Clinical Oral Investigations
https://doi.org/10.1007/s00784-020-03623-8

ORIGINAL ARTICLE

The effects of a novel herbal toothpaste on salivary lactate dehydrogenase as a measure of cellular integrity

Prem K. Sreenivasan[1] · Veera Venkata Prasad Kakarla[2] · Shweta Sharda[2] · Yogitha Setty[2]

Received: 4 October 2019 / Accepted: 6 October 2020

Abstract

Objective Lactate dehydrogenase (LDH) is a critical intracellular enzyme responsible for anaerobic respiration in pyruvate metabolism which becomes detectable in extracellular spaces after cellular breakdown. This clinical investigation examined the effects of brushing with a test toothpaste containing natural ingredients, i.e., clove (*Syzygium aromaticum*), aloe vera (*Aloe barbadensis*), amla (*Emblica officinalis*), neem (*Azadirachta indica*), tulsi (*Ocimum basillicum*), and honey (from *Apis mellifera*), and 0.96% zinc (zinc oxide, zinc citrate) and 0.76% SMFP (1000 ppm F) in a calcium carbonate base formulated with natural ingredients (Ved Shakti, Colgate Palmolive India) and a fluoride toothpaste containing 0.76% SMFP (1000 ppm F) in a calcium carbonate base (Colgate Cavity Protection, Colgate Palmolive; henceforth control) on salivary LDH in conjunction with the assessments of dental plaque and gingivitis representing oral hygiene parameters.

Materials and methods This double-blind, two-cell study enrolled 70 adults (age range 20–59 years). Subjects completed a washout and provided baseline saliva samples for LDH analysis and clinical assessments of dental plaque and gingivitis using the Turesky Modification of Quigley-Hein and Loe-Silness methods respectively. Subjects were randomly assigned to brush their teeth with either the test or control. Post-treatment sample collection and clinical evaluations were conducted after 3 weeks, 6 weeks, and 12 week sof brushing with all assessments conducted 12 h after hygiene. Statistical analyses were conducted independently for each parameter by *t*-test for within treatment evaluation and analysis of covariance (ANCOVA) for between treatment comparisons.

Results At baseline, treatment groups demonstrated no significant differences for LDH or dental plaque and gingival index scores. Brushing with the test demonstrated progressive reductions in salivary LDH, plaque and gingival index scores over the study duration in comparison to the control. The test demonstrated reductions in LDH of 9.5–15.4% over the study period in comparison to the control representing statistically significant effects ($p < 0.05$). The test also demonstrated reductions in dental plaque that ranged between 6.4 and 16.2% over the study period and gingivitis reductions that ranged between 8.2 and 23.8% representing statistically significant results ($p < 0.05$).

Conclusions Brushing with a novel herbal toothpaste demonstrated significant reductions in salivary LDH representing improvements in cellular integrity with concurrent reductions in dental plaque and gingivitis as compared to the control dentifrice.

Clinical relevance Salivary LDH measurements offer a non-invasive and objective measurement of mucosal cellular integrity complementing other evaluations and clinical assessments such as plaque and gingival index scores.

Keywords Barrier damage · Clinical study · Cellular integrity · Dental plaque · Dentifrice · Gingivitis · Lactate dehydrogenase · Mucosa · Subjects · Toothpaste · Toothbrushing

Introduction

The human mouth exposed to the external environment represents a complex ecosystem shaped by its distinct intra-oral niches harboring large densities of endogenous organisms found as biofilms on the surfaces of the teeth, tongue, and mucosa. Microbial utilization of dietary residues augments the growth and proliferation of these organisms releasing

✉ Veera Venkata Prasad Kakarla
kakarlap@hotmail.com

[1] Department of Oral Biology, Rutgers School of Dental Medicine, Newark, NJ 07103, USA

[2] Public Health Dentistry SDM College of Dental Sciences and Hospital SDM College of Dental Sciences & Hospital, Affiliated to SDM University, Dharwad, Karnataka, India

Published online: 16 October 2020

metabolic by-products and cellular factors some of which have antigenic or immunological features [1, 2, 3]. Optimal routine oral hygiene representing an important component of self-care is needed to sufficiently cleanse the teeth and the mouth to reduce the influences of these inflammatory components. Epidemiological evidence indicates that inflammation of the gums and tissues supporting the teeth resulting in gingivitis is widespread and is commonly attributed to the lack of effective oral hygiene to cleanse the teeth and the mouth [4]. The inception and advancement of gingivitis to other destructive conditions such as periodontitis are widely attributed to a microbial etiology that may lead to tooth loss [1].

Mucosal surfaces such as those in the mouth, nose, and gastrointestinal tract form an interface between the host and the external environment including the organisms endogenous to these regions [2, 5, 3]. The oral epithelium forms the outermost cellular barrier of the host offering protection from pathogens, their by-products, exogenous substances, mechanical stresses and other factors. Defined laboratory models offer an important avenue to clarify the interactions between the epithelial cells and exogenous substances resident in the human mouth including the endogenous organisms [2]. The presence of oral bacteria, their by-products, and other substances has an effect on these mucosal cells representing an area relevant to oral health and hygiene but with limited literature representation. In general, damage to mucosal cells due to microbial toxins or other factors in laboratory analyses have been evaluated by studying the release of intracellular content and cellular enzymes [6–11, 12]. Lactate dehydrogenase (LDH) is a constitutive enzyme found in all cells of the human body [7, 13, 14] with reported stability [15] and an extensive literature [8]. The extracellular localization of LDH is reportedly associated with epithelial tissue injury [16] and related to the oral clinical status [14, 17, 12]. Laboratory investigations report relationships between LDH release, ATP levels, and oxidative phosphorylation with descriptions of other parameters of cellular damage such as glycolysis needed to maintain membrane function and integrity [18]. These efforts suggest a progression of steps leading to membrane damage and the release of intracellular contents. Progress in mucosal barrier research has offered a broader perspective with reference to analysis of gastrointestinal status associated with health and clinical disease [5].

