STUDY OF BLOOD PRESSURE LOWERING EFFECTS OF *LABISIA PUMILA* (BLUME) MERZ VAR. *ALATA* EXTRACTS AND ITS MECHANISM OF ACTIONS IN SPONTANEOUSLY HYPERTENSIVE RATS

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by

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LIST OF SYMBOLS & ABBREVIATIONS

%	percentage
<	less than
α	alpha
β	beta
&	and
AA	arachidonic acid
AC	adenylyl cyclase
ACh	acetylcholine
AngII	angiotensin II
ANOVA	analysis of variance
ANS	autonomic nervous system
ARASC	Animal Research and Service Centre
ATP	adenosine triphosphate
BF-LPWE	n-butanol fraction of *Labisia pumila (Blume) Merz* var. *alata* water extract
BP	blood pressure
b.w.	body per weight
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
CAM	calmodulin
cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
CK	creatine kinase
cm	centimetre
CNS	central nervous system
CO	cardiac output
COX	cyclooxygenase
CVD	cardiovascular disease

DAG	diacylglycerol
DBP	diastolic blood pressure
EAF-LPWE	ethyl acetate fraction of *Labisia pumila (Blume) Merz* var. *alata* water extract
EC_{50}	concentration which gives 50% response of the maximum
EDCF	endothelium-derived contracting factor
EDHF	endothelium-derived hyperpolarising factor
EDNO	endothelium-derived nitric oxide
EDRF	endothelium-derived relaxing factor
EGTA	ethylene glycol-bis (β-aminoethylether)-N,N,N',N'-tetraacetic acid
e.g.	for example
eNOS	endothelium nitric oxide synthase
et al.	et alii, others
g	gram
g/L	gram per litre
GC	guanylyl cyclase
GTP	guanosine triphosphate
Inc.	Incorporation
IP_3	inositol trisphosphate
i.p.	intraperitoneal
i.u.	international unit
iNOS	inducible nitric oxide synthase
i.v.	intravenous
kg	kilogram
KCl	potassium chloride
KH_2PO_4	potassium dihydrogen orthophosphate
K^+	potassium ion
L	litre
L-NAME	N^{ω}-nitro-L-arginine methyl ester hydrochloride

LPCHLOR	*Labisia pumila (Blume) Merz* var. *alata* chloroform extract
LPMEOH	*Labisia pumila (Blume) Merz* var. *alata* methanol extract
LPPET	*Labisia pumila (Blume) Merz* var. *alata* petroleum ether extract
LPWE	*Labisia pumila (Blume) Merz* var. *alata* water extract
M	molar
MAP	mean arterial pressure
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulphate
MLCK	myosin light chain
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
mg	milligram
ml	millilitre
mm	millimetre
mmHg	millilitre mercury
mM	millimolar
mAChRs	muscarinic acetylcholine receptors
n	numbers of animals
nAChRs	nicotinic acetylcholine receptors
NaCl	sodium chloride
$NaHCO_3$	sodium bicarbonate
NE	norepinephrine
NIBP	non-invasive blood pressure
NHMS	The National Health and Morbidity Survey
nNOS	neuronal nitric oxide synthase
NOAEL	no-adverse-effect-level
NOS	nitric oxide synthase

NTS	nucleus tractus solitarius
ODQ	1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one
PE	phenylephrine
PGH_2	prostaglandin H_2
PGI_2	prostacyclin
PIP_2	phosphatidylinositol 4, 5-bisphosphate
PKA	cAMP-dependent protein kinase
PKC	protein kinase C
PKG	cGMP-dependent protein kinase
PLC	phospholipase C
PNS	peripheral nervous system
$P <$	statistically significant if less than
RAAS	renin-angiotensin-aldosterone system
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
R_{max}	maximum relaxation
ROCC	receptor-operated Ca^{2+} channels
SBP	systolic blood pressure
S.E.M.	standard error of mean
sGC	soluble guanylate cyclase
SHR	spontaneously hypertensive rat
SNP	sodium nitroprusside
SOCC	store-operated Ca^{2+} channels
SV	stroke volume
TPR	total peripheral resistance
USM	Universiti Sains Malaysia
µL	microlitre
µM	micromolar
µg	microgram
VGCC	voltage-gated calcium channels
VPR	volume pressure recording

VSMC	vascular smooth muscle cells
vs.	versus
WF-LPWE	water fraction of *Labisia pumila (Blume) Merz* var. *alata* water extract
WHO	World Health Organization

LIST OF APPENDICES

KAJIAN KESAN PENURUNAN TEKANAN DARAH OLEH EKSTRAK *LABISIA PUMILA* (BLUME) MERZ VAR. *ALATA* DAN MEKANISME TINDAKANNYA DI DALAM TIKUS BERHIPERTENSI SPONTAN

ABSTRAK

Hipertensi ialah risiko utama komplikasi kardiovaskular seperti strok, infarksi miokardium, penyakit jantung iskemik dan kegagalan jantung. Ubat-ubatan herba adalah elemen penting untuk rawatan alternatif atau penemuan ubat baru untuk hipertensi. Secara tradisinya, *Labisia pumila* (Blume) Merz var. *alata* digunakan dalam menjaga kesihatan wanita terutamanya dalam keadaan selepas haid dan selepas bersalin. Dalam kajian ini, kesan vasorelaksan ekstrak *Labisia pumila* (Blume) Merz var. *alata* pada tekanan darah tikus berhipertensi spontan (SHR) telah dikaji. *Labisia pumila* (Blume) Merz var. *alata* diekstrak secara berurutan dalam pelarut dengan kekutuban yang berbeza. SHR dirawat secara oral sekali setiap hari selama 28 hari dengan ekstrak eter petroleum *Labisia pumila* (Blume) Merz var. *alata* (LPPET), ekstrak kloroform (LPCHLOR), ekstrak metanol (LPMEOH) dan ekstrak air (LPWE) pada dos 500 mg/kg/hari. Tekanan darah sistolik (SBP) diukur secara tidak invasif dalam SHR sedar dengan kaedah “tail-cuff”. Kesan penurunan tekanan darah oleh *Labisia pumila* (Blume) Merz var. *alata* dan mekanismenya dijalankan dalam SHR yang dibius, SHR yang di"pithed" dan persediaan cincin aorta terasing SHR. Agonis dan antagonis (L-NAME, indometasin, biru metilen, ODQ, atropina, heksametonium, prazosin dan propranolol) digunakan untuk kajian mekanisme. LPWE mengurangkan SBP SHR dengan ketara ($P < 0.05$) setanding dengan verapamil selepas 28 hari rawatan dan dengan itu dipilih untuk difraksinasikan menggunakan n-

