



**“EFFICACY OF HERBAL AND NON-HERBAL TOOTHPASTE IN THE REDUCTION OF PLAQUE, GINGIVITIS AND SALIVARY NEUTROPHIL COUNT”- A RANDOMIZED CLINICAL STUDY**

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**ABSTRACT**

**Introduction:** Periodontal disease is a continuum of oral diseases of polymicrobial origin, characterized by a bacterial plaque induced gingival inflammation which if left untreated, may lead to chronic infection and loss of attachment. Before the clinical signs of disease become evident, neutrophils start appearing, acting as a first line of defense against the invading microbes. Therefore, plaque control using toothbrush and toothpaste play a key role in the prevention of these diseases. But some of the substances of conventional toothpastes show undesirable side effects such as tooth staining and altered taste sensation. This had led to paying increased attention on using natural ingredients in herbal dentifrices. **Aim:** To compare the efficacy of herbal toothpaste (Dantkanti) and non-herbal toothpaste (Colgate Total) in the reduction of plaque, gingivitis and salivary neutrophil count. **Method:** 40 dentate subjects who fulfil the inclusion and exclusion criteria were enrolled. At the baseline visit they were randomly assigned to herbal toothpaste group (Dantkanti group) and non-herbal toothpaste group (Colgate Total group) according to their baseline gingival scores followed by estimation of plaque score, salivary neutrophil count. All subjects were instructed to use their respective products and recalled after 4 weeks for re-evaluation. **Results:** Both at baseline and after 30 days, there was no significant difference ( $p > 0.05$ ) between both the groups in reduction of plaque, gingivitis and salivary neutrophil count. **Conclusion:** Herbal toothpaste was as effective as non herbal toothpaste in reduction of plaque, gingivitis and salivary neutrophil count.

**KEYWORDS:** Herbal toothpaste, Non herbal toothpaste, Gingivitis, Plaque, Neutrophil count.

**INTRODUCTION**

Dental plaque, which is considered as the precursor of dental caries and periodontal diseases, is a microbial biofilm which is continuously present on the hard and soft tissues of the oral cavity and it contains various microorganisms.<sup>[1]</sup> Periodontal disease is characterized by a bacterial plaque induced gingival inflammation which, if left untreated, may lead to chronic infection and loss of attachment. Clinically, disease begins with gingivitis, which is inflammation of the gingiva in response to the bacterial biofilm on adjacent teeth. Before the clinical signs of gingivitis become evident, neutrophils start appearing, acting as a first line of defense against the invading microbes.<sup>[2]</sup> Neutrophils are White Blood Cells that circulate in the blood stream and migrate to the site of the infection to kill the invading microbes.<sup>[2]</sup> These cells migrate through the gingival crevices also into the oral cavity and act against

microbes. Even with optimal plaque control, the junctional epithelium is never sterile and neutrophils exit the gingival microvasculature and enter into the periodontal tissue.<sup>[3]</sup> Neutrophil count in the gingival exudates reflects its degree of inflammation.<sup>[15]</sup> Thus neutrophil count increases in the gingival sulcus during the course of gingivitis.<sup>[4]</sup> Therefore prevention and control of periodontal disease is dependent on optimal plaque control. Many mechanical aids are used worldwide to remove or control plaque, including tooth brushes, dental floss, mouth rinses and dentifrices.<sup>[5]</sup> But plaque control using toothbrush and toothpaste play a key role in the prevention of these diseases.<sup>[6]</sup> Hence, various chemical formulations were tried in dentifrices to prevent plaque and gingivitis. But some of these substances show undesirable side effects such as tooth staining and altered taste sensation.<sup>[7]</sup> This had led to paying increased attention on using natural ingredients in

herbal dentifrices which have fewer side effects. There are very few studies related to efficacy of herbal and non herbal toothpaste in reduction of neutrophil count, which is an inflammatory biomarker for many oral diseases including periodontal diseases. Therefore, an attempt was made to compare the efficacy of herbal toothpaste (Dantkanti) and a triclosan based non herbal toothpaste (Colgate total) which is one of the toothpaste available in the market, in reduction of not only plaque and gingivitis but also salivary neutrophils count.

