ELUCIDATION OF THE VASORELAXATION MECHANISMS INDUCED BY *Syzygium polyanthum* LEAVES AQUEOUS EXTRACT

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by

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

%	Percentage
α	Alpha
δ	Delta
σ	Sigma
≤	Less than or equal to
<	Less than
°C	Degree Celsius
hr	Hour
g	Gram
g/mL	Gram per millilitre
M	Molar
mg/kg	Milligram per kilogram
mg/mL	Milligram per millilitre
min	Minute
mm	Millimetre
mmHg	Millimetres of mercury
mM	millimolar
mL	Millilitre
nM	Nanomolar
V	Volt/voltage
µg/mL	Microgram per millimetre
µL	Microliter
µM	Micromolar
ACC	American College of Cardiology
ACE	Angiotensin-converting enzyme

ACEIs	Angiotensin-converting enzyme inhibitors
Ach	Acetylcholine
AESP	Aqueous extract of *Syzygium polyanthum* leaves
AHA	American Heart Association
Ang I	Angiotensin I
Ang II	Angiotensin II
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ARASC	Animal Research and Service Centre
ARBs	Angiotensin II receptor blockers
BBs	Beta blockers
Ca^{2+}	Calcium
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
CCBs	Calcium channel blockers
CVDs	Cardiovascular diseases
cGMP	Cyclic guanosine monophosphate
CKD	Chronic kidney disease
CO_2	Carbon dioxide
COX	Cyclooxygenase
DBP	Diastolic blood pressure
DMSO	Dimethyl sulfoxide
EC_{50}	Half-maximal of effective concentration
+EC	Endothelium-intact
-EC	Endothelium-denuded
EDCFs	Endothelium-derived constricting factors
EDHF	Endothelium-derived hyperpolarizing factor

EDRFs	Endothelium-derived relaxing factors
E_{max}	Maximum response
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FRIM	Forest Research Institute Malaysia
GTP	Guanosine-5-triphosphate
IACUC	Institutional Animal Care and Use Committee
IM	Indomethacin
IPH	Institute Public Health
JNC 8	Eighth Joint National Committee
K^+	Potassium
K^+_{Ca}	$Ca2^+$-dependent potassium
KCl	Potassium chloride
KH_2PO_4	Potassium dihydrogenphosphate
KHS	Kreb's Henseleit solution
L-NAME	N-ω-Nitro- l-Arginine Methyl Ester
MB	Methylene blue
MLCK	Myosin light chain kinase
MR	mineralocorticoid receptor
Na^+	Sodium
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NCDs	Non-communicable diseases
NHANES	National Health and Nutrition Examination Survey
NHMS	National Health and Morbidity Survey
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs

$MgSO_4$	Magnesium sulfate
O_2	Oxygen
ODQ	1H-[1,2,4]oxadiazolo[4,3-alpha]quinoxalin-1-one
PE	Phenylephrine
PGI_2	Prostaglandin
PGH_2	Prostaglandin H_2
PKA	Protein Kinase A
PKC	Protein Kinase C
PKG	Protein Kinase G
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SEM	Standard error mean
sGC	Soluble guanylate cyclase
SHR	Spontaneous hypertensive rats
SNS	Sympathetic nervous system
TXA_2	Thromboxane A_2
US	United States
VOCC	Voltage-operated Ca^{2+} channels
VSMCs	Vascular smooth muscle cells
WHO	World Health Organization
WKY	Wistar Kyoto

PENENTUAN MEKANISME PEMVASOKENDURAN YANG DIDORONG OLEH EKSTRAK AKUAS DAUN *Syzygium polyanthum*

ABSTRAK

Kajian yang lepas menunjukkan bahawa ekstrak akuas daun *Syzygium polyanthum* (AESP) mempunyai aktiviti pengeduran-vaso. Akan tetapi, masih ada kekurangan maklumat dari segi mekanisma terhadap aktiviti terus pengenduran-vaso nya. Oleh itu, kajian ini dibentuk untuk menentukan pengenduran bergantung-endotelia dan menyiasat mekanisme pengeduran-vaso oleh AESP. Kaedah *in-vitro* digunakan, gegelang aorta dipasang kepada miograf, pengecutan dengan phenylphrine (1 µM) dan dikaji berdasarkan protokol perencatan agonis-antagonis. Pengenduran bergantung-endotelia dikaji ke atas gegelang torasik aorta endotelia-utuh dan -tanpa yang diisolasi dari tikus Wistar-Kyoto (WKY). Mekanisma pengenduran-vaso oleh AESP dinilai ke atas gegelang torasik aorta endotelia-utuh. Kajian ini menunjukkan pengenduran AESP adalah bergantung pada endotelia. Pengenduran AESP disekat oleh L-NAME (perencat nitrik osid sintase/eNOS, 100 µM) dan ODQ (perencat guanilat siklas larut/sGC, 10 µM). Akan tetapi, metilina biru (agen penurunan guanosin monofosfat siklik/cGMP, 10 µM) dan indometacin (perencat COX tidak selektif, 10 µM) tidak merencat pengenduran AESP dengan ketara. Secara keseluruhan, penemuan ini menunjukkan pengenduran AESP adalah bergantung kepada endothelia melalui laluan NO/eNOS dan sGC.

