicrobial agent as an adjunct due to its substantive and antibacterial properties. It is commonly used after periodontal surgeries to maintain oral hygiene. Various available published literatures suggest that it is effective against gram-positive and gram-negative bacteria, yeasts, dermatophytes and even some lipophilic viruses [7,8]. Hyaluronic Acid (HA) has gathered increasing attention in recent past due to it's wound healing properties, along with remarkable anti-inflammatory and anti-bacterial action supporting its potential in treating inflammatory conditions across various medical fields, including dentistry[9].As an antiseptic agent, CHX has proven efficacy in curbing the infections in open wounds that may help in faster recovery, however it has no direct role in wound healing. HA, on the other hand possesses good antiinflammatory, antioxidative, antiedematous and hygroscopic properties and is often recommended for faster wound healing. The adjunct of HA may improve soft tissue healing in the early post-operative period.Short term use of the combination of CHX and HA may help in reducing pain and inflammation and faster healing of the wound site[10].

The novel product to be tested in this study is an aqueous vehicle mouth rinse, enriched with pharmacological agents viz. HA(0.1%) and CHX (0.2%). The rationale of the development of a novel formulation is to integrate the anti-infective property of CHX and wound healing property of HA and to attain the beneficial effects of the combination on faster wound healing. The study was intended to evaluate and establish the test product as a potential aid in faster wound healing and improving overall gum health in patients of post oral surgical procedures. In addition, the safety and acceptability of the mouth rinse was also evaluated.

II. MATERIAL AND METHODS

2.1. Study Design

The present study was a prospective, open label, randomized, double arm, multi-center, comparative clinical study, aimed to evaluate the test product as a potential aid in improving overall gum health in patients of post oral surgical procedures. The study consisted of 05 visits (Figure 1) including 1 screening & enrollment visit, 3 evaluation visits on Day 03, 07, 15 and one follow-up visit on Day 30 was scheduled for safety evaluation. Demographic details, medical history, wellbeing, physical and dental examination, concomitant medication etc. were recorded during the screening visit. The enrolled subjects were treated either with the test product or placebo as per randomization schedule. All subjects were equally divided in each arm. All eligible subjects underwent oral assessment, photographs and

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software-based assessment and subjective assessment. Safety was assessed throughout the study by monitoring of adverse events.

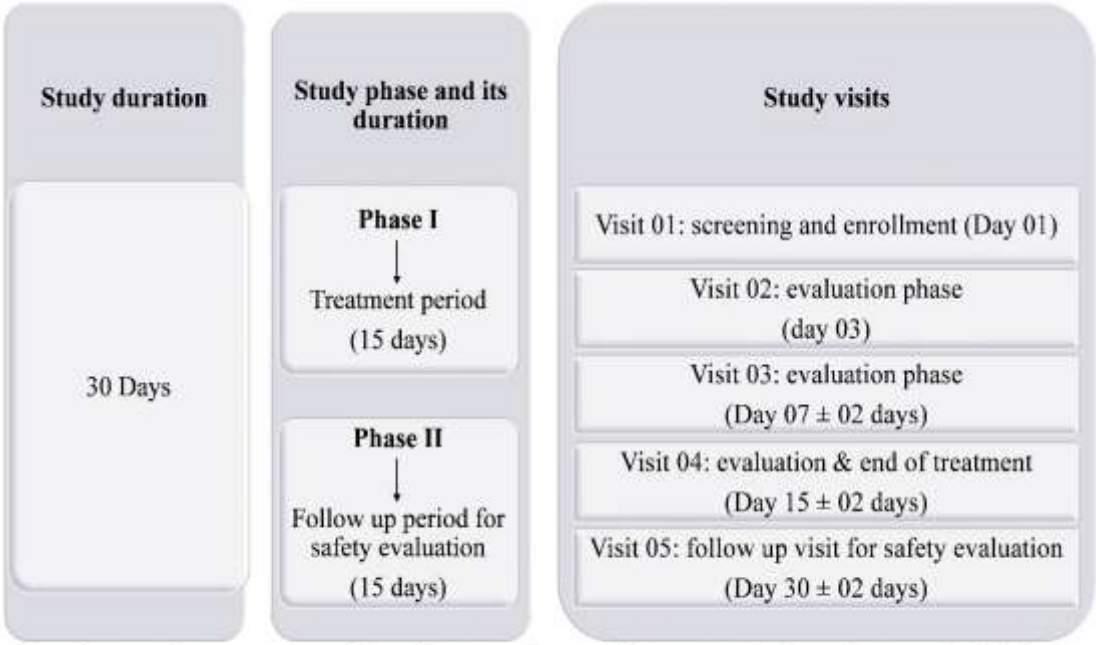

Figure 1: Study visits: schematic diagram.

