ANGIOTENSIN CONVERTING ENZYME INHIBITION ACTIVITY AND ANTIHYPERTENSIVE EFFECT OF THAI MEDICINAL PLANTS

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE
(BIOPHARMACEUTICAL SCIENCES)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2005

ISBN 974-04-6180-8

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was submitted to the Faculty of Graduates Studies, Mahidol University
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on
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ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my advisor, Associate Professor Suwan Thirawarapan, for her supervisions, guidance, kindness and constructive critisms which enable me to carry out this thesis succesfully.

I wish to express my gratitude to Associate Professor Wisuda Suvitayavat, who provided invaluable helps. I wish to express my appreciation to Professor Nuntavan Bunyapraphatsara, for her kind during my work.

I would like to thank Associate Professor Leena Suntornsuk, Associate Professor Opa Vajragupta and Assistant Professor Nongluk Ruangwises for their invaluable advice and technical instruction in fluorometrical analysis. I also would like to thank Associate Professor Chongkol Tiangdah for her invaluable suggestions and comments. Moreover, I wish to thank Associate Professor Wongsatit Chuakul for his suggestion and help with the medicinal plant collection and identification from Siriruckhachati Medicinal Plant Garden.

I thanked to Mrs. Tippawan Hanamornset, Miss Ratana Narksanga and Mrs. Chuleeporn Nammesri from Department of Physiology for their kind assistance. I would like to extend my thanks to everybody who gave me assistance and friendships during my study. Mrs. Ubonwan Bunpeng from the Department of Pharmaceutical Botany and Mr.Mana Tarploha have been of technical assistance.

Furthermore, the Department of Pharmaceutical Chemistry, the Department of Microbiology, the Department of Pharmaceutical Botany and the Department of Physiology are heartily thanked for permission to use facilities.

Finally, I owe unending gratitude to my family for their warm love, understanding and entirely encounrage me in graduation, without them, I would never came to this point.

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ABSTRACT

Renin angiotensin system plays a major role in blood pressure regulation. The inhibition of angiotensin converting enzyme is a major action of the drug used for the treatment of hypertension. Presently, there have been substantial reports from various countries demonstrating that several medicinal plants had angiotensin converting enzyme inhibition (ACEI) activity.

The present study aimed to evaluate the *in vitro* ACEI activity of Thai medicinal plants that had pharmacological activity and/or claimed in traditional medicine to lower blood pressure, by using fluorometric assay. In addition, the mangrove plants with antioxidant activity were also investigated for this activity. From 49 specimens of 30 plant species, the ACEI activity of greater than 50 % were determined in 9 specimens of 6 species of ethyl acetate extract of mangrove plants and 4 specimens from 3 species of water extract of medicinal plants. Among the water extract of medicinal plants, *Alstonia scholaris* (L.) R.Br. or Sattaban's bark, which had the highest ACEI activity (52.89 %) was used to examine the antihypertensive and vasodilating actions.

The antihypertensive action was examined in two-kidney, one-clip hypertensive rats. The blood pressure was measured by tail cuff method every 15 minutes for 90 minutes after single oral feeding with *A. scholaris* bark extract at the doses of 0.75, 1.5, 3 and 6 g of dried bark/kg compared to captopril 25 mg/kg. The isolated aortic rings of these hypertensive rats were used to examine the effect of the extract at the doses of 12.7, 25, 51, 102 mg of dried bark/ml on contractile response to norepinephrine (NE) compared to captopril 0.42 mg/ml. This was also examined in the isolated aortic ring from normal rats.

The *A. scholaris*'s bark extract had significant antihypertensive effects in a dose-independent manner with lesser effect compared to captopril. The extract slightly decreased heart rate, while captopril had no effect. In addition, the extract also decreased contractile response of isolated aortic ring to NE with the greater effect in those from normal than hypertensive rats.

The results from this study indicated that some Thai medicinal plants and mangrove plants had ACEI activity, and *A. scholaris*'s bark water extract with ACEI activity can decrease blood pressure and caused vasodilatation in hypertensive rats.

KEY WORDS : *ALSTONIA SCHOLARIS* / ANGIOTENSIN CONVERTING ENZYME INHIBITION / ANTIHYPERTENSIVE / MEDICINAL PLANTS / MANGROVE PLANTS
118 P. ISBN 974-04-6180-8

ฤทธิ์ยับยั้ง ANGIOTENSIN CONVERTING ENZYME และลดความดันโลหิตของสมุนไพรไทย (ANGIOTENSIN CONVERTING ENZYME INHIBITION ACTIVITY AND ANTIHYPERTENSIVE EFFECT OF THAI MEDICINAL PLANTS)

สุรวุฒิ ยิ่งสุขไพศาล 4337557 PYBS / M

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บทคัดย่อ

ระบบ renin angiotensin มีบทบาทสำคัญในการควบคุมความดันโลหิต การใช้ยาที่มีฤทธิ์ยับยั้ง angiotensin converting enzyme (ACE) จึงเป็นแนวทางสำคัญในการรักษาโรคความดันโลหิตสูง ในปัจจุบันมีรายงานฤทธิ์ยับยั้ง ACE ของพืชสมุนไพรหลายชนิดจากหลายประเทศ

ในการศึกษานี้จึงประเมินฤทธิ์ยับยั้ง ACE ในหลอดทดลอง โดยการวิเคราะห์เชิงฟลูออโรเมตรีของสมุนไพรไทยที่มีการใช้ในตำรายาไทยและ/หรือที่มีรายงานฤทธิ์ลดความดันโลหิต และพืชป่าชายเลนที่มีรายงานฤทธิ์ต้านออกซิเดชั่น จากการศึกษาพืช 30 ชนิด รวม 49 ตัวอย่าง พบว่า 9 ตัวอย่างจากสารสกัดเอธิล อะซิเตทของพืชป่าชายเลน 6 ชนิด และ 4 ตัวอย่างจากสารสกัดน้ำของสมุนไพร 3 ชนิด มีฤทธิ์ยับยั้ง ACE ได้มากกว่า 50 % สำหรับสารสกัดน้ำของสมุนไพรเปลือกต้น *Alstonia scholaris* (L.) (สัตตบรรณ) มีฤทธิ์ยับยั้งสูงสุดที่ 52.89 % จึงใช้ในการทดสอบฤทธิ์ลดความดันโลหิตและการหดรัดหลอดเลือดต่อไป

การศึกษาฤทธิ์ต่อความดันโลหิตใช้หนูขาวที่ชักนำให้เกิดภาวะความดันโลหิตสูง ด้วยวิธี two kidney-one clip ทำการวัดความดันโลหิตที่หางหนูด้วยวิธี tail cuff method ทุก 15 นาที เป็นเวลา 90 นาที ภายหลังการป้อนสารสกัดครั้งเดียว ขนาด 0.75, 1.5, 3 และ 6 กรัม/กก เปรียบเทียบกับ captopril 25 มก/กก หลังจากนั้นใช้หลอดเลือดแดง aorta ทดสอบผลของสารสกัดขนาด 12.7, 25, 51 และ 102 มก/มล และ captopril 0.42 มก/มล ต่อการหดรัดของ norepinephrine (NE)

สารสกัดน้ำของเปลือกต้นสัตตบรรณ ลดความดันโลหิตได้อย่างมีนัยสำคัญทางสถิติโดยไม่ขึ้นกับขนาดที่ใช้ แต่ลดได้น้อยกว่า captopril โดยมีผลลดอัตราการเต้นของหัวใจเล็กน้อย นอกจากนี้สารสกัดยังลดผลการหดรัดหลอดเลือดแดง aorta ของ NE โดยให้ผลกับหลอดเลือดจากหนูปกติมากกว่าหนูความดันโลหิตสูง

ผลจากการศึกษาแสดงว่าสมุนไพรและพืชป่าชายเลนของไทยบางชนิดมีฤทธิ์ยับยั้ง ACE และสารสกัดของเปลือกต้นสัตตบรรณมีฤทธิ์ยับยั้ง ACE มีผลลดความดันโลหิตและขยายหลอดเลือดของสัตว์ทดลองที่มีความดันโลหิตสูง

118 หน้า ISBN 974-04-6180-8

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LIST OF ABBREVIATIONS

ACE	=	angiotensin converting enzyme
ACEI	=	angiotensin converting enzyme inhibition
ADH	=	antidiuretic hormone
ANP	=	atrial natriuretic peptide
Ang I	=	angiotensin I
Ang II	=	angiotensin II
BP	=	blood pressure
CO_2	=	carbondioxide
g	=	gram
Hip-His-Leu	=	hippuryl-l-histidine-l-leucine
His-Leu	=	histidine-l-leucine
h	=	hour
HPLC	=	high performance liquid chromatography
kg	=	kilogram
KH solution	=	kreb-henseleit solution
l	=	liter
M	=	molar
mg	=	milligram
min	=	minute
mM	=	millimolar
mm	=	millimeter
mm Hg	=	millimeter mercury
ml	=	millilitre
NE	=	norepinephrine
nm	=	nanometer
OPA	=	*o*-pthaldehyde
O_2	=	oxygen
RAS	=	renin-angiotensin system

LIST OF ABBREVIATIONS (continued)

u	=	unit (rotation per minute)
%	=	percent
ºC	=	degree celcius
2K1C	=	2 kidneys, 1 clip
µM	=	micromolar

CHAPTER I
INTRODUCTION

Hypertension is the most common cardiovascular disorder which has a major contribution in coronary artery disease, heart failure, renal insufficiency, stroke and dissecting aneurysm of the aorta. The renin-angiotensin system (RAS) plays an important role in the control of cardiovascular homeostasis, affecting both blood pressure and fluid volume and is one of the most important ethiological candidates in hypertension (1). The RAS was an endocrine system, in which angiotensinogen of hepatic origin is secreted into the systemic circulation and cleaved by renin and subsequently angiotensin converting enzyme (ACE), to produce the active peptide angiotensin II (Ang II).

The ACE is an important component of the renin angiotensin system). It has been known that, arterial ACE is high both in genetic (2,3) and experimental (4,5) models of hypertension and that, blood pressure is correlated with basal arterial ACE activity (2). Moreover, ACE inhibitors are one of the widely used antihypertensive drugs in treatment of patients with essential and renovascular hypertension (6). Randomized, placebo-controlled trials have shown that ACE inhibitors are effective in lowering blood pressure and in the treatment of left ventricular dysfunction (7,8). The peptides from the venom of the South American snake *Bothrops jararaca* are among the most important natural products identified as ACE inhibitors (9), and were the starting point for the development of captopril and other synthetic ACE inhibitors (7,10). Several screening studies reported that many higher plants possessed ACE inhibitory activity (11-18). However, there were no report of *in vitro* ACE inhibitory activity of plants in Thailand. The search for the potential of plants as a natural source of ACEI will lead to the study for active fractions and possible to replace the synthetic ACEI which are imported from the other countries. This study is aimed at a preliminary *in vitro* screening of ACE inhibitory activity of Thai medicinal plants which had traditional use or pharmacological

action involved the cardiovascular functions. The mangrove plants with antioxidant activity were also screened for this activity. Moreover, a selected plant with ACE inhibitory activity was used for further study to clarify the action on cardiovascular function in renovascular hypertensive rats. Specifically the effect on blood pressure and heart rate were studied in rat by oral administration, and the effect on arterial vasculature were studied in isolated rat aortic ring.