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ABSTRACT

Previous studies have demonstrated that aqueous extract of *Syzygium polyanthum* leaves (AESP) has vasorelaxation activity. However, there is still very little information on its mechanism of action on direct vasorelaxation activity. Therefore, the present study was designed to determine endothelium-dependant relaxation of the AESP and investigated the mechanism of vasorelaxation. The *in vitro* method was utilised, mounting the aortic ring into myograph, precontracted with phenylephrine (1 µM) and studied based on the agonist-antagonist inhibition protocols. The endothelium-dependant was studied on endothelium intact and denuded thoracic arterial ring isolated from Wistar-Kyoto (WKY) rats. The vasorelaxation mechanisms of AESP were evaluated on the endothelial-intact aortic rings. This study shown that the AESP relaxation was endothelium-dependant. The vasorelaxant effect of AESP was attenuated by L-NAME (endothelial nitric oxide synthase/eNOS inhibitor, 100 µM) and ODQ (soluble guanylate cyclase/sGC inhibitor, 10 µM). However, methylene blue (cyclic guanosine monophosphate/cGMP lowering agent, 10 µM) and indomethacin (non-selective COX inhibitor, 10 µM) did not produce significant inhibition on the AESP relaxation. Taken together, the findings indicated that vasorelaxation of AESP was endothelium-dependent, through NO/eNOS and sGC pathways.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Non-communicable diseases (NCDs) have emerged as the world's leading cause of death and become one of the century's most critical health issues. In 2016, the World Health Organization (WHO) estimated that NCDs caused 57 million deaths worldwide, accounting for 71% of fatalities (WHO, 2018). In Malaysia, NCDs have been gradually increasing and were projected to be responsible for 74% of all mortality with cardiovascular diseases (CVDs). It is the primary cause of mortality with 17.9 million deaths – accounting for 44% of global NCD-related deaths), followed by cancers (9 million; 22%), chronic respiratory diseases (3.8 million; 9%) and diabetes (1.6 million; 4%%) (WHO, 2018). This trend is expected to continue for many years, putting a significant strain on the world's healthcare services.

According to the National Health and Morbidity Survey (NHMS) 2019 (IPH, 2020), three main metabolic risk factors are associated with CVDs; hypertension, diabetes, hyperlipidemia, or high cholesterol. Out of the major risk factors, hypertension is the most prevalent trigger for CVDs and is known as the "silent killer" since no warning signs or symptoms appeared. It also causes several co-occurring conditions, such as stroke, heart disease, kidney failure, and cerebrovascular disease (WHO, 2019). Whether alone or combined with other metabolic disorders, high blood pressure independently increases the risk of CVDs (Forouzanfar et al., 2016). Blood pressure and the risk of cardiovascular events have a constant and continuous

association independent of other risk factors. The higher the blood pressure, the more likely people to have CVDs.

Hypertension is defined as a persistent increase in blood pressure measurement; a systolic blood pressure (SBP) of ≥140 mmHg and diastolic blood pressure (DBP) of ≥90 mmHg (Hall et al., 2012). A rise in total peripheral resistance, particularly resistance to blood flow in the systemic circulation, is the leading cause of high blood pressure. Increased peripheral resistance is frequently linked due to environmental and genetic factors, defects in the sympathetic nervous system, the renin-angiotensin-aldosterone system, and impaired functioning of vascular relaxing substances like nitric oxide (NO) and prostaglandin (PGI_2) due to endothelial dysfunction, all of which can contribute to and perpetuate hypertension (Suzanne et al., 2018). Hypertension is categorized into two main types; primary (essential) and secondary hypertension. Essential hypertension is defined by a lack of identifiable cause for blood pressure rise, accounts for approximately 95% of all cases. Since its unknown etiology may be more difficult to ascertain or establish, essential hypertension is more difficult to manage. Interestingly, the percentage of patients with essential hypertension far exceed those with secondary hypertension. Secondary hypertension accounts for the remaining 5% of cases caused by identifiable causes such as kidney illness and tumor, and thus has a relatively higher chance of being treated (Hall et al., 2012; Behrens and Leitzmann, 2013).

It has been suggested that the pathophysiology of hypertension is closely related to endothelial dysfunction. Furchgott and Zawadzki's pioneering research has established the importance of the endothelium in controlling vascular smooth muscle responses to acetylcholine. Various pharmacological (e.g., acetylcholine and bradykinin) and physical (e.g., shear stress) trigger that act through endothelial cells

had been found to release the vasoactive substances and have subsequently become major research tools in understanding endothelial function (Furchgott and Zawadzki, 1980a). In healthy endothelium, both endothelium-derived relaxing factors (EDRFs) including nitric oxide (NO) and prostaglandins (PGI_2), and endothelium-derived contracting factors (EDCFs) are maintained and produced their effect via interaction between the endothelium and vascular smooth muscle cells. When there is an imbalance between these opposing factors, the decrease of NO bioavailability with concurrent increased production of reactive oxygen species (ROS) may result in endothelial dysfunction and eventually lead to hypertension (Konukoglu and Uzun, 2016).

In general, endothelial dysfunction predicts the onset of anatomically visible vascular disease, which is strongly linked to hypertension. Endothelial dysfunction, however on the other hand, can be improved with herbal therapies (Disi et al., 2015). A wide range of ethnomedicinal plants and their particular metabolites can influence signaling pathways involved in cardiovascular physiology. These plants are not only vasculo-protective, but they may also be able to reverse hypertension alterations. The ethnomedicinal herbs' hypotensive effects have been demonstrated in few studies. Several bioactive chemicals extracted from the plants have been studied scientifically and proved effective in treating chronic disorders including hypertension. As a result, herbs and medicinal plants are gaining popularity due to their potential for healing as natural goods that are also proven to benefit one's health.

Most ethnomedicinal plants mediate the vasorelaxation effect through endothelium-dependent relaxation modulation to decrease blood pressure. According to Luna-Vázquez et al. (2013), the endothelium-dependent relaxation pathways involved in the vasodilation of blood vessels to produce an antihypertensive effect

such as NO/eNOS, sGC, cGMP and PGI_2/COX are the most commonly used in studying the mechanism of action of tested compounds, since both of these pathways are well-studied EDRFs. In sum, EDRFs play a key role in the mechanisms of action of these vasodilators extracted from the plants or the metabolites themselves.

