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Clinical and microbiologic effects of commercially available gel and powder containing *Acacia arabica* on gingivitis

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ABSTRACT

Background: There is a need for an anti-plaque agent that can be used on a daily basis without the side effects of antibacterial chemicals such as chlorhexidine. The present study was designed to evaluate the clinical and microbiologic effects of commercially available gel and powder containing *Acacia arabica* in subjects with gingivitis.
Methods: One hundred and twenty subjects with chronic generalized gingivitis were selected and randomly divided into four groups: Group 1 – placebo group; Group 2 – *Acacia arabica* gel group; Group 3 – *Acacia arabica* powder group; and Group 4 – 1% chlorhexidine gel group. Microbial counts of plaque samples, the gingival index of Loe and Silness and the plaque index were evaluated at baseline, 6 weeks, 12 weeks and 24 weeks. Microbial counts of plaque samples were evaluated at all visits.
Results: *Acacia arabica* gel and powder showed significant clinical improvement in gingival and plaque index scores as compared to a placebo. This improvement was comparable to 1% chlorhexidine gel. The difference between gel and powder with regard to clinical and microbiological parameters was not found to be significant at any time interval.
Conclusions: Both *Acacia arabica* gel and powder may be useful herbal formulations for chemical plaque control in subjects with gingivitis.

Keywords: Antimicrobial, antioxidant, clinical trial, gingivitis, herbal medicine.

Abbreviations and acronyms: ANOVA = analysis of variance; GI = gingival index; PI = plaque index.

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INTRODUCTION

Periodontal diseases are chronic infectious diseases characterized by a bacterial challenge that can provoke a destructive host response, leading to periodontal attachment loss and ultimately possible tooth loss.[1,2] It is well established that supragingival plaque is the cause of gingivitis and plays a primary role in the initiation of periodontitis.[1] The removal of microbial plaque leads to resolution of gingival inflammation and cessation of plaque control leads to recurrence of inflammation.

Mechanical plaque removal using a toothbrush and other oral hygiene aids has been found to be an effective way to control gingival inflammation.[3] The human limitations associated with adequate mechanical plaque removal have resulted in widespread occurrence of gingivitis.[4,5]

The widespread occurrence of gingivitis provides the rationale for supplementing toothpastes with anti-gingivitis agents that augment mechanical plaque removal.[6] Many chemical agents have been tested as adjuncts to mechanical methods which can reduce plaque and its associated gingivitis. Several antibacterial chemicals, like chlorhexidine, have been used. However, side effects such as discolouration of teeth and unpleasant taste can occur when these chemicals are prescribed for an extended period.[7] There is still a need for an anti-plaque agent that can be used on a daily basis with minimal side effects.

The gum of *Acacia arabica* has been an article of commerce for thousands of years. The tree with its gum is pictured during the reign of Ramses III and later inscriptions of ancient Egypt. It was exported to the gulf of Aden in 1700 BC and was mentioned by Theophastus in the third century BC under the name of

'Egyptian gum'.[8] *Acacia* gum consists primarily of *Arabica*, a complex mixture of calcium, magnesium and potassium salts of Arabic acid. It contains tannins which are reported to exhibit astringent, haemostatic and healing properties.[9] It also contains cyanogenic glycosides in addition to several enzymes such as oxidases, peroxidases and pectinases, all of which have been shown to exhibit antimicrobial properties.[8,10] In some African and Asian countries, *Acacia* products are used to treat stomach disturbances, to cover inflamed and burned surfaces, and to stop nasal bleeding. The bark constituents of *Acacia catechuit* are used to treat stomatitis, gingival bleeding, improve appetite and to give a pleasant taste.[10] The boiling product of *Acacia rrugineais* is used as a rinse for gingivitis in general and mouth ulcers in particular.[10]

Clark *et al*. has reported the antibacterial and anti-protease activities of *Acacia arabica*.[11] Gazi concluded that *Acacia* gum has the potential to inhibit early plaque formation although the long-term effect may not be there.[12]

The short-term clinical effects of a commercially available prescription gel containing *Acacia arabica* in the reduction of plaque and gingival inflammation in subjects with gingivitis were assessed and it was found that its efficacy is comparable to chlorhexidine.[13] Apart from this, *Acacia arabica* gel can be used for an extended period of time unlike chlorhexidine which causes tooth discolouration and has an unpleasant taste.