butanol dan etil asetat untuk mendapatkan fraksi n-butanol (BF-LPWE), fraksi etil asetat (EA-LPWE) dan fraksi air sisa (WF-LPWE). Fraksi dengan polariti tertinggi (WF-LPWE) menghasilkan kesan penurunan tekanan darah yang paling besar dalam SHR yang telah dibius, SHR yang di "pithed" dan vasorelaksasi di dalam cincin aorta SHR terasing ($P < 0.05$). Kesan penurunan tekanan darah yang disebabkan oleh WF-LPWE berlaku melalui penglibatan reseptor kolinergik muskarinik, oksida nitrik dan laluan siklooksigenase, perencatan reseptor α_1-adrenergik dan penghalangan reseptor ganglion nikotinik dalam persekitaran keseluruhan haiwan yang utuh. Walaupun dalam pendekatan cincin aorta *in vitro*, hanya pembebasan Ca^{2+} intraselular serta pengambilan Ca^{2+} ekstraselular, dan laluan sGC/kitaran GMP telah dikenalpasti memainkan kesan vasorelaksan WF-LPWE yang penting, peranan mereka dalam persekitaran keseluruhan haiwan yang utuh tidak boleh dinilai kerana sifat prosedur yang digunakan. Peranan kawalan barorefleks pusat telah diakui dengan keterlibatan reseptor adrenergik α_1 dalam kesan penurunan tekanan darah WF-LPWE dengan pergantungan pada CNS yang utuh. Tambahan pula, peranan reseptor adrenergik β dalam memperantarakan kesan penurunan tekanan darah WF-LPWE mungkin telah ditindas oleh kawalan barorefleks pusat yang utuh dalam SHR yang dibius. Sebatian aktif dalam WF-LPWE yang ditentu menggunakan RP-HPLC ialah asid galik dan katekin yang boleh menyumbang kepada kesan penurunan tekanan darah WF-LPWE. Kesimpulannya, *Labisia pumila* (Blume) Merz var. *alata* mempamerkan potensi kesan penurunan tekanan darah dalam persekitaran hidup haiwan yang utuh.

STUDY OF BLOOD PRESSURE LOWERING EFFECTS OF *LABISIA PUMILA* (BLUME) MERZ VAR. *ALATA* EXTRACTS AND ITS MECHANISM OF ACTIONS IN SPONTANEOUSLY HYPERTENSIVE RATS

ABSTRACT

Hypertension is major risk for cardiovascular complications such as stroke, myocardial infarction, ischemic heart disease and cardiac failure. Herbal medicines is an important element for possible alternative treatments or novel drug discovery for hypertension. Traditionally, *Labisia pumila (Blume) Merz* var. *alata* was used in maintaining women's health especially in the post-menstrual and post-partum's conditions. In this study, vasorelaxant effects of *Labisia pumila (Blume) Merz* var. *alata* extracts on blood pressure of spontaneously hypertensive rats (SHR) were studied. *Labisia pumila (Blume) Merz* var. *alata* were extracted sequentially in solvents with different polarity. SHR were treated once orally daily for 28 days with *Labisia pumila* (Blume) Merz var. *alata* petroleum ether extract (LPPET), chloroform extract (LPCHLOR), methanol extract (LPMEOH) and water extract (LPWE) at the dose of 500 mg/kg/day. The systolic blood pressure (SBP) was non-invasively measured in conscious SHR by tail-cuff method. The blood pressure lowering effects of *Labisia pumila (Blume) Merz* var. *alata* and its mechanisms were further carried out in anaesthetised-SHR, pithed-SHR and isolated aortic ring of SHR. Agonists and antagonists (L-NAME, indomethacin, methylene blue, ODQ, atropine, hexamethonium, prazosin and propranolol) were used for the mechanism studies. LPWE significantly ($P < 0.05$) reduced SBP of the SHR as comparable as verapamil after 28 days of treatment and was thus selected for fractionation

using n-butanol and ethyl acetate to obtain n-butanol fraction (BF-LPWE), ethyl acetate fraction (EA-LPWE) and the residue water fraction (WF-LPWE). The most polar fraction (WF-LPWE) produced the greatest blood pressure lowering effects in anaesthetised-SHR, pithed-SHR and vasorelaxation in isolated aortic rings of SHR ($P < 0.05$). WF-LPWE-induced blood pressure lowering effects were exerted through involvement of muscarinic cholinergic receptors, nitric oxide and cyclooxygenase pathways, inhibition of α_1-adrenergic receptor and blockage of nicotinic ganglionic receptors in an intact whole animal environment. Although in the *in vitro* aortic ring set up, only intracellular Ca^{2+} release as well as extracellular Ca^{2+} uptake, and sGC/cyclic GMP pathway were established to play important significant vasorelaxant effects of WF-LPWE, their roles in the intact whole animal environment were not able to be evaluated due to the nature of the procedure utilised. The role of central baroreflex control was acknowledged with the involvement of α_1-adrenergic receptor in the blood pressure lowering effects of WF-LPWE with dependency on an intact CNS. Furthermore, the role of β-adrenergic receptor in mediating the blood pressure lowering effects of WF-LPWE could have been suppressed by the intact central baroreflex control in the anaesthetised-SHR. The active compounds in WF-LPWE determined using RP-HPLC were gallic acid and catechin which may contribute to the blood pressure lowering effects of WF-LPWE. In conclusion, *Labisia pumila* (Blume) Merz var. *alata* exhibited a potential of blood pressure lowering effects in an intact whole animal living environment.

CHAPTER 1

INTRODUCTION

1.1 Overview of the usage of herbal plants as traditional medicines

Herbal plants or natural products are used as folk remedies for generations. Medicinal herbs were the primary health care agents for many centuries before the development of modern medicines. Despite growth in synthetic chemistry, natural products are still used as sources for new drugs. It is due to the influence of culture in the use of traditional medicine and also the potential of the plants themselves. There has been an increasing usage of herbal medicines due to the potency of bioactive compounds in these natural products. Traditional medicines are found in almost every country in the world and the demand is increasing. More countries have gradually accepted the contributions that traditional medicines can make to the well-being of individuals and improve their healthcare systems. Governments and consumers are interested in herbal medicines and are now beginning to consider aspects of traditional medicines practices integrated into health service delivery. As such, in 2002, WHO developed strategies and policies to support the development of traditional medicine usage and practices, and the strategies were strengthened in WHO Traditional Medicine Strategy, 2014 – 2023 (WHO, 2013).