CHAPTER II
LITERATURE REVIEW

Cardiovascular regulation

Homeostatic mechanisms regulate cardiovascular activity to ensure that tissue blood flow, or called tissue perfusion, is the demand for oxygen and nutrients. When a group of cells becomes active, the circulation to that region must increase to deliver the necessary oxygen and nutrients and to carry away the waste products and carbon dioxide that they generate. Then, the goal of cardiovascular regulation is to ensure that these blood flow changes occur at an appropriate time, in the right area, and without drastically altering blood pressure and blood flow to any vital organs.

Factors involved in the regulation of cardiovascular function include the followings (19) :

Neural control of blood pressure and blood flow

The nervous system is responsible for adjusting cardiac output and peripheral resistance to maintain adequate blood flow to vital tissues and organs. The cardiovascular centers responsible for these regulatory activities via the cardiac centers and vasomotor centers of the medulla oblongata. The cardiac centers include a cardioacceleratory center that increases cardiac output through sympathetic innervation and a cardioinhibitory center that reduces cardiac output through parasympathetic innervation. As the vasomotor center of the medulla oblongata primarily controls the diameters of the arterioles. Inhibition of the vasomotor center leads to vasodilation, that reduces peripheral resistance. Stimulation of the vasomotor center causes vasoconstriction that increases peripheral resistance. Moreover, the cardiovascular center detect

changes in tissue demanded by monitoring arterial blood, especially blood pressure by the baroreceptor reflexes and pH by chemoreceptor reflexs.

Baroreceptor Reflexes

Baroreceptor reflexes are autonomic reflexes that adjust cardiac output and peripheral resistance to maintain normal arterial pressures. When blood pressure elevatess, the increased output from the baroreceptors sents to the medulla oblongata, where it inhibits the cardioacceleratory center, stimulates the cardioinhibitory center, and inhibits the vasomotor center resulting in decreasing sympathetic outflow. In addition, the vagus nerves release acetylcholine, which reduces the rate and strength of the cardiac contractions, lowering cardiac output. This pattern is reversed if blood pressure becomes abnormally low. The three major baroreceptor populations respond to alterations in blood pressure at key locations within the cardiovascular system.

1. Aortic baroreceptors are located within the aortic sinuses, pockets in the walls of the ascending aorta adjacent to the heart.

2. Carotid sinus baroreceptors are located in the walls of the carotid sinuses, expanded chambers near the bases of the internal carotid arteries of the neck. Because pressure changes at this location affect the blood flow to the brain, the carotid sinus reflex is both extremely sensitive and quite important.

3. Atrial baroreceptors, in the wall of the right atrium, monitor the blood pressure there and at the venae cavae, the end of the systemic circuit. The responses produced by the atrial reflex differ from those of the aortic and carotid reflexs. Under normal circumstances, the heart pumps arterial blood into the aorta at the same rate that it is arriving at the right atrium. When blood pressure rises in the atrium, the atrial baroreceptors stimulate the cardioacceleratory center, increasing cardiac output to remove venous blood and atrial pressure returns to normal.

Chemoreceptor reflexes

The chemoreceptor reflexes respond to changes in the carbon dioxide levels, oxgen levels, or pH in the blood and cerebrospinal fluid. The chemoreceptors involved are sensory neurons found in the carotid bodies, located in the neck near the carotid sinus, and the aortic bodies, situated near the arch of the aorta. Any of these changes leads to a stimulation of the cardioacceleratory and vasomotor centers. This elevates arterial pressure and increases blood flow through peripheral tissues. Chemoreceptor output also affects the respiratory centers in the medulla oblongata. As a result, a rise in blood flow and blood pressure is associated with an elevated respiratory rate. Coordination of cardiovascular and respiratory activity is vital, because accelerating tissue blood flow is useful only if the circulating blood contains adequate oxygen. In addition, a rise in the respiratory rate accelerates venous return through the action of the respiratory pump.

Hormones

The endocrine system provides both short-term and long-term regulation of cardiovascular performance.

Epinephrine (E) and norepinephrine (NE)

E and NE from the adrenal medullae increase cardiac output and peripheral vasoconstriction similar to sympathetic activation.

The renin-angiotensin system (RAS)

The kidney provides the long-term control of blood pressure by altering the blood volume. The renin-angiotensin system preserves end – organ perfusion by regulating extracellular fluid volume, sodium and water balance, and cardiovascular activity. Thus, this system is important for blood pressure

stability and extracellular fluid volume homeostasis (20). Baroreceptors in the kidney respond to reduced arterial pressure (and to sympathetic stimulation of β-adrenoceptors) by releasing the enzyme renin, from juxtaglomerular cells. Renin is a step – limiting enzyme whose sysnthesis is influenced by the hydrostatic pressure sensed at the glomerular afferent arterioles, angiotensin II levels, and the quantity of sodium delivered to the macula densa. Renin acts to cleave angiotensinogen synthesized by the liver, forming angiotensin I (Ang I). Angiotensin-converting enzyme (ACE) then converts angiotensin I to angiotensin II (Ang II) (21). Ang II is acted in an autocrine and paracrine manner (22). The actions of Ang II on intrarenal hemodynamics are critical to the blood pressure – raising effect (23). Ang II exerts its actions in target organs and tissues by binding to both Ang II type 1 and 2 (AT_1 and AT_2 receptors). Ang II plays a mandatory role in the induction of hypertrophy by directly inducing the molecular events of early cardiac growth (24 – 26). Its synthetic machinery is upregulated in hypertrophied rat (27) and human (28) myocardium, and it seems to be required for the growth of stretched neonatal myocytes *in vitro* (29). In the kidney, Ang II cause sodium and water retension (30) and efferent arteriolar vasoconstriction (31). It also stimulates the secretion of ADH (32) by the pituitary and aldosterone (33) by the adrenal cortex, and the two complement one another. At the cellular level, Ang II promotes proliferation (34). Most of these effects of Ang II appear to be mediated through the AT_1 receptor (35).

Angiotensin-converting enzyme (ACE)

Angiotensin-converting enzyme is a zinc metalloenpeptidase, which functions as a C-terminal peptidyl dipeptidase, present as a membrane-bound in endothelial cells, in epithelial or neuroepithelial cells and in the brain and as a soluble form in blood and numerous body fluids (36). ACE converts Ang I to Ang II by removal of the C-terminal dipeptide, His-Leu. Thus, ACE is strategically poised to regulate the vasocontrictive, salt-retentive and hypertrophic properties of Ang II.

In the treatment of hypertension, inhibition of the ACE is established as one modern therapeutic principle. Angiotensin-converting enzyme inhibitor (ACEI) have achieved widespread usage. They lower blood pressure and decrease total peripheral vascular resistance (TPVR) by interfering with the formation of Ang II, usually with little effect on heart rate or cardiac output (37). ACEI have an established role, either as monotherapy or in combination, particularly with diuretics, in the management of essential hypertension. They are the preferred initial drug treatment in patients with hypertension with coexistent heart failure, myocardial infarction with systolic dysfunction, type 1 diabetes with proteinuria and renal insufficiency in the absence of bilateral renal artery stenosis (38). Then, ACEI were developed as therapeutic agents targeted for the treatment of hypertension because are considered effective, safe, and well tolerated (39).

Antidiuretic hormone (ADH)

In addition to its water – conserving effect on the kidneys, ADH also responds to an increase in the osmotic concentration of the plasma. The immediate result is a peripheral vasoconstriction that elevates blood pressure.

Atrial natriuretic peptide (ANP)

ANP release is stimulated by increased blood pressure. Action of ANP is reduction of blood volume and blood pressure by (1) promoting the loss of sodium ions and water at the kidneys, (2) increasing water losses at the kidneys by blocking the release of ADH and aldosterone, (3) reducing thirst, (4) blocking the release of E and NE, and (5) stimulating peripheral vasodilation.

Mechanism of decreasing blood pressure

Arterial blood pressure is regulated within a narrow range to provide adequate perfusion of the tissues without causing damage to the vascular system, particularly the arterial intima. Arterial blood pressure is directly proportional to the product of the cardiac output and the peripheral vascular resistance. In both normal and hypertensive individuals, cardiac output and peripheral resistance are controlled mainly by two overlapping control mechanisms: the baroreflexes mediated by the sympathetic nervous system, and the renin-angiotensin system. Most antihypertensive mechanisms are reducing of cardiac output and/or decreasing of peripheral resistance (40). In accordance with mechanisms of action, the antihypertensive mechanisms can be classified as follows:

(1) Increasing sodium and water excretion

Inhibiting tubular reabsorption of ions and water causes a decrease in extracellular volume, resulting in a decrease in cardiac output and renal blood flow.

(2) Interfering the sympathetic control of arterial pressure (41)

Many mechanisms can reduce sympathetic tone by acting at the following sites:

a. afferent pathways by increasing the sensitivity of peripheral receptors to pressor stimuli

b. central nervous system by controlling sympathetic activity and consequently arterial pressure

c. sympathetic ganglia by reducing the transmission of impulses

d. sympathetic nerve endings by reducing the release of norepinephrine

e. vascular α-adrenoceptors by blocking the vasoconstrictor effect of norepinephrine

f. cardiac β-adrenoceptors by blocking cardiac stimulant effects of sympathetic stimulation

(3) Vasodilation

The direct-acting smooth muscle relaxation of vascular smooth muscle decreases vascular peripheral resistance and therefore decreases blood pressure.

The inhibition of ACE inhibited the production of Ang II resulted in alleviating the increased blood pressure effect of renin-angiotensin system.

The inhibition effect of ACE involves not only decreasing Ang II but also increasing bradykinin. Because ACE is identical with kininase II, the enzyme controlling the breakdown of bradykinin. The inhibition of ACE is capable of enhancing the vasdodilation effect of bradykinin (42).

Animal models of hypertension

In cardiovascular research, animal models have allowed the study of cardiovascular diseases in the early stages, as well as the investigation of the mechanisms of the pathogenesis of cardiovascular diseases and the effects of drug intervention. The use of rats as animal models is rational from the economic viewpoint and many techniques have been developed to measure relevant functional parameters. These are the mimic of human hypertension in rat models.