Acacia arabica powder is a polyherbal formulation with a similar composition to *Acacia arabica* gel. Thus, the present study was designed to evaluate the short-term clinical and microbiologic effects of commercially available prescription gel and powder containing *Acacia arabica* in the reduction of plaque and gingival inflammation in subjects with gingivitis, and to compare their affects with the 1% chlorhexidine gel.

MATERIALS AND METHODS

After ethical approval was granted, 120 dentate subjects (60 males, 60 females, mean age 27.37 years) who reported to the Department of Periodontics, Government Dental College and Research Institute, Bangalore, India were recruited for the study conducted from November 2010 to April 2011. Informed consent was obtained from all potential subjects. Group sample sizes were decided by power analysis with 95% power and a significance level of 0.05.

Subjects diagnosed with chronic generalized gingivitis, aged 25–40 years, having at least 20 natural teeth, with no history of periodontal therapy or previous use of antibiotics or anti-inflammatory medication within the preceding six months were included in the study. All patients fulfilled the clinical criteria of the gingival index (Loe and Silness)[14] >1, pocket probing depth ≤3 mm, clinical attachment loss = 0, with no evidence of radiographic bone loss. Subjects with known allergies to the constituents of the formulation, haematological disorders or other systemic illness, pregnant and lactating females, undergoing orthodontic treatment and with smoking habits were excluded.

Each subject was randomly assigned by computer generated number sequence to one of the four groups (30 subjects in each group): Group 1 – placebo (Charak Pharma Pvt Ltd, India); Group 2 – *Acacia arabica* gel (Charak Pharma Pvt Ltd, India); Group 3 – *Acacia arabica* powder (Charak Pharma Pvt Ltd, India); Group 4 – 1% chlorhexidine gel (Hexidine gel, ICPA Health Products Ltd, India).

The main ingredient of both the polyherbal gel and powder was *Acacia arabica*, and the others being *Barleria prionitis, Emblica officinalis, Terminalia chebula, Terminalia belerica, Vitex negundo, Quercus infectoria, Melia azadirachta*, *Acacia catechu*, *Messua ferrea* and *Embelia ribes*.

Each 40 gm of the gel contained 0.8 w/w % *Acacia arabica*, 0.4 w/w % *Barleria prionitis,* 0.24 w/w % *Emblica officinalis,* 0.24 w/w % *Terminalia chebula,* 0.24 w/w % *Terminalia belerica,* 0.2 w/w % *Vitex negundo,* 0.08 w/w % *Quercus infectoria,* 0.04 w/w % *Melia azadirachta*, 0.24 w/w % *Acacia catechu*, 0.02 w/w % *Messua ferrea and* 0.02 w/w % *Embelia ribes*. Similarly, each 40 gm of powder contained 8 gm *Acacia arabica*, 4 gm *Barleria prionitis,* 2.4 gm *Emblica officinalis,* 2.4 gm *Terminalia chebula,* 2.4 gm *Terminalia belerica,* 2 gm *Vitex negundo,* 0.8 gm *Quercus infectoria,* 0.4 gm *Melia azadirachta*, 2.4 gm *Acacia catechu*, 0.2 gm *Messua ferrea and* 0.2 gm *Embelia ribes*.

