EVALUATION OF ANTI-HYPERTENSIVE AND VASORELAXANT EFFECTS OF *PHALERIA MACROCARPA* EXTRACTS

By

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IN THE NAME OF ALLAH

THE MOST BENEFICENT AND MERCIFUL

THIS THESIS IS DEDICATED

TO

MY FATHER ALTAF HUSSAIN (LATE)

WHOSE PERISHING WISH WAS TO

CELEBRATE THESE LUCRATIVE

MOMENTS WITH ME IN FULL BLOOM

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LIST OF ABBREVIATIONS

ACE	Angiotensin converting enzyme
ACEIs	Angiotensin converting enzyme inhibitors
ANP	Atrial natriuretic peptide
AVP	Arginine vasopressin
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
α	Alpha receptors
AT-1	Angiotensinogen-1
ARBs	Angiotensin receptor blockers
ALE	Artichoke leaf extract
AC	Adriamycin cyclophosphamide
APAF	Apoptotic protease activating factor
Akt	Protein kinase B
APCs	Antigen presenting cells
AECUSM	Animal Ethics Committee of Universiti Sains Malaysia
As	Absorbance of test sample
Ac	Absorbance of control sample
Ach	Acetylcholine
ANOVA	Analysis of variance
BP	Blood pressure
BH4	Tetrahydrobioptin

BSEP	Bile salt export pump
Bcl	B-cell lymphoma
DPPH	Dimethyl Sulfoxide
BAX	B-cell lymphoma-2 associated X proteins
BMI	Body mass index
CE	Chloroform extract
CSF	Cerebrospinal fluid
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic guanosine monophosphate
CARD	Caspase activation and recruitment domain
CXCR4	Chemokine receptor type-4
CXCL-12	Chemokine ligand-12
COX	Cyclooxygenase
DOPA	Dihydroxyphenylalanine
DADS	Diallyl disulphide
DATS	Diallyl trisulphide
DIZ	Diameter of inhibitory zone
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDRF	Endothelium derived relaxing factor
EDVC	Endothelium derived vasoconstriction
eNOS	Endothelial Nitric oxide synthase
ET	Endothelin
ERK	Extracellular signal regulated kinase

ELISA	Enzyme linked immunosorbent assay
EGTA	Ethylene glycol tetraacetic acid
FRAP	Ferric reducing antioxidant power
GABA	Gamma amino butyric acid
GAE	Gallic acid equivalent
GCMS	Gas chromatography mass spectrometry
HO-1	Hemoxygenase-1
HMG-CoA	H3-hydroxy-3-methylglutaryl-coenzyme A
HUVEC	Human umbilical vein endothelial cells
HbA1c	Glycated hemoglobin
HPLC	High-performance liquid chromatography
HPR	Horseradish peroxidase
IFN	Interferon
KKS	Kallikrein-kallidin system
KRG	Korean red ginseng
KTRs	Killer triggering receptors
NIBP	Noninvasive blood pressure
OSA	Obstructive sleep apnea
OECD	Organization of Economic Cooperation Development
PAI	Plasminogen activating factor
PCSK-9	Proprotein convertase subtilisin/kexin-type-9
RVLM	Rostral ventrolateral medulla
RAAS	Renin angiotensin aldosterone system

SSLA	Lyophilized salvia salt of lithospermic acid power of injection
SkCa	Calcium sensitive small conductance potassium channels
SREBP-2-TF	Sterol regulatory element binding protein transcription factor
TPA	12-O-tetradecanoylphorbol-13-acetate

PENILAIAN KESAN ANTI-HIPERTENSI DAN VASORELAKSASI EKSTRAK *PHALERIA MACROCARPA*

ABSTRAK

Kajian ini menyiasat kesan anti-hipertensi ekstrak *Phaleria macrocarpa* pada tikus hipertensi spontan (SHR), tikus Wistar Kyoto (WKY) dan kesan ekstrak terhadap vasorelaksasi di dalam cincin aortik terasing dari pada tikus Sprague Dawley menggunakan pendekatan bioaktiviti. Buah kering telah diekstrak secara berperingkat dengan pelarut-pelarut yang berkepolaran berbeza. Kesan anti-hipertensi terhadap beberapa peringkat dos ekstrak telah dinilai dengan menggunakan kaedah tekanan darah sistolik bukan invasif. Ekstrak eter petroleum (PE) dan ekstrak air (WE) menurunkan tekanan darah pada hari ke-14 (15.9 dan 27.7% masing-masing, $p<0.05$), menunjukan kedua-dua ekstrak mempunyai kesan aktiviti anti-hipertensi. WE menurunkan kedua-dua tekanan arteri purata (MAP) dan kadar denyutan jantung (HR) pada tikus WKY sebanyak 16.3 dan 4.3% masing-masing ($p<0.05$). WE menghalang peningkatan aruhan-norepinefrina (NE) terhadap MAP dan HR dalam WKY dengan mengurangkan kesan isoprenalina terhadap tindak balas presor ($p<0.05$), mencadangkan ada kemungkinan terdapatnya kesan antagonis-alfa dan penghalang saluran kalsium. WE merencat peningkatan MAP, HR dan halaju gelombang denyutan (PWV) ($p<0.05$) dengan ketara. SHR yang telah diberi secara oral selama 14 hari dengan WE menurunkan secara signifikan tahap plasma enzim penukar angiotensin ($p<0.05$). Selain itu, ia juga meningkatkan tahap plasma nitrik oksida (NO) dengan meyokong kesannya ke atas PWV secara signifikan ($p<0.05$), memandangkan NO secara langsung

