AN EXPERIMENTAL STUDY ON ANTIHYPERTENSIVE ACTIVITY OF GARCINIA ATROVIRIDIS FRUIT EXTRACTS

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AN EXPERIMENTAL STUDY ON ANTIHYPERTENSIVE ACTIVITY OF *GARCINIA ATROVIRIDIS* FRUIT EXTRACTS

by

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This thesis is dedicated to
my beloved family & husband

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LIST OF ABBREVIATIONS

%	Percent
±	Plus minus
°C	Degree Celcius
µg	Microgram
µmol/L	Micromoles per litre
α	Alpha
β	Beta
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ARASC	Animal Research & Service Centre
ARB	Angiotensin-II receptor blocker
AT	Angiotensin
BP	Blood pressure
BPM	Beat per minute
bw	Body weight
Ca^{2+}	Ion calcium
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
cm	Centimeter
CNS	Central nervous system
CO	Cardiac output
CO_2	Carbon dioxide
CoA	Coenzyme A
DAG	Diacylglycerol
DP	Diastolic blood pressure
EDHF(s)	Endothelium-derived hyperpolarizing factor(s)
eNOS	Endothelial nitric oxide synthase
EPI	Epinephrine
g	Gram
g/L	Gram per litre
GA	Garcini atroviridis
GACE	*Garcinia atroviridis* chloroform extract
GAME	*Garcinia atroviridis* methanol extract
GAPET	*Garcinia atroviridis* petroleum ether extract
GAWE	*Garcinia atroviridis* water ether extract
GPCR	G-protein coupled receptor
h	Hour
HCA	Hydroxycitric acid
HCAL	Hydroxycitric acid lactone
HCTZ	Hydrochlorothiazide
HR	Heart rate
iNOS	Inducible isoform nitric oxide synthase
IP_3	Inositol- 1,4,5-trisphosphate
ISOP	Isoprenaline
K^+	Potassium ion

KCl	Potassium chloride
kg	Kilogram
KH_2PO_4	Potassium dihydrogen phosphate
KPS	Kreb's physiological solution
LDL	Low density lipoprotein
L-NAME	N^{ω}-nitro-L-arginine methyl ester
M	Molar
M	Muscarinic
MAP	Mean arterial pressure
mg	Milligram
$MgSO_4$	Magnesium sulphate
ml	Milliliter
mmHg	Milimeter of mercury
Na^+	Sodium ion
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NE	Norepinephrine
ng	Nanogram
Nm	Nicotinic-muscle
Nn	Nicotinic-nerve
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
O_2	Oxygen
PE	Phenylephrine
PR	Peripheral resistance
s	second
SD	Sprague Dawley
SEM	Standard error of mean
SHR	Spontaneous hypertensive
SNS	Sympathetic nervous system
SP	Systolic blood pressure
UK^+	Absolute urine potassium
UNA^+	Absolute urine sodium
-ve	Negative
w/w	Weight by weight
WKY	Wistar Kyoto

SATU KAJIAN TENTANG AKTIVITI ANTIHIPERTENSI EKSTRAK BUAH *GARCINIA ATROVIRIDIS*

ABSTRAK

Tumbuhan *Garcinia atroviridis* (GA) digunakan dalam perubatan tradisional untuk merawat sakit telinga, sakit tekak, batuk, kelumumur, sakit perut yang berkaitan dengan kehamilan dan juga penyakit darah tinggi. Dalam kajian ini, kesan antihipertensi berpandukan ekstraksi dan fraksi buah GA dijalankan untuk mengetahui komponen kimia yang paling aktif. Buah kepada pokok ini telah dikeringkan, dikisar halus dan diekstrak dengan menggunakan pelarut bersiri iaitu petroleum eter, klorofom, metanol dan air. Ekstrak telah dikeringkan di bawah tekanan yang rendah dan kemudian dibeku-kering. Kesan kesemua ekstrak tersebut telah diuji ke atas gegelung aorta tikus. Petroleum eter ekstrak didapati sebagai ekstrak yang paling aktif dalam pengenduran fenilefrina (PE) pra-kontraksi pada gegelung aorta. Kemudian, ekstrak petroleum eter difraksi menggunakan pelarut n-heksana dan diklorometana. Fraksi n-heksana (2 mg ml^{-1}) adalah yang paling aktif dalam pengenduran gegelung aorta. Penyingkiran endotelium pada gegelung aorta tidak menghapuskan kesan pengenduran ekstrak petroleum eter GA dan dengan ini ianya dicadangkan kesan pengenduran ekstrak ini tidak bergantung kepada endotelium. Tikus hipertensi yang diberi rawatan oral pada dos 1 g kg^{-1} mengurangkan tekanan sistolik darah dan kadar denyutan jantung secara signifikan dan ini menguatkan lagi kesan antihipertensi buah GA. Pada tikus yang dibius, suntikan ekstrak metanol dan air GA mengurangkan tekanan arteri mean (MAP), sistolik (SP), diastolik (DP) dan kadar denyutan jantung (HR) pada tikus normal

mengikut dos yang diberi. Suntikan ekstrak petroleum eter dan klorofom menyebabkan kenaikan MAP, SP, DP dan HR pada tikus normal.

Mekanisma yang terlibat dalam pengurangan tekanan darah pada tikus dibius juga telah dikaji menggunakan fenilefrina (α-agonis), isoprenalina (β-agonis) dan asetilkolina (agonis kolinergik). Hasil kajian mendapati kenaikan MAP yang dirangsang oleh fenilefrin direncat secara signifikan oleh ekstrak air GA dan dicadangkan disebabkan oleh sekatan pada reseptor α-adrenergik. Kesan diuretik ekstrak metanol dan air juga dikaji. Tiada kesan signifikan pada pengeluaran air kencing dan pengambilan air, tetapi, kenaikan pengeluaran natrium yang signifikan telah didapati pada kumpulan tikus yang diberi ekstrak air GA. Oleh itu, kesan penurunan tekanan darah oleh ekstrak GA dicadangkan disebabkan oleh kesan vasodilasi dan aktiviti α-antagonis. Analisis kimia kualitatif pada ekstrak air GA menunjukkan kehadiran alkaloid, flavonoid, terpenoid, steroid, saponin dan glikosida kardiak.

