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EFFECTS OF ALPHA-MANGOSTIN ON INFLAMMATION AND GROWTH FACTORS RELEASE BY WOUND HEALING CELLS EXPOSED TO HIGH GLUCOSE ENVIRONMENT

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ABSTRACT

Poor wound healing is a serious complication of diabetes mellitus, culminating in chronic, non-healing diabetic foot ulcers (DFU). The arises from disruptions in cellular and molecular pathways involved in wound healing. Diabetes impacts cell migration, cytokines release, growth factors, and proteases that are crucial for regulating and sustaining the healing process. Natural products like alpha (α)-mangostin, a xanthone from Garcinia mangostana Linn (GML) pericarp, are traditionally used for wound healing. However, their potential in diabetic wound healing is underexplored. This study investigates α-mangostin's effects on inflammation, proliferation, and remodelling phases of wound healing under diabetic conditions in vitro. Human monocytic cells (THP-1), human coronary artery endothelial cells (HCAEC), and human dermal fibroblast cells (HDF) were exposed to 35 mM glucose for 72 hours to simulate diabetic conditions. These cells were then treated with α-mangostin (0.15, 2.5, and 5 µg/mL) for 24 hours. Carboxymethylcellulose (5 µg/mL) was used as a positive control. Cells incubated with glucose alone (35mM) served as a negative control called glucose control. A scratch assay was performed and the rate of cell migration was calculated. The protein and gene expression of IL-6, MMP-9, TIMP-2, and growth factors (PDGF, TGF-β, CTGF, VEGF, and BFGF) were measured by standard ELISA kits and quantitative real-time PCR. The study assessed α-mangostin's effects on IL-6, MMP-9, TIMP-2, and key growth factors across wound healing phases using HCAEC, THP-1, and HDF cells under a high glucose environment. In the inflammatory phase, α-mangostin reduced IL-6 gene expression in HCAEC and THP-1 at all concentrations ($p<0.05$), with reduction of protein secretion found in HDF (0.15 µg/mL ($p<0.001$) and 5 µg/mL ($p<0.0001$). In the proliferative phase, 0.15 ug/mL α-mangostin accelerated HCAEC ($p<0.001$) and HDF ($p<0.01$) migration compared to glucose controls. For growth factors, in HCAEC cells, α-mangostin elevated PDGF gene expression (0.15 and 5 µg/mL ($p<0.05$), VEGF protein secretion (5 µg/mL, $p<0.05$), and BFGF gene/protein levels (0.15 and 2.5 ug/mL, $p<0.01$). In HDF cells, α-mangostin increased PDGF secretion (2.5 and 5 µg/mL, $p<0.05$), TGF-β gene/protein levels (0.15 and 2.5 ug/mL, $p<0.05$), and enhanced CTGF gene expression at all concentrations ($p<0.05$). For THP-1 cells, α-mangostin significantly increased PDGF gene/protein levels at all concentrations ($p<0.05$). Additionally, there was an increment of TGF-β, CTGF, VEGF and BFGF protein/gene levels in THP-1 incubated with α-mangostin at various concentrations. For remodelling phase, various concentrations of α-mangostin significantly reduced both the MMP-9 gene and/or protein levels in HCAEC, HDF and THP-1 cells ($p<0.05$). Conversely, α-mangostin increased TIMP-2 gene/protein levels in HCAEC and THP-1 at various concentrations ($p<0.01$). In conclusion, α-Mangostin reduced IL-6 gene expression and protein secretion in various cell types during the inflammatory phase. It also speeds up cell migration in the proliferative phase and increased the expression of growth factors like PDGF, VEGF, BFGF, TGF-β, and CTGF. In the remodeling phase, α-mangostin lowered MMP-9 levels and increased TIMP-2 expression. This study demonstrates that α-mangostin has beneficial effects on accelerating diabetic wound healing *in vitro.* Therefore, the findings from this study indicate the novel potential of α-mangostin in accelerating diabetic wound healing *in vitro*. This may lead to its development as a therapeutic agent for conditions characterised by high glucose levels, particularly diabetic foot ulcers.

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“Believe that you are capable of doing what you want to do. I know what you can do”.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Diabetes mellitus (DM) is a chronic disease resulting from genetic and environmental factors, culminating in reduced insulin secretion or defective insulin receptor function. This leads to a hyperglycaemic state with accompanying abnormalities in carbohydrate, protein, and fat metabolisms. As outlined in the 2019 World Health Organisation's (WHO) Global Diabetes Report, the number of adults with diabetes has nearly quadrupled since 1980 to 422 million and is projected to reach 680 million by 2045 (Artasensi et al., 2020).

According to the International Diabetes Federation Survey in 2021, 20% (4,431,500) of Malaysians suffer from diabetes, and the number is predicted to increase in the coming years. It is anticipated that 7 million Malaysian adults aged 18 and older will be diagnosed with diabetes by the year 2025, posing a significant threat to the nation's health services (Akhtar et al., 2022). Diabetes complications occur when patients are unable to meet clinical goals, leading to additional diseases and resulting in increasingly more difficult and complex management (Chin et al., 2017). One of the most common complications is poor wound healing, which can lead to diabetic foot ulcers (Mariam et al., 2017).

Normal wound healing consists of stages: haemostasis, inflammation, proliferation, and remodelling stages. Each stage is crucial; failure in any of them can interrupt the wound healing process and result in chronic wounds (Tyavambiza et al., 2022). The process of haemostasis, which involves blood clotting to stop bleeding, starts immediately after an injury. Within hours of injury, the inflammatory phase begins. White blood cells are drawn to the wound site to clear away any cell debris, bacteria, or any offending agents present at the site. The leukocytes also release growth factors to promote the next phase. Granulation tissue is produced during the proliferation phase to "fill in" the tissue that has been injured and removed during the inflammatory phase. In addition, the epithelium also proliferates to close the gap between injured surface cells. After the gap has been closed, this healed tissue is strengthened by the reorganisation of the ground substances such as collagen in the remodelling phase (Wilkinson & Hardman, 2020).