IN-VITRO CARDIOVASCULAR EFFECTS OF *GYNURA PROCUMBENS* (LOUR.) AND *ORTHOSIPHON STAMINEUS* (BENTH.)

BY

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In the name of ALLA H

The most beneficent and merciful

THIS THESIS IS DEDICATED

TO

MY *WIFE* AND MY SONS *AHMAD* AND *AKKRM*

FOR THEIR LOVE, PATIENCE, DEVOTION AND

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LIST OF ABBREVIATIONS

AV	Atrioventricular node
B*f*	n-butanol sub-fraction
• C	Degree Centigrade
Ca^{2+}	Calcium ion
cAMP	cyclic Adenosine monophosphate
C*f*	Chloroform sub-fraction
CO_2	Carbon dioxide
EDRF	"The endothelium-derived relaxation factor"
Fig.	Figure
g	Gram
Hz	Hertz
K^+	Potassium
Kg	Kilogramme (s)
ISP	Isoprenaline
log	Logarithm
mg	Milligram
ml	Milliliter
µl	Micro liter
n	Number of cases
N^+	Sodium ion
NA	Noradrenaline

NP/PEG	Natural product- Polyethylene glycol
NO	Nitric oxide
O_2	Oxygen
PSS	Physiological salt solution
R*f*	Retardation factor
SA	Sinoatrial nodes
S.E.M	Standard error of mean
SD rat	Sprague-Dawley rat
TLC	Thin layer chromatography
UV	Ultra violet
w/v	Weight per volume
WHO	World Health Organization

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KESAN *IN-VITRO* KARDIOVASKULAR *GYNURA PROCUMBENS* DAN *ORTHOSIPHON STAMINEUS*

ABSTRAK

Gynura procumbens dan ***Orthosiphon stamineus*** digunakan di dalam perubatan tradisional untuk mengubati penyakit darah tinggi. Kajian menunjukkan kedua-dua *Gynura procumbens* dan *Orthosiphon stamineus* merencat aktiviti kardiak dan menyebabkan vasodilatasi, dua mekanisme yang menyumbang kepada penurunan tekanan darah. Aktiviti kardiak dinilai menggunakan sediaan terasing *in-vitro* atrium kanan yang berdenyut secara spontan dan atrium kiri yang diberi rangsangan elektrik berturutan dan aktiviti vasodialatasi menggunakan sediaan terasing jaluran aorta tikus Sprague Dawley. Serbuk kering daun *Gynura procumbens*, tumbuhan pertama yang diselidiki diekstraksi secara bersiri dengan eter petroleum dan diikuti dengan metanol. Ekstrak metanol didapati merencat kesan inotropi yang diaruhkan oleh isoprenalin pada atrium kanan lebih baik berbanding ekstrak eter petroleum. Ekstrak metanol seterusnya difraksikan kepada fraksi kloroform, etil asetat, n-butanol dan air. Fraksi n-butanol didapati menyebabkan perencatan paling kuat kedua-dua aktiviti atrium kanan dan kiri. Fraksi n-butanol kemudiannya di sub-fraksikan menggunakan kromatografi kilat turus kering dan menghasilkan tiga sub-fraksi (Bf_1-Bf_3). Hasil percubaan menunjukan subfraksi Bf_3 menyebabkan perencatan terkuat kesan inotropi yang diaruhkan oleh isoprenalin pada kedua-dua sediaan atrium kanan dan kiri dan menyarankan sebatian yang mempunyai aktiviti kardiak terdapat dalam fraksi Bf_3.

Antara fraksi-fraksi yang didapatkan daripada ekstrak metanol diatas, fraksi kloroform didapati merencat paling kuat penguncupan jaluran aorta yang diaruhkan oleh

noradrenalin. Fraksi kloroform kemudiannya disubfraksikan dengan kromatografi kilat turus kering dan menghasilkan lima sub-fraksi (Cf_1-Cf_5). Keputusan percubaan menunjukan sub-fraksi Cf_3 menyebabkan perencatan kontraksi aruhan noradrenalin yang paling kuat.

