Effect of A Natural Extract Toothpaste on the Bacteria Colonies of Initial Dental Plaque Colonizers

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Effect of A Natural Extract Toothpaste on the Bacteria Colonies of Initial Dental Plaque Colonizers

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Abstract: *To determine the effect of a natural extract toothpaste Parodontax®on the numbers of initial dental plaque colonizers.*
Methods: *AQuasi- clinical trial type of study was conducted on 48 participants (age between 18 and 30 years). The study participants were students attending a technical training institute in Nairobi County, Kenya.Streptococcus mutans, Lactobacilli and Staphylococcus aureus were the three microorganisms chosen for culture and study. All visible supragingival dental plaque was collected using sterile swabs at baseline and at the end of three weeks. The plaque samples were then cultured and the colony forming units of Streptococcus mutans, Lactobacilli and Staphylococcus aureus were calculated before and after commercially available Parodontax® use. The plaque index of Silness and Loe (1964) was used to determine oral health status while the gingival index (Loe and Silness, 1963) was used to determine gingival health.*
Results: *There was a reduction in the mean gingival index from 1.10 ± 0.47 beforeParodontax® use to 0.58 ± 0.37 after Parodontax® use. Average plaque score before Parodontax® use was 1.33± 0.58and 0.68 ± 0.37 afteruse. Use of natural extract toothpaste was found to reduce the colony forming units of Streptococcus mutans by49.14 ± 14.24, Lactobacilli by 31.40 ± 23.76,while that of Staphylococcus aureus reduced by 3.82 ± 12.97.*
Conclusion: *Within the limits of this study, a natural extract toothpaste, Parodontax® significantly reduced the colony forming units of the initial dental plaque colonizers. Gingival index was found to decrease with the reduction of the colony forming units of the early dental plaque colonizers suggesting a possible link between the two.*
Keywords: *gingivitis, Streptococcus mutans, Lactobacilli and Staphylococcus aureus,colony forming units.*

I. Introduction

Plaque associated oral disease affects a considerable proportion of the population and is considered one of the major causes of tooth loss. Tooth brushing is an effective method used to remove accumulated dental plaque on the tooth surface. This leads to the reduction of putative pathogens and thus a shift of the microbial environment to normal flora. In the presence of normal flora, the periodontal condition is usually stable. However poor brushing habits are common and are reflected in the high worldwide prevalence of gingivitis and other oral conditions [1]. Tooth brushing alone only removes 50% of dental plaque [2]therefore, additional mechanical and antimicrobial measures are necessary to further reduce the bacterial load [3]. In an endeavor at arriving at the ideal toothpaste formulation that significantly reduces plaque bacteria, various ingredients have been tried. The use of natural extract toothpastes may provide an alternative to the use of conventional synthetic ingredients such as triclosan used for their antimicrobial properties in toothpastes. Natural extract toothpastes contain substances that are extracted from nature and then isolated and purified by various environmentally sound techniques such as: filtration, fermentation, distillation, expressing and other like processes [4]. Some dental experts encourage the use of toothpastes containing natural ingredients such as plant extracts which achieve antibacterial effects and are not carcinogenic. It is also important to bear in mind that unless these natural extract toothpastes have been proven through research, to accomplish antibacterial effects, switching from conventional toothpastes to natural extract may not be necessary. The brand names and label composition are not enough reasons to make a switch from conventional toothpastes to natural ones.

Okpalugoet al.(2009) [5]found that some toothpaste brands significantly increased bacterial counts. The reason for this is not clear but may be due to the ingredients (sodium saccharin and other sweeteners) in the toothpastes. The natural extract toothpaste under study does not contain these sweeteners and may therefore be effective in dental plaque reduction. The toothpastes are formulated such that they contain medicinal andprophylactic components which ensure reduction of oral bacteria and thus prevent some forms of periodontal

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diseases. However, the brushing technique and frequency of brushing is also important in the reduction of oral micro-organisms [6].