Randomization was prepared by biostatistician and shared with pharmacist/ designee only. The randomization schedule was generated, using SAS® statistical software (Version: 9.4 or higher; SAS Institute Inc., USA) ensuring the treatments were balanced. The personnel involved in the dispensing of products were accountable for ensuring compliance to randomization schedule.

2.2 Study Participants

A total of 50 otherwise healthy subjects(25 subjects in each arm) who had planned for oral surgical procedures like tooth extraction, implant surgeries or periodontal surgerieswere enrolled in this study.

2.3 Ethics

The clinical study was carried out in accordance with "The code of ethics of the world medical association" (Declaration of Helsinki), The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)-GCP (E6 R2) and other applicable ethical guidelines for experiments involving humans. Central Drugs Standard Control Organization (CDSCO) registered Institutional Ethics committee reviewed and approved the Protocol and study documents. The trial was prospectively registered with Clinical Trial Registry of India (CTRI)with reference no. CTRI/2023/06/054218 on 20 June 23prior to study initiation. Signed Informed Consent Form (ICF) was taken from the volunteers and a copy of the same was also provided to them. Subject's identity was kept confidential and the data generated in the study was handled as per in-house Standard Operating Procedures (SOPs) and applicable regulations.

2.4 Test Product(s)

The test product (ClohexMouth Rinse containing CHX(0.2%) and HA (0.1%) of Dr. Reddy's Laboratories, India), or Placebo product (Saline) was assigned to the subjects as per randomization schedule. On Day 01, Investigator dispensed 10 ml of product (i.e., test or placebo) on the sterile gauze/cotton and kept it at the wound site for 60 seconds prior to the final dressing. From Day 02, subjects used the product themselves at home following the instructions. 10 ml of product had to be taken to rinse the mouth for 30 seconds. While rinsing, the product had to be moved inside the mouth side to side from one cheek to the other. Instruction was given to spit out the product after rinsing. The product was used twice daily (morning & evening) for treatment period, i.e. 15 days. The participants were instructed to report on scheduled

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visits without using the product and refrain from using other mouth rinses during the study.

2.5 Inclusion Exclusion Criteria

Males and non-pregnant/non-lactating female subjects (age 18 to 50 years)who were willing to provide written informed consent for participating in the study and underwent oral surgical procedures like tooth extraction, implant surgeries and periodontal surgeries, and who were willing and able to follow the study protocol, were enrolled.

The key exclusion criteria of study were as follows: Pregnant or breastfeeding females or who were planning pregnancy during the study period; subjects who were receiving steroid medications, which would have compromised the study objective; subjects who were having history of diabetes mellitus, acute cardiac and circulatory diseases, HIV, hepatitis etc.; subjects with known allergy or sensitivity to ingredients viz. CHX and HA; subjects who were having problems of hemostasis and coagulation; subjects who were alcohol addicts; subjects who smoked cigarettes or consumed any other form of tobacco; who had participated in a similar clinical study within the previous 30 Days; any other condition which could have warranted exclusion from the study, as per the investigator's discretion.

2.6 Efficacy Endpoint(s)

2.6.1 Primary Endpoint(s)

Primary outcome of the study was to assess the effect of test product on wound healing using Healing index scale [11]from baseline (i.e. Day 01 before product administration) to Day 03, Day 07 and Day 15 and in comparison to placebo. Based on tissue color, response to palpation (bleeding or non-bleeding), Granulation tissue (present or absent) and Incision margin (Connecting tissue exposure), the healing index was scored on a 5-point scale (Table 1).

Table 1: Healing Index Scale (After Landry et al., 1988)

Score	Clinical findings					Interpretation
	Tissue colour	Response to palpation	Granulation tissue	Incision margin	Suppuration	
1	≥50% of gingiva red	Bleeding	Present	Not epithelialized, with loss of epithelium beyond incision margin	Present	Very poor
2	≥50% of gingiva red	Bleeding	Present	Not epithelialized, with connective tissue exposed	-	Poor
3	≥25% and<50% of gingiva red	No bleeding	None	No connective tissue exposed	-	Good
4	<25% of gingiva red	No bleeding	None	No connective tissue exposed	-	Very good
5	All tissues pink	No bleeding	None	No connective tissue exposed	-	Excellent

Assessment of wound healing using Percentage healing index (PHI) was the other primary endpoint. PHI was done only for subjects who underwent tooth extraction and was not carried out in the subjects of post implant surgeries and periodontal surgeries, for which, only digital

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photographs were taken. For PHI calculation, the photographs of the wound were taken at all timepoints and were analyzed for measuring the area of wounds using ImageJ® software developed by Wayne Rasband of the Research Services Branch, National Institute of Mental Health (Bethesda, Maryland, USA). Percentage Unhealed Index (PUI) was calculated (PUI = T1/T0 X 100) for the wound that represented the percentage of the lesion that was not healed over the period. The PHI was then calculated by the formula PHI = 100– PUI for the given timepoint. The analysis was carried out in triplicates and the average was used to calculate the PHI.Needless to mention that since its inception, the ImageJ program has grown significantly. It is a domain Java image processor and image analyzer software.It is an open-source software that allows users to visualize, inspect, quantify, and validate scientific image data. With advancements in digital dentistry, ImageJ software is playing significant role as an image processing tool for cliniciansin the field of dentistry as well [12].