mengurangkan kekakuan arteri yang ditentukan oleh nilai PWV. Dalam kajian *in vitro*, PE dan WE merencat pengecutan aruhan-NE pada cincin aortik tikus sebanyak 40 dan 58% masing-masing (p<0.05). Fraksi aktif WE, WF-4 menunjukkan kesan vasorelaksasi yang besar (42.8%, p<0.05). Sub-fraksi SF-2 dari fraksi WF-4 merencat pengecutan aorta tikus sehingga 44.5% (p<0.05). SF-2 menghasilkan kesan vasorelaksasi pada kedua-dua cincin aortik tanpa endotelium dan dengan kehadiran endotelium. Walau bagaimanapun, inkubasi cincin aortik dengan kehadiran endotelium, L-NAME dan indometasin menyekat kesan vasorelaksasi SF-2 dengan ketara, menunjukkan penglibatan laluan NO dan prostasiklin, masing-masing. Malahan, SF-2 didapati menunjukkan kesan vasorelaksasi terhadap pra-pengecutan-NE dalam cincin aortaik tanpa kehadiran endotelium melalui perencatan pengecutan aruhan kalsium di dalam larutan Kreb bebas kalsium, mencadangkan sekatan influk kalsium melalui reseptor kendalian (ROCC) saluran kalsium (p<0.05). Apabila dilakukan analisis kromatografi cecair berprestasi tinggi (HPLC), SF-2 didapati mengandungi rutin, mangiferin dan asid galik (0.04%, 0.21% dan 0.04% masing-masing). Kajian ini menyokong potensi penggunaan ekstrak *Phaleria macrocarpa* untuk merawat hipertensi dalam perubatan tradisional.

EVALUATION OF ANTI-HYPERTENSIVE AND VASORELAXANT EFFECTS OF *PHALERIA MACROCARPA* EXTRACTS

ABSTRACT

The present study investigated the anti-hypertensive effect of *Phaleria macrocarpa* extracts in spontaneously hypertensive rats (SHR), Wistar Kyoto (WKY) rats and the vasorelaxant effect of the extracts in isolated rat aorta of Sprague Dawley rats using bioactivity guided approach. The dried fruits were extracted sequentially in solvents with different polarity. The anti-hypertensive effect of the graded doses of crude extracts was evaluated in SHR by non-invasive blood pressure method. Petroleum ether (PE) and water extracts (WE) significantly reduced the blood pressure on 14^{th} day of treatment (15.9 and 27.7% respectively, $p<0.05$) suggesting profound anti-hypertensive activity of these two extracts. WE reduced both the mean arterial pressure (MAP) and heart rate (HR) in WKY by 16.3 and 4.3% respectively ($p<0.05$). WE inhibited norepinephrine (NE) induced elevation of MAP and HR in WKY with less effects against the pressor responses to isoprenaline ($p<0.05$) suggesting its possible alpha-antagonistic and calcium channel blocking effect. WE significantly inhibited MAP, HR and pulse wave velocity (PWV) when compared with control group ($p<0.05$). An oral administration of WE to SHR for 14 days significantly inhibited the plasma level of angiotensin converting enzyme ($p<0.05$). Moreover, it elevated plasma nitric oxide (NO) level with respect to control group ($p<0.05$) supporting its effect on PWV as NO directly reduces the stiffness in arteries determined by the values of PWV. In

the *in vitro* study, PE and WE inhibited NE induced contraction in rat aortic rings by 40 and 58% respectively ($p<0.05$). The active WE fraction WF-4 showed considerable vasorelaxant effect (42.8% relaxation, $p<0.05$). The sub-fraction SF-2 of WF-4 inhibited contraction in rat aorta up to 44.5% ($p<0.05$). SF-2 elicited vasorelaxation in aortic rings with both denuded as well intact endothelium. However, incubation of intact aortic rings with L-NAME and indomethacin significantly blocked the vasorelaxant effect of SF-2, suggesting the involvement of NO and prostacyclin pathway respectively. Moreover, SF-2 was found to exhibit its vasorelaxant effect in denuded aortic rings pre-contracted with NE by inhibiting calcium induced contraction in calcium free Kreb's solution suggesting blockage of calcium influx through receptor operated calcium channels (ROCC) ($p<0.05$). When subjected to high performance liquid chromatography (HPLC) analysis, SF-2 was found to contain rutin, mangiferin and gallic acid (0.04%, 0.21% and 0.04% respectively) in SF-2. This study supports the potential of use of *Phaleria macrocarpa* extracts to treat hypertension in traditional medicine.

CHAPTER 1
INTRODUCTION

1 Hypertension

1.1 Introduction to Hypertension

Hypertension is defined as sustained increase in the arterial blood pressure, owing to a greater risk for cardiovascular diseases prevalent in advanced age (Awoke et al., 2012; Brunton et al., 2007; Themistocles, 2007). Although systolic blood pressure disproportionately rises greater in elders as a result of decreased compliance in blood vessels caused by aging and arteriosclerosis, it is equally affecting the health of middle-aged people where it has no apparent cause (Brunton et al., 2007). Hypertension may manifest major risk for coronary artery disease, myocardial infarction, cardiac failure and many other cardiovascular complications (Awoke et al., 2012). It is a serious global health problem enticing the urge for advanced research to ensure decrement of this fatal disease (Themistocles, 2007; Tripathy, 2011). Physiologically normal blood pressure is considered to be within the range of 120/80 mm Hg, the systolic and diastolic blood pressure respectively.

In hypertensive patients the hypertrophy and hyperplasia of arterial and ventricular walls cause stiffness and reduced compliance, thus increasing the resistance to blood flow which precede the onset of high blood pressure and other cardiovascular health hazards (Fukutomi et al., 2010; Michael, 2001). With advanced age, lack of elasticity and decreased compliance in arterial walls increase resistance to blood

flow due to which heart has to pump hard to push blood through small arterioles to maintain continuous and rhythmic contractions. When pressure in the arteries remain high for longer period of time, it establishes a hypertensive state (Tripathy, 2011) common in elderly as well as young. Along with many others, cerebral stroke is the highest risk as an outcome of sustained high blood pressure because of inadequate blood flow in brain. Several factors contributing to the rise in blood pressure include advancing age, life-style prone to develop high blood pressure, chain smoking, metabolic syndrome as obesity, diabetes, renal function abnormalities, various structural changes as increased peripheral vascular resistance or arterial rigidity (Koliaki et al., 2013). Generally life style modifications such as regular exercise, maintenance of body weight, increased water intake, alcohol and salt restriction as well to adapt the healthy dietary patterns (Koliaki et al., 2013) can have a profound effect to lower blood pressure (Michael, 2001).