1.2 Potential of *Labisia pumila* (Blume) Merz var. *alata* in the management of blood pressure

Labisia pumila, locally called as Kacip Fatimah, from the family of Myrsinaceae, is a popular herb in South East Asian for treating a variety of illnesses (Karimi *et al*.,

2013). *Labisia pumila* has been used since decades ago among Malay women especially in maintaining and improving their well-being afterbirth. The extracts and constituents of *Labisia pumila* have been shown to possess anti-cancer, anti-oxidant, anti-osteoporosis, anti-inflammatory properties (Nadia *et al*., 2012; Fathilah *et al*., 2013). Hypertension postpartum is defined as blood pressure 140/90 mm Hg or greater afterbirth. Severe postpartum hypertension will lead to a maternal heart attack and stroke (Sharma and Kilpatrick, 2017). Vascular dysfunction associated with the complex pathological stage of cardiovascular diseases (CVD) leads to chronic, abnormal elevation of vascular resistance and results in hypertension (Giles *et al*., 2012). An important means of treating CVD relates to the induction of vasorelaxation. Al-Wahabi *et al*. (2007) reported that phytoestrogenic property of *Labisia pumila* could maintain the elastic lamellae architecture of the aorta in ovariectomised rats. Moreover, the protective effect of this plant in modulating post-menopausal cardiovascular risk was comparable to estrogenic replacement therapy. *Labisia pumila* water extract also contains a common polyphenol, gallic acid (GA) which possesses anti-oxidant activity, thus have a great potential in reducing blood pressure. Several strong anti-oxidants including the flavones (Woodman *et al*., 2005) have been found to induce vasorelaxation.

1.3 Research problem statement

Cardiovascular diseases (CVD) become global noncommunicable diseases (NCD) that causes 17.9 million deaths per annum (WHO, 2016). Hypertension contributes to 45% of deaths due to heart disease and 51% of deaths due to stroke (WHO, 2013). In 2015, the overall prevalence of hypertension among Malaysian adults of 18 years old and above was 30.3% as reported by the National Health and Morbidity Survey 2015 (NHMS). There

was a general increasing trend in prevalence with age, reaching a peak of 75.4% among the 70 - 74 years old (NHMS, 2015). Approximately 1.56 billion people (29% of the world's adult population) are predicted to have hypertension by 2025 (Kearney *et al.*, 2005). These unpleasant facts showed that hypertension remained at peril. To lessen the impact of CVD on individuals and society, a comprehensive approach is required to prevent and control the rises of the number of the prevalence of hypertension among Malaysian as well as world's populations.

The use of herbal medicines as an alternative treatment is currently increasing among the world's population. *Labisia pumila* has been widely used for the women's related illnesses in traditional practices and was reported to possess anti-cancer, anti-oxidant, anti-osteoporosis, and anti-inflammatory properties. Al-Wahaibi *et al*. (2007) has reported that phytoestrogenic property of *Labisia pumila* could maintain the elastic lamellae architecture of the aorta in ovariectomised rats. They claimed that protective effect of this plant in modulating post-menopausal cardiovascular risk was comparable to estrogenic replacement therapy. However, scientific reports and evidence on blood pressure lowering effects of *Labisia pumila* (Blume) Merz var. *alata* remain elusive and there is no report so far on its vascular activities. Therefore, this study aimed to investigate the potential of *Labisia pumila* (Blume) Merz var. *alata* blood pressure lowering effects and its vascular mechanisms in spontaneously hypertensive rats (SHR).

1.4 Significance of studies

Traditionally, the decoction of *Labisia pumila* (Blume) Merz var. *alata* is widely used in facilitating childbirth delivery and improving post-partum health. Despite maintaining the health of women reproductive system, Sarawak male also consume this

herbal plant for their stamina. *Labisia pumila* (Blume) Merz var. *alata* was reported to have phytoestrogenic effects, anti-oxidant, anti-ageing, anti-cancer, anti-inflammatory as well as anti-fungal activities. There was no traditional claim on its antihypertensive effects, but study has reported that phytoestrogenic property of *Labisia pumila* (Blume) Merz var. *alata* promotes the elasticity of aorta in ovariectomised rat in modulating post-menopausal cardiovascular risk. Taken together all these claims, the potential of blood pressure lowering effect of *Labisia pumila* (Blume) Merz var. *alata* and its mechanisms at the vascular level was explored. This study investigated the effects of *Labisia pumila* (Blume) Merz var. *alata* extracts on the blood pressure regulation by the peripheral vascular system in SHR, the most common genetic animal models of hypertension. Perhaps, new understanding on blood pressure lowering effect and its mechanisms of *Labisia pumila* (Blume) Merz var. *alata* extracts and its fractions would be established.

1.5 Research objectives

The objectives of this study were:

1. To study the blood pressure lowering effects of *Labisia pumila* (Blume) Merz var. *alata* extracts in spontaneously hypertensive rats using non-invasive tail-cuff blood pressure measurement system.
2. To study the blood pressure lowering effects of *Labisia pumila* (Blume) Merz var. *alata* extracts and fractions using invasive blood pressure measurement *in vivo* in anaesthetised spontaneously hypertensive rats.
3. To elucidate the mechanisms of blood pressure lowering effects of *Labisia pumila* (Blume) Merz var. *alata* fractions in anaesthetised and pithed spontaneously hypertensive rats.
4. To study the mechanisms of vasorelaxation effects of *Labisia pumila* (Blume) Merz var. *alata* extracts and fractions by using isolated aortic ring of spontaneously hypertensive rats.
5. To identify the main bioactive compounds responsible for the blood pressure lowering and vasodilation effects in the most active fraction of *Labisia pumila* (Blume) Merz var. *alata* using RP-HPLC.

1.6 Research hypothesis

This study postulated that *Labisia pumila* (Blume) Merz var. *alata* extracts might potentially have blood pressure lowering effects involving the peripheral vascular mechanisms.

1.7 Summary of study

Figure 1.1 illustrates the summary of the study. Firstly, *Labisia pumila* (Blume) Merz var. *alata* extraction processes were carried out successively with petroleum ether, chloroform, methanol, and water by using maceration extraction method. The *in vivo* and *in vitro* studies were carried out on the *Labisia pumila* (Blume) Merz var. *alata* extracts in order to choose the extract with the most blood pressure lowering activities, which was then further fractionated. The selected *Labisia pumila* (Blume) Merz var. *alata* fraction was subjected to the mechanism studies.