In Malaysia, several ethnomedicinal plants are famous in lowering blood pressure. Although many claims have been made about the ability of several Malay medicinal plants to lower blood pressure, it is clear that these claims need to be verified scientifically. *Syzygium polyanthum* (Wight) Walp. Var. *Polyanthum* belonged to the *Myrtaceae* family, is one of the medicinal plants that has been traditionally established as an alternative treatment for hypertension (Rahim et al., 2018; Wan Ahmad et al., 2017). *S. polyanthum* plant is well known among Malaysians as "salam", "serai kayu", or "samak kelat". The decoction of the leaves is traditionally consumed to treat hypertension. Phytochemical constituents in the aqueous extract including gallic acid and α-pinene were responsible for lowering blood pressure through vasorelaxation or vasodilation effects (Ismail and Wan Ahmad, 2019). However, the underlying mechanisms of the vasorelaxation effect induced by *S. polyanthum* leaves remain unclear. Therefore, the present study was carried out to investigate the mechanism of vasorelaxation effect of aqueous extract of *S. polyanthum* leaves (AESP) on Wistar-Kyoto (WKY) rats using *in vitro* myograph study.

1.2 Scope of study

The present study was undertaken to assess the endothelium-dependent vasorelaxation effect of AESP and its possible underlying mechanisms involved. The study was evaluated using thoracic aorta rings that isolated from WKY rats. Loh et al. (2016) stated that with 67% majority of the past studies, used the aorta ring as the assay model to evaluate an endothelium function involving vasodilation activity and cell signalling mechanisms *in vitro*, followed by mesenteric artery, coronary artery, pulmonary artery, renal artery, carotid artery, basilar artery and femoral artery. This approach was cost-effective, rapid, and simple. It is known as the "golden instrument" in pharmacological studies (Rameshrad et al., 2016).

In this study, adult WKY rats were euthanised and dissected to retrieve the thoracic aorta. The vasorelaxation activity through NO/eNOS, SGC, cGMP and PGI_2/COX pathway among the endothelium-dependent relaxation pathway are investigated in the present study. In conjunction, the respective blockers or inhibitors such as N(ω)-Nitro-L-Arginine Methyl Ester (L-NAME), 1H-1,2,4 Oxadiazolo 4,3-a Quinoxalin-1-One (ODQ) and methylene blue were used to block NO, SGC and cGMP activities; respectively. Whereas, indomethacin was use to inhibit the PGI_2 molecules by COX activity (Tan and Yam, 2018). An *in vitro* functional myograph study approach was conducted by measuring the force from a contracting smooth muscle of a rats aorta. The generated force then was translated into an electrical output displayed and analyzed on a computer in the unit of a gram of contraction. The ability of AESP to induce the vasorelaxation effects on isolated aorta rings was evaluated based on the changes of the isometric force of contraction that was pre-contracted with phenylephrine.

1.3 Research problem

The high blood pressure in hypertensive individuals had been uncontrolled for many years. Kearney et al. (2005) stated that the proportion of people with hypertension would climb to 1.56 billion by 2025, which predicts a future, incurable burden of the disease in the global population. As a result of the ongoing trend, effective hypertension therapy is becoming increasingly important. Lowering blood pressure with routinely used antihypertensive medication reduces the risk of CVD and all-cause death without a doubt. Several clinical trials have demonstrated that reducing blood pressure reduces myocardial infarction risk by 20% to 2%, stroke risk by 35% to 40%, and heart failure risk by 50% (Ettehad et al., 2016).

However, conventional antihypertensive drugs are frequently associated with adverse drug responses, which can limit treatment options and decrease patient compliance, causing blood pressure control to be compromised. The use of two or more antihypertensive drugs is normally required to achieve blood pressure management; however, increasing the number of antihypertensive medications in a regimen may result in even more side effects (Sudhakar et al., 2015). Furthermore, the prescribed drugs are expensive and the delayed treatment often involved higher costs (Al Disi et al., 2016). The costly cost and side effects implicated from conventional antihypertensive drugs thus enforce the new alternative sources of medicines that should be cheaper and more effective with minor side effects.

As can be seen recently, a lot of work has explored local herbs or medicinal plants with hypotensive and antihypertensive medicinal properties. Particular medicinal plant's extracts are proven to exhibit antihypertensive properties through specific mechanisms and pathways. For example, *Orthosiphon stamineus* and *Tiliacora triandra* plant induced vasorelaxation activity through multiple mechanisms,

including endothelium-dependent relaxation pathways such as NO/eNOS and latter through sGC and cGMP pathway, and also PGI_2/COX (Panthiya et al., 2019; Tan and Yam, 2018a). Recently, *S. polyanthum* is one of the proven antihypertensive medicinal plants currently studied for its therapeutic values. Based on a previous finding, it has been suggested that ethanolic leaves extract of *S. polyanthum* plant has modulated the lowering blood pressure activity through inhibition of angiotensin-converting enzyme (ACE) activity (Muthia et al., 2017). In addition, aqueous and methanolic extracts of *S. polyanthum* leaves demonstrated a significant vasorelaxation effect on thoracic aorta rings isolated from WKY and SHR through the modulation of autonomic receptors and NO pathway (Ismail and Ahmad, 2017). Above all findings related to antihypertensive properties by *S. polyanthum* leaves, no attempt yet was made on AESP leaves in elucidating whether it is endothelium-dependent and the possible underlying vasorelaxation mechanisms contributed by the endothelium-dependent via NO/eNOS, sGC, cGMP and PGI_2/COX pathways. Therefore, this has urged the present study to seek out the mechanisms of action of AESP in the hope this scientific knowledge can be a crucial element for planning and developing new therapeutic drug strategies.

