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Original Article

# Impact of red-lime and white-lime betel quid on oral cell lines: Cytotoxicity and effects on fibronectin and type I collagen expression

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**KEYWORDS**
Betel quid;
Periodontal ligament fibroblasts;

**Abstract** *Background/purpose:* Chewing betel quid is linked to an increased risk of oral cancer. This study investigates the effects of red-lime and white-lime betel quid extracts on oral cell lines, focusing on cytotoxicity and their influence on fibronectin and Type I collagen expression, which were crucial for oral tissue integrity and cancer development.

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Cytotoxicity;
Type I collagen;
Red-lime

*Materials and methods:* Four oral cell lines, human gingival fibroblasts, tongue squamous cell carcinoma cells, human periodontal ligament fibroblasts, and human fetal osteoblasts, were treated with red-lime and white-lime betel quid extracts. Cytotoxicity assays and Western blotting were used to assess cell viability and protein expression.
*Results:* Both red-lime and white-lime betel quid extracts exhibited dose-dependent effects on all tested cell lines, with variations in sensitivity observed among cell types. Notably, red-lime betel quid exerted stronger cytotoxic effects on human gingival fibroblasts and human fetal osteoblasts. Red-lime betel quid increased fibronectin and Type I collagen in periodontal ligament fibroblasts but reduced both proteins in fetal osteoblasts. White-lime betel quid extract generally elevated fibronectin and decreased Type I collagen across cell lines.
*Conclusion:* This study reveals a nuanced, concentration-dependent impact of betel quid extracts on oral cells, with significant variability in cytotoxicity and changes in fibronectin and Type I collagen expression. These findings suggest that abrupt cessation of betel quid chewing can lead to dental issues such as mobile teeth. Red-lime betel quid uniquely affects periodontal ligament fibroblasts by increasing both fibronectin and Type I collagen.


## Introduction

Chewing betel quid is a significant risk factor for oral cancer, with studies indicating substantial risks compared to individuals who have never used it. The International Agency for Research on Cancer classifies it as a Group 1 carcinogen.[1] Betel quid chewing is prevalent in South Asia, Southeast Asia, and the Pacific Islands and is a major factor in the development of oral cancer due to arecoline, the primary carcinogenic ingredient in areca nut.

In Taiwan, two major types of betel quid products are available (Fig. 1 and Supplement Fig. 9): red-lime betel quid and white-lime betel quid. Red-lime betel quid known as "Chhi$^{n}$-á" in Taiwanese, involves splitting a fresh areca fruit, stuffing it with pieces of *Piper betle* Linn. inflorescence, and adding red lime. Red lime betel quid consists mainly of slaked lime (calcium hydroxide) mixed with ingredients like licorice powder, traditional Chinese medicine, and spices. White-lime betel quid, or "hióh-á," involves wrapping a fresh areca fruit with betel leaves coated with white lime (calcium hydroxide).

Before 2000, red-lime betel quid dominated the Taiwanese market. Recently, however, rumors among the public and oral cancer patients suggest that red-lime betel quid irritates the oral mucosa, increasing the risk of oral ulcers and cancer. Consequently, white-lime betel quid has become more popular. Although betel quid consumption is decreasing, the incidence of oral squamous cell carcinoma is declining slowly.

Current research primarily focuses on the carcinogenic effects of arecoline, with limited attention to the impact of betel quid products on periodontal ligaments and bone. The effects of betel quid on various oral cell lines have not been adequately studied, and the influence on extracellular matrix (ECM) proteins like fibronectin and Type I collagen remains unknown.

Our study aimed to investigate the cytotoxicity of red-lime and white-lime betel quid extracts on different oral cell lines and explored their effects on fibronectin and Type I collagen expression. This research was significant as it explored fibronectin levels in betel quid-related oral health issues, potentially guiding interventions or preventive measures.

This manuscript examined the effects of different betel quid products on in vitro cultured human gingival fibroblasts, squamous cell carcinoma cells, human periodontal ligament fibroblasts, and human fetal osteoblasts. We assessed the cytotoxicity and the expression of collagen type I and fibronectin through the western blotting, providing insights that may spark future studies on betel quid.

## Materials and methods

### Cell culture

This in vitro study utilized four cell lines: HGnF (human gingival fibroblasts), SCC-25 (tongue squamous cell carcinoma), HPLF (human periodontal ligament fibroblasts), and hFOB1.19 (human fetal osteoblasts). HGnF Cells were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA) and cultured in low glucose Dulbecco's Modified Eagle Medium (DMEM; GeneDireX, Taoyuan, Taiwan) with 4 mM L-glutamine, 1000 mg/L glucose, 110 mg/L sodium pyruvate, penicillin/streptomycin, and 10 % fetal bovine serum (FBS; Thermo Fisher Scientific, Waltham, MA, USA). SCC-25 Cells were procured from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan) and cultured in DMEM/F12 with 25 mM HEPES, 400 ng/ml hydrocortisone (Sigma—Aldrich, Merck, Darmstadt, Germany), and 10 % FBS. HPLF Cells were purchased from ScienCell Research Laboratories and cultured in low glucose DMEM supplemented with 4 mM L-glutamine, 1000 mg/L glucose, 110 mg/L sodium pyruvate, penicillin/streptomycin, and 10 % FBS. hFOB1.19 Cells were obtained from Bioresource Collection and Research Center (BCRC) and cultured in DMEM/F12 without phenol red, supplemented with 0.3 mg/ml G418 (Sigma—Aldrich, Merck, Darmstadt, Germany) and 10 % FBS.