Hypertension is considered to be the major risk factor for cardiovascular morbidity and mortality (Katzung et al., 2009) including coronary heart disease, ischemia and congestive heart failure etc. (Koliaki et al., 2013). On the basis of blood pressure, there are four classes of blood pressure that are mentioned in Table 1.1 (Craig et al., 2004).

1.2 Types of hypertension and its causes

Hypertension is a common disease with a wide range of causes. It's a leading risk factor for cardiovascular diseases worldwide, with a high morbidity, mortality and considerable drug therapy (Agyemang et al., 2015; Santiago et al., 2014). Hypertension has been divided into different types to seek its basic and underlying effects to achieve proper health level.

Table 1.1 Classification of Blood Pressure (Katzung et al., 2009)

Blood Pressure		**Class**
Systolic Pressure	**Diastolic Pressure**	
120	80	Normal
120-135	80-89	Pre-hypertension
140	90	Hypertension
140-159	90-99	Stage I
160 or above	100	Stage II

1.2.1 Essential/Primary Hypertension

Hypertension that has no specific cause is termed as essential, primary or idiopathic hypertension (Katzung et al., 2009; Ritter et al., 2008) also commonly referred as silent killer. It may go unnoticed for a long period of time or may exhibit its effects in late age by producing cardiovascular disorders (Tripathy, 2011). Cardiac output and peripheral vascular resistance are the primary determinants of essential blood pressure. Increased peripheral resistance is attributed to combination of several

abnormalities as genetic disposition, stress, environmental, dietary factors (increased salt or decreased calcium and potassium intake) (Koliaki et al., 2013), humoral abnormalities as hyperinsulinemia (Kahleová et al., 2002), natriuretic hormone or renin-angiotensin-aldosterone system, increased production of vasoconstrictor substances (angiotensin II and endothelin I) and decreased production of vasodilating substances (prostacyclin, bradykinin, nitric oxide) in vascular endothelium (Wells et al., 2011). Approximately 30% cases of essential hypertension underlie the hereditary abnormalities as mutation of functional abilities of certain genes as angiotensin, angiotensin converting enzyme (ACE), beta-2 adrenoceptor and alpha-adducin. Life-style modifications, proper dietary intake and reduced stress can help to treat these symptoms (Tripathy, 2011).

1.2.2 Secondary Hypertension

The hypertension that occurs because of a specific underlying cause is categorized as secondary hypertension. In fact due to the underlying disorders as kidney failure, tumors, thyroid problems, hyperaldosteronism, iatrogenic or drug-induced, birth defect or sleep apnea (Tripathy, 2011), Cushing's disease (Barna et al., 2012; Tripathy, 2011), contraction of aorta or renal artery etc., sometimes surgical treatments are obligatory (Katzung et al., 2009). Pheochromocytoma is a tumor of adrenal gland tissue that stimulates release of epinephrine and norepinephrine in large amounts. Though low in prevalence (0-5%) it can give disastrous outcomes. Chronic kidney diseases as renal artery stenosis (unilateral/bilateral) in which renal artery gets narrow due to atherosclerosis may disrupt blood flow to target kidney

creating hypertension. Cushing's disease may be an underlying cause of persistent hypertension as it exhibits vasoconstriction through hormonal effects.

1.2.3 Malignant Hypertension

Diastolic blood pressure that exceeds 130 mmHg gives rise to malignant state. Target organs easily affected by malignant hypertension are kidney, heart and eyes (retinopathy) raising the requirement of immediate medical attention (Roach et al., 2006). Major symptoms include numbness of extremities, vision disturbance, anxiety, fatigue and seizures. It can be regarded sometimes as a medical emergency as stroke or suffocation can arise all of a sudden reaching to dangerous level. Blood pressure rises quickly to a higher level sometimes without any previous signs and symptoms creating urgency in the situation.

1.2.4 Isolated Systolic Hypertension

Consistent systolic blood pressure above 160mmHg and diastolic pressure below 90mmHg is considered as isolated systolic hypertension, a major health problem in aging society (Wells et al., 2011). It occurs mostly in elderly people ≥ 60 years of age (Brunton et al., 2007) and due to poor dietary intake. Age-related stiffness in arteries and arteriosclerosis causing loss of elasticity in arteries give rise to isolated systolic hypertension (Craig et al., 2004; Fukutomi et al., 2010). In young subjects with elastic arteries, the arterial pulse pressure travels at a slow speed and returns in the diastole of the cardiac cycle from the peripheral site. In older subjects, elastin fibres are decreased and collagen fibres are increased, therefore the arteriolar walls

are thickened and stiffened with loss of elasticity due to endothelial dysfunction and vascular remodeling, thus the arterial wave travels faster and returns back in the late systole thereby amplifying the systolic peak pressure and attenuating the diastolic pressure consequently presenting the isolated systolic hypertension. Left ventricular hypertrophy may develop due to increased arteriolar load as a result of isolated systolic hypertension leading to cardiac failure. Cardiovascular morbidity and mortality are more related to isolated systolic blood pressure than diastolic one (Duprez, 2012).