Tumbuhan kedua yang diselidiki adalah *O. stamineus*. Serbuk kering daun *O. stamineus* diekstraksi secara bersiri dengan eter petroleum, kloroform, metanol dan akhirnya dimaserasi dengan air. Ekstrak kloroform didapati menyebabkan perencatan paling kuat penguncupan aorta arohan noradrenalin. Ekstrak kloroform kemudiannya difraksikan dengan kromatografi kilat turus kering dan menghasilkan lima sub-fraksi (Cf_1-Cf_5). Sub-fraksi Cf_2 didapati menyebabkan perencatan paling kuat penguncuapan aruhan noradrenalin. Analisis fitokimia awal menggunakan kromatografi lapisan nipis menggunakan reagen semburan spesifik menunjukan subfraksi Bf_3 dan Cf_3 *G. procumbens* dan fraksi Cf_2 *O. stamineus* mengandungi flavonoid, terpenoid dan kumarin. Berdasarkan dari kajian penyelidik terdahulu, flavonoid dilaporkan boleh mengaruhkan vasodilatasi. Oleh itu, besar kemungkinan vasodilatasi yang diaruhkan oleh *G. procumbens* dan *O. stamineus* adalah disebabkan oleh kandungan flavonoidnya. Perencatan aktiviti kardiak dan vaskular tumbuhan-tumbuhan ini mungkin menerangkan penggunaan tumbuhan-tumbuhan ini untuk merawat penyakit darah tinggi dalam perubatan tradisional.

ABSTRACT

Gynura procumbens and ***Orthosiphon stamineus*** have been used as traditional medicine to treat hypertension. This study has shown that both *Gynura procumbens* and *Orthosiphon stamineus* inhibit cardiac activity and cause vasodilatation, the two mechanisms which contribute to the lowering of blood pressure. The cardiac activity was evaluated using isolated spontaneously beating right atria and electrically paced left atria, whereas vasodilating activity was studied using aortic strip preparations isolated from Sprague Dawley (SD) rats. The dried pulverized *G. procumbens* leaves were serially extracted with petroleum ether and followed by methanol. The methanol extract was found to inhibit the isoprenaline-induced inotropic activity of right atria more than the petroleum ether extract. The methanol extract was then fractionated into chloroform, ethyl acetate, n-butanol and water fractions. Fraction n-butanol was found to cause the strongest inhibition for both cardiac right and left atria activities. Fraction n-butanol was then fractionated again using dry flash column chromatography to afford three sub-fractions: (Bf_1- Bf_3). The results showed that, sub-fraction Bf_3 caused the strongest inhibition of the inotropic effect induced by isoprenaline in both right and left atria which suggest the compound (s) with cardiac activity is in sub-fraction Bf_3.

Among the fraction obtained from methanol extract above the chloroform fraction was found to cause the strongest inhibition on noradrenaline induced contraction of aortic strip. The chloroform extract was then fractionated again using dry column chromatography to afford five sub-fractions (Cf_1- Cf_5). Our results show that, sub-fraction Cf_3 caused the strongest inhibition of the contraction induced by noradrenaline. The second plant studied, was *O. stamineus*. Dried powdered leaves of *O. stamineus*

were serially extracted with petroleum ether, chloroform, methanol and lastly macerated in water. The chloroform extract was found to cause the strongest inhibition of noradrenaline-induced contraction in aortic strip prepararion. The chloroform extract was then fractionated using dry flash column chromatography to afford five fractions (Cf_1- Cf_5). Fraction Cf_2 was found to cause the strongest inhibition of contraction induced by noradrenaline.

Preliminary phytochemical analysis by thin layer chromatography using specific spray reagents showed that sub-fraction Bf_3 and Cf_3 of *G. procumbens* and fraction Cf_2 of *O. stamineus* contained flavonoids, terpenoids and coumarins.

Based on previous studies by Duarte *et al.* (2001), flavonoids have been known to cause Therefore, it is seems likely that the vasodilatation caused by *G. procumbens* and *O. stamineus* extracts were attributed to flavonoids (TLC studies). The inhibition of the cardiac and vascular activity of the plants may explain their use for treatment of high blood pressure in traditional medicine.

CHAPTER ONE / INTRODUCTION

1.1 Hypertension

High blood pressure, termed "hypertension," is a condition that afflicts more than 50 million Americans and is a leading cause of morbidity and mortality. Hypertension is much more than a "cardiovascular disease" because it affects other organ systems of the body such as kidney, brain, and eye. Tens of millions of Americans are not even aware of being hypertensive because it is usually asymptomatic until the damaging effects of hypertension (such as stroke, myocardial infarction, renal dysfunction, etc.) are observed.

