EVALUATION OF AQUEOUS EXTRACT OF *Syzygium polyanthum* LEAVES AS ANTI-HYPERTENSIVE AGENT IN SPONTANEOUS HYPERTENSIVE RAT (SHR)

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

ACE	angiotensin-converting enzyme
ACEI/s	angiotensin-converting enzyme inhibitor/s
AESP	aqueous extract from *S. polyanthum*
Ang II	angiotensin II
Animal (K_m)	constant used for the animal
ANOVA	analysis of variance
ARASC	Animal Research and Service Centre
ARBs	angiotensin receptor blockers
BBs	beta blockers
BHT	butylated hydroxytoluene
BP	blood pressure
BSA	body surface area
C	concentration of gallic acid from the calibration curve
c	concentration of quercetin from the calibration curve
CAE	caffeic acid equivalent
CAT	catalase
CCBs	calcium channel blockers
CE	catechin equivalent
CVD	cardiovascular disease
DBP	diastolic blood pressure
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DPX	distyrene plasticizer xylene
EA	ethyl acetate
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
FBC	full blood counts
FE	flavonol equivalent

FRAP	ferric reducing antioxidant power
FRIM	Forest Research Institute Malaysia
GA	gallic acid
GAE	gallic acid equivalent
GCMS	gas chromatography and mass spectrometry
GP_X	glutathione peroxidase
HA	hippuric acid
HCL	hydrochloride acid
4-HNE	4-hydroxynonenal
H.E.D.	human equivalent dose
H_2O_2	hydrogen peroxide
HHL	Hippuryl-Histidyl-Leucine
H&E	hematoxylin and eosin
HPLC	high-performance liquid chromatography
HRP	horseradish peroxidase
Human (K_m)	constant for human
IC_{50}	half inhibitory concentration
IMR	Institute for Medical Research
IP	intraperitoneal .
LC-MS	liquid chromatography-mass spectrometry
M	mass of dry extract
MDA	malondialdehyde~~s~~
Meth	methanol
mg GAE/1g	mg gallic acid equivalents per 1g of plant material
mM FE /1 mg	mM Ferrous equivalents per 1mg of the dry extract
mg QE/1g	mg quercetin equivalents per 1g of plant material
NaCl	sodium chloride
NCD	non-communicable disease
NHMS	National Health and Morbidity Surveys
NOS	nitric oxide synthase

$O_2^{\cdot -}$	superoxide anion radical
OD	optical density
OH	hydroxyl radical
QE	quercetin equivalent
RAAS	renin –angiotensin –aldosterone system
RE	rutin equivalent
R_f values	retention factor
ROS	reactive oxygen species
R^2	regression coefficient
S. polyanthum	*Syzygium polyanthum*
SBP	systolic blood pressure
S.D.	standard deviation
SEM	scanning electron microscope
S.E.M.	standard error of the means
SGPT/ ALT	serum glutamic pyruvic transaminase/ Alanine transaminase
SHR	spontaneously hypertensive rats
SOD	superoxide dismutase
T&CM	traditional and complementary medicine
TAC	total antioxidant capacity
TBA	thiobarbituric acid
TFC	total flavonoid content
TLC	thin-layer chromatography
TPC	total phenolic content
TPTZ	2,4,6-Tris (2-pyridyl)-s-triazine
UAE	ultrasound-assisted extraction
UV	ultraviolet
UV-Vis	ultraviolet-visible
V	volume of the extract

VSMC	vascular smooth muscle cell
WHO	World Health Organization
WKY	wistar Kyoto
X	distance of solvent front travelled from its origin
XO	xanthine oxidase
Y	distance of spot travelled from its origin
α	type 1 error probability
β	power of study
δ	the difference in means for selected parameter
σ	within group standard deviation
m	ratio of control to experimental group
ppm	parts per million

PENILAIAN EKSTRAK AKUEUS DAUN *Syzygium polyanthum* SEBAGAI AGEN ANTI-HIPERTENSI DALAM TIKUS BERHIPERTENSI SPONTAN (SHR)

ABSTRAK

Hipertensi berkait dengan morbiditi dan mortaliti yang ketara. Penggunaan herba perubatan sebagai perubatan alternatif untuk menguruskan hipertensi meningkat secara exponen. *Syzygium polyanthum* (S. polyanthum), telah diakui sebagai agen antihipertensi secara tradisional. Kajian ini bertujuan untuk mengenalpasti kesan antihipertensi daripada ekstrak akueus daun *S. polyanthum* (AESP) dan mekanisme dengan menggunakan tikus berhipertensi spontan (SHR). Profil fitokimia, pelbagai ciri antioksidan dan aktiviti antihipertensi telah dinilai. Lima puluh ekor SHR jantan telah dibahagikan sama rata kepada 5 kumpulan: SHR tidak-terawat, Losartan-terawat, 3 kumpulan dos AESP-terawat yang berbeza (1500 mg/kg, 1750 mg/kg dan 2250 mg/kg) dan 10 WKY jantan sebagai kawalan. Semua rawatan diberi secara oral selama 12 minggu. Tekanan darah sistolik (SBP) diukur setiap 2 minggu. Analisa biokimia, penanda tekanan oksidatif, dan tahap enzim *Angiotensin-converting* (ACE) telah dinilai pada penghujung kajian. Histologi aorta toraks dan ginjal telah dinilai menggunakan pewarnaan hematoksilin & eosin (H&E), dan mikroskop imbasan elektron (SEM). AESP mengandungi flavonoid dan fenol, dengan asid galik telah dikenalpasti. AESP menunjukkan aktiviti antioksidan dan perencat-ACE *in vitro* tinggi. Dalam SHR AESP-terawat; SBP menurun secara ketara, fungsi ginjal dan penanda tekanan oksidatif ditambahbaik. Walau bagaimanapun, hanya AESP (2250 mg/kg) menurunkan kepekatan ACE secara ketara. Terdapat juga penambahbaikan histologi aorta toraks dan ginjal dalam SHR AESP-terawat. Oleh itu, kajian