AN EXPERIMENTAL STUDY ON ANTIHYPERTENSIVE ACTIVITY OF *GARCINIA ATROVIRIDIS* FRUIT EXTRACTS

ABSTRACT

Garcinia atroviridis (GA) plant has been used as a traditional medicine for treating earache, throat irritation, cough, dandruff and stomach disorders associated with pregnancy and hypertension. In the present study, the anti-hypertensive effects-guided extraction and fractionation of GA fruit were carried out in an attempt to find the most active chemical component. The fruit of this plant was dried, ground and serially extracted with petroleum ether, chloroform, methanol and water. The extracts were dried under reduced pressure and later freeze-dried. The vasodilator effect of the extracts was examined on isolated rat aortic ring preparation. The petroleum ether extract was found to be the most potent in relaxing PE pre-contracted aortic rings. The petroleum ether extract was then fractionated with n-hexane and dichloromethane. The n-hexane fraction (2 mg ml^{-1}) was found to be the most active in relaxing the aortic ring. Removal of the endothelium of the aortic ring did not abolish the relaxing property of petroleum ether extract which suggests that the relaxing effect of the extract is not endothelium dependent. Orally administered all of GA extracts (1 g kg^{-1}) significantly reduced the systolic blood pressure and heart rate of spontaneous hypertensive (SH) rats which support the antihypertensive effect of GA fruit. In anaesthetized rats, the intravenous administration of methanol and water extracts of GA dose-dependently reduced mean arterial pressure (MAP), systolic pressure (SP), diastolic pressure (DP) and heart rate (HR) of SD rats. The intravenous administration of petroleum ether and chloroform extracts caused an increase in MAP, SP, DP and HR in normotensive rats. The mechanism involved in

reducing blood pressure of anesthetized normotensive rats was examined using phenylephrine (an α-agonist), isoprenaline (a β-agonist) and acetylcholine (a cholinergic agonist). It was found that the increase in MAP induced by phenylephrine was significantly inhibited by water extract of GA. It suggests that the water extract of GA possess α-adrenergic receptor blocker activity. The diuretic effect of methanol and water extracts of GA was investigated. There was no significant increase in urine output and water intake but there was a significant increase in urinary sodium excretion in orally administered water extract of GA. Therefore, it can be suggested that the lowering of blood pressure effect of GA is due to it's the vasodilator and α-antagonist activities. Qualitative chemical analysis suggests that the water extract of GA contained alkaloids, flavonoids, terpenoids, steroids, saponins and cardiac glycosides.

CHAPTER ONE

INTRODUCTION

1.1 The cardiovascular system

The cardiovascular system composes of a set of tubes, blood vessels for the flowing of blood, and a pump which is the heart that produces the flow. In 1628, the experimental science of physiology begun when William Harvey presented that the entire system forms a circle in which the blood is continuously being pumped out of the heart via one set of vessels and returning via a different set. Blood is pumped through the pulmonary circulation, from the right half of the heart via the lungs and back to the left of the heart (Figure 1.1). The second circuit (systemic circulation) pumped the blood from the left half of the heart through all the tissues of the body except the lungs and then back to the right half of the heart. The vessels carrying blood away from the heart are called the arteries. The aorta is a single large artery whereby blood left the half left of the heart. From the aorta, branching arteries conduct blood to various organ and tissues and it is further divided into smaller branches which are called arterioles (Vander et al., 1970).

The chambers of the heart normally contract in a coordinated manner, pumping blood efficiently by a route determined by the valves. The heart consists of three layers which are the epicardium, the myocardium and the endocardium. The autonomic nervous system of the heart is composed of dual innervation from the sympathetic and parasympathetic divisions. These nerves produce neural regulation of cardiac function via conduction tissue (Maximilian Buja, 2007).