2.6.2 Secondary Endpoint(s)

Oral Assessment by investigator for overall gum health (in terms of Color, Consistency, Texture & Bleeding) by dental examination was the secondary outcome and was assessed from baseline (i.e. Day 01 before product administration) to Day 03, Day 07 and Day 15 and in comparison to placebo. In addition, Subject Satisfaction Questionnaire (pertaining to reduction in post-operative pain and teeth staining, improvement in wound healing and gum health) after test product administration on Day 03, Day 07 and Day 15, along with Subject Response Index (Perception about Product for appealing taste, appearance and overall likeability) after product administration at Day 15 were the other secondary endpoints.

Safety was assessed by incidence of undesirable /adverse event (AE) or Serious Adverse Event (SAE) during scheduled study visits or self-reported throughout the study duration.

2.7 Statistical analysis

Continuous variables were summarized using tables of descriptive statistics: number of subjects with recorded observations, mean, standard deviation, median, minimum and maximum. Categorical variables were summarized using counts and percentages.For continuous variables, within-treatment analyses were conducted to compare baseline to post-treatment data using paired t-test. For categorical variables, the within-treatment analysis was conducted to compare baseline to post-treatment analysis using Wilcoxon signed rank test. The analysis was also done between both the arms (i.e., test arm and placebo arm) for all parameters except Subject Satisfaction Questionnaire and Subject Response Index (Perception about Product). All statistical tests were performed using SAS software of 5% level of significance (Version: 9.4; SAS Institute Inc., USA).

III. RESULTS

3.1. Participant characteristics/ Subject Demography

In each arm, total 25 subjects (9 males and 16 females) were enrolled. Age of the subjects ranged between 19 to 49 years with average being 35.2 years. All 50 subjects completed the study and there was no drop-out. Both the groups demonstrated no statistical significant difference in Age (P=0.4104), Gender (P=1.0000), Race (P=NE) and baseline characteristics of endpoints viz. HI (P=1.0000) and PHI (P=0.9004)(Table 2).

Table 2: Demographic and Baseline Characteristics

Category/Statistics	Test (N = 25) n (%)	Placebo (N = 25) n (%)	p value (Test vs. Placebo)
Age (Completed Years)			
n	25	25	0.4104[a]
Mean ± SD	34.2 ± 7.73	36.2 ± 8.92	
Median	35.0	38.0	
Min, Max	19, 49	21, 49	
Gender [n (%)]			

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Male	9 (36%)	9 (36%)	1.0000[b]
Female	16 (64%)	16 (64%)	
Predominant Race [n (%)]			
Asian	25 (100%)	25 (100%)	NE
Healing Index Scale (HI) (n = 25)	-	-	1.0000[c]
Percent Healing Index (PHI) (n = 20)	-	-	0.9004[a]

Abbreviation(s): Max = maximum; Min = minimum; N = number of subjects in the specified treatment arm; NE = not evaluable; n = number of subjects in the specified category; SD = standard deviation.
[a]p-value is calculated using two-sample t-test.
[b]p-value is calculated using chi-square test.
[c]p-value is calculated using Wilcoxon rank sum test.

3.2 Efficacy Assessments

3.2.1 Assessment of Wound Healing using Healing Index Scale

For Test and Placebo Group, the mean wound healing score was 1.0 at baseline. No significant difference (P=1.0000) was observed between both the groups at baseline. The score was increased to 2.2, 3.4 and 4.2 in test group and 2.0, 2.7 and 3.4 in placebo group on Day 03, Day 07 and Day 15 respectively. After using the test product, wound healing demonstrated significant improvement on Day 03, Day 07 and Day 15 (P<0.0001), as compared to baseline. Healing in the test group was significantly more than the placebo group on Day 07 (P=0.0011) and Day 15 (P=<0.0001) (Table 3).