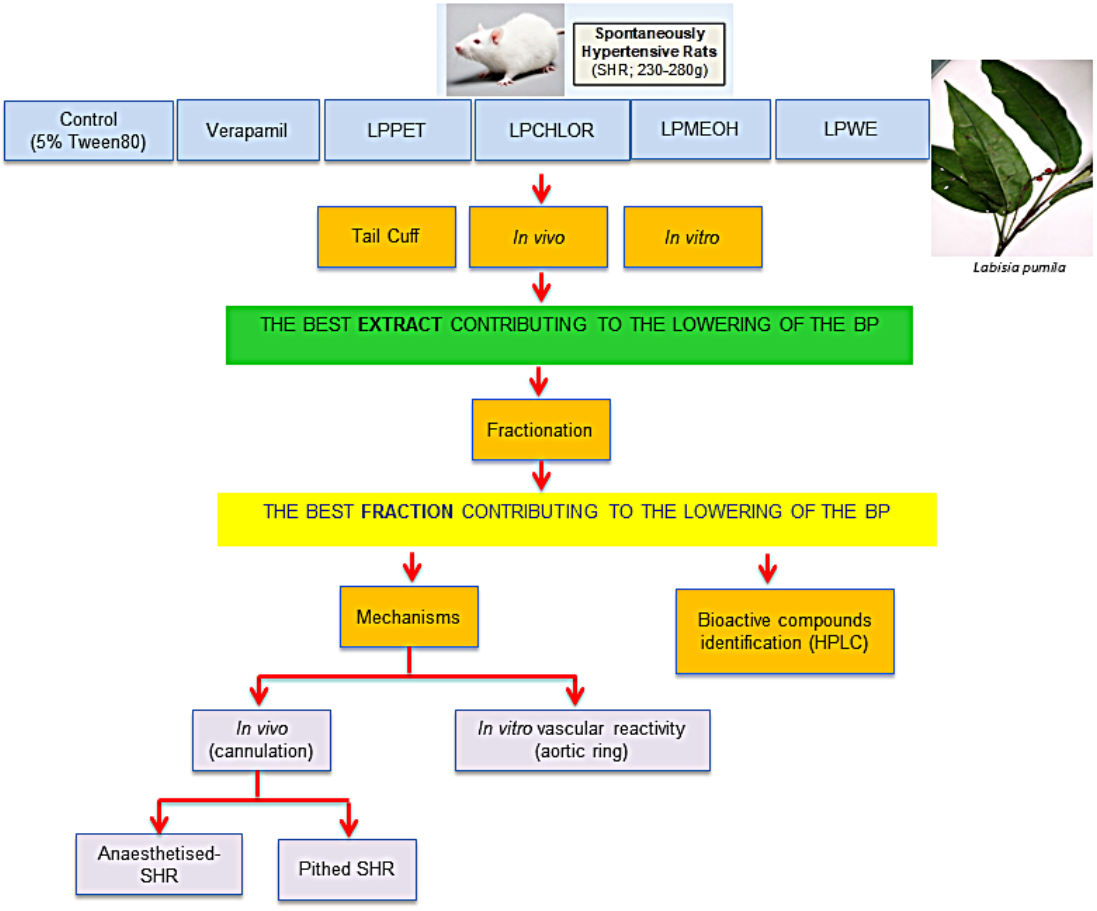

Figure 1.1 Experimental overview of the study.

CHAPTER 2

LITERATURE REVIEW

2.1 Hypertension

Hypertension is diagnosed when the blood vessels have persistently risen in systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg (WHO, 2013). Hypertension leads to major risk for cardiovascular complications such as stroke, myocardial infarction, ischemic heart disease and cardiac failure (Awoke *et al*., 2012). The risk factors for hypertension are ageing, gender, weight, body mass index (BMI), waist circumference, sedentary lifestyle, smoking and alcohol intake (Singh *et al.*, 2011).

2.1.1 Prevalence and risk factors

Cardiovascular diseases (CVD) become a serious global illness that causes 17.9 million deaths per annum, representing 31% of global mortality (WHO, 2016). Hypertension contributes to 45% of deaths due to heart disease and 51% of deaths due to stroke (WHO, 2013). In 2015, 1.13 billion people worldwide were reported to have hypertension with upwards of 1 in 4 men and 1 in 5 women (WHO, 2015). As reported by the National Health and Morbidity Survey in 2015, the overall prevalence of hypertension among Malaysian adults of 18 years old and above was 30.3%. There was a general increasing trend in prevalence with age, reaching a peak of 75.4% among the 70 - 74 years old (NHMS, 2015). Malaysian Community Salt Study (MyCoSS) found that people with older age, BMI, and diabetes were at higher risk to have hypertension (Zaki *et al*., 2021). Genetic factors including family history of hypertension, age over 65 years,

and co-existing diseases such as diabetes or kidney disease (Ranasinghe *et al*., 2015). Ho *et al*. (2020) reported that female, elder persons who were unemployed or retired, and low socioeconomic status with poorer access to care, lack of knowledge and awareness of the consequence of uncontrolled hypertension had a higher prevalence of hypertension in 2006 and 2015. Today's lifestyle has become one of the risk factors for hypertension including excessive salt intake, smoking, alcohol consumption, renal impairment, and the contraceptive pill (Pilakkadavath and Shaffi, 2016).

2.1.2 Blood pressure regulation

Blood pressure (BP) is the pressure inside the blood vessels when the blood passes through them, expressed as systolic/diastolic in millimetres of mercury (mmHg). Systolic blood pressure (SBP) is a peak pressure of the aortic pulse, indicating the amount of pressure that blood exerts when the heart is contracting whereas diastolic blood pressure (DBP) is the pressure when the heart relaxes, the pressure falling toward minimal value. The elasticity, compliance and capacity of vascular components regulate the flow of blood by three main determinants: blood volume, cardiac output (CO) and total peripheral resistance (TPR). Total peripheral resistance (TPR) is defined as the total resistance to blood flow provided by the entire vascular system (Blood *et al*., 2007). The blood pressure measuring is affected if the flow of blood is interrupted by any resistance due to the changes of the diameter or length of the blood vessels or changes in the blood viscosity. The following formula of blood pressure describes how it works:

$$BP = CO \times TPR$$

Thus, the blood pressure is regulated by both factors, CO and TPR. When the TPR or CO increases, blood pressure will go up, and *vice versa*. In the normal physiology stage, homeostasis, such as baroreceptor reflex, maintains the stability of blood pressure regulation.

Mean arterial pressure (MAP) is clinically important to assess vascular function. MAP becomes the most important in order to describe the blood pressure. MAP is the pressure that drives blood into the tissues, averaged over the entire cardiac cycle and the mean pressures. As such, the MAP of the large arteries is identical (Widmaier *et al*., 2006). MAP is calculated as the following formula:

$$MAP = DBP + 1/3\ (SBP - DBP)$$

Irregular blood pressure regulation leads to pathophysiological stage of hypertension.