mencadangkan pelbagai ciri antihipertensi AESP disebabkan oleh antioksidannya (terutamanya asid galik) dan aktiviti perencat ACE. Kajian juga mencadangkan mekanisma antihipertensi *S. polyanthum* melakukan aktiviti perencatan melalui perencatan tindakan ACE yang mungkin melibatkan laluan RAAS.

EVALUATION OF AQUEOUS EXTRACT OF *Syzygium polyanthum* LEAVES AS ANTI-HYPERTENSIVE AGENT IN SPONTANEOUS HYPERTENSIVE RAT (SHR)

ABSTRACT

Hypertension is associated with significant morbidity and mortality. The use of medicinal herbs as alternative medicines to manage hypertension is increasing exponentially. *Syzygium polyanthum*, has been claimed traditionally as an antihypertensive agent. This study aimed to determine the antihypertensive effects of the aqueous extract of *S. polyanthum* (AESP) leaves and its mechanisms using spontaneously hypertensive rats (SHR). The phytochemical profiling, antioxidant properties and antihypertensive activity were evaluated. Fifty male SHR were divided equally into 5 groups; untreated-SHR, Losartan-treated, 3 groups of AESP-treated with different dosages (1500 mg/kg, 1750 mg/kg and 2250 mg/kg), and 10 male WKY rats as control. All treatments were given orally for 12th weeks. Systolic blood pressure (SBP) was measured biweekly. Whereas, the biochemical analysis, oxidative stress markers and angiotensin-converting enzyme (ACE) level were evaluated at the end of the study. The histology of thoracic aorta and kidney were assessed using haematoxylin & eosin (H&E) staining, and scanning electron microscope (SEM). AESP contains flavonoids and phenols with gallic acid detected. AESP showed high *in vitro* antioxidant and ACE-inhibitory activities. In AESP-treated SHR; SBP reduced significantly, improved renal function and oxidative stress markers. However, only AESP (2250 mg/kg) significantly reduced ACE concentration. There was also histology improvement of the thoracic aorta and renal in AESP-treated SHR. Hence, the study suggests that antihypertensive properties of AESP are due to its antioxidant

(mainly gallic acid) and ACE inhibitory activity. Thus, this study reveals the antihypertensive mechanism of AESP exerted inhibitory activity through suppression of the ACE action which might involved RAAS pathway.

CHAPTER 1
INTRODUCTION

1.1 Background of the study

Natural products have been widely used as the primary materials and have played a crucial role in the development of a new drug as a lead compound. The use of medicinal herbs as alternative medicines to manage hypertension is increasing exponentially. The exponential is due to the conservative claim that these products are safe and free from side effects as used in ancient traditional Chinese, Greek-Unani and Ayurvedic medicines (Pandey *et al.,* 2013).

According to the World Health Organization (WHO, 2013a), around 80% of the total world population still relies on herbal products as primary health care. National Health and Morbidity Survey (NHMS) 2015 reported that 0.3% of hypertensive patients chose traditional and complementary medicine (T&CM) as their primary method of treatment (Institute for Public Health, 2015a). Another NHMS 2015 report (Volume IV) on T&CM showed that 40.4% of Malaysian respondents had chosen T&CM as their primary treatment prior to seeking conventional treatment (Institute for Public Health, 2015b). Eighteen point three percent (18.3%) used T&CM practices solely as alternative treatment without seeking conventional treatment.

Many antihypertensive drugs are used to control hypertension, ranging from diuretics, renin inhibitors, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin receptor blockers, calcium channel blockers, α-adrenergic blockers, β-adrenergic blockers to vasodilators. Due to the undesirable side effect of several synthetic drugs

and higher cost of antihypertensive drugs (Kiriyama *et al.,* 2016), more experiments have now been concentrated on herbs and medicinal plants as potential hypertensive medicines. The trend was due to reports that medicinal plants contain thousands of bioactive compounds with therapeutic applications (Pan *et al.,* 2013); i.e. phenolics, flavonoids, diterpenoids, and anthocyanins were thought to contribute to cardiovascular and hypertensive pharmacological potential (Fraga *et al.,* 2019). Hence, people are more aware nowadays in seeking natural-based product approaches to replace the role of synthetic products for their treatment of hypertension and other diseases.

Malaysia, a tropical country in Southeast Asia, has a rich diversity of flora and fauna, mostly herbs and medicinal plants. Malay people especially like to eat raw herbs in their daily diet as a condiment (ulam). Malaysia has more than 120 traditional ulam species available, ranging from shrubs to large trees (Abdul Raji *et al.,* 2017). *Syzygium polyanthum* (Wight) Walp or locally known as 'Serai kayu' is one of the favourite ulam that has been consumed for ages. The plant is commonly grown at home and can be found abundantly on the local market. In addition to eating as ulam, the plant's leaves are usually added to "nasi kerabu" and "Kelantanese laksam" among Malay people. Besides, the plant has traditionally been used to treat various diseases such as hypertension, diabetes mellitus, diarrhoea, gastritis, skin diseases and hypercholesterolemia (Sumono and Wulan, 2008). The plant also has multi-biological properties such as antioxidant (Kusuma *et al.,* 2011), antihypertensive (Ismail *et al.,* 2018), antihyperglycaemic (Widyawati *et al.,* 2015a), and antimicrobial properties (Hamad *et al.,* 2017).

