S. POLYANTHUM LEAVES AQUEOUS EXTRACT AS POTENTIAL ANTIHYPERTENSIVE AGENT IN SPONTANEOUS HYPERTENSIVE RAT MODEL

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by

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In the name of Allah, the Most Gracious, the Most Merciful

All gratifications are referred to Allah.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

AESP	Aqueous Extract of *Syzygium polyanthum*
ATII	Angiotensin II
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DVR	Descending Vasa Recta
AVR	Ascending Vasa Recta
eNos	Endothelial NO synthase
GBM	Glomerular Basement Membrane
H&E	Haematoxylin and Eosin
NHMS	National Health and Morbidity Survey
NO	Nitric oxide
MESP	Methanolic Extract of *Syzygium polyanthum*
WKY	Wistar Kyoto rat
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SHR	Spontaneous Hypertensive rat
SEM	Scanning Electron Microscope
UAE	Ultrasound-assisted Extraction
VR	Vascular remodelling
RAAS	Renin–Angiotensin–Aldosterone System
CKD	Chronic Kidney Disease
VSMC	Vascular Smooth Muscle Cell
RFT	Renal Function Test
FRAP	Ferric Reducing Antioxidant Power
TPC	Total Phenolic Content
ARB	Angiotensin Receptor Blocker

ABSTRAK

Hipertensi adalah salah satu punca utama yang menyumbang kepada penyakit kardiovaskular. Salah satu tumbuhan perubatan tempatan yang dilaporkan mempunyai potensi sebagai anti-hipertensi adalah *Syzygium polyanthum* (serai kayu). Oleh itu, kajian ini dijalankan untuk mengkaji ekstrak akuas *S. polyanthum* (AESP) sebagai ejen antihipertensi yang berpotensi dalam model tikus hipertensi spontan (SHR). Kajian ini menggunakan 35 ekor tikus jantan SHR yang telah dibahagikan kepada 5 kumpulan; SHR yang tidak dirawat, rawatan dengan Losartan, rawatan dengan 3 dos AESP yang berbeza (1500mg/kg, 1750mg/kg dan 2250mg/kg) bersama dengan 7 ekor tikus jantan WKY sebagai kawalan normal dirawat secara oral selama 92 hari. Hasil rawatan dengan AESP dinilai dengan mengukur pengurangan tekanan darah sistolik (SBP) menggunakan teknik tidak invasif (tail-cuff). Sementara itu, perubahan morfologi torasik aorta dan ginjal tikus dinilai melalui pemerhatian pada pewarnaan hematoksilin & eosin serta mikroskop imbasan electron (SEM). Ujian Fungsi Renal (RFT), aktiviti antioksidan (FRAP) dan jumlah kandungan fenolik terkumpul (TPC) di dalam AESP juga telah dinilai dalam kajian ini. Hasil kajian mendapati, AESP menurunkan bacaan SBP tikus SHR dengan ketara, dan hampir setanding dengan kumpulan kawalan normal pada minggu ke-8 untuk kumpulan yang dirawat, manakala pada minggu ke-10 setanding dengan kumpulan rawatan dengan Losartan. Pada penghujung minggu ke-12, bacaan SBP untuk kumpulan yang dirawat dengan AESP bersamaan dengan kumpulan WKY dan lebih rendah dari kumpulan yang dirawat dengan Losartan. Dos terendah (1500mg/kg) telah dipilih sebagai dos efektif. Tambahan pula, AESP juga telah berjaya menambahbaik struktur morfologi torasik aorta dan ginjal tikus. Penambahbaikan struktur morfologi organ yang dikaji boleh dikaitkan dengan kandungan fenolik dan kadar antioksidan yang tingi dijumpai di dalam AESP. Tiada perbezaan yang ketara dalam RFT antara kumpulan kajian. Adalah dicadangkan bahawa biarpun terdapat perubahan morfologi ginjal yang ketara disebabkan oleh kesan hipertensi selama 3 bulan, namun tiada kesan kepada fungsinya. Jadi, kajian ini mencadangkan bahawa *S. polyanthum* mempunyai potensi sebagai ejen antihipertensi dan boleh mengurangkan kesan kerosakan organ akhir dalam penyakit hipertensi.

ABSTRACT

Hypertension remains a major modifiable risk factor for cardiovascular disease (CVD). One of the local medicinal plant reported could be potentially served as antihypertensive agent is *Syzygium polyanthum* (serai kayu). Therefore, this study aims to evaluate the aqueous extract of *S. polyanthum* (AESP) as potential antihypertensive agent in spontaneous hypertensive rat model (SHR). This study employed 35 male SHR which then was further divided into 5 group; untreated-SHR, Losartan-treated, 3 AESP-treated with different dosages (1500 mg/kg,1750 mg/kg and 2250 mg/kg) along with 7 male WKY rats as normal control orally treated for 92 days. The effects of AESP were evaluated by measuring the reduction of systolic blood pressure (SBP) using non-invasive tail-cuff method. Meanwhile, morphology changes of the thoracic aorta and kidney was assessed using haematoxylin & eosin staining observation as well as Scanning Electron Microscope (SEM). Renal function test (RFT), antioxidant activity (FRAP) and total phenolic content (TPC) of AESP were also evaluated in this study. The results of the study suggested that, all three doses of AESP significantly reduced SBP of SHR rat comparable to normal WKY rat at 8^{th} week, while at 10^{th} week comparable to Losartan-treated group. At the end of 12^{th} week, SBP of the AESP-treated group are similar to WKY group and lower than Losartan-treated group. The lowest dosage (1500mg/kg) was chosen as the effective dose. In addition, AESP also manage to improve the morphology structure of thoracic aorta and kidney of the rat. The morphology improvement of the organ of interest structure can be attributed to the high antioxidant property and phenolic compound found in AESP. There was no significant difference in RFT among the experimental groups. This suggested that despite there were significant morphological changes on the kidney due to 3 months effects of hypertension, their function remains intact. Thus, this study strongly suggests that *S. polyanthum* possess potential as antihypertensive agent and can alleviate the effect of end organ damage in hypertension.