This study is a useful guide for determining the effectiveness of natural extract toothpaste on the bacterial colonies of the initial dental plaque colonizers. The aim of this study was to determine the effectiveness of Parodontax®herbal toothpaste(GlaxoSmithKline Consumer Healthcare, Registered in England and Wales No. 3888792. Registered Office: 980 Great West Road, Brentford, Middlesex, TW8 9GS, United Kingdom. Manufacture by Demiclen, Slovakia) on the bacterial colony forming units of the early dental plaque colonizers andinvestigate its relationship with gingivitis.This particular herbal toothpaste is available commercially in Nairobi, Kenya. The active ingredients of the brand used in this study were; sodium bicarbonate, purified water, glycerine, cocamidopropyl betaine, rhatany tincture, peppermint oil, cornmint oil terpeneless #28, coneflower expressed juice stabilizer, xanthan gum, myrrh tincture, tincture of chamomile, sodium fluoride USP (0.31% w/w), oil of sage, sodium saccharin USP, iron oxide red E172 (contains at least 70% w/w specialized ingredients).The individual components are reputed to have a variety of medicinal properties which have been shown to control diseases of the periodontium such as gingivitis [7].Sodium bicarbonate (45%) acts as an abrasive. Sage (Salvia officinalis) is known for its antibacterial, tissue strengthening and deodorizing effects. Coneflower (Echinacea purpurea) is added into the toothpaste formulation for its immunomodulatory action and effectiveness in the treatment of chronic infections. Rhatany (Krameria triandra) is a good astringent. Coneflower, rhatany and sage have been shown by Willerhausen et al.(1991) [8] to have anti-inflammatory and antiseptic effect. Chamomile (Matricaria recutita) provides antibacterial and anti-inflammatory effects and is used for its wound healing properties. Myrrh (Commoiphora molmol) possesses tissue strengthening and styptic properties. Kitagaki et al. (1983) [9] were able to demonstrate antibacterial effect of chamomile and myrrh.

II.   Materials And Methods

This was a quasi – clinical trial to investigate bacterial colony forming units of the early dental plaque colonizers before and after three weeks of Parodontax®use. There were no controls in this study since it was not a randomized clinical trial and alternate explanations were not being sort as it is only the effect of the toothpaste on specific micro-organisms namely Streptococcus mutans, Lactobacilli and Staphylococcus aureusthat was being investigated. This was an observational study where all participants underwent the same experimental protocol. This being a pretest – posttest within participant design which is explained by Shadish et al. in 2001 [10]. It is an inexpensive method used to collect preliminary data.The study analyses look at within subject effects (before and after).

The study introduced natural extract toothpaste (Parodontax®) to individuals who had never used it before and assessed its effect on the specific organisms named above. The colony forming units of the specific bacteria was determined before the intervention and then again after three weeks.Individual bacterial counts were not done, rather, the counts were of the colony forming units. The commercially available Parodontax® was used in the study. The samples that were used in this study were obtained from the free samples distributed by GSK Kenya (GSK, Likoni Rd, Industrial Area, P.O.Box 78392 00507, Kenya) to patients attending the University of Nairobi Dental Hospital, Kenya as a marketing strategy. The formulation of Parodontax® used in this study is different than the commercially available Parodontax® described at http://www.parodontax.com and sold in other countries.

2.1 Subjects

Forty-eight participants with age ranging from 18-30 years with a mean of 22.50 ± 2.52 were recruited voluntarily into the study and all gave written consent. 29 (60.4%) were males while 19 (39.6%) were females. They were selected from a population of 327 students comprising of 197 males and130 females who were currently in session at the time of the study. The sample size was calculated from a formula described by Gorstein in 2007 [11] during a survey of vitamin and mineral status of populations.

$$n = \frac{Z\alpha^2 p(1-p)(DEFF)}{d^2}$$

$$n = \frac{1.96^2 \times .5 \times .5(0.5)}{.1^2} = 48.02$$

Where:
1. $Z\alpha$ - Z-score at the level of precision (1.96)
2. P is the estimate of the expected proportion of colony count reduction (0.5).
3. d is the desired level of absolute precision (0.1)
4. DEFF is the Estimated design effect (50% - 0.5) ml

Therefore, a sample of 48 respondents was used to assess the intervention.

Systematic convenience sampling was employed during sample selection. The convenience sampling was based on availability of the participants on the specific days the data was collected. Every 5th student who fit the inclusion criteria was selected given the student population in order to get the desired sample size. All students who satisfied the inclusion criteria and gave consent were given an equal chance to participate in the study (as long as they were the 5th candidate) to give students in the various levels equal opportunity to participate in the study.Fig 1 explains the process followed.

The inclusion criteria were students who were willing to brush every 12 hours with natural extract toothpaste for a period of three weeks and gave written consent to voluntarilyparticipate in the study. The exclusion criteria included;students with any visible tooth or mouth infection as this would have an effect on microbial load, any individual suffering from systemic diseases such as diabetes, hypertension or suffering from transmissible diseases. The immunity of such individuals tends to be compromised and this can affect the results of the study, students not willing to brush 12hourly with Parodontax®.Students already using natural extract toothpaste and those with clinical attachment loss were also excluded.This study only examined effect on gingival inflammation.All the participants recruited into this study were found to have some form of gingivitis.

