MORPHOLOGICAL IMPROVEMENTS OF HYPERTENSIVE RAT'S THORACIC AORTA & KIDNEY WITH *SYZYGIUM POLYANTHUM*

by

NOR 'ATIQAH BINTI MUHAMAD

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List of Abbreviations & Symbols

ACEIs	Angiotensin converting enzyme inhibitors
Ang	Angiotensin
ARBs	Angiotensin receptor blockers
AT_1	Type-1 angiotensin II receptor
AT_2	Type-2 angiotensin II receptor
CAM	Complementary and alternative medicine
CCB	Calcium channel blockers
CMC	Carboxymethylcellulose
CPD	Critical point dryer
CVD	Cardiovascular diseases
DBP	Diastolic blood pressure
DPX	Distrene plasticizer xylene
ESRD	End stage renal disease
GFR	Glomerular filtration rate
GPCR	G-protein coupled receptor
H&E	Haematoxylin & eosin
HDL	High density lipoprotein
HPLC	High performance liquid chromatography
hs-CRP	High sensitivity c-reactive protein
LDL	Low density lipoprotein
MESP	Methanolic extract of *S. polyanthum*
RAAS	Renin angiotensin-aldosterone system
SBP	Systolic blood pressure
SEM	Scanning electron microscope

SHR	Spontaneous hypertensive rats
USAE	Ultrasonic-assisted extraction
WKY	Wistar-Kyoto
%	Percent
≥	More or equal to
<	Less than
>	More than
°C	Degree celcius
±	Plus minus
d	Deci
g	Gram
mg	Miligram
mL	Mililiter
mmHg	Milimeter mercury

ABSTRAK

Bancian Kesihatan dan Morbiditi Kebangsaan (2015) melaporkan bahawa kelaziman hipertensi di Malaysia untuk 18 tahun ke atas adalah lebih tinggi berbanding dengan negara-negara jiran dan piawaian antarabangsa. Antara penyebab utama ialah ketidakpatuhan pengambilan kepada drug antihipertensi sintetik disebabkan kesan sampingan. Oleh itu, masyarakat pada masa kini lebih cenderung untuk mendapatkan rawatan berasaskan herba alternatif yang biasa kepada mereka. *Syzygium polyanthum* atau "serai kayu" adalah salah satu herba yang dimakan mentah oleh masyarakat Kelantan. Daunnya telah digunakan secara tradisional untuk mengurangkan tekanan darah dalam pesakit hipertensi. Kajian sebelum ini menunjukkan yang ekstrak daun *S. polyanthum* mengurangkan tekanan darah pada tikus hipertensi. Objektif kajian ini adalah untuk menentukan kesan ekstrak metanol oral daun *S. polyanthum* (MeSP) dalam histopatologi aorta dan ginjal tikus hipertensi spontan (SHR). MeSP telah disediakan oleh proses sonikasi. Tempoh kajian ini adalah 4 minggu (sub-akut) di mana 15 SHR jantan telah dibahagikan sama rata kepada 3 kumpulan: Kumpulan 1 (MeSP 2000 mg/kg), Kumpulan 2 (*Losartan* 10 mg/kg), Kumpulan 3 sebagai SHR yang tidak dirawat (kawalan negatif untuk tekanan darah tinggi). Lima tikus jantan normotensif Wistar Kyoto (WKY) ditetapkan sebagai kawalan negatif (Kumpulan 4). Semua tikus telah dieutanasia pada akhir kajian. Aorta toraks dan ginjal tikus telah dikaji menggunakan pewarnaan hematoxylin dan eosin (H&E); juga dengan mikroskop pengimbas elektron (SEM). Aorta SHR yang dirawat dengan MeSP dan *Losartan* menunjukkan selaput adventitia dan lapisan otot licin (selaput media) berada dalam keadaan hampir normal. Perubahan yang sama dalam aorta juga ditumjukkan dalam SEM. Bagi ginjal yang dirawat dengan MeSP dan *Losartan*, glomerulus dan kapsul Bowman kekal dalam keadaan baik dalam kedua-dua H&E dan SEM. Kesimpulannya,

pemberian ekstrak *S. polyanthum* secara oral berupaya menambah baik perubahan morfologi dalam aorta dan ginjal yang berkait dengan perubahan hipertensif menjanjikan rawatan alternatif untuk hipertensi.