CHAPTER 1: INTRODUCTION

1.1 Background

Non-Communicable Diseases (NCDs) contribute to an estimated 73% of total deaths worldwide, with the biggest contributor being cardiovascular diseases (CVD). CVD is responsible for 31% of all death worldwide (World Health Organization, 2017). The burden of mortality, morbidity and disability due to CVD is currently high and continue to grow. The Second Burden of Disease Study for Malaysia, published by the Institute for Public Health in 2012, ranked hypertension, smoking, diabetes, high cholesterol and high body mass index (BMI) as the biggest contributors to CVD and deaths. Data from the previous National Health and Morbidity Surveys (NHMS) showed an increasing trend for all NCD risk factors. An analysis of NHMS 2015 showed that at least 63% of adults aged 18 years and above had at least one NCD risk factor either overweight or obesity, high blood pressure, high blood sugar or high blood cholesterol. An estimated 35% of deaths occur in individuals aged less than 60 years, which are mainly due to CVD. The World Health Report in 2013 estimated that 29% of the world's adult population, or about 1.56 billion people, will have hypertension by the year of 2025. In short, Malaysia now has a “sick” or “at risk” population. More alarmingly, our children are just as vulnerable to the risk of NCDs right from fetal development and increase further during childhood with the exposure to unhealthy diets, lack of exercise as well as smoking and excessive alcohol consumption (World Health Organization, 2013).

Hypertension is an increasingly important medical and public health issue because of its high prevalence and its detrimental sequelae (Whitworth, 2003; Ezati *et*

al., 2002). Essential hypertension (also called primary hypertension or idiopathic hypertension) is the most common type of hypertension, affecting 95% of hypertensive patients (Hall *et al.,* 2006; Oparil *et al.,* 2003), it tends to be familial and is likely to be the consequence of an interaction between environmental and genetic factors. Prevalence of essential hypertension increases with age, and individuals with relatively high blood pressure at younger ages are at increased risk for the subsequent development of hypertension (Nandhini, 2014). NHMS in 2015 has shown that the prevalence of hypertension in Malaysia for adults aging from 18 years old and above has increased from 32.2% in 2006 to 32.7% in 2015. Meanwhile, for those individual aging above 30 years old, the prevalence has increased from 42.6% to 43.5%. In terms of the main ethnic groups, the Bumiputera from Sabah & Sarawak have the highest prevalence at 36.4%, followed by the Malays at 34.0%, Chinese at 32.3% and lastly the Indians at 30.6% (Institute for Public Health, 2015).

An analysis of NHMS (Volume III) in 2015 data also has shown that 63% of Malaysians had at least one cardiovascular risk factor. Hypertension remains the number one risk factor with a prevalence rate of 42.6% in adults above 30 years of age, followed by central obesity with 37% prevalence rate, hypercholesterolaemia with 24% prevalence rate and hyperglycaemia is the lowest prevalence rate with 15% (Goff *et al.,* 2014). The relationship between blood pressure and risk of cardiovascular events is continuous, consistent and independent of other risk factors. The higher the blood pressure, the greater the chance of myocardial infarction, heart failure, stroke and kidney diseases. The presence of each additional risk factor, such as dyslipidaemia, diabetes mellitus or smoking status, compounds the risk. Therefore, the main aim of identifying and treating hypertension is to reduce these risks of end organ damage or end organ complications. This disease is defined as persistent elevation of

systolic blood pressure of 130-139 mmHg and diastolic blood pressure of 80-89 mmHg (Table 1.1) (Whelton et al., 2017).

Evidence from randomized control trials (RCTs) has showed that a small reduction in blood pressure may result in a large reduction in the risk of stroke and myocardial infarction (Ward *et al.*, 2009; Schmieder, 2005). The antihypertensive treatment has made great progress in modern medicine. The therapeutic drugs include six classes of antihypertensive agents and fixed compound preparation (Wang & Xiong, 2012; Jia *et al.*, 2011; Xu & Chen, 2007). However, there is concern that the benefits demonstrated in RCTs of antihypertensive medication are not implemented in everyday clinical practice and that the long-term use of western medicine will produce some side effects, even produce resistance and affect therapeutic efficacy, only 53% of patients treated for hypertension had blood pressure actually controlled to ≤140/90 mm Hg (Yoon *et al.*, 2015). Therefore, seeking for a new effective decompression method is an important subject of hypertension treatment. Complementary and alternative medicine (CAM) is recognized and accepted in Europe and America that have developed a high degree of modern medicine, as an important complement to the western mainstream medicine system (Wang & Xiong, 2012; Su & Li, 2011). Recent researches showed that CAM could be regularly recommended for lowering elevated blood pressure (Wang & Xiong, 2012; Xiong *et al.*, 2012; Xu et al., 2012).

Table 1.1: Definition of hypertension according to blood pressure level in adult adapted from ACC/AHA guideline (2017)

Blood Pressure Category	SBP		DBP
Normal	<120 mmHg	and	<80 mmHg
Elevated	120-129 mmHg	and	<80 mmHg
Hypertension			
Stage 1	130-139 mmHg	or	80-89 mmHg
Stage 2	≥ 140 mmHg	or	≥ 90 mmHg

Traditional medicine treatment had been practiced for centuries to cure numbers of illness, one of them is hypertension. Amazing number of medicinal properties in the nature are being used in preparing the medicine to fight against this disease. Active components in the plant are the chemical compound which exists naturally and being modified into form of capsules, tablets, liquid, essential oil, ointment or teas to correct a lot of physical imbalance that lead to health complications (Obidike & Salawu, 2013). Natural products have been widely used as the main materials and played a crucial role as the lead compound in the development of new drug. Recently, the exploitation on medicinal herbs as alternative medicines in curing various type of diseases and infections had increased exponentially. This is due to the research claim that these products are safe and free from side effects has been applied in the ancient traditional Chinese, Greek- Unani and Ayurveda medicines.

In this modern era, people are more aware of their health status that leads them to find natural-based products to substitute the role of various synthetic products which are available in market. According to the World Health Organization (WHO), about 60-80% of people from developing countries consume traditional medicine for the

treatment of various diseases (World Health Organization, 2013). Therefore, herbs and medicinal plants are gaining interest because of their potential in healing as natural products, which are also known to be good sources to provide better health. Continuous research and studies of potential herbs and medicinal plants are important as natural products from plant origin will continue to be in demand. In this modern era, people are more aware of their health status that leads them to find natural-based products to substitute the role of various synthetic products which are available in market.