Although it is reported that the plant is useful for treating hypertension and other diseases, there is still limited scientific evidence of its antihypertensive activity. Therefore, it is worth scientifically determining the potential benefits of oral administration of this plant in the management of hypertension and its complications. Consideration is needed because the plant's young shoot has been claimed as a supplement to lower the blood pressure (decoction) among hypertensive people for many years.

Later studies by Dafriani (2016) and Aris (2019) confirmed that the water decoction from *S. polyanthum* leaves in hypertensive patients, were able to reduce the blood pressure. In addition, recent animal studies (*in vivo*) have also been successful in demonstrating a further reduction in systolic blood pressure (SBP) over a short-term period of six weeks (Ahmad *et al.,* 2017; Ismail *et al.,* 2018). Even though both studies have confirmed *S. polyanthum* leaves have been able to lower blood pressure (Ahmad *et al.,* 2017; Ismail *et al.,* 2018), the mechanism for the lowering blood pressure is still not clear to date, and none of the long-term studies to confirm this finding have been conducted. This long-term study period (12 weeks of oral administration-*in vivo*) was therefore conducted to reconfirm the anti-hypertensive effect contributed by *S. polyanthum* leaves extract from low to high doses (1500 mg/kg, 1750 mg/kg and 2250 mg/kg) as well as its possible anti-hypertensive grounds for determination.

The reduction of blood pressure could be due to the high presence of antioxidant compounds (particularly gallic acid), a group of phenolic compounds in the leaves (Ismail *et al.,* 2018). There is considerable evidence supporting the view that dietary intake of antioxidants and polyphenols may have an effect in preventing or reducing

hypertension (Baradaran *et al.,* 2014), which further support the leaves are capable of lowering blood pressure. Some studies have also argued that the presence of angiotensin-converting enzyme inhibitor (ACEI) in the plant (Muthia *et al.,* 2017; Ismail and Ahmad, 2019) may also be one of the potential reasons for reducing blood pressure. Yet less is known about *S. polyanthum* leaves' effect on the Renin Angiotensin Aldosterone System (RAAS) and blood pressure pathways. In order to confirm the possible bioactive compounds and the exact pathway responsible for this antihypertensive activity, an extensive study must be carried out.

Because hypertension is associated with damage to target organs (especially blood vessels and kidneys) (Rahimmanesh *et al.,* 2012; Renna *et al.,* 2013), biochemical study, histological studies and oxidative stress activity were also essential to assess in hypertensive research (Siti *et al.,* 2015; Allison, 2016; Ramli *et al.,* 2017, Ramli *et al.,* 2018). In this current study, these three parameters would be done to assess the hypertensive progression and health-enhancing effects of supplying plant extracts.

1.2 Problem statement

Hypertension is one of the major public health problems due to its rapid increase and high prevalence worldwide. The disease can lead to numerous complications, such as heart disease, stroke and kidney failure, which increase the risk of death if not treated early. Although various antihypertensive drugs have been used to control hypertension, there is still insufficient absolute control of hypertension in Malaysia to date. In addition, these medications have been known to cause some detrimental side effects such as depression, delirium, sexual dysfunction, insomnia, and foetal anomalies, which influence people seeking natural plant-based antihypertensive agents (herbs); which are believed to have a wealth of antioxidants to combat cardiovascular risk and its complications. More scientific research is therefore needed to verify the efficacy and to clarify the safety profile of these herbal medicinal products for their antihypertensive potential.

1.3 Objectives of the study

1.3.1 General objective

To evaluate the therapeutic effects of aqueous extract of *S. polyanthum* (AESP) leaves as antihypertensive in the hypertensive rat model.

1.3.2 Specific objectives

1. To determine the phytochemical compounds present in AESP.
2. To determine the *in vitro* antioxidant properties and angiotensin-converting enzyme (ACE) activity in AESP
3. To determine the effects of daily oral administration of AESP for 12 weeks on blood pressure in hypertensive rats.
4. To determine the effects of daily oral administration of AESP for 12 weeks on biochemical parameters (renal function test, full blood count) and angiotensin-converting enzyme level in hypertensive rats after 12 weeks.
5. To determine the effects of daily oral administration of AESP for 12 weeks on histopathology of kidney and descending thoracic aorta in hypertensive rats after 12 weeks.
6. To determine the effects of daily oral administration of AESP for 12 weeks on oxidative stress markers serum levels in hypertensive rats after 12 weeks.

1.4 Significance of the study

The present study provides a fundamental basis for further exploring the possible mechanism of action of the compound (especially gallic acid) found in *S. polyanthum* leaves as an antihypertensive agent with additional benefits for the improvement of the damaged target organ (kidney and blood vessel). This distinctive initiative should promote the use of an agricultural product of *S. polyanthum* as an innovative, practical food product that will help the health of the global community as more citizens become

more aware of their wellness. It will also reduce the country's dependence on imported vegetation and fruit while helping to improve the socio-economic status of local farmers in our country. In addition, citizens will also benefit from various health benefit from a number of pharma-nutritional medicinal products obtained from *S. polyanthum*, and part thereof. This diversification will enhance people's quality of life in line with the Malaysia's New Economic Model national government agenda; deliver a high impact model and sustainability.