A pilot study was conducted at the polytechnic to confirm validity and reliability of the questionnaires. The principal investigator was calibrated by EGW an experienced periodontist. Cohen's Kappa score was used to calculate inter-examiner and intra-examiner reliability of the periodontal examination. The principal investigator re-examined every 5th subject to assess intra-examiner variation.
Only calibrated laboratory equipment was used and a scheduled list of calibration maintained. The Kenyatta National Hospital laboratory maintains a strict calibration schedule as required by the manufacturers.

Authority to carry out this study was sought from Ethics and Standards Committee of KNH and University of Nairobi and the permit number is KNH-ERC/A/306. Written authority to carry out the research at the Kinyanjui technical training institute was sought and given by the institute's management. Written consent was given by every subject included in the study. A signature was accepted as proof for voluntary consent.

2.2 Assessment of oral and gingival health
The oral health status was assessed using the plaque index by Silness and Loe, 1964 [12]. This scores for the amount of plaque on the surfaces of the index teeth. The gingival index by Loe and Silness, 1963 [13] was used to assess for the amount of gingival inflammation. The index teeth 16, 11, 25, 36, 41 and 45 were scored for plaque and gingival index; if an index tooth was missing then the tooth distal to it was scored.

2.3 Plaque collection and laboratory technique.
Forty-eight plaque samples were collected from the students around the same time midmorning just before lunch by the principal investigator. This was done under natural light on a normal college chair. All visible supragingival plaque was collected using sterile swabs from the lingual and buccal aspects of all the teeth. The bacteria were then cultured and grown on agar plates and bacteria colonies counted. Each study participant used Parodontax® toothpaste 12-hourly every day as the only dentifrice, for a period of three weeks.Supragingival plaque was then sampled again from the buccal and lingual surfaces of all teeth using sterile swabs after Parodontax® use.The dental plaque samples were kept in air tight containers and delivered to the laboratory at the earliest opportunity and within one hour. The swabs were washed in 10ml of sterile normal saline. Ten-fold serial dilutions of washed swab was made up with sterile normal saline and plated using the pour plate technique. The diluted sample (0.5ml) was delivered by pipette into 19.5 ml of blood agar and MacConkey plates. The plates were then incubated upside down in an incubator at 37°C for 24 h and colony forming units of oral bacterial flora counted after 24 hr. Bacterial colonies of only initial plaque colonizers were counted. These are the Streptococcus mutans, Lactobacilli and Staphylococcus aureusspecies. Percent bacterial reduction was calculated from the difference in bacterial counts on the agar plates before and after Parodontax® use.

2.4 Statistical analysis
Statistical Package for the Social Scientists (SPSS version 20.0) was used to perform the tests. Levene's test of homogeneity was used to test for equality of variances among the different parameters. The percent reduction in bacterial colonies of streptococcus mutans, lactobacilli and staphylococcus aureus was calculated before and after use of Parodontax®. When there was normal distribution, the relationship between oral microbial colonies and gingival status was analyzed using chi-square and t-test. In the current study, there was normal distribution. Statistical significance was set at $p < 0.05$.

III. Results

The mean results for inter-examiner reproducibility, of the Cohen's Kappa were 1.00 for plaque score and 1.00 for gingivitis. A total of 9 participants were re-examined and then Cohen's Kappa scores were obtained as 1.00 for plaque score and 1.00 for gingivitis. An alpha level of 0.05 was used for all statistical tests. There was homogeneity of variance between the two groups as far as age is concerned and the Levene's test of homogeneity of variances showed a non-statistically significant difference in variances for age by gender (F = 0.075, p = 0.785). An Independent t- test was subsequently run on the data and showed a non-statistically significant difference in participants' age between females (22.84± 2.65) and males (22.28± 2.45), t(46) = 0.758, p = 0.452. The participants in this study (68.75%) mainly brushed their teeth once a day.23(79.3%) of the male participants brushed once daily while 6 (20.7%) brushed twice a day. On the other hand,10(52,6%) of the female participants brushed once a day while 9 (47.4%) of them brush twice daily.

3.1 Oral health status

The prevalence of plaque was 100% with the mean plaque score beforeParodontax ® use being 1.33±0.58. All participants were found to have gingival inflammation and the mean gingival index was1.10±0.47 beforeParodontax®.