Malaysia, a tropical Southeast Asia country, is gifted with a rich diversity of flora and fauna, particularly, herbs and medicinal plants. Malay people, especially, like to consume traditional vegetables, which also known as *ulam* in their daily diet. This is due to the taste (Abas *et al.*, 2006) and aroma which will help in enhancing their appetite and promoting a better health for the people. There are more than 120 species of traditional *ulam*, varying from shrubs to large trees, which can be found in Malaysia. They are usually grown at home and could be found abundantly in local market. One of the favorite *ulam* that have been consumed for ages is *Syzygium polyanthum* (Wight) Walp.–There are other previous studies which report that *S. polyanthum* also possesses biological activities such as antioxidant (Raden *et al.*, 2009), antibacterial (Sumono & Agustin, 2008), antimicrobial, anti-inflammation (Wientarsih *et al.*, 2007) and antifungal (Noveriza & Miftakhurohmah, 2010).

1.2 Scope of Study

This study was conducted in orders to investigate the antioxidant activity and hypotensive effects of *Syzygium polyanthum* leaves aqueous extract (AESP) in Spontaneous Hypertensive Rat (SHR) for a long-term period (sub-chronic) which is 92 days. The previous study on hypotensive effect had been done by our group but on a short-term basis which were acute (24 hours) and sub-acute study (28 days).

In this experiment, *in-vivo* approached has been employed on SHR. AESP was prepared using sonication method. The total phenolic content in AESP was evaluated using colorimetric method which are Total Phenolic Content (TPC). In current study, the antioxidant activity of AESP was determined using Ferric Reducing Antioxidant Power (FRAP) assay. Phenolic compounds and antioxidants are known to contribute in lowering the blood pressure in previous studies. Apart from measurement of blood pressure, the effects of AESP was also evaluated by assessing the changes in morphology of kidney and thoracic aorta of the rat.

As a summary, this study investigated the total phenolic content, antioxidant activity and antihypertensive effects of *S. polyanthum* leaves aqueous extract using hypertensive-rat model (SHR).

1.3 Research Problem.

According to the World Health Organization (2013), medicinal plant would be the best source to obtain a variety of drug and it was estimated that there were almost 80% of people in the world still using the traditional herbal drug in daily life to maintain their health. Traditional medicine pioneered interventions like healthy diet, exercise, herbal remedies, and ways to reduce everyday stress. Traditional medicine has much to offer, especially as a contribution to primary health care and universal coverage, and most especially at a time when chronic non-communicable diseases was depicted as the world's biggest killer. Despite the growing global demand of herbal medicine, there are still concerns associated with not only their use, but their safety. Herbal remedies are generally referred to as safe and are presented to the public as being "natural" and completely "safe" due to their long history of use (Afolabi *et al.*, 2012). Although there has been reported increase in the ethnopharmacological investigations of medicinal plants in literature, the growing number of herbal product users around the globe and lack of scientific data on the safety profile of herbal products make it necessary to conduct deeper and more precise study of herbal products (Van Wyk *et al.*, 2000).

Besides that, hypertension remains a major modifiable risk factor for CVD despite important advances in our understanding of its pathophysiology and the availability of effective treatment strategies. High blood pressure increases the risk of CVD for millions of people worldwide, and there is evidence that the problem is only getting worse. The incidence of end-stage renal disease and the prevalence of heart failure have also increased. A major contributor to these trends is inadequate control of BP in the hypertensive population (Greene *et al.*, 2013).

1.4 Aim of the Research

This study was aimed to achieve the following general and specific objectives.

1.4.1 General objective

The general objective of this study is to evaluate the *S. polyanthum* leaves aqueous extract as potential antihypertensive agent in spontaneous hypertensive rat model (SHR).

1.4.2 Specific objectives

- To evaluate the antioxidant properties of *S. polyanthum* aqueous extract (AESP).
- To evaluate the hypotensive effects of sub-chronic repeated-oral AESP on Spontaneous Hypertensive rat's (SHR) blood pressure using Tail-Cuff method.
- To evaluate the effects of AESP on SHR's kidney function using biochemical parameters.
- To evaluate the effects of AESP on morphological changes of SHR's kidney and aorta.

1.5 Research hypothesis

- AESP has significant antioxidant property.
- AESP significantly reduce SHR's blood pressure.
- AESP improves SHR's kidney function.
- AESP improves SHR's kidney and thoracic aorta morphology.

CHAPTER 2: LITERATURE REVIEW

2.1 Hypertension

Hypertension or High Blood Pressure, physiological condition involving increased pressure on the arterial walls. The American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines in 2017 defined and classified hypertension in adults which is the diagnosis of hypertension is made based on an average of more than 2 careful readings of blood pressure obtained on more than 2 occasions, the systolic blood pressure measurements is higher than 130-139 mmHg and the diastolic blood pressure readings is between 80-89 mmHg. Blood pressure is the force of blood pushing against blood vessel walls as the heart pumps out blood. Abnormal increase in the amount of blood pressure force on blood vessels as it moves through the body is called as high blood pressure or hypertension (National Institute of Health, 2014).

Most people without chronic health conditions have a normal blood pressure if it stays below than 120 mmHg for systolic and below 80 mmHg for diastolic. Elevated blood pressure is a systolic pressure of 120 mmHg to 129 mmHg and a diastolic pressure less than 80 mmHg. Stage 1 high blood pressure is a systolic pressure of 130mmHg to 139mmHg or a diastolic pressure of 80mmHg to 89mmHg or above while, stage 2 high blood pressure is systolic pressure is equal and higher than 140mmHg or the diastolic blood pressure is equal and higher than 90mmHg (Whelton *et al.,* 2017). Many people have a condition known as labile hypertension, in which blood pressure is elevated on initial examination but registers normal on subsequent measurements (Trott & Harrison, 2014). For this reasoning, a diagnosis of hypertension requires elevated blood pressure readings on several occasions. Elevated arterial blood pressure indicates increased arterial resistance to blood flow, but in 90

percent of patients no cause for this increased resistance can be identified. These cases are called essential hypertension (Natekar *et al.,* 2014). Essential hypertension also could be defined as high blood pressure in which secondary causes such as renovascular disease, renal failure, pheochromocytoma, aldosteronism, or other causes of secondary hypertension are not present (Marques *et al.,* 2015). This type of hypertension accounts for 95% of all cases of hypertension. It is a heterogeneous disorder, with different patients having different causal factors that lead to high blood pressure. Essential hypertension needs to be separated into various syndromes because the causes of high blood pressure in most patients presently classified as having essential hypertension can be recognized. Indeed, in Stage 1 hypertension, treatment of hypertension (systolic 130–139 mmHg, diastolic 80-89 mmHg), reduces the prevalence of left ventricular hypertrophy, a predictor of future morbidity and mortality (Whelton *et al.,* 2017; Egan *et al.,* 2014; Ehret & Caulfield, 2013; Coffman, 2011). There is also a 42% reduction of the risk of stroke and a reduction in the risk of dementia. Essential hypertension remains a major modifiable risk factor for CVD despite important advances in our understanding of its pathophysiology and the availability of effective treatment strategy.