### AIM

To compare the efficacy of herbal toothpaste (Dantkanti) and non-herbal toothpaste (Colgate Total) in the reduction of plaque, gingivitis and salivary neutrophil count.

### OBJECTIVES

To determine the mean gingival, plaque scores and salivary neutrophil count among people using herbal and non herbal toothpaste at baseline and after 30 days of usage and to compare percentage changes in the mean gingival, plaque scores and the salivary neutrophil count among these two groups after 30 days of usage.

### MATERIALS AND METHOD

The present study was a randomized, 2-cell parallel design study, conducted in the Department of Public health Dentistry, SDM College of Dental Sciences and Hospital, Dharwad. Prior to the start of the study Ethical clearance was obtained by the Ethical Review Committee, S.D.M. College of Dental Sciences and Hospital, Dharwad.

#### Sample size determination

Sample size is calculated using the formula

$$n_0 = Z^2 \sigma^2 / e^2$$

Z = Standard variate value (1.96 at 5% and 2.58 at 1% level of significance)

e = Acceptable error (0.05)

$\sigma$  = SD of the sample/population

A study was done to evaluate the effectiveness of two different herbal toothpaste formulations in the reduction of plaque and gingival inflammation in patients with established gingivitis. The standard deviation (SD) of the mean gingival score at 30 days interval in Colgate Herbal group (i.e. SD = 0.11) was considered to determine the sample size.<sup>[8]</sup>

$$n_0 = \frac{(1.96)^2 \times (0.11)^2}{(0.05)^2} = 18.58$$

Therefore sample size for the present study was 38 (19 subjects each in the two groups).

#### Source and number of subjects

In this study forty healthy subjects aged above 18 years belonging to both the Genders, who fulfilled the inclusion exclusion criteria and signed the informed

consent form were taken into the study and their demographics were recorded.

#### Inclusion criteria

People aged above 18 years, belonging to both the genders and in good general health, who are capable to read, understand and sign the informed consent form, with Scorable facial and lingual surfaces of a minimum of 20 sound natural teeth, not more than 4 pockets and pockets less than 6 mm and a Gingival and plaque Index score of  $\geq 1.0$  and  $\geq 1.5$  respectively and subjects should be available for the entire study duration.

#### Exclusion criteria

Subjects were excluded if they have destructive periodontal disease, Significant soft tissue pathology, severe gingivitis/systemically related gingival enlargement, History of diabetes, hepatic, renal disease or other serious medical condition and transmissible disease, Orthodontic appliance or any kind of fixed or removable appliances, History of allergies to dental products or their ingredients, Pregnant and breast feeding women, History of adverse habits like smoking and tobacco chewing, Patients under antibiotics, steroid therapy or any anti inflammatory drugs in the previous month, Oral prophylaxis in the preceding month or periodontal treatment in the preceding 3 months or participation in any other plaque and gingivitis clinical study involving oral products within the last 30 days