1.4 Objectives of study

1.4.1 General objective

The general objective of the study is to elucidate the possible underlying mechanisms of the vasorelaxation effects by AESP using WKY rat model.

1.4.2 Specific objectives

- To evaluate the endothelium-dependent relaxation effects of AESP
- To determine the possible involvement of NO/eNOS pathway in vasorelaxation effect of AESP
- To figure out the possible involvement of the sGC pathway in the vasorelaxation effect of AESP
- To investigate the possible involvement of the cGMP pathway in the vasorelaxation effect of AESP
- To find out the possible contribution of the PGI_2/COX pathway in vasorelaxation effect by AESP

1.5 Research hypothesis

- AESP exhibit the vasorelaxation effects through endothelium-dependent pathways.
- AESP mediate the vasorelaxation mechanism via the NO/eNOS, sGC, cGMP and PGI_2/COX pathways.

1.6 Significant of study

To date, the vasorelaxation mechanism of lowering blood pressure by *S. polyanthum* leaves extracts has been established to involve the NO activity using L-NAME, an eNOS inhibitor (Ismail and Ahmad, 2017). In order to address this gap of information, the present study was conducted to further elucidate the vasorelaxation activity by the contribution of endothelium-dependent relaxation pathway via sGC, cGMP and PGI_2/COX activity in mediating the lowering of blood pressure and vasorelaxation effects by *S. polyanthum* leaves extracts. By understanding its possible mode of action in-depth, the concomitant of adverse effects due to the herbal and drugs interactions can be prevented. In addition, by providing the evidence-based study, it is hoped that herbal research in Malaysia on local herbs, will govern the future of our traditional medicines in achieving a more acceptable standard before being commercialized in the market. Furthermore, these herbal researches will eventually help the economy of the villagers known to *S. polyanthum* plant at their places and promotions throughout the country to enhance the healthy lifestyle by consuming the leaves as a fresh salad or more known as' 'ulam' by locals.

CHAPTER 2

LITERATURE REVIEW

2.1 Hypertension

According to WHO (2019), hypertension is diagnosed if, when the readings are taken twice on two different occasions, the systolic and/or diastolic blood pressure readings on both days is ≥140 mmHg and/or ≥90 mmHg, respectively. The American College of Cardiology/American Heart Association (ACC/AHA) guidelines in 2017 on hypertension can be summarised in Table 2.1. Hypertension can be classified as either "Elevated" (120-129 and less than 80) or "Stage I Hypertension" (130-139 mmHg or 80-89 mmHg). A person with blood pressure of 140/90 mmHg is classified as "Stage 2 Hypertension". The prevalence of high blood pressure is expected to triple among men under age 45 and double among women under 45 (Whelton et al., 2018).

Table 2.1. Updated guidelines- definition of hypertension according to blood pressure level in adults from the ACC/AHA (2017) (adapted from Whelton et al., 2018).

Blood Pressure Category	**Systolic (mmHg)**		**Diastolic (mmHg)**
Normal	Less than 120	and	Less than 80
Elevated	120 – 129	and	Less than 80
Stage 1 Hypertension	130 – 139	or	80 – 89
Stage 2 Hypertension	140 or higher	or	90 or higher
Hypertensive Crisis	Higher than 180	and/or	Higher than 120

Hypertension can be further classified into either essential (primary) or secondary hypertension. Essential hypertension, defined by a lack of identified cause for blood pressure rise, accounts for approximately 95% of all cases. Secondary hypertension, caused by various medical problems such as renal illness and tumours, accounts for the remaining 5% of patients (Hall et al., 2012). The prevalence of hypertension is expected to rise by more than 50% in the next 30 years, resulting in a massive illness burden for society. As a result of this trend, effective hypertension therapy is becoming increasingly important.

2.1.1 Prevalence

According to the NHMS in 2019, the prevalence of hypertension in people aged 18 and above has fallen to 30.0% (IPS, 2019). Yet, the percentage of people with hypertension aware of their condition has grown from 35.6% to 50% since 2011 (Table 2.2). The prevalence was also greater in rural regions than in urban areas (32.8% vs 29.2%). A closer look at the group of people who are entirely unaware of their hypertension discloses important information. It is more common in rural regions (15.5% vs 13.7%). Males and females also have different percentages (16.2% vs 11.9%). Surprisingly, 15.8% of those respondents have used herbal or traditional supplementary medicines. 9.3% of overall prevalence hypertension had it alone (with no other co-morbidities), 9.9% had hypercholesterolemia, 8.1% had hypercholesterolemia plus diabetes, and 2.7% had diabetes. The control of hypertension in individuals on medication has increased from 34.3% in 2011 to 45.0% in 2019 (Table 2.2). Consequently, despite advances, hypertension control remains below 50% of those on therapy, with some cohorts behaving worse than others (Yusoff et al., 2021).

Table 2.2. Prevalence, awareness, treatment and control of hypertension in Malaysia between 2011 to 2019 in adults above 18 years old (adapted from Yusoff et al., 2021)

Year	Prevalence of hypertension (%)	Awareness among all hypertensives (%)	Treatment among those aware (%)	Control among treated (%)
2011	33.6	40.7	77.5	34.3
2015	35.3	37.5	83.2	37.4
2019	30.0	50.0	89.4	45.0

2.1.2 Risk factors

Age and gender are all interconnected non-modifiable risk factors for hypertension. In both sexes, blood pressure levels and the prevalence of hypertension rise with age. Women have higher mean blood pressure and hypertension prevalence than males by the age of 60 (Singh et al., 2012). Race and ethnicity are important risk factors for hypertension as well. For example, in National Health and Nutrition Examination Survey (NHANES) 2015–2016 data for 4,821 US adults aged 20 years found that non-Hispanic Black people had a significantly higher age-standardized prevalence of hypertension (57.3%) than non-Hispanic White people (43.8%) and Hispanic-Americans (44.7%) (Dorans et al., 2018). Family history or genetic factor is also an important non-modifiable risk in hypertension.