CHAPTER 2
LITERATURE REVIEW

2.1 Non-communicable disease (NCD)

Non-communicable disease (NCD), also known as chronic diseases, can be life-threatening and lead to mortality if adequate treatment is not provided. NCD is non-infectious and cannot be transmitted between individuals, and is the result of a combination of genetic, environmental, physiological, and behavioural factors (WHO, 2018).

The four main categories of NCDs are cardiovascular disease (CVD), cancer, chronic respiratory diseases, and diabetes. According to the World Health Organisation (2018), NCD is reported to have killed approximately 41 million people worldwide per year, equivalent to 71% of total deaths, with the largest contributor being CVD. Deaths due to CVD are estimated at 17.9 million people each year, and more than half were due to hypertension, followed by cancers (9.0 million), respiratory diseases (3.9 million) and diabetes (1.6 million) (WHO, 2018).

In Malaysia, NCD caused 73% of the total deaths in Malaysia (NHMS, 2015). The Second Burden of Disease Study for Malaysia, published by the Institute for Public Health (2012), ranked hypertension, high cholesterol, diabetes, high body mass index (BMI), and smoking among the major contributors to CVD and death. Hypertension is also known to be one of the metabolic risk factors associated with NCD that caused premature death worldwide (WHO, 2018).

2.2 Hypertension

2.2.1 Definition

Hypertension, also known as elevated blood pressure (BP), is commonly associated with increased cardiovascular disease. It is often referred to as a 'silent killer' because the symptoms are not too noticeable (Sheih *et al.*, 2009). This is because hypertension can develop into more severe complications that are insidiously undetected. Based on the WHO report (2013b), the risk of death often increases when these complications are left untreated.

Hypertension can be classified as primary hypertension and secondary hypertension. Primary hypertension can be defined as high BP with no known underlying condition. It accounts for 95% of all cases of hypertension. Primary hypertension is a heterogeneous disorder in which different patients have different causal factors leading to high BP (Carretero and Oparil, 2000). Secondary hypertension, on the other hand, can be defined as high BP with known aetiology, including renal parenchymal hypertension, primary aldosteronism, renovascular hypertension, and sleep apnea syndrome. Secondary hypertension was reported to account for 10% of hypertensive patients (Puar *et al.*, 2016).

2.2.2 Blood pressure categories

Normal individuals have systolic blood pressure (SBP) of less than 120 mmHg and

a diastolic blood pressure (DBP) of less than 80 mmHg, i.e. 120/80 mmHg (Unger *et al.,* 2020) (Table 2.1). A further increase of SBP between 120 and 129 mmHg is classified under "Elevated Blood Pressure." Whereas SBP falls between 130 and 139 mmHg, and DBP falls between 80 and 89 mmHg, it is considered to be "Hypertension Stage I." In "Hypertension Stage II" the SBP is more than 140 mmHg, and the DBP is higher than 90 mmHg. If blood pressure continues to rise above 180 mmHg for SBP and above 120 mmHg for DBP, the situation is referred to as "Hypertensive Crisis;" where emergency treatment is needed.

Table 2.1: Blood Pressure Categories. (Unger *et al.,* 2020).

Blood Pressure Category	**Systolic mmHg (upper number)**		**Diastolic mmHg (lower number)**
Normal	Less Than 120	and	Less Than 80
Elevated	120-129	and	Less Than 80
High Blood Pressure (Hypertension) Stage 1	130-139	or	80-89
High Blood Pressure (Hypertension) Stage 2	140 or Higher	or	90 or Higher
Hypertensive Crisis (consult your doctor immediately)	Higher Than 180	and/ or	Higher Than 120

2.2.3 Prevalence

2.2.3(a) Worldwide

Every year, the incidence of people with hypertension is rising rapidly worldwide. Currently, 1.13 billion people worldwide are living with hypertension (WHO, 2019). The overall prevalence was estimated at around 30% (NHMS, 2019). In the year 2015, one in four men and one in five women had hypertension, and it is expected that the number of people with hypertension will continue to rise to 1.56 billion by 2025 (Tabrizi *et al.,* 2016). Of the most worrying, hypertension is also reported to cause a total of 7.5 million deaths per year (WHO, 2019). Annual mortality is predicted at 23.5 million people by 2030 (WHO, 2013).

2.2.3(b) Malaysia

The 2015 National Health and Morbidity Survey (NHMS) reported about 6.1 million people or about one in three adults with hypertension in Malaysia. Based on the available reports, the prevalence of hypertension differs between countries, countries and subgroups of a country's populations (Picon *et al.,* 2012). The prevalence was higher in our neighbouring countries, Thailand (23.6%) and Singapore (16%). Based on NHMS 2015, 30.8% of males and 29.7% of females were affected by hypertension. There was also a generally increasing prevalence trend by age, from 6.7% in the 18-19 age group to a peak of 75.4% in the 70-74 age group. The prevalence started to plateau from 2011 (32.6%) to 2015 (30.3%) (NHMS, 2019). For the main ethnic groups, Sabah & Sarawak Bumiputera has the highest prevalence at 36.4%, followed

by Malays at 34.0%, Chinese at 32.3% and Indians at 30.6% (IPH, 2011). Among these, Kelantan, along with Kedah, ranked the highest prevalence at 23.2% of undiagnosed hypertension. The prevalence remained high, as there is still no significant improvement made by the community despite policies and task forces implemented by the government of Malaysia (IPH, 2011; MOH, 2014).