ABSTRACT

National Health and Morbidity Survey (2015) reported that the prevalence of hypertension in Malaysia for 18 years old and above is higher as compared to our neighboring countries and international standards. Among others, this can be attributed to poor compliance to synthetic antihypertensive drugs due to their side-effects. Hence, people nowadays are seeking the alternative herb-based treatment that is familiar to them. *Syzygium polyanthum* or "*serai kayu*" is one of the herbs eaten raw by Kelantanese. The leaves are traditionally used to reduce blood pressure in hypertensive patient. Previous study has shown the *S.polyanthum* leaves extract significantly reduced the blood pressure in hypertensive rats. The objective of this study is to determine the effects of oral methanolic extract of *S.polyanthum* leaves (MESP) on histopathology of spontaneous hypertensive rat's (SHR) aorta and kidney. MESP was prepared by sonication process. The period of the study was 4 weeks (sub-acute) where 15 male SHR were equally divided into 3 groups: Group 1 (MESP 2000 mg/kg), Group 2 (Losartan 10 mg/kg), Group 3 as untreated SHR (negative control for hypertension). Five male normotensive Wistar Kyoto Rats (WKY) served as a negative control (Group 4). All rats were euthanized at the end of the study. Rat's thoracic aorta and kidney were examined by hematoxylin and eosin (H&E) staining; also with scanning electron microscope (SEM). In aorta of MESP-treated, the tunica adventitia and smooth muscle layer (tunica media) of and Losartan-treated SHR's aorta was near-normal condition. Similar improvements in aorta were also noted on SEM. As for kidney, in MESP-treated SHR and Losartan-treated showed well-preserved glomerulus and well capsulated of Bowman's capsule in both H&E and SEM. In conclusion, oral administrations of *Sygyzium polyanthum* extract was able to improve the morphological

changes in aorta and kidney that are associated with hypertensive changes suggesting that a promising alternative treatment for hypertension.

CHAPTER 1

INTRODUCTION

1.1 *Syzygium polyanthum*

S. polyanthum (Wight) Walp. Var. *polyanthum* is a plant belonging to Myrtaceae, is widely used in Indonesian and Malaysian cuisines. It also known as "*serai kayu*" or "*salam*," and is consumed by Malays as a traditional remedy for hypertension and it leaves are well-known as traditional medication for various illnesses such as cataract, gastritis, hypercholesterolemia, skin diseases, and diabetes mellitus (Sumono & Agustin, 2008).

Tropilab (2016) stated that *Eugenia polyantha* is synonym to *S. polyanthum*. This species is more familiar with names "serah or kelat samak" among Malaysian people. *S. polyanthum* plant can reach a height of 90 feet although 60 feet is more common (Figure 1.1). The flowers are pink and somewhat fragrant while the fruits are round; red at first, later brown (Figure 1.2). Their seeds are small and brown.

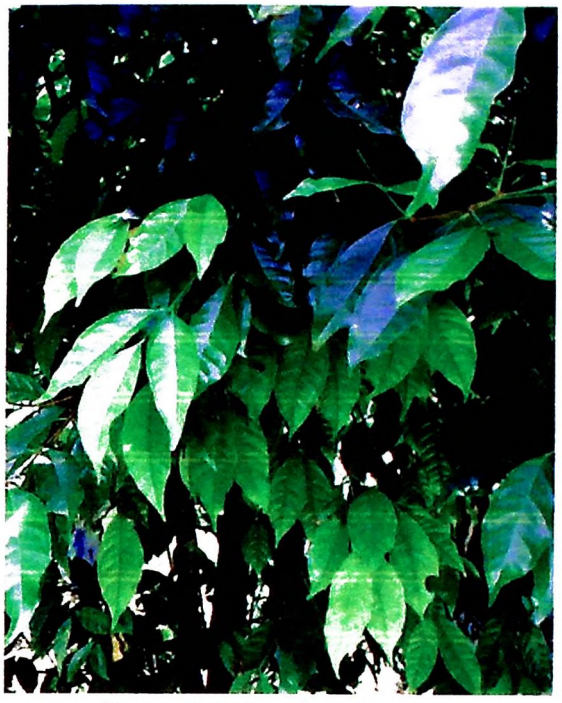

Figure 1.1: *S. polyanthum* tree

Figure 1.2: *S. polyanthum* fruit

Each part of the plant has its own benefits and uses. The leaves of the plant are widely used as spice due to its flavor (Lemmens *et al.*, 1995). Besides being used as spices for cooking purposes, the leaf and bark of *S. polyanthum* have been used as traditional remedies to treat diarrhea, rheumatism and anti-hyperuricemia (Burkill, 1966 & Haque *et al.*, 2004). Sumono & Agustin (2008) reported that its roots and fruits extract have the ability to neutralize hang over caused by too much alcohol consumption.

