

Article

Bactericidal Activity of Crevicular Polymorphonuclear Neutrophils in Chronic Periodontitis Patients and Healthy Subjects under the Influence of Areca Nut Extract: An In Vitro Study

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Featured Application: The current study is an attempt to explore the host–bacterial interaction of periodontal pathogens in the oral environment under experimental conditions that may be influenced by areca nut extracts. The function of neutrophils differs in peripheral blood and gingival crevicular fluid. This study observed a reduction in the bactericidal activity of crevicular polymorphonuclear neutrophils isolated from chronic periodontitis patients and healthy subjects in the presence of areca nut extracts. This could be one of the mechanisms by which the areca nut compromises periodontal health.

Abstract: Arecanut chewing is an established risk factor for oral submucous fibrosis (OSMF), but its role in periodontal disease has not yet been defined. This study aimed to assess the effect of areca nut extracts (ANE) on the bactericidal activity of crevicular polymorphonuclear neutrophils (cPMNs) in healthy subjects and chronic periodontitis (CP) patients. An in vitro study was designed with an equal number of ($n = 30$) gingival crevicular fluid (GCF) samples collected from CP patients and healthy subjects. Bactericidal activity and hydrogen peroxide (H_2O_2) assays were performed with the GCF samples pre-treated with extracts of two varieties of areca nut: ripe and tender. Simultaneously, controls were also carried out with Hank's balanced salt solution (HBSS) and catechin. Independent t-test and one-way analysis of variance (ANOVA), along with post-hoc analysis, were employed for statistical analysis. In both study groups, a significant reduction ($p < 0.01$) in the bactericidal activity was noted when the samples treated with the ripe areca nut (rANE) were compared with the tender variant (tANE). Similarly, H_2O_2 levels were significantly reduced ($p < 0.001$) in the rANE in contrast to tANE for both study groups. The above results were significant within the group but were found to be non-significant between the study groups, except when it was treated with HBSS ($p < 0.001$). In the present study, it was found that there was a reduction in the bactericidal activity and H_2O_2 production of cPMNs in both healthy subjects and CP patients in the presence of areca nut extract. Moreover, the effect of rANE on cPMNs was more detrimental than tANE.

Keywords: neutrophils; areca nut; periodontitis; antibacterial activity; reactive oxygen species

1. Introduction

The main etiological factor for periodontal disease is the disruption in the homeostasis of the host–bacterial interplay. Such an imbalance creates a conducive milieu for commensal and pathogenic bacteria to flourish, thus triggering an inflammatory cascade [1,2]. It is a well-documented fact that polymorphonuclear neutrophils (PMNs), commonly referred to as neutrophils, are the foremost players in the immunological arena. The destruction of periodontal supporting tissues will be favoured if the functions of PMNs are compromised [3,4]. Periodontal diseases are not only affected by immune cells, but various environmental risk factors can also act as a catalyst for the worsening of the condition. Smoking has already been established to have a causal relationship [5], whereas the influence of areca nut chewing has not been explored much in the context of gingival and periodontal diseases.

Areca nut has been rated as the fourth most widely abused substance, and is mainly consumed in East and South-East Asian countries in various forms [6]. Its users amount to 600 million people worldwide, which comprises of 10–20% of the total population of the world [7]. In India, tender areca nuts are mainly consumed in the southern and north-eastern regions, while the ripe form is consumed in almost every part of the country. The ripe variant of the areca nut is commonly consumed as a preparation in piper betel leaves with or without additives. However, in Taiwan, tender areca nut is more often consumed without any accompaniment, like tobacco [8].

In the past, investigations have been carried out to study the deleterious impact of areca nut on the gingival epithelium [9,10], fibroblast [11], and cementoblast [12]. Insights have also shown radiographic changes in oral bone tissues [13]. These findings led us to contemplate that areca nut usage could compromise gingival and periodontal health. Although periodontal disease is prevalent in areca nut chewers compared to non-chewers [13], its exact mechanism is nevertheless not well understood.