#### Method

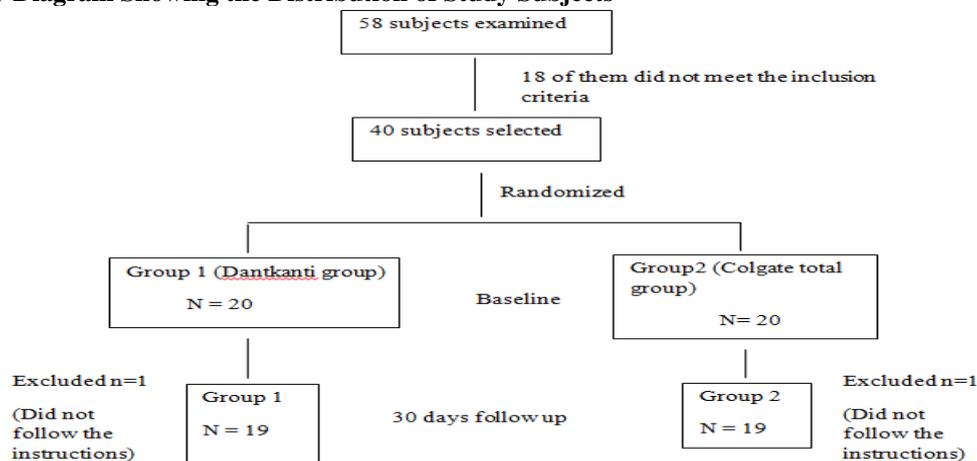
A total 58 subjects were examined for the present study. 40 of them were selected, since 18 of them did not meet the inclusion criteria. For standardization purpose all the subjects who were selected underwent a washout period for 7 days. All were given a similar washout toothpaste and a toothbrush (Colgate MaxFresh toothpaste and a medium bristled Colgate toothbrush) for the purpose of uniformity and were asked to use it for 7 days and were recalled on the 8th day for the baseline visit without brushing and rinsing. On the day of baseline visit, subjects were given 10 ml of saline and were asked to rinse with it for 30 seconds and expectorate it into a 50 ml falcon tube. This oral rinse was collected for the purpose of oral neutrophil estimation, which was examined using fluorescent microscope under blue light. The collected samples were vortexed for 30 secs for the proper dispersion of neutrophil cells. 500  $\mu$ l of the sample was then transferred into a vial and to it 500  $\mu$ l of acridine orange dye was added. Then 0.1  $\mu$ l of this solution was introduced into the Neubauer chamber using a pipette. Neubauer chamber was then placed on the fluorescent microscope stage. Microscope was then focused until sharp image of the cells are seen. Cell counting was started from the first grid square, likewise neutrophil count was done in all the 4 grid squares of both the chambers. Then the total cell count was divided by 2 and the final count was calculated per ml. After the collection of saliva sample, gingival and plaque scores of the subjects were recorded using Loe and Silness

gingival index<sup>[9]</sup> (modified) and Quigley and Hein plaque index<sup>[10]</sup> (1962) modified by Turesky et al (1970) respectively. Subjects were randomized into two study groups - Group 1: Herbal toothpaste group (Dantkanti group) and Group 2: Non Herbal toothpaste group (Colgate total group) based on their baseline mean gingival scores. Their washout toothpastes and toothbrushes were taken back and their respective study products were issued and instructed to use similar sort of brushing technique. Subjects were recalled after 30 days of their baseline visit to the clinic without brushing and rinsing. Gingival and plaque scores and neutrophils counts were recorded using the above mentioned indices and also toothpastes were weighed to check the compliance of subjects.

### Compliance

All the subjects were given a reminder related to the usage of their products and their visits through a phone call and through text messages at interval of one week, during the study period. Subjects were asked to get their respective products on each visit. Products were weighed and compared with the actual weight of the product, so as to get to know how much product had been used. If any subject did not use the product in required amount or did not follow the instruction, he was again instructed regarding the usage. The investigator considered the subjects as drop out if they did not follow the instructions.

### Consort Flow Diagram Showing the Distribution of Study Subjects



### Statistical Analysis

The gingival scores, plaque scores and the oral neutrophil count of the subjects at each visit were entered into the computer (MS-Office 2007, Excel data sheet). The data was subjected to statistical analysis using the statistical package (SPSS version 20). Shapiro-Wilk test was done to assess Normality. Since the majority of the variables followed normal distribution, parametric test was applied. Comparison of the gingival scores, plaque scores and the oral neutrophil count at baseline and final visit, within the group was done using paired t test and between the groups were done using unpaired t test.