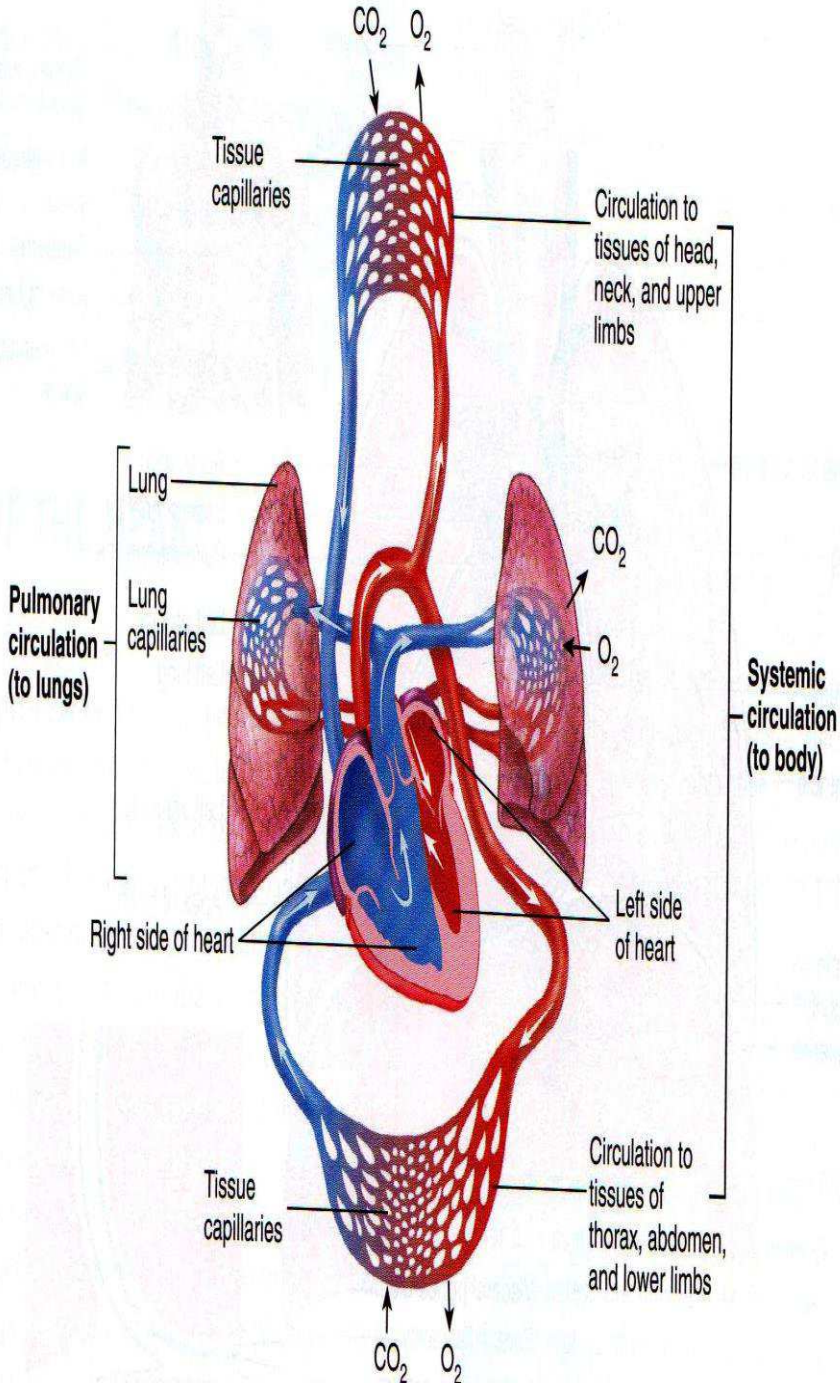

Figure 1.1 : Systemic and pulmonary circulation of the heart (Adapted from McKinley and O'Louglin, 2007).

1.1.1 Arteries and arterioles

Blood is released with each heartbeat from the left ventricle into the aorta, where it flows quickly to the organs through large conduit arteries. Successive branching leads via muscular arteries to arterioles and capillaries, where gas and nutrient exchanges occur (Rang and Dale, 2007). Essential hypertension is related with increased peripheral vascular resistance to blood flow which is cause by reduction in the caliber and/or number of small arteries and arterioles which are the main resistance vessels (Intengan and Schiffrin, 2000; London and Guerin, 1999). The contractility of vascular smooth muscle cells in the walls of small arteries and arterioles determined the arterial blood pressure (Moosmang et al., 2003). The arterial system has two functions; first as a conduit function to supply blood flow to peripheral tissues and organs on the basis of a pressure gradient and second as cushioning function to transform the pulsatile flow produced by the intermittent ventricular ejection into a continuous flow of blood in the periphery (Safar et al., 2003; London and Guerin, 1999).

The key cause of increased peripheral resistance is a decrease in lumen diameter (Intengan and Schiffrin, 2000). The vascular alterations that are involved in the decreased lumen size may be influenced by several distally located structural, mechanical and functional factors. These include eutrophic and hypertrophic remodeling of arterial and arteriolar vessels. In eutrophic remodeling, the outer diameter and the lumen are decreased and the cross-sectional area of the media is unaltered, which produce a greater media-lumen ratio. This type of remodeling is usually found in mild, essential hypertensive patients. Meanwhile hypertrophic remodeling includes a thickening of the media that encroaches on the lumen which narrowed it and therefore increased the media-lumen ratio and medial cross-sectional

area (Intengan and Schiffin, 2000). This type of remodeling is found in renovascular hypertension patients. Moreover, changes of the microenvironment (especially sodium and other cations), reduced endothelium-mediated vasodilation and genetically mediated modifications of vasomotor tone, resulting from smooth muscle or endothelial cells are also the factors that decrease arterial cross-sectional area (Safar et al., 2003).

1.1.1.1 Anatomy of the arteries

There are three layers in the arteries (Figure 1.2) :

i) Tunica intima or interna is the inner most layer of the artery wall, and has intimate contact with the blood. It is composed of an endothelium and a subendothelial connective tissue separated from a circumferentially-arranged smooth muscle cells called the internal elastic lamina.

ii) Tunica media which is the middle layer of the vessel is the thickest layer in arteries. It is a circularly arranged elastic fiber, connective tissue and polysaccharide substances. Sympathetic innervation causes the smooth muscle to contract while relaxation of the fibers causes vasodilation.

iii) Last but not least is tunica adventitia or externa, the outermost. It is fully made of loosely woven collagen, connective tissue fibrils and sympathetic nerves (McKingley and O'Loughlin, 2007).

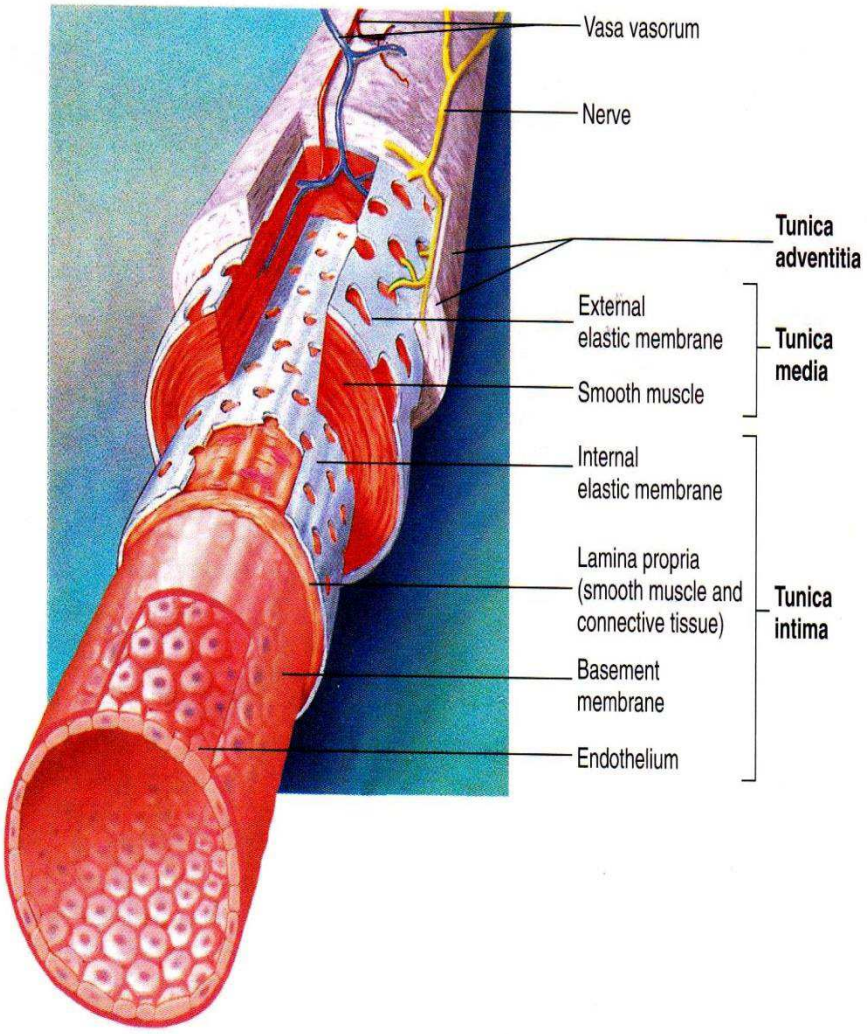

Figure 1.2 : The layers of the blood vessel include intima, media and adventitia (Adapted from Seeley, 2008).