Secondary hypertension can be the outcome of pregnancy, vascular or kidney diseases, or endocrine tumours. Hypertension related to a specific aetiology like this is markedly differing from essential hypertension, of which the aetiology cannot be identified, in the condition and therapeutic strategies. Secondary hypertension is often resistant hypertension, for which a target blood pressure is difficult to achieve by standard treatment. It has been recognized that secondary hypertension accounts for almost 10% of hypertensive patients (Forman *et al.,* 2008). As secondary hypertension

is often resistant to treatment, the incidence of secondary hypertension in patients with a resistance-resistant hypertension may be higher.

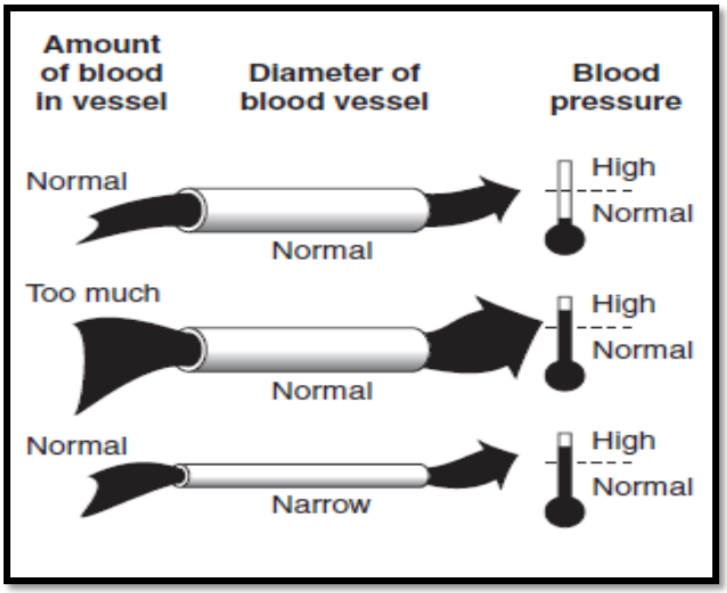

Figure 2.1: The changes in the diameter of blood vessel with different amount of blood pumped through it (National Institute of Diabetes, Digestive and Kidney Disease, 2014).

2.1.1 Aetiology of Hypertension

Aetiological factors correlated with hypertension in adults have also been associated with blood pressure elevations in youth. In addition to that, lack of physical activity or sedentary lifestyle may increase the risk of developing hypertension by 20-50% (Carretero & Oparil, 2000). Besides that, intrauterine malnutrition, family history of hypertension, obesity in particularly excess abdominal fat, insulin resistance, high dietary sodium intakes, low dietary intakes of calcium, potassium and magnesium, physical inactivity, high alcohol intakes, tobacco use, drug use (cocaine, ecstasy, anabolic steroids), emotional stress, diet pill use, oral contraceptives are many other factors associated with development of hypertension (Nandhini, 2014; Carretero & Oparil, 2000).

An inadequate supply of nutrients may program changes in foetal structure and metabolism, increasing the risk of hypertension and other diseases in later life. Hyperinsulineamia and insulin resistance are also associated with the development of hypertension which leads to many problems (Nandhini, 2014; Contreras *et al.*, 2000). Frequent etiological factors for secondary hypertension include renal parenchymal hypertension, primary aldosteronism (PA), renovascular hypertension and sleep apnea syndrome. Renal parenchymal hypertension is caused by glomerular diseases, such as chronic glomerulonephritis and diabetic nephropathy, interstitial kidney diseases, such as chronic pyelonephritis, and polycystic kidney disease (PKD) (Oparil *et al.*, 2003). As other etiological factors for secondary hypertension, the following conditions have been reported: in endocrine hypertension, phaeochromocytoma and Cushing's syndrome are related to an excessive production of catecholamines and cortisol, respectively. Hypothyroidism, hyperthyroidism, hyperparathyroidism and acromegaly are also etiologically involved in hypertension (Bartosh & Aronson, 1999).

2.1.2 Pathophysiology of hypertension.

Hypertension is a chronic elevation of blood pressure that, in the long-term will causes end-organ damage and results in increased morbidity and mortality. Blood pressure is the product of cardiac output and systemic vascular resistance. Patients with arterial hypertension may have an increase in cardiac output or systemic vascular resistance, or both (Cain & Khalil, 2002). In the younger age group, the cardiac output is often elevated, while in older patients increased systemic vascular resistance and increased stiffness of the vasculature play a dominant role. Vascular tone may be elevated because of increased α-adrenoceptor stimulation or increased release of peptides such as angiotensin or endothelins (Prys-Roberts, 2002).

The final pathway is an increase in cytosolic calcium in vascular smooth muscle causing vasoconstriction. Several growth factors, including angiotensin and endothelins, cause an increase in vascular smooth muscle mass termed vascular remodelling. Both an increase in systemic vascular resistance and an increase in vascular stiffness augment the load imposed on the left ventricle, this induces left ventricular hypertrophy and left ventricular diastolic dysfunction (Yusuf *et al.*, 2000). In youth, the pulse pressure generated by the left ventricle is relatively low and the waves reflected by the peripheral vasculature occur mainly after the end of systole, thus increasing pressure during the early part of diastole and improving coronary perfusion (Osmond & Barker, 2000). With ageing, stiffening of the aorta and elastic arteries increases the pulse pressure. Reflected waves move from early diastole to late systole. This results in an increase in left ventricular afterload, and contributes to left ventricular hypertrophy (Williams et al., 2014; Howell *et al.*, 1997). The widening of the pulse pressure with ageing is a strong predictor of coronary heart disease.