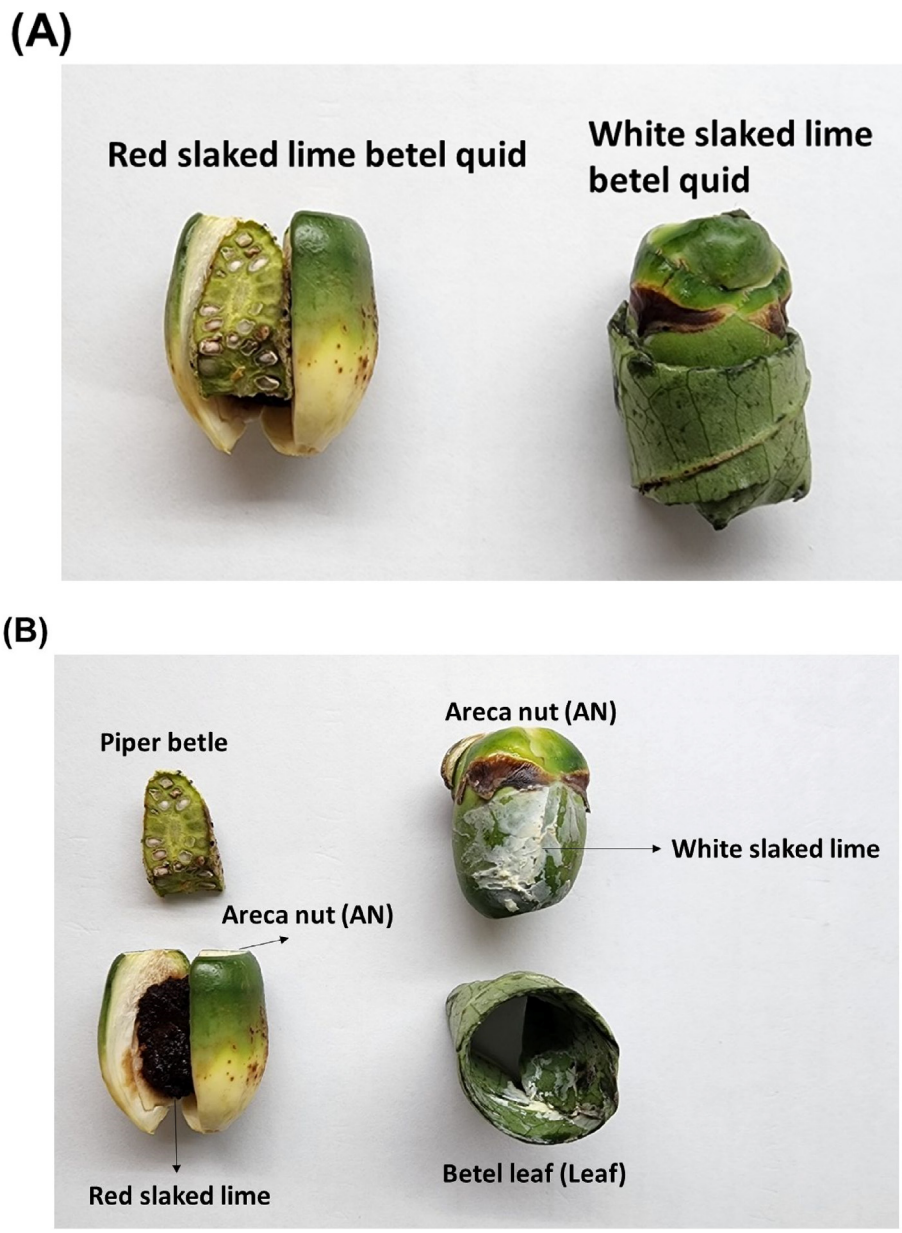


**Figure 1 Commercially available red-lime and white-lime betel quid in Taiwan**: (A) The image on the left depicts a red-lime betel quid, while the image on the right shows a white-lime betel quid; (B) The left panel illustrates the ingredients of the red-lime betel quid, which include areca nut (AN), red-lime, and piper betle. The right panel shows the ingredients of the white-lime betel quid, consisting of areca nut (AN), white slaked lime, and betel leaf.

All cell lines were maintained at 37 °C with 95 % air and 5 % CO2. Subculturing was performed using 0.05 % Trypsin—EDTA (GeneDireX) when cells reached 80—90 % confluence.

## Betel quid extraction

Red-lime betel quid (containing areca nut, kudzu, and red lime) and white-lime betel quid (containing areca nut, betel leaves, and white lime) were used (Fig. 1). Juice extraction involved blending the betel quid with double-distilled water (ddH2O), collecting the mixture, and extracting it overnight at 4 °C. After centrifuging at 4000 RCF for 15 min, the supernatant was filtered and dried into powder at 50 °C. The powder was dissolved in ddH2O and stored at −20 °C for research.

## Treatment

Cells were seeded to 80—90 % confluence and treated with varying betel quid extract concentrations (0—800 μg/mL) for up to seven days. Samples were collected on days 1, 3, 5, and 7 for cytotoxicity analysis and to determine appropriate concentrations for subsequent experiments.[2,3]

### Cytotoxicity assay

$1 \times 10^4$ cells/well were seeded in a 96-well plate and treated with betel quid extracts for 1, 3, 5, and 7 days. Following the Cell Counting Kit-8 (Biotools Co., New Taipei City, Taiwan) protocol, the CCK-8 reagent was added, and plates were incubated at 37 °C, 5 % CO2 for 1.5 h. Absorbance was measured at 450 nm.

### Protein extraction

$1 \times 10^6$ cells/well were seeded in a 10 cm dish. After seven days of treatment, cells were washed with 1xPBS, scraped, centrifuged, and lysed on ice with cell lysis buffer (Pro-prep protein extraction solution, iNtRon Biotechnology, Kirkland, WA, USA). The lysate was centrifuged, and the supernatant was collected for protein concentration measurement using the SMART BCA protein assay kit (iNtRon Biotechnology). Samples were stored at −80 °C.