The main culprit of gingival and periodontal disease is plaque, comprising colonies of microorganisms. With the advancement of the disease, there is a growth in colonies with the addition of various microbes, thus transforming the composition of the plaque and local environment [1,2]. Concurrently, the body's immune mechanism is also preparing to combat the microbial intrusion by recruiting various progenitor and immune cells that will reach the site to impart their action [14]. During the progression of periodontal disease, various inflammatory cytokines play important roles [15]. PMNs, being the first favoured among line of defence mechanisms, come into effect immediately. This makes them highly crucial in the initial inflammatory response [16]. This rationale supports the idea that situations in which PMN actions are curbed will lead to the destruction of tissues far beyond the rate of the body's physiological repair. PMNs, which are available to the oral cavity, commonly known as oral PMNs, are distinct from their counterparts seen in the peripheral blood [17]. Subsets of oral PMNs that exist in the gingival crevice, known as crevicular PMNs (cPMNs), are of paramount importance with regard to the gingival and periodontal diseases because of their proximity to the diseased tissues [18,19].

PMNs derived from the peripheral blood of healthy subjects have been used to assess the effect of areca nut extract (ANE) on its various function such as chemotaxis, phagocytosis and bactericidal activity [20–22]. However, only the phagocytic activity of cPMNs has been assessed [23]. Additionally, the emphasis has been placed on areca nut usage in the development of potentially malignant disorders (oral submucous fibrosis) and oral cancer. However, the due attention has not been directed towards its role in periodontal diseases. Based on the literature search, no *in vivo* longitudinal studies have evaluated the bactericidal activity of cPMNs under the impact of areca nut. Thus, the current study is a preliminary *in vitro* study to assess the effects of ANE on the bactericidal activity of cPMNs in healthy subjects and CP patients.

The null hypothesis states that areca nut extract has no effect on the bactericidal activity of crevicular PMNs in healthy and chronic periodontitis patients.

2. Materials and Methods

2.1. Study Description

An analytical *in vitro* study was designed, and ethical approval was obtained from the institutional ethical committee of the KLE'S Institute of Dental Science, Belgaum, India (ethical approval no. 327). From a total of ninety-five patients who were screened, only 60 subjects were recruited for the study. As per American Academy of Periodontology classification (1999) of periodontal disease [24], thirty patients ($n = 30$) above the age of 30 years with the sites having clinical evidence of ≥ 1 mm of clinical attachment loss (CAL) were categorized as CP patients and recruited to "study group B". Patients found to have any systemic diseases (including the conditions that compromise the neutrophils number or activity) or any lesions in the oral cavity were not recruited for the study. Pregnant women, smokers, areca nut or betel quid chewers or subjects with a history of antibiotics intake in the last six months were not considered for the study. An equal number ($n = 30$) of age- and gender-matched subjects whose marginal gingiva was free of inflammation and did not bleed on probing with probing depth ≤ 3 mm were considered as healthy subjects and made up "study group A". Informed consent was procured from the subjects, who voluntarily agreed to participate in the study.

2.2. Study Protocol

Two independent examiners were engaged for the purpose of recruitment and sample collection from subjects in both study groups. At the beginning of the study, examiners underwent a training session to standardize the methodology. Additionally, inter- and intra-examiner reliability was calculated with Cronbach's alpha coefficient values of 0.91 and 0.93, respectively, showing a high degree of agreement. Patient histories, including medical and personal histories, were recorded along with each patient's identification data. A detailed clinical examination was carried out with an emphasis on bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment loss (CAL), gingival index (Löe and Silness, 1963), and plaque index (Turesky's modification of Quigley and Hein plaque index, 1970). During patient allocation, the subjects were given a card with a written code designated for each study group. The gingival crevicular fluid (GCF) was collected by two well-trained examiners using the gingival crevicular washing (GCW) technique [25,26]. Subsequently, all samples were treated for the estimation of biochemical and microbiological parameters.

2.3. Sample Collection

cPMNs were procured from the gingival crevice by deploying the above-mentioned technique [25,26]. Rinsing of the oral cavity for about 60 s was advised before collecting the sample. With the application of a gentle blow of compressed air, the site was allowed to dry, and sustained isolation was achieved with cotton rolls. For every subject, fifteen sequential washings of the gingival crevice were taken with Hank's balanced salt solution (HBSS) (Product ID No. TL1010, HIMEDIA Laboratories, Mumbai, India), using a 2 mL disposable syringe, and the washing was collected in conventional 5 μ L pipettes [25,26]. The sample was centrifuged at 2000 revolutions per minute (RPM) for ten minutes. The cPMNs were washed with HBSS two times, and later they were counted using a Neubauer chamber.