The autonomic nervous system plays an important role in the control of blood pressure. In hypertensive patients, both increased release of, and enhanced peripheral sensitivity to, norepinephrine can be found. In addition, there is increased responsiveness to stressful stimuli (Loscalzo, 2008). Another feature of arterial hypertension is a resetting of the baroreflexes and decreased baroreceptor sensitivity. The renin–angiotensin system is involved at least in some forms of hypertension and is suppressed in the presence of primary hyperaldosteronism (Jürgens & Graudal, 2004). The protease renin cleaves angiotensin to yield converted into an active octapeptide, angiotensin II by the angiotensin-converting enzyme (ACE) (Osmond & Barker, 2000). Though the renin–angiotensin system is widespread in the body, the main source of renin is the juxtaglomerular apparatus of the kidney. This apparatus senses the renal perfusion pressure and the sodium concentration in the distal tubular fluid (MacGregor *et al.,* 2006) High angiotensin II concentrations suppress renin secretion via a negative feedback loop. Angiotensin II acts on specific angiotensin AT1 and AT2 receptors causing smooth muscle contraction and the release of aldosterone, prostacyclin, and catecholamines (Anand, 1999; Nakao *et al.*, 1999). The renin–angiotensin–aldosterone system (RAAS) plays an important role in the control of arterial pressure including the sodium balance. Elderly or black patients tend to have low-renin hypertension. Others have high-renin hypertension and these are more likely to develop myocardial infarction and other cardiovascular complications (Sever & Poulter, 1989; Beilin, 1988).

In human essential hypertension, volume regulation and the relationship between blood pressure and sodium excretion (pressure natriuresis) are abnormal (INTERSALT, 1988). The primary cause of sodium and water retention may be an abnormal relationship between pressure and sodium excretion resulting from reduced

renal blood flow, reduced nephron mass, and increased angiotensin or mineralocorticoids (Gates et al., 1996). Considerable evidence indicates that resetting of pressure natriuresis plays a key role in causing hypertension. Sodium and water retention are associated with an increase in blood pressure. It is postulated that sodium, via the sodium–calcium exchange mechanism, causes an increase in intracellular calcium in vascular smooth muscle resulting in increased vascular tone (Findling, 1997; Hansson, 1995). In patients with essential hypertension, resetting of pressure natriuresis is characterized either by a parallel shift to higher blood pressures and salt-insensitive hypertension, or by a decreased slope of pressure natriuresis and salt sensitive hypertension (Mune *et al.*, 1995).

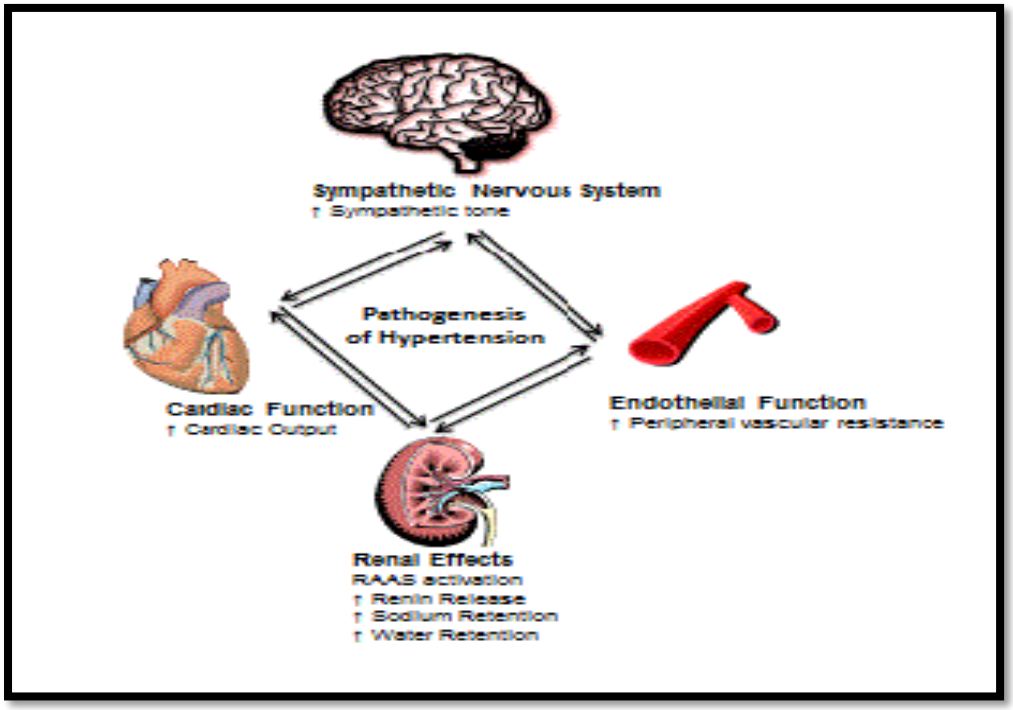

Figure 2.2: System that involved in the developement and maintainance of hypertension (Delacroix *et al.*, 2014).

2.2 Hypertension and kidney

The kidneys are two bean-shaped organs, each about the size of a fist. They are located just below the rib cage, one on each side of the spine (NIDDK, 2012). Every day, the two kidneys filter about 120 to 150 quarts of blood to produce about 1 to 2 quarts of urine, composed of wastes and extra fluid (Campese *et al.*, 2006). The urine flows from the kidneys to the bladder through tubes called ureters. The bladder stores urine. When the bladder empties, urine flows out of the body through a tube called the urethra, located at the bottom of the bladder (Levey *et al.*, 2003). In men the urethra is long, while in women it is short. Kidneys work at the microscopic level. The kidney is not one large filter (Casas *et al.*, 2005). Each kidney is made up of about a million filtering units called nephrons. Each nephron filters a small amount of blood. The nephron includes a filter, called the glomerulus, and a tubule. The nephrons work through a two-step process (Gudbjartsson, 2010). The glomerulus lets fluid and waste products pass through it; however, it prevents blood cells and large molecules, mostly proteins, from passing. The filtered fluid then passes through the tubule, which sends needed minerals back to the bloodstream and removes wastes (Kottgen *et al.*, 2009). The final product becomes urine.

