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MECHANISMS OF ANTI-HYPERTENSIVE EFFECT OF STANDARDIZED AQUEOUS-ETHANOLIC EXTRACT OF *FICUS DELTOIDEA KUNSTLERI* IN SPONTANEOUSLY HYPERTENSIVE RATS

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ABSTRACT

In Malaysia, the prevalence of hypertension has increased over the last 10 years. Considering the current trends in the prevalence of hypertension in Malaysia, it can be speculated that there will be further increases in the number of individuals with cardiovascular diseases, leading to increased healthcare related economic burden. Among the factors that contribute to the poor control of hypertension is poor compliance to treatment. On that note, herbs, which occupy an important place in society have been actively investigated for their pharmacological potential. In this regard, *Ficus deltoidea* plant has attracted attention of researches over the last few years. It has been scientifically proven to possess wide range of pharmacological activities, which include anti-diabetic, wound healing and anti-oxidant properties. The data on the effect of this plant on blood pressure are non-existent. Hence, this study investigated the blood pressure lowering effect of standardized aqueous-ethanolic extract of leaves of *Ficus deltoidea Kunstleri* (FDK extract) in spontaneously hypertensive rats (SHR). The possible mechanisms of its anti-hypertensive effect were also investigated. Firstly, the dose dependent effect of FDK extract was determined using 4 different doses (500, 800, 1000, 1300 mg. Kg^{-1}) that were administered orally for 4 weeks to SHR. Blood pressure was measured weekly using tail-cuff plethysmography. Besides blood pressure, body weight, urine output, food and water intake were also measured weekly. Subsequently, using the most effective dose, the effect of FDK extract on renin-angiotensin-aldosterone system (RAAS), endothelial function and anti-oxidant status was examined. Urine was subjected to ^{1}H-NMR metabolomics and kidney tissue for gene expression studies. Systemic effects of FDK extract were studied by measuring liver and kidney functions as well as morphological features of kidney, aorta and heart. In the dose-response study, 1000 $mg.kg^{-1}$ dose had the largest blood pressure lowering effect, based on area under time versus response curve (AUC). When compared to the control group, FDK extract treated rats showed lower concentration of angiotensin I, angiotensin II, aldosterone and angiotensin converting enzyme (ACE) activity. In addition, FDK extract treated rats showed higher concentration of ACE2 and angiotensin 1-7 in the serum. RT-PCR showed significantly greater ACE2 gene expression in the kidney of FDK extract treated group compared to vehicle treated group. FDK extract treated rats had improved endothelial function as evidenced from the higher concentration of eNOS in this group compared to vehicle treated group. FDK extract treated rats also exhibited higher serum total anti-oxidant capacity (TAC) compared to that in the vehicle treated group. Additionally, urinary metabolomic studies revealed altered levels of amino acids, ketone bodies and substances involved in energy metabolism and oxidative stress in the FDK extract treated group, indicating its antioxidant properties in its anti-hypertensive effect. The gene expression for FOXO3a, SOD2 and catalase were significantly greater in the kidney of FDK extract treated group compared to vehicle treated group. No changes were observed in liver and kidney functions as well as in the morphology of kidney, heart and aorta of FDK extract treated group, indicating absence of significant adverse effects of FDK extract at the dose used. In conclusion, administration of 1000 $mg.kg^{-1}$ of standardized aqueous-ethanolic extract of leaves of FDK reduces blood pressure in SHR by reducing RAAS activity and improving endothelial function and redox status. These effects of FDK extract seem to be associated with changes in several metabolic pathways, which include amino acid metabolism, synthesis and degradation of ketone bodies and energy metabolism.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Hypertension is one of the commonest non-communicable diseases (NCD) worldwide. It is also the highest contributor to disability life-years (DALYs) for both males and females (Ministry of Health Malaysia, 2018) The prevalence of hypertension among adults aged 18 years and above in Malaysia was estimated at 35.3% in 2015 (Ab Majid *et al.,* 2018). This has increased from the last few years whereby it was 30.3% based on the National Health and Morbidity Survey 2015 (Ministry of Health Malaysia, 2015). It is noteworthy that non-communicable diseases accounted for 74% of all deaths in Malaysia in the year 2016, whereby 35% of deaths were attributed to cardiovascular diseases (World Health Organization, 2018).

Primary or essential hypertension contributes to 95% of the cases of hypertension. Unlike secondary hypertension, the cause of essential hypertension is unknown. It is believed that essential hypertension involves complex pathophysiological mechanisms. Extensive research has been conducted in order to determine the precise pathophysiological mechanism of hypertension and then accordingly design therapeutic options for its treatment. Role of genetics in essential hypertension is considered complex as no single gene has been identified that contributes to the development of this disease. Polymorphism of several genes has been shown to correlate with the development of essential hypertension (Y. Chen *et al.,* 2019; Jhuo *et al.,* 2019). In the Malaysian population, close relationship was found between polymorphism of Ala589ser of WNK4 gene with increased blood pressure (Ghodsian *et al.,* 2016). Though the role of genetics in human hypertension is increasingly recognized much however still needs to be identified.

Sympathetic nervous system plays an integral part in the regulation of blood pressure. Alteration in the autonomic control was documented in the hypertensive rat model (Berg & Jensen, 2011). The altered autonomic control causes increased excitability of nucleaus tractus solitarii (NTS) of the hypothalamic paraventricular nucleus (HPN), probably involving a disruption in the balance between glutamatergic and GABAergic inputs (J. J. Zhou *et al.,* 2019). Interestingly, exercise has been shown