Oral hygiene with a toothpaste and a toothbrush are commonly used to cleanse the mouth and reduce the undesirable influences of the resident bacteria and their damaging by-products. Herbal ingredients including clove, aloe vera, amla, neem, tulsi and honey representing natural ingredients [19–22] and zinc salts [20] have a long history of use in oral hygiene due to their record of safety and efficacy. These ingredients are reported to demonstrate effects on oral bacteria [23] with honey reducing the levels of salivary mutans streptococci [24] and formulations effective on dental plaque in clinical evaluations [25]. Several clinical studies identify the effects of herbal formulations on dental plaque and gingivitis [26, 27], microbiological outcomes [27], and among orthodontic patients for home-based use [28].

A toothpaste formulated with herbs and zinc has demonstrated reductions in dental plaque and gingivitis along with effects on oral bacteria including gram-negative organisms of the dental plaque, cheek, and tongue (manuscript in prep). Studies with zinc in conjunction with dietary ingredients report clinical improvements in the integrity of gastrointestinal mucosa of children [29] with favorable attributes of other dietary ingredients reported in other models [30]. Based on the large surface area of the oral mucosa and its exposure to the environment and resident stresses, the effect of oral hygiene on mucosal integrity likely represents an important patient relevant outcome. However, investigations evaluating the effects of routine oral hygiene on parameters of mucosal health remain limited. Accordingly, the purpose of this study was to evaluate salivary LDH as a measure of mucosal cellular integrity after oral hygiene with this toothpaste. Included in the study were assessments of dental plaque and gingivitis to examine the effects of the issued treatments on well-identified clinical parameters.

Materials and methods

This single-center clinical study was conducted at the SDM Dental College and Hospital, Dharwad, India, after approval of the study protocol by the ethical board of the college. The study was a randomized, single-center, double-blind parallel design study. A total of eighty adult male and female subjects were enrolled based on the following inclusion and exclusion criteria. Inclusion criteria included (1) subjects providing voluntary informed consent and in good general health between the age of 18 and 70 years with at least 20 natural teeth (excluding third molars), (2) subjects available for the study period, and (3) subjects presenting a mean gingival index score of at least 1.0 as determined by the Loe-Silness gingival index and an initial mean plaque index score of 1.5 or more by the Turesky Modification of the Quigley-Hein plaque index.

Exclusion criteria included (1) subjects who presented with orthodontic bands, partial removable dentures, oral ulcers, advanced periodontal disease, and five or more carious lesions requiring immediate restorations; (2) subjects who reported allergies to consumer products including ingredients used commonly in personal care formulations; (3) subjects reporting ongoing pregnancy, lactation, systemic conditions, or reporting prescription medications; (4) subjects who had undergone dental or medical treatments in the month prior to the screening visit or had participated in a clinical study or test panel; and (4) subjects who were unable to refrain from food or drink for a period of four hours or reported the use of antibiotics in the 4 weeks preceding the screening visit.

Study enrollment was restricted to those who completed an informed consent form, met the study criteria based on evaluations, and were available for the study duration.

Procedures

Enrolled subjects completed a washout phase with a commercially available fluoride toothpaste and toothbrush. During the washout phase, subjects performed oral hygiene with the provided washout articles and refrained from using any other oral hygiene formulations. After the washout phase, subjects returned to the dental clinic for their baseline evaluations. At the baseline visit, subjects arrived for their scheduled visit prior to oral hygiene and provided a saliva sample. Saliva was collected in a sterile wide-mouth tube marked with subject relevant identification details. Approximately 5 ml of saliva was collected from each subject. A clinical evaluation for gingival index and dental plaque were conducted during the baseline visit as described in the section below. Subjects were randomized to a treatment group and assigned their treatments. Test toothpaste assignment was blinded with these articles overwrapped and provided a unique code. To maintain blinding, treatment assignments were conducted in an area separate from clinical evaluations or other activities by personnel unaffiliated with any other study functions. All subjects were informed to brush twice daily with their assigned toothpastes using the provided soft-bristled toothbrush. The test toothpaste was formulated with natural ingredients, i.e., clove (*Syzygium aromaticum*), aloe vera (*Aloe barbadensis*), amla (*Emblica officinalis*), neem (*Azadirachta indica*), tulsi (*Ocimum basillicum*), and honey (from *Apis mellifera*), and 0.96% zinc (zinc oxide, zinc citrate) and 0.76% SMFP (1000 ppm F) in a calcium carbonate base (Ved Shakti, Colgate Palmolive India) and a fluoride toothpaste containing 0.76% SMFP (1000 ppm F) in a calcium carbonate base (Colgate Cavity Protection, Colgate Palmolive) without the natural ingredients or zinc served as a control.

Test toothpaste assignment was blinded with these articles overwrapped and provided a unique code. A commercially available soft-bristled toothbrush was provided to all subjects. Subjects were informed to brush twice daily with provided toothpaste and toothbrush and not share the provide articles with anyone, refrain from using any other oral hygiene products for the duration of the study, and inform study personnel in the event of any change in their health status. Subjects returned to the dental clinic for sampling and examinations similar to the baseline visit after 3 weeks, 6 weeks, and 12 weeks of brushing with their assigned toothpaste. At each visit, a dentist completed an oral examination, and subjects were interviewed for their health status and experiences with provided test articles.

Clinical assessments for dental plaque and gingivitis

Assessments of dental plaque and gingivitis were conducted using established clinical indices represented by the Turesky Modification of Quigley-Hein and the Loe-Silness index respectively [31, 32].