2.4. Cell Viability Assay

After the treatment of cPMNs with HBSS, ripe areca nuts extract without husk (rANE), tender areca nuts extract (tANE), and catechin (catechin product ID No. C1251 G, Sigma-Aldrich Laboratories, Bangalore, India) were checked for cell viability by trypan blue dye exclusion assay. The technique used to assess the cell viability was adopted from Hung et al. 2000 [20].

2.5. Preparation of Areca Nut Extracts

A fresh areca nut with husk was dried at room temperature. Ten grams of each ripe and tender areca nut were sliced and mixed with 250 mL of distilled water, and it was then freeze-dried. The after-extraction yield was about 26% for tANE and 12% for rANE. The extract was diluted by adding distilled water, and concentrations of 50 µg/mL for rANE and 400 µg/mL for tANE were made [18].

2.6. Bactericidal Assay

Freshly isolated cPMNs (10^7 cells/mL) were incubated with rANE (50 µg/mL), tANE (400 µg/mL), and catechin (1250 µg/mL) for 30 min at 37 °C on a rotator. cPMNs treated with HBSS alone served as a control. *Aggregatibacter actinomycetemcomitans*, ATCC 33384 (Promochem, Bangalore, India) was combined with treated and control cPMNs in a microfuge tube and was slowly rotated at 37 °C for one hour. Bactericidal assay and the determination of bacteria in colony-forming units (CFU) were performed as described by Hung et al. 2000 [20].

2.7. Microassay for H₂O₂ Production—Phenol Red Oxidation

The oxidation of phenol red (product ID No. P5530, Sigma Aldrich Laboratories, India) by HRPO (Horseradish peroxidase product ID No. P8250, Sigma Aldrich Laboratories, Bangalore, India) uses H₂O₂ as a substrate, which reflects a colour change. In the presence of an excess of HRPO, the change in colour was proportional to the concentration of H₂O₂. The assay was set up in microtiter plates, and optical density was read on an enzyme-linked immunosorbent assay (ELISA) reader at 600 nm [27]. The microassay for H₂O₂ production—Phenol red oxidation—was performed as described by Rajkovic IA et al. (1984) [28].

2.8. Statistical Analysis

Regarding sample size, post hoc analysis was performed using G Power 3.1.9.2 software (Heinrich-Heine-Universität Düsseldorf, Northrhine-Westphalia, Germany, 2007) with a confidence interval (α) of 0.05 and an effect size of 0.8. The sample size achieved a statistical power of 0.92.

The data were collected and later entered into a Microsoft Excel spreadsheet for the purpose of data editing and coding. Sample characteristics are summarized in the form of percentages and means with standard deviations under descriptive statistics. Variables like bactericidal activity and H₂O₂ assay were compared between and within study groups using independent t-test and one-way ANOVA (analysis of variance) with post-hoc analysis, respectively. The parametric test was applied with a confidence interval of 95%, and $p < 0.05$ was considered statistically significant. Data analysis was performed using version 21 of the Statistical Package for the Social Sciences (SPSS IBM, Chicago, IL, USA).

3. Results

3.1. Sample Distribution

Based on inclusion and exclusion criteria, a total of 60 samples with thirty (30) sampling units in each group were recruited (Figure 1). Both groups had subjects with comparable mean age and gender distribution (Table 1).

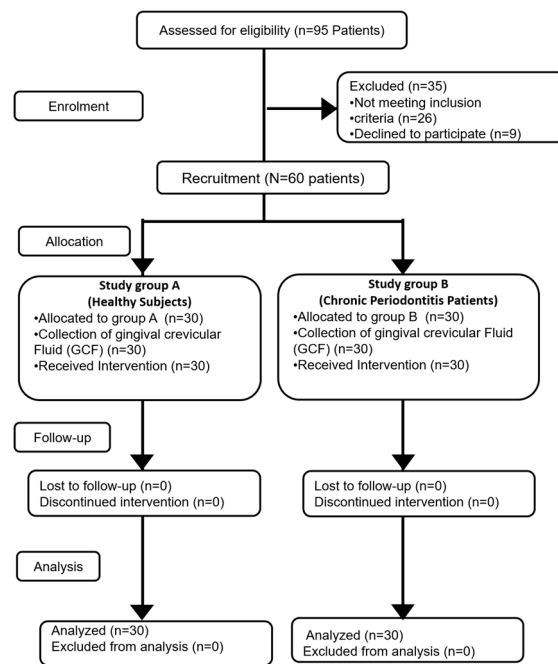


Figure 1. Consort flowchart.