Table 1. Some rat models of hypertension (43)

Hypertension	Model
Systemic	Spontaneously Hypertensive Rats (SHRs)
	Mineralocorticoids (DOCA – salt)
	NO synthase inhibition (L – NAME administration)
	Transgenics (TGR)
	Diabetic hypertensive rats (STZ – SHR, Zucker)
Pulmonary	Monocrotaline
	Hypoxia (normobaric, hypobaric)
Renal	Renal artery occlusion (1K1C, 2K1C)

Systemic hypertension

- Spontaneously hypertensive rats (SHRs)

The SHR is a useful model because the compounds which lower blood pressure in SHR also lower blood pressure in hypertensive humans. The SHR is a chronic stable model producing symptoms which are predictable and controllable and avoiding difficult or life-threatening technical interventions (43).

- Mineralocorticoid (DOCA-salt)

This model showed a markedly depressed renin-angiotensin system. Selye *et al* (44) was the first to demonstrate that deoxycorticosterone acetate (DOCA) produces hypertension in rats. There is increased DOCA-induced reabsorption of salt and water leading to increased blood volume and hence increased BP. There is also increased secretion of vasopressin leading to water retention and vasoconstriction.

- NO synthase inhibition (L-NAME administration)

SHRs when given a non-selective nitric oxide synthase inhibitor, Nω-nitro-L-argininemethyl ester (L-NAME), for 4 weeks develop time and dose-dependent hypertension (45).

- Transgenic rats (TGR)

Several transgenic rat lines expressing candidate genes for hypertension have been produced. Introducing an additional renin gene, the murine Ren-2 gene, into the germ line of rats results in transgenic hypertensive rat strain, TGR (mREN2) 27, with an overexpression of renin. Genetic linkage studies have shown that the components of RAS are associated with this type of hypertension. TGR model may be useful for studying the role of local RAS in hypertension (46).

- Diabetic hypertensive rats

Rapid injection of streptozotocin to adult rats produces many of the characteristic cardiovascular and renal features of human with uncontrolled insulin-dependent diabetes (47), even though these rats can survive without exogenous insulin for at least 12 weeks and are usually normotensive. Streptozotocin administration to SHR produces a model of hypertensive insulin-

dependent diabetic humans. These rats show progressive cardiac deterioration (48) and have been used to measure responses of antihypertensive drugs on hypertrophy and vascular permeability (49).

Pulmonary hypertension

In human pulmonary hypertension, pulmonary artery systolic and mean pressures exceed 30 and 20 mm.Hg, respectively, at rest or pulmonary artery mean pressure exceeds 30 mm.Hg during excerise (50).

- Monocrotaline

Subcutaneous administration of a single dose of the Crotalaria alkaloid, monocrotaline (60-105 mg/kg), to rats has been used as a non-invasive, slowly-developing, haemodynamically relevant model for primary pulmonary hypertension leading to right ventricular hypertrophy (51,52).

- Hypoxia

Secondary pulmonary hypertension caused by hypoventilation can be produced in rats as a model for humans by prolonged exposure to normobaric hypoxia (for example, 2 week with 10% O_2) (53). Hypoxia in rats produces characteristic morphological changes in the pulmonary vasculature, especially an extension of smooth muscle into more peripheral pulmonary arteries and reduction in their number (54).

Renovascular hypertension

Renovascular hypertension develops in response to renal ischaemia; models involve restricting blood flow by clips on the renal arteries (55,56).

2 Kidney, 1 Clip (2K1C) Goldblatt hypertension

The first animal model of hypertension is developed by Harry Goldblatt, the renal artery of a dog was clipped to produce a secondary form of hypertension (56), thereby initiating the development of a wide array of animal

models for hypertension. The model of two - kidney, one - clip (2K1C) Goldblatt hypertension, wherein one renal artery is constricted and the contralateral kidney is left intact. In the presence of hemodynamically sufficient unilateral renal artery stenosis, the kidney distal to the stenosis is rendered ischemic, activating the renin-angiotensin system, and producing high levels of Ang II, causing a "vasoconstrictor" type of hypertension. The degree of renal artery stenosis necessary to produce hemodynamically significant reductions in perfusion, triggering renal ischemia and activation of the renin-angiotensin system, generally dose not occur until a reduction of 80% or more in both lumen diameter and cross – sectional area of the renal artery takes place. Lesser degrees of renal artery constriction do not initiate this sequence of events. The model of 2K1C Goldblatt hypertension implies that the contralateral (nonaffected) kidney is present, and that its renal artery is not hemodynamically significantly narrowed. As illustrated, the "contralateral kidney" demonstrates suppressed renin production and undergoes a pressure natriuresis, presumably because of Ang II – initiated vasoconstriction and sodium retention, leading to systemic elevation of blood pressure that results in suppression of renin release and enhanced excretion of sodium (57).

The course of experimental 2K1C hypertension may be divided into three sequential phases.

In phase I, renal ischemia and activation of the renin-angiotensin system are of fundamental importance, and in this early phase of experimental hypertension, the blood pressure elevation is renin – or Ang II – dependent. Several days after renal artery clamping, renin levels fall, but blood pressure remains elevated. This second phase of experimental 2K1C hypertension may be viewed as a pathophysiologic transition phase.

In phase II, during this transition phase salt and water retention are observed as a consequence of the effect of hypoperfusion of the stenotic kidney, augmented proximal tubular reabsorption of sodium and water and Ang II – induced stimulation of aldosterone secretion contribute to this sodium and water retention. In addition, the high levels of Ang II stimulate thrist, which further augments expansion of the extracellular fluid volume. The expanded

extracellular fluid volume results in a progressive suppression of peripheral renin activity.

In phase III, after several weeks, a chronic phase ensues wherein unclipping the renal artery of the experimental animal does not lower the blood pressure. This failure of "unclipping" to lower the blood pressure in this chronic phase (III) of 2K1C hypertension is due to widespread arteriolar damage to the "contralateral kidney" consequent to prolonged exposure to high blood pressure and high levels of Ang II. In this chronic phase of 2K1C renovascular hypertension, extracellular fluid volume expansion and systemic vasoconstriction are the main pathophysiologic abnormalities. The pressure natriuresis of the "contralateral kidney" blunts the extracellular fluid volume expansion caused by the "stenotic kidney" but as the "contralateral kidney" suffers vascular damage from extended exposure to elevated arterial pressure, its excretory function diminishes and extracellular fluid volume expansion persists (58). This hypertensive animal model is suggested to represent renovascular hypertension occurred in human (59).

Screening of ACE inhibition activity

The ACE inhibition assay constitutes a good model for the rapid screening of plant extracts, as the inhibition of ACE is established as a therapeutic principle for the treatment of hypertension. A number of *in vitro* screening studies have been made on the ACE inhibitory activities of higher plants by measurement the amount of products cleaved from substrate analog by ACE, using spectrophotometry (60,61), fluorometry (17,18,62), and HPLC (10-16,63,64).

Yun *et al* (60) investigated 27 plant species from Korean folk medicine used to treat hypertension or related diseases by using spectrophotometric assay modified from the method of Cushman and Cheung (65) which used to study the activity of *in vitro* ACE from rabbit lung. This method measured the production of hippuric acid from hippuryl-L-histidyl-L-leucine (hip-his-leu) by ACE activity. Because of the insufficient spectral difference between hip-his-leu

and hippuric acid, the amount of hippuric acid formed could be spectrophotometry determined after seperation from unreacted hip-his-leu (65).

Friedland and Silverstein (66) developed the spectrofluorometric method from the spectrophotometric assay, which was not very sensitive and required a long incubation and fastidious techniques, for measuring ACE activity in serum. Similar substrate, hip-his-leu, was used, but the amount of his-leu, instead of hippuric acid, was measured by forming fluorescence of histidyl moiety with the aldehyde group of *o*-phthaldialdehyde. Inokuchi *et al* (17) applied the method of Friendland and Silverstein to investigate the *in vitro* ACE inhibitory activity of the plants by using benzyl-gly-his-leu as substrate. The amount of his-leu from the cleaving activity of ACE after incubation with the plant extracts were measured. They investigated 50 % methanol extracts of 65 crude drugs from 60 plant species, used in Japanese or Chinese folk medicine to treat hypertension. Fourteen from 65 plant extracts showed ACEI activity greater than 50 % at the concentration of 20 μg/ml (17). They also investigated the effect of the tannin fractions of plants with high ACEI activity on other peptidase activity. It was found that the plant extracts at dose 20 μg/ml showed inhibiting effect on the activity of carboxypeptidase B, leucine aminopeptidase, trypsin and chymotrypsin, but in lesser extent as compared to the effect on ACE. However, it had no significant effect on the activity of kallikrein (17,18).

Elbl and Wagner (63) developed an *in vitro* assay for the detection of ACE inhibitors in plant extracts using chromophore- and fluorophore-labelled tripeptide dansyltriglycine as substrate, which is cleaved by ACE into dansylglycine and diglycine. The product dansylglycine and unreacted substrate are separated and quantified by reversed phase of HPLC with UV detection. This method was claimed to reduce the interference from plant constituents by UV absorption or fluorescence emission. Hansen *et al* (11) modified the technique of Elbl and Wagner by developing elution system which decreased the time for seperating dansylglycine and dansyltriglycine and reported that the crude extract of water, 96 % ethanol and acetone of 7 from 31 plant species from India, China and Chile, exhibited more than 50 % ACEI activity. This method was used to screen for *in vitro* ACEI activity of plants from several

countries included Reunion Island (12), India (13,14), South Africa (15) and Brazil (16). From these studies, several medicinal plants had more than 50 % ACEI activity.

Recently, an *in vitro* screening of ACE inhibitor from plant extracts using colorimetric assay was compared with HPLC assay described above. This colorimetric assay used hippuryl-glycine-glycine as the substrate. The cleaved product, glycine-glycine, by ACE was subsequent reacted with trinitrobenzenesulfonic acid and the absorbance was determined. The colorimetric method showed good correlation with HPLC assay with correlation coefficients 0.9935 and 0.9034, respectively, for captopril and plant extracts. The colormetric method involved only inexpensive reagents and equipments and required shorter time assay than HPLC method (67).

Several classes of compound in plants have been shown *in vitro* ACEI activity. These are proanthocyanidins (68,69) flavonoids (64,68-71), xanthones (72), tannin (62,73), triterpenoids (74,75), benzoquinones (76), alkaloid (77).

The tannin fractions isolated from seeds of *Areca catechu* L. which exhibited high *in vitro* ACEI activity was investigated for blood pressure lowering effect. Both oral (100 mg/kg) and intravenous (10 mg/kg) administration of the extract, showed the reduction in blood pressure in spontaneous hypertensive rats (SHR). In normotensive rats, using the same doses as in SHR, oral administration did not affect the blood pressure, while intravenous administration reduced blood pressure but in a lesser amount than SHR (62).

Alstonia scholaris R.Br.