Unhealthy lifestyle behaviours such as smoking, diabetes, dyslipidaemia, overweight, physical inactivity, and unhealthy diet are among the modifiable risk factors for hypertension (WHO, 2019). Several studies also have indicated that even when hypertensive patients are educated about their diagnosis, they do not modify their lifestyle habits, resulting in the prevalence and incidence of hypertension continuing to rise in most countries due to an ageing population (Chobanian et al., 2003; Y. Kim and Kong, 2015). However, on the plus side, lifestyle modifications can reduce the risk of hypertension; smoking cessation, healthy eating, reducing dietary sodium and alcohol intake, regular physical activity, and achieving a healthy body weight effectively lower blood pressure.

2.1.3 Pathophysiology

Maintaining physiological blood pressure levels requires a complex interplay of various elements of an integrated neurohumoral system. Renin is a circulating enzyme (protease) that contributes to extracellular volume maintenance and arterial vasoconstriction. Renin converts angiotensinogen to angiotensin I (Ang I). Angiotensin-converting enzyme (ACE) converts Ang I into angiotensin II (Ang II), the most vasoactive peptide and a potent blood vessel constrictor (the centre of the pathogenetic role of the renin-angiotensin-aldosterone system (RAAS) in hypertension). Ang II is linked to endothelial dysfunction and has profibrotic and pro-inflammatory actions, mediated in part by increased oxidative stress and resulting in renal, cardiac, and vascular damage (Suzanne et al., 2018). Aldosterone roles are also important in adverse cardiovascular effects. In response to sodium deficiency and decreased extracellular fluid volume, Ang II promotes aldosterone. It is bound to the mineralocorticoid receptor (MR) at non-epithelial sites in the brain, heart, and vasculature, which is consistent with the idea that aldosterone directly affects these organs causing endothelial dysfunction, vasoconstriction, and hypertension.

The heart, much like the kidney, is critical in controlling salt and water balance. This action is primarily mediated by a cardiac hormone known as atrial natriuretic peptide (ANP). It has significant natriuretic and vasodilator effects, allowing them to maintain Na^+ equilibrium and blood pressure during Na^+ loading (Kerkelä et al., 2015). ANP is produced from the heart when blood salt levels and pressure rise. It binds to its receptor in the kidney and blood vessels, causing salt excretion, blood volume reduction, and vasorelaxation. Physiological investigations have long demonstrated the autonomic sympathetic nervous system (SNS) roles in regulating cardiovascular

functioning. The SNS play critical role in blood pressure regulation, particularly in responding to transitory variations in arterial pressure via baroreflex mechanisms. Increased SNS activity causes the alpha-1 adrenergic receptor to trigger endothelial dysfunction, vasoconstriction, vascular smooth muscle proliferation, and increased arterial stiffness, all of which contribute to the development and maintenance of hypertension (Toshiro, 2014).

Inflammatory and immunological components have been demonstrated to play an essential part in the processes underlying hypertension development. Increased vascular permeability and the production of potent mediators such as ROS, NO, cytokines, and metalloproteinases are all connected with the inflammatory process. Notably, cytokines influence renal tubular function by increasing the local angiotensinogen and Ang II production and enhancing Na^+ and volume retention in hypertension. Infiltration of innate and adaptive immune cells in the kidney and artery wall and other inflammatory processes are additional indications of hypertension and cardiovascular disease. Innate immunological responses mediated by macrophages have been related to hypertension caused by antagonism to Ang II, aldosterone, and NO meanwhile T lymphocyte-mediated adaptive immune responses have also been associated with the development of hypertension and the harm it causes to target organs (Bernardo, 2016). In an animal study conducted by Mattson et al. (2013), there are a reduction of mature lymphocytes alleviated hypertension and renal damage caused by a high-salt diet. Both pro-inflammatory and regulatory T cell aberrations are linked to hypertension and organ damage because they regulate the inflammatory processes in the kidney and vasculature.

2.1.4 Sign and symptoms and complications

According to WHO (2019), since hypertension typically does not present any warning signs or symptoms, many people are unaware that they have it. As a result, blood pressure must be checked frequently. An annual blood pressure check is recommended for people with normal blood pressure (AHA, 2016b). Headaches and nosebleeds are not typical symptoms of normal high blood pressure most of the time. If the blood pressure is 180/120 mm Hg or above, it might happen. Early morning headaches, nosebleeds, abnormal heart rhythms, visual problems, and buzzing in the ears are symptoms that might arise. Uncontrolled, severe hypertension can lead to symptoms such as tiredness and nausea. It can also lead to confusion, anxiety, chest discomfort, and muscle tremors (WHO, 2019).

High blood pressure complications can be divided into short-term and long-term effects (Fuchs and Whelton, 2020). Heart attacks and strokes are usually acute events mainly caused by a blockage that prevents blood from flowing to the heart or brain. The resulting damage from hypertension causes the coronary arteries in the heart to become narrowed slowly by plaque, a condition called atherosclerosis. As arteries harden with plaque, blood clots become more likely to form. There is also a strong relationship between hypertension and chronic kidney disease (CKD), such that persistent hypertension can lead to a reduction in kidney function, while a continuous kidney function loss may exacerbate blood pressure management (AHA, 2016a). Volume overload, sympathetic overactivity, salt retention, endothelial dysfunction, and hormonal system changes contribute to CKD hypertension (Elaine et al., 2019).