Subjects accepted to participate in the study returned for a baseline examination. Subjects were assessed for plaque using the plaque index (PI) (Tureskey *et al.* modification of Quigley Hein Index)[15,16] and gingival inflammation using the gingival index (GI) (Loe and Silness),[14] as well as for oral soft tissue status. Following the assessments, all subjects received a supragingival prophylaxis and polishing to remove plaque, calculus and extrinsic stain. After prophylaxis, patients were given the respective sample along with a diary to record product usage and daily oral hygiene activities by the investigator (PB). Subjects were instructed to apply half a teaspoonful of powder or gel on the gums, to be rubbed on the gums and teeth with the index finger and left for about 5 minutes. Regular use in the morning and evening after brushing was advised. All samples were covered in plain white packets to ensure blinding. Subjects were asked to refrain from all other unassigned forms of oral hygiene, including dental floss, chewing gum or oral rinses during the study. Subjects were assessed for gingivitis using the GI[14] and for plaque, using the PI[15,16] in the

same dental unit under identical conditions at baseline, 6 weeks, 12 weeks and 24 weeks (ARP).

At baseline and at each visit, the dental plaque sample was collected from each subject (EA). Each volunteer was asked to gargle with saline to remove any food debris. Taking all aseptic measures, the plaque was collected from the marginal gingiva of the lower first molar tooth using a sterile paper point so that the standardized length of the paper point (coloured area) touched the tooth for 5 seconds. This specimen was immersed in 1 ml of phosphate buffered saline (PBS). These plaque specimens were vortexed for 10 seconds and subcultures were immediately performed on mitis salivarius (MS) agar for streptococcus species and GMC medium for *Actinomyces* species, taking 5 ml of plaque in PBS.

The colonies of *Streptococcus sanguis*, *Streptococcus mitis*, *Streptococcus intermedius*, *Streptococcus orali*, *A. viscosus* and *A. naeslundii* were identified based on colony morphology. Colonies with similar morphology were counted using a colony counter, their numbers recorded and the total number taken into account.

Apart from clinical and microbiologic evaluation, subjective evaluation was also undertaken at each visit using a questionnaire relating to the taste and flavour or any adverse effect experienced after use.

Statistical analysis

Analysis of data was carried out using SPSS 10.5 (SPSS, Chicago, IL, USA). The values of different parameters collected are expressed as means + standard Deviation (SD). Normality of continuous data was tested using the Kolmogorov–Smirnov test. Mean together with percentage change from baseline at 6 weeks, 12 weeks and 24 weeks were calculated. Comparison between the three treatment groups and within each treatment group was performed using one-way analysis of variance (ANOVA). Pairwise comparisons of groups were made using the Bonferroni procedure if the ANOVA statistics were significant at the 0.05 level.

RESULTS

Seven subjects did not complete the study and were excluded from the analysis (Fig. 1). There was no significant difference between the groups with respect to any parameter at baseline. There was a gradual decrease in the PI, GI scores and microbial counts by the 6-week, 12-week and 24-week time interval, respectively, in all the groups (Table 1).

A significant percent reduction was observed in PI in all groups at all time intervals except in group 1 between 6 and 12 weeks and 12 and 24 weeks. A significant reduction was also observed in GI in all groups at all time intervals except in between 12 and 24 weeks. Microbial counts also showed a significant reduction in all groups at all time intervals except in group 1 between 12 and 24 weeks (Table 2 and 3).

No significant difference was found between group 4 and group 3 for any parameter except for GI at 12 weeks. Also, no significant difference was found between group 4 and group 2 and between group 2 and group 3 for any parameter at any interval. However,

Table 1. PI, GI scores and microbial counts of all groups at different follow-ups