2.2.4 Pathophysiological factors of hypertension development

Numerous pathophysiological factors have been involved in the development of hypertension. Factors include neurohormonal factors, dietary factors, vascular factors, cellular mechanisms, oxidative stress, and other factors (Schulz *et al.,* 2011; Acelajado *et al.,* 2012). Numerous evidence has shown that oxidative stress is the predominant factor that leads to an increase in blood pressure (Ceriello, 2008; Rodrigo *et al.,* 2011; Tsiropoulou *et al.,* 2016). Details of pathophysiological factors that may contribute to hypertension are provided in Table 2.2.

Table 2.2: Pathophysiological factors contribute to hypertension. (Schulz *et al.*, 2011; Acelajado *et al.*, 2012).

Pathophysiologic Factor	Mechanism (Increased or decreased activity)
Neurohormonal Mechanism	
SNS activity	Increased
RAAS	Increased
Production of sodium retaining hormones	Increased
Production and expression of vasoconstrictors	Increased
Production and expression of vasodilators	Decreased
Kallikrein-kinin system activity	Decreased
Dietary Factors	
Sodium intake	Increased
Potassium and calcium intake	Decreased
Vascular Factors	
Peripheral resistance	Increased
Vascular stiffness	Increased
Endothelial dysfunction	Increased
Cellular Mechanisms	
Cellular ion transport	Increased or decreased
Adrenergic receptor activity	Increased or decreased
Others	
Inflammation	Increased
Psychosocial stress	Increased
Oxidative stress	Increased
Genetic	Increased
Environment	Increased

2.3 Oxidative stress in hypertension

Oxidative stress is generally known to cause many types of disease. One of these is the key factors that make progress towards hypertension (Rodrigo *et al.*, 2011; Montezano and Touyz, 2012) (Figure 2.1). This occurs when an imbalance exists between the production of reactive oxygen species (ROS) and the antioxidant defence system. In normal physiological conditions, ROS plays an important role in the maintenance of vascular function and its structure. However, under pathophysiological conditions (due to radical exposure, ageing, etc.), ROS overproduction will promote endothelial dysfunction, vascular remodeling and inflammation, ultimately leading to vascular damage and other complications, including kidney damage (Ozbek, 2012; Dikalov and Nazarewicz, 2013; Tsiropoulou *et al.*, 2016).

There are several types of ROS that can be detected within the vessel, including superoxide anion radical ($O_2^{\cdot -}$), hydroxyl radical (·OH), singlet oxygen, hypochlorite radicals, nitric oxide radicals and various lipid peroxides (Landmesser and Harrison 2001; Chandra *et al.*, 2012). Due to the high amount of ROS and the simultaneous reduction of the antioxidant defence system, ROS will alter their molecules to become more toxic radicals. This toxic radical will promote various negative effects on cellular functions such as protein and lipid synthesis disruption, alteration of transcription factors which, in turn, influence various complications, including vascular function (Touyz *et al.*, 2003; Touyz and Schiffrin, 2004) and kidney function and structure (Sachse and Wolf, 2007; Palm and Nordquist, 2011).

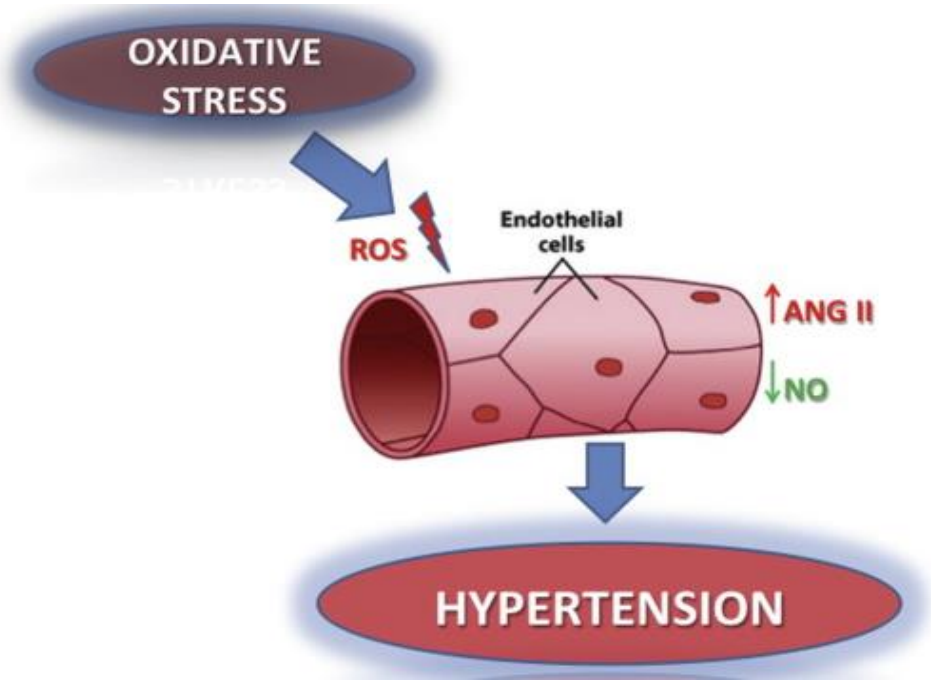

Figure 2.1: Oxidative stress is a key factor that leads to hypertension (Santilla *et al.,* 2015). Overproduction of ROS promotes the reduction of NO and at the same time generates high ANG II level. This condition had caused endothelial dysfunction, which lastly promoting hypertension. NO: nitric oxide, ROS: reactive oxygen species, ANG: angiotensin.