2.1.3 Pathophysiology of hypertension

Several factors involved in the regulation of normal blood pressure include the sympathetic nervous system, renin-angiotensin-aldosterone system, endothelial functions, genetics, as well as sodium and water intake. As such, the disorders of these factors lead to the development of hypertension (Carretero & Oparil, 2000). Cardiac output and peripheral resistance are two components in the regulation of blood pressure. Sympathetic dysfunction triggers the increase in cardiac output. Studies have demonstrated that sympathetic over activity is a core component in the pathophysiology of hypertension (Mark, 1996). Renin-angiotensin-aldosterone system (RAAS) also triggers the release of aldosterone from the adrenal glands that increases salt reabsorption coupled with water retention resulting in further increase of blood pressure (Silva *et al*., 2012). The major

underlying mechanism for endothelial dysfunction seen in hypertension is the decrease in the availability of nitric oxide (NO), a consequence of increased oxidative stress in hypertensive patients (Sander *et al*., 1999).

2.1.4 Management of hypertension

The management of hypertension includes both pharmacological and non-pharmacological interventions. Body weight control, sodium intake control, avoidance of alcohol consumption, regular physical exercise, healthy foods, cessation of smoking and relaxation therapies are the non-pharmacological management scheduled for hypertensive patients. The antihypertensive drugs such as α-blockers, β-blockers, diuretics, angiotensin-converting enzymes to inhibitors, angiotensin II-receptor blockers and calcium channel blockers, are the most commonly used drugs to treat hypertension (Table 2.1) (Chobanian *et al*., 2003; Weber *et al*., 2014).

Table 2.1 List of antihypertensive drugs commonly used in the management of hypertension.

Drug	Mechanism of action	Side effects	References
Diuretics (thiazides, loop diuretics, potassium-sparing diuretics)	Diuretics promote urine production and regulate hypertension by inhibiting Na^+ ions reabsorption from distal convoluted tubules in the kidney. Thiazides also enhance the reabsorption of Ca^{2+}. Blood flow reduces due to the loss of H_2O and sodium in the urine. A decrease in CO decreases blood pressure.	Hypovolemia, hyponatremia, hypokalaemia, hyperkalaemia, hyperuricemia, metabolic alkalosis, metabolic acidosis, and hyperuricemia.	(Guignard, 2021)
Beta-blockers (metoprolol, bisoprolol)	In the kidney, beta-blockers reduce renin secretion by blocking β_1-receptors and therefore reduce renin-angiotensin-aldosterone activity. In the heart, blockage of $beta_1$-receptors in the sino-atrial node reduces the stroke volume and heart rate (negative chronotropic effect), which reduces the CO, and blockage of $beta_1$- receptors in the myocardium decreases cardiac contractility (negative inotropic effect).	Asthma, heart failure, hypoglycaemia, bradycardia, heart block, intermittent claudication, and Raynaud's phenomenon.	(Frishman, 1988; Gorre and Vandekerckhove, 2010)

Calcium channel blockers (CCB) (verapamil, diltiazem)	A decrease in contraction, known as a negative inotropic effect, occurs when it acts on the heart (cardiac muscles), resulting in a negative chronotropic effect or a slower heart rate. Consequently, CO decreases blood pressure, and vasodilation occurs. The CCB interferes with the flow of Ca^{2+} via the Ca^{2+} pathway. They reduce the heart's contraction by operating on the vascular smooth muscle. The arterial diameter increases, and the TPR decreases as the blood vessel dilates, allowing blood pressure to decline.	Dizziness, fatigue, headaches, flushing sensation, palpitation, peripheral oedema, and fatigue.	(Russel, 1988; Scholz, 1997)
Angiotensin receptor blocker (ARB) (losartan, irbesartan, valsartan)	ARB blocks angiotensin II receptors type I, directly induces vasodilation, decreases aldosterone production, and decreases Na^{+} reabsorption. Together, they can improve blood pressure by lowering CO. ARB has very similar effects to ACE inhibitors. ACE inhibitors prevented the development of angiotensin II, while ARB blocks AT_1 receptors in blood vessels. Losartan reduces blood pressure in both essential and experimental hypertension. Losartan, which selectively inhibits the angiotensin II-mediated vascular pressor effects on AT_1, was later introduced into oral antihypertensive treatment.	Dizziness, light-headedness, swelling of the lips, tongue, or face. Some will experience troubled breathing.	(Smith *et al*., 1992; Timmermans *et al*., 1992; Timmermans *et al*., 1993; Cachofeiro *et al*., 1995; Navar *et al*., 1996; Terra, 2003; Kobori *et al*., 2007)

α_1-blockers (doxazosin, prazosin)	α_1-blockers suppress α_1-adrenergic receptors in the arteries, smooth muscles, and the central nervous system tissues. Generally, it induces relaxation of vascular smooth muscle tone (vasodilation) by reducing the peripheral vascular and blood pressure resistance.	Dizziness, fatigue, headache, nausea, and lethargy are the most frequently reported. Other than that, patients will experience light-headedness, nasal congestion, headache, reflex tachycardia, and orthostatic hypotension.	(Carruthers, 1994; Chung *et al*., 1999)
Angiotensin-converting enzyme (ACE) inhibitors (captopril, ramipril, enalapril, perindopril, lisinopril)	ACE inhibitor controls blood pressure by regulating the volume of fluids in the body. It decreases blood vessel tension and blood volume, thereby reducing blood pressure. The ACE inhibitor also decreases activity of the renin-angiotensin-aldosterone pathway. The amount of CO and stroke volume decreases, and resistance in the blood vessels of the kidney decreases, which contributes to an increase in natriuresis (excretion of sodium in the urine).	Common adverse drug reactions include cough, skin rash, hyperkalaemia, hypotension, headache, dizziness, fatigue, nausea, and renal impairment.	(Warren and O'connor, 1980; Rossi, 2003; Lubanski and McCullough, 2009)

Direct vasodilators (sodium nitroprusside, organic nitrates, hydralazine)	Vasodilators operate directly on the smooth muscles of the arteries thus the blood flows through them freely. Furchgott's discovery of NO in 1983 contributed to the idea of a "nitric oxide donor," indicating vasodilation induced by other antihypertensive drugs through a different mechanism.	Many other adverse effects of hydralazine, such as drug fever, skin eruptions, and gastrointestinal disturbances. Produce a combination of tachycardia, reactive renin response, and sodium retention.	(Koch-Weser, 1974; Furchgott, 1983; Armario *et al*., 1994)

2.2 Nervous system

The nervous system controls and coordinates all the body's functions. The nervous system consists of two main parts: central nervous system (CNS) and peripheral nervous system (PNS).