1.2 Prevalence of Hypertension

Hypertension is an important risk factor for CVD and a common disease causing significant mortality and morbidity. James *et al.* (2014) reported that hypertension is the most common medical condition seen in primary health-care setting which can lead to myocardial infarction, stroke, renal failure and premature death if not detected and treated early. Hypertension has been identified as the leading risk factor for global disease burden (Lim *et al.*, 2012).

According to American Society of Hypertension (ASH), raised blood pressure is a condition in which the blood vessels have a persistently raised pressure. Blood is carried from the heart to all parts of the body through the vessels. Each time the heart beats; it pumps blood into the vessels. Blood pressure is created by the force of the blood pushing against the walls of blood vessels (arteries) as the heart pumps it. The higher the pressure, the harder the heart has to pump. Chobanian *et al.* (2003) stated that hypertension in adults is defined as resting systolic blood pressure (SBP) of 140 mmHg or grater and/or a diastolic blood pressure (DBP) of 90 mmHg or greater in adults who are not taking antihypertensive medication.

Hypertension is reported to have affected millions worldwide. The prevalence of this disease varies from country to country and between populations. The National Health and Morbidity Survey (NHMS) (2015) has shown that the prevalence of hypertension in Malaysia for adults ≥18 years is 30.3% where 13.1% are known to have hypertension whereas 17.2% are previously undiagnosed with hypertension. This is higher by comparison to Thailand (23.6%), United Kingdom (20.3%) and Singapore (16%). According to Ong *et al.* (2008), almost one-third of the U. S adult population suffered with hypertension. Data from the National Health and Nutrition Examination

Survey, 2011-2012 showed that the age–adjusted prevalence of hypertension among adults in United States aged 18 and over was 29.1% in 2011-2012, similar to the prevalence in 2009-2010. The prevalence of hypertension was similar for men and women at nearly one-third. The prevalence of hypertension increases with advancing age to the point where more than half of people 60-69 years of age and approximately three-fourths of those 70 years of age and older are affected (Burt *et al.*, 1995). In Malaysia, the prevalence rises with increasing age. This upward trend was also reported in other parts of the world (Chow *et al.*, 2013; Ezzati *et al.*, 2008; Kearney *et al.*, 2004; Naing & Aung, 2014; Picon *et al.*, 2012).

The increasing prevalence of hypertension is also influenced by socioeconomic position. The socioeconomic conditions of the places where people live and work have an even more substantial influence on health than personal socioeconomic position (Macintyre *et al.*, 2002; Ross & Mirowsky, 2008). Educational attainment and income are the indicators that are most commonly used to measure the effect of socioeconomic position on health in United States. Research indicates that substantial educational and income disparities exist across many measures of health (Krieger *et al.*, 2003; Singh & Hiatt, 2006). Ma *et al.* (2013) reported that low educational attainment might be a proxy for low health literacy. Patients with adequate health literacy can read, understand, and act on health-care information (Hertz *et al.*, 2005). Low socioeconomic status could have led to limited access to health care, and to ignorance of the complications of uncontrolled hypertension. Differences in personal values, beliefs, attitudes, outlook on life, and behavior could also contribute to these differences. World Health Organization (WHO) (2013) also highlighted that hypertension disproportionally affects populations in low- and middle-income countries where the health system is weak especially in African Region.

In Kelantan, hypertension is a common disease and it is associated with multiple risk factors for cardiovascular disease. The prevalence is likely to increase with increasing affluence and to become a major health problem causing significant morbidity and mortality in the near future (Mafauzy *et al.*, 2003).

Hypertension is a common disorder which if not effectively treated, results in greatly increased probability of coronary thrombosis, stroke and renal failure. In order to treat hypertension, there are many classes of synthetic drugs available in pharmaceutical industry such as diuretics, beta-blockers, CCBs, ARBs and ACEIs. However, depending on the synthetic drugs therapy to reduce blood pressure is very costly (WHO, 2003).