1.2 The vascular endothelium

In earlier days it had been thought until 1981 that the vascular endothelium acts as a passive barrier between plasma and extracellular fluid. Nowadays, new discovery has found out that it is also a source of numerous potent mediators such as prostanoids, nitric oxide, peptides and endothelium-derived hyperpolarising factor(s) (EDHF(s)) (Rang and Dale, 2007) which regulate the vascular tone. Vascular endothelium lines the circulatory system and is comprised of a monolayer of endothelial cells. There are three types of endothelial cells based on their intercellular junctions which are continuous, fenestrated or discontinuous and they are very adaptable to the specific requirements of an individual organ (Pasyk and Jakobczak, 2004). The phenotype differs between species, different organs and also in a specific organ itself. For example, in the kidney, the endothelial cells are fenestrated in peritubular capillaries, discontinuous endothelium in glomerullar capillaries and continuos in other parts (Risau, 1995). The other important role of endothelium of the cardiovascular system includes the synthesis and secretion of various molecules, haemostasis and coagulation, inflammatory responses, vasculogenesis and angiogenesis (Pries and Kuebler, 2006).

Furchgott and Zawadzki, (1980) discovered an endothelium-derived relaxing factor in a study on the ability of acetylcholine to elicit relaxation of isolated strips of rabbit aorta which was entirely dependent on the presence of the endothelium, which was later identified as nitric oxide (NO) (Palmer et al., 1987). It was found out that the endothelial NO was synthesized by NOS from L-arginine, a semi-essential amino acid through an N^G hydroxy-L-arginine intermediate yielding L-citrulline (Palmer et al., 1988). Nitric oxide produced by the vascular endothelium is a major regulator of vascular homeostasis and if the vascular function is impaired, it can lead to the

development of a number of clinical conditions (Moncada and Higgs, 2006). For example, changes in endothelial function is the first step towards the development of atherosclerosis, a disorder of large and medium arteries and contribute to their perpetuation and to the clinical manifestations of vascular diseases (Higashi et al., 2009; Sima et al., 2009). Moreover, the production of NO is also due to the shear stress produced by the flowing blood and pulsatile stretch of the vascular wall (Fleming and Busse, 2003).

NO is a highly reactive signaling molecule that is made in a wide variety of cells, mostly neurons, skeletal muscle, endothelial cells and certain immune system cells and regulates physiologic and pathophysiologic processes including cardiovascular, inflammation, immune and neurol functions (Paige and Jaffrey, 2007). In these cells, NO is synthesized by NO synthase (NOS) isoenzymes which consist of two constitutive and one inducible isoform, each named for the initial cell type which it was isolated and encoded by a separate gene. NO which are present in endothelial cell are call endothelial NOS (eNOS) and neuronal NOS (nNOS) for NO present in neurons, both are constitutive isoform. The inducible isoform (iNOS) is present in macrophages and smooth muscle cells as a vital inflammatory mediator (Spieker et al., 2006).

A study (Huang et al., 1995) has discovered the role of eNOS in vascular function whereby they disrupted the eNOS gene in mice. Their result showed that the eNOS mutant mice are hypertensive due to the lack of vascular response to acetylcholine. Other than that, a similar study done by Shesely et al., (1996) discovered similar result and they also observed reduction in the heart rates of the eNOS mutant mice (-/-) significantly compared to +/+ and +/- mice. In 1998, Miyamoto et al., have investigated the molecular involvement of the eNOS gene in essential hypertension

and they found that there was a significant association of the Glu298 Asp polymorphism in the eNOS gene with essential hypertension in Japanese patients. All these studies concluded that eNOS is important for the regulation and maintenance of normal blood pressure. Furthermore, other studies discovered that eNOS -/- mice developed bicuspid aortic valve (Lee et al., 2000), heart failure and congenital septal defects (Feng et al., 2002) and major defects in lung morphogenesis (Han et al., 2004).

1.3 Autonomic nervous system

The motor portion of the nervous system consists of two major subdivisions: autonomic and somatic. The autonomic nervous system activities are under direct unconscious control or independent. The two major divisions of autonomic nervous system are sympathetic nervous system (SNS) and parasympathetic nervous system. Meanwhile somatic division is mainly concerned with consciously controlled functions, for example respiration, posture and movement.

The increase of SNS activity is the primary cause of hypertension in humans and animal models (Wys, 1993). There are many studies that described the correlation between sympathetic activation and essential hypertension (DiBona, 2004). The abnormal renal excretory function is vital for the initiation and development of primary hypertension. The kidneys respond to changes in arterial pressure by changing the urinary water and sodium excretion. This happen through pressure natriuresis, the homeostasis of renal body fluid feedback mechanisms couples the long-term regulation of arterial pressure to extracellular volume. In normal regulation, an increase in arterial blood pressure will result in an increased in urinary

sodium and water excretion and thus blood volume is reduced and the blood pressure is returned to normal. Based on computer modeling studies, a sustained increase in arterial pressure will only happen if there is a chronic decrease in renal excretory function (DiBona, 2004).

Moreover, nitric oxide (NO) deficiency may increase the SNS contribution towards some forms of hypertension (Wyss, 1993). For instance, in a study of L-NAME, (a NO synthesis inhibitor) treated rats with glucose infusion produced hypertension compared to responses to glucose and L-NAME alone that is not affected by combined α- and β-adrenoceptor blockade. The inhibition of NO by L-NAME increases the hypertensive effects and tachycardiac responses. These results suggests that NO may protect against hypertension during chronic glucose infusion through suppression of sympathetic activity (Claxton and Brands, 2003).