The term "hypertension" can be applied to elevations in mean arterial pressure, diastolic pressure, or systolic pressure. Hypertension is often defined as a diastolic pressure of 90 mmHg or above, or a systolic pressure of 140 mmHg or above or both. A diastolic pressure of 80 to 89 mmHg and a systolic pressure of 120-139 mmHg is termed prehypertension. Elevations in either diastolic or systolic pressure represent a significant risk factor to the patient (Richard, 2002).

Mean arterial pressure is the average value for arterial pressure. It is the product of cardiac output and vascular resistance. It is usually not discussed in the context of hypertension because it is not normally measured in a patient. However, changes in either cardiac output or systemic vascular resistance will increase not only diastolic and systolic pressures, but also mean arterial pressure. The term "mean arterial pressure" is usually spoken in the context of the arterial pressure that is responsible for organ perfusion.

1.2 Arterial blood pressure

Ejection of blood into the aorta by the left ventricle results in a characteristic aortic pressure pulse. The peak of the aortic pressure pulse is termed the systolic pressure ($P_{systolic}$), and the lowest pressure in the aorta, which occurs just before the ventricle ejects blood into the aorta, is termed the diastolic pressure ($P_{diastolic}$). The difference between the systolic and diastolic pressures is the aortic pulse pressure. The mean aortic pressure (P_{mean}) is the average pressure (geometric mean) during the aortic pulse cycle.

As the aortic pressure pulse travels down the aorta and into distributing arteries, there are characteristic changes in the systolic and diastolic pressures, as well as in the mean pressure. As the pressure pulse moves away from the heart, the systolic pressure rises and the diastolic pressure fall. There is also a small decline in mean arterial pressure as the pressure pulse travels down distributing arteries due to the resistance of the arteries. Therefore, when arterial pressure is measured using a sphygmomanometer (i.e., blood pressure cuff) on the upper arm, the pressure measurements represent the pressure within the brachial artery which will be slightly different than the pressure measured in the aorta or the pressure measure in other distributing arteries.

In most patients (90-95%) presenting with hypertension, the cause is unknown. This condition is called essential (or primary) hypertension. The remaining 5-10% of hypertensive patients have hypertension that results secondarily from renal disease, endocrine disorders, or other identifiable causes. This form of hypertension is called secondary hypertension (Richard, 2002).

Regardless of the origin of hypertension, the actual increase in arterial blood pressure is caused by either an increase in systemic vascular resistance (SVR) or an increase in cardiac output (CO). Therefore, in order to understand how arterial blood pressure can become elevated, it is first necessary to understand the mechanisms that regulate both SVR and CO (Neal, 1992)

1.3 Essential hypertension

Essential (or primary) hypertension accounts for approximately 90-95% of patients diagnosed with hypertension. Unlike secondary hypertension, there is no known cause of essential hypertension. Despite many years of active research, there is no unifying hypothesis to account for the pathogenesis of essential hypertension. There is a natural progression of this disease that suggests early elevations in blood volume and cardiac output might initiate subsequent increase in the systemic vascular resistance. This increase in blood volume was suggested to be due to inability of the kidney to adequately handle sodium as a basic underlying defect. In chronic, long-standing hypertension, blood volume and cardiac output are often normal and the hypertension is sustained by an elevation in systemic vascular resistance rather than by an increase in cardiac output. This increased resistance is caused by a thickening of the walls of resistance vessels (i.e. arteries) and by a reduction in lumen diameters. There is also an evidence for increased vascular tone that could be mediated by enhanced sympathetic activity or by increased circulating levels of angiotensin II. In recent years, considerable evidence has suggested that changes in vascular endothelial function may cause the increase in vascular tone. For example, in hypertensive patients, the vascular endothelium produces less nitric oxide and the vascular smooth muscle is less sensitive to the actions of this powerful vasodilator.

There is also an increase in endothelin production, which can enhance vasoconstrictor tone. There is compelling evidence that hyper-insulinemia and hyperglycemia in type 2 diabetes (non-insulin dependent diabetes) causes endothelial dysfunction by enhanced oxygen free radical mediated damage and decreased nitric oxide bioavailability (Richard, 2002).