1.2.5 White Coat Hypertension

It is also called anxiety-induced hypertension or isolated office hypertension (Fukutomi et al., 2010). This type of hypertension only rises in doctor's clinic and remains normal outside the clinic prevalent in 30-40% of hypertensive subjects (Pierdomenico et al., 2004). It is stress- related occurring in the vicinity of clinics only, accounting to the clinical setting, increasing patient's anxiety (Alper et al., 1999). The difference between office blood pressure and daytime blood pressure is known as white coat effect (WCE). Decreased arterial compliance with increased wall stiffness in advanced age may be a profound risk factor for exaggerated blood pressure in the proximity of a specific environment. Regular follow-ups to the clinic may help to lower the chances to develop this hypertension (Wells et al., 2011).

1.2.6 Resistant Hypertension

Blood pressure systolic or diastolic, obstinately stagnant on 140/90 irrespective of the triple-drug regime is considered resistant (Acelajado et al., 2012). At least four medications are recommended for its treatment (Dudenbostel et al., 2012). Resistant hypertension leads to refractory hypertension which remains uncontrolled even with maximum drug therapy and needs special medical care, though its estimated prevalence opts to be less than 5% of general population with high blood pressure (Alper et al., 1999). It needs ≥4 medication for treatment (Segura et al., 2010). Patients with refractory hypertension are more prone to cardiovascular risk factors than the patients with controlled hypertension (Sim et al., 2013) (Acelajado et al., 2012). Obesity, excessive alcohol intake, excessive dietary salt intake and initially mismanaged treatment of hypertension may give rise to resistant hypertension (Ahmed et al., 2009). Non-compliance to treatment can also be a cause of resistant hypertension. Few underlying causes and symptoms of secondary hypertension left untreated or undiagnosed for a long period of time may result in the onset of stage referred as resistant hypertension. Included in these may be obesity, pheochromocytoma, chronic kidney diseases, primary aldosteronism and few exogenous substances. Gain in weight leading to obesity gives rise to many health complications including insulin resistance and hyperlipidemia. As obesity is correlated to increase in blood pressure therefore it may create a hindrance in treatment of blood pressure prompting to become resistant to therapy and develop resistant hypertension. Stress- free and peaceful sleep is a blessing and an important factor to restore blood pressure to its normal range (Ahmed et al., 2009).

Obstructive sleep apnea (OSA) is a common symptom of patients with refractory hypertension (Dudenbostel et al., 2012). Somehow obesity and OSA are correlated with resistant hypertension. Obese patients have more visceral fat that stimulates aldosterone secretion and increases adrenergic activation thereby disturbing sleep cycle and increasing anxiety, thus contributing to resistant hypertension (Alper et al., 1999). Pheochromocytoma is a tumor of adrenal gland tissue that stimulates release of epinephrine and norepinephrine in large amounts. These are hormones that contribute to increase in blood pressure and heart rate. Though low in prevalence (0-5%) it can give disastrous outcomes. Chronic kidney diseases such as renal artery stenosis (unilateral/bilateral) in which renal artery gets narrow due to atherosclerosis may disrupt blood flow to target kidney creating hypertension and making it resistant to treatment. Primary aldosteronism due to adrenal adenoma or adrenal hyperplasia may be a profound obstruction in treatment of resistant hypertension (Segura et al., 2010). Exogenous substances such as non-steroidal anti-inflammatory drugs (NSAIDS), dietary salt, alcohol consumption, smoking habits, nicotine or caffeine intake may play their role to resist treatment therapy of hypertension. NSAIDS inhibit prostaglandins and thus vasodilation giving rise to vasoconstriction as a secondary effect. Every cup of coffee provides an augmentation of 0.8mmHg in systolic while 0.5mmHg in diastolic blood pressure (Alper et al., 1999).

Patients with resistant hypertension should be encouraged to monitor blood pressure daily and away from the vicinity of clinic to counteract the doubt of presence of

white coat hypertensive effects if present. Discrepancy between home and clinic readings should be assessed to start the antihypertensive therapy (Alper et al., 1999).

1.3 Factors affecting the regulation of blood pressure

Normal pressure to maintain the flow of blood in a rhythmic manner throughout the body is a necessary element of general physiology of our body. Increase in the blood pressure (BP) either in elderly or young ones is a complex mechanism influenced by genetic and environmental factors (Kahleová et al., 2002). Systolic blood pressure is the pressure in heart to push oxygenated blood to whole system and diastolic blood is the pressure under which heart relaxes and fills up the blood under a normal physiological process. Whenever there is a rise or fall in blood pressure due to any underlying reason or some environmental factor or advancing age, the body has inbuilt ability to counteract the effect either increased or decreased blood pressure to bring it back to its normal physiological state. This ability is provided by different systems dominant in body which become active as soon as any alarming signal goes to them indicating changes beyond the normal range of blood pressure. The elasticity, compliance and capacity of vascular components helps to regulate the flow of blood by three main determinants i.e blood volume, cardiac output (CO) and total peripheral resistance (TPR) (Mannfred, 1997, 2003).

Blood volume is determined by the rate at which blood flows in and out of vascular compartment. Cardiac output is influenced by the rate at which blood is pumped from heart to arteries and back to veins, both covering total blood flow. Venous return is determined by TPR. Combined resistance in systemic circulation is directly proportional to the rate at which blood leaves arteries and arterioles.

$$BP = CO \times TPR$$

Where BP stands for blood pressure, CO stands for cardiac output and TPR presents total peripheral resistance (Tripathy, 2011).

Resistance (R) is inversely proportional to one fourth of the radius of lumen of vessel.

$$F = Pa - \frac{Pv}{R}$$

Whereas F stands for "Flow of blood in circulatory system",

Pa stands for "Pressure in arteries"

Pv stands for "Pressure in veins", (Tripathy, 2011)

These all determinants working in a coordinated manner control and maintain physiological homoeostasis, thus conducting the entire perfusion system properly.

Regulation of blood pressure is maintained by two major systems, remote control system and local control system. These systems along with their division of classes and subclasses are shown in Figure 1.1.

1.3.1 Remote Control System

The system by name represents an automatically controlled system which is further divided into two main classes as nervous system and humoral system.