Table 1. Descriptive analysis for sample characteristics.

		Study Group (A)-Healthy Subjects (n = 30)	Study Group (B)-Chronic Periodontitis (n = 30)	p-Value
Age (Mean ± SD)		40.90 ± 4.964	40.07 ± 4.135	0.483
Gender	Male	13 (43.3)	14 (46.7)	0.795
	Female	17 (56.7)	16 (53.3)	

SD—Standard Deviation.

3.2. Comparative Analysis of Biochemical and Microbiological Parameters

Two comparative analyses were carried for each parameter, i.e., within (Table 2) and between study groups (Figure 2). First, cell viability was considered for evaluation. There was a non-significant difference ($p > 0.05$) in both comparative analyses for study groups A and B.

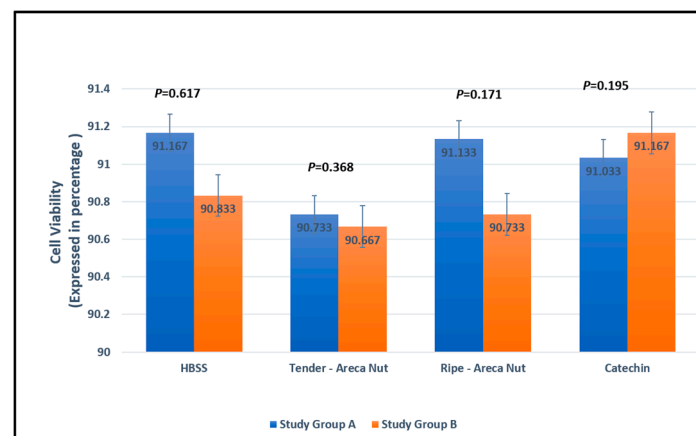


Figure 2. Intergroup comparative analysis of cellular viability of cPMNs.

Table 2. Intragroup comparative analysis of microbiological (cell viability and bactericidal activity) and biochemical (hydrogen peroxide) parameters.

Parameter	Type of Intervention	Study Group A	<i>p</i> Value	Study Group B	<i>p</i> Value
Cell Viability (Expressed as percentage of cells)	HBSS	91.167 ± 2.841	0.927	90.833 ± 2.560	0.922
	tANE	90.733 ± 3.433		90.667 ± 2.695	
	rANE	91.133 ± 2.556		90.733 ± 3.814	
	Catechin	91.033 ± 1.991		91.167 ± 2.841	
Bactericidal Activity (Expressed as colony-forming units –CFU –Cells × 10 ⁶)	HBSS	3.127 ± 0.758 × 10 ⁶	0.000 *	2.010 ± 0.240758 × 10 ⁶	0.000 *
	tANE	4.140 ± 0.681758 × 10 ⁶		4.100 ± 0.661758 × 10 ⁶	
	rANE	5.513 ± 0.549758 × 10 ⁶		5.337 ± 0.586758 × 10 ⁶	
	Catechin	6.427 ± 0.482758 × 10 ⁶		6.227 ± 0.563758 × 10 ⁶	
Hydrogen Peroxide (H ₂ O ₂) (Expressed as µM)	HBSS	35.967 ± 6.594	0.000 *	46.733 ± 5.8659	0.000 *
	tANE	29.633 ± 5.255		29.567 ± 7.180	
	rANE	22.600 ± 4.709		25.067 ± 3.841	
	Catechin	17.167 ± 4.291		15.667 ± 3.632	

Note: Results expressed as Mean ± SD; * *p*-value < 0.001; SD—Standard Deviation; tANE—Tender areca nut extract; rANE—Ripe areca nut extract; HBSS—Hank's balanced salt solution.

With the confirmation of the survival of neutrophils, its bactericidal action and H₂O₂ assay were scrutinized. The comparison was principally focused on rANE and tANE. The other two chemicals, HBSS and catechin, were examined as negative and positive controls, respectively. The outcome values of HBSS and catechin for each parameter were interpreted as the upper and lower benchmark values, as applied per case.