Table 3: Healing Index Scale

Parameters	#Visit 01 (Day 01)		Visit 02 (Day 03)			Visit 03 (Day 07 ± 02 Days)			Visit 04 (Day 15 ± 02 Days)		
	Mean ± SD	Median	Mean ± SD	CFB	Median	Mean ± SD	CFB	Median	Mean ± SD	CFB	Median
Test (n=25) (p-value)	1.0 ± 0.00	1.0	2.2 ± 0.37	1.2 ± 0.37	2.0	3.4 ± 0.76	2.4 ± 0.76	3.0	4.2 ± 0.47	3.2 ± 0.47	4.0
	-		-	<0.0001[a]		-	<0.0001[a]		-	<0.0001[a]	
Placebo (n=25) (p-value)	1.0 ± 0.00	1.0	2.0 ± 0.00	1.0 ± 0.00	2.0	2.7 ± 0.75	1.7 ± 0.75	3.0	3.4 ± 0.71	2.4 ± 0.71	3.0
	-		-	<0.0001[a]		-	<0.0001[a]		-	<0.0001[a]	
Test vs. Placebo (p-value)	1.0000[b]		-	0.1099[b]		-	0.0011[b]		-	<0.0001[b]	

Abbreviation(s): CFB = change from baseline; Min = Minimum; Max = Maximum; n = number of subjects; SD = standard deviation.
Baseline visit
[a]p-value is calculated using Wilcoxon sign rank test.
[b]p-value is calculated using Wilcoxon rank sum test.

3.2.2 Assessment of Wound Healing using Percentage Healing Index (PHI)

For Test group, the mean percentage healing index (PHI) of the wound was 22.7% on Day 03, which exhibited improvement by 50.7% and 68.4% on Day 07 and Day 15 respectively. On the other hand, the mean percentage healing index (PHI) of the wound for placebo group was 15.1%, 36.6% and 53.7% on Day 03, Day 07 and Day 15 respectively. After using the test product, PHI of the wound showed significant improvement on Day 07 (P<0.0001) and Day 15 (P<0.0001), as compared to baseline. PHI was significantly higher in the test group than the placebo group on Day 07 (P=0.0032) and Day 15 (P=0.0005) (Table 4; Figure 3).

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Table 4: Percentage Healing Index (PHI)

Parameters	#Visit 02 (Day 03)	Visit 03 (Day 07 ± 02 Days)		Visit 04 (Day 15 ± 02 Days)	
	%Mean ± SD	%Mean ± SD	CFB	%Mean ± SD	CFB
Test (n=20)	22.7 ± 3.80	50.7 ± 8.20	28.0 ± 7.11	68.4 ± 5.90	45.7 ± 6.85
(p-value)	-	-	<0.0001[a]	-	<0.0001[a]
Placebo (n=20)	15.1 ± 4.15	36.6 ± 5.55	21.5 ± 6.03	53.7 ± 3.14	38.6 ± 4.59
(p-value)	-	-	<0.0001[a]	-	<0.0001[a]
Test vs. Placebo	-7.6 ± 3.98	-	-6.6 ± 6.59	-	-7.1 ± 5.83
(p-value)	<0.0001[b]	-	0.0032[b]	-	0.0005[b]

Abbreviation(s): CFB = change from baseline; SD = standard deviation.
The assessment was done only for the subjects of tooth extraction.
Baseline visit
[a]p-value is calculated using paired t-test.
[b]p-value is calculated using two-sample t-test.