1.3.1.1 Nervous System (NS)

Two divisions of autonomic nervous system that mainly control and regulate all functions and responses of body from time to time are sympathetic nervous system (SNS) and parasympathetic nervous system (PNS) (Brunton et al., 2007). SNS innervate blood vessels primarily while heart is innervated by both SNS and PNS. Nervous system possesses neural control or baroreflex system which regulates the pressure of blood flow in the body.

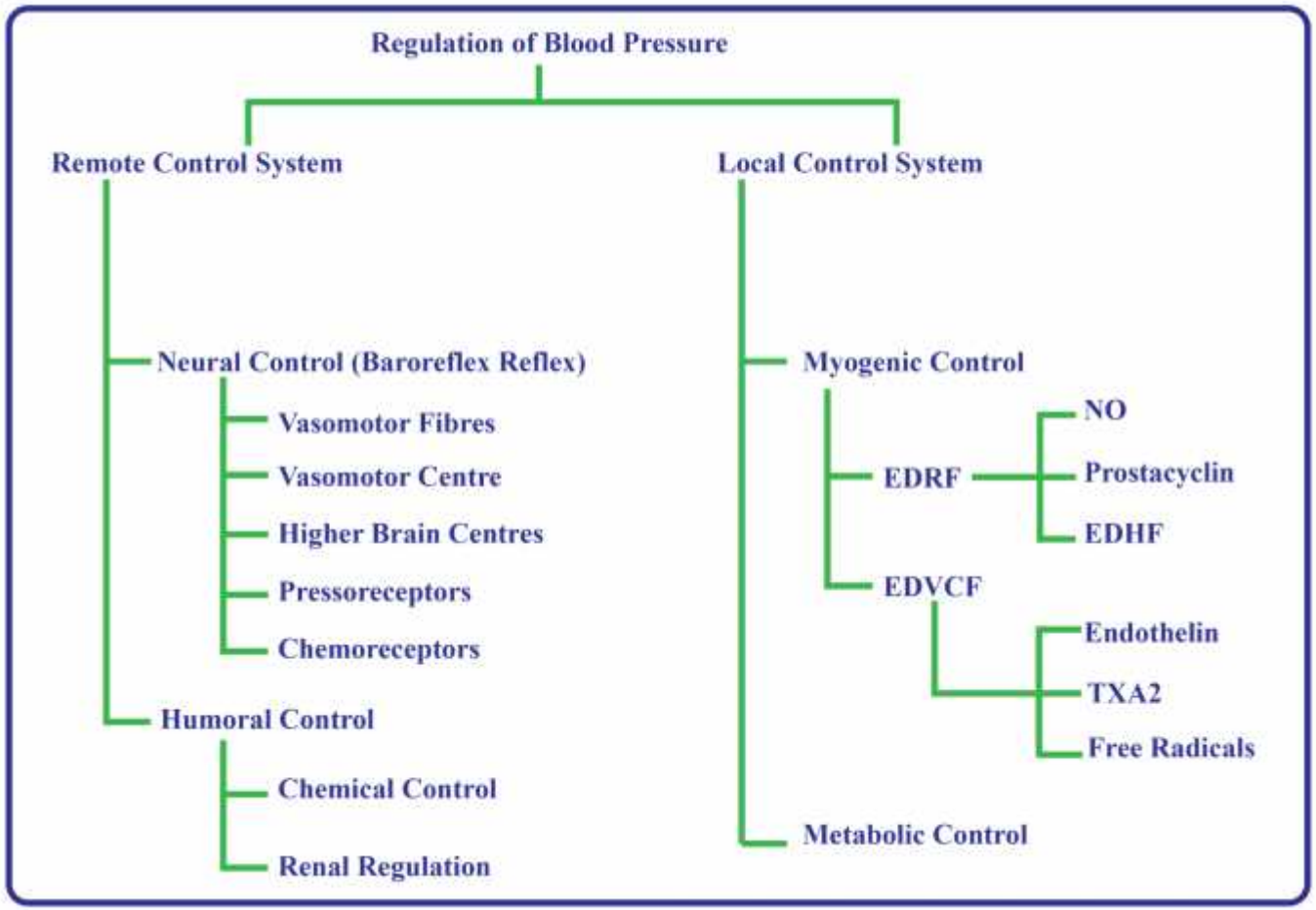

Figure 1.1 Factors regulating the blood pressure. Abbreviations NO: Nitric oxide; EDRF: Endothelium derived relaxing factor; EDVCF: Endothelium derived vasoconstricting factor; EDHF: Endothelium derived hyperpolarizing factor; TXA2: Thromboxane A2.

1.3.1.2 Neural Control (Baroreflex System)

Baroreceptor reflex system has mechanosensitive receptors in aorta and carotid sinus that give rise to afferent nerve fibre which relay impulses to central nervous system (Katzung et al., 2009). A diagrammatic presentation of baroreflex system is shown in Figure 1.2. Signals are being processed in central nervous system and transmitted to heart and vasculature through efferent nerves conducting through sympathetic and para sympathetic fibers. Contractile state of the vessels is being regulated by sympathetic innervation in alpha-receptors of arterioles. Increased sympathetic tone augments resistance in vessels thus enhancing blood pressure causing vasoconstriction and vice versa (Richard et al., 2011). Sinoatrial node (SA) of heart is being innervated by sympathetic (β1) and para sympathetic (vagal) nervous system. Activation of sympathetic nerves in SA-node on one hand causes positive chronotropic effect by increasing discharge rate of SA-pacemaker cells thus enhancing heart rate and on the other hand leads to positive inotropic effect by innervating β1-receptors on cardiac ventricles thus enhancing stroke volume.