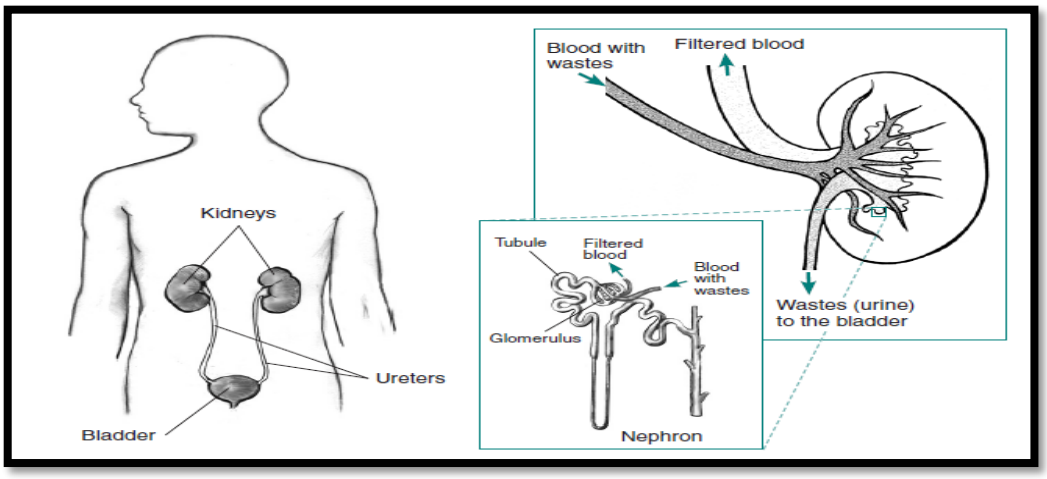

Figure 2.3: Kidney and nephron anatomy in human body (USA National Institute of Health, 2015).

The incidence of end-stage renal failure has increased despite advances in antihypertensive treatment, but has recently decreased slightly (Araujo & Wilcox, 2014). Patients in whom haemodialysis was initiated in 2011, the most frequent underlying disease was diabetic nephropathy with percentage of 44.3%, with chronic glomerulonephritis being the second most frequent with 20.2% and nephrosclerosis being the third with 11.8%. Most of these chronic kidney diseases (CKDs) induce hypertension, but hypertension promotes the progression of kidney damage and establishes a vicious circle leading to end stage renal failure (NIDDK, 2012).

As there is no radical treatment for CKD at present, blood pressure control by antihypertensive drug therapy primarily using RA system inhibitors (angiotensin II receptor blockers (ARBs) or angiotensin-converting enzyme (ACE) inhibitors) is extremely important for the prevention of end-stage renal failure (Zhu *et al.*, 2009). As there is a close relationship between CKD and hypertension, it is often difficult to determine which came first, CKD or hypertension, if they are concurrent. If abnormal findings have been obtained on urinalysis, or renal dysfunction has appeared before hypertension, or if the presence of hypertension, proteinuria or renal dysfunction from an early phase of pregnancy can be confirmed, hypertension is likely to be caused by CKD (Pallone *et al.*, 2012). Also, if hypertension is mild relative to abnormal urinary findings or kidney damage, or if there are few hypertensive cardiovascular complications concurrent with the kidney disorders, CKD is considered to underlie the hypertension (Hanssons *et al.*, 2000). Urinalysis and measurement of the serum creatinine concentration should be performed in all hypertensive patients, and, if an abnormality persists, kidney morphology must be evaluated using abdominal ultrasonography or CT.

2.3 Antioxidants, Oxidative Stress and Reactive Oxygen Species (ROS).

Since the late 19th and early 20th century, chemists have studied antioxidants, a loosely defined group of compounds characterized by their ability to be oxidized in place of others compounds present. Biologists realized the importance of antioxidants in health with the 1960s publications of vitamins and flavanoids, followed by later research in the 1970s on ascorbic acid (vitamin C), cancer, and the common cold (Cameron & Pauling, 1976). Scientists actively researching and discussing on antioxidants as protecting agent (Ganesan *et al.,* 2015; Shilpa *et al.,* 2014; Srimuwarni, 2005) explanations for the effects of antioxidants on cancer susceptibility and overall health expanded rapidly in subsequent decades with research into mechanisms, molecular targets, and molecular interactions (American Institute of Cancer Research, 2007).

Antioxidants are agents that at low concentrations prevent or inhibit oxidation of oxidisable biomolecules, such as DNA, lipids, and proteins. In biological systems, enzymatic and non-enzymatic systems have evolved to protect against oxidative damage. Major enzymatic antioxidants are SOD, catalase, glutathione peroxidases, thioredoxin, and peroxiredoxin (Gongora *et al.,* 2006). Non-enzymatic antioxidants include ascorbate, tocopherols, glutathione, bilirubin, and uric acid (Chen *et al.,* 2001). Low antioxidant bioavailability promotes cellular oxidative stress and has been implicated in cardiovascular and renal oxidative damage associated with hypertension. During normal cellular metabolism, several enzymes are capable of transferring electrons from an electron donor to molecular oxygen (Araoujo & Wilcox, 2014). These include the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, mitochondrial electron transport, and, under certain circumstances, nitric oxide (NO) synthase (Lushchack, 2014; Harrison & Dikalov, 2005). The 1-

electron oxidation product of oxygen yields superoxide (O_2^-), while a 2-electron reduction of oxygen results in formation of hydrogen peroxide (H_2O_2). These are referred to as ROS, and they can undergo numerous subsequent reactions leading to production of many other ROS (San Martin & Griendling, 2014).

The formation of ROS is a natural consequence of aerobic metabolism and is integral for maintaining tissue oxygen homeostasis (Castro & Freeman, 2001). Oxygen homeostasis which is the balance between constitutive oxidants and antioxidants, is maintained through a natural series of reduction–oxidation (redox) reactions involving the transfer of electrons between two chemical species: compounds that lose electrons (oxidised) and those that gain electrons (reduced) (Ray *et al.,* 2012). When oxygen homeostasis is not maintained, the cellular environment becomes oxidatively stressed. Approximately, 1–3% of oxygen consumed by the body is converted into ROS (Finkel & Holbrook, 2000).Three of the major ROS which are superoxide radical, hydrogen peroxide and hydroxyl radical are normal metabolic byproducts that are generated continuously by the mitochondria in growing cells (Seifried *et al.,* 2006; Lopaczynski & Zeisel, 2004; McCord, 2000) Potentially damaging oxidative stress can be generated by excess ROS, which are kept in check by endogenous cellular antioxidant mechanisms. Increase of ROS levels influence activity of signal transduction pathways leading to cell proliferation, or to apoptosis or necrosis, depending on the dosage and duration of ROS and also on cell type. Typically, low doses of ROS can be mitogenic, whereas medium doses lead to temporary or permanent growth arrest which also could be called as replicative senescence and high doses usually result in cell death either by apoptosis or necrosis (Holbrook & Ikeyama, 2002). The chemistry of these reactions as they pertain to vascular biology and hypertension has recently been reviewed in depth.