Nowadays, the usage of dietary and medicinal plants as complementary and alternative medicines (CAM) are widely used compared with conventional treatment. According to Maghrani *et al.* (2005), this is based on the claim of minor side effects and has well therapeutically performance. In Malaysia, one of the local herbs known which is known as *S. polyanthum* is said to be one of the potential alternative medicine due to its biological properties (Kusuma *et al.*, 2011).

1.3 Scope of the Research

S. polyanthum (Wight) Walp. var. *polyanthum* leaves are consumed as a traditional Malay treatment of hypertension. It also can be used for diabetic, diarrhea, gastritis, drunks, and skin diseases (Sumono & Agustin, 2008). Besides, it has other benefits such as diuretic and analgesic effect (Ismail *et al.*, 2013).

Less study has been carried out with *S. polyanthum* and there was no previous scientific study has reported its effects on blood pressure. Since *S. polyanthum* has been used as the traditional medicine for hypertension, thus it is possible to determine scientifically the possible benefits of oral administration of this plant on cardiovascular system (Mohamed *et al.*, 1996). The other concern is the ability of this plant to produce the toxicological effects of conventional drugs used by hypertensive patients. Therefore, it is important to determine the toxicity on acute and sub-acute oral ingestions of these extracts.

To further validate the safe consumption of this plant extracts, a pharmacological and toxicological evaluation in animal studies were carried out. In this research, *in vivo* approach has been employed to determine the overall effect of this medicinal plant extract on both Wistar–Kyoto (WKY) and spontaneous hypertensive rat (SHR) morphologies.

1.4 Aim of the Research

Generally, the objective of this study is to determine the morphological improvements of rat's thoracic aorta and kidney in responds to MESP leaves in SHR.

The specific objectives of this study are:

i. To compare the extraction process using the ultrasonic-assisted extraction (USAE) method with Soxhlet's method from previous study.
ii. To determine the histological/morphological changes of rat's thoracic aorta and kidney using hematoxylin and eosin (H&E) staining.
iii. To determine the histological/morphological changes of rat's thoracic aorta and kidney using scanning electron microscope (SEM).
iv. To determine the effects of blood glucose on administration to the extract.

1.5 Hypothesis

MESP leaves are able to improve histopathological changes associated with hypertension.

CHAPTER 2

LITERATURE REVIEW

2.1 *Syzygium polyanthum*

2.1.1 Background of *Syzygium polyanthum*

Scientifically, *S. polyanthum* is named as *Eugenia polyantha Wight* and the synonyms are *Eugenia lucidula miq* (Dalimartha, 1999). *S. polyanthum* or known as "serai kayu" or "samak" among Malaysian and bay leaf among Indonesian; has been known since long time ago as a species that can be used for therapy. *S. polyanthum* leaves has been used as an alternative treatment for hypertension, diabetic, diarrhea, gastritis, drunks and skin diseases. The plant also has other benefits such as diuretic and analgesic effect (Utami & Tim, 2005). It also recognized as one of the popular medicinal plants that being used as spice for cooking purposes.

Dalimartha (1999) reported that this plant is mostly grown in the forest, but they may be planted in the garden. It can be found in lowlands until 1.400 meters above sea level and this plant may grow about 25 meters in height, have large straight root, round trunk and smooth surface (Wijayakusuma, 2002). Bay trees have small, white, and fragrant flowers. Their leaf has 2.5-8.0 centimeters long leaf with flat margins, the tip is blunt and the base of the leaf stretch along length and tight (Figure 2.1) (Utami & Tim, 2005). Chinese therapy books describe this plant has fragrant smell and has astringent to the taste (Winarto & Karyasari, 2003).

2.1.2 *Syzygium polyanthum* as Therapeutic Agent

S. polyanthum extracts were proven to possess antibacterial activity against *Staphylococcus aureus*, antifungal activities against *Alternaria alternata* and *Colletotrichum capsici* (Mackeen *et al.*, 1997), antinematodal activity against the pine wood nematode, *Bursaphelenchus xylophilus* (Ali *et al.*, 2000), anti-tumor promoting activity (Lelono *et al.*, 2009) and antioxidant activity (Kusuma *et al.*, 2011; Perumal *et al*,. 2012; Wong *et al*, 2006). Har and Ismail (2012) reported that the extracts of *S. polyanthum* leaves showed a mild antioxidant activity due to both detected phenolic acids, gallic and caffeic acids.