On comparing bactericidal action within the group, it was found to be significantly reduced (*p* = 0.001) with rANE when compared to tANE (Table 2). This becomes evident with the significant increase in CFUs in rANE in comparison to tANE. Similar results were noticed for both the groups (Table 2). With the intention of exploring further, a post-hoc analysis was performed for both groups. The CFUs register a significant gradual increase along the sequence of chemicals/extracts, starting with HBSS, followed by tANE, rANE and catechin. Statistically significant (*p* < 0.001) differences were seen between all possible combinations of pairs in the post-hoc analysis. Both study groups shared similar results (Tables 3 and 4). However, a non-significant reduction (*p* > 0.05) in bactericidal activity was noticed in the case group when compared with its healthy counterpart for all reagents and extracts, except for HBSS (*p* < 0.001) (Figure 3).

Table 3. Post-hoc analysis of microbiological (bactericidal activity) and biochemical (hydrogen peroxide) parameters for group (A)—healthy subjects.

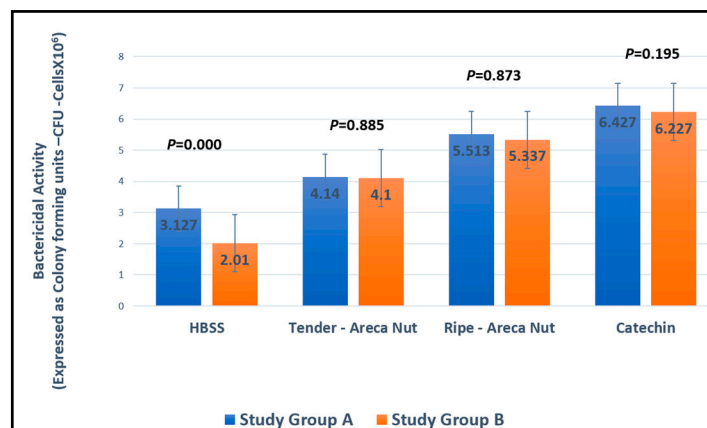
Parameter	Study Group (A)—Values Expressed as Mean ± Standard Deviation			
	HBSS	tANE	rANE	Catechin
Bactericidal Activity	3.127 ± 0.758 × 10 ⁶ b*c*d*	4.140 ± 0.681 × 10 ⁶ b*c*d*	5.513 ± 0.549 × 10 ⁶ b*c*d*	6.427 ± 0.563 × 10 ⁶ b*c*d*
H ₂ O ₂	35.967 ± 6.594 b*c*d*	29.633 ± 5.255 a*c*d*	22.600 ± 4.709 a*b*d*	17.167 ± 4.291 a*b*c*

Note: a = compared to HBSS; b = compared to tenderareca nut; c = compared to ripeareca nut; d = compared to catechin; * *p* < 0.001; SD—standard deviation; H₂O₂—hydrogen peroxide; tANE—tender areca nut extract; rANE—ripe areca nut extract.

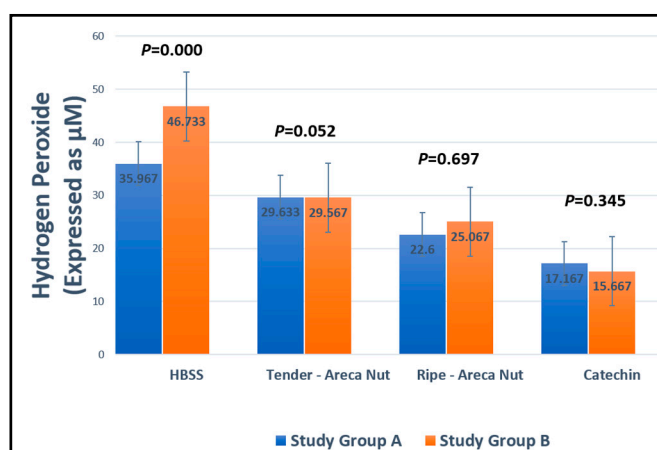
Table 4. Post-hoc analysis of microbiological (bactericidal activity) and biochemical (hydrogen peroxide) parameters for group (B)—chronic periodontitis.