Botanical description

Alstonia scholaris R.Br.(Apocynaceae), popularly known as Devils tree, Tin pet or Phaya sattaban in Thai common name, is 13 – 26 m in hight with an erect trunk, grey bark and copiously lenticellate, glabrous and whorled branches. Its leaves is simple, whorled, usually 5 – 7 in a whorl at the end of

an year's growth, 8.7 – 16.2 cm long, 3 – 5.5 cm broad, oblong or obovate – lanceolate, acute at base, obtuse, rounded or emarginate at apex, very faintly crenate, glabrous, bright green and shining above, paler with a white "bloom" beneath, rather thick, lateral veins numerous, slender, node prominent, petioles very short with a blunt hooked fleshy process on the upper surface at the base. Its flowers are greenish – white, regular, bisexual, nearly sessile in small, cymose clusters, terminating whorls or umbellate branches of erect, pubescent panicles which are 7.5 – 10 cm long ; bracts ovate, pubescent ; sepals 5, segments imbricate, pubescent ; petals 5, fused into a cylindrical, wide corolla – tube, 6 mm long, pubescent, hairy within about 2/3 way down, lobes truncate, overlapping to the left, convolate ; disk absent ; stamens 5, inserted in the upper part of the corolla - tube, alternating with its lobes ; anthers distinct, pointed, introrse, dehiscing longitudinally ; ovary superior, 2 – carpellary, carpels distinct, hairy ; style single, filifrom, thickened at the summit ; fruit follicles 30 -45 cm long, very slender, cylindrical, pendulous, becoming completely everted after dehiscence ; seeds numerous, oblong, flat with a fringe of hair at both ends (78).

Chemical components of the *Alstonia scholaris* R.Br. bark

The major constituents of the bark of *A. scholaris* are the alkaloids B-6-7-seco-angustilobine, 17-*O*-acetyl-echitamine, N-(B)-demethyl-echitamine, losbanine, tubotaiwine (79), ditaine (80), ditamine, echitenine (81), echitamidine (81,82), echitamine (81-84), echitamine chloride (85), porphyrine (86).

The studied on the alcoholic extract of the root bark of *A. scholaris* found macralstonine, *O*-methylmacralstonine, pleiocarpamine, talcarpine, villalstonine (87). In addition, *O*-acetylmacralstonine, alstomacroline, alstomacrophylline, alstomerine, alstonerine, alstophylline, 20-epi-antirhine, macrocarpamine, N_b-oxide-villalstonine, alkaloids others are presented in *A. macrophylla* (88). Another non-nitrogenous bitter, alstonine, has been reported from *A. constricta* (89).

Moreover, the bark of *A. scholaris* contained terpenes, β-amyrin acetate (85), betulinic acid (90), lupeol acetate (91), 3-acetoxy oxandran (92). In the root bark of *A. boonei,* α-amyrin, α-amyrin linoleate, α-amyrin palmitate, lupeol, lupeol palmitate, lupeol linoleate, are presented (93).

Vashi and Patel (84) found amino acid (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, methionine, phenylalanine, proline, hydroxyproline, threonine, tryptophan) in the bark of *A. scholaris*.

A. scholaris bark has others compounds, caffeic acid, sucrose (94), β-sitosterol (91).

Ethnopharmacology activities of *Alstonia scholaris* R.Br. bark

Alstonia scholaris has been used as a folk medicine in Thailand to treat several diseases. The decoction of *A. scholaris* bark of this plant are used for relieving dysentery, vermifugel, coryza, bronchitis (95-100), fever, diarrhea, enteropathy (100,101) haemogogic, malaria, cough, diabetes (100). It has also been used to treat heart diseases and hypertension (102), leucorrhoea (103) in India.

Pharmacological activities of *Alstonia scholaris* R.Br. bark

The 50 % alcoholic extract of stem bark had anticancer activity to human sarcoma in the embryonated egg (104). *A. scholaris* was reported as cytotoxic against the human cancer cell lines (88), antitumoral (105). It also was found to possess pronounced activity in human lung adenocarcinoma and large cell carcinoma cell lines (87). It also had antineoplastic activity in HeLa cells (106). It had been reported to have hepatoprotective activity by inhibiting liver injuries induced by carbon tetrachloride, β-D-galactosamine, acetaminophen and ethanol (107). In addition, it had antihypertensive activity (104,108). The effect on cardiovascular function of *A. scholaris* have been reported from 2 screening studies. Dhar Ml *et al.* (104), screening for various biological activities of Indian plants, reported that 50 % alcoholic extract of stem bark had hypotensive effect

in anesthesized dog when injected intravenously at the dose of 50 mg/kg. The screening for pharmacological activity of Thai medicinal plants, including *A. scholaris*, which are the ingredients in traditional cardiovascular preparations, showed that 50 % of alcoholic extract of bark given to anesthesized dog by intravenous administration at the dose of 100 mg of plant material/kg caused moderate decrease in heart rate with slight increase in blood pressure (108).

The medicinal plants that have been reported to have the blood pressure lowering effects are *Alpinia galanga* (L.), *Artocarpus heterophyllus* Lam., *Hibiscus rosa-sinensis* L. (109), *Alstonia scholaris* (L.) R.Br. (104), *Centella asiatica* (L.) Urb. (110-112), *Hibiscus sabdariffa* L.(113-116), *Jasminum sambac* (L.) Aiton (108,117) *Lagerstroemia speciosa* (L.) Pers. (109,118) *Nelumbo nucifera* Gaerth. (108) *Ocimum tenuiflorum* L. (108,119) *Pandanus amaryllifolius* Roxb. (120) *Piple betle* L. (121,122). Some of them are also claimed to use as diuretic and decreased blood pressure in traditional preparation. These are *C. asiatica* (95-98,123-125), *H. sabdariffa* L.(100,124), *L. speciosa* (96,100), *N. nucifera* (126) and *P. amaryllifolius* (95,97,123,126).

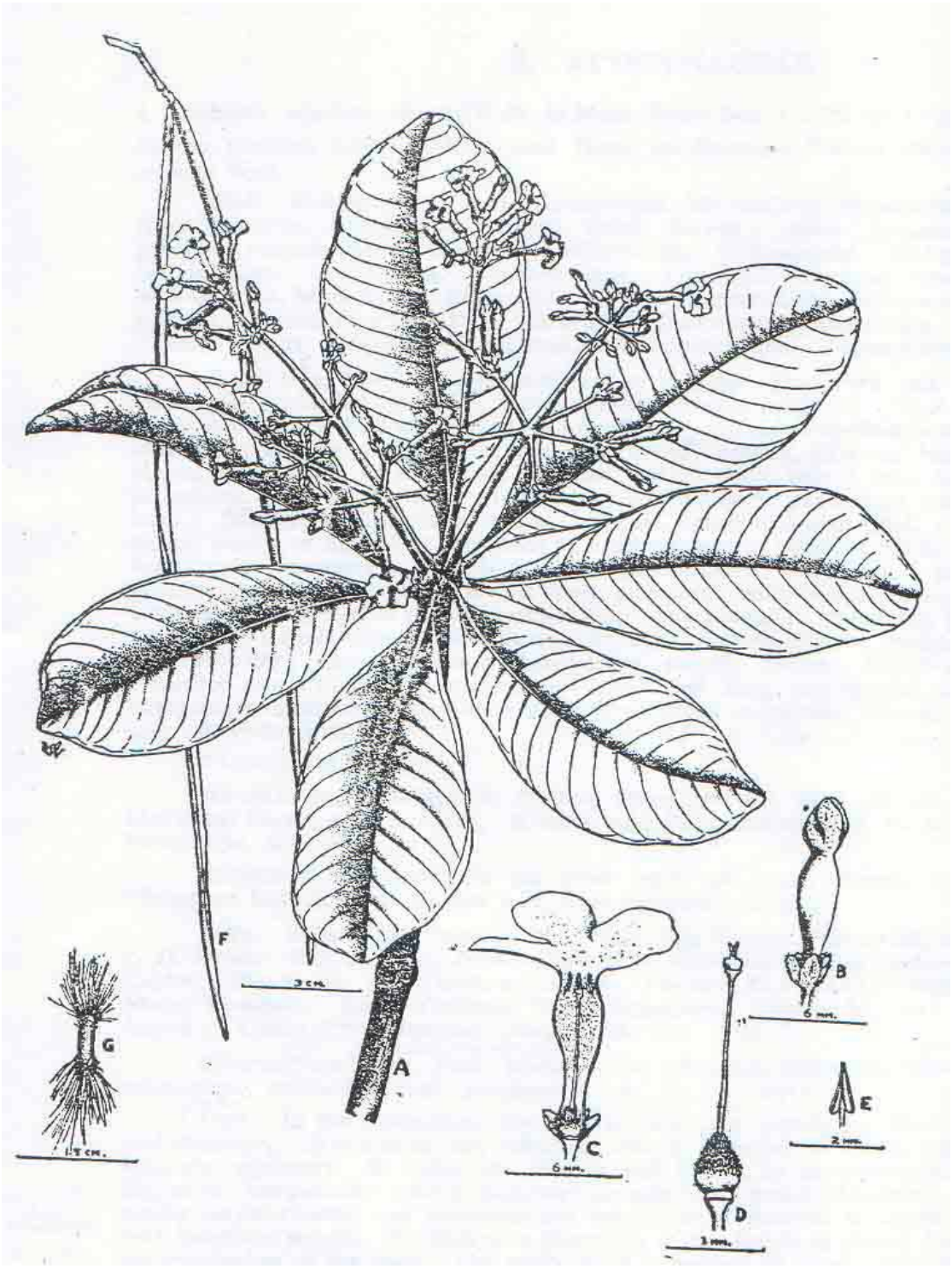

Figure 1. Line drawing of *Alstonia scholaris* R.Br.(78).
A = branch with leaves and panicle, B = flower bud, C = longitudinal section of a flower, D = pistil, E = stamen, F = fruit, G = seed

CHAPTER III
MATERIALS AND METHODS

Materials

1. Animals

Male Wistar rats (The National Laboratory Animal Center, Salaya, Mahidol University)

2. Chemicals

Ascorbic acid	Merck, Germany
Angiotensin converting enzyme	Sigma Chemical Co., U.S.A.
Calcium chloride dihydrate	Carlo ebar reagenti, France
Captopril	Sigma Chemical Co., U.S.A.
D-glucose anhydrous	Ajax Finechem, Australia
Dimethylsulphoxide	Labscan Asia Co., Ltd., Thailand
Dipotassium phosphate	Sigma Chemical Co., U.S.A.
Hippuryl-L-Histidine–L-Leucine	Sigma Chemical Co., U.S.A.
Magnesium sulphate heptahydrate	May & Baker Ltd, Englland
Norepinephrine	Sigma Chemical Co., U.S.A.
o-Phthaldialdehyde	Sigma Chemical Co., U.S.A.
L-Histidine-L-Leucine	Sigma Chemical Co., U.S.A.
Potassium chloride	Riedel-De HaËn ag, Germany
Sodium hydrogen carbonate	Carlo ebar reagenti, France
Sodium chloride	APS finechem Co., Australia
Sodium hydroxide	Mallinckrodt Baker, Mexico
Sodium pentobarbital	Ceva Sante Animale, France

3. Equipments

Air pump	
Blood pressure recorder	Panlab s.l., Spain
Centrifuge apparatus	Hettich, Germany
Disposable syringe 1, 2.5, 5, 10 ml.	
Elastic rubber	
Esophageal tube feeding	
Fluorophotometer	Perkin Elmer, U.K.
Force – displacement transducer FT03G	Glass Instrument, U.S.A.
Grinder machine	Arthur H. Thomas, U.S.A.
Isolated organ bath chamber	Ugo basile, Italy
Lamp 100 watt	
Lyophilizer	
Magnetic stirrer	Ikamag®, Germany
Micropipettes (100 – 1000 μl)	Pipetman, France
Micropipettes (20 – 200 μl)	Pipetman, France
Micropipettes (1 – 10 μl)	Socorex, Switzerland
Mixture	Vortex, U.S.A.
Organ bath	
pH meter	Orion 420A, U.S.A.
Porcelain dish	
Rat restrainer	
Shaking water bath	Heto, Denmark
Surgical operating set	
Ultrasonic bath	Elma Transsonic 660/H, Germany
Windo Graf Electrophysiology Monitor	Gould Instrument System, U.S.A.

