

## **A pilot study on the evaluation of the genotoxic effects of betel quid on human buccal cells using the DNA comet assay in a Sri Lankan cohort**

G. S. C. Prabhashani, C. Udukumbura, H. W. Dilanthi, M. D. M. Fernando

*Center for Natural Product Research and Biotechnology for Integrative Health, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo, Sri Lanka*

Betel quid (BQ) chewing, a prevalent habit in South Asia, is classified as a Group I carcinogen for oral cancer (OC). In Sri Lanka (SL), 92% of tea estate workers are habitual BQ chewers and exhibit a threefold increased risk of developing OC. Despite this, direct evidence of BQ-induced DNA damage in buccal epithelial cells remains limited, particularly within the Sri Lankan population. This pilot study aimed to assess the genotoxic effects of BQ in human buccal cells using the Comet assay (CA), which is a widely used technique to detect genotoxicity by DNA damage in eukaryotic cells. The cross-sectional study included 30 adults (15 BQ chewers and 15 non-chewers) aged > 18 years, from Maradana, Sri Lanka. Ethical approval was obtained from the Ethics Review Committee, Faculty of Medicine, University of Colombo. Buccal cells were collected, and DNA damage was quantified via comet tail length (TL). Statistical analysis was performed using independent t-tests and ANOVA. Significantly higher levels of DNA damage were observed in BQ chewers (mean TL:  $34.49 \pm 1.32$  px) compared to non-chewers ( $13.93 \pm 0.64$  px;  $p < 0.001$ ), confirming BQ's genotoxic potential. No significant differences in TL were observed based on age, gender, smoking status, or tobacco co-use ( $p > 0.05$ ). Despite variability in chewing frequency (daily: 40%; weekly: 6.67%; monthly: 33.3%; occasionally: 20%) and duration (mean: 5 years), no dose-response relationship was detected, possibly due to sample size limitations. The CA effectively detected genotoxicity among habitual BQ chewers in the group, even in the absence of clinical lesions. These findings support the utility of CA for early risk assessment in high-risk populations. Given the established association between BQ use and oral precancerous lesions, urgent public health measures are needed to promote BQ cessation and reduce the OC burden.

**Keywords:** *Betel quid, Genotoxicity, Comet assay, Oral cancer, Sri Lanka*