There are also other factors that contribute to SNS activation which result in resistant hypertension such as obesity, obstructive sleep apnea and excess of aldosterone (Tsioufis et al., 2011). Resistant hypertension is uncontrolled blood pressure despite treatment with three antihypertensive agents.

The role of SNS in the pathophysiology of hypertension and its complications means that the regulation of sympathetic activity should be a vital target of antihypertensive treatment. In addition, further investigation on mechanisms that leads to SNS activation is also very helpful in understanding and managing hypertension.

1.3.1 Adrenergic receptors

The adrenergic receptors play an important role in regulating sympathetic nervous system activity and also as a site of action for many therapeutics agents. They are members of the G-protein coupled receptor superfamily (GPCR). α and β are the two families of receptors that were initially identified based on their responses to the adrenergic agonists epinephrine, norepinephrine and isoproterenol. The use of specific blocking drugs and the cloning of genes has revealed the molecular identities of a number of receptor subtypes.

1.3.1.1 α-adrenergic receptors

The α-adrenoceptors show a weak response to the synthetic agonist isoproterenol, but they are responsive to the naturally occurring catecholamines, epinephrine (EPI) and norepinephrine (NE). Both EPI and NE produce different effects. EPI relax smooth muscle and is only produced in the adrenal medulla while NE does not relax smooth muscle. NE acts as a neurotransmitter in the central nervous system and in the sympathetic nervous system at postganglionic neuro effector junctions (Insel, 1996). The α-adrenoceptors are further classified into two subgroups, α_1 and α_2 based on their affinities for α agonists and blocking drugs. α_1 receptors are located on smooth muscle membrane of arteries, veins and sphincters of the urinary and gastrointestinal tract and involve in constriction of smooth muscle. The mechanism of action to activate the α-receptors is via G protein activation of phospholipase C that leads to the production of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) from phosphatidylinositol. IP_3 promote the release of Ca^{2+} from the endoplasmic reticulum into the cytosol and DAG turns on other proteins within the cell.

α_2 receptors are present in presynaptic nerve endings. They are stimulated by EPI and NE to activate a negative feedback mechanism that reduces and modulates the release of additional NE. The effects of binding at α_2 receptors are mediated by inhibition of adenylyl cyclase and a fall in the levels of intracellular cAMP. The α-receptors are further categorized into α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, and α_{2C}-. This classification is required for understanding the selectivity of some drugs (Harvey, 2012; Hitner and Nagle, 2012).

1.3.1.2 β-adrenergic receptors

Initially, there were at least two subtypes of β-receptors, designated as β_1 and β_2. β_1 receptors have approximately equal affinity for EPI and NE, while β_2 receptors have higher affinity for EPI than for NE. β_3 was identified subsequently and was found in cardiac tissue and was reportedly to induce negative inotropic effect (Gauthier et al., 1996). The β-adrenergic signaling pathway plays an important role in stimulation of the heart (β_1) and bronchodilation (β_2). Stimulation of β-adrenergic receptors increases heart rate, force of cardiac contraction, rate of cardiac relaxation and automaticity. These effects happen when this receptor is activated by adrenergic agonists or sympathetic neuronal stimulation (Post et al., 1999).

The mechanism of action of β-adrenergic involves the stimulation of adenylyl cyclase by stimulatory G protein which increases the levels of cAMP and then, the phosphorylation of proteins through cAMP dependent protein kinase. The examples of these proteins are phospholamban, calcium channels and contractile proteins which after phosphorylation results in a functional response.

Isoproterenol is one example of drug that produces both β_1 and β_2 effects. This drug causes overstimulation of the heart along with bronchodilator effect. Because of this, researchers have investigated and discovered β drugs that would only stimulate β_2 receptors without causing excessive stimulation of β_1 receptors in the heart. This is important for the treatment of bronchodilator. This is one example that shows the importance in knowing the function of receptor subtypes.

1.4 Adrenergic antagonists

The adrenergic antagonists (blockers or sympatholytic agents) bind to adrenoceptors but do not trigger the usual receptor-mediated intracellular effects. These drugs act by either reversibly or irreversibly attaching to the receptors, therefore preventing its activation by endogenous catecholamines. The antagonists are categorized according to their affinities for α and β receptors in the peripheral nervous system. Many adrenergic antagonists have important functions in clinical medicine, mainly to treat cardiovascular diseases (Harvey, 2012).

1.4.1 α-adrenergic antagonist

Drugs that block α-adrenoceptors significantly affect blood pressure. Since arteriolar and venous tone are determined by α-receptors on vascular smooth muscle, blockade of these receptors reduces the sympathetic tone of the blood vessels, leads to decrease peripheral vascular resistance which results in lower blood pressure. This also promotes a reflex tachycardia and postural hypotension (Bai, 2008; Katzung, 2007).

Some examples of α-antagonists are phentolamine, prazosin, doxazosin, terazosin and tamsulosin. Phentolamine has been used in the treatment of pheochromocytoma and male erectile dysfunction. Prazosin, doxazosin and terazosin are beneficial in the treatment of hypertension. These drugs are selective competitive blockers of α_1-receptors. Meanwhile, tamsulosin are useful for the treatment of benign prostatic hypertrophy (Katzung, 2007).

1.4.2 β-adrenergic antagonist

All the clinically available β-blockers are competitive antagonists. Non-selective β-blockers act at both β_1- and β_2-receptors, whereas cardioselective β-antagonists block β_1-receptors. β-blocking drugs lower blood pressure in hypertensive patients but do not induced hypotension in normal patients. This is because the α-adrenoceptors remain functional which means the normal sympathetic control of the vasculature is maintained. β-receptor antagonists are primarily important in the treatment of angina, chronic heart failure and myocardial infarction (Harvey, 2012).

One example of β-blocker is propranolol. This drug blocks the action of isoproterenol on the cardiovascular system. Hence, in the presence of propranolol, isoproterenol does not exert its effects which are increased heart rate or reductions in mean arterial pressure and diastolic pressure. Other examples of other β-blocker drugs are nadolol, timolol, pindolol and sotalol (Katzung, 2007).