2.1.5 Treatments

Hypertension treatment aims to reduce the blood pressure to <140/90 mmHg. However, in individuals with hypertension and diabetes or renal illness, the blood pressure goal is much lower, targeted at <130/80 mmHg. The use of non-pharmacologic treatments is recommended for all hypertensive individuals. As long as lifestyle adjustments are implemented early, the risk of various diseases can be reduced and prevent the need for medication therapy. Therefore, every person should be encouraged to adopt lifestyle changes, such as eating a healthier diet, stopping smoking and getting more exercise to avoid high blood pressure (Arnett et al., 2019).

In addition to lifestyle modifications, medication treatment may be used to treat high blood pressure. In the Eighth Joint National Committee (JNC 8) guidelines, there are several types of antihypertensive medicines used to treat hypertension; the most commonly prescribed groups include diuretics, calcium channel blockers (CCBs), angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin II receptor blockers (ARBs) (Armstrong, 2014). Diuretics, most notably chlorthalidone and indapamide, are more effective at preventing cardiovascular disease at a lower cost. These drugs should be initiated as first-line hypertension therapy unless the patient has evidence of chronic kidney disease (CKD) where (ACEIs) or ARBs is indicated. Same as diuretics, CCBs, either dihydropyridines or non-dihydropyridines types are recommended as a first-line treatment alone or in combination with other antihypertensives in all patients with hypertension regardless of age and race, except patients with CKD where ACE inhibitors or ARBs are the recommended first-line treatment. However, an exception to beta-blockers (BBs) is not indicated as a first-line treatment for hypertension unless there is a specific indication of heart failure and myocardial infarction.

2.2 Endothelial function

In 1980, the endothelium's role in regulating the vascular tone and cardiovascular homeostasis was recognized (Furchgott and Zawadzki, 1980b). It is realized that healthy endothelium is important in maintaining normal blood fluidity and fibrinolysis, vascular tone, angiogenesis, monocyte/leukocyte adhesion and platelet aggregation. In endothelium-dependent vasorelaxation mechanism, endothelium releases substance became known as the endothelium-derived relaxing factors (EDRFs). The most well-studied EDRFs are NO and PGI_2. As endothelium can cause vasorelaxation, it also can cause vasoconstriction by endothelium-derived contracting factors (EDCFs) such as endothelin-1 (ET-1), thromboxane A_2 (TXA_2), angiotensin II (Ang II) and reactive oxygen species (ROS) (Loh et al., 2016). In healthy endothelium, both productions of EDRFs and EDCFs are balanced. Figure 2.1 presenting the balance between vasorelaxant and vasoconstricting factors in the normally functioning endothelium.

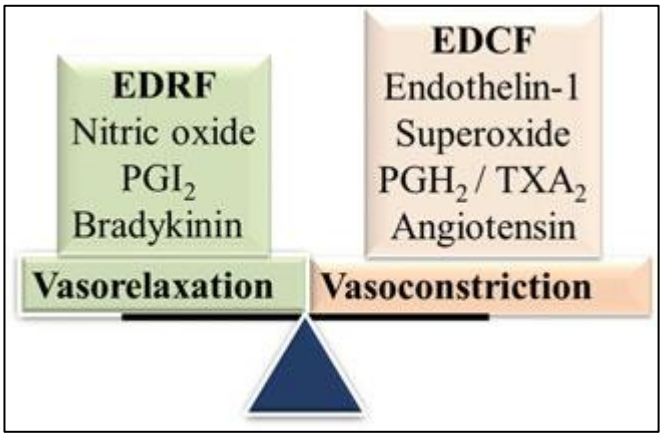

Figure 2.1. The balance between vasorelaxant and vasoconstricting factors ought to be maintained for normal endothelial function (Konukoglu and Uzun, 2016).

2.2.1 Signalling mechanism of vasorelaxation

2.2.1 (a) Direct vasorelaxation mechanisms

NO/eNOS pathway is majorly known for promoting the direct vasorelaxation effect through endothelium-dependent contribution. NO is a powerful vasorelaxant substance of smooth vascular muscles that affect the endothelium by their regulatory effects. Reduced NO levels have been involved in atherosclerosis and hypertension pathogenesis. As shown in Figure 2.2, NO is synthesized from an L-arginine by eNOS as a free radical and activates the soluble guanylate cyclase (sGC). sGC enzyme converts guanosine 5′-triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), which activates protein kinase G (PKG), leading to decreasing cytosolic calcium concentrations. A reduction of intracellular calcium concentrations indirectly causing the vasorelaxation of VSMCs (Konukoglu and Uzun, 2016). These eNOS action and NO production can be triggered by sensing the shear stress, acetylcholine, bradykinin and histamine by both Ca^{2+} -dependent and -independent pathways.

