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IN VITRO EVALUATION OF WOUND HEALING POTENTIAL OF BAJONG LN RICE EXTRACTS: ON CELLULAR ACTIVITIES AND PROTEIN EXPRESSION IN HUMAN GINGIVAL FIBROBLASTS, HGF CELLS

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ABSTRACT

Oral wound healing is a complex and dynamic process that remains underexplored despite its clinical importance. Fibroblasts, particularly human gingival fibroblast (HGF) cells play a central role in this process through their involvement in cell proliferation, migration, extracellular matrix (ECM) remodelling and growth factor signalling. While rice *{Oryza sativa L.)* extracts have demonstrated regenerative potential in dermal and gastrointestinal tissues, their therapeutic relevance and mechanistic roles in oral fibroblast-mediated healing are poorly understood. Notably, Malaysia's indigenous Bajong Lubok Nibong (Bajong LN) rice, traditionally used for treating skin and oral lesions has yet to be scientifically validated in an oral-specific wound healing model. To address this knowledge gap, this study aimed to investigate the cellular and molecular effects of Bajong LN rice methanolic (BLN-ME) and aqueous (BLN-A) extracts on HGF cells, focusing on their potential to promote oral wound healing through modulation of cell viability, proliferation, migration and key signalling pathways. This study employed a structured three-phase *in vitro* approach. In Phase 1, five crude extracts (hexane, ethyl acetate, ethanol, methanol and aqueous) were prepared via successive maceration and screened for cytocompatibility using an optimised MTS assay. BLN-ME and BLN-A were selected for their significant viability-promoting effects on HGF cells without cytotoxicity. Phase 2 evaluated the cellular effects of optimal concentrations 250 ug/ml for BLN-ME and 500 ug/ml for BLN-A on HGF cells; and obtained IC50 as well as ICso on ORL-48 oral squamous carcinoma cells. The functional cellular assays included short- and long-term proliferation (MTS, TBE, clonogenic assays), wound closure (scratch assay) and cell migration (Transwell migration assay). Both extracts enhanced HGF regeneration without inducing malignant cell growth, suggesting therapeutic selectivity for oral wound healing. In Phase 3, ELISA-based molecular profiling revealed upregulation of key autocrine/paracrine growth factors (IGF-1, PDGF-BB, bFGF and EGF) which facilitated receptor tyrosine kinase (RTK) signalling and activation of the downstream PI3K/AKT/mTOR pathway, as confirmed via LY294002 and rapamycin inhibition ($p < 0.05$). Parallel increases were observed in wound-associated effector proteins (FAK, MMP-9, COL-1, MMP-1, TFMP-1 and fibronectin) with a regulated MMP-l/TFMP-1 ratio maintained below 1.0, indicating balanced ECM turnover. Collectively, the findings demonstrated that both BLN-ME and BLN-A act as selective, multi-targeted modulators of regenerative activity in HGF cells, with BLN-ME showing slightly higher potency across assays. Notably, these extracts did not stimulate ORL-48 oral cancer cell growth, thereby affirming their cyto-selective safety profile. These results provide mechanistic clarity and position Bajong LN rice extracts as promising candidates for oral wound healing, with translational potential for future *in vivo* and clinical applications.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Wound healing in the oral cavity is a complex and tightly regulated biological process that restores tissue integrity following injury. This multi-overlapping phased process involves the orchestration of cellular events including haemostasis, inflammation, proliferation, migration, extracellular matrix (ECM) remodelling and re-maturation, primarily driven by fibroblasts (Wallace et al., 2019; Pereira et al., 2022). Among various types of fibroblasts, human gingival fibroblast (HGF) cells demonstrate a unique regenerative phenotype, contributing to the scarless healing observed in oral mucosa compared to skin tissue. HGF cells as the principal mesenchymal cells in the gingival connective tissue, play a pivotal role in orchestrating wound healing events by producing ECM components, secreting growth factors, supporting cellular proliferation and migration, as well as signalling molecules essential for tissue repair (Mah et al., 2014; Ahangar et al., 2020).

In the context of oral health, the capacity for rapid fibroblast-mediated wound healing is crucial in the recovery from traumatic injury, periodontal therapy, surgical interventions and oral ulceration. The biological activities of fibroblasts are regulated by multiple signalling cascades, among which the phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin (PI3K/AKT/mTOR) pathway plays a central role in governing intracellular processes related to cell survival, proliferation and matrix biosynthesis in response to upstream growth factor stimulation (Jere et al., 2022; Kasowanjete et al., 2023; Wang et al., 2023).

The complexity of wound healing highlights its importance in medicine, where understanding how it works is essential for successful treatment. Despite ongoing advancements, the development of affordable, biocompatible and safe wound healing agents remains an ongoing challenge. Many currently available commercial formulations lack selectivity, carry adverse effects and prohibitively expensive for long-term use. Even with progress in medical care, wound healing remains a challenge (Krizanova et al., 2022; Monika et al., 2022). As such, attention has increasingly turned to natural product-based therapeutics, particularly plant-derived compounds due to their