1.5 Cholinergic receptors

The cholinergic drugs act on receptors that are activated by acetylcholine (ACh). These drugs act by either stimulating or blocking receptors of the parasympathetic (cholinergic) nervous system. Neurotransmission in cholinergic neurons include six sequential steps which are synthesis, storage, release, binding of ACh to a receptor, degradation of the neurotransmitter in the synaptic cleft and recycling of choline and acetate. The synthesis of ACh involves choline transportation from the extracellular fluid into the cytoplasm. Choline acetyltransferase catalyzes the reaction of choline with acetyl coenzyme A (CoA) to form ACh in the cytosol. Acetyl CoA is derived from the mitochondria and is develop by the pyruvate oxidation and fatty acid oxidation. ACh is then stored into presynaptic vesicles by an active transport process coupled to the efflux of protons. Here, ACh is protected from degradation in the vesicle. The release of ACh happens when an action potential propagated by voltage-sensitive sodium channels arrives at a nerve ending, causing an increase in the concentration of intracellular calcium. Then, ACh binds to postsynaptic cholinergic receptors which are classified into two; muscarinic and nicotinic. Afterwards, ACh is hydrolyzed by acetylcholinesterase in the synaptic cleft. Last but not least, choline is taken up by the neuron, acetylated into ACh and stored. These processes will be repeated after activation of subsequent action potential (Harvey, 2012).

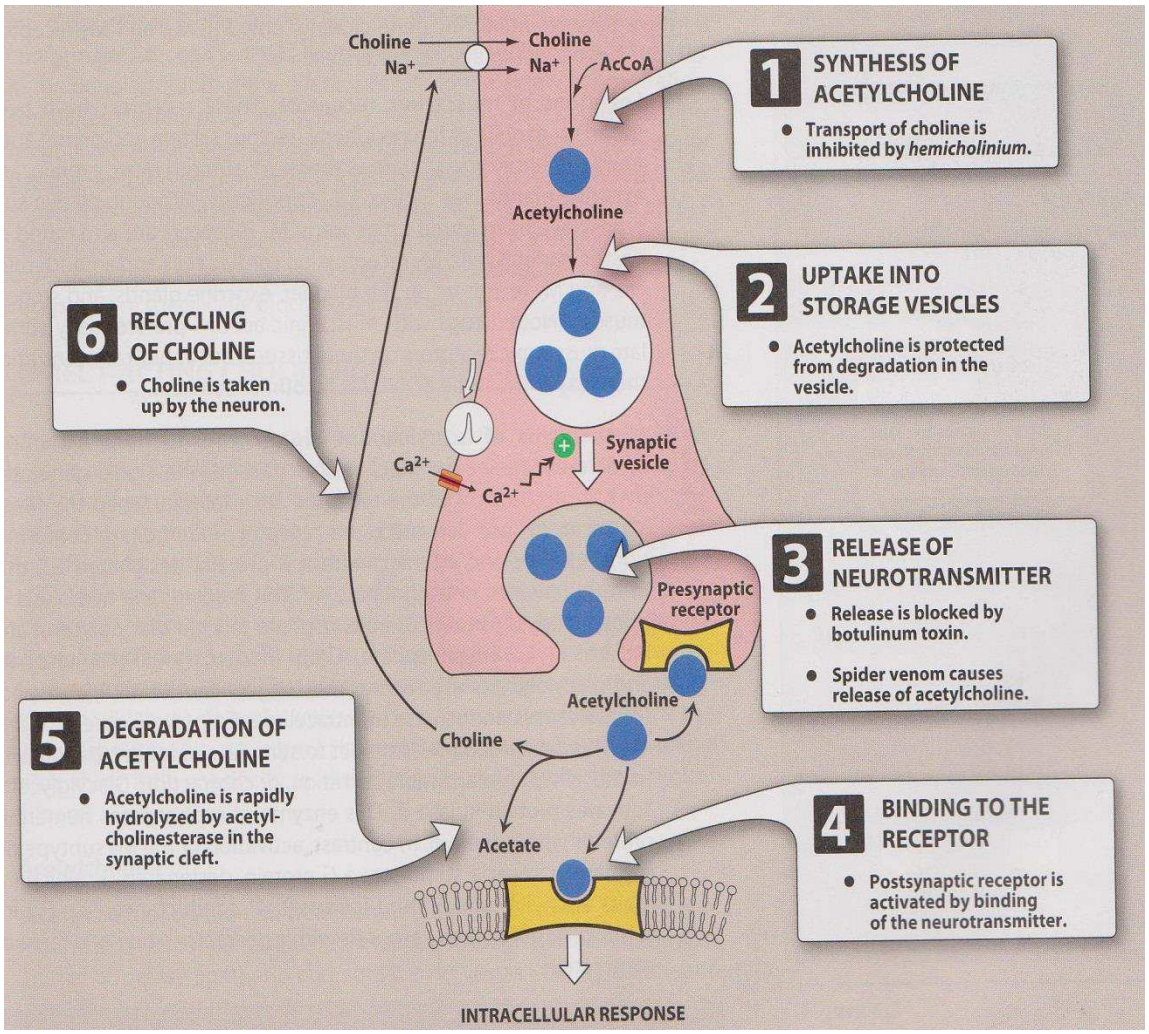

Figure 1.3: Synthesis and release of acetylcholine from the cholinergic neuron. (Adapted from Harvey, 2012).

1.5.1 Muscarinic receptors

Muscarinic receptors belong to the class of G protein-coupled receptors. Five subclasses of muscarinic receptors have been identified: M_1, M_2, M_3, M_4 and M_5. Nevertheless only M_1, M_2 and M_3 have been functionally characterized. These receptors are located on ganglia of the peripheral nervous system and on the autonomic effector organs, for example the heart, smooth muscle, brain and exocrine

glands. M_1-receptors are also found on gastric parietal cells, M_2-receptors on cardiac cells and smooth muscle while M_3 receptors on the bladder, exocrine glands and smooth muscle.