### Western blotting

Cell lysate was mixed with 5*x* protein loading dye (iNtRon Biotechnology), and proteins were separated using 8 % SDS-PAGE, then transferred to PVDF membranes (Cytiva, Amersham, UK). Membranes were blocked with 2 % BSA (Sigma−Aldrich, Merck, Darmstadt, Germany) and incubated overnight with primary antibodies for fibronectin (Abcam, Cambridge, UK), collagen I (Proteintech, Rosemont, IL, USA), and β-actin (Abcam). After washing, membranes were incubated with secondary antibodies (Abcam), and signals were developed using ECL-HRP substrate (Biotools). Images were captured using the Bio-rad ChemiDoc XRS + system (Bio-rad, Hercules, CA, USA) and analyzed with Bio-rad Image Lab Software.

### Statistical analysis

Data are expressed as mean ± SE. Statistical analysis was performed using Prism8 software (GraphPad Software, San Diego, CA, USA). Differences between groups were assessed using one-way ANOVA or Unpaired t-tests, with $P < 0.05$ considered statistically significant.

## Results

### Effective dosage of red-lime betel quid on various cell lines

**HGnF cells:** Treatment with red-lime betel quid showed a dose-dependent reduction in cell survival. At 800 μg/ml for 1 day, only 65 % of cells survived. For 3, 5, and 7 days at >200 μg/ml, survival rates were 65 %, 55 %, and 46 %, respectively. We used 200 μg/ml for 7 days for further experiments (Fig. 2A and Table 1).

**SCC-25 cells:** A 100 μg/ml dose stimulated cell proliferation for up to 5 days, and 200 μg/ml for 7 days also promoted growth. However, doses >400 μg/ml inhibited growth (Fig. 2B and Table 1).

**HPLF cells:** At 800 μg/ml for 1 day, only 65 % survived. Doses >400 μg/ml for 3 days also inhibited growth. A 100 μg/ml dose for 7 days was used for further experiments (Fig. 2C and Table 1).

**hFOB1.19 cells:** Treatment with 800 μg/ml for 1 day left only 19 % of cells alive. Doses >400 μg/ml for 3 and 5 days reduced survival to 70 % and 46 %. We used 100 μg/ml for 7 days in further experiments (Fig. 2D and Table 1).

### Effective dosage of white-lime betel quid on various cell lines

**HGnF cells:** A dose of 800 μg/ml for 1 day resulted in 51 % survival. Doses > 400 μg/ml for 3, 5, and 7 days showed survival rates of 79 %, 68 %, and 54 %. We used 400 μg/ml for 7 days for further experiments (Fig. 3A and Table 2) (see Table 3).

**SCC-25 cells:** A 400 μg/ml dose for 1 day stimulated cell proliferation. A 200 μg/ml dose for 7 days was used for further experiments (Fig. 3B and Table 2).

**HPLF cells:** Treatment with 800 μg/ml for 1 day left 70 % of cells alive. Doses > 400 μg/ml for 3, 5, and 7 days resulted in survival rates of 64 %, 56 %, and 56 %. We used 400 μg/ml for 7 days for further experiments (Fig. 3C and Table 2).

**hFOB1.19 cells:** A dose of 800 μg/ml for 1 day resulted in 23 % survival. Doses > 400 μg/ml for 3, 5, and 7 days showed survival rates of 69 %, 53 %, and 42 %. We used 400 μg/ml for 7 days in further experiments (Fig. 3D and Table 2).

### Effects of red-lime betel quid on protein expression

**HGnF cells:** 200 μg/ml red-lime betel quid increased fibronectin but decreased Type I collagen expression (Fig. 4A).

**SCC-25 cells:** 200 μg/ml red-lime betel quid increased fibronectin but decreased Type I collagen expression (Fig. 4B).

**HPLF cells:** 100 μg/ml red-lime betel quid increased both fibronectin and Type I collagen expression (Fig. 4C).

**hFOB1.19 cells:** 100 μg/ml red-lime betel quid decreased both fibronectin and Type I collagen expression (Fig. 4D).

### Effects of white-lime betel quid on protein expression

**HGnF cells:** 400 μg/ml white-lime betel quid increased fibronectin but decreased Type I collagen expression (Fig. 5A).

**SCC-25 cells:** 200 μg/ml white-lime betel quid increased fibronectin but decreased Type I collagen expression (Fig. 5B).

**HPLF cells:** 400 μg/ml white-lime betel quid increased fibronectin but decreased Type I collagen expression (Fig. 5C).