Laboratory analysis of saliva samples

Saliva samples collected from subjects were transported to a laboratory and analyzed upon receipt. LDH assessments were conducted by examining the reaction between pyruvate and NADH to produce NAD^+ and lactate based on described principles [8] utilizing a commercial kit (DIALAB, Austria) and conducted as per provided procedures. Saliva samples were centrifuged at 3,000 RPM for 5 min, and the supernatants utilized for tests based on established principles [33]. Saliva (20 μl) was incubated with pyruvate substrate (1 ml) and incubated for 1 min at room temperature, prior to the addition of 0.25 ml of NADH. Samples were read for absorbance at 340 nm at 30-s intervals for up to 120 s. LDH levels (IU/L) were derived from change in absorption per minute. Controls included reactions that lacked each reagent of the test and those with no added samples.

Statistical analysis

A sample size of 40 subjects per treatment group was determined to detect a difference of approximately 0.2 units between treatments for gingival index scores. Calculations were based on standard deviations of 0.3 with α of 0.05 and statistical power of 80%.

Statistical analyses were conducted separately for each evaluation including the gingival index, plaque index, and LDH. Treatment groups were compared with respect to gender using a Chi-square and an independent *t*-test for age. Within treatment comparisons from the baseline to each post-treatment assessment was conducted using a paired *t*-test. Independent *t*-tests compared baseline scores for each parameter between treatment groups. The effect of treatments at each follow-up examination was determined by analysis of covariance (ANCOVA) using baseline adjusted scores. Statistical analyses were two-sided with significant differences determined at $p < 0.05$.

Results

A summary of demographic characteristics of subjects completing the study is shown in Table 1. Seventy subjects completed the entire study with 34 subjects in the test and 36 subjects in the control group providing evaluable results as shown in CONSORT form (Fig. 1). Twenty women and fifty

Table 1 Demographics of subjects who completed the entire study

	Total (n = 70)	Control (n = 36)	Test (n = 34)
Age			
Mean (SD)[†]	38.07 (9.34)	38.9 (8.25)	37.2 (10.42)
Age range	20–59	20–56	20–59
Gender			
Male	n = 50	n = 25	n = 25
Mean (SD)	37.86(10.13)	38.8 (9.22)	36.92 (11.09)
Age range	20–59	20–56	20–59
Female	n = 20	n = 11	n = 9
Mean (SD)	38.6 (7.17)	39.18 (5.84)	37.89 (8.85)
Age range	20–58	27–49	26–59

[†] No significant differences between treatment groups for age by ANOVA ($p > 0.05$)

No significant differences between treatment groups for gender by Chi-square analysis ($p > 0.05$)

men completed the study with an age range of 20–59 years and an average age of 38.07 years. Analyses indicate that the mean age in the control and test groups were 38.9 and 37.2 years respectively with no significant differences by ANOVA ($p > 0.05$). Similarly, there were no significant differences between the two treatment groups for gender by Chi-square analysis ($p > 0.05$). No adverse events were reported by the subjects or dental examiners over the study period.

Results from the clinical evaluations conducted over the study period from subjects who completed the entire study are presented in Tables 2, 3, 4, and 5. Table 2 provides a summary of results as mean and SD from all evaluations conducted 12 h after oral hygiene. Average baseline levels of LDH in the treatment groups were 242.1 and 240.2 in the test and control groups respectively and recorded average gingival index scores of 1.2 and dental plaque index scores of 2.58 at baseline. Analyses indicate no significant differences between treatment groups for any recorded clinical outcome ($p > 0.05$) but progressive reductions in post-treatment scores over the study period.

Analysis of the 3-week post-treatment results is presented in Table 3. All treatments demonstrating significant reductions from their corresponding baselines for clinical outcomes were evaluated ($p < 0.05$). Analysis by ANCOVA comparing the two treatment groups indicates significant greater reductions in the test group in comparison to the control ($p < 0.05$). Baseline-adjusted LDH scores for the test and control were 213 and 236 respectively representing a difference of 9.5% between the treatment groups. At the 3-week post-treatment evaluation, the gingival index scores for test and control groups were 0.89 and 0.97 respectively for a 9.5% difference between treatments ($p < 0.05$). Plaque index scores for the test and control were 2.33 and 2.49 respectively after the 3-week use of assigned test treatments. A significant difference of 6.4% in dental plaque scores was observed with lower plaque levels observed in the test group in comparison to the control ($p < 0.05$).

Shown in Table 4 is a summary of the analysis of results from the 6-week post-treatment evaluations. Both treatment groups demonstrated reductions from their corresponding baseline by paired t-tests for each evaluated outcome measure ($p < 0.05$). The test group demonstrated significantly greater reductions than the control by ANCOVA for LDH and the clinical indices evaluating gingivitis and dental plaque ($p < 0.05$). Baseline-adjusted LDH scores for the test and control were 146 and 169 respectively representing a statistically significant difference of 13.9% between the treatment groups ($p < 0.05$). Gingival index scores for the test and control were 0.67 and 0.84 respectively representing a 20% difference between treatment groups ($p < 0.05$). At the 6-week assessment, plaque index scores for the test and control were 1.86 and 2.22 respectively. A significant difference of 16.2% in dental plaque scores were observed between the treatment groups with lower scores registered with the test group in comparison to the control ($p < 0.05$).

Analysis of the 12-week results is presented in Table 5. Treatment groups demonstrated reductions for each outcome from their corresponding baselines ($p < 0.05$) with significant differences between treatment groups ($p < 0.05$). LDH levels in the test and control were 146 and 173 respectively representing a statistically significant difference of 15.4% between these treatment groups ($p < 0.05$). Gingival index scores for the test and control were 0.61 and 0.80 representing a 23.8% difference between treatment groups ($p < 0.05$). Plaque index scores for the test and control groups were 1.41 and 1.72 respectively ($p < 0.05$). Analyses indicate that the test group registered a statistically significant 18% lower dental plaque score than the control ($p < 0.05$).