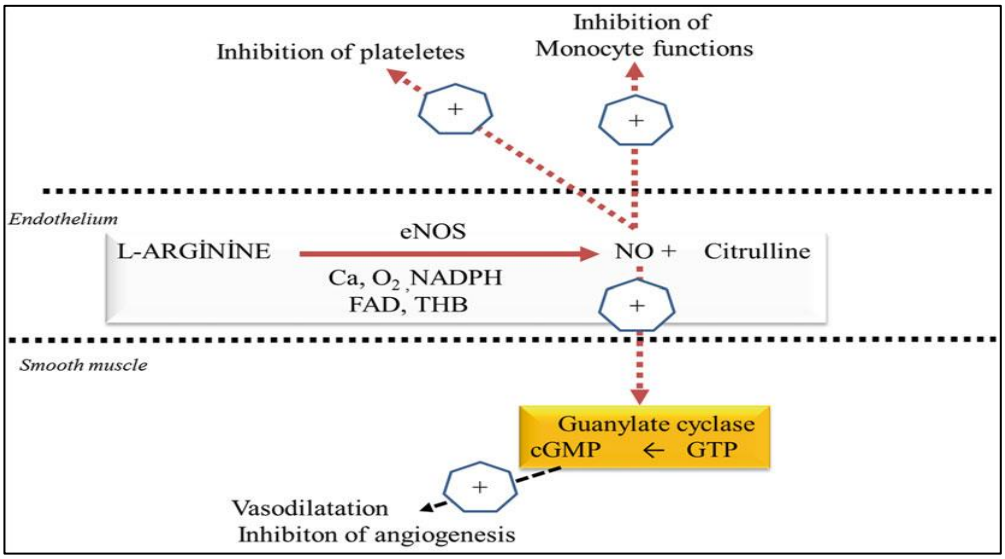

Figure 2.2. The synthesis of NO by eNOS can activate the sGC to produce cGMP. The increase of cGMP reduces the intracellular Ca^{2+} levels and thus leads to the vasorelaxation of VSMCs (Konukoglu and Uzun, 2016).

Known as the PGI_2/COX pathway, the direct vasorelaxation is induced by another potent vasodilator known as PGI_2, which belongs to the eicosanoid family of lipid molecules and is synthesized from arachidonic acid via cyclooxygenase (COX) enzymes in healthy endothelium. PGI_2 binds to the endothelial prostacyclin receptors and increases the cytosol's cyclic adenosine monophosphate (cAMP) levels. cAMP activates the protein kinase A (PKA) which leads to the dephosphorylation and inhibition of the myosin light chain kinase (MLCK) (Konukoglu and Uzun, 2016). This causes the relaxation of the smooth muscle relaxation and vasodilation (Figure 2.3).

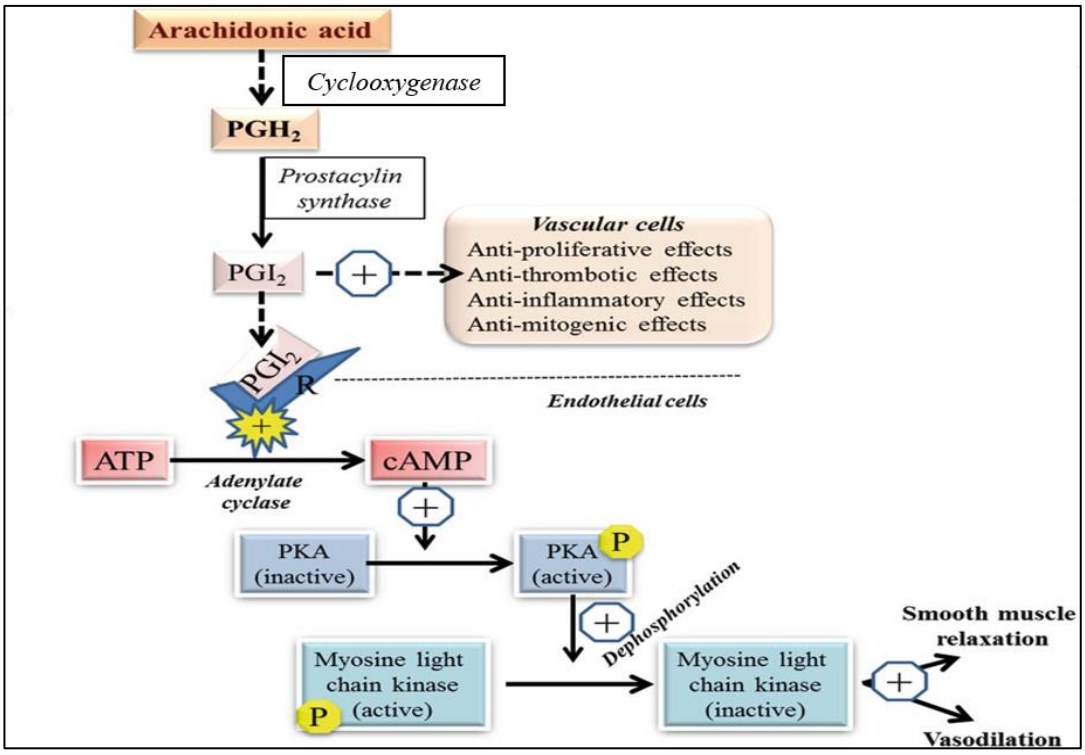

Figure 2.3. The synthesis of PGI_2 from arachidonic acid by COX enzymes. The production of cAMP levels in cytosol increased, resulting in PKA activation and inhibiting the MLCK, leading to the relaxation of VSMC (Konukoglu and Uzun, 2016).

Other than that, the endothelium can also produce hyperpolarization. Endothelial cells were able to produce hyperpolarization by showing the endothelial-dependent relaxation cannot be eliminated by eNOS inhibitors. The term endothelium-derived hyperpolarizing factor (EDHF) represents a mechanism rather than specific factors, which main function is to hyperpolarize the VSMCs causing the cells to relax. EDHF react through the activation of the potassium (K^+) and calcium (Ca^{2+}) channels which initiates the VSMCs and triggers the vasorelaxation (Luksha et al., 2009).