Vagal innervation produces exactly opposite effect thus lowering conduction velocity and heart rate. Similarly increase in blood pressure activates baroreceptor activity leading to inhibition of sympathetic impulses resulting in vasorelaxation through effect on blood vessels and decreased contractility and heart rate through effect on heart, while parasympathetic impulses are enhanced inferring decreased heart rate and contractility (Craig et al., 2004; Mannfred, 1997, 2003).

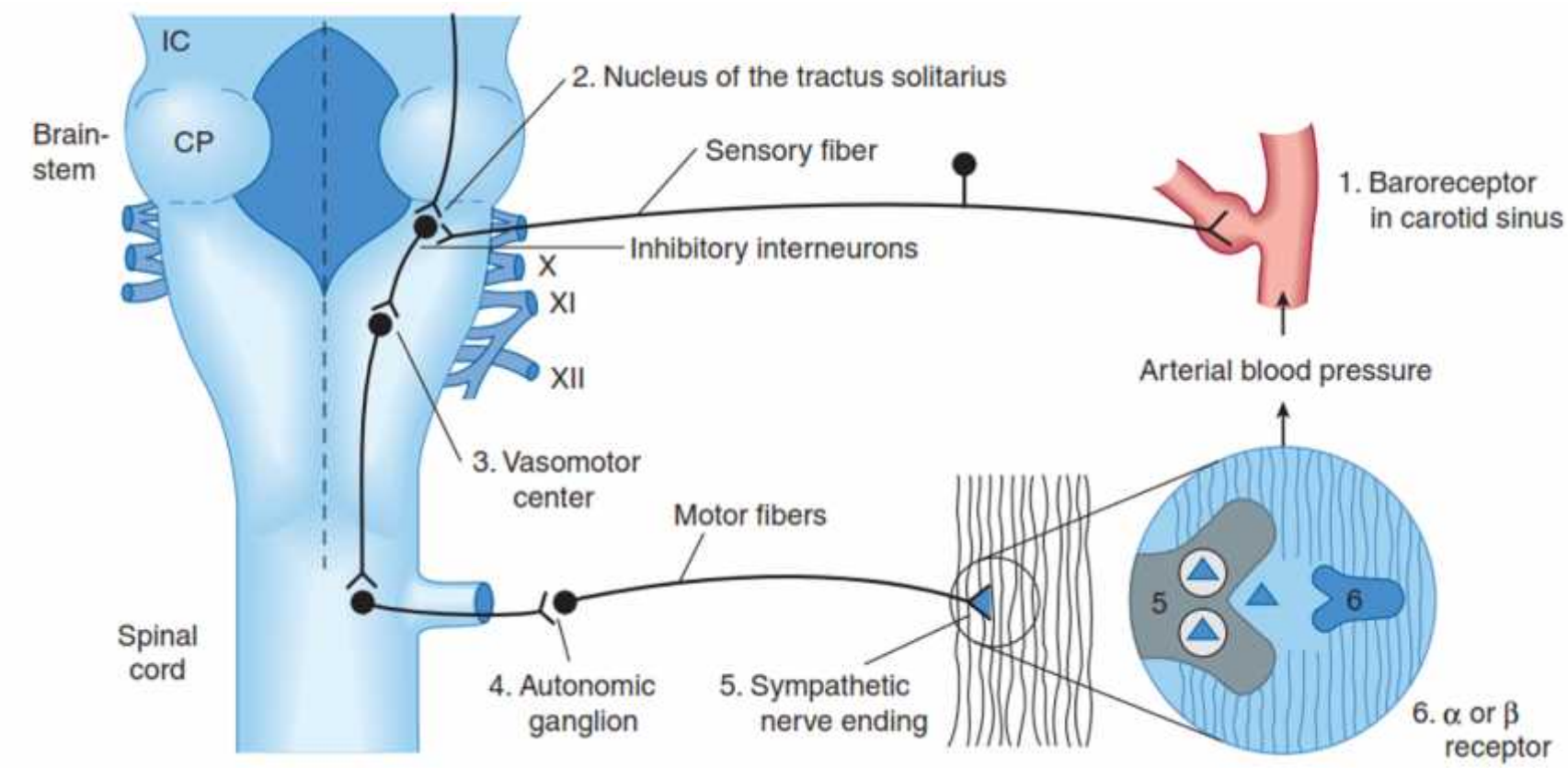

Figure 1.2 Presentation of baroreflex system. Adopted from Basic and clinical pharmacology (Katzung et al., 2009).

1.3.1.2.a Vasomotor Fibres and Vasomotor Centre

Vasomotor center is located in reticular substance of medulla and lower third of pons. It transmits parasympathetic impulses through vagus nerve to heart and sympathetic impulses through spinal cord. It contains several important centers, vasoconstrictor area, vasodilator area and sensory area. The vasoconstrictor area distributes its fibres to all levels of spinal cord to excite vasoconstrictor neurons of sympathetic nervous system. The fibres from the neurons of vasodilator area project upward to vasoconstrictor area and causes vasodilation. The sensory area controls activities of both vasoconstrictor and vasodilator areas, for example baroreceptor reflex controlling blood pressure (Tripathy, 2011).

1.3.1.2.b Pressoreceptors

An acute control and regulation of BP is nicely undertaken by pressoreceptors or baroreceptors present in large arteries of thorax, neck, carotid and aortic sinuses. If arterial pressure rises, the afferent impulses from pressoreceptors decrease heart rate and cardiac contractility by modulating cardiac inhibitory center, turning down the mean arterial pressure (MAP) and activating low pressure receptors (Rau et al., 2001). These low pressure receptors activate rostral ventrolateral medulla (RVLM) or medulla pressor which stimulates SNS to release NE, with ultimate effect of increasing blood pressure to normal physiological level (Tripathy, 2011).