S. polyanthum leaves extract is also non-cytotoxic to normal mammalian cell lines (Perumal *et al*,. 2012). According to Lelono *et al.* (2009), this extracts exhibited potential antidiabetic effects through the inhibition of alpha glucosidase activity and lowering glucose blood level due to the presence of active fraction compounds indicated phenolic acid with benzoic acid moiety; 3, 4, 5-trihydroxy benzoic acid, 4-hydroxy 3-methoxy benzoic acid and 4-hydroxy 3, 5-dimethoxy benzoic acid.

The leaves can be eaten raw as a salad (Figure 2.2) or used as a medicine. The extract is usually used to stop diarrhea, cure gastritis, control blood sugar in diabetes mellitus, reduce itchiness, as astringent, and treat scabies (Wijayakusuma, 2002). He also stated that the leaf has lower side effect compared to synthetic drugs. To be consumed as drugs, the extract of *S. polyanthum* leaves should be boiled, while as an ointment, the leaves is crashed and applied on the affected skin. Other than that, *S. polyanthum* leaves can also be used to treat patients with high uric acids. In conjuction, Apriono *et al.*, (2008) reported that infuse of *S. polyanthum* leaves at 0.5 mg increase the excretion of uric acid in urine of Wistar male rat.

Figure 2.1: *S. polyanthum* leaf

Figure 2.2: *S. polyanthum* leaves eaten as raw salad

2.1.3 Chemical Properties of *Syzygium polyanthum*

Winarto & Karyasari (2003) reported that *S. polyanthum* has a lot of chemical compunds; which consist of terpenoids, phenols, tannins, avonoids, and alkaloids. Steroids were found in the crude ethanolic extract of the leaves and the ripe fruits whereas saponins were found in the unripe fruits, whereas carbohydrates were present in both the ripe and unripe fruits (Kusuma *et al.*, 2011).

On the other hand, the chemical constituents of the essential oil from *S. polyanthum* leaves are extensively studied. According to Winarto & Karyasari (2003), only 0.05% of essential oils present in *S. polyanthum* including citric acid and eugenol. Eugenol is one of the compounds presents in *S. polyanthum* leaves. Gautier *et al.* (2013) reported that eugenol, has reputed ability as a vasorelaxant compound that causes vasodilation *in vitro* (Damiani *et al.*, 2003; Interaminense *et al.*, 2007) and reduces blood pressure and heart rate of rats *in vivo* (Lahlou *et al.*, 2004). Among the compounds, the presence of □-pinene which belongs to terpenoid family is notable since it was associated with hypotension in both the non-anaesthetized (Menezes *et al.*, 2010) and the urethane–anaesthetized rats (Eh, 2003).

Essential oil is mainly consisting of terpenoid compound with atomic carbon framework of five. The characteristics of essential oil are highly evaporated in room temperature without decomposition, bitter, sweet smell in accordance with plant that produce it and soluble in organic solvent but not water soluble. According to Sumono & Agustin (2008), another compound that form essential oil including phenilpropane biosynthetic is phenol compound such as eugenol, khavikol and khavibetol. Essential oil in some plants has biological activity as antibacterial and antifungal, thus it can be

used as food preservatives and natural antimicrobial. Besides antiseptic and antioxidant activity, it also has activity to inhibit the growth of some bacteria and fungi.

Tannins, a liquid glycoside derived from polypeptide and ester polymer, which can be hydrolyzed by the secretion of bile (3, 4, 5-trinidrokside benzoic acid) and glucose. It is isolated from some part of plants can be found in market and it is cream-colored powder, amorf, astringent taste and aromatic. Tannins is used as astringent for gastrointestinal tract or skin and it also can make precipitation of the cell membrane protein and have a little penetration activity, so it can influence the permeability of cell membranes (Rahardjo, 1996).

Flavonoid is a genetic term used for aromatic heterocyclic oxygen compound which is derived from 2-phenilbenzopiran or its 2, 3-dehydro. Flavonoid is one of natural phenolic compound present in most plant and usually found in seeds and fruits. Sabir (2013) reported that flavonoid is synthesized in small amount about 0.5-1.5% and can be found in almost every part of a plant. Anthocyannis is one of a subgroup of flavonoid that is responsible to give yellow, red and blue pigment. Flavonoid is classified based on the level of oxidation level into catechin, leukoanthocianidine, flavanol, flavon and antocianidine.