Oxidative stress is a state of imbalance between oxidants or also called ROS and antioxidants in favour of the oxidants, leading to damaging effects (Makino *et al.*, 2002; Zalba *et al.*, 2001; Lacy *et al.*, 2000). Physiologically, our bodies' own antioxidant defence systems are capable of adapting to the changing levels of oxidants in order to maintain the oxidant-antioxidant balance (Sies, 2007) This antioxidant defence system includes enzymes such as superoxide dismutase, glutathione peroxidase and catalase, non-enzyme molecules including albumin, bilirubin and glutathione (Wang *et al.* 2013), the molecular regulatory mechanism by Nrf2/ARE-mediated antioxidant gene expression (Mann *et al.* 2007) as well as micronutrients and vitamin (Sies, 2007). However, in pathophysiological conditions, the production of ROS exceeds the natural antioxidant defence of the cells, causing the active ROS to attack and producing cellular alterations (Majzunova *et al.* 2013; Vaziri, 2008). Oxidative stress and inflammation are closely interrelated, since oxidative stress can cause inflammation, which in turn can induce oxidative stress (Ishibashi *et al.*, 2013). Both oxidative stress and inflammation cause injury to cells (Onuf, 2012) and giving rise to endothelial dysfunction (McCord, 2000).

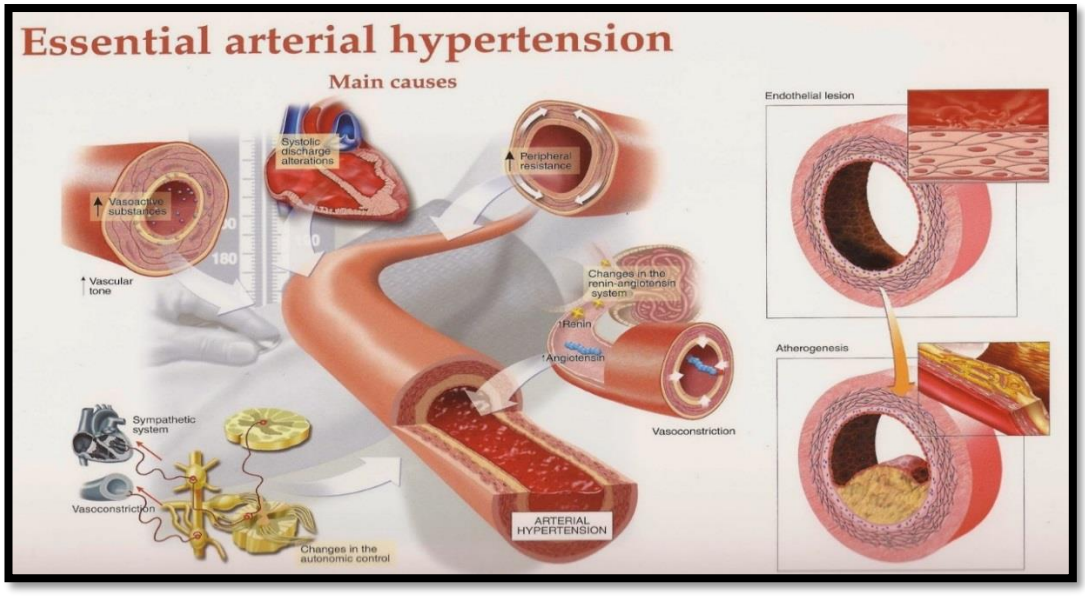

Figure 2.4: Main causes of essential hypertension and its complications, from O'Shaughnessy (2001).

2.4 Vascular Remodelling

Pathophysiological mechanisms that contribute to high blood pressure are complex and involve many biological systems. One of the most importance biological system is the vascular system, which exhibits characteristic changes notably endothelial dysfunction, vascular remodelling, and vascular inflammation (Montezano *et al.,* 2014; Savoia *et al.,* 2011). These changes are the cause and consequence of high blood pressure and are therefore attractive targets for antihypertensive therapy. Endothelial dysfunction is characterized by a state of reduced vasodilation, increased vasoconstriction, adhesion of pro-inflammatory monocytes with coagulation, and loss of integrity of the endothelial monolayer (Figure 2.9) (Xiao *et al.,* 2014; Drummond *et al.,* 2011). Associated with these functional changes are structural alterations of arterial remodelling, characterized by an increased media to lumen ratio (Schiffrin, 2012). Common to these processes are alterations in endothelial cells and vascular smooth muscle cells (VSMCs) to a vasoconstrictor, mitogenic, profibrotic, promigratory and pro-inflammatory, phenotype influenced by oxidative stress or increased bioavailability of ROS (Vukelic & Griendling, 2014; Xu *et al.,* 2009).

Results from a meta-analysis showed that the DASH diet, rich in antioxidants from fruits and vegetables, is associated with significant blood pressure lowering and a reduction of 13% in the 10-year Framingham risk score for cardiovascular disease (Hoagland *et al.*, 2003). Increasing antioxidant capacities through foods and drinks that contain flavonoids has also been shown to improve endothelial function in some patients with hypertension (Xiao *et al.,* 2014; Xu *et al.,* 2009). Studies in hypertensive patients demonstrated that consumption of dark chocolate, black tea, and red wine increased flow mediated dilation and improved endothelial function (Diplock, 2000; Löliger, 1991) Epidemiological studies have linked low dietary intake of antioxidant