Thus, in order to prevent uncontrolled ROS from rising, the redox equilibrium system becomes an important key to maintaining the correct functionality of cellular functions (Schieber and Chandel, 2014; Kurutas, 2016). Redox systems include enzymatic antioxidants (naturally produced by our body, such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) and non-enzymatic antioxidants (externally supplied from other sources, such as vitamins A, C and E, glutathione and several antioxidants present in food). Relatively, enzymatic antioxidants have been considered to be the best known antioxidant defence system for ROS normalisation compared to non-enzymatic antioxidants (Li *et al.,* 2016; Aziz *et al.,* 2019) and have thus far been used as oxidative stress biomarkers in many studies (Sáez *et al.,* 2004; Ahmad *et al.,* 2013).

Various methods have been developed to measure and evaluate oxidative stress in hypertension, including markers for lipid peroxidation, protein oxidation and antioxidant status (Tsiropoulou *et al.,* 2016). The most commonly used assays are lipid peroxidation (such as malondialdehydes (MDA) assay) and antioxidant status (such as total antioxidant capacity (TAC) assay, SOD assay, CAT assay and GPx assay). On the basis of literature, these assays are important to measure in order to determine the biological redox status, at the same time to assess the hypertensive state and progression and the health-enhancing effects of the antioxidant supply in hypertensive people (Marrocco *et al.,* 2017). This is because many studies have shown that hypertensive people usually have higher levels of MDA (Ahmad *et al.,* 2013; Ekeanyanwu *et al.,* 2016) as well as lower levels of SOD activity (Sáez *et al.,* 2004; Ahmad *et al.,* 2013; Baradaran *et al.,* 2014), CAT levels (Sáez *et al.,* 2004; Ahmad *et al.,* 2013) and TAC levels (Onuoha *et al.,* 2013).

2.4 Complications and consequences of hypertension

Hypertension due to oxidative stress is well documented to cause damage to the end organ (Simão *et al.*, 2011). Several important organs, such as the heart, brain, kidneys, blood vessels, and eyes, are the major target organs that are commonly affected during uncontrolled hypertension (Schmeider, 2010; WHO, 2019). Growing evidence has shown that damage to blood vessels and kidneys have had a major impact (Rahimmanesh *et al.*, 2012; Lyle and Taylor., 2019). The total damage to the hypertensive-induced end organ is summarised in Table 2.3.

Table 2.3: End organ damage in arterial hypertension. (Schmeider, 2010).

End organ damage in hypertension	
Vasculopathy • Endothelial dysfunction • Remodeling • Generalized atherosclerosis • Arteriosclerotic stenosis • Aortic aneurysm	**Cerebrovascular damage** • Acute hypertensive encephalopathy • Stroke • Intracerebral haemorrhage • Lacunar infarction • Vascular dementia • Retinopathy
Heart disease • Left ventricular hypertrophy • Atrial fibrillation • Coronary microangiopathy • CHD, myocardial infarction • Heart failure	**Nephropathy** • Albuminuria • Proteinuria • Chronic renal insufficiency • Renal failure

2.4.1 Blood vessel

2.4.1(a) Normal structure of blood vessel

The blood vessel consists of three layers; tunica intima, media and external (Zhao *et al.,* 2015) (Figure 2.2). Tunica intima, the innermost layer, is composed of a single layer of endothelial. The lining is separated from the tunica medium by an internal elastic lamina. Whereas, the middle layer; the tunica media mainly consists of a large number of smooth muscle cells, a large number of elastic fibres and connective tissues. The layer of the media is distinguished from the external layer by the external elastic lamina. Apart from that, the outer layer; the outer tunica consists of various elements, including connective tissues, vasa vasorum, fibroblasts and collagen fibres, which help to retain the structure of the vessel. The layer is also composed of nerve ends and perivascular adipose tissue.

In normal vessels, two important components of the blood vessels responsible for maintaining the tone of the vessel (vasoconstriction and vasorelaxation) are vascular smooth muscle cells and endothelial cells. The lumen diameter of the blood vessel is controlled by the smooth muscle cells in the tunica media. It regulates the tone of the vessel by expanding circularly around the lumen (Sandoo *et al.,* 2010). In contrast, endothelial cells found on the surface of the endothelial layer regulate the tone of the blood vessel by releasing vasoconstrictors and vasodilators factors (Vanhoutte *et al.,* 2009; Rajendran *et al.,* 2013) including nitric oxide, prostacyclin, angiotensin II (Ang II), endothelin-1, leukotrienes, and reactive oxygen species (ROS) when endothelial cell alterations occur (Vanhoutte *et al.,* 2009).