**hFOB1.19 cells:** 400 μg/ml white-lime betel quid decreased both fibronectin and Type I collagen expression (Fig. 5D).

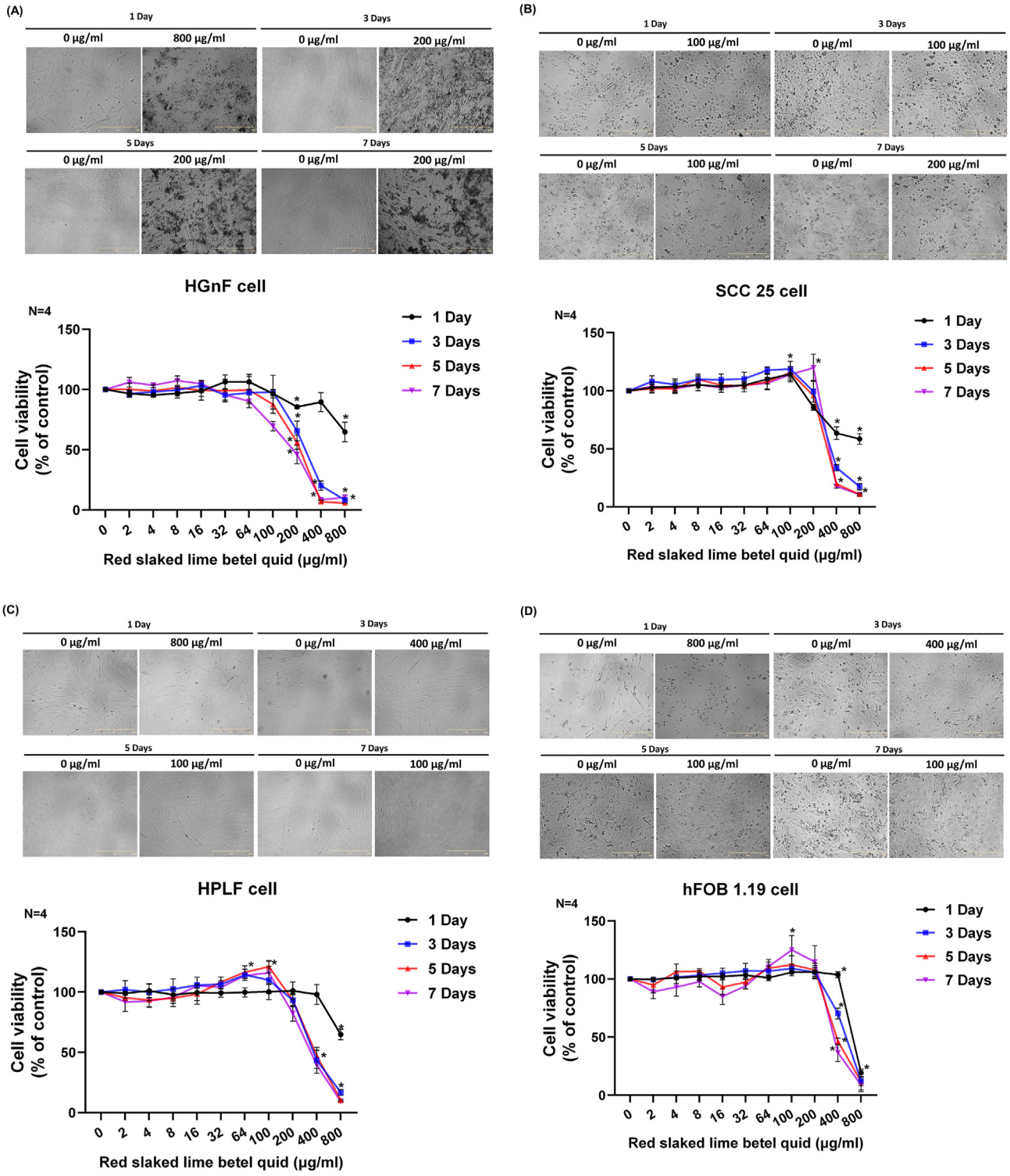


**Figure 2 Effective concentrations of red-lime betel quid on various human cell lines.** (Detailed data are shown in the Supplementary Figures) Eleven different concentrations of red-lime betel nut were administered to cultured cells for 1, 3, 5, and 7 days. Cell proliferation was analyzed using the CCK-8 assay to determine the effective concentration and treatment duration for subsequent experiments: (A) Human gingival fibroblast (HGnF) cells treated with 200 μg/ml red-lime betel quid for 7 days; (B) Squamous cell carcinoma (SCC-25) cells treated with 200 μg/ml red-lime betel quid; (C) Human periodontal ligament fibroblasts (HPLF) cells treated with 100 μg/ml red-lime betel quid; (D) Human fetal osteoblast cell line (hFOB1.19) cells treated with 100 μg/ml red-lime betel quid.

**Table 1** Summary table of effective doses for cells treated with red-lime betel quid (n = 4). There was a significant difference between the experimental groups and the control group (0 μg/ml). Statistical analysis was performed using one-way ANOVA. * indicates $P < 0.05$, indicating a significant difference. ** indicates $P < 0.005$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$.

| Red slaked lime betel quid | 1 day | 3 days | 5 days | 7 days |
|---|---|---|---|---|
| **HGnF** | | | | |
| Concentration (μg/ml) | 800 | 200 | 200 | 200 |
| Cell viability (%) | 65 | 66 | 56 | 46 |
| *P value* | **** | **** | **** | **** |
| **SCC-25 cell** | | | | |
| Concentration (μg/ml) | 200 | 200 | 200 | 200 |
| Cell viability (%) | 114 | 119 | 116 | 120 |
| *P value* | ** | **** | *** | *** |
| **HPLF cell** | | | | |
| Concentration (μg/ml) | 800 | 400 | 100 | 100 |
| Cell viability (%) | 65 | 43 | 121 | 116 |
| *P value* | **** | **** | **** | ** |
| **hFOB cell** | | | | |
| Concentration (μg/ml) | 800 | 400 | 400 | 100 |
| Cell viability (%) | 19 | 70 | 46 | 125 |
| *P value* | **** | **** | **** | ** |

## Discussion

The results of our study highlight the differential cytotoxic effects of red-lime and white-lime betel quid extracts on various oral cell lines. The observed dose-dependent cytotoxicity underscores the variable sensitivity among cell types, particularly noting that human gingival fibroblasts and human fetal osteoblasts exhibited higher sensitivity to red-lime betel quid extract. This aligns with previous findings that have documented the cytotoxic nature of betel quid components and their potential to induce cell death and impair cellular functions in oral tissues.[1–6]