vitamins (vitamin C, vitamin E, ß-carotene, polyphenol) with cardiovascular disease. Antioxidant vitamins influence endothelial function and vascular contractility. Intra-arterial administration of high doses of vitamin C improved endothelium-dependent vasodilation in the forearm microcirculation of hypertensive patients, by increasing NO bioavailability (Aruoma, 1991; Gutteridge & Halliwell, 1989; Southorn & Powis, 1988). Proteins that contain cysteine residues are highly sensitive to oxidative modification. These oxidative modifications lead to changes in structure, activity, and function of target proteins (Andersen, 2004). Proteins that are redox-sensitive include ion transporters, receptors, kinases, phosphatases, transcription factors, structural proteins, and matrix metalloproteases, all of which are important in regulating endothelial and VSMC function (Becker, 2007; Baud & Karin, 2001). ROS also regulate prostaglandin production and signalling, important in regulating vascular function. In hypertension, oxidative stress induces increased production of prostanoids by constitutive (COX-1) and inducible (COX-2) cyclooxygenases, which lead to increased vasoconstriction and reduced endothelium-dependent vasodilation (Berry & Hare, 2004). H_2O_2 stimulates production of vasoconstrictor prostanoids thromboxane, prostacyclin and prostaglandin E_2, which cause vasoconstriction in hypertension.

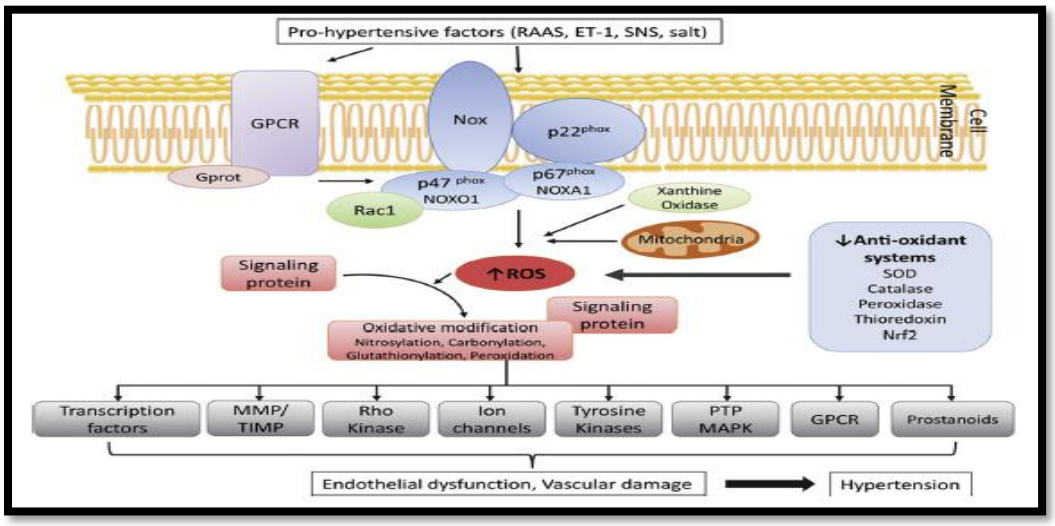

Figure 2.5: Mechanism of ROS which cause the occurrence of endothelial dysfunction and eventually lead to hypertension (Montezano, 2014).

2.5 Spontaneous Hypertensive Rats (SHR)

A great expansion of experimental research began with the development of rat strains with genetically inherited hypertension. The SHR offers specific and uniform genetic predisposition, thus allowing the study of the causes, mechanisms, and pathology of hypertension, as well as its behavioural consequences, and the comparison of the efficacy of proposed therapeutic interventions in relation to existing clinical treatments (Kannel, 1993). Moreover, central neurohormonal mechanisms which constitute the dominating trigger influence in SHR and provide a model of hypertension that allows the study of the combined influence of both aging and hypertension on cognitive and physical functions on different developmental stages (Hanes *et al.,* 1996; Weindruch, 1995). Notwithstanding, the SHR is a genetic model, and the appearance of neural disturbances could be a parallel genetic phenomenon and not necessarily or exclusively related to the elevated blood pressure (Bohr *et al.*, 1991). Normal aging also produces a slow decline in neuron population, tissue distensibility, basal metabolic rate, and oxygen consumption, thus affecting cardiovascular performance (Meneses & Hong, 1998). There is an extensive range of rat models, most mimic some aspects of the relevant human disease. No model mimics exactly all the symptoms of the human disease, partly because many of changes in the human disease are not thoroughly understood (Doggrell & Brown, 1998).

SHRs are descendants of an outbred Wistar male with spontaneous hypertension from a colony in Kyoto, Japan; mating with a female with an elevated blood pressure, and then brother X sister mating continued with selection for spontaneous hypertension, defined as a systolic blood pressure of over 150 mmHg persisting for more than one month (Okamoto & Aoki, 1963). From 1968, this inbred strain of SHRs was further developed in the USA (Kurtz & Morris, 1987). The various

colonies of SHR are pre-hypertensive for the first 6–8 weeks of their lives with systolic blood pressures around 100–120 mmHg (Adams *et al.*, 1989) and then hypertension develops over the next 12–14 weeks (McGuire & Tweitmeyer, 1985). As in humans, hypertension develops more rapidly and becomes more severe in male than female SHR (Iams & Wexler, 1979). *In vivo* studies have shown that, in the early stages of hypertension, SHRs have an increased cardiac output with normal total peripheral resistance. As the SHR progresses into the established hypertension state, the cardiac output returns to normal and the hypertrophied blood vessels produce an increase in the total peripheral resistance (Smith & Hutchins, 1979).

The male SHR is commonly used as a model of established human hypertension (Takata & Kato, 1996), and to test new antihypertensive medication, for example felodipine (Lund, 1990). Human hypertension is difficult to study as there is substantial individual variation in the two triggering elements of hypertension, polygenetic disposition and excitatory environmental factors, leading to many variations in the direct and indirect effects on the cardiovascular system that are difficult to differentiate. Researchers in hypertension have commonly resorted to the use of SHRs which have, within each colony, uniform polygenetic disposition and excitatory factors which produce uniform changes in the indirect and direct effects on the cardiovascular system. This lack of inter-individual variation is one of the major advantages of the SHR (Lindpaintner *et al.*, 1992).

Another advantage of the SHR is that it follows the same progression of hypertension as human hypertension with pre-hypertensive, developing and sustained hypertensive phases with each phase lasting at least several weeks. In addition, the normal life spans of normotensive and SHRs are comfortably short, which is 2.5–3 years and 1.5–2.5 years, respectively, to make it relatively easy to follow ‘cradle to