1.3.1.2.c Chemoreceptors

The chemoreceptors play their part in BP regulation during respiration giving response to changes in chemical constituents of blood. They are found in aortic arch and in large arteries of neck. Peripheral chemoreceptors in carotid bodies respond to hypoxia whereas central ones located on medulla and brainstem respond to hypercapnia (Pitsikoulis et al., 2008). If oxygen content of blood is decreased and hydrogen ion level is increased, then it is being sensed immediately by chemoreceptors and impulse is sent to vasomotor center though vasomotor fibres. Ultimately BP is increased by vasoconstriction effect (Tripathy, 2011).

1.3.1.2.d Higher Brain Centers

Higher brain centers include hypothalamus and cortex. Fight or flight situation stimulates higher brain centers to relay information to medullary center. Medullary center contains reflexes to control BP, so it regulates either fall or rise in BP. Any changes in blood pressure detected by stretch receptors in aortic and carotid bodies influence medullary centre to send nerve impulses through sympathetic fibres either to sinoatrial node to enhance cardiac muscle contraction or to vagus nerve to lessen and slow down cardiac muscle contraction. It also helps to increase stroke volume of heart to regulate cardiac output thus blood pressure (O'Donohoe et al., 2013).

1.3.1.3 Humoral System

It controls and regulates blood pressure by modulating chemical stimuli and renal response causing vasodilation, vasoconstriction, release of chemicals and hormones and creating alteration of blood volume.

1.3.1.3.a Chemical Controls

Chemicals such as epinephrine, norepinephrine, atrial natriuretic peptide (ANP) and arginine vasopressin control and regulate BP on short term basis. They act directly on VSMC or vasomotor center to alter BP.

1.3.1.3.b Adrenal Medulla Hormones

Epinephrine and norepinephrine are catecholamines secreted by adrenal medulla in response to stress or excitement. These bind to adrenergic receptors present on target cells such as heart and blood vessels to give positive chronotropic, inotropic effects and vasoconstricting effects respectively (Tripathy, 2011).

1.3.1.3.c The Peptide Hormones

1.3.1.3.c.i Atrial Natriuretic Peptide (ANP)

It is a powerful vasodilator with a short half -life. It is secreted from myocytes of atria when high blood pressure is detected by stretch receptors as a result of increased venous return (O'Donohoe et al., 2013). ANP binds to choroid plexus of epithelial cells and generates cyclic guanosine monophosphate causing alteration in

ion transport thus slowing cerebrospinal fluid production (Johanson et al., 2006). Antagonizing the effect of catecholamines it reduces blood volume by increasing renal sodium and water clearance.

1.3.1.3.c.ii Antidiuretic Hormone (ADH)

Antidiuretic peptide hormone also known as arginine vasopressin (AVP) acts on V_1 receptors in VSMC and V_2 receptors on renal collecting duct. It is released in response to hypovolemic shock. ADH increases water permeability through cAMP-dependent pathway and decreases urine formation. This effect increases blood volume, CO and arterial pressure. ADH causes vasoconstriction via IP_3 –signal transduction pathway and results in increased arterial pressure as well as peripheral vascular resistance (PVR). Its levels in blood increases fivefold in hypovolemic shock and it exerts its pressor effect by stimulating V_2 receptors (Johanson et al., 2006).

1.3.1.3.d Ouabain and Dihydro-ouabain Like Factors

Endogenous ouabain (11-epimer) of plant ouabain and dihydro-ouabain were discovered in 1991. They are synthesized by adrenal cortex and inhibit sodium-pump (Na-K-ATPase) in cell membrane, thereby increasing sodium level and vascular tone (Schoner et al., 2005). During essential hypertension they induce natriuresis to lower down blood volume and ultimately decrease blood pressure (Tripathy, 2011).

1.3.1.3.e Renin Regulation

Kidney is a vital organ of our body which is involved in the maintenance of normal blood pressure through interference in the total blood volume in body fluids. It regulates blood pressure in long term manner by keeping in control the blood volume by two parameters i.e. the renin-angiotensin-aldosterone system and the kallikerin-kallidin system (Katzung et al., 2009).

1.3.1.3.f Renin-angiotensin-aldosterone system (RAAS)

The direct role of RAAS is to increase blood pressure on long term basis (Richard et al., 2011). Renin is a hormone that is released by the kidney into systemic circulation. Angiotensin-I is formed by action of renin on angiotensinogen (an α-2 globulin). Inactive angiotensin-I is converted to active angiotensin-II which is a potent vasoconstrictor and is a major octapeptide hormone of RAAS. It acts on AT_1-receptors of vasculature and also secrete aldosterone from medullary cortex (Ritter et al., 2008). Both of these actions help to increase blood pressure. Indirectly in baroreceptor reflex action, during SNS- activation, angiotensin-II increases sympathetic outflow and increases heart rate and blood pressure. RAAS-pathway blockade serves consequential improvement in cardiovascular health by reduction in BP and restoration of normal endothelial and renal function.

1.3.1.3.g Kallikrein-kallidin System (KKS)

Bradykinin is an important inflammatory mediator involved in the maintenance of normal cardiovascular function. It is formed from high molecular weight kininogen

by plasma kallikerin. Bradykinin induces increased intracellular calcium concentrations that activates intracellular cytosolic phospholipase A2. Combined actions of phospholipase A2, cyclooxygenase and prostaglandin synthases produce prostaglandins such as PGE2 which induces vasodilation and inhibits smooth muscle cell proliferation (Bradbury et al., 2004). Bradykinin augments nitric oxide (NO) and prostacyclin generation to improve endothelial dysfunction. Kallidin is released from kininogens from tissue kallikrein (Tripathy, 2011).

1.3.2 Local Control System

Vascular perfusion pressure is maintained by a regulated flow of blood in tissues or in regional vascular beds. Two most prominent pillars of this system are myogenic control and metabolic control.