Parameter	Study Group (B)-Values Expressed as Mean $\pm$ Standard Deviation			
	HBSS	tANE	rANE	Catechin
Bactericidal Activity	$2.010 \pm 0.240 \times 10^6$ b ^a c ^a d ^a	$4.100 \pm 0.661 \times 10^6$ b ^a c ^a d ^a	$5.337 \pm 0.586 \times 10^6$ b ^a c ^a d ^a	$6.227 \pm 0.482 \times 10^6$ b ^a c ^a d ^a
H ₂ O ₂	46.733 ± 5.865 b ^a c ^a d ^a	29.567 ± 7.180 a ^a c ^a d ^a	25.067 ± 3.841 a ^a b ^a d ^a	15.667 ± 3.632 a ^a b ^a c ^a

Note: a = compared to HBSS; b = compared to tender arec nut; c = compared to ripe areca nut; d = compared to catechin; * $p < 0.001$; SD—standard deviation; H₂O₂—hydrogen peroxide; tANE—tender areca nut extract; rANE—ripe areca nut.

**Figure 3.** Intergroup comparative analysis of bactericidal activity.

To unwind the machinery of bactericidal action, the H₂O₂ assay was assessed. Within the study group, it showed a significant decrease ($p < 0.001$) in the levels of rANE in contrast to tANE. Identical results were seen for both groups (Table 2). Significant ($p < 0.001$) results were seen in the post-hoc analysis, with the highest level of H₂O₂ seen in HBSS followed by tANE and rANE, with lastly catechin being recorded with the minimum. Analogous results were seen for both groups (Tables 3 and 4). The comparison of H₂O₂ assay between the groups yielded non-significant ($p > 0.05$) results with all reagents/extract used in the study, except for HBSS ($p < 0.001$) (Figure 4).

**Figure 4.** Intergroup comparative analysis of hydrogen peroxide production.

4. Discussion

The oral ecosystem is a niche for many commensal and pathogenic bacteria, whether in health or disease. *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), and *Fusobacterium nucleatum* (Fn),

and *Aggregatibacter actinomycetemcomitans* (Aa), belongs to various complexes of dental plaque (DP). These bacteria are identified as secondary colonizers in the subgingival plaque [1,29]. These bacteria are killed continuously by various innate immunological cells like PMNs, which account for more than 90% of GCF cells. The cPMNs are considered to be the frontiers for this battle, as they form a barrier between the junctional epithelium and sub-gingival biofilm [18,19,30]. It is believed that any defect and/or delay in PMN recruitment and its microbicidal action will compromise periodontal health [31]. Periodontal disease becomes even more compromised in the presence of adverse habits. It is well documented that tobacco consumption is a risk factor for developing periodontal disease [32], while the influence of areca nut is still not apparent. Thus, the aim of our study was to explore areca nut as a probable risk factor that can alter the bactericidal activity of neutrophils in healthy and CP patients.

In the present study, the cellular viability of cPMNs was found to be in the range of 90–92% in both groups, with no significant differences between intergroup and intragroup comparisons. This implies that the concentration of areca nut and reagents did not affect the cellular viability of cPMNs. The cellular viability of oral PMNs without any additives was found to be approximately 95% [33]. However, 84–85% of cellular viability of cPMNs was reported when treated with ANE [23]. Studies with peripheral blood PMNs reported a cellular viability of 85–95% when treated with ANE and arecoline [20].

An association has been reported between areca nut chewing and clinically active periodontal disease sites showing bleeding on probing (BOP) [34], as well as clinical attachment loss (CAL) [8]. The subgingival plaques at these sites have been recorded to harbour periodontal pathogens such as Aa and Pg [35]. Nevertheless, Aa is also found in the buccal mucosa of 36% of the healthy population [36]. The serotype c of Aa (AATCCC 33384) is prevalent in the Indian population of healthy (16.7%) and CP patients (41.2%) [37]. Thus, in the current study, this strain was employed to check the bactericidal activity of cPMNs in health and CP.

Most of the methods employed to measure bactericidal activity were indirect, as they evaluated the metabolic burst. Hirsch et al. 1964 [38] reported a direct way of measuring bactericidal activity. This was achieved by plating the bacteria recovered from neutrophils after phagocytosis, and was measured in CFU. The same technique was employed in our study to assess bactericidal activity.