Interestingly, red-lime betel quid extract increased the expression of fibronectin and Type I collagen in periodontal ligament fibroblasts, a response that could be indicative of an attempt by these cells to maintain extracellular matrix integrity under stress conditions induced by the extract. In contrast, the same extract led to a reduction of these proteins in human fetal osteoblasts, suggesting a detrimental effect on bone-forming cells, which might impair bone integrity and regeneration. This dichotomy in response emphasizes the complex interaction between areca nut components and cellular pathways involved in extracellular matrix production and turnover.[3] In a large Taiwanese population follow-up study, Kuo found that betel nut chewing is associated with decreased calcaneus ultrasound T-Score.[7] Interestingly, Mishra et al. observed that betel leaf extract and its major component, hydroxychavicol, promote osteogenesis and alleviate glucocorticoid-induced osteoporosis in rats.[8]

White-lime betel quid extract demonstrated a more consistent pattern of elevating fibronectin while decreasing Type I collagen across the tested cell lines. The reduction in Type I collagen, a major structural component of the extracellular matrix, could compromise tissue integrity and promote a microenvironment conducive to cancer progression, as previously reported by other studies.[9] This pattern suggests a potential mechanism by which white-lime betel quid could contribute to the development and progression of oral cancers, by altering the extracellular matrix composition and dynamics in a way that favors malignant transformation and invasion.

Our findings provide significant insights into the potential mechanisms by which betel quid chewing contributes to oral pathology. Specifically, the distinct impacts on fibronectin and Type I collagen expression suggest that the components of red-lime and white-lime betel quid may differentially influence the structural integrity of oral tissues, potentially leading to conditions that favor cancer development. The increase in fibronectin, for instance, has been linked to enhanced cell adhesion and migration, processes that are crucial in cancer metastasis. Additionally, the observed decrease in Type I collagen could disrupt tissue homeostasis, leading to a weakened extracellular matrix that is more susceptible to neoplastic changes. Kondaiah et al. found that arecoline down-regulated the expression of collagens 1A1 and 3A1 in human primary gingival fibroblasts; however, these collagens were induced by arecoline in the presence of a spent medium of cultured human keratinocytes.[10] Arecoline interacts with phosphodiesterase 4 A (PDE4A) to exert its effects through modulating PDE4A activity but not PDE4A expression.[11] Tsai et al. noticed that the arecoline-induced buccal myofibroblast activities, including collagen gel contractility, cell motility, and wound healing were all suppressed by paeonol treatment.[12] Existing studies suggest that MMPs influence the expression of Fibronectin and Type I Collagen, thereby facilitating the invasion of OSCC into surrounding cells.[13,14]

Research indicates that fibronectin can regulate the adhesion of gingival fibroblasts to bone.[15] Studies indicate that fibronectin affects cell adhesion, migration, and wound healing and may play a critical role in the interaction between periodontal ligament (PDL) cells and the extracellular matrix (ECM), potentially influencing the