2.2 Hypertension

2.2.1 Definition of Hypertension

Hypertension is one of the most common disorders in the world, affecting one billion individuals, and contributing to approximately 7.1 million deaths per year (Prado *et al.*, 2007). Hypertension is a major risk factor for stroke, myocardial infarction, vascular disease, and chronic kidney disease. Despite its high prevalence, control of the disease is far from adequate. Data from 2005-2008 show that only 46% to 51% of hypertensive individuals actively control blood pressure, defined as a level below 140/90 mmHg (Chobanian *et al.*, 2003). According to the American Heart Association (AHA) in ("Hypertension: Practice Essentials, Background, Pathophysiology," n.d.), approximately 75 million adults in the United States are affected by hypertension, which is defined as a systolic blood pressure (SBP) of 140 mmHg or more or a diastolic blood pressure (DBP) of 90 mm Hg or more or taking antihypertensive medication.

2.2.2 Stages of Hypertension

According to a report by American Society of Hypertension (ASH), normal individuals are individuals with optimal blood pressure levels and have no identifiable early markers of CVD. Resting average blood pressure levels are usually <120/80 mmHg, but these individuals may experience occasional elevated blood pressures (even to levels 140/90 mmHg).

The cardiovascular (CV) status of an individual decides the individual is either normal or hypertensive. The progression of hypertension from early to advanced can be categorized to stages 1, 2, and 3 hypertensions (Table 2.1). Each stage of hypertension is characterized by the cumulative presence or absence of markers of hypertensive CVD and evidence of target organ damage regardless of the blood pressure level such as progression includes such parameters as microalbuminuria or evidence of left ventricular hypertrophy (Giles *et al.*, 2009). The progression of the disease can be clearly indicated in a more advanced category by the occurrence of a major CV event. These stages are classified based on their own markers of CVD such as blood pressure, cardiac, vascular, renal, retinal, vasculature and cerebrovascular (Giles *et al.*, 2009).

Table 2.1: Definition and classification of hypertension adapted from Giles *et al.* (2009). Definition and Classification of Hypertension: An Update, *11* (11), 612.

DEFINITION & CLASSIFICATION OF HYPERTENSION				
Classification	**Normal**	**Stage 1 Hypertension**	**Stage 2 Hypertension**	**Stage 3 Hypertension**
Descriptive Category	Normal blood pressure or rare blood pressure elevations and no identifiable cardiovascular disease[a]	Occasional or intermittent blood pressure elevations and early cardiovascular disease[a]	Sustained blood pressure elevations or progressive cardiovascular disease[a]	Marked and sustained blood pressure elevations or advanced cardiovascular disease[a]
Cardiovascular Risk Factors (Table II)	None or few	Several risk factors	Many risk factors present	Many risk factors present
Early Disease Markers (Table III)	None	Usually present	Overtly present	Overtly present with progression
Target Organ Disease (Table IV)	None	None	Early signs present	Overtly present with or without CVD events
Definition and classification of hypertension by classifying individuals by blood pressure level or cardiovascular status; however, priority is given to cardiovascular status. [a]Cardiovascular disease designation is determined by the constellation of risk factors, early disease markers, and target organ disease as listed in Tables II–IV.				

Stage 1 hypertension is indicated by early CVD markers and it is the earliest identifiable stage of hypertensive disease. It generally arises from circulatory, vascular or renal adaptations due to the response to environmental or genetic stimuli. This stage is usually characterized by early signs of functional or structural changes in the heart or small arteries. In this stage, blood pressure levels are above 115/75 mmHg and it may be elevated particularly due to the stimuli from environmental stress. Patients often have more than one CV risk factor (Table 2.2). The individuals with early disease markers exhibit the characteristics of this stage (Table 2.3) and they do not show any evidence of target organ damage (Table 2.4). Stage 1 hypertension is an important stage to further investigation on two fronts. The first is to bring specific and sensitive cost-effective tests that can detect early CVD markers (Table 2.3) in the clinical settings. Secondly, it is to determine whether early vascular derangements can be weakened or reversed before the onset of target organ damage or overt CVD (Giles *et al.*, 2009).

Stage 2 hypertension is marked by diffused disease markers. Stage 2 hypertension patients frequently have sustained resting 140/90 mmHg blood pressure level, 140/90 mmHg, with much higher elevations in response to physiologic or psychological stressors. However, this group also includes individuals, regardless of blood pressure levels, with numerous disease markers (Table 2.3) or limited evidence of early target organ damage such as left ventricular hypertrophy (Table 2.4). Individuals with stage 2 hypertension suffer a progressive disease that has developed as a consequence of persistent functional and structural changes in blood pressure control mechanisms and in the heart and vasculature. Specialized or research studies can detect some of the early target organ damage characteristics of this stage of hypertension which should be evaluated further to determine their potential utility and cost effectiveness in clinical settings. Risk factors that are associated with stage 2

hypertension, if not modified, continue to contribute to progressive target organ disease (Giles *et al.*, 2009).