Discussion

Common oral diseases including gingivitis and periodontal disease are chronic conditions reported globally [1, 4]. Whereas these conditions are attributed to the presence of endogenous bacteria and their metabolic by-products [2], these may work in conjunction with other exogenous substances to exert toxic effects on the exposed mucosa and the epithelial cells. Whilst emerging technologies have augmented assessments of oral health status, the corresponding microbiomes, their prevalence in distinct oral niches andthe effects of interventions, fewer investigations have examined the oral mucosa nor its integrity in the transition from health and disease or treatmenteffects [34]. The purpose of this study was to extend our knowledge on examining the cellular status of the oral mucosa

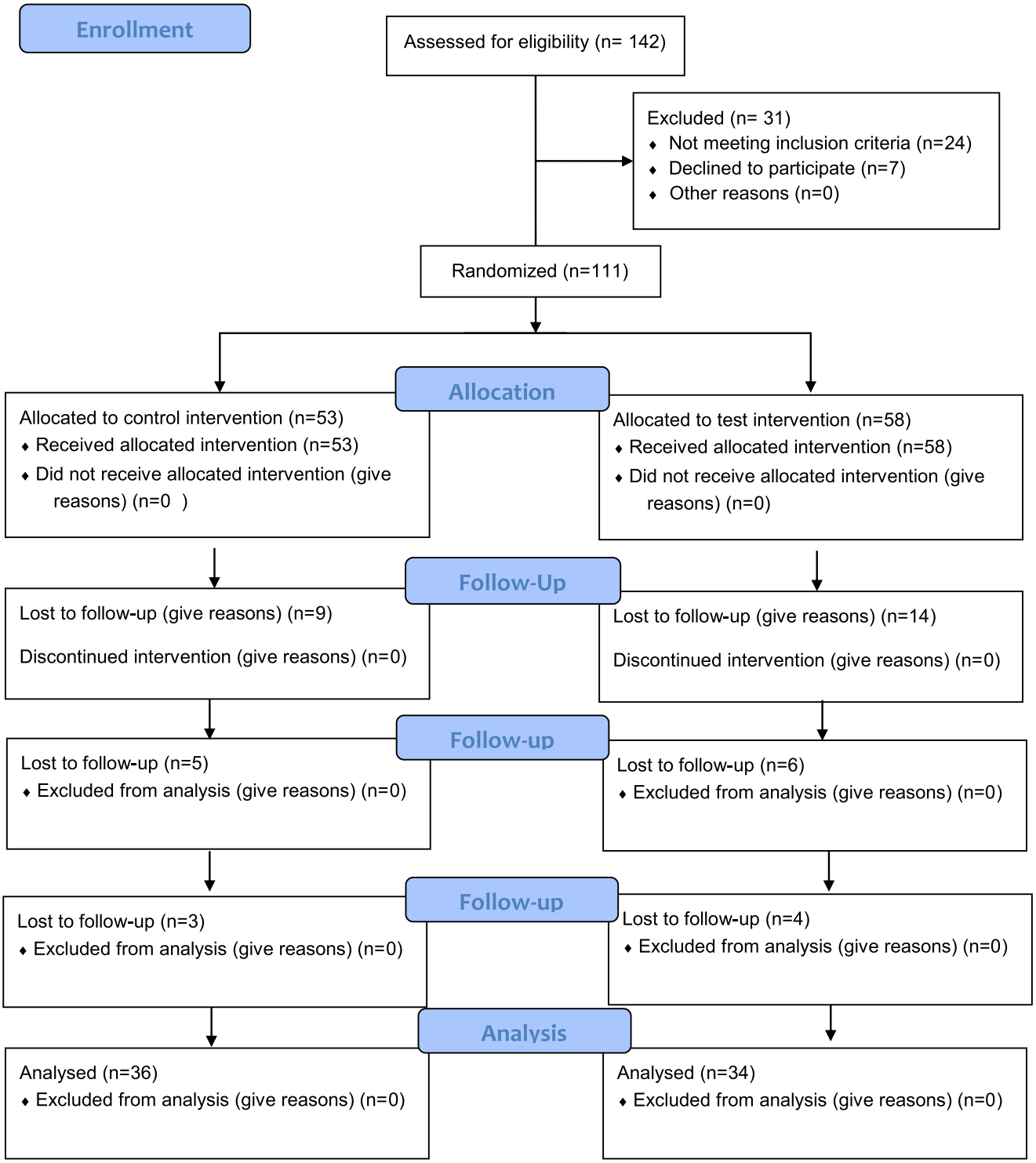

Fig. 1 CONSORT flow diagram

and mucosal barrier and evaluate any changes following oral hygiene. Included in the clinical study design was an assessment of oral health status using commonly utilized indices for dental plaque and gingivitis to provide a biological measure of the efficacy features of evaluated toothpastes.