Methods

1. Preparation of plant extracts

Forty nine specimens from 30 plant species were used in this study. They were divided into 2 groups, the medicinal and mangrove plants. The name of the plants and their yields were summarized in Appendix A.

1.1 Water extract of medicinal plants

Fifteen specimens from 12 medicinal plants were used. *O. tenuiflorum* and *P. betle* were bought from local market, Chan road, Bangkok, Thailand. The other eleven specimens from 8 medicinal plants, except *C. asiatica* juice and *H. sabdariffa* extract received from Miss. Sompong Muangnongwa (110) and Professor Nuntavan Bunyapraphatsara, respectively, were collected from Siriruckachati Medicinal Plant Garden, Mahidol University, and identified by Associate Professor Wongsatit Chuakul, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University

All plants were dried in hot air oven at 50°C more than 24 hours. The dried material was powdered by grinding machine and extracted with water. Plants powder was extracted with distilled water (1 g:10 ml), boiling for 15 minutes and let stand until reaching the room temperature. Then, the decoction was filtered through a layer of cotton wool on muslin cloth. The filtrate (plant extract) was freezedried by lyophilizer. The lyophilized extract was kept in a tight container at temperature -20°C until used.

1.2 Ethyl acetate extract of mangrove plants

The ethyl acetate extract of thirty four specimens from 18 species of mangrove plants received from Professor Nuntavan Bunyapraphatsara were used. All mangrove extracts had antioxidant activity. They were collected from the mangrove areas in Nakorn Srithammarat, Samutsongkram, and Samutsakorn

Province. The plant material was refluxed with methanol for 1 h, then the extract was filtered through filter paper, and the filtrate was extracted by partition with several portions of ethyl acetate in separatory funnel until the color of the ethyl acetate portion is pale yellow. All of the ethyl acetate extract were combined and dried under vacuum. The dried crude extract was kept in refrigerator until use.

2. Animal preparation

Male Wistar rats weighing between 100 –120 grams were obtained from the National Laboratory Animal, Salaya, Mahidol University. The animals were housed in the animal room of Faculty of Pharmacy, Mahidol University, which controlled temperature at 20 - 22 °C and 12-hour cycle light for at least one week before being used in the experiment. They were fed with regular rat chow and water *ad libitum.*

The experimental protocal for studying in animals was approved by the committee on animal use for research and education of Faculty of Pharmacy, Mahidol University.

2.1 Preparation of (2K1C) Goldblatt hypertensive rats

Rats were anesthesized with sodium pentobarbital (50 mg./kg. intraperitoneal). The left kidney was exposed through a small flank incision in the left side of abdomen. The renal artery of the left kidney was separated from the renal vein. A u-shaped silver clip (approximated size 1 x 7 x 1 mm. with 6 mm. cleft and 1 x 0.3 mm opening width) was placed on the exposed left renal artery. The kidney was then gently pushed back into the retroperitoneal cavity. The muscle layer was sutured, and the skin incision was sewed with sterile thread (127-128). Systolic blood pressure (SBP) was measured weekly after surgery. The rats were considered as hypertensive rats when SBP were over 180 mm Hg. The experiments in hypertensive rats were performed usually within 4-6 weeks after placement of the clip. At the end of experiment the rats were

killed and the silver clip was removed and cleaned, so that it can be used repeatedly.

2.2 Isolated aortic ring preparation

The isolated aortic ring experiment was performed on the thoracic aorta taken from the rats anesthezied with ether. The thoracic cavity was opened by initiating an incision above the diaphragm and below the xiphoid process. The skin and ribs were cut through an anteriority. The ribs cage was lifted up and folded over anteriorly to reveal the thoracic cavity. The heart and lung were lifted to expose the thoracic aorta lying atop the spinal cord. The area above aortic arch was marked by the arterial clamp for excision a segment of aortic ring by cutting above the clamp and the opposite end of the thoracic aorta. The thoracic aortic ring was removed rapidly and placed in an aerated porcelain dish containing Kreb-Henseleit solution. (KH solution composed of 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4.7H_2O$, 1.1 mM KH_2PO_4, 24 mM $NaHCO_3$, 2.5 mM $CaCl_2.6H_2O$ and 4.5 mM glucose. The pH was adjusted to a range of 7.35 to 7.45 with diluted HCl solution) (129). The thoracic aortic ring was cleaned of adhering fat and connective tissue and cut into 7 mm segment. The hook was made by passing two small iron strings into the lumen of aortic ring under a resting tension of 0.5 g. One iron string was made the lower hook and fastened to the fixed hook in the terminal of the stiring rod. The other string was made the upper hook and fastened to the force-displacement transducer which was connected to the Graf Electrophysiology monitor. The thoracic aortic segment was allowed to equilibrate for 45 minutes in organ bath contained 30 ml KH solution continually aerated with a 95% O_2 and 5% CO_2 mixture. The temperature was kept constant at 37 °C by the isolated organ bath chamber.

3. Measurement

3.1 Angiotensin converting enzyme inhibition activity

The screening of inhibitory action on ACE activity were determined by spectrofluorometric method described by Inokuchi *et al* (17). In brief, Histidyl – L - Leucine (his - leu), the product from the conversion of the substrate analogue, Hippuryl -L - Histidyl - L – Leucine, were measured by formation of a fluorescent adduct with OPA. (see Appendix B) The fluorescence was read in triplicate at excitation wavelength at 360 nm and emission wavelength at 485 nm by the Fluorophotometer (Figure 2).

The measured fluorescence intensity from formation of the fluorescent adduct of OPA and his - leu refered to the activity of the ACE. The amount of his - leu produced by the enzyme was obtained from the standard curve of his–leu which was obtained from the same assay protocal except adding the various concentration of his - leu solution instead of substrate solution in the enzyme incubation. The ACEI activity of plant extract was determined by substracting the amount of his – leu produced in the absence of sample solution. (see Appendix B) The level of inhibition was calculated by comparison with the amount of his - leu obtained from the control.

The ACEI activity of the plant extract was compared to captopril by using the standard curve of captopril which was obtained from enzyme incubation with captopril instead of sample solution. (see Appendix B)

3.2 Blood pressure by tail cuff method

The systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MAP) were measured by indirect blood pressure measurement of caudal artery in conscious rats using indirect blood pressure recorder (tail cuff method) which were shown in Figure 3. Tail cuff is inflated and then deflated. Pulsation disappear when cuff is inflated. When cuff is deflated pulsations start appearing when pressure in the cuff equals systolic pressure. The cuff is attached to a

tail cuff sphymomano-meter and pressure transducer and BP is recorded on a chart. Prior to the experiment, the rats were regularly trained at least one week to get used to the tail cuff method by placing them in rat restrainers to restrict their movement and giving the tail cuff pressure for familization of the animal with these procedures. On the experimentation, the rats were prewarmed about 30 minutes by two 100 watt lamps for sufficient vasodilation of caudal artery, then the rats were fixed in restrainers and waited for 2 minutes for reducing the stress from warming, before starting the measurement. Training the animal and warming the tail are required for this method (130-132).

3.3 Heart rate

The heart rate was determined from pulse rate obtained stimultaneously in blood pressure measurement by tail cuff method.

3.4 Tension of isolated aortic ring

The ring of thoracic aorta was given force 1 g and equilibrated for 1 hr. The KH solution was replaced every 30 min interval during the equilibration period. The force of contraction was measured by a force-displacement transducer with appropriate preamplifier and recorded on chart paper of the Graf Electrophysiology recorder which were shown in Figure 4.

4. Experimental procedure

Part 1. *In vitro* screening for ACEI activity

All of the plant extracts were redissolved in 99.5 % DMSO. The lyophilized powder of *C. asistica*, *H. rosa-sinensis*, *H. sabdariffa*, *J. sambac O. tenuiflorum* and *P. betle* were also dissolved in distilled water. Then, the extract solution were centrifuge at 5100 u for 10 minutes, and the supernatant were used for assay.

The angiotensin converting enzyme inhibitor activity of plant extract were evaluated by determining the formation of conjugated his - leu and OPA and expressed as percentage of ACE inhibition.

Part 2. Effect of water extract of *A. scholaris* on cardiovascular function in 2K1C hypertensive rats

The dose of *A. scholaris* extract per kg which showed ACEI activity at the same level of captopril at dose 25 mg/kg was used as the starting dose to study the cardiovascular function in 2K1C hypertensive rats. If these was no response on SBP, the dose of the extract was increased gradually. From the preliminary study, the lowest dose which showed SBP reduction was 0.75 g of dried bark/kg. Thus the dose 0.75, 1.5, 3 and 6 g of dried bark/kg were used for study in experiment 2.1 These doses were also used in experiment 2.2. The concentration of captopril and the *A. scholaris* extract in the organ bath were calculated from the concentration of those in the estimated blood volume from body weight of rat.

Experiment 2.1 Effect on blood pressure and heart rate

The lyophilized powder were redissolved in distilled water.

Captopril (25 mg / kg) was used as the positive control. It was administered by esophageal intubation in conscious rat and the blood pressure and heart rate was detected at 0, 15, 30, 45, 60 and 90 minutes after administration. The experiment was repeated in the same rat by giving *A. scholaris* extract at the doses of 0.75, 1.5, 3 and 6 g. of dried bark/kg after at least 3 day wash out period.

Experiment 2.2 Effect on the contraction of isolated aortic ring

The lyophilized powder was redissolved in distilled water

The response of isolated aortic rings to norepinephrine and *A. scholaris* extract were examined as followed.