### Study Products

All the subjects in both the groups (Dantkati and Colgate total group) received their respective toothpastes and toothbrushes throughout the study. Toothbrush used was a medium bristled Colgate toothbrush.

### Prohibited / allowable medications or precautions

Subjects were advised to not to use any other mouth rinse, dentifrice or oral hygiene devices other than the study materials during the study. Due to any health issue if the subject underwent any medication, he had to inform the investigator regarding medication so that the investigator could drop the subject from the study or else it may interfere with the results.

### Subject progress and discontinuation

Subjects were considered to have completed the study if they were followed up throughout the duration of the study. Subjects could be dropped out if they failed to substantially comply with the protocol requirement, failed to report for a scheduled examination, if they received emergency dental or medical treatment or any medication that may interfere with the parameters under study, developed serious adverse reactions, if they chose to terminate participation in the study and discontinued treatment or relocated.

Statistical significance was recorded if the P-value was 0.05 or less.

### RESULTS

This study was conducted among 40 subjects for a period of 30 days. They were randomly allocated into two groups of 20 subjects each. Group 1: Herbal toothpaste group (Dantkanti Group), Group 2: Non Herbal toothpaste group (Colgate Total Group). The subjects were followed for a period of 30 days after issuing the respective products. At the end of 30 days, a total of 38 subjects were available for the follow up, with 19

subjects in each group with an overall attrition rate of 5% (n=2). [Table 1].

Independent sample t test was applied, which showed that there was no significant difference between the two groups in the mean Gingival scores, Plaque scores and oral neutrophil count at the baseline visit ( $p>0.05$ ). [Table 2].

After 30 days, the comparison of gingival scores, plaque scores and neutrophils count was done between two groups using Independent sample t test. The p value states that even after one month there was no significant

difference between both the groups with respect to all the three parameters ( $p>0.05$ ). [Table 3].

Statistically there was no significant difference in the percentage change in mean gingival score, plaque score and neutrophil count between the two groups in 30 days interval ( $p>0.05$ ). [Figure 1].

Comparison of gingival scores, plaque scores and neutrophil counts at baseline visit and after 1 month within the groups using paired t test showed a significant difference ( $p<0.05$ ) in both the groups. [Table 4].

**Table 1: Duration of the study and number of subjects dropped out.**

Group	Subjects at the beginning of the study	Duration of the study (weeks)	Number of study subjects at the end of study period (n)	Number of drop outs	Percentage of drop outs
Group 1 Herbal toothpaste group (Dantkanti Group)	20	4	19	1	5%
Group 2 Non Herbal toothpaste group (Colgate Total Group)	20	4	19	1	5%
Total (N)	40	4	38	2	5%

**Table 2: Comparison of gingival scores, plaque scores and neutrophil counts at baseline visit between the two groups.**