2.2.1 (b) Indirect vasorelaxation mechanism

The vasorelaxation effect on the VSMCs also can be induced with the absence of the endothelium. The vasorelaxation mechanism is indirectly induced by a variety of ways such as; the inhibition of extracellular Ca^{2+} influx through the transmembrane Ca^{2+} channels and/or inhibition of voltage-operated Ca^{2+} channels (VOCCs) by opening K^{+} channels, and hence induction of hyperpolarization (Chen et al., 2009; Rameshrad et al., 2016). Therefore, the hyperpolarization of the VSMCs causing the cells to relax. In addition to MLCK dephosphorylation to induce vasorelaxation, the protein kinase C (PKC) also contributes to vascular regulation. In most smooth muscles, PKC has vasocontraction effects through the phosphorylation of Ca^{2+} channels or other proteins that regulate cross-bridge formation of actin and myosin (Webb, 2003). Thus, vasorelaxation can be indirectly induced by inhibiting the PKC activity in the contractile steps by the PKC inhibitor that could interact with the PKC molecule, interfere with PKC binding to its substrates, decrease PKC synthesis, or counteract the effects of PKC (Ringvold and Khalil, 2017). Moreover, VSMCs relaxation can occurs by the inhibition of Ca^{2+} release from intracellular stores as the rise of cytosolic Ca^{2+} mediated by the release of intracellular stores activates the Ca^{2+}/calmodulin-dependent MLCK for VSMCs contraction (Schlossmann et al., 2003).

2.2.2 Endothelial dysfunction in hypertension

Most commonly, impaired endothelium-dependent vasorelaxation is a hallmark of endothelial dysfunction characterized by imbalanced EDRFs and EDCFs, elevated ROS and as well as deficiency of NO bioavailability, which is responsible for a variety of cardiovascular disorders especially hypertension. The elevation of vascular production of ROS has caused the increasing activity of oxidative stress and has been linked to the disturbance in NO metabolism such as elevated degradation NO levels. In general, oxidative stress can be defined as an excess formation or incomplete removal of highly reactive molecules of ROS that promotes vasocontraction and thus increase the systemic vascular resistance in hypertension (Santilli et al., 2015). Along with the concomitant of the reduced levels of NO, as the most common vasodilator substance in the endothelium, the vasorelaxation activity in VSMCs become impaired in hypertension. Figure 2.4 summarised the impaired response of endothelium to vasocontraction in hypertension.

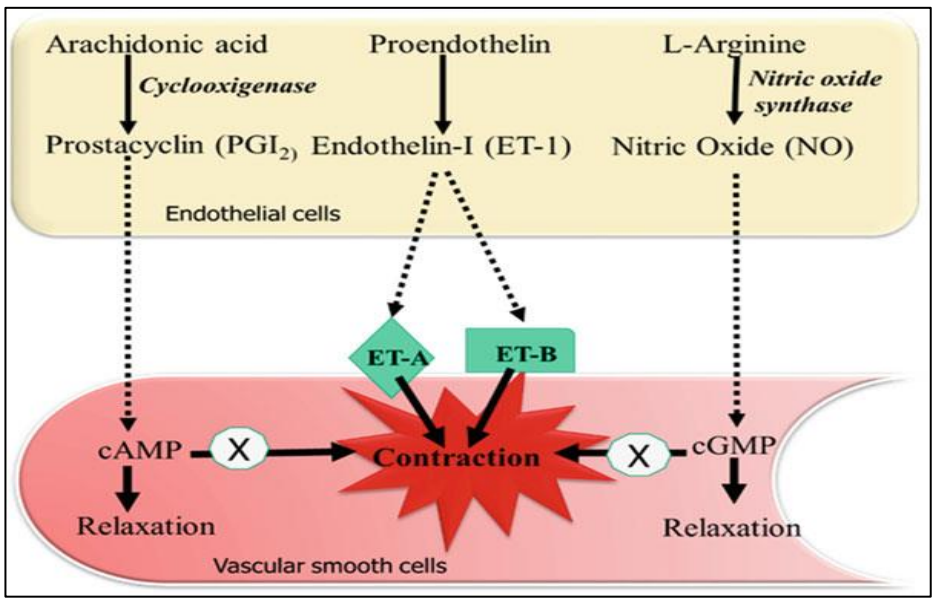

Figure 2.4. Impaired response of endothelium to the vasocontraction in hypertension (Konukoglu and Uzun, 2016).

2.2.3 Antihypertensive therapy and endothelial dysfunction

Some antihypertensive pharmacologic compounds can reverse endothelial dysfunction. The antihypertensive drugs currently have endothelial protective effects and enhanced the production of NO which can neutralize the free radicals, for example, Angiotensin-II type 1 receptor blockers (ARBs); olmesartan and losartan (Matavelli and Siragy, 2015). ARBs are generally prescribed for people who cannot tolerate Ang-converting enzyme inhibitors (ACEIs). ACEIs have similar effects to ARBs, another kind of blood pressure medication, but they act differently. ACEIs enhance NO bioavailability by inhibiting Ang-II production and improve the action of the hyperpolarizing factor produced from endothelium under specific circumstances. Short and long-term use of ACEIs also enhance coronary and peripheral endothelial function. This impact might differ according to the chemical administered, risk factors, and hereditary variables in existence (Mendoza Torres et al., 2015).

Beta-blockers (BBs), including propranolol and atenolol, have a protective endothelium impact and improve the vasorelaxation reactions based on the endothelium by boosting NO release. The combination of BBs with an ACEI was also indicated to have a more favourable impact on the endothelial function than monotherapy in hypertensive individuals with obesity. Ca^{2+} channel blockers (CCBs) like dihydropyridine also protect against endothelial cell death produced by ROS (Yasu et al., 2013). However, a reduction in blood pressure does not always mean that these antihypertensive medicines enhance the function of the endothelium. Because endothelial and oxidative stress play key roles in cardiovascular and metabolic disorders, different diet patterns, calorie restriction, and nutritional supplementation with physical activity behaviours may have major consequences for disease prevention.