- Response to norepinephrine

Norepinephrine was added in cumulative concentration of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M. The force of contraction were continuously recorded until getting the maximum contraction. The tissue was washed with KH solution for several times and waited for 20 minutes or until returning to basal level. A submaximal dose of NE, 10^{-6} M, was selected for further study.

- Response to captopril

The response to NE 10^{-6} M were measured until getting maximal contraction. The tissue was washed with KH solution for several times and waited for 20 minutes or until returning to basal level. The response to NE 10^{-6} M was repeated after adding captopril 0.42 mg/ml for 5 minutes. The force of concentration was continuously recorded until getting the maximum contraction. Then, the tissue was washed with KH solution for several times and waited for 20 minutes or until returning to basal level.

- Response to *A. scholaris* extract

The experiment were repeated as above in the same tissue by adding *A. scholaris* extract at the concentrations 12.7, 25, 51 and 102 mg of dried bark/ml, respectively.

Statistical analysis

The data were expressed as mean ± the standard error of mean (SEM). The paired t-test was used to compare the effects of *Alstonia scholaris* at various time points with the pre-administration. The unpaired t-test was used to compare between normal and hypertensive groups. The comparison between the response to NE alone and NE in the presence of captopril or *A. scholaris* extract of isolated aortic ring contraction were made with a paired t-test. One way analysis of variance (ANOVA) of Tukey was used to compare the values for more than two groups.

The p-value of less than 0.05 ($p < 0.05$) was considered to be statistically significant difference.

Figure 2. Fluorophotometer (Perkin Elmer, U.K.).

Figure 3. Equipments for studies on the systolic blood pressure and heart rate in rats by Blood pressure recorder (Panlab s.l., Spain).

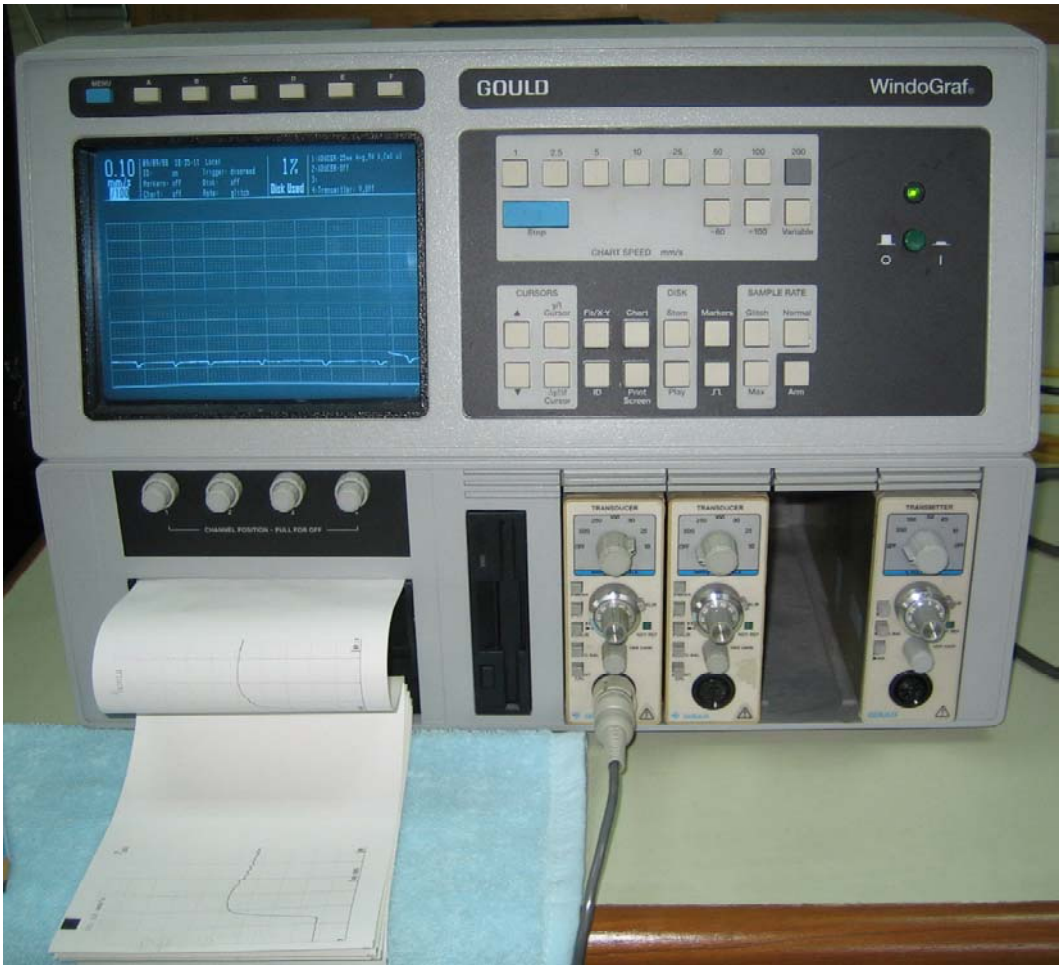

Figure 4. Windo Graf Electrophysiology Monitor (Gould Instrument System, U.S.A).

CHAPTER IV
RESULTS

Part 1. In vitro screening for the ACE inhibition activity

The percentage inhibition of ACE activity by medicinal and mangrove plant extracts were shown in Table 2. In this study, 4 medicinal plants with 5 part used and 9 mangrove plants with 13 part used exhibited the ACE inhibition activity. The water extract of *A. galanga* rhizome, *A. scholaris* bark and *L. speciosa* bark showed 51.37, 52.89 and 51.17 % inhibition of ACE activity, respectively. The ethyl acetate extract of mangrove plants which had ACE inhibition activity greater than 50 % were *A. alba* branches and fruits, *B. maunwongyathiae* bark and seeds, *B. gymorrhiza* leaves, *B. parviflora* leaves, *P. paludosa* young pods and *X. granatum* leaves and stem bark.

The medicinal plant extract was selected for further study in examining the actions on cardiovascular function due to their good water solubility. Among those the bark extract of *A. scholaris* was used because it had highest ACE inhibitor activity.

Table 2. The percentage of ACE inhibition activity of water extract of medicinal plants and ethyl acetate extract of mangrove plants, compared to captopril

Plant species	part screened[a]	local name	solvent[b]	inhibition of ACE activity (%)[c]	1 mg extract equivalent to captopril (mg)
I Medicinal plants (water extract)					
Alpinia galanga (L.) Willd.	Rh	Kha daeng	d	51.37	0.0091
	Rh	Kha lueang	d	0.00	-
Alstonia scholaris (L.) R.Br.	Bk	Phaya sattaban	d	52.89	0.0094
Artocarpus heterophyllus Lam.	L	Khanun	d	0.00	-
Centella asiatica (L.) Urb.	L	Bua bok	w	0.00	-
Hibiscus rosa-sinensis L.	L	Chaba	w, d	0.00	-
Hibiscus sabdariffa L.	C	Krachiep	w, d	0.00	-
Jasminum sambac (L.) Aiton	L	Mali	w, d	0.00	-
Lagerstroemia speciosa (L.) Pers.	Bk	Inthanin	d	51.17	0.0091
	L		d	28.13	0.0048
Nelumbo nucifera Gaertn.	R	Bua khao	d	0.00	-
	R	Bua daeng	d	0.00	-
Ocimum tenuiflorum L.	Ar	Ka phrao	w, d	0.00	-
Pandanus amaryllifolius Roxb.	L	Toei hom	d	10.23	0.0018
Piper betle L.	L	Phlu	w, d	0.00	-

Table 2. The percentage of ACE inhibition activity of water extract of medicinal plants and ethyl acetate extract of mangrove plants, compared to captopril (continued)

Plant species	part screened[a]	local name	solvent[b]	inhibition of ACE activity (%)[c]	1 mg extract equivalent to captopril (mg)
II Mangrove plants (ethyl acectate extract)*					
Acanthus ebracteatus Vahl.	L	Ngueak plamo dok khao	d	0.00	-
Avicennia alba Blume.	B	Samae khao	d	55..91	0.0100
	Fl		d	0.00	-
	Fr		d	96.39	0.0170
	L		d	0.00	-
Avicennia marina (Forsk.) Vierth	B	Samae thale	d	0.00	-
	Fl		d	15.63	0.0027
	L		d	47.65	0.0085
Barringtonia maunwongyathiae	Bk	Chik dong	d	89.07	0.0158
	L		d	0.00	-
	S		d	73.00	0.0127
Bruguiera gymorrhiza (L.) Savigny	B	Phangka hua sum dok daeng	d	0.00	-
	Fl		d	0.00	-
	L		d	51.76	0.0091
Bruguiera parviflora Wight & Am.ex Griff.	L	Thua dam	d	73.65	0.0130

Table 2. The percentage of ACE inhibition activity of water extract of medicinal plants and ethyl acetate extract of mangrove plants, compared to captopril (continued)

Plant species	part screened[a]	local name	solvent[b]	inhibition of ACE activity (%)[c]	1 mg extract equivalent to captopril (mg)
Flagellaria indica L.	Fr	Wai ling	d	0.00	-
Lumnitizera racemosa Willd.	Fr	Fat dok khao	d	49.67	0.0091
Molineria latifolia Herb.	L	Phrao nok nhum	d	19.70	0.0033
	R		d	0.00	-
Nypa fruticans Wurmb.	Sm	Chak	d	0.00	-
Phoenix paludosa Roxb.	Py	Peng	d	94.80	0.0167
Rhizophora apiculata Blume.	B	Kong kang bai lek	d	0.00	-
	L		d	0.00	-
Rhizophora mucronata Poir.	L	Kong kang bai yai	d	0.00	-
Sonneratia caseolaris (L.) Engl.	L	Lam phu	d	0.00	-
Suaeda maritina (L.) Dum.	P	Cha khram (green leaves)	d	0.00	-
	P	Cha khram (red leaves)	d	0.00	-
Xylocarpus granatum Koen.	L	Tabun khao	d	73.28	0.0130
	Sb		d	71.90	0.0127
Xylocarpus rumphii (Kostel.) Mabberley	B	Taban	d	0.00	-
	E		d	0.00	-

Table 2. The percentage of ACE inhibition activity of water extract of medicinal plants and ethyl acetate extract of mangrove plants, compared to captopril (continued)

Plant species	part screened[a]	local name	solvent[b]	inhibition of ACE activity (%)[c]	1 mg extract equivalent to captopril (mg)
	L		d	0.00	-
	Sd		d	0.00	-
Wedelia biflora (L..) DC	Fl	Ben chamat thale	d	0.00	-

[a]Ar=Aerial part, Bk=Bark, B=Branches, C=Calyx, E=Exocarp, Fl=Flowers, Fr=Fruits, L=Leaves, P=Pods, Py=Young pods, Rh=Rhizome, R=Roots, Sm=Stamen, Sd=Seeds, S=Stem, Sb=Stem bark.