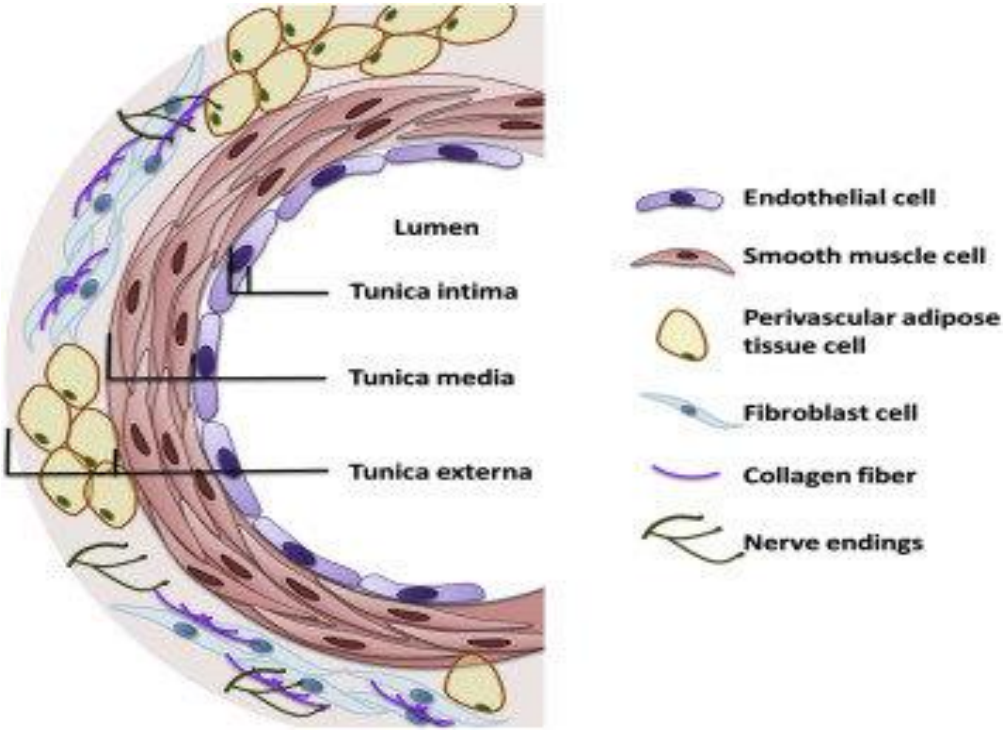

Figure 2.2: Blood vessel layers. The layers consist of tunica intima, tunica media, and tunica externa. (Zhao *et al.*, 2015).

Other than that, endothelial cells are also responsible for maintaining the homeostasis process (Durand and Gutterman, 2013; Wang *et al.,* 2015), controlling the flow of blood, regulating the autocrine-paracrine mechanism (Sena *et al.,* 2013), preventing thrombosis (Verhamme and Hoylaerts, 2006; Vanhoutte *et al.,* 2009), regulating the balance between coagulation and fibrinolysis, reducing platelets-leucocytes adhesion (Verhamme and Hoylaerts, 2006; Vanhoutte *et al.,* 2009), and producing inflammatory mediators to prevent inflammation activity (Calvin *et al.,* 2014). Details of the function of endothelial cells are shown in Figure 2.3.

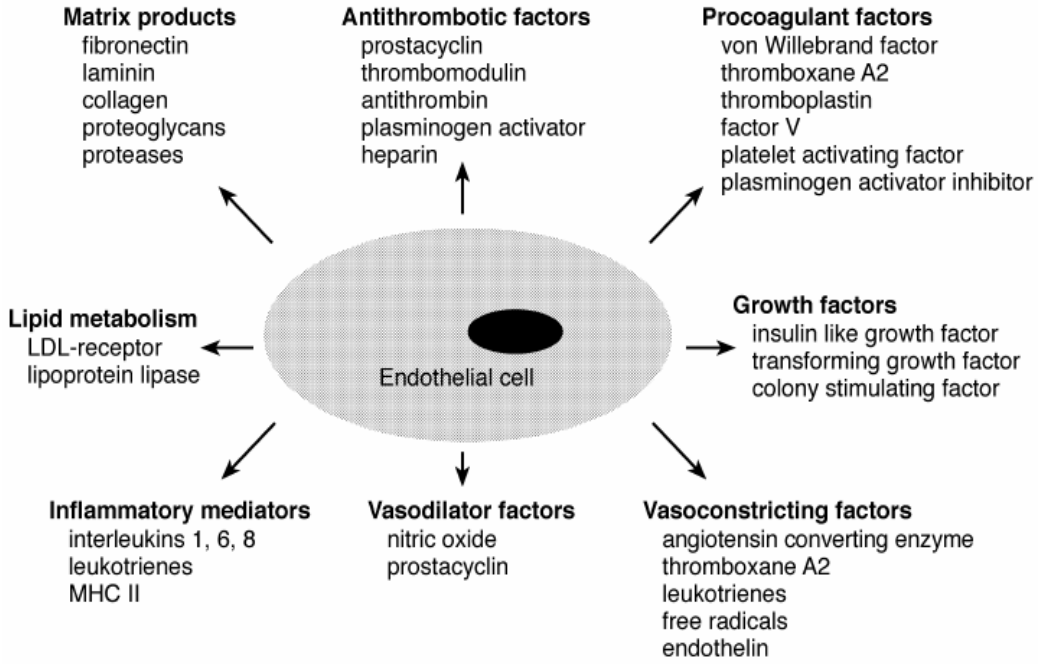

Figure 2.3: Roles of endothelial cell. (Galley, 2004).

2.4.1(b) Alteration of vascular components

A small decrease in the lumen size of the blood vessel due to hypertensive oxidative stress significantly increases the resistance to blood flow. This prolonged blood resistance will alter the structure and function of the vessel (Lyle & Taylor, 2019). The alterations include vascular remodeling, endothelial dysfunction, increased vascular stiffness, and inflammation (Rodrigo *et al.,* 2011; Touyz, 2012; Renna *et al.,* 2013; Montezano *et al.,* 2014). The most common hypertensive complications reported are vascular remodeling and endothelial dysfunction (Savoia *et al.,* 2011; Rahimmanesh *et al.,* 2012; Renna *et al.,* 2013).