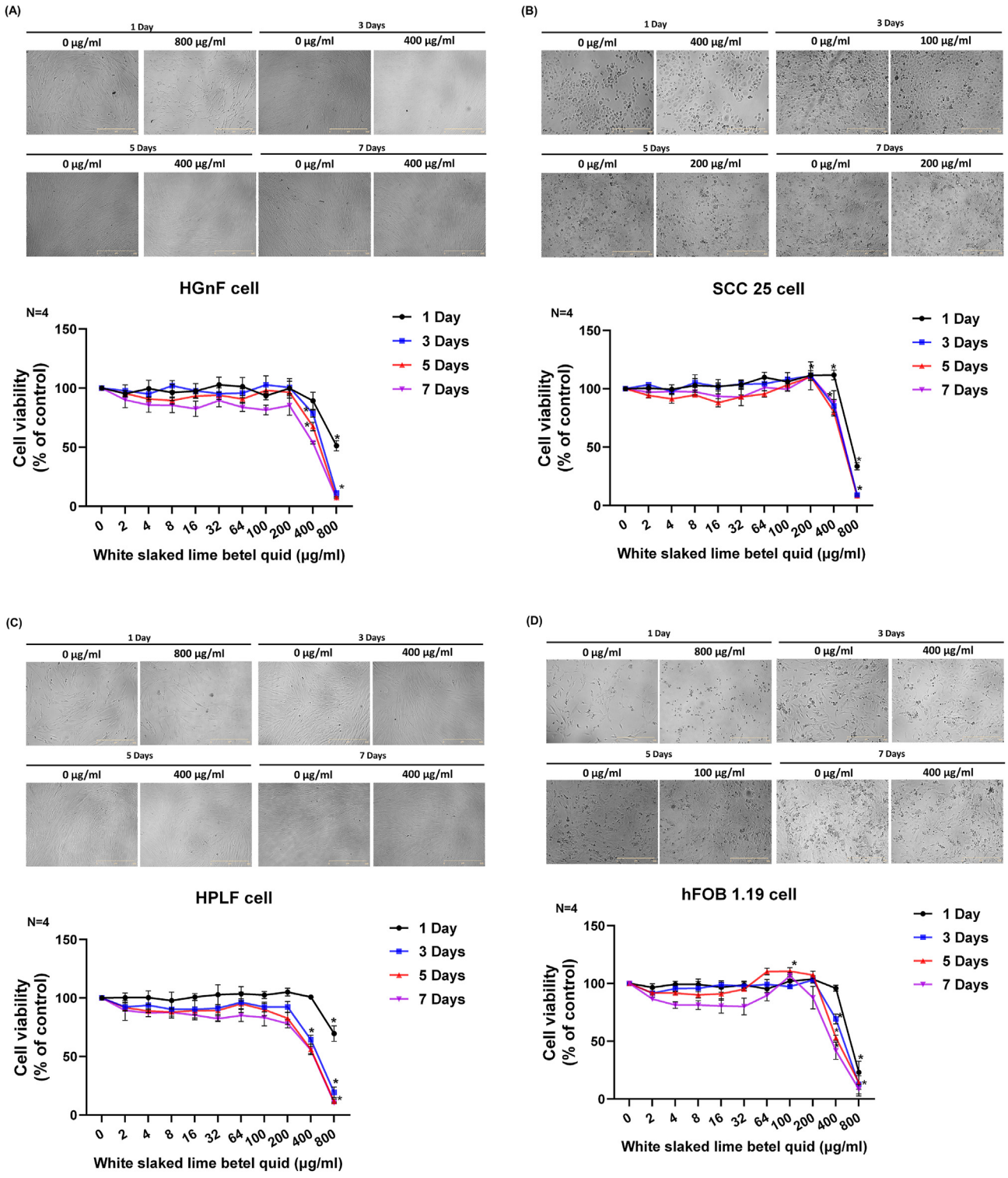


**Figure 3 Effective concentrations of white-lime betel quid on various human cell lines.** (Detailed data are shown in the Supplementary Figures) Eleven different concentrations of white-lime betel quid were administered to in vitro cultured cells for 1, 3, 5, and 7 days. Proliferation was analyzed using the CCK-8 assay to determine the effective concentration and duration for subsequent research: (A) Human gingival fibroblast (HGnF) cells treated with 400 μg/ml white-lime betel quid for 7 days; (B) Squamous cell carcinoma (SCC-25) cells treated with 200 μg/ml white-lime betel quid; (C) Human periodontal ligament fibroblast (HPLF) cells treated with 400 μg/ml white-lime betel quid; (D) Human fetal osteoblast (hFOB1.19) cells treated with 400 μg/ml white-lime betel quid. All cell images were captured using the JuLI FL cell history recorder at 4× magnification. There was a significant difference between the experimental and control groups (0 μg/ml). Data are expressed as mean ± SE. Statistical analysis was performed using one-way ANOVA. * indicates $P < 0.05$, indicating statistical significance.

**Table 2** Summary table of effective doses for cells treated with white-lime betel quid (n = 4). There was a significant difference between the experimental groups and the control group (0 μg/ml). Statistical analysis was performed using one-way ANOVA. * indicates $P < 0.05$, indicating a significant difference. ** indicates $P < 0.005$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$.

| White slaked lime betel quid | 1 day | 3 days | 5 days | 7 days |
|---|---|---|---|---|
| **HGnF cell** | | | | |
| Conc. (μg/ml) | 800 | 400 | 400 | 400 |
| Cell viability (%) | 51 | 79 | 68 | 54 |
| *P value* | **** | **** | **** | **** |
| **SCC-25 cell** | | | | |
| Conc. (μg/ml) | 400 | 100 | 200 | 200 |
| Cell viability (%) | 112 | 108 | 110 | 111 |
| *P value* | *** | ns | *** | *** |
| **HPLF cell** | | | | |
| Conc. (μg/ml) | 800 | 400 | 400 | 400 |
| Cell viability (%) | 70 | 64 | 56 | 56 |
| *P value* | **** | **** | **** | **** |
| **hFOB cell** | | | | |
| Conc. (μg/ml) | 800 | 400 | 400 | 400 |
| Cell viability (%) | 23 | 69 | 53 | 42 |
| *P value* | **** | **** | **** | **** |

**Table 3** Summary of protein expression in human gingival fibroblast (HGnF) cells, squamous cell carcinoma (SCC-25) cells, human periodontal ligament fibroblast (HPLF) cells, and human fetal osteoblast (hFOB) cells affected by red-Lime and white-lime betel quid.

| | Red slaked lime betel quid | | White slaked lime betel quid | |
|---|---|---|---|---|
| | Fibronectin | Collagen I | Fibronectin | Collagen I |
| HGnF | ↑ | ↓ | ↑ | ↓ |
| SCC-25 cell | ↑ | ↓ | ↑ | ↓ |
| HPLF cell | ↑ | ↑ | ↑ | ↓ |
| hFOB 1.19 cell | ↓ | ↓ | ↓ | ↓ |

Both red-lime and white-lime betel quid increased the expression of fibronectin in human gingival fibroblast (HGnF) and squamous cell carcinoma (SCC-25) cells, while they decreased collagen type I expression. In human periodontal ligament fibroblast (HPLF) cells, both red-lime and white-lime betel quid enhanced fibronectin expression; however, red-lime betel quid increased collagen type I expression, whereas white-lime betel quid decreased it. In human fetal osteoblast (hFOB) cells, both red-lime and white-lime betel quid decreased the protein expression of fibronectin and collagen type I.

regeneration of periodontal tissues.[16] Additionally, it is known that Type I collagen affects the development of PDL cells and tooth movement.[17,18] Fibronectin regulates the differentiation of osteoblasts.[19] The consequences of reduced fibronectin and Type I collagen expression in hFOB1.19 cells warrant exploration in the context of bone health. It is currently understood that fibronectin and TGF-β1 may influence bone formation.[20]

Future research should further explore the molecular pathways modulated by these extracts to develop effective interventions. Understanding the specific components responsible for these effects could lead to the development of targeted therapies that neutralize the harmful effects of areca nut while preserving oral tissue integrity. Previous literature has shown that employing Type I or III collagen as a matrix can modulate the growth and/or differentiation of human osteoblasts.[21] Our findings corroborate that inhibition of Type I collagen expression in hFOB1.19 cells occurs with both the red and white betel quid treatments.