[b]The solvent used in dissolving the extract : w = distilled water, d = 99.5 % DMSO.

[c]Final concentration of the extract solution in the ACE inhibition assay was 0.33 mg extract/ml. Data presented are the mean of three replicates.

*water soluble fraction of ethyl acetate extract.

Part 2. Effect of water extract of *Alstonia scholaris* on cardiovascular function in 2K1C hypertensive rat

Weekly SBP, DBP and MAP of rats after hypertensive induction were shown in Table 3 – 5 and Figure 5-7. At the fourth week of surgery, 70 % of rats had SBP greater than 180 mm.Hg. The mean increased SBP, DBP and MAP from week 0 to week 4 were 124 ± 4 to 190 ± 4, 71 ± 3 to 123 ± 4, and 88 ± 3 to 150 ± 4, respectively. On contrary, weekly heart rate significantly decreased at week 3 and 4 (Table 6 and Figure 8).

All rats had progressively increase in weekly body weight during hypertensive induction (Table 7 and Figure 9).

Table 3. Weekly systolic blood pressure (SBP) of rats before (week 0) and after surgical hypertensive induction

No	Week						
	0	1	2	3	4	5	6
1	110	137	160	163	186	-	-
2	135	138	164	169	204	-	-
3	127	146	178	186	197	-	-
4	141	138	190	146	188	-	-
5	133	162	159	133	174	182	-
6	110	141	167	156	174	198	-
7	116	130	176	154	199	-	-
8	110	163	167	145	177	161	198
9	129	220	170	201	209	-	-
10	128	153	188	167	192	-	-
Mean	124	153^{aa}	172^{aaa}	162^{aaa}	$190^{aaa,bb,cc,ddd}$	180^{a}	198
± SEM	4	8	3	6	4	11	0

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-hypertensive induction (week 0).

$^{bb}(p < 0.01)$: significant difference from values at week 1.

$^{cc}(p < 0.01)$: significant difference from values at week 2.

$^{ddd}(p < 0.001)$: significant difference from values at week 3.

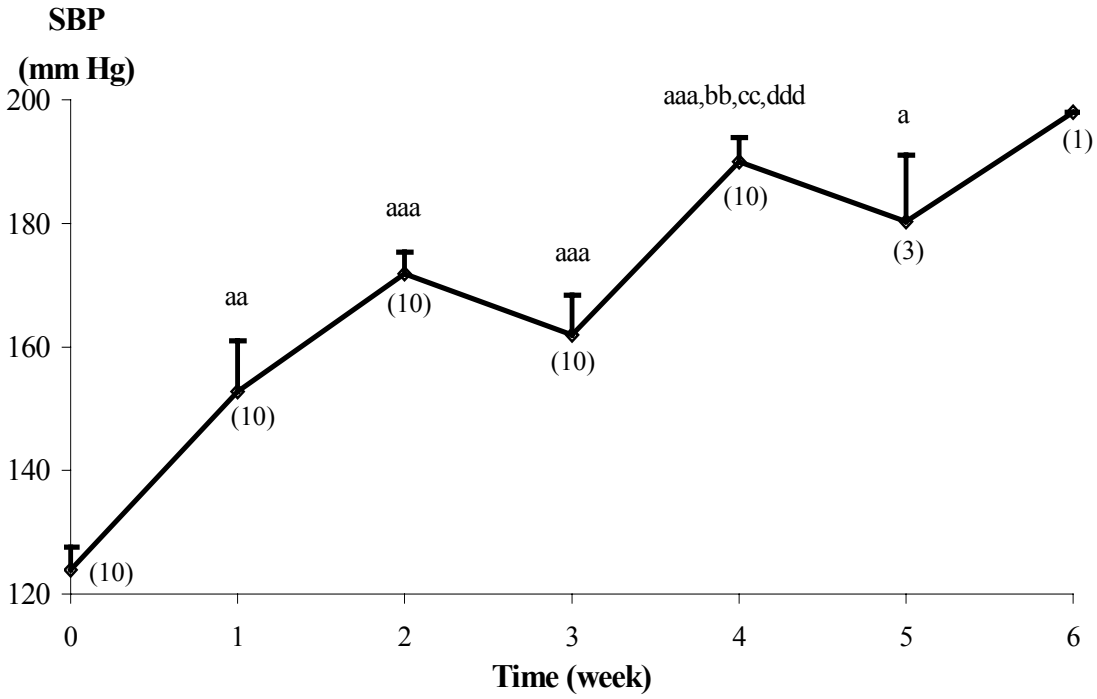

Figure 5. Weekly systolic blood pressure (SBP) of rats before (week 0) and after surgical hypertensive induction. Number in parenthesis represents the number of animal.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-hypertensive induction (week 0).

$^{bb}(p < 0.05)$: significant difference from values at week 1.

$^{cc}(p < 0.05)$: significant difference from values at week 2.

$^{ddd}(p < 0.05)$: significant difference from values at week 3.

Table 4. Weekly diastolic blood pressure (DBP) of rats before (week 0) and after surgical hypertensive induction

No	Week						
	0	1	2	3	4	5	6
1	69	89	101	110	133	-	-
2	87	80	108	100	143	-	-
3	65	97	107	117	113	-	-
4	80	82	130	83	125	-	-
5	68	83	107	78	110	104	-
6	63	86	118	114	105	125	-
7	72	80	118	112	127	-	-
8	63	101	112	76	118	116	135
9	77	166	99	150	116	-	-
10	67	85	112	85	135	-	-
mean	71	95^{a}	111^{aaa}	103^{aa}	$123^{aaa,b,c,d}$	115^{a}	135
± SEM	3	8	3	7	4	6	0.00

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre – hypertensive induction (week 0).

$^{b}(p < 0.05)$: significant difference from values at week 1.

$^{c}(p < 0.05)$: significant difference from values at week 2.

$^{d}(p < 0.05)$: significant difference from values at week 3.

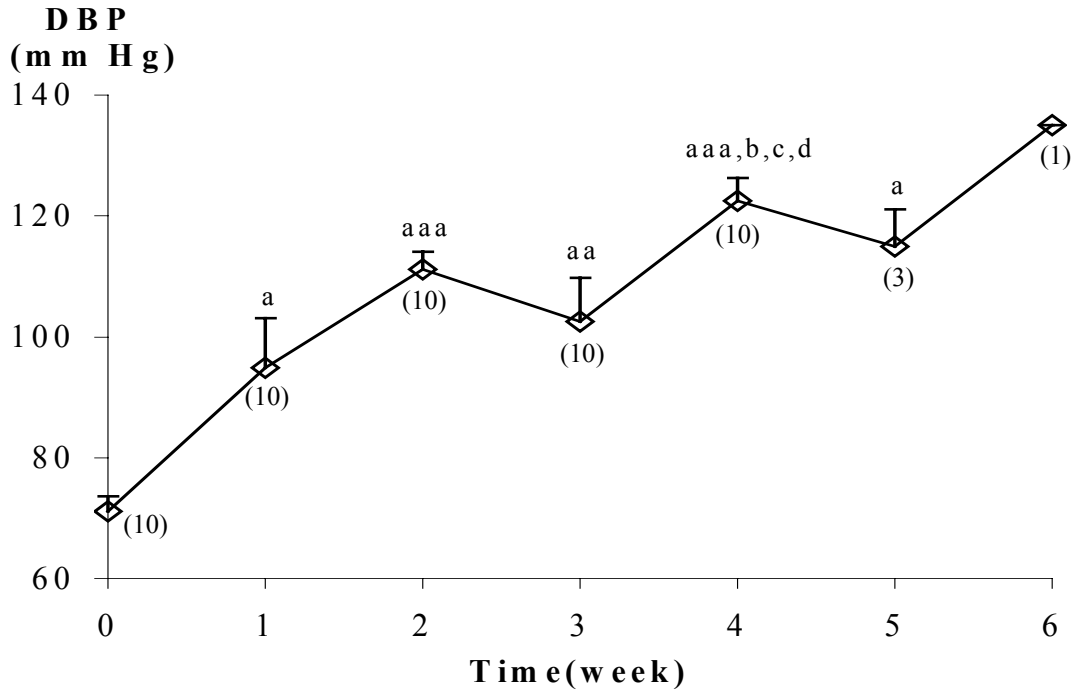

Figure 6. Weekly diastolic blood pressure (DBP) of rats before (week 0) and after surgical hypertensive induction. Number in parenthesis represents the number of animal.

All values were expressed as mean ± sem.

[a]($p < 0.05$), [aa]($p < 0.01$), [aaa]($p < 0.001$) : significant difference from their pre-hypertensive induction (week 0).

[b]($p < 0.05$) : significant difference from values at week 1.

[c]($p < 0.05$) : significant difference from values at week 2.

[d]($p < 0.05$) : significant difference from values at week 3.

Table 5. Weekly mean arterial pressure (MAP) of rats before (week 0) and after surgical hypertensive induction

No	Week						
	0	1	2	3	4	5	6
1	82	105	120	127	150	-	-
2	103	99	126	123	163	-	-
3	85	113	130	140	141	-	-
4	100	100	150	104	146	-	-
5	89	109	124	96	131	144	-
6	78	104	134	128	128	155	-
7	86	96	137	136	151	-	-
8	76	121	130	99	137	131	156
9	94	184	122	167	147	-	-
10	87	107	137	112	154	-	-
mean	88	114^{a}	131^{aaa}	123^{aa}	$145^{aaa,bb,c,d}$	$143^{a,d}$	156
± SEM	3	8	3	7	3	7	0

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-hypertensive induction (week 0)

$^{bb}(p < 0.01)$: significant difference from values at week 1.

$^{c}(p < 0.05)$: significant difference from values at week 2.

$^{d}(p < 0.05)$: significant difference from values at week 3.