The mechanism of action of M_1- and M_3-receptors involves the interaction with G protein which then activates phospholipase C. This result to the hydrolysis of phosphatidylinositol-(4,5)-bisphosphate to produce diacylglycerol (DAG) and inositol (1,4,5)-trisphosphate. Inositol (1,4,5)-trisphosphate causes an increase in intracellular Ca^{2+} while DAG activates protein kinase C. Ca^{2+} stimulates or inhibits enzymes or produce hyperpolarization, secretion or contraction while protein kinase C phosphorylates many proteins within the cell. This is the opposite to the M_2-mechanism of action which inhibits adenylyl cyclase and increase K^+ conductance. The heart responds with a decrease in rate and force of contraction (Harvey, 2012).

1.5.2 Nicotinic receptors

Nicotinic receptors are found in the CNS, adrenal medulla, autonomic ganglia and the neuromuscular junction. These receptors recognize nicotine with only a limited affinity for muscarine. Nicotinic receptors belong to the superfamily of receptor-gated ion channels and are composed of five subunits (Hosey, 1992). Two types of nicotinic receptors are identified: nicotinic-nerve (Nn) which is found at both the parasympathetic and sympathetic ganglia and nicotinic-muscle (Nm) which is located on cell membranes of skeletal muscle (Hitner and Nagle, 2012).

The binding of two ACh molecules produce a conformational change that permits the entry of sodium ions, causing the depolarization of the effector cell. Low

concentration of nicotine stimulates the receptor while higher concentration blocks the receptor (Harvey, 2012).

1.6 Blood pressure (BP) classification

Blood pressure is a result of the factors that modulate cardiac output (CO) and peripheral resistance (PR). The formula for BP is

$$BP = CO \times PR$$

Cardiac output is the amount of blood that is pumped out of the heart per minute. Peripheral resistance is the resistance that the arterioles have against the flow of blood. The increasing of these factors, heart rate, stroke volume or peripheral resistance will increase blood pressure and stimulation of the sympathetic nervous system will increase all the three factors. There are many factors that contribute to high blood pressure which include weight problem, high intake of sodium, smoking, lack of exercise and stress. Although these factors may not be the primary cause, controlling of these factors may result in modest decreases in BP (Hitner and Nagle, 2012).

Table 1.1: Blood pressure classification. For classification of normal blood pressure, the requirements for both systolic and diastolic pressure must be met; for the remaining categories, either the systolic or the diastolic requirement must be met (Williams et al., 2009).

Classification	Systolic	Diastolic
	mmHg	
Normal	<120	<80
Prehypertension	120-139	80-89
Stage 1 hypertension	140-159	90-99
Stage 11 hypertension	≥160	≥100

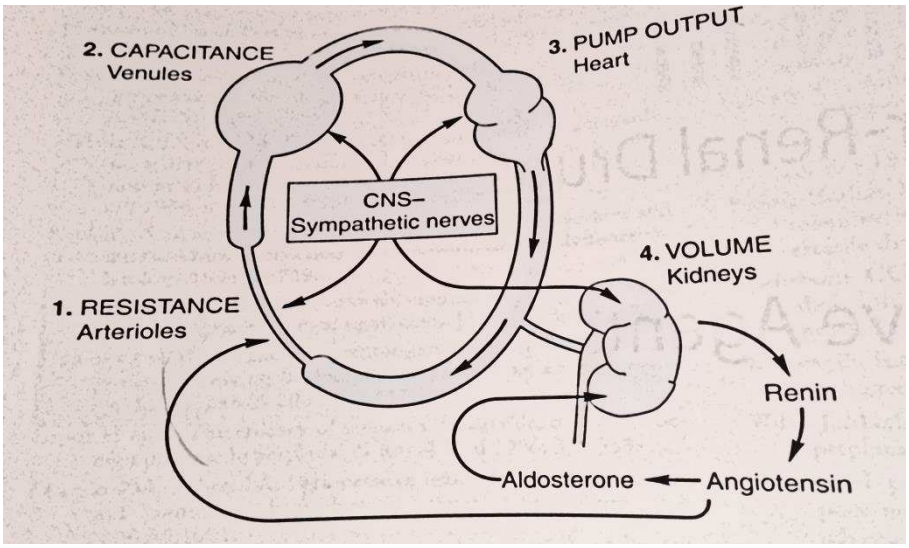

Figure 1.4: Anatomic sites of blood pressure control (Adapted from Katzung, 2007).

1.7 Hypertension

The most common cardiovascular disease is hypertension. Arterial blood pressure is an important indicator of a person's state of health. If a person has low blood pressure, it is a medical emergency while blood pressure elevation directly indicates the risks of damage to kidney, heart and brain (Perloff et al., 1993). Mild hypertension (blood pressure 140/90 mmHg) also increases the risk of eventual end organ damage (Katzung, 2007). The gold standard for measurement of arterial pressure is with a catheter through direct intra-arterial measurement. However, as this technique is not practical for repeated measurements and large scale public health screening, the indirect method of measurement is commonly used. This technique required the use of sphygmanometer to measure the pressure that collapse the artery in the upper arm or leg (an occluding cuff, stethoscope and manometer). The cuff is inflated to a level above arterial pressure (as showed by obliteration of the pulse). As the cuff is gradually deflated, the pressure is noted through a series of sounds (Williams et al., 2009). The direct method measures pressure and the indirect method is more indicative of flow, therefore the results will be similar. The indirect method is generally less reproducible and less accurate (Perloff et al., 1993). However, it is claimed to be sufficiently accurate because it is practical, simple, cost effective and non-invasive.

1.8 Synthetic drugs for the treatment of hypertension

All antihypertensive agents act at one or more of the four anatomic control sites which are arterioles, venules, heart and kidneys and their effects are produced by interfering with normal mechanisms of blood pressure regulation (Katzung, 2007).