2.2.1 Central nervous system

CNS comprises the brain and spinal cord. CNS controls all parts of the human body. Located in the forebrain, all voluntary actions in the human body are controlled by the motor cortex in the frontal lobe of the cerebrum. Hindbrain (medulla oblongata, pons, and cerebellum) and midbrain control involuntary actions like breathing, salivation, and digestion. Medulla oblongata is located in the brainstem. It controls the involuntary functions such as respiration, cardiac function, blood pressure and reflexes. Blood pressure is particularly regulated by regions in the medulla called nucleus tractus solitarius (NTS), rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM) (Colombari *el al*., 2001).

2.2.2 Peripheral nervous system

The PNS branches out nerves from the CNS, connecting the CNS and other parts of the body. The PNS is divided into somatic and autonomic nervous systems (ANS). The afferent nerves (sensory neurons) bring the stimuli from the peripheral receptors to the CNS, whereas the efferent nerves (motor neurons) carry impulses from CNS to the effectors. The somatic nervous system is a "voluntary" nervous system which controls the skeletal muscles. The ANS is an "automatic" nervous system which is involuntary

controlled. For example, the heart rate is “automatically” increases during excitement. That is how ANS controls body's response to increase blood supply in excited condition. The ANS is important in regulating involuntary physiologic processes including heart rate, blood pressure, respiration, digestion, and sexual arousal. The ANS is divided into two main divisions (sympathetic and parasympathetic) and one extensive enteric nervous system (ENS) branches of the nervous system. ENS functions are independent of the remainder of the nervous system (Karemaker, 2017). The sympathetic nervous system (SNS) governs “fight-or-flight” response where body needs oxygen-rich blood during intense demand such as physical activities, dangerous or stressful situations (McCorry *et al*., 2007). For example, increase the heart rate to increase blood supply and improve the delivery of oxygen to other parts of body during physical activities (Alshak & Das, 2023). This reaction primarily achieved by regulating blood vessels tone. An increase in sympathetic signals leads to vasoconstriction (Waxenbaum *et al*., 2021). “Para-” means adjacent to, thus, “parasympathetic” was named. It refers to the location of the ganglia near the wall of target tissue where the presynaptic parasympathetic fibre synapses (Karemaker, 2017). The parasympathetic nerve fibres exit the brainstem via cranial nerves (CN) III, VII and IX and X, as well as sacrum (S) 2-4 nerve roots. 75% of the PNS are the vagal nerves (CN X). The vagus nerve promotes the “rest and digest” processes that occur when the body is at rest, including salivation, tears, urination, digestion, sexual arousal and defecation. The vagus nerve stimulates cardiac relaxation in several aspects of function. Primarily, it reduces conduction speed through the atrioventricular node. The PNS relies solely on ACh to be well-functioned. ACh is a primary neurotransmitter released by a postganglionic neuron in the PNS (Waxenbaum *et al*., 2021). It causes an action potential or activates a secondary messenger system. Acetylcholine released from

parasympathetic nerve terminals will activate ganglionic nicotinic receptors, hence, activates muscarinic receptors at the cellular level (Olshansky *et al*., 2008) (Figure 2.1).

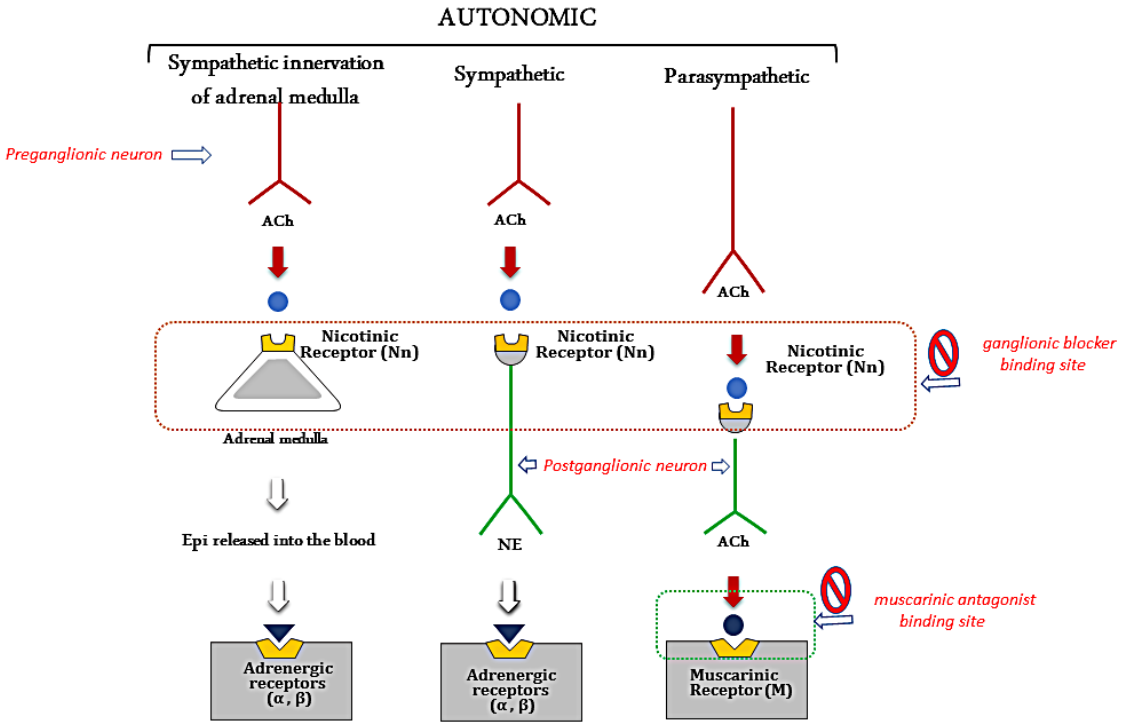

Figure 2.1 Autonomic nervous system (modified from Richard, 2011). ACh = acetylcholine, Epi = Epinephrine, NE = Norepinephrine, α = alpha adrenergic, β = beta adrenergic.

2.2.3 Central baroreceptor reflex

The central baroreceptor reflex is a short-term regulator of blood pressure homeostatic mechanisms (Fernandez *et al*., 2015). The baroreflex provides a rapid negative feedback loop in which increased blood pressure causes a decrease in heart rate (Wehrwein and Joyner, 2013).