Group	PI scores at baseline and different follow-ups			
	Baseline	6 weeks	12 weeks	24 weeks
1	4.435 ± 0.704	3.574 ± 0.763	3.327 ± 0.178	3.110 ± 0.734
2	4.575 ± 0.666	3.602 ± 0.727	2.916 ± 0.716	2.348 ± 0.666
3	4.609 ± 0.545	3.760 ± 0.664	3.085 ± 0.667	2.651 ± 0.608
4	4.435 ± 0.649	3.585 ± 0.806	2.972 ± 0.745	2.474 ± 0.720
	GI scores at baseline and different follow-ups			
	Baseline	6 weeks	12 weeks	24 weeks
1	1.913 ± 0.379	1.527 ± 0.339	1.260 ± 0.285	1.206 ± 0.326
2	2.158 ± 0.499	1.349 ± 0.270	0.969 ± 0.195	0.707 ± 0.273
3	2.024 ± 0.413	1.463 ± 0.285	1.198 ± 0.244	0.873 ± 0.254
4	2.102 ± 0.393	1.355 ± 0.563	2.102 ± 0.393	0.778 ± 0.372
	Microbial counts at baseline and different follow-ups (× 1000000)			
	Baseline	6 weeks	12 weeks	24 weeks
1	30.60 ± 2.12	26.13 ± 2.78	24.32 ± 3.12	24.20 ± 3.31
2	30.16 ± 3.10	19.29 ± 3.54	14.23 ± 2.59	10.68 ± 2.30
3	29.93 ± 3.20	19.42 ± 3.52	14.23 ± 2.59	10.68 ± 2.30
4	29.95 ± 2.79	20.85 ± 2.91	15.06 ± 2.51	11.15 ± 2.02

Table 2. Percent reduction in PI, GI and microbial counts at different follow-ups

Group	% reduction in PI scores at different follow-ups		
	6 weeks	12 weeks	24 weeks
1	−19.73 ± 9.32	−24.74 ± 11.80	−30.17 ± 11.45
2	−21.82 ± 7.04	−36.94 ± 9.26	−49.28 ± 10.1
3	−18.83 ± 7.58	−33.35 ± 10.08	−42.67 ± 9.98
4	−19.58 ± 13.53	−33.31 ± 13.75	−44.63 ± 13.57
	% reduction in GI scores at different follow-ups		
	6 weeks	12 weeks	24 weeks
1	−19.75 ± 10.61	−33.52 ± 10.41	−35.68 ± 18.15
2	−35.06 ± 15.45	−53.20 ± 11.93	−65.89 ± 13.78
3	−26.73 ± 9.92	−39.69 ± 11.38	−55.85 ± 12.70
4	36.45 ± 23.78	54.26 ± 21.32	62.94 ± 17.59
	% reduction in microbial counts at different follow-ups (× 1000000)		
	6 weeks	12 weeks	24 weeks
1	−14.57 ± 7.52	−20.49 ± 9.02	−20.92 ± 9.40
2	−36.11 ± 9.40	−52.80 ± 7.33	−64.56 ± 6.68
3	−35.18 ± 9.17	−52.40 ± 7.44	−64.24 ± 6.90
4	−30.52 ± 6.75	−49.74 ± 7.32	−62.67 ± 6.34

Table 3. Intragroup comparison at various follow-ups (Bonferroni test)

Visit	Comparison between weeks	% reduction from baseline					
		PI		GI		Microbial count	
		Mean diff.	'p' value	Mean diff.	'p' value	Mean diff.	'p' value
Group 1	6 vs 12	5.011	0.237	13.774	<0.001*	5.918	0.03*
	6 vs 24	10.442	0.001*	15.932	<0.001*	6.352	0.017*
	12 vs 24	5.431	0.172	2.157	1.000	0.434	1.000
Group 2	6 vs 12	15.114	<0.001*	18.139	<0.001*	16.686	<0.001*
	6 vs 24	27.455	<0.001*	30.829	<0.001*	28.452	<0.001*
	12 vs 24	12.341	<0.001*	12.690	0.002	11.765	<0.001*
Group 3	6 vs 12	14.520	<0.001*	12.964	<0.001*	17.221	<0.001*
	6 vs 24	23.845	<0.001*	29.121	<0.001*	29.062	<0.001*
	12 vs 24	9.324	0.001*	16.156	<0.001*	11.841	<0.001*
Group 4	6 vs 12	13.734	0.001*	17.809	0.005*	19.221	<0.001*
	6 vs 24	25.050	<0.001*	26.490	<0.001*	32.149	<0.001*
	12 vs 24	11.316	0.005*	8.680	0.342	12.928	<0.001*

*Statistically significant.