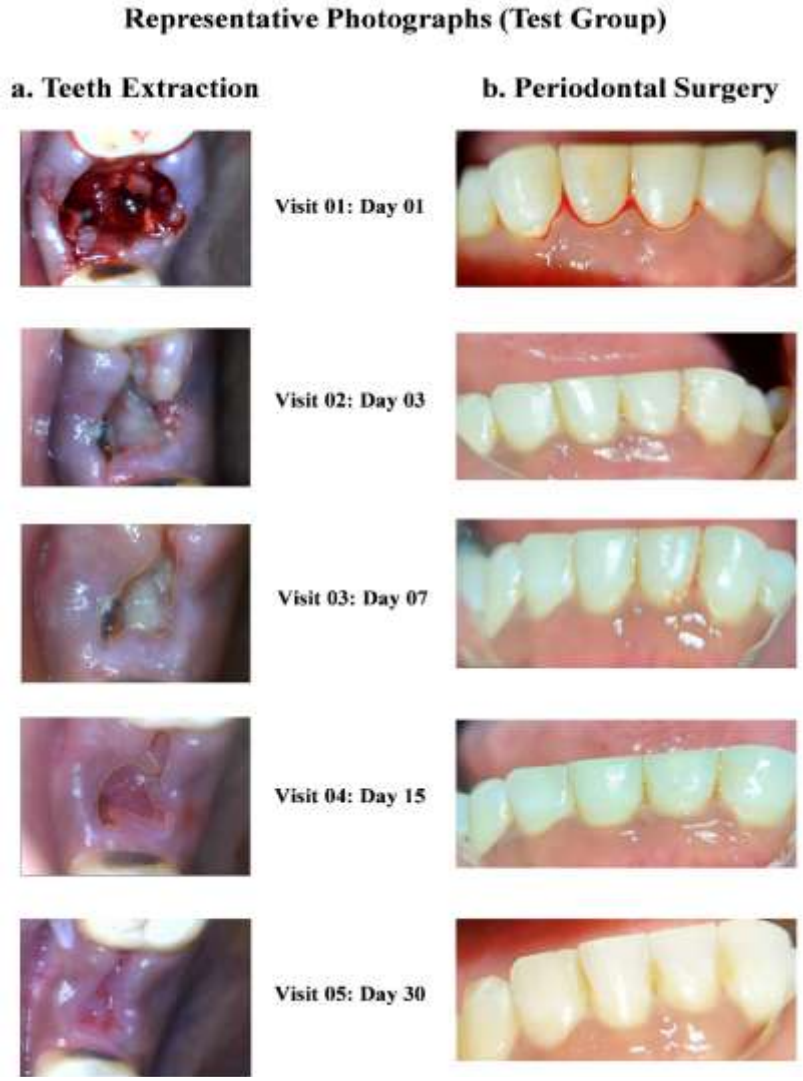

Figure 3: Representative photographs showing oral wound healing.

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3.2.3 Assessment of Overall Gum Health by Dental Examination

At baseline (i.e., Day 01), 100% subjects exhibited abnormal gum condition in terms of bleeding, colour, consistency and texture in both the study arms. On Day 03, 100% subjects showed normal gum tissue in terms of bleeding,where change was statistically significant (P=0.0002) in test group, whereas, the ratio of normal gum tissues in placebo group was only 56%. Likewise, 80% subjects showed normal gum tissue whereas 20% subjects showed abnormal gum condition in terms of colour [change was statistically significant (P<0.0001)] in test group. This ratio was very low in placebo group where only 20% subjects exhibited normal gum tissues.

In terms of consistency and texture, 96% subjects showed normal gum tissue whereas 4% subjects showed abnormal gum condition [change was statistically significant (P<0.0001)] in test group. In control group, on the other hand, only 8% and 16% subjects showed normal gum tissue in terms of consistency and texture respectively.

However, on Day 07 and Day 15, 100% subjects demonstrated normal gum tissue in terms of bleeding, colour, consistency and texture in both the study arms (Table 5).

Table 5: Overall Gum Health

Parameter	Treatment Group	Visit 1 (Day 01)		Visit 2 (Day 03)		Visit 3 (Day 07)		Visit 4 (Day 15)	
		Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal
Bleeding	Test	25 (100)	0 (0.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
				p = 0.0002[a]					
	Placebo	25 (100)	0 (0.00)	11 (44.00)	14 (56.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
Color	Test	25 (100)	0 (0.00)	5 (20.00)	20 (80.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
				p <0.0001 [a]					
	Placebo	25 (100)	0 (0.00)	20 (80.00)	5 (20.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
Consistency	Test	25 (100)	0 (0.00)	1 (4.00)	24 (96.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
				p <0.0001 [a]					
	Placebo	25 (100)	0 (0.00)	23 (92.00)	2 (8.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
Texture	Test	25 (100)	0 (0.00)	1 (4.00)	24 (96.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)
				p <0.0001 [a]					
	Placebo	25 (100)	0 (0.00)	21 (84.00)	4 (16.00)	0 (0.00)	25 (100)	0 (0.00)	25 (100)

Test (N = 25), n (%); Placebo (N = 25), n (%)
Abbreviation(s): N = number of subjects in specified treatment; n = number of subjects in specified category.
[a]p-value is calculated using chi-square/fisher's exact test (if any cell frequency less than 5).

3.2.4 Assessment of Subject Satisfaction Questionnaire

Response on the Subject Satisfaction Questionnaire was recorded in a 5-point likert scale (1 = Strongly disagree, 2 = Disagree, 3 = Neither agree nor disagree, 4 = Agree and 5 = Strongly agree). After 03 days, all study subjects (100%) agreed to strongly agreedfor reduction in post-operative pain and wound healing in test group. 84% study subjects agreed to strongly agreed that overall gum health hadimproved. 28% study subjects agreedfor reduction in teeth staining.However, none of the subjects from placebo group agreed to these parameters. After 07 days, all study subjects (100%) strongly agreedfor reduction in post-operative pain, wound healing and reduction in teeth staining in test group. All study subjects (100%) also agreed to strongly agreed for improvement in overall gum health. From placebo group, only 64% study subjects agreed for post-operative pain reduction and wound healing, whereas 68% and 4% participants agreed for improvement in overall gum health and teeth staining.After 15 days, all study subjects (100%) agreed to strongly agreedfor reduction in post-operative pain and teeth staining and improvement in overall gum health in the test group. All study subjects (100%) strongly agreed that the wound seems to have healed. Even in placebo group, reduction in post-operative pain was reported in all study subjects (100%) and 96% study subjects agreed to strongly agreedfor wound healing and improvement in overall gum health. For reduction in teeth staining, the percent was equal to Day 7 i.e. 4% (Table 6, Figure 2).