Baroreceptors refer to the pressure receptors, responding to blood pressure changes and so called “stretch-sensitive mechanoreceptors”. Located in carotid sinuses

and aortic arch, baroreceptor forms a negative feedback (mechanism of homeostasis) to restore blood pressure to normal stage. It escalates the afferent nerve fibres which transmit impulses to CNS (Katzung *et al.,* 2009). Baroreceptors are stimulated when the contractile forces strengthen, thus increasing the heart rate and peripheral vascular resistance (PVR) and CO. The baroreceptors will reset at higher levels which lead to the hypertension condition if the spiking of BP persists *(*Kougias *et al*., 2010).

Both parasympathetic (vagal) and sympathetic (β_1) nerves are responsible in the changes of BP by baroreflex, and both have opposing effects on the BP. Activation of sympathetic nerves in SA-node increases the TPR and CO by increasing heart rate and stroke volume, thus increasing BP. Baroreceptor action potentials are conveyed to the NTS. Increased activation of the solitary nucleus inhibits the vasomotor centre and stimulates the vagal nerves. The sensory information of the carotid sinus and the aortic arch are conveyed from baroreceptors to the medulla via vagal nerve (Lovic *et al*., 2014). Parasympathetic activation produces the opposite effect. It decreases the heart rate and CO, resulting in vasodilation. Inhibition of sympathetic innervation causes a drop in peripheral resistance while parasympathetic reduces heart rate and contractility. Both activities decrease blood pressure. Vice versa, sympathetic activation with parasympathetic inhibition increases blood pressure (Figure 2.2).

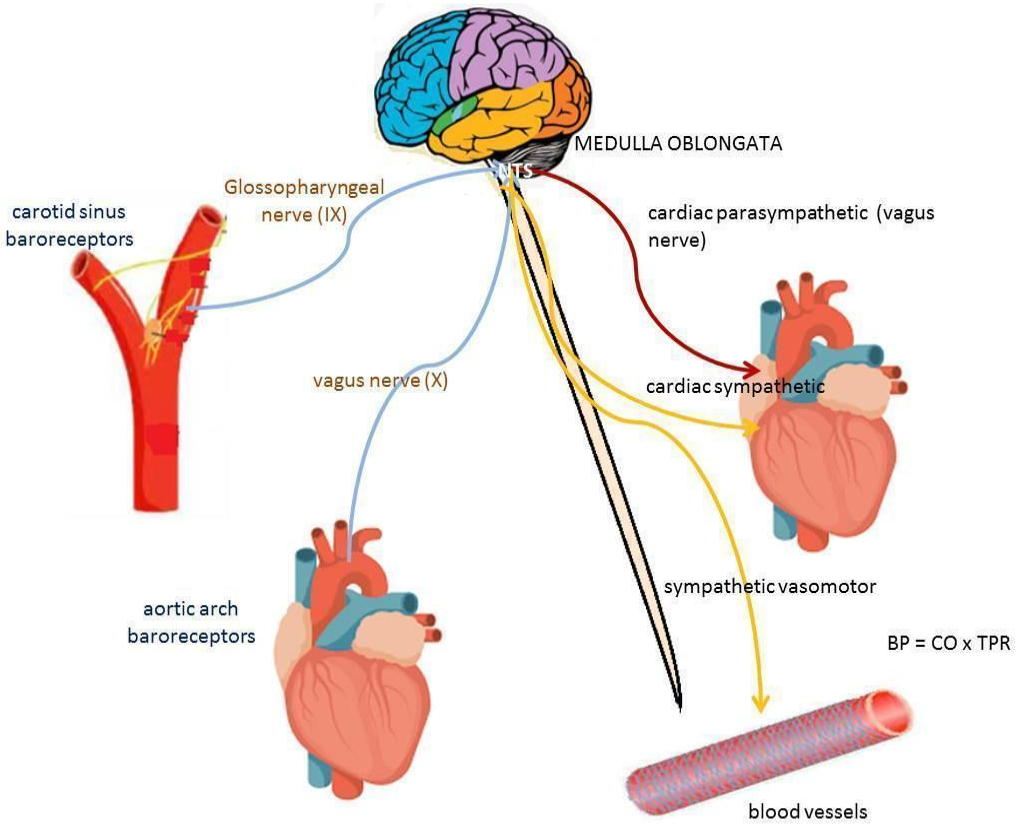

Figure 2.2 Baroreceptor reflex mechanisms (modified from Rovere and Christensen, 2015). BP; blood pressure, CO; cardiac output, TPR; total peripheral resistance.

2.3 Cardiovascular System

Cardiovascular system consists of the pulmonary circulation and systemic circulation. Heart is an organ which drives the blood through the entire organs of the body. The heart receives blood from venous blood vessels (venous return) to the right atrium via superior and inferior vena cava. Blood from the right atrium flows to the right ventricle then ejects into the pulmonary artery during ventricular contraction. Blood passes through the pulmonary circulation within the lungs for the exchange of the gases. The blood leaving the lungs enters the left atrium via the pulmonary veins and flows to the left

ventricle passively. During left ventricular contraction at high pressure (100 - 140 mmHg), blood is ejected into the systemic arterial system via aorta to the entire organs of the body.

In systemic circulation, the large artery exits the left side of the heart via the aorta. The large vein reaches the right side through the superior vena cava and inferior vena cava. Microcirculation involves blood flow in the smallest blood vessels like arterioles, capillaries, and venules (Brownley *et al*., 2000).

2.3.1 Vascular system

Vascular system transports blood to and away from organs and the exchange of gases, nutrients and fluid between blood and tissue. Blood vessels carry blood through the body. Blood vessels regulate arterial and capillary blood pressure, conduct the blood flow, and distribute blood volume throughout the body during constriction and dilation process. Activation of vascular smooth muscle within the vascular wall causes changes in vascular diameters. Factors that activate vascular smooth muscle are autonomic nerves, metabolites and biochemical signals, and vasoactive substances released by endothelial cells lining the blood vessels (Tortora and Derrickson, 2006).

The primary resistance vessels are small arteries and arterioles. Exchange occurs in capillaries and venules. Veins and venules serve as capacitance and vena cava as collection. In a normal adult, the highest mean blood pressure is in the aorta and the pressure drops as blood flows away from the heart. There is a large fall (approximately 50 - 70%) in mean arterial blood pressure when the blood flows through small arteries and arterioles.

This is because these vessels have a high resistance of relative to their flow, as applied to the equation:

$$\Delta P = F \times R$$

Where ΔP = drop in pressure
F = flow
R = resistance

The capillaries pressure is relatively low in order to avoid fluid leak through the thin walls of the capillaries. The pressure continues to drop when the blood reaches the venules, vein and very close to zero within the thoracic vena cava (Richard, 2011).