The classifications of these agents according to the principal regulatory site or mechanism on which they act include the following:

i) Diuretics: Lower blood pressure by reducing blood volume through urinary excretion of water and electrolytes. Electrolytes are ion such as sodium (Na^+), calcium (Ca^{++}), chloride (Cl^-) and potassium (K^+). There are different diuretics drugs with different mechanisms. Below are the examples of the drugs and how they act:

   a) Thiazide diuretics: For examples, hydrochlorothiazide and chlorthalidone. These drugs lower blood pressure by increasing sodium and water excretion. This results in a decrease in extracellular volume which leads to decrease in cardiac output and renal blood flow.

   b) Loop diuretics: For examples, furosemide, bumetanide and torsemide. Loop diuretics cause decreased renal vascular resistance and increased renal blood flow. These drugs decrease blood potassium levels; act by inhibiting sodium and chloride reabsorption in the loop of Henle and distal tubule. This type of drug is the most efficacious of the diuretic drugs.

   c) Potassium-sparing diuretics: For examples, amiloride, eplerenone, spironolactone and triamterene. These drugs act in the collecting tubule to inhibit Na^+ reabsorption and K^+ excretion.

ii) β-adrenoceptor-blocking agents: β-blockers acts primarily by decreasing cardiac output. They may also decrease sympathetic outflow from the central nervous system and inhibit the release of renin from the kidneys,

therefore decreasing the formation of angiotensin II and the excretion of aldosterone. They are divided into these classes:

a) Drug which acts at both β_1 and β_2 receptors, for example, propranolol.
b) Selective blockers of β_1 receptors such as metoprolol, atenolol and nebivolol.

iii) Angiotensin Converting Enzyme (ACE) inhibitors: lower blood pressure by decreasing peripheral vascular resistance without increasing cardiac output, rate or contractility. These drugs block or decrease the production or action of angiotensin II from angiotensin I via angiotensin-converting enzyme. Angiotensin II is one of the most potent natural vasoconstrictors known. By reducing angiotensin II levels, the secretion of aldosterone, a hormone from the adrenal gland that increases sodium reabsorption in the kidney, is also decreased. The increase in sodium reabsorption causes the body to retain water that raises blood volume and increases blood pressure. The decreasing blood pressure by blocking the effect of angiotensin II is through two mechanisms: dilating arteries and decreasing blood volume.

iv) Angiotensin II-receptor blockers (ARBs): These drugs are alternatives to the ACE inhibitors. For examples, candesartan, eprosartan, irbesartan, losartan, valsartan etc. These drugs act by blocking the AT1 receptors and thus decreasing the activation of AT1 receptors by angiotensin II. Their effects are similar to ACE inhibitors in which they block aldosterone

secretion, hence lowering blood pressure and decreasing salt and water retention.

v) Calcium-channel blockers: Calcium-channel antagonists block the inward movement of calcium by binding to L-type calcium channels in the heart and in smooth muscle of the coronary and peripheral arterial vasculature. This results in relaxation of smooth muscle, dilating arterioles. These blockers are categorized into three chemical classes, each with different pharmacokinetic properties and clinical purpose:

a) Diphenylalkylamines: For example, verapamil. This drug exerted its effects on both cardiac and vascular smooth muscle cells. Hence, heart rate, contractility and blood pressure decrease.
b) Benzothiazepines: For example, diltiazem. The effects is similar like verapamil except it has less pronounced negative inotropic effect on the heart compared to that of verapamil.
c) Dihydropyridines: These include nifedipine, amlodipine, felodipine, isradipine, nicardipine and nisoldipine. These drugs have much higher affinity for vascular calcium channels than for calcium channels in the heart. These drugs functions mainly as arteriolar vasodilator, thus decreasing blood pressure.

vi) α-adrenoceptor-blocking agents: For examples, prazosin, doxazosin and terazosin. These three drugs are selective α_1-blocker. They act by

decreasing peripheral vascular resistance and lower arterial blood pressure via arterial and venous smooth muscle relaxation.

vii) β-adrenoceptor blocking agents: For examples, propranolol, metoprolol, atenolol, nebivolol. These drugs reduce blood pressure mainly by decreasing cardiac output. They might also decrease sympathetic outflow from the central nervous system (CNS) and inhibit the release of renin from the kidneys, hence decreasing the formation of angiotensin II and the secretion of aldosterone.

viii) Centrally acting adrenergic drugs: Some examples of these drugs are clonidine, guanabenz, guanfacine and methyldopa. The mechanism of action involve the stimulation of inhibitory α_2 receptors in the vasomotor center of the medulla oblongata which results in decrease of sympathetic stimulation to the heart, kidneys and blood vessels. For methyldopa, this α_2 agonist forms α-methylnorepinephrine (false transmitter) to diminish adrenergic outflow from the CNS.

ix) Vasodilators: Hydralazine and minoxidil are two examples of vasodilators drugs. Hydralazine causes direct vasodilation which act on arteries and arterioles. Meanwhile minoxidil dilates arterioles but not venules (Harvey et al., 2012; Holland and Adams, 2007).

1.9 The use of plant/herbal medicines in cardiovascular diseases

The use of medicinal plants in treating, preventing or alleviating diseases has been of importance lately. Herbal or medicinal plants have been investigated extensively and it has been found that some of them have therapeutic effect including in cardiovascular diseases. Usually, these are folkloric medicines that have been used for generations. New technology in research has successfully proven their effectiveness and identified the compound(s) which exert its effects. Plants comprise of complex mixtures of metabolites and the products come in different form such as liquid, semi-solid or dry powder for internal or external use. Different methods of plant extractions are employed to obtain the therapeutically desired portions using specific solvent. These include maceration, infusion, hot continuous extraction (Soxhlet), decoction, super-critical fluid extraction, microwave-assisted extraction and hydrodistillation techniques (Tiwari et al., 2011).

One example of medicinal plant that has been proven to have cardiovascular effects is aqueous extract of ginger which was reported to possess hypotensive, vasodilator, cardio suppressant and stimulant effects (Ghayur et al., 2005). Garlic (Allium sativum) also is widely known for its cardiovascular effects. A study by Matsuura, (2001) discovered that the saponins fractions from garlic lowered total plasma and low-density lipoprotein (LDL) cholesterol in a hypercholestrolemic animal model. Moreover, garlic extract has also been shown to relax endothelium dependent and independent pulmonary arteries and to inhibit endothelin-1 induced contraction (Kim-Park and Ku, 2000). The effect of garlic juice on reducing heart rate has also been reported. However at higher dosages, undesirable effect was obtained (Yadav and Verma, 2004). Water extract of garlic containing glutamylpeptides has the ability to inhibit angiotensin-converting enzyme (ACE) in-vitro which indicates that this