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Table 6: Subject satisfaction questionnaire

Group (Arm)	Test (N = 25) n (%)	Placebo (N = 25) n (%)	Test (N = 25) n (%)	Placebo (N = 25) n (%)	Test (N = 25) n (%)	Placebo (N = 25) n (%)
Response	Strongly disagree or disagree		Neither agree nor disagree		Agreeorstrongly agree	
Question 1	**Reduction in post-operative pain**					
Visit 2 (Day 03)	0 (0.00)	6 (24.00)	0 (0.00)	19 (76.00)	25 (100.00)	0 (0.00)
Visit 3 (Day 07)	0 (0.00)	0 (0.00)	0 (0.00)	9 (36.00)	25 (100)	16 (64.00)
Visit 4 (Day 15)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	25 (100.00)	25 (100.00)
Question 2	**Wound seems to have healed**					
Visit 2 (Day 03)	0 (0.00)	6 (24.00)	0 (0.00)	19 (76.00)	25 (100.00)	0 (0.00)
Visit 3 (Day 07)	0 (0.00)	0 (0.00)	0 (0.00)	9 (36.00)	25 (100)	16 (64.00)
Visit 4 (Day 15)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.00)	25 (100)	24 (100.00)
Question 3	**Improvement in overall gum health**					
Visit 2 (Day 03)	0 (0.00)	7 (28.00)	4 (16.00)	18 (72.00)	21 (100.00)	0 (0.00)
Visit 3 (Day 07)	0 (0.00)	0 (0.00)	0 (0.00)	8 (32.00)	25 (100.00)	17 (68.00)
Visit 4 (Day 15)	0 (0.00)	1 (4.00)	0 (0.00)	0 (0.00)	25 (100.00)	24 (96.00)
Question 4	**Reduction in teeth staining**					
Visit 2 (Day 03)	0 (0.00)	8 (32.00)	18 (72.00)	17 (68.00)	7 (28.00)	0 (0.00)
Visit 3 (Day 07)	0 (0.00)	1 (4.00)	0 (0.00)	23 (92.00)	25 (100)	1 (4.00)
Visit 4 (Day 15)	0 (0.00)	1 (4.00)	0 (0.00)	23 (92.00)	25 (100.00)	1 (4.00)

Abbreviation(s): N = number of subjects in specified treatment; n = number of subjects in specified category.
Note: Percentages is based on the number of subjects in the specified treatment arm.