Table 4. Intergroup comparison at various follow-ups (Bonferroni test)

Visit	Comparison between groups	% reduction from baseline					
		PI		GI		Microbial count	
		Mean diff.	'p' value	Mean diff.	'p' value	Mean diff.	'p' value
6 weeks	4 vs 3	−0.7502	1.000	−9.722	0.119	4.66	0.189
	4 vs 2	2.247	1.000	−1.389	1.000	5.594	0.061
	4 vs 1	0.148	1.000	−16.706	0.001*	−15.948	<0.001*
	2 vs 3	−2.997	1.000	−8.332	0.271	−0.934	1.000
	2 vs 1	−2.009	1.000	−15.317	0.002*	−21.543	<0.001*
	3 vs 1	0.898	1.000	−6.984	0.554	−20.608	<0.001*
12 weeks	4 vs 3	0.036	1.000	−14.567	0.001*	2.659	1.000
	4 vs 2	3.627	1.000	−1.059	1.000	3.06	0.793
	4 vs 1	−8.575	0.025*	−20.741	<0.001*	−29.251	<0.001*
	2 vs 3	−3.591	1.000	−13.507	0.003*	−0.400	1.000
	2 vs 1	−12.20	<0.001*	−19.681	<0.001*	−32.311	<0.001*
	3 vs 1	−8.611	0.024*	−6.174	0.604	−31.911	<0.001*
24 weeks	4 vs 3	−1.955	1.000	−7.091	0.052	1.572	1.000
	4 vs 2	4.652	0.695	2.95	1.000	1.897	1.000
	4 vs 1	−14.46	<0.001*	−27.264	<0.001*	−41.745	<0.001*
	2 vs 3	−6.607	0.158	−10.041	0.089	−0.324	1.000
	2 vs 1	−19.11	<0.001*	−30.214	<0.001*	−43.643	<0.001*
	3 vs 1	−12.50	<0.001*	−20.173	<0.001*	−43.318	<0.001*

*Statistically significant.

significant difference was found with respect to reduction in PI, GI and microbial counts in group 1 as compared to group 2, 3 and 4 at the end of 24 weeks (Table 4).

On subjective evaluation, all subjects gave positive responses regarding the taste and flavour of the *Acacia arabica* gel and powder and placebo. Adverse reactions like discolouration of teeth, alteration of taste or paraesthesia were not reported after use. However, about 46% of subjects reported an unpleasant taste and discolouration of teeth following the use of chlorhexidine gel. Patient compliance was checked with the assistance of diaries kept by the subjects and instructions were reinforced at each visit.

DISCUSSION

The purpose of this investigation was to determine the clinical and microbiologic effects of polyherbal gel and powder containing *Acacia arabica* on plaque and gingival inflammation in subjects with gingivitis and to compare these effects to 1% chlorhexidine gel. Both the *Acacia arabica* gel and *Acacia arabica* powder group showed significant improvement in the PI and GI scores at all time intervals. Microbial counts were significantly reduced from baseline to 24 weeks and the results were comparable to 1% chlorhexidine gel.

Chlorhexidine is an efficient anti-plaque agent. Its regular use is hindered by the unsightly staining of teeth

and tongue when prescribed for extended periods.[7] Therefore, there is a need for an anti-plaque agent that can be used on a daily basis with minimal side effects. The discolouration of teeth after the use of chlorhexidine gel may have affected the blinding to a certain extent. A non-conventional approach was used for the application of the commercially available chlorhexidine gel so that direct comparison can be made with the herbal gel and powder.

