UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF *FICUS DELTOIDEA* ON ATHEROGENESIS: *IN VITRO* AND *IN VIVO* STUDIES

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ABSTRACT

Coronary artery disease (CAD), mainly caused by atherosclerosis, remains the leading cause of morbidity and mortality in Malaysia and worldwide. *Ficus deltoidea* (FD), a medicinal plant with potent anti-inflammatory and antioxidant properties, may provide protection against atherosclerosis. This study evaluated the *in vitro* anti-atherogenic potential of four FD varieties and their bioactive constituents (vitexin and isovitexin), followed by *in vivo* assessment of safety and efficacy in high cholesterol diet (HCD)-induced atherosclerotic rabbits using crude and bioenhanced (BE) extracts. Human coronary artery endothelial cells (HCAEC) were stimulated with lipopolysaccharide (LPS) and treated with aqueous ethanolic extracts (5–40 µg/ml) of FD var. *kunstleri* (FDK), *trengganuensis* (FDT), *intermedia* (FDI), and *deltoidea* (FDD). Effects on inflammation, endothelial activation, oxidative stress, and monocyte adhesion were assessed through protein and gene expression analysis (ELISA and QuantiGene plex), reactive oxygen species (ROS) production (DCFH-DA assay), and monocyte binding (Rose Bengal assay). Vitexin (125–1000 nM) and isovitexin (12.5–100 nM) were also evaluated for their effects on protein inflammation and endothelial activation markers. The most efficacious extract, FDK, was tested *in vivo* in high cholesterol diet (HCD)-fed New Zealand White rabbits to compare the effects of crude FDK extract and its bio-enhanced (BE) form (FDK-CMCS hydrogel; carboxymethyl chitosan (CMCS)). Rabbits were divided into two groups, G1 and G2, which represent early (fed 1% HCD for 4 weeks) and established (fed 1% HCD for 8 weeks) atherosclerosis. Rabbits were then randomised into treatment subgroups: FDK700 (700 mg/kg/day FDK), BEFDK125 (125 mg/kg/day BEFDK), BEFDK250 (250 mg/kg/day BEFDK), FDK-free, simvastatin (2.5 mg/kg/day) and placebo. G2 included an additional FDK800 (800 mg/kg/day FDK). Treatments were given for 8 weeks, followed by a normal diet. The body weight and blood pressure were recorded. Blood samples were collected for lipid and biochemical parameters. Aorta and vital organs were harvested at the end of the experiment. FDK extract exhibited the highest inhibition of inflammation, endothelial and oxidative markers, along with significant reductions in ROS and monocyte adhesion *in vitro.* Vitexin and isovitexin had comparable effects. For *in vivo*, both FDK and BEFDK in G1 and G2 significantly reduced total cholesterol, LDL-C, and HDL-C. Only BEFDK and FDK-free groups showed significant plaque regression in G1; no regression was observed in G2. No adverse biochemical or histopathological effects was observed. In conclusion, FD, particularly FDK, exhibits potent anti-inflammatory, anti-monocyte endothelial adhesion, anti-endothelial activation, antioxidant effects *in vitro,* with vitexin and isovitexin contributing to these effects. *In vivo,* both FDK and BEFDK produced significant improvements in lipid profiles; however, these effects were not fully translated at the tissue level. Both BEFDK (up to 250 mg/kg) and FDK extract (up to 800 mg/kg) are safe for use in atherosclerotic rabbits. Further research is required before these findings can be translated into therapeutic strategies for human studies.

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CHAPTER 1
INTRODUCTION

1.1 Research Background

Atherosclerosis, an accumulation of fatty deposits in the inner wall of the blood vessels, is a public health concern due to the high global incidence and prevalence of cardiovascular disease (CVD). In 2019, CVD caused 32% of deaths worldwide, with an estimated 17.9 million lives lost to the disease. Among the many forms of CVD, ischaemic heart disease tops the cardiovascular fatalities (World Health Organization, 2021), mainly attributed to a fixed blockage of medium-sized arteries that supply blood to the heart muscles. This disease imposes an exorbitant burden on the social, financial, and health ecosystems universally (Amini *et al.*, 2021).

Atherosclerosis, which can be partially reversed by treating the underlying causes and leading a healthy lifestyle to control the risk factors (Schade *et al.*, 2021), is exacerbated by hyperlipidaemia, one of the strong risk factors for both atherosclerosis and CVD. Fortunately, several effective advances made in recent years to slow down disease progression and its cardiovascular consequences, to some extent, have mitigated this grim plight. Treatment for cardiovascular disease includes statins, ezetimibe, β-blockers, aspirin, angiotensin receptor blockers and recently proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor. Currently, statins are the first-line treatment in primary and secondary prevention of CVD (Paparodis *et al.*, 2024).

Despite significant breakthroughs in our understanding of the pathogenesis of atherosclerosis, the treatment of cardiovascular disease is largely aimed at addressing hypertension and hyperlipidaemia rather than addressing the root cause of the disease: Atherosclerosis; specifically, the vascular wall chronic inflammation, which is the active driver of plaque development.

In addition, statins, the drug used in the treatment of hyperlipidaemia, have been linked to unfavourable side effects such as muscle pain, digestive problems, diabetes, and liver damage (Karahalil *et al.*, 2017; Thompson *et al.*, 2016). Atherosclerosis develops over many years, so any anti-atherosclerosis therapy is most likely a long term or even a lifelong treatment. Thus, alternative strategies with minimal harmful effects are urgently needed. In Malaysia, there are an array of herbal plants with medicinal