The human mouth has diverse microenvironments including shedding and non-shedding surfaces. Numerous factors including nutrient availability, mechanical stress, microbial growth, and others that remain incompletely characterized likely play a role in maintaining the health and integrity of the mucosal barrier [2]. In the current investigation, an

Table 2 Summary of subject mean (SD) for salivary LDH, dental plaque, and gingival index scores at all examinations for subjects who completed the entire study

Parameter	Treatment	Baseline Mean (SD)*	3 Weeks Mean (SD)	6 Weeks Mean (SD)	12 Weeks Mean (SD)
Salivary LDH	Test	242.1 (165.6)	213.6 (100.2)	146.2 (69.5)	146.7 (75.3)
	Control	240.2 (115.9)	236 (107.4)	169.9 (76.6)	173.4 (75.2)
Gingival index	Test	1.28 (0.14)	0.88 (0.21)	0.66 (0.16)	0.61 (0.16)
	Control	1.20 (0.14)	0.97 (0.18)	0.83 (0.13)	0.80 (0.14)
Plaque index	Test	2.58 (0.45)	2.32 (0.48)	1.85 (0.45)	1.41 (0.39)
	Control	2.58 (0.57)	2.49 (0.48)	2.21 (0.45)	1.72 (0.46)

*No statistically significant difference was indicated between the two treatment groups at baseline for all evaluated parameters ($p > 0.05$)

assessment of salivary LDH was utilized as an indicator of damage to the oral mucosa and its breakdown. Based on its extensive history, salivary LDH has been evaluated in periodontal research [14] with reports elucidating changes in its levels from health to disease [17]. Other research has examined cellular integrity and membrane function including assessments of biocompatibility of implants for application in dentistry and orthopedics [35] or changes in cellular activity during orthodontics [36]. LDH comprising an enzyme from the host is reportedly more sensitive than measures of other leaked cellular contents including analysis of cellular DNA [35]. Its cytoplasmic localization, abundance, and ubiquity within the living cell offer features appropriate for analysis. The sequences of events relating LDH release to changes in cellular glycolysis and intracellular ATP release or changes in membrane function and integrity are relevant in determining the steps in the initiation, progression, and potential repair of cellular damage [18].

Other features of salivary LDH that need consideration are its relationship to oral indices and clinical status [17] but without a relationship to serum LDH [37] or other physiological changes [38, 39] offering oral region specific assessments unaffected by general health status. For example, tear LDH is reportedly an established and objective approach to examine the status of corneal epithelial surface. Clinical investigations designed to improve the safety features of daily wear or extended use contact lens have utilized tear LDH as a measure of the effects of hypoxia and/or mechanical damage due to these lenses [16]. Additional investigations report the utilization of LDH as a cellular marker to compare the effects of marketed multipurpose contact lens solutions on the corneal epithelium [40]. The toxicity of ingredients considered for food [41] and cosmetic applications [42] were reported using LDH analysis [12]. To design effective therapeutics, analysis of nasal lavage fluid LDH has examined the integrity of nasal respiratory epithelium to evaluate if cell penetrating peptides can enhance the delivery of insulin without damaging nasal mucosa [43]. The significance of maintaining the gut barrier function [5], its alterations in disease, and dietary approaches to augment the barrier has been reported in clinical evaluations [29]. Other investigations indicate barrier enhancements by dietary supplements [30] or the negative influences of food intolerances and its impact on gut barrier function [44].

Table 3 Summary of baseline-adjusted subject mean (SE) for salivary LDH and gingival and plaque index at the 3-week post-treatment evaluation for subjects who completed the entire study

Parameter	Treatment	Adj. 3 week Mean (SE)	Within treatment comparisons*	Between treatment comparisons¶
Salivary LDH (IU/L)	Test	213.6 (100.2)	11.8%	9.5%
	Control	236.0 (107.4)	-15.6%	
Gingival index	Test	0.89 (0.22)	30.5%	8.2%
	Control	0.97 (0.18)	19.2%	
Plaque index	Test	2.33 (0.49)	9.7%	6.4%
	Control	2.49 (0.49)	3.5%	

*Percent change exhibited by the 3-week mean relative to the baseline mean. A positive value indicates a reduction in the parameter by a paired *t*-test. Statistically significant at $p < 0.05$

¶Percent differences between the test and control treatments evaluated by ANCOVA utilizing baseline-adjusted means. Statistically significant at $p < 0.05$

Table 4 Summary of baseline-adjusted subject mean (SE) for salivary LDH and gingival and plaque index at the 6-week post-treatment evaluation for subjects who completed the entire study

Parameter	Treatment	Adj. 6 Weeks Mean (SE)	Within treatment comparisons*	Between treatment comparisons
Salivary LDH (IU/L)	Test	146.2 (69.5)	39.6%	13.9%
	Control	169.9 (76.6)	16.8%	
Gingival index	Test	0.67 (0.17)	47.7%	20.2%
	Control	0.84 (0.14)	30.0%	
Plaque index	Test	1.86 (0.46)	27.9%	16.2%
	Control	2.22 (0.45)	13.9%	

*Percent change exhibited by the 6-week mean relative to the baseline mean. A positive value indicates a reduction in the parameter by a paired *t*-test. Statistically significant at $p < 0.05$

¶Percent differences between the test and control treatments evaluated by ANCOVA utilizing baseline-adjusted means. Statistically significant at $p < 0.05$

Analysis of saliva samples in this study is consistent with the increasing focus placed on this sample for research and diagnosis [45]. A range of markers representing oral and systemic conditions including metabolites, heavy metals, toxins, hormones, proteins, and enzymes are evaluable in saliva [46]. In this investigation, saliva was evaluated biochemically since it becomes contaminated with LDH due to mucosal cellular injury representing a breakdown of the oral barrier. The flexibility offered by saliva has led to other investigations that seek to optimize collection procedures, examine sample banking, automation [14, 47], and other biochemical test formats that may aid longitudinal evaluations or advance clinical practice. While sample stability has been identified as an important feature influencing results [6], the present investigation sought to reduce the influences of experimental variables by analyzing samples upon receipt. Additionally, sampling was conducted in the morning (between 7 AM and 9 AM) from all subjects prior to oral hygiene or breakfast for standardized assessments. LDH analyses were conducted upon sample receipt with appropriate controls. These steps were taken to minimize loss of enzyme activity consequent to utilized methodology or other sample variables including bacterial activity, sample pH, dietary influences, and other interfering constituents in saliva such as exogenous enzyme activities that may lead to sample denaturation including physical parameters and the effects of freezing and thawing.