Figure 2.4 shows the schematic representation of vascular remodeling in arteries in response to hypertension. Vascular remodeling is indicated by high proliferation and hypertrophy of the smooth muscle cell, migration of monocytes, reduction in elastin levels, high inflammatory cell count, increased apoptosis and increased fibrosis (collagen, fibronectin and extracellular matrix deposition), resulting in thickening of the vascular media and narrowing of the vascular lumen (Intengan and Schiffrin, 2001; Touyz and Schiffrin, 2004; Paneni *et al.,* 2017).

In addition, vascular remodeling can also be found on the endothelial surface of the blood vessel. In normal levels of shear stress (15 - 40 dynes/cm2), endothelial cells line the endothelial layer; normally elongate, align in the direction of blood flow and maintain the barrier function. Too much blood flow, however, will trigger abnormal shear stress. This pressure will cause high friction on the endothelial surface; the endothelial surface will be damaged (Resnick *et al.,* 2003; Yang *et al.,* 2014).

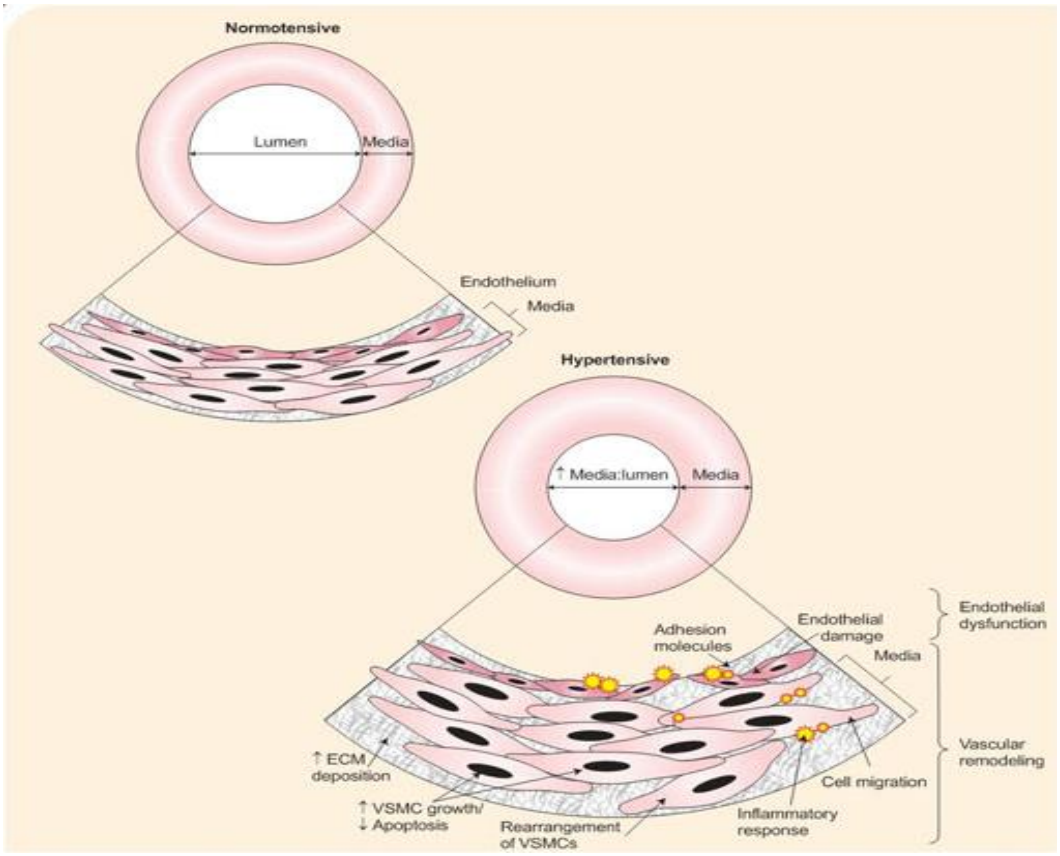

Figure 2.4: The blood vessel underwent vascular remodeling in response to increased resistance to blood flow. Cellular growth of vascular smooth muscle cells (VSMCs), cell migration, rearrangement of VSMCs, extracellular matrix (ECM) deposition, inflammation and endothelial damage (Paneni *et al.*, 2017) are observed.

In addition, damage to the vessel can also be assessed by its functional alterations. In pathological conditions, the function of the endothelial cell will be compromised as it releases a high number of vasoconstrictor factors. This endothelial dysfunction will reduce vascular relaxation and increase vascular contractile activity (Paneni *et al.,* 2017). Many other characteristics of endothelial dysfunction include cell proliferation, fibrosis and adhesion molecules on the wall of the blood vessel (Rahimmanesh *et al.,* 2012).

2.4.2 Renal

2.4.2(a) Normal structure of the kidney

The kidney plays a number of crucial functions. It filters approximately 120 to 150 quarts of blood to produce 1 to 2 quarts of urine, consisting mainly of waste and extra fluid (NIDDK, 2014). A kidney can be divided into three main inner regions (Figure 2.5); renal cortex (outer part of the kidney), renal medulla (inner part of the kidney) and renal pelvis (vessel and nerve of the kidney). There are millions of functional nephronic units or nephron that responsible for filtering the blood, removing waste materials and toxins such as urea, creatinine and uric acid in the form of urine. The nephron itself consists mainly of the renal corpuscle and the renal tubule. There are two main components in the renal corpuscle; the glomerulus and its surrounding Bowman capsule.

The glomerulus is characterised as having a tight capillary ball that supported by a cytoplasmic bundle of actin-like filaments; mesangial cells. These mesangial