2.3.2 Vascular endothelium

Vascular endothelium is a thin layer of cells that line all blood vessels. Endothelium is important in the regulation of the vascular tone and structure (Versari *et al*., 2009). Endothelial cells (EC) synthesise nitric oxide (NO) and prostacyclin (PGI_2) which play a vital role in regulating the smooth muscle function (Richard, 2011). The presence of EC is important for vasorelaxation by acetylcholine (ACh) (Furchgott and Zawadzki, 1980). EC's function is attenuated in the pathological stage of cardiovascular diseases and is regained once pathological stimuli are removed (Alexander *et al*., 2021). In hypertension, vascular remodelling alters the vascular structures which include thickening of vessel wall, narrowing lumen diameter and increasing the ratio of vessel wall to lumen (Intengan and Schiffrin, 2001).

2.3.3 Endothelium-derived vasodilators

The innermost layer of blood vessels is the vascular endothelium. It is a source of several potent mediators that control the contractile and dilator tone of the underlying smooth muscles which play an important role in blood pressure regulation (Alexander *et al*., 2021).

2.3.3(a) Endothelium-derived relaxing factors

2.3.3(a)(i) Nitric oxide and cGMP pathways (NO/cGMP pathways

In the 1980s, Furchgott and Zawadski discovered nitric oxide (NO) as an endothelium-derived relaxing factor (EDRF). NO is synthesised from amino acid L-arginine by enzyme NO synthase (NOS). There are three isoforms of NOS: eNOS, iNOS and nNOS (Moncada *et al*., 1997). eNOS is a calcium-dependent, releases NO in response to receptor-stimulated NO formation such as acetylcholine, bradykinin, and substance-P (Richard, 2011). nNOS, which is present in the brain, spinal cord, and peripheral nervous system, responsible for the calcium-dependent release from neurons. The inducible NO (iNOS) is calcium-independent, expressed in response to inflammatory factors such as lipopolysaccharide and cytokines (Nathan and Xie, 1994). iNOS plays an important role in the immune system and wound healing.

NO is important in the regulation of blood pressure. NO physiologically governs the blood pressure, released in a vessel which causes endothelium-dependent vasorelaxation (Ignarro *et al*., 1987; Palmer *et al*., 1987). NO is synthesised by endothelial cells, then rapidly diffuses into the vascular smooth muscle cells (VSMC) and binds to guanylyl cyclase (GC). Activation of GC catalyses the dephosphorylation of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), a second messenger.

cGMP is a phosphate-stimulating phosphorylation of several cellular proteins that leads to smooth muscle relaxation (Ritter *et al*., 2008).

2.3.3(a)(ii) Cyclooxygenase (COX) pathway

Prostacyclin (PGI_2) is a member of the eicosanoid, metabolised from endogenous arachidonic acid (AA) via cyclooxygenase (COX) pathway in the endothelial cells (Ritter *et al*., 2008). PGI_2 serves as a vasodilator and inhibits platelet aggregation in the cardiovascular system (Smyth *et al.,* 2009; Stitham *et al.*, 2011). PGI_2 inhibits VSMC proliferation and de-differentiation (Fetalvero *et al.*, 2006 & 2007). COX converts AA to prostaglandin H_2 (PGH_2) and produces PGI_2 by enzyme prostacyclin synthase. PGI_2 involves G-protein coupled receptors, mediated by the PGI_2 receptor (IP) (Funk *et al*., 1993). PGI_2 binds to the IP and triggers the activation of the G-protein thus signals adenylyl cyclase (AC) to produce cAMP, which activates protein kinase A (PKA). PKA continues phosphorylating and inhibiting myosin light-chain kinase (MLCK), which leads to smooth muscle relaxation and vasodilation (Figure 2.3). Studies also have reported that PGH_2 and PGI_2 excite baroreceptor neurons, as well as cardiac vagal afferents (Schultz, 2001).

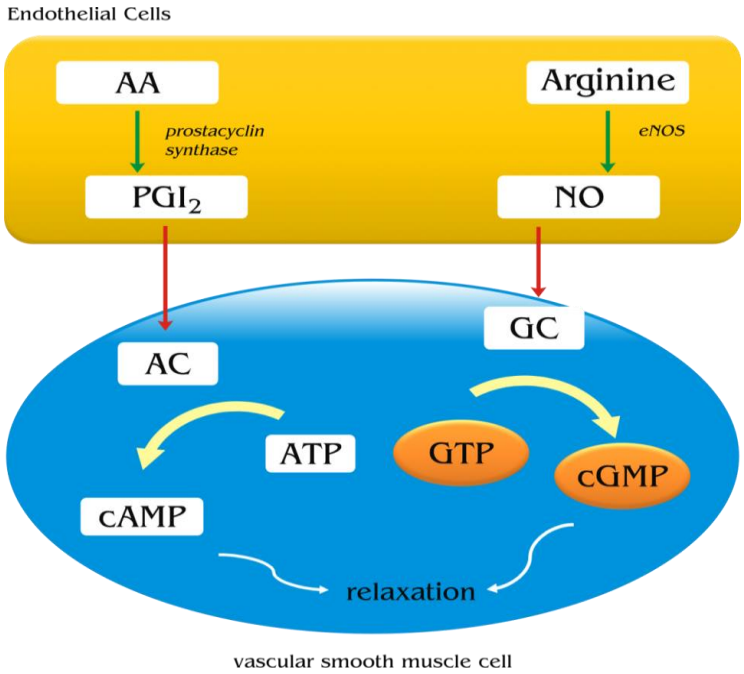

Figure 2.3 Vascular smooth muscle relaxation (modified from Vanhoutte, 1998; Sanders *et al*., 2000). AA; arachidonic acid, PGI_2; prostacyclin, NO; nitric oxide, AC; adenylyl cyclase, GC; guanylyl cyclase, ATP; cAMP; cyclic adenosine monophosphate, GTP; guanosine triphosphate, cGMP; cyclic guanosine monophosphate.

2.3.3(b) Endothelium-derived hyperpolarising factors (EDHF)

Despite NO and PGI_2, the presence of EDHF to endothelium-dependent relaxation is currently appreciated. Study reported that when NO and PGI_2 are inhibited, there is still another factor causing the vessels to dilate and was associated with hyperpolarisation of the vascular smooth muscle and the entity was called "endothelium-derived hyperpolarising factor" (Luksha *et al*., 2009; Garland *et al*., 2011). Chen *et al*. (1988) clearly showed that endothelium-dependent hyperpolarisation was distinct from the action of EDRF (Parkington *et al*., 2004; Garland *et al*., 2011).