1.4 Secondary hypertension

Secondary hypertension accounts for approximately 5-10 % of all cases of hypertension. Secondary hypertension has an identifiable cause. Regardless of the cause, arterial pressure becomes elevated either due to an increase in cardiac output, an increase in systemic vascular resistance, or both. When cardiac output is elevated, it is generally due to either increased neurohumoral activation of the heart or increased blood volume. Some of the causes for secondary hypertension are listed below:

- Renal artery stenosis
- Chronic renal disease
- Primary hyperaldosteronism
- Stress
- Sleep apnea
- Hyper- or hypothyroidism
- Pheochromocytoma
- Preeclampsia
- Aortic coarctation

1.5 Systemic vascular resistance

Systemic vascular resistance (SVR) refers to the resistance to blood flow offered by all of the systemic vasculature, excluding the pulmonary vasculature. It is sometimes referred as total peripheral resistance (TPR). Systemic vascular resistance (SVR) is therefore determined by those factors that influence vascular resistance in individual vascular bed. Mechanisms that cause vasoconstriction will increase SVR, and those that cause vasodilatation will decrease SVR. The actual change in SVR in response to neurohumoral activation, for example, depends upon the degree of activation and vasoconstriction, the number of vascular beds involved, and the relative in series and parallel arrangement of these vascular beds to each other. Although SVR is primarily determined by changes in blood vessel diameters, changes in blood viscosity also affect SVR.

Systemic vascular resistance can be calculated if cardiac output (CO), mean arterial pressure (MAP), and central venous pressure (CVP) are known.

$$\mathbf{SVR = (MAP - CVP) \div CO}$$

Because CVP is normally near 0 mmHg, the calculation is often simplified to:

$$\mathbf{SVR = MAP \div CO}$$

It is very important to note that SVR can be calculated from MAP and CO, but it is not determined by either of these variables. A more accurate way to view this relationship is that at a given CO, if the MAP is very high, it is because SVR is high. Mathematically, SVR is the dependent variable in the above equations; however,

physiologically, SVR and CO are normally the independent variables and MAP is the dependent variable (Richard, 2002).

1.6 Arterial tone

Arterial tone is an important factor in the regulation of blood pressure. Arterial tone is determined by the interaction of the endothelium and the smooth muscle.

1.6.1 Endothelium

The vascular endothelium is highly active endocrine organ covering the inner surface of the arteries and veins. The endothelium is an important regulator of arterial tone because it secretes various vasodilating and vasoconstrictive substances (Figure 1).

1.6.1.1 Vasodilatory factors

1.6.1.1.1 Nitric oxide

In 1980 Furchgott and Zawadzki showed that the endothelium must be intact for acetylcholine (Ach) to induce arterial smooth muscle relaxation. A substance originating from a vessel with an intact endothelium caused relaxation in an arterial ring with a denuded endothelium; it was named "the endothelium-derived relaxation factor" (EDRF). Later the EDRF was confirmed to be nitric oxide (NO) (Ignarro *et al.,* 1987; Palmer *et al.*, 1987). NO is a gaseous free radical which is synthesized from the amino acid L-arginine by a family of NO syntethases (NOSs). NO relaxes vascular smooth muscle cells (VSMCs) by increasing the production of cyclic guanosine 3′, 5′- monophosphate (cGMP). A normal endothelium constantly releases small amounts of NO. Extra NO is released in response to physiological stimuli such as increased shear stress and reduced oxygen tension, and to substances

such as acetylcholine (Ach), bradykinin, histamine, thrombin, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and the substance P. So far, NO is the most potent vasodilator known (Umans and Levi, 1995).

1.6.1.1.2 Prostacyclin

Prostacyclin (PGI_2) is formed from arachidonic acid (AA) by the enzyme cyclo-oxygenase. The endothelial cells are the highest producers of PGI_2, but VSMCs and fibroblast are also able to synthesize PGI_2. Prostacyclin (PGI_2) is produced in response to shear stress and to substances that stimulate NO formation. The contribution of PGI_2 to vasodilation is less than that of NO (Mikkola *et al.*, 1996).

1.6.1.1.3 Endothelium-derived hyperpolarizing factor (EDHF)

Endothelium-dependent relaxations and hyperpolarizations can be partially or totally resistant to the inhibitors of cyclo-oxygenase and NO synthetase, suggesting the existence of an additional endothelial relaxing mechanism. These NO- and PGI_2-independent relaxations appear to be without an increase in the intracellular levels of cyclic nucleotides in smooth muscle cells and the relaxations are antagonized by apamin and charybdotoxin (ChTX), the inhibitors of Ca^{2+} sensitive K^+ channels (K_{ca}) (Feletou and Vanhoutte, 1999). It has been suggested, therefore, that the hyperpolarization of smooth muscle cells caused by the opening of K^+ channels is responsible for these relaxations, and the relaxing agent is called an endothelium-derived hyperpolarizing factor (EDHF).