1.3.2.1 Myogenic Control

Smooth muscles contribute to the structure of walls of arteries, arterioles, veins and venules. Circulating hormones and mediators released locally from endothelial cells and sympathetic nerve terminals control the contractile state of vessels by regulating the amount of calcium in smooth muscle cells (Rang et al., 2011). Rise in calcium (Ca^{2+}) initiates contraction in smooth muscle cells which either activates myosin light-chain kinase causing myosin phosphorylation or inhibits myosin phosphatase to sensitize myofilaments to Ca^{2+} (Ritter et al., 2008).

Contraction of smooth muscle cells is excited by three mechanisms through agents

a) Inositol triphosphate causes release of intracellular calcium causing contraction.

b) Membrane depolarization opens the voltage-gated calcium channels increasing entry of calcium thus causing contraction.

c) Increasing sensitivity to Ca^{2+} by myosin light-chain kinase.

Relaxation can be caused by these three mechanisms

a) Direct inhibition of voltage-gated calcium channels

b) Indirectly by membrane hyperpolarization

c) Increased intracellular cyclic guanosine monophosphate (cGMP) or cyclic adenosine monophosphate (cAMP) which either opposes agonist-induced increase in Ca^{2+} or inactivates myosin light-chain kinase facilitating Ca^{2+}-efflux thus causing relaxation.

Tissues of arteries and veins self-regulate and accommodate to changes in pressure with which blood enters, its flow pattern and the level of resistance present. Due to such adjustment phases systemic blood pressure and tissue perfusion are maintained and regulated by myogenic control.

1.3.2.1.a Endothelium Derived Vasodilators

A passive barrier between plasma and extracellular fluid lining the innermost layer of blood vessels is the vascular endothelium (Tripathy, 2011) which is also a source of several potent mediators that control the contractile and dilator tone of underlying smooth muscles (Ritter et al., 2008; Spieker et al., 2000). It plays a

crucial role to control hypertension (Spieker et al., 2000). These mediators include the followings:

1.3.2.1.a.i Prostacyclin

Prostacyclins (PGI_2) is a member of eicosanoid family (Ritter et al., 2008). It acts on prostanoid receptors and relax smooth muscles (Tripathy, 2011). PGI_2 is synthesized from COX by arachidonic acid in response to shear stress (Spieker et al., 2000). It increases the level of cGMP by binding with G- protein coupled receptors, through protein kinase A activation it phosphorylates few proteins which help to relax vascular smooth muscle cells by inhibiting myosin light chain kinase (Ritter et al., 2008). PGI_2 also inhibit platelet aggregation by activating adenylyl cyclase and inhibits the increase in intracellular level of calcium in smooth muscle cells. Dynamic balance between endothelial dilators and constrictors is disrupted during cardiovascular disease states. Bioactivity of NO or PGI_2 decreases in endothelial dysfunction and activity of contractile factors increase. PGE_2 is synthesized by microvessels of endothelial cells which either act as direct vasodilators or inhibit norepinephrine (NE) release from sympathetic nerve terminals. PGG_2 or PGH_2 are also known as prostaglandin endoperoxide intermediates. These are contracting factors which are derived from endothelium and act via thromboxane receptors.

1.3.2.1.a.ii Nitric oxide (NO)

Nitric oxide, previously known as endothelium-derived relaxing factor is an endothelium derived vasodilator (Spieker et al., 2000). Nitric oxide synthase (NOS) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydrobioptin (BH_4) synthesizes NO from L-arginine. Of all the three types discovered, the endothelial NOS (eNOS) and neuronal NOS (nNOS) are vascular endothelial types and need calcium for their activation. The third type, inducible NOS (iNOS) is not calcium dependent and inflammation stimuli trigger its formation. NO is synthesized by eNOS present in vascular endothelial cells. It is released from endothelium in the vascular lumen from where it binds to heme moiety of soluble guanylate cyclase (sGC) in adjacent VSMC. This action increases formation of cGMP. cGMP is a phosphate stimulating phosphorylation of several cellular proteins which exhibits vasodilation and relax in VSMC. Endothelial dysfunction is a major problem in essential hypertension due to which NO level in these patients is decreased to a significant amount. It can lower down the blood pressure caused by effects of NA, serotonin, endothelin or angiotensin-II. Its continuous release in vessels causes vascular relaxation thus controlling blood pressure physiologically, (Ritter et al., 2008) as well it inhibits vascular smooth muscle proliferation, platelet aggregation, monocyte adhesion (Tripathy, 2011) and migration thus preventing atherosclerosis and thrombosis.

1.3.2.1.a.iii Endothelium-derived hyperpolarizing factors (EDHFs)

Generally NO and PGI_2 hyperpolarize smooth muscle cells due to their relaxant effects (Spieker et al., 2000). Even if synthesis of NO and PGI_2 is inhibited, endothelium-dependent relaxation persists in some vessels in response to few mediators as acetylcholine (Ach) or bradykinin, this dilatation effect can be attributed to EDHFs. These include epoxyeicosatrienoic acids (EETs-derived from cytochrome P450 enzymes), hydrogen peroxide (H_2O_2), carbon monoxide (CO), C-type natriuretic peptide (CNP), few lipoxygenase (LOX) products and calcium-activated potassium channels (Tripathy, 2011).

1.3.2.1.b Endothelium Derived Vasoconstrictor Factors (EDCFs)

Diseased state of the body imbalances the normal ratio of contractile and dilatory mediators released by endothelium. The production of NO is decreased in a distinguishable amount that pushes the contractile mediators to come in action and give vasoconstricting effect in VSMC and thus serves to augment BP.

1.3.2.1.b.i Endothelin

Endothelin (ET) is abundantly found in most of the vascular tissues. Isomers of endothelin are ET-1, ET-2 and ET-3. Physiologically it is a regulator of blood pressure but during pathological conditions ET constricts blood vessels predominantly. It has two types of receptors named ET_A and ET_B . ET_A-receptors couple with phospholipase-C(PLC), protein kinase-C(PK-C) and causes mitogenesis as well as aldosterone secretion and release of intracellular Ca^{2+}