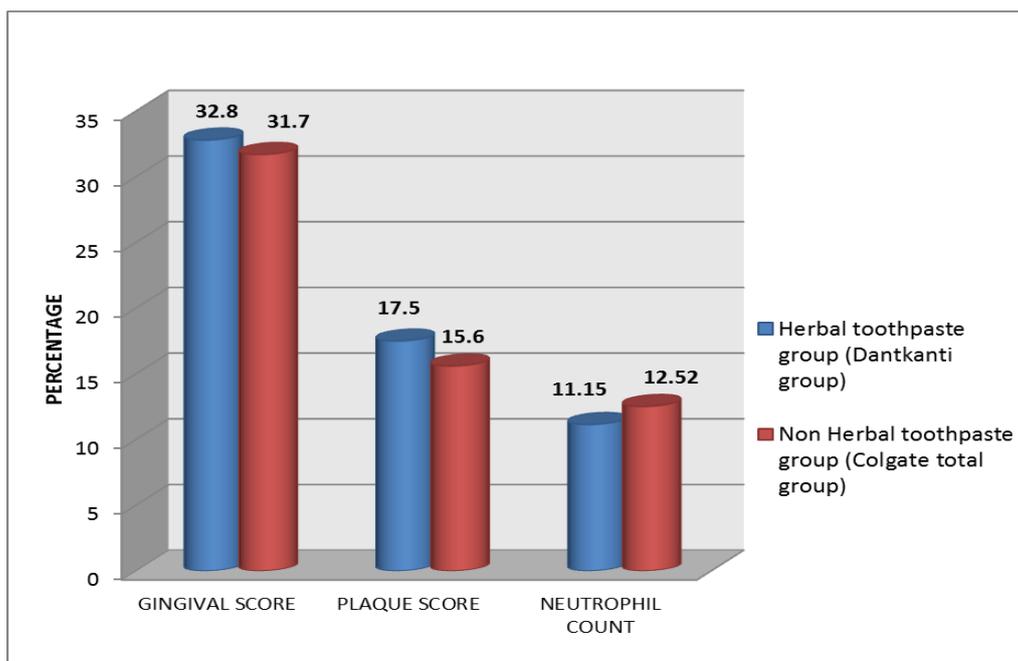
Variables	Herbal toothpaste group (Dantkanti group)	Non Herbal toothpaste group (Colgate total group)	P value*
	Mean $\pm$ S.D	Mean $\pm$ S.D	
Gingival score	1.253 $\pm$ 0.302	1.277 $\pm$ 0.282	<b>0.792</b>
Plaque score	2.757 $\pm$ 0.294	2.867 $\pm$ 0.384	<b>0.315</b>
Neutrophil count in lakhs	0.727 $\pm$ 0.395	0.661 $\pm$ 0.402	<b>0.604</b>

SD- Standard Deviation,  $p\leq 0.05$ .

**Table 3: Comparison of gingival scores, plaque scores and neutrophil counts after 30 days between the two groups.**

Variables	Herbal toothpaste group (Dantkanti group)	Non Herbal toothpaste group (Colgate total group)	P value*
	Mean $\pm$ S.D	Mean $\pm$ S.D	
Gingival score	0.902 $\pm$ 0.328	0.932 $\pm$ 0.284	<b>0.762</b>
Plaque score	2.396 $\pm$ 0.302	2.56 $\pm$ 0.368	<b>0.143</b>
Neutrophil count in lakhs	0.647 $\pm$ 0.373	0.563 $\pm$ 0.364	<b>0.487</b>

SD- Standard Deviation,  $p\leq 0.05$ .



Graph 1: Percentage reduction in mean gingival scores, plaque scores and neutrophil count among the two groups in 30 days interval.

Table 4: Comparison of gingival scores, plaque scores and neutrophil counts at baseline visit and after 1 month within the two groups.

Variables	Herbal toothpaste group (Dantkanti group)		Non Herbal toothpaste group (Colgate total group)	
	Baseline	1 month	Baseline	1 month
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Gingival score	1.253 ± 0.302	0.902 ± 0.328	1.277 ± 0.282	0.932 ± 0.284
P value*	0.000		0.000	
Plaque score	2.757 ± 0.294	2.396 ± 0.302	2.867 ± 0.384	2.56 ± 0.368
P value*	0.000		0.000	
Neutrophil count in lakhs	0.727 ± 0.395	0.647 ± 0.373	0.661 ± 0.402	0.563 ± 0.364
P value*	0.000		0.000	

SD- Standard Deviation,  $p \leq 0.05$ .

## DISCUSSION

This randomized double blind clinical study was done to check the efficacy of herbal (Dantkanti) and non-herbal toothpaste (Colgate Total) in the reduction of plaque and gingivitis in addition with salivary neutrophil count.

The results of this study showed that at base line both the gingival score and plaque score in both the groups were  $>1$  and  $>1.5$  respectively, which fulfils our inclusion criteria. And at the base line there was no significant difference ( $P < 0.05$ ) between both the groups regarding all the 3 clinical parameters, which states that subjects were randomly distributed in both the groups.