3.2 Changes after using Parodontax ®

There was a reduction in the mean gingival index from 1.10 ± 0.47 before Parodontax ® use to 0.58 ± 0.37after Parodontax ® use. Average plaque score before Parodontax ® use was 1.33 ± 0.58 and 0.68 ± 0.37 after Parodontax ® use as shown in Table 1.

3.3 Colony forming units

Use of natural extract toothpaste was found to reduce the colony forming units of Streptococcusmutans by49.14 ± 14.24, Lactobacilli by 31.40 ± 23.76while that of Staphylococcus aureus reduced by3.82 ± 12.97 as shown in Table 2.

3.4 Relationship between plaque scores and gingivitis

Results of this study showed a statistically significant association between participants' plaque scores and their gingival index. Fig2 shows a curve estimation oflinear regression model which elicited a statistically significant association between pre-test plaque scores and pre-test gingival scores, $F(1, 46) = 156.166$, $R^2 = 0.773$, n = 48, p =0.001. This showed that there was a positive increase in gingival index as the plaque score increased. A curve estimation linear regression model elicited a statistically significant association between post plaque scores and post gingival scores as shown in Fig 3.

IV. Discussion

The study population consisted of 48 college students of African descent who were attending a technical training institute mostly after high school. Out of the 48 participants, 29 (60.4%) were males while 19 (39.6%) were females. This could be explained by the fact that males are more inclined naturally to technical courses such as the diploma in electrical engineering, diploma in plumbing and civil engineering that are a majority of the courses offered at the technical training institute. The age of the participants in the present study ranged between 18 – 30 years with a mean of 22.50 and a narrow standard deviation of 2.52. This narrow age range was attributed to the fact that a majority of the participants were young adults seeking tertiary education after completing secondary education. This meant that the age range would not be diverse since majority of the students join the institution after completing high school education. The participants in this study mainly brushed their teeth once a day. The frequency of tooth brushing was not found to be significantly associated with the oral health status of the study participants.There was no significant difference between the age of the participants and their plaque score. In the current study plaque score correlated with gingival index. The mean plaque score in this urban population before Parodontax® use was 1.33 ± 0.58despite majority of the participants (68.75%) reporting brushing only once daily.

In this study, only early plaque colonizers were under investigation. The detection of the bacterial colonies of Streptococcus mutans, Lactobacilli and Staphylococcus aureus in this study was lower than previously reported in other studies [14]. This may be due to the use of the less sensitive culture technique. Other methods like polymerase chain reaction which uses actual DNA extraction to detect microorganisms is more sensitive and will yield higher levels of detection. The mean gingival index measured by the Loe-Silness index (1963) [13] was 1.10 ± 0.47 before Parodontax® use and 0.58 ± 0.37after Parodontax® use. The mean gingival index was not statistically significant when males and females were compared. This finding is different from that reported in a Tanzanian population where the male gender was described as a risk factor for periodontal disease [15]. There was correlation between plaque score and gingival index in these participants

with gingival index increasing with increase in plaque scores. The results of this open – label randomized clinical trial demonstrated the tested natural extract toothpaste was effective and led to an improvement in oral hygiene and also in the gingival status (as assessed using the Gingival Index and plaque score) in the study participants. These results were similar to those by Makarem in 2007 [16]. After 21 days use of natural extract toothpaste significant reduction in dental plaque accumulation on the tooth surfaces was shown. This was a double blinded clinical trial among boys of 11 – 12years with the same socio-economic status. Final values of plaque scores were significantly lower at 56% reduction for the natural extract tooth paste containing barberry extracts as opposed to 18.5% for the placebo compared to baseline values.

Using the commercially available natural extract toothpaste Parodontax ®increased the effectiveness of plaque control, among the study participants as elicited by the decrease in plaque scores after Parodontax® use. Probably, active ingredients of the herbal extracts penetrate the biofilm and prevent plaque accumulation [17]. Results of other clinical studies confirm the long-term plaque- and gingival bleeding-reduction properties of natural extract toothpastes [18, 19]. In this study, Parodontax ® tooth paste was effective in reducing of dental plaque. This may be due to the three pillars of strength in Parodontax ®toothpaste which include attack, defend, and fortify. Signs of gingival inflammation reduced significantly. Parodontax® toothpaste was found to decrease the colony forming units of the initial dental plaque colonizers Streptococcus mutans, Lactobacilli and Staphylococcus aureus. The results of this study clearly demonstrated that natural extract toothpaste could inhibit the growth of several cariogenic and pathogenic bacteria, however, the effectiveness varied against the different tested microorganisms. Study results are in agreement with Darmani et al. 2006 [20] who examined the effects of miswak extracts on the growth of the various cariogenic microorganisms including Streptococcus mutans. The result showed inhibition in growth of Streptococcusmutans.