The predominant gram-positive species associated with gingivitis include *S. sanguis, S. mitis, S. intermedius, S. oralis, A. viscosus* and *A. naeslundii.*[17] Therefore, these organisms were specifically cultured to assess the microbiologic effects.

In our study, a significant reduction in PI and GI scores at all time intervals was observed with the use of both gel and powder, as well as a significant reduction in microbial counts. The positive clinical and microbiological effects of both gel and powder can be attributed to their various ingredients.

The reduction in plaque and gingivitis scores in Group 1 (placebo) can be attributed to the Hawthorne effect (i.e. patients frequently appear to improve merely from the effects of being placed in a clinical trial).[18]

Scaling and root planing was performed at the baseline so that all subjects had similar minimal levels of plaque and calculus. This might be the reason for the less obvious clinical affects noted during the study and may have also caused the small difference between the placebo and the positive control group.

The inhibitory effect of acacia gum can be attributed to active constituents like arabica, cyanoglycosides, oxidases, peroxidase and pectinases present in *Acacia*.[8,10] Tannins are also found to be present in *Acacia*, leading to its astringent and haemostatic effects.[9] The potential to inhibit early plaque formation was found in *Acacia arabica* gum as compared to sugar free gum although the long-term effect may not be there.[12] Certain *Acacia arabica* chewing sticks are found to be active against several types of cariogenic bacteria frequently found in the human oral cavity.[19] Antioxidant activity of ethyl acetate soluble fraction of *Acacia arabica* bark has been found in rats.[20] All these properties may be responsible for the antimicrobial, anti-gingivitis and anti-plaque effects of *Acacia arabica*.

The properties of various constituents of the gel and powder which can give rise to their anti-plaque and anti-gingivitis effects are listed in Table 5.

A defined role for reactive oxygen species and oxidative stress in the tissue destruction that characterizes periodontitis has been described.[21] Therefore, except for the antibacterial and anti-inflammatory