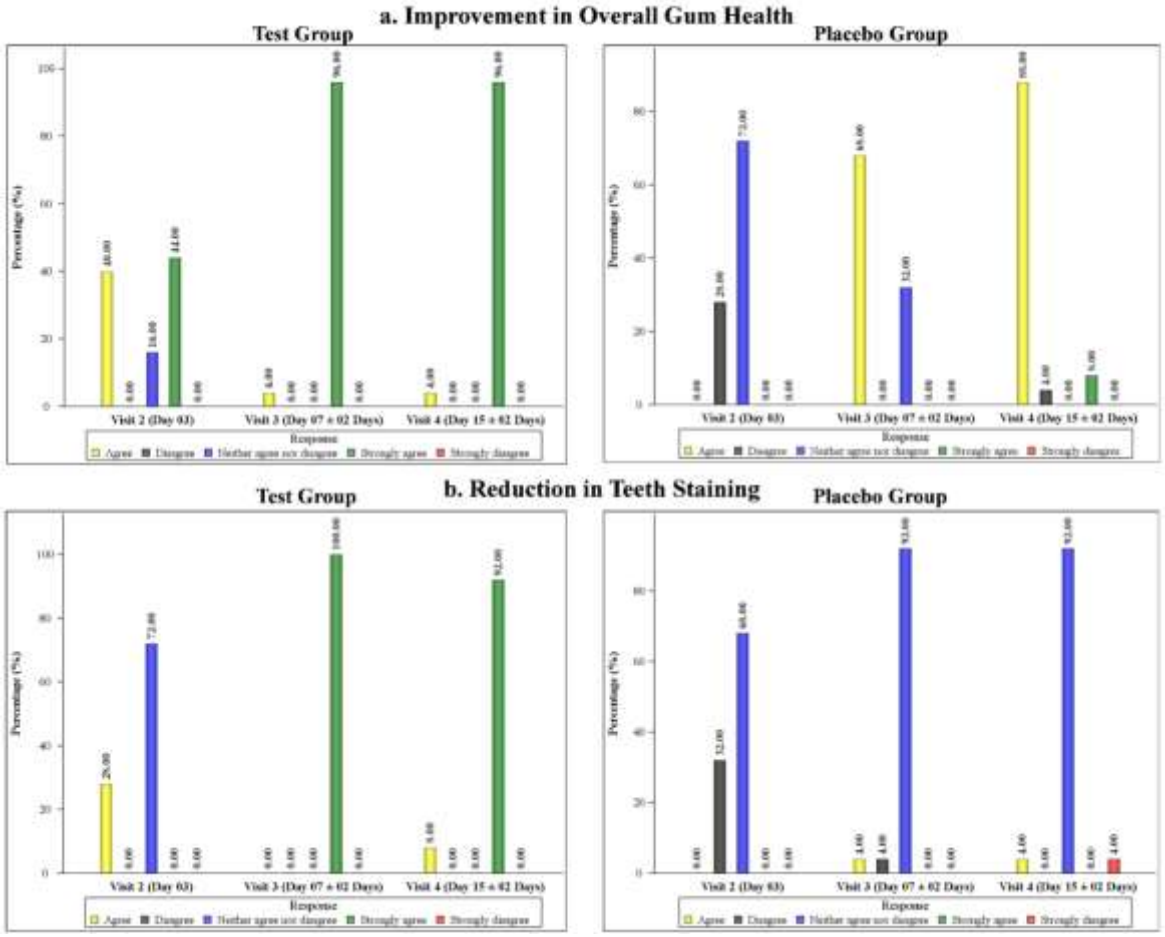

Figure 2: Graphical representation of Subjective Satisfaction Questionnaire.

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3.2.5 Assessment of Subject Response Index (Perception about Product)

At the end of treatment, i.e. Day 15, all study subjects (100%) strongly agreed that the product had an appealing taste. All the study subjects (100%) either strongly agreed or agreed that the product had an appealing appearance. For overall likeability, the ratio was 100% in test group. Whereas, the ratio in the placebo group was 64.00%, 68.00% and 72.00% for appealing taste, appealing appearance and overall likeability respectively (Table 7).

Table 7: Subject response index

Sr. No	Question	Visit	Response	Test (N = 25) n (%)	Placebo (N = 25) n (%)
1	The product has appealing taste.	Visit 4 (Day 15)	Strongly disagree	0 (0.00)	3 (12.00)
			Disagree	0 (0.00)	5 (20.00)
			Neither agree nor disagree	0 (0.00)	1 (4.00)
			Agree	0 (0.00)	15 (60.00)
			Strongly agree	25 (100)	1 (4.00)
2	The product has appealing appearance.	Visit 4 (Day 15)	Strongly disagree	0 (0.00)	6 (24.00)
			Disagree	0 (0.00)	2 (8.00)
			Neither agree nor disagree	0 (0)	0 (0)
			Agree	1 (4.00)	16 (64.00)
			Strongly agree	24 (96.00)	1 (4.00)
3	Overall did you like this product?	Visit 4 (Day 15)	Yes	25 (100)	18 (72.00)
			No	0 (0.00)	7 (28.00)

Abbreviation(s): N = number of subjects in specified treatment; n = number of subjects in specified category.
Note: Percentages is based on the number of subjects in the specified treatment arm.

3.3 Safety Assessments

During the conduct of present study, no local intolerances were observed in any of the subjects,suggestive of favorable safety profile of the test product.No adverse events (AEs) or Serious Adverse Events (SAEs) were reported, or observed during the course of the study.

IV. DISCUSSION

Healing of oral wounds created after tooth extraction procedure or other periodontal surgeries is comparatively slower than the usual dermal wounds and needs due care to avoid secondary infections that further delays the complete wound healing process. Topical antimicrobials, including mouth rinse are commonly prescribed post-surgical procedures.

The beneficial effects of CHX in the field of periodontology have been extensively studied. Often, it has been considered the gold standard and has served as a potent broad-spectrum antiseptic agent in medicine. CHX is capable of electrostatically binding to the negatively charged surfaces of bacteria due to its cationic nature. By which, it damages the outer layers of the cell wall and rendering it permeable[13]. It exhibits significant antimicrobial effect against both Gram-negative and Gram-positive bacteria, as well as certain fungi and viruses [14, 15], which attributes the healing of tissues by protecting from developing infections. Aqueous CHX solution has a wide-spectrum antimicrobial activity, even at low concentrations, and has been found effective against Candida albicans - the most common fungal species of the oral cavity [16]. This suggests that CHX exhibits good antimicrobial activity that can support the recovery and regeneration of damaged tissues by preventing infections.