Ingredients in the test toothpaste include zinc and several herbal components utilized in oral hygiene due to their record of safety and efficacy. A considerable literature documents the favorable application of zinc salts in oral hygiene [20] and herbal ingredients [19, 21, 22] including clove extracts [48]. Improvements in clinical and microbiological outcomes were reported among subjects randomized to an aloe vera toothpaste formulation in comparison to control [27], and laboratory and clinical studies have examined the effects of other ingredients. In comparison to a placebo, a neem mouthrinse provides significant reductions in plaque and gingival indices [49] with similar effects reported for a toothpaste used as a home-based adjunct by orthodontic patients [28]. Amla and tulsi (holy basil) have fewer investigations. Tulsi inhibited

Table 5 Summary of baseline-adjusted subject mean (SE) for salivary LDH and gingival and plaque index at the 12-week post-treatment evaluation for subjects who completed the entire study

Parameter	Treatment	Adj. 12 Weeks Mean (SE)	Within treatment comparisons*	Between treatment comparisons
Salivary LDH (IU/L)	Test	146.7 (75.3)	39.4%	15.4%
	Control	173.4 (75.2)	15.1%	
Gingival index	Test	0.61 (0.16)	52.3%	23.8%
	Control	0.80 (0.14)	33.3%	
Plaque index	Test	1.41 (0.39)	45.3%	18.0%
	Control	1.72 (0.47)	33.3%	

*Percent change exhibited by the 12-week mean relative to the baseline mean. A positive value indicates a reduction in the parameter by a paired *t*-test. Statistically significant at $p < 0.05$

¶Percent differences between the test and control treatments evaluated by ANCOVA utilizing baseline-adjusted means. Statistically significant at $p < 0.05$

both *Prevotella intermedia* and *Fusobacterium nucleatum* in laboratory studies, and a tulsi rinse reduced dental plaque in a 4-day clinical evaluation [50]. Whereas a cariogenic effect of honey was reported in laboratory evaluations using a rat model [51], honey has a history of medical applications in respiratory infections, wound healing, and improving skin conditions [52] along with dental literature reporting laboratory and clinical effects [21, 22]. For instance, in conjunction with green tea, honey reduces salivary organisms [23]. Amongst children assigned honey, reductions in salivary Streptococcus mutans were noted over a 21-day period [24]. Additionally, dental plaque inhibition were observed amongst children after use of mouthwash formulated with honey [53]. In investigations with 437 young adults [25] and dental students [-54] favorable outcomes were reported after use of a honey mouthwash.

Results from the present investigation demonstrate similar scores at baseline between treatment groups for all evaluated parameters but progressive reductions in these outcomes over the study. Oral LDH levels in the control group decreased from 240 to 236 over the study period representing no significant differences; however, the effects of the test group were substantially higher with a 11% difference. The test group consistently demonstrated statistically significant reductions from the control with a 9.5% noted after 3 weeks that increased to 13% at 6 weeks and 15% at 12 weeks. The inclusion of conventional clinical indices in the evaluations was aided in relating the observed results with those noted with LDH.

Subjects who volunteered for study participation reported a domiciliary status representing both urban and rural habitation. While these subjects were not seeking either medical or dental care, they reported limited utilization of dental services due to factors commonly reported from such populations [55]. Notably, these community dwelling individuals had no prior exposure or participation in any clinical studies. While they were not instructed on brushing techniques or to change their dietary habits, subjects were instructed to brush twice daily and refrain from other oral hygiene formulations during the study period. Study compliance was evaluated by periodic contact with the subjects and during the post-treatment recall visits. The present data evaluated samples collected from subjects with gingivitis and no clinical signs of periodontal disease and were free of systemic conditions including diabetes based on a self-reported health status during the screening visit. Future studies can consider expanding on these results by examining other populations to determine the effects of interventions and other parameters. In summary, the results from this investigation indicates that salivary LDH represents a convenient and non-invasive method to evaluate mucosal integrity and likely amenable to point-of-care diagnostics along with the flexibility for remote sampling applications. Furthermore, LDH evaluation is established in laboratory examination of ingredients with the present efforts representing a translation to clinical application. Taken together, results from this investigation provides an approach to examine the effects of dental interventions such as oral hygiene formulations on assessing cellular integrity or breakdown and injury to provide a whole mouth assessment.

Funding The work was supported by the Department of Public Health Dentistry, SDM College of Dental Sciences and Hospital, Dharwad, Karnataka, India.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Claydon NC (2008) Current concepts in toothbrushing and interdental cleaning. Periodontol 48:10–22
2. Groeger SE, Meyle J (2015) Epithelial barrier and oral bacterial infection. Periodontol 69(1):46–67
3. Gendron R, Grenier D, Maheu-Robert L (2000) The oral cavity as a reservoir of bacterial pathogens for focal infections. Microbes Infect 2(8):897–906
4. Figuero E, Nóbrega DF, García-Gargallo M, Tenuta LM, Herrera D, Carvalho JC (2017) Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review. J Clin Periodontol 44(Suppl 18):S116–S134
5. Oshima T, Miwa H (2016) Gastrointestinal mucosal barrier function and diseases. J Gastroenterol 51(8):768–778
6. Barranco T, Cerón JJ, López-Jornet P, Pastor J, Carrillo JM, Rubio M, Tornel PL, Cugat R, Tecles F, Tvarijonaviciute A (2018) Impact of saliva collection and processing methods on aspartate aminotransferase, creatin kinase and lactate dehydrogenase activities. Anal Sci 34(5):619–622
7. Bonnekoh B, Farkas B, Geisel J, Mahrle G (1990) Lactate dehydrogenase release as an indicator of dithranol-induced membrane injury in cultured human keratinocytes. A time profile study. Arch Dermatol Res 282(5):325–329
8. Cummings BS, Wills LP, Schnellmann RG (2012) Measurement of cell death in Mammalian cells. Curr Protoc Pharmacol Chapter 12: Unit12.8. https://doi.org/10.1002/0471141755.ph1208s56
9. Hecker H, Johannisson R, Koch E, Siegers CP (2002) In vitro evaluation of the cytotoxic potential of alkylphenols from Ginkgo biloba L. Toxicology 177(2-3):167–177
10. Kastelova A, Dimova S, Nemery B (2003) Propranolol cytotoxicity in rat and human lung in vitro. Methods Find Exp Clin Pharmacol 25(7):509–515
11. McKarns SC, Hansch C, Caldwell WS, Morgan WT, Moore SK, Doolittle DJ (1997) Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity. Fundam Appl Toxicol 36(1):62–70