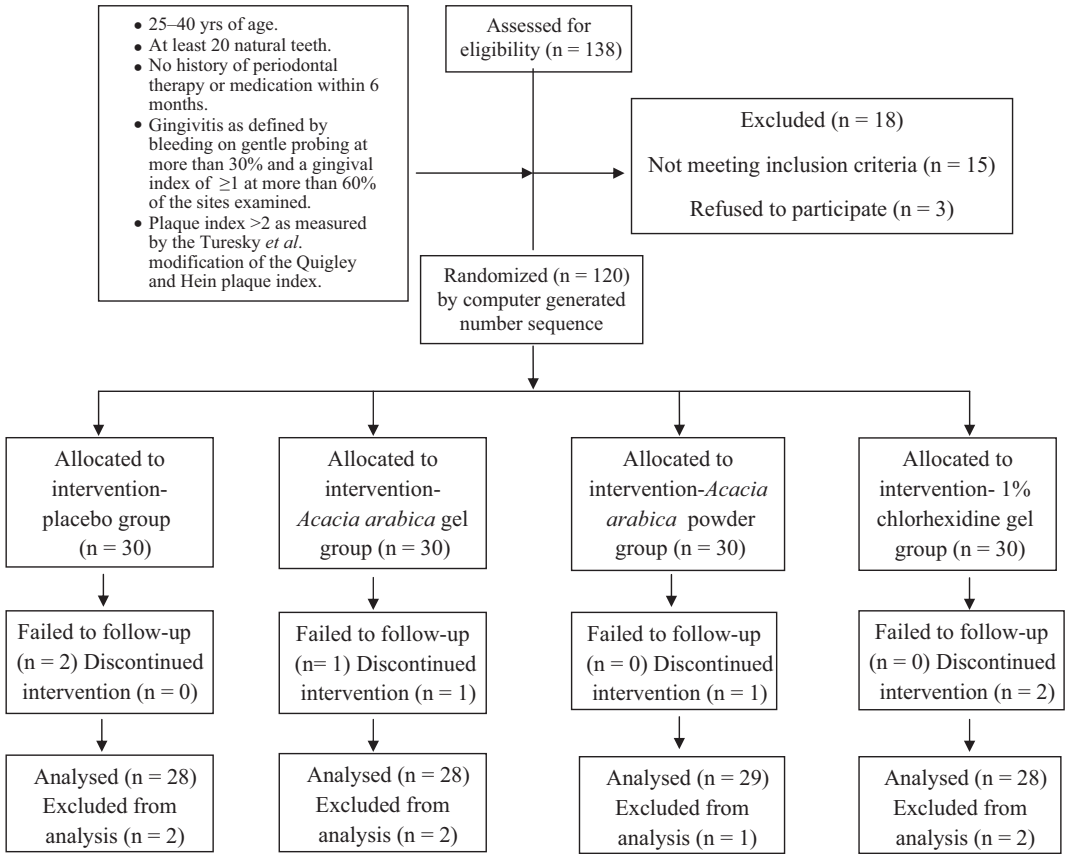

Fig. 1 Study flow.

Table 5. The various ingredients of gel and powder and their properties

Ingredient	Properties and references
Barleria prionitis	anti-inflammatory activity against different inflammagens like carrageenan, histamine and dextran along with the inhibition of vascular permeability[22]
Emblica officinalis	anti-inflammatory properties[23]
Terminalia chebula	strongly inhibits growth, sucrose induced adherence and glucan induced aggregation of *Streptococcus mutans*[24]
Vitex negundo Linn	Non-steroidal anti-inflammatory drugs (NSAIDs) like activity,[25] fresh leaves of *Vitex negundo* have been suggested to possess anti-inflammatory and pain suppressing activities possibly mediated via prostaglandin synthesis inhibition, antihistaminic, membrane stabilizing and antioxidant activities[26]
Quercus infectoria	anti-bacterial activity against the dental pathogens like *Streptococcus mutans, Streptococcus salivarius* and *Streptococcus sanguis*.[27] *Quercus infectoria* galls possess antioxidant activity and abrogates oxidative stress-induced functional alterations in murine macrophages[28]
Melia azadirachta	significant reduction of plaque and gingivitis scores compared to the placebo.[29] Tetraterpenoid mahmoddin present in *Melia azadirachta* is responsible for its antibacterial properties,[30] while nimbidin and sodium nimbidinate are responsible for its anti-inflammatory activity[31]
Acacia catechu, Messua ferrea, Embelia ribes	antioxidant properties[32,33]

effects, the role of the various constitutents as herbal antioxidants might have also resulted in improvement in gingival status. These formulations might also have an anti-caries affect which may be due to certain constituents like *Terminalia chebula* and *Quercus infectoria*. Further long-term longitudinal studies should be conducted, taking into account these two constituents as the main ingredients of the gel or powder to assess the anti-caries affect.

Although both the gel and powder have been found to be effective in improving gingival status and resulted in significant improvement in clinical and microbiological parameters, gel was found to be more effective than powder despite a similar composition. However, the difference between them was not significant. The probable reason for this finding could be because the retention of gel may be more on the gingival tissues as compared to the powder as the vehicle used in the gel is glycerin. Further long-term longitudinal studies are required to confirm the findings of this study.

CONCLUSIONS

The widespread occurrence of gingivitis provides the rationale for supplementing toothpastes with anti-gingivitis agents that augment mechanical plaque removal. *Acacia arabica* gel and powder showed significant clinical improvement in gingival and plaque index scores as compared to a placebo. This improvement was comparable to 1% chlorhexidine gel. Thus, this study suggests that both *Acacia arabica* gel and powder may be useful herbal formulations for chemical plaque control in subjects with gingivitis.

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