Within individual groups, HBSS-treated cPMNs showed the least impact on CFU. Meanwhile, CFU were less numerous in the CP group than in the healthy group treated with HBSS. This observation could be because of the hyperactivity of the bactericidal activity of PMNs observed in the CP group [39,40]. In both groups, the rANE-treated cPMNs showed a higher number of CFU on plating compared to those treated with tANE. The probable reason for this is the difference in the concentration of alkaloid/phenolic compounds. It was found that the concentration of catechin was 16 times higher in rANE than that in tANE, but investigations into the concentrations of other phenolic compounds in rANE and tANE are still underway [22]. Thus, it cannot be said affirmatively that these constituents of areca nut act synergistically or have an independent function in retarding the bactericidal activity. Nevertheless, similar results were obtained with peripheral blood neutrophils [20]. A comparable reduction in CFU, although non-significant, was seen with the different extracts of areca nut (rANE, tANE) and catechin between the groups (Figure 3). This suggests that the areca nut and catechin altered the activity of cPMNs, thereby impairing their ability to kill microorganisms.

PMNs may kill bacteria through several mechanisms, including the release of toxic oxygen metabolites (superoxide anions, H_2O_2 , etc.) and the extracellular release of antimicrobial substances [41]. We decided to undertake H_2O_2 production for the assessment of the oxidative killing of microorganisms.

On performing the intergroup comparison, H_2O_2 production by neutrophils treated with tANE, rANE and catechin showed a non-significant difference. This can be interpreted as the neutrophils acting in a similar way, irrespective of the status of the subject. It is interesting to note that a similar effect of H_2O_2 production was shown in the presence of areca nut extracts when different study groups were compared. Thus, it has a detrimental effect on the bactericidal activity via oxidative burst. A similar phenomenon was seen when stimulated and unstimulated neutrophils were compared under

the influence of smoke [42]. It was interpreted that phorbolmyristate acetate (PMA) was stimulated in cells exposed to smoke, and there was a time-related inhibition of both superoxide and H_2O_2 production. Thus, it can be assumed that tobacco smoke and areca nut extract work in a similar fashion, but studies are needed to test and validate the above results.

When study groups were compared with HBSS alone, there was a significant difference in H_2O_2 . This can be easily attributed to the fact that cPMNs might be primed or have shown hyperactivity in CP patients, thus indicating more oxidative burst in terms of H_2O_2 . On the contrary, a significant difference in the H_2O_2 production of cPMNs treated with rANE and tANE was observed in the intragroup comparison. However, in another study, when unstimulated cells were exposed to smoke, there was a time-related increase in the release of superoxide and H_2O_2 [42]. Less H_2O_2 production was seen in both of the groups treated with rANE. This observation could be understood by deciphering the oxygen-dependent pathway. In this pathway, NADPH oxidase catalyzes the production of superoxide anions, and subsequently, superoxide dismutase converts it into H_2O_2 , leading to oxidative burst [41]. A similar pattern was observed by Hung et al. (2000) [20] when they compared the superoxide dismutase (SOD) activity of peripheral blood neutrophils in healthy patients treated with ANE. There was also a reduction in SOD activity, and rANE surpassed the tANE. Thus, the reduced production of H_2O_2 could be one of the possible mechanisms seen in our study for reduced bactericidal activity.

As the areca nut extracts reduced the bactericidal activity of the crevicular PMNs of healthy subjects and chronic periodontitis patients, we therefore reject the null hypothesis.

This is the first study of its kind, probing the role of cPMNs isolated from CP patients and healthy subjects under the influence of ANE. In our study, a similar reduction in the bactericidal activity of cPMNs was seen in both study groups when treated with tANE and rANE. Based on these results, it can be assumed that the areca nut would have potentiated the initiation of the disease in health status and progression in CP patients. This could have implications in the determination of prognosis and treatment planning. With the limitation of a small sample size, *in vivo* and longitudinal studies are warranted to validate the observations. Furthermore, it would be relevant to expand on the current study model by taking more periodontal pathogens into consideration, such as *P. gingivalis*, *T. denticola*, and *F. nucleatum*, as well as a newer species, *Filifactora locis* [43]. It would be interesting to conduct studies to better understand the role of areca nut on other immune and circulating progenitor cells of periodontally healthy and diseased subjects.

5. Conclusions

A decreased bactericidal activity and H_2O_2 production of cPMNs were reported in both healthy subjects and patients with CP when treated with tANE and rANE. This implies that the areca nut influences the cPMNs, thus reducing their efficiency at eliminating bacteria from the periodontal environment. It could be one aspect of areca nut in compromising the oral health of chewers.

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