Stage 3 hypertension is also synonym to overt CVD. Individuals suffering with stage 3 hypertension usually have sustained resting blood pressure level, 140/90 mmHg, and they often experience marked elevations to levels >160/100 mmHg. This category includes all individuals with clinical evidence of overt target organ damage (Table 2.4) or CVD, as well as those who have already sustained CV events regardless of blood pressure levels. In Stage 3 hypertension, overt target organ damage is demonstrable and CV events may have already occurred or are imminent. It is an advanced stage of the hypertensive continuum. If aging and the persistence of other identifiable risk factors together with a blood pressure elevation are present, it may exacerbate and accelerate the risk of morbidity and mortality. Vigorous attempts at blood pressure lowering as well as aggressive management of other CVD risk factors must be started promptly and sustained in these individuals to prevent or delay further progression (Giles *et al.*, 2009).

Table 2.2: Cardiovascular risk factors adapted from Giles *et al.* (2009). Definition and Classification of Hypertension: An Update, *11*(11), 612.

Increasing age
Elevated blood pressure[a]
High heart rate
Overweight/obesity
Increased body mass index
Central obesity
Increased abdominal circumference
Increased abdominal adiposity (waist-to-hip ratio)[a]
Dyslipidemia
Elevated LDL or non-HDL cholesterol
Low HDL cholesterol[a]□
Elevated triglycerides[a]
Elevated blood glucose, insulin resistance, or diabetes[a]
Chronic kidney disease□
Smoking□
Family history of premature CVD (< age 50 y in men, < age 60 y in women)
Sedentary lifestyle
Psychosocial stressors
Elevated hs-CRP
Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; non-HDL cholesterol, total cholesterol) HDL cholesterol. [a]Components of the metabolic syndrome.

Table 2.3: Early markers of hypertensive cardiovascular disease adapted from Giles *et al.* (2009). Definition and Classification of Hypertension: An Update, *11*(11), 613.

System	Physiologic Alteration
Blood pressure	▪ Loss of nocturnal blood pressure dipping ▪ Exaggerated blood pressure responses to exercise or mental stress ▪ Salt sensitivity ▪ Widened pulse pressure□
Cardiac	▪ Left ventricular hypertrophy (mild) ▪ Increased atrial filling pressure ▪ Decreased diastolic relaxation ▪ Increased natriuretic peptide□
Vascular	▪ Increased central arterial stiffness or pulse wave velocity ▪ Small artery stiffness ▪ Increased systemic vascular resistance ▪ Increased wave reflection and systolic pressure augmentation ▪ Increased carotid intimal-media thickness ▪ Coronary calcification or stenosis by computed tomographic angiography ▪ Endothelial dysfunction ▪ Capillary rarefaction
Renal	▪ Microalbuminuria (urinary albumin excretion of 30–300 mg/d)[a]□ ▪ Elevated serum creatinine ▪ Reduced estimated GFR (60–90 mL/min)
Retinal	Hypertensive retinal changes

Abbreviation: GFR, glomerular filtration rate. [a]Also a marker of microcirculatory disease.

Table 2.4: Hypertensive target organ damage and overt cardiovascular disease adapted from Giles *et al.* (2009). Definition and Classification of Hypertension: An Update, *11* (11), 613.

System	Evidence Of Target Organ Damage & Cardiovascular Disease
Cardiac	▪ Left ventricular hypertrophy (moderate to severe) ▪ Systolic or diastolic cardiac dysfunction ▪ Symptomatic heart failure ▪ Myocardial infarction ▪ Angina pectoris ▪ Ischemic heart disease or prior revascularization
Vasculature	▪ Peripheral arterial disease ▪ Carotid arterial disease ▪ Aortic aneurysm ▪ Wide pulse pressure (> 65 mmHg)
Renal	▪ Albuminuria (urinary albumin excretion > 300 mg/d)□Chronic kidney disease (estimated ▪ GFR < 60 mL/min) or ESRD
Cerebrovascular	▪ Stroke ▪ Transient ischemic attack ▪ Decreased cognitive function ▪ Dementia ▪ Loss of vision
Abbreviations: ESRD, end-stage renal disease; GFR, glomerular filtration rate.	