12. Li L, Lau BH (1993) A simplified in vitro model of oxidant injury using vascular endothelial cells. In Vitro Cell Dev Biol Anim 29A(7):531–536
13. Carlson RP, Lefer AM (1976) Hepatic cell integrity in hypodynamic states. Am J Phys 231(5 Pt. 1):1408–1414
14. De La Peña VA, Diz Dios P, Tojo Sierra R (2007) Relationship between lactate dehydrogenase activity in saliva and oral health status. Arch Oral Biol 52(10):911–915
15. Dos Santos DR, Souza RO, Dias LB, Ribas TB, de Oliveira LCF, Sumida DH, Dornelles RCM, Nakamune ACMS, Chaves-Neto AH (2018) The effects of storage time and temperature on the stability of salivary phosphatases, transaminases and dehydrogenase. Arch Oral Biol 85:160–165. https://doi.org/10.1016/j.archoralbio.2017.10.016
16. Ladage PM, Yamamoto K, Ren DH, Li L, Jester JV, Petroll WM, Cavanagh HD (2001) Effects of rigid and soft contact lens daily wear on corneal epithelium, tear lactate dehydrogenase, and bacterial binding to exfoliated epithelial cells. Ophthalmology. 108(7):1279–1288
17. Ekuni D, Yamane-Takeuchi M, Kataoka K, Yokoi A, Taniguchi-Tabata A, Mizuno H, Miyai H, Uchida Y, Fukuhara D, Sugiura Y, Tomofuji T, Morita M (2017) Validity of a new kit measuring salivary lactate dehydrogenase level for screening gingivitis. Dis Markers 2017:9547956
18. Kristensen SR (1989) A critical appraisal of the association between energy charge and cell damage. Biochim Biophys Acta 1012(3):272–278
19. Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL (2013) Emerging trends of herbal care in dentistry. J Clin Diagn Res 7(8):1827–1829
20. Sanz M, Serrano J, Iniesta M, Santa Cruz I, Herrera D (2013) Antiplaque and antigingivitis toothpastes. Monogr Oral Sci 23:27–44
21. Seal M, Rishi R, Satish G, Divya KT, Talukdar P, Maniyar R (2016) Herbal panacea: the need for today in dentistry. J Int Soc Prev Community Dent 6(2):105–109
22. Taheri JB, Azimi S, Rafieian N, Zanjani HA (2011) Herbs in dentistry. Int Dent J 61(6):287–296
23. Abdelmegid F, Al-Agamy M, Alwohaibi A, Ka'abi H, Salama F (2015 Fall) Effect of honey and green tea solutions on Streptococcus mutans. J Clin Pediatr Dent 39(5):435–441
24. Rupesh S, Winnier JJ, Nayak UA, Rao AP, Reddy NV, Peter J (2014) Evaluation of the effects of manuka honey on salivary levels of mutans streptococci in children: a pilot study. J Indian Soc Pedod Prev Dent 32(3):212–219
25. Nayak PA, Nayak UA, Mythili R (2010) Effect of Manuka honey, chlorhexidine gluconate and xylitol on the clinical levels of dental plaque. Contemp Clin Dent 1(4):214–217
26. Abhishek KN, Supreetha S, Sam G, Khan SN, Chaithanya KH, Abdul N (2015) Effect of neem containing toothpaste on plaque and gingivitis–a randomized double blind clinical trial. J Contemp Dent Pract 16(11):880–883
27. Pradeep AR, Agarwal E, Naik SB (2012) Clinical and microbiologic effects of commercially available dentifrice containing aloe vera: a randomized controlled clinical trial. J Periodontol 83(6):797–804
28. Tadikonda A, Pentapati KC, Urala AS, Acharya S (2017) Anti-plaque and anti-gingivitis effect of Papain, Bromelain, Miswak and Neem containing dentifrice: a randomized controlled trial. J Clin Exp Dent 9(5):e649–e653
29. Lima AA, Anstead GM, Zhang Q, Figueiredo ÍL, Soares AM, Mota RM, Lima NL, Guerrant RL, Oriá RB (2014) Effects of glutamine alone or in combination with zinc and vitamin A on growth, intestinal barrier function, stress and satiety-related hormones in Brazilian shantytown children. Clinics (Sao Paulo) 69(4):225–233
30. Jiao N, Wu Z, Ji Y, Wang B, Dai Z, Wu G (2015) L-Glutamate enhances barrier and antioxidative functions in intestinal porcine epithelial cells. J Nutr 145(10):2258–2264
31. Löe H, Silness J (1963) Periodontal disease in pregnancy. Acta Odontol Scand 21:533–551
32. Turesky S, Gilmore ND, Glickman I (1970) Reduced plaque formation by the chloromethyl analogue of vitamin C. J Periodontol 41:41–43
33. Thomas L (1998) Clinical laboratory diagnostics: use and assessment of clinical laboratory results. TH-Books, Frankfurt, pp 89–94
34. Wade WG (2013) The oral microbiome in health and disease. Pharmacol Res 69(1):137–143
35. Velasco-Ortega E, Alfonso-Rodríguez CA, Monsalve-Guil L, España-López A, Jiménez-Guerra A, Garzón I, Alaminos M, Gil FJ (2016) Relevant aspects in the surface properties in titanium dental implants for the cellular viability. Mater Sci Eng C Mater Biol Appl 1(64):1–10
36. Perinetti G, Serra E, Paolantonio M, Bruè C, Meo SD, Filippi MR, Festa F, Spoto G (2005) Lactate dehydrogenase activity in human gingival crevicular fluid during orthodontic treatment: a controlled, short-term longitudinal study. J Periodontol 76(3):411–417
37. Yoshie H, Tai H, Kobayashi T, Oda-Gou E, Nomura Y, Numabe Y, Ito K, Kurihara H, Kamoi KJ (2007) Salivary enzyme levels after scaling and interleukin-1 genotypes in Japanese patients with chronic periodontitis. Periodontol 78(3):498–503
38. Kugahara T, Shosenji Y, Ohashi K (2008) Screening for periodontitis in pregnant women with salivary enzymes. J Obstet Gynaecol Res 34(1):40–46
39. Musumeci V, Cherubini P, Zuppi C, Zappacosta B, Ghirlanda G, Di Salvo S (1993) Aminotransferases and lactate dehydrogenase in saliva of diabetic patients. J Oral Pathol Med 22(2):73–76
40. Cavet ME, Harrington KL, VanDerMeid KR, Ward KW, Zhang JZ (2009) Comparison of the effect of multipurpose contact lens solutions on the viability of cultured corneal epithelial cells. Cont Lens Anterior Eye 32(4):171–175
41. Alavizadeh SH, Hosseinzadeh H (2014) Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem Toxicol 64:65–80
42. Contado C, Mejia J, Lozano García O, Piret JP, Dumortier E, Toussaint O, Lucas S (2016) Physicochemical and toxicological evaluation of silica nanoparticles suitable for food and consumer products collected by following the EC recommendation. Anal Bioanal Chem 408(1):271–286
43. Khafagy E-S, Morishita M, Isowa K, Imai J, Takayama K (2009) Effect of cell-penetrating peptides on the nasal absorption of insulin. J Control Release 133(2):103–108
44. Hippe B, Remely M, Bartosiewicz N, Riedel M, Nichterl C, Schatz L, Pummer S, Haslberger A (2014) Abundance and diversity of GI microbiota rather than IgG4 levels correlate with abdominal inconvenience and gut permeability in consumers claiming food intolerances. Endocr Metab Immune Disord Drug Targets 14(1):67–75
45. Ngamchuea K, Chaisiwamongkhol K, Batchelor-McAuley C, Compton RG (2017) Chemical analysis in saliva and the search for salivary biomarkers - a tutorial review. Analyst 143(1):81–99
46. Hofman LF (2001) Human saliva as a diagnostic specimen. J Nutr 131(5):1621S–1625S
47. Alonso de la Peña V, Diz Dios P, Lojo Rocamonde S, Tojo Sierra R, Rodríguez-Segade S (2004) A standardised protocol for the quantification of lactate dehydrogenase activity in saliva. Arch Oral Biol 49(1):23–27
48. Cecchini C, Silvi S, Cresci A, Piciotti A, Caprioli G, Papa F, Sagratini G, Vittori S, Maggi F (2012) Antimicrobial efficacy of Achillea ligustica All. (Asteraceae) essential oils against reference and isolated oral microorganisms. Chem Biodivers 9(1):12–24
49. Chatterjee A, Saluja M, Singh N, Kandwal AJ (2011) To evaluate the antigingivitis and antipalque effect of an Azadirachta indica