The disruption of the initial dental plaque colonizers with natural extract toothpaste will then prevent maturation of the microbial biofilm by interfering with the attachment of late colonizers and consequently reducing gingival inflammation. Within the limitations of this study, we were able to demonstrate the relationship between the number of the colony forming units of the early dental plaque colonizers and gingival index. It was observed that there was an increase in gingival index of the participants as their plaque scores increased.

4.1 Limitations of the study

There were no controls in this study. Controls are normally used to eliminate alternate explanations of the experimental results. In this study, alternate explanations were not being sort as it is only the effect of the toothpaste on specific micro-organisms that was under investigation.Therefore, cause and effect cannot be conclusively claimed to be due to the intervention. However, the data provides good information for future experimentation. Home-use toothpaste studies are often influenced by a number of factors which can mask the superiority of the test agent. One factor that may influence the outcome of these investigations is the Hawthorne effect. Participants in clinical trials may experience some improvement associated not to the therapeutic properties of the test agent but rather related to a behavior modification, as a consequence of the sheer participation in the trial. Subjects participating in oral hygiene studies improve their tooth brushing, irrespective of the product they receive.

V.   Conclusion

There was statistically significant difference between the number of the colony forming units of Streptococcus mutans, Lactobacilli and Staphylococcus aureusat baseline and at the end of 3 weeks of Parodontax ® use. Association was drawn between the colony forming units and gingival index.

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TABLES

Table 1. Pretest-Posttest analysis of participants' clinical characteristics and microbial colony forming units

Characteristics	n (%)	M+SD	95% CI		df	T		p-value
Plaque scores								
Pretest	48 (100.0)	1.33+0.58	0.49 – 0.81		47	8.030**		<0.001
Posttest	48 (100.0)	0.68+0.37						
Gingival scores								
Pretest	48 (100.0)	1.10+0.47	0.37 – 0.68		47	6.748**		<0.001
Posttest	48 (100.0)	0.58+0.37						
Strep mutans								
Pretest	48 (100.0)	531.89+105.84	245.24 – 299.34		47	20.251**		<0.001
Posttest	48 (100.0)	259.58+81.55						
Lactobacillus								
Pretest	48 (100.0)	8.06+4.48	4.43 – 7.07		47	8.765**		<0.001
Posttest	48 (100.0)	2.31+1.98						
Staph aureus								
Pretest	48 (100.0)	0.42+0.99	0.10 – 0.53		47	2.894*		0.006
Posttest	48 (100.0)	0.10+0.37						

Paired-Samples t-test was used.
** p< 0.001
* p< 0.05

Table2. Descriptive characteristics of percentage change in the colony forming units for Streptococcus mutans, Lactobacillus and Staphylococcus aureus

% change	n (%)	Mean ± SD	Range	Minimum	Maximum	Mode
		Descriptive characteristics				
Strep mutans	48 (100.0)	49.14 ± 14.24	80.00	0.00	80.00	50.00
Lactobacillus	48 (100.0)	31.40 ± 23.76	75.00	0.00	75.00	0.00
Staph aureus	48 (100.0)	3.82 ± 12.97	50.00	0.00	50.00	0.00

Descriptive statistics (means, SD, range and mode) analysis was used.

Table 3. Correlation between percentage change of Streptococcus mutans, Lactobacillus and Staphylococcus aureus with gingival Index and plaque scores

Characteristics	n (%)	Strep mutans		Lactobacillus		Staph epidermidis	
		R	p-value	r	p-value	R	p-value
Plaque scores							
Pretest	48 (100.0)	-0.183	0.214	0.106	0.471	0.285*	0.049
Posttest	48 (100.0)	0.125	0.396	0.120	0.418	0.253	0.083
Gingival scores							
Pretest	48 (100.0)	-0.278	0.056	0.253	0.083	0.371*	0.009
Posttest	48 (100.0)	0.100	0.498	0.026	0.858	0.280	0.054