2.2.3 Mechanism of Hypertension

There are four main regulatory of arterial blood pressure mechanisms, which are nervous system mechanism (short term regulation), renal mechanism (long term regulation), hormonal mechanism (mediators) and local regulation (Figure 2.3). All these mechanisms play some important roles in regulation of blood pressure.

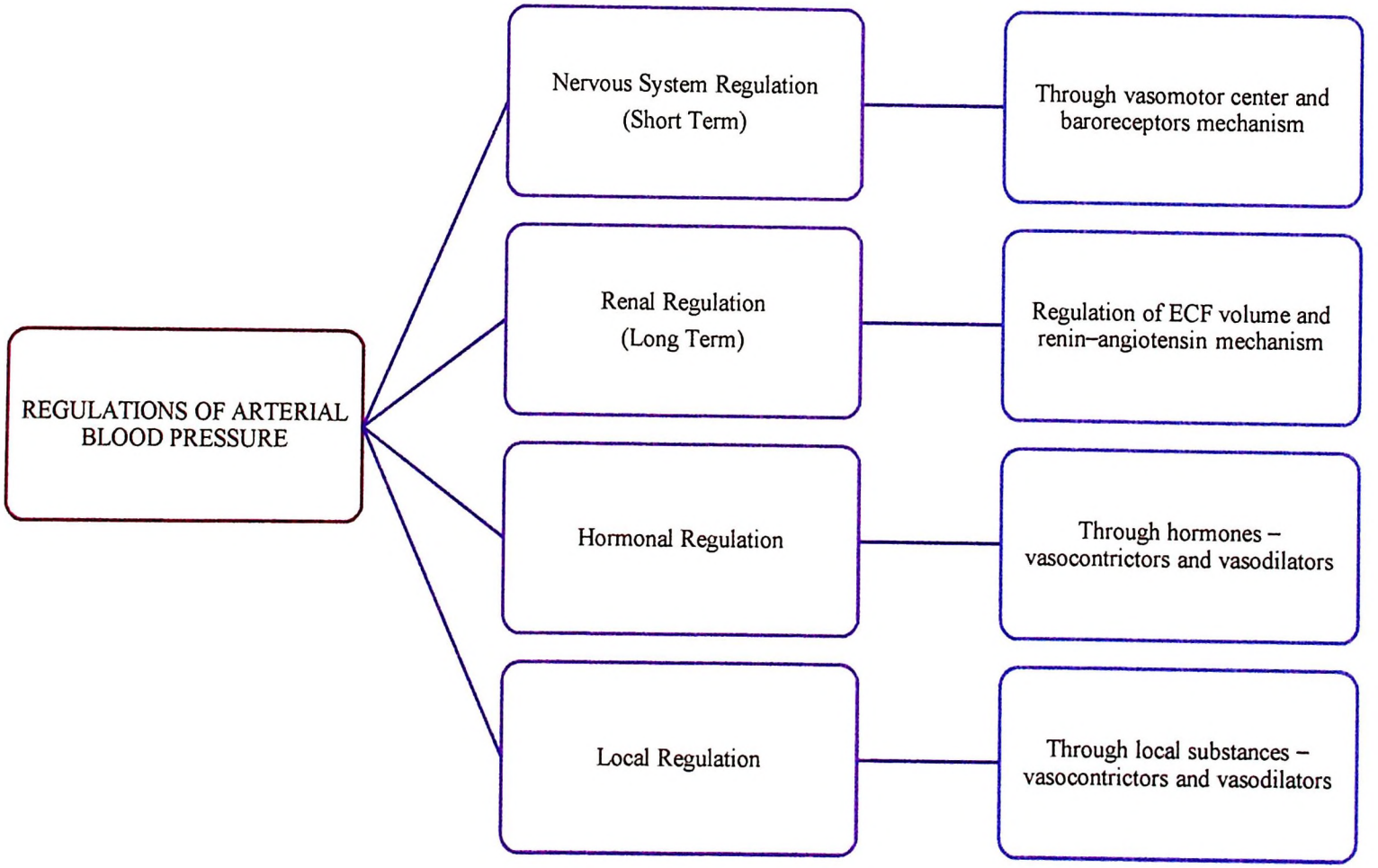

Figure 2.3: Summary of arterial blood pressure regulation