By the end of 30 days it was noticed that, both herbal and non herbal toothpaste groups showed **17.5%** and **15.6%** reduction of plaque scores respectively. Reduction of plaque was statistically not significant between the groups at the end of 30 days ( $P = 0.143$ ) [Table 2]. This is in accordance with the reports of Ozaki *et al.* (19.9% and 18.3% reduction, respectively, for herbal and non-herbal dentifrices)<sup>[11]</sup> and de Oliveira *et al* with the herbal product Aloe vera in plaque reduction.<sup>[7]</sup> It shows that herbal toothpaste is as effective as non herbal toothpaste in the reduction of plaque.

By the end of 30 days, both the herbal and non herbal toothpaste groups showed **32.8%** and **31.7%** reduction of gingival scores respectively. Reduction of gingivitis

was not statistically significant between the groups ( $P=0.762$ ). This is in agreement with the report by George *et al*, 28.4% and 36.3% reduction in gingivitis, respectively.<sup>[12]</sup> Whereas Singh *et al* found significant reduction in gingivitis in herbal (Dantkanti) group as compared to non herbal group ( $P<0.05$ ).<sup>[13]</sup> This difference may be due to their different control product.

Neutrophil count was used as another clinical parameter in this study to check the efficacy of herbal and non herbal toothpaste, since it appears before the clinical signs of gingivitis appear and it can be used as salivary biomarker in diagnosis of periodontal diseases in early stage. By the end of 30 days, both herbal and non herbal group showed **11.15%** and **12.52%** reduction of neutrophils count respectively. This reduction was not significant between the group ( $P= 0.487$ ). It shows that herbal toothpaste is as effective as non herbal toothpaste in reduction of neutrophil count.

The results of paired t-test showed, within the group there was a significant reduction in plaque, gingivitis and salivary neutrophil count in 30 days in both the herbal and non herbal toothpaste groups. This significant difference may be due to changed attitude of the patients towards toothbrushing because of continuous motivation during the study period.

No adverse reactions to dentifrice products were observed in the present study and there was no significant difference in the dentifrice tube weights between both the groups after 30 days usage, which shows that all the subjects equally used the toothpastes.

Colgate total toothpaste contains triclosan and fluoride. Triclosan has antibacterial property and it is well effective against plaque and gingivitis. Fluoride has anti caries properties.<sup>[14]</sup> Although in this 30 days period no adverse reactions were noticed in the non herbal toothpaste group, but studies have shown that conventional toothpastes have some undesirable side effects like staining of teeth, altered taste sensation with a long term usage.<sup>[5]</sup> Therefore herbal products may be advised to use, which are also equally effective.

The herbal toothpaste (Dantkanti) contains Akarkara (anacyclus pyrethrum), Neem (azadirachta indica), Babool (acacia arabica), Pudina (menthe spicata), Long bud (syzygium aromaticam), Tomar (xan-thoxylum alatum), Haldi (curcuma longa), Pilu (salvadora persica), Bakul (mimusops elengi), Vidang (Embelia ribes) etc. The above herbal ingredients have the known effect of antibacterial, anti in-flammatory and anti cariogenic properties.<sup>[13]</sup>

#### Limitations of the study

Since the study was conducted for 30 days and for a noticeable change in gingivitis, it takes longer time, so a clinical trial for a longer duration may give a more coherent picture of the effectiveness of herbal dentifrice

in the reduction of plaque, gingivitis and neutrophils count.

#### CONCLUSION

Based on the results obtained, it can be concluded that, clinically herbal dentifrices are as effective as non-herbal (conventional) dentifrices in the reduction of plaque, gingivitis and salivary neutrophils count.

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Nil.

#### Conflicts of interest

There are no conflicts of interest.

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