Mode of action behind antibacterial activity of CHX suggests that it is a positively charged hydrophobic and lipophilic molecule. It enters the cell through active or passive transport mechanism post-interacting with phospholipids and lipopolysaccharides of the cell membrane of bacteria [17]. In a randomized controlled clinical trial, 0.2% CHX gel significantly improved oral wound healing in the test group who applied the gel every 12 hours for 7 days after extraction and a

greater wound closure and significant clinical healing (p-value < 0.05) was achieved [18]. Irrespective of the established wound healing effects and clinically proven efficacy of CHX, there is increasing need to have more ingredients that can aid the healing process aside from maintaining aseptic conditions [19].

In recent years, HA has garnered increasing attention for its potential in treating inflammatory conditions across various medical fields, including dentistry [20]. Numerous studies conducted on the effects of HA in periodontal wound healing suggest that there is direct advantage in wound healing. HA has few peculiar physiochemical and biological properties that makes it suitable for treatment of the inflammatory process in medical areas [9]. The available data of different clinical studies collectively highlight the diverse therapeutic properties of HA in periodontal health. In dentistry, HA is often used for the treatment of periodontal diseases due to itswound healing, anti-inflammatory and anti-bacterial action.

Various studies have established the efficacy and it could be applied as an adjunct to mechanical therapy in the treatment of periodontitis. It has excellent hygroscopic property. According to Sutherland (1998), when HA is incorporated into aqueous solution, hydrogen bonding occurs between adjacent carboxyl and N-acetyl groups[21]. It lets HA to keep conformational stiffness and to retain water. HA possesses good anti-inflammatory, antioxidative, and physical barrier effects that leads to promote tissue healing and preventing tissue damage [22]. Apart from anti-inflammatory action, antiedematous property is supposed to be the factorfor faster wound healing.In a randomized double-blind study for the treatment of plaque-induced gingivitis, HA containing gel had a significant beneficial effect on plaque indices starting from day 4 [23]. In a short-term randomized controlled clinical trial, significantly additional anti-oedematigenous effect in early healing was observed in CHX+HA mouthwash when compared to CHX group alone. However, no difference was seen in antiplaque and antigingivitis activities between both the groups [10]. In another clinic-microbiological study, it was established that using HA and CHX mouthwashes improves periodontal health in patients with periodontitis, with HA having a stronger effect, and can be utilized as a potential therapeutic intervention in treating periodontitis patients[22].

It is therefore evident from the above discussion that the products having combination of CHX and HA can be utilized prospectively for retaining oral hygiene post-surgical process. Results of the present study also substantiate the fact, where the test product containing CHX and HA in aqueous vehicle was evaluated for faster wound healing in the patients post oral surgical procedures. The product demonstrated significant improvement in wound closure rate on Day 03, Day 07 and Day 15 (P<0.0001) within group leading to better wound healing. Wound healing in the test group was significantly more than the placebo group on Day 07 (P=0.0011) and Day 15 (P<0.0001), indicating effectiveness of the test product in faster healing of the wound caused due to oral surgical procedures. PHI of the wound also demonstrated the same trend, where significant improvement on Day 07 and Day 15 (P<0.0001) was recorded. PHI was significantly higher in the test group than the placebo group on Day 07 (P=0.0032) and Day 15 (P=0.0005). It also exhibited symptoms of early healing and improving overall gum health (in terms of color, consistency, texture and bleeding) in the patients and helped in reducing the redness and swelling of gums by providing antiseptic environment to check the infections and microbial growth. In addition, the trial participants also endorsed the test product for reducing the post-operative pain and teeth staining, aiding the healing of the wound and improving overall gum health. The product was well-perceived by the subjects in terms of its appealing taste, appearance and overall likeability. Safety of the test product was also established based on no apparent or experienced discomfort, any intolerance or adverse skin reactions/events reported during the study conduct.

We acknowledge few limitations of the present clinical study. This study was limited by lower sample size and placebo controlled study design with no active control group to compare. Further evidences are required to determine the true impact of test product in larger population with different types of oral wound by comparing with the available treatment options. The authors have an opinion that study conduction on a larger patient pool and controlled comparative trial design will certainly remediate the aforesaid limitations and will be helpful in establishing the safety and efficacy of the mouth rinse test product as an adjuvant therapy to the patients of oral post-surgical procedures.

V. CONCLUSION

In this clinical study, the mouth rinse formulated with CHX and HA was found safe and significantly more effective than the placebo in aiding the healing of the wound caused during oral

surgical procedures, including tooth extraction. It also aided in improving overall gum health (in terms of color, consistency, texture and bleeding) and reduced the post-operative pain, inflammation and teeth staining in patients. The product was well-perceived by the subjects in terms of its appealing taste, appearance and overall likeability. Based on the results, the test product can be recommended as an adjuvant to the patients of oral post-surgical procedures.

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