The clinical implications of our study are significant, particularly in regions where betel quid chewing is prevalent. Given the complex effects observed in this study, abrupt cessation of betel quid chewing, especially with red-lime betel quid, might result in adverse dental issues such as mobile teeth due to sudden changes in extracellular matrix composition. Therefore, gradual cessation strategies coupled with supportive dental care might be more effective in mitigating these risks.

Despite the significant findings, our study has several limitations. The in vitro nature of the experiments limits the ability to fully extrapolate these results to in vivo conditions. The cellular responses observed in a controlled laboratory environment might differ from those in a complex living organism where multiple factors interact. Additionally, the study did not examine the long-term effects of betel quid extracts on oral cells, which would be critical for understanding chronic exposure impacts. Variations in betel quid composition and preparation methods

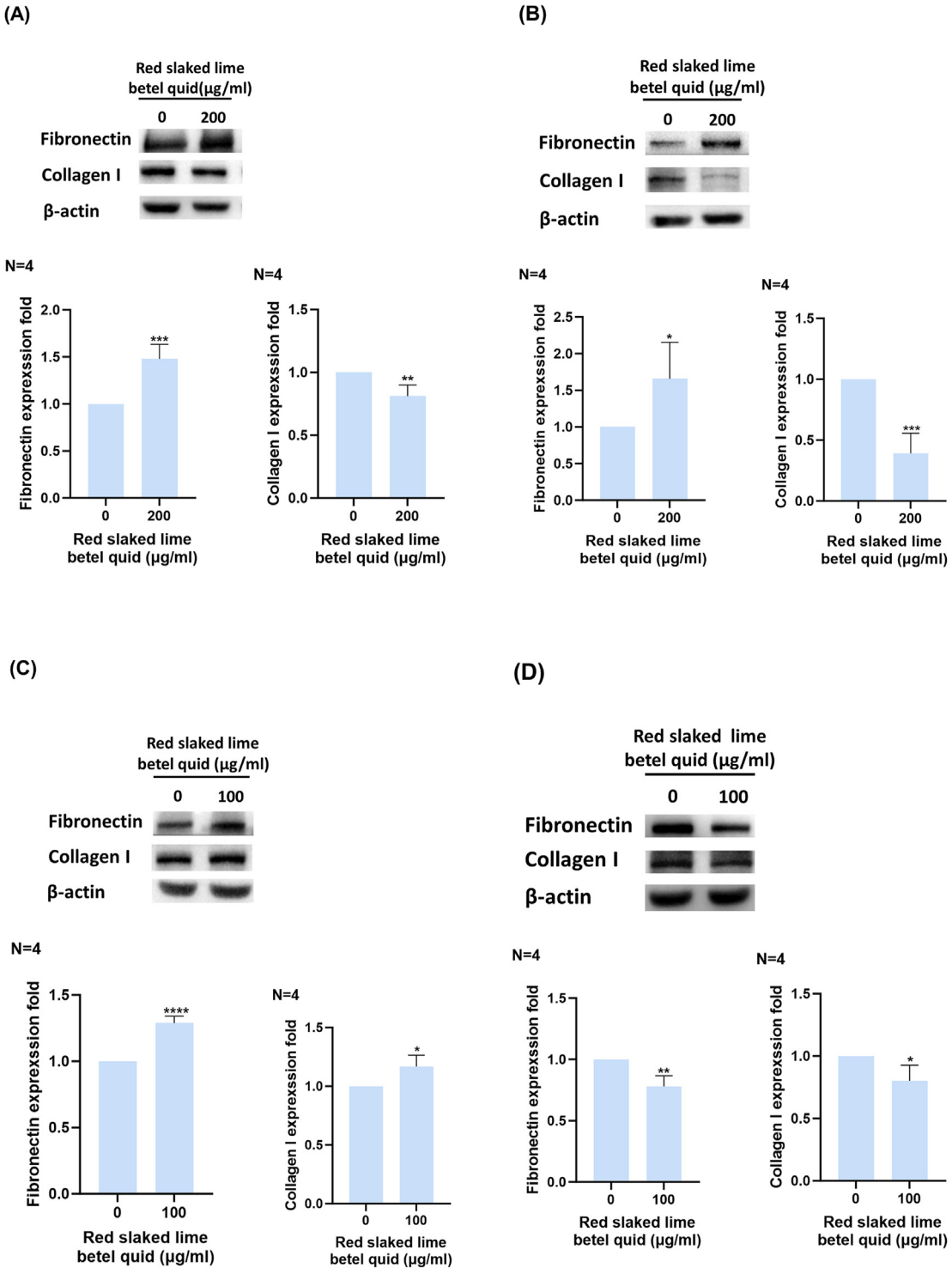


**Figure 4 Effects of red-lime betel quid on protein expression of fibronectin and Type I collagen in various human cell lines.** Western blotting was used to analyze the protein expression of fibronectin and Type I collagen in cells treated with red-lime betel quid (n = 4): (A) Red-lime betel quid stimulated fibronectin expression and inhibited Type I collagen expression in human gingival fibroblast (HGnF) cells; (B) Red-lime betel quid stimulated fibronectin expression and inhibited Type I collagen expression in squamous cell carcinoma (SCC-25) cells; (C) Red-lime betel quid enhanced the protein expression of both fibronectin and Type I collagen in human periodontal ligament fibroblast (HPLF) cells; and (D) Red-lime betel quid reduced the protein expression of fibronectin and Type I collagen in human fetal osteoblast (hFOB) cells. Data are presented as mean ± SE and were compared with the control group (0 μg/ml). Statistical analysis between the two groups was conducted using the unpaired *t*-test. **P* < 0.05, ***P* < 0.005, ****P* < 0.001, indicating statistical significance.