(neem) mouthrinse on plaque induced gingivitis: a double-blind, randomized, controlled trial. Indian Soc Periodontol 15(4):398–401
50. Hosamane M, Acharya AB, Chhavi V, Trivedi D, Shetty SB, Thakur SL (2014) Evaluation of holy basil mouthwash as an adjunctive plaque control agent in a four day plaque regrowth model. J Clin Exp Dent 6(5):e491–e496
51. Bowen WH, Lawrence RA (2005) Comparison of the cariogenicity of cola, honey, cow milk, human milk, and sucrose. Pediatrics. 116(4):921–926
52. Negut I, Grumezescu V, Grumezescu AM (2018) Treatment Strategies for Infected Wounds. Molecules 23(9):2392
53. Singhal R, Siddibhavi M, Sankeshwari R, Patil P, Jalihal S, Ankola A (2018) Effectiveness of three mouthwashes - manuka honey, raw honey, and chlorhexidine on plaque and gingival scores of 12-15-year-old school children: a randomized controlled field trial. J Indian Soc Periodontol 22(1):34–39
54. Jain A, Bhaskar DJ, Gupta D, Agali C, Gupta V, Gupta RK, Yadav P, Lavate AB, Chaturvedi M (2015) Comparative evaluation of honey, chlorhexidine gluconate (0.2%) and combination of xylitol and chlorhexidine mouthwash (0.2%) on the clinical level of dental plaque: A 30 days randomized control trial. Perspect Clin Res 6(1): 53–57
55. Pham TA, Ueno M, Shinada K, Yanagisawa T, Wright FA, Kawaguchi Y (2011) Periodontal disease and related factors among Vietnamese dental patients. Oral Health Prev Dent 9(2):185–194