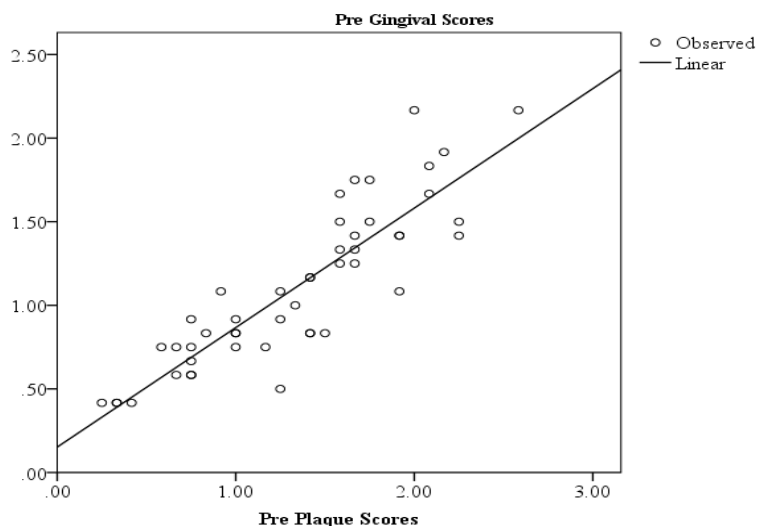Pearson Correlation Coefficient r analysis was used.
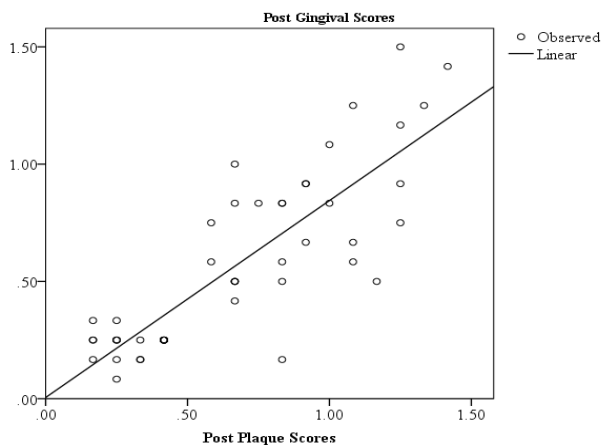* p< 0.05

Figures
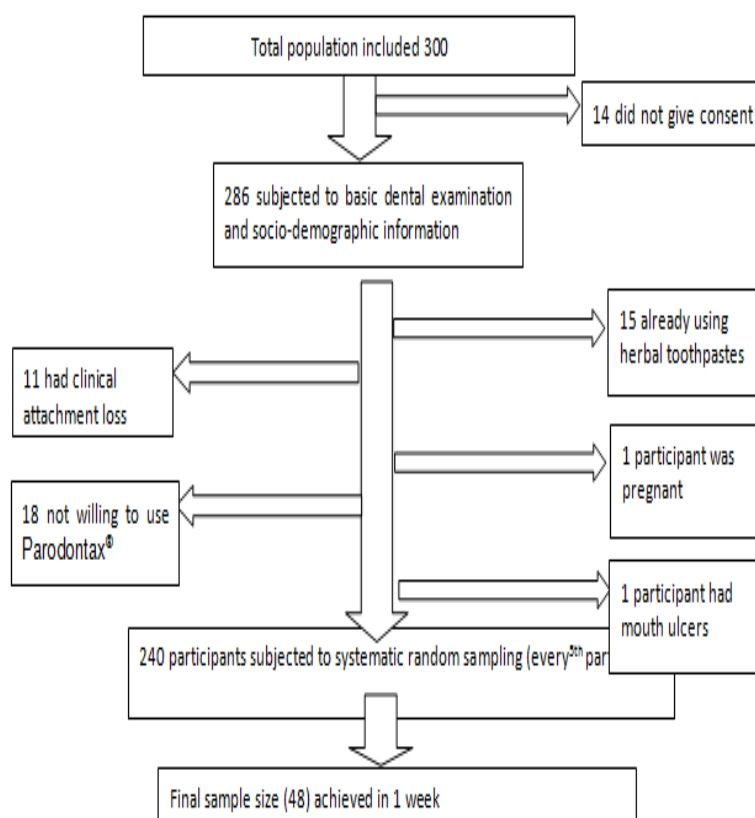
Figure 1: Participant recruitment schematic

Figure 2: Plot model of pre-test plaque scores and pre-test gingival scores the probability of the plaque score given the gingival score.

Figure 3: Plot model of post plaque scores and post gingival scores.

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