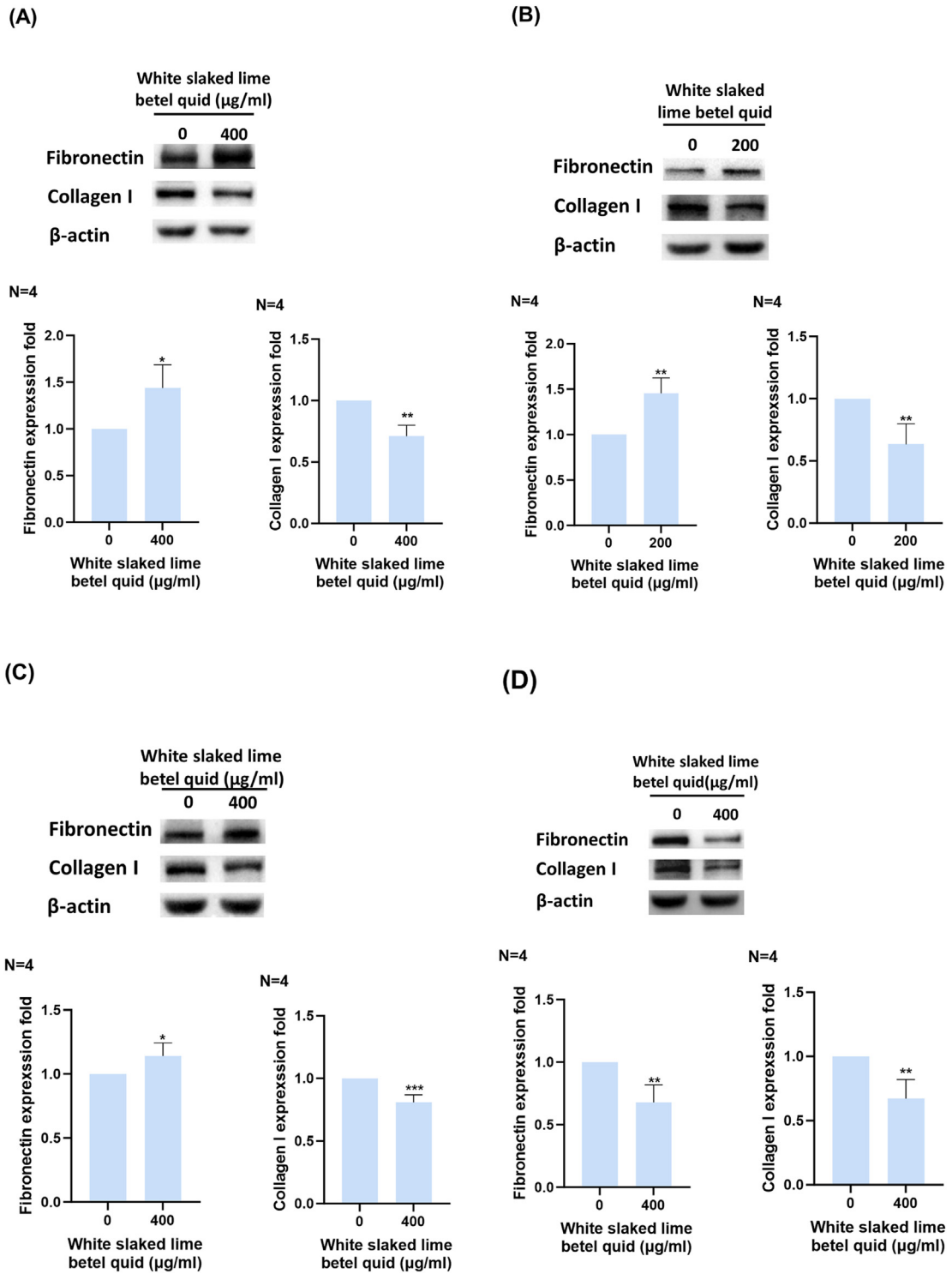


**Figure 5 Effects of white-lime betel quid on the protein expression of fibronectin and Type I collagen in various human cell lines.** Western blotting was used to analyze the effects of white-lime betel quid on fibronectin and Type I collagen protein expression in cells (n = 4): (A) White-lime betel quid enhanced fibronectin expression and inhibited Type I collagen expression in human gingival fibroblast (HGnF) cells; (B) White-lime betel quid enhanced fibronectin expression and inhibited Type I collagen expression in squamous cell carcinoma (SCC-25) cells; (C) White-lime betel quid enhanced fibronectin expression and inhibited Type I collagen expression in human periodontal ligament fibroblast (HPLF) cells; and (D) White-lime betel quid reduced the protein expression of both fibronectin and Type I collagen in human fetal osteoblast (hFOB) cells. Data were presented as mean ± SE and were compared with the control group (0 μg/ml). Statistical analysis between the two groups was conducted using the unpaired *t*-test. **P* < 0.05, ***P* < 0.005, ****P* < 0.001, indicating statistical significance.

across different cultural practices were not accounted for, which could influence the generalizability of the findings.

This study underscores the differential cytotoxicity of red-lime and white-lime betel quid extracts on various oral cell lines, highlighting their distinct impacts on fibronectin and Type I collagen expression. Red-lime betel quid extract exhibited higher toxicity, particularly affecting human gingival fibroblasts and human fetal osteoblasts. Both extracts altered extracellular matrix protein levels, potentially compromising tissue integrity and promoting cancer progression. These findings emphasize the need for public health initiatives to raise awareness about the risks of betel quid use and for developing targeted therapies to mitigate its harmful effects. Future research should focus on the long-term impacts and molecular mechanisms underlying these effects to support effective intervention strategies for populations where betel quid chewing is prevalent.

## Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jds.2024.09.011.

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