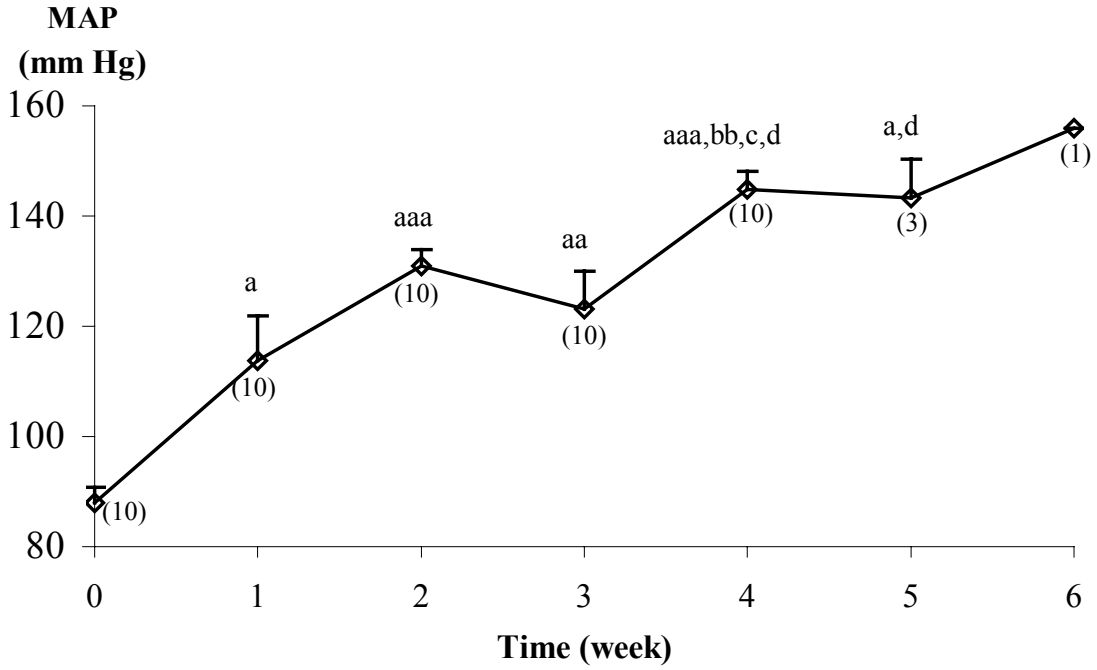

Figure 7. Weekly mean arterial pressure (MAP) of rats before (week 0) and after surgical hypertensive induction. Number in parenthesis represents the number of animal.

All values were expressed as mean ± sem

[a]($p < 0.05$), [aa]($p < 0.01$), [aaa]($p < 0.001$) : significant difference from their pre-hypertension induction (week 0).

[bb]($p < 0.01$) : significant difference from values at week 1.

[c]($p < 0.05$) : significant difference from values at week 2.

[d]($p < 0.05$) : significant difference from values at week 3.

Table 6. Weekly heart rate of rats before (week 0) and after surgical hypertensive induction

No	Week						
	0	1	2	3	4	5	6
1	443	498	387	372	359	-	-
2	418	414	349	371	320	-	-
3	408	386	356	347	363	-	-
4	402	420	362	357	286	-	-
5	412	382	383	381	332	370	-
6	445	389	397	353	324	347	-
7	413	383	362	359	299	-	-
8	513	381	375	388	324	411	391
9	297	411	332	350	345	-	-
10	318	362	389	376	380	-	-
Mean	407	403	369^{b}	$365^{a,b}$	$333^{a,bb,cc,d}$	376	391
± SEM	19	12	6	4	9	19	0

[a]($p < 0.05$) : significant difference from their pre – hypertensive induction (week 0).

[b]($p < 0.05$), [bb]($p < 0.01$) : significant difference from values at week 1.

[cc]($p < 0.01$) : significant difference from values at week 2.

[d]($p < 0.05$) : significant difference from values at week 3.

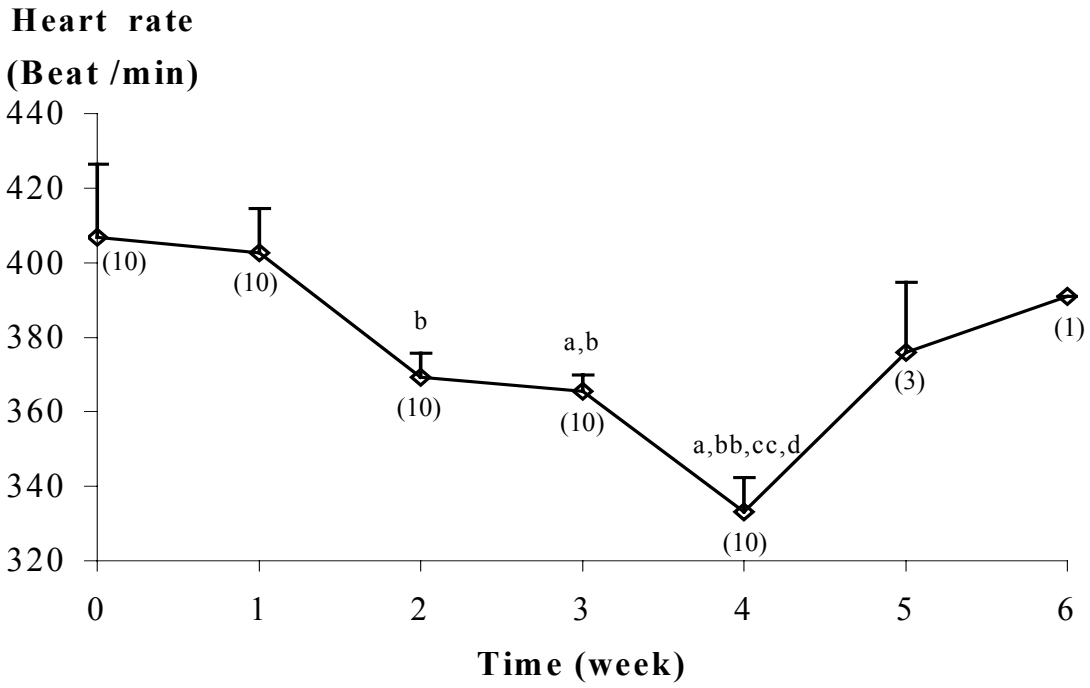

Figure 8. Weekly heart rate of rats before (week 0) and after surgical hypertensive induction. Number in parenthesis represents the number of animal.

All values were expressed as mean ± sem.

[a]($p < 0.05$) : significant difference from their pre – hypertensive induction(week 0).

[b]($p < 0.05$), [bb]($p < 0.01$) : significant difference from values at week 1.

[cc]($p < 0.01$) : significant difference from values at week 2.

[d]($p < 0.05$) : significant difference from values at week 3.

Table 7. Weekly body weight of rats before (week 0) and after surgical hypertensive induction

No	Week						
	0	1	2	3	4	5	6
1	135	207	250	286	298	-	-
2	172	233	273	298	305	-	-
3	176	239	269	294	309	-	-
4	159	204	230	255	249	-	-
5	228	252	286	314	318	368	-
6	240	248	287	318	315	325	-
7	225	261	283	319	319	-	-
8	192	214	247	292	297	349	371
9	292	263	279	303	365	-	-
10	265	299	320	335	352	-	-
mean	208	242^{aa}	$272^{aaa,bbb}$	$301^{aaa,bbb,ccc}$	$313^{aaa,bbb,ccc}$	$347^{a,b}$	371
± SEM	16	9	8	7	10	12	0

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-hypertensive induction (week 0).

$^{b}(p < 0.05)$, $^{bbb}(p < 0.001)$: significant difference from values at week 1.

$^{ccc}(p < 0.001)$: significant difference from values at week 2.

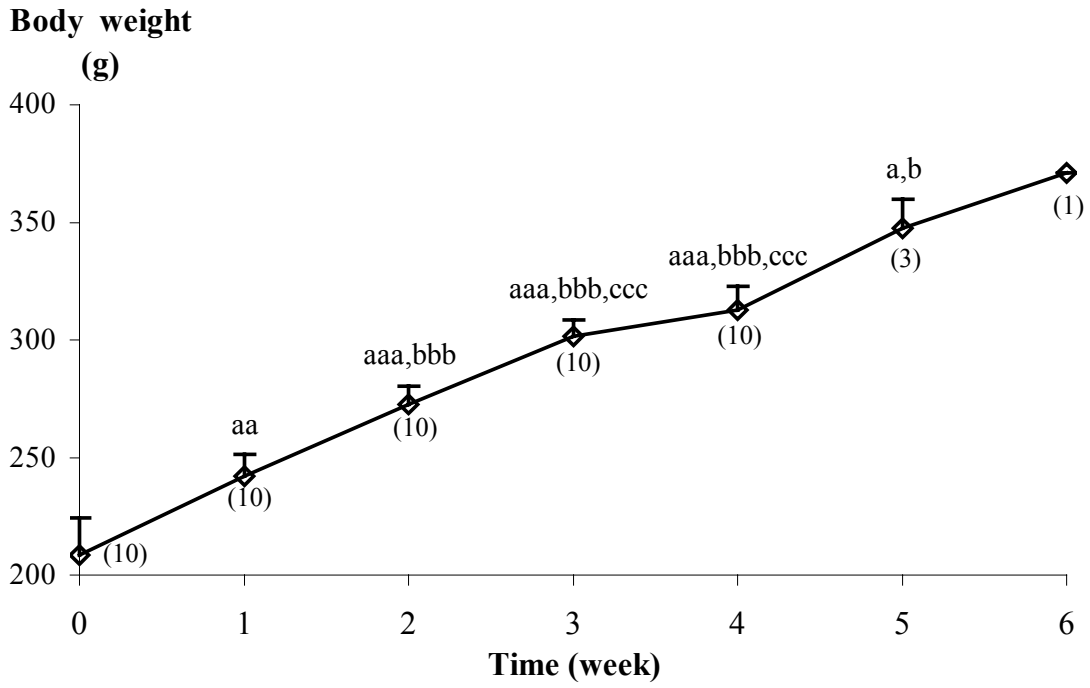

Figure 9. Weekly body weight of rats before (week 0) and after surgical hypertensive induction. Number in parenthesis represents the number of animal.

All values were expressed as mean ± sem.

[a]($p < 0.05$), [aa]($p < 0.01$), [aaa]($p < 0.001$) : significant difference from their pre-hypertensive induction (week 0).

[b]($p < 0.05$), [bbb]($p < 0.001$) : significant difference from values at week 1.

[ccc]($p < 0.001$) : significant difference from values at week 2.

Experiment 2.1 Effect on blood pressure and heart rate

2.2.1. Blood pressure

- Systolic blood pressure (SBP)

The effect of *A. scholaris* extract at the doses of 0.75, 1.5, 3, and 6 g of dried bark/kg, and captopril at dose of 25 mg/kg on SBP in hypertensive rats were shown in Table 8 and Figure 10. The percentage of SBP compared to the pre-administration levels were also summarized in Table 9 and Figure 11.

All five groups of rats had comparable basal hypertensive level of SBP.

Captopril at the dose of 25 mg/kg significantly decreased SBP throughout 90 minutes of study, with maximal effect at 90 minutes. These decreased SBP were significantly lower than all *A. scholaris* treated groups at the corresponding times from 30 to 90 minutes. The percentage SBP of captopril at 90 minutes was 72 ± 1 % of pre-administration level.

All 4 doses of *A. scholaris* extract had significant decreasing effect on SBP. *A. scholaris* extract at dose of 0.75 g/kg decreased SBP at 15, 60 and 90 minutes with maximal effect at 15 minutes (89 ± 2 %). The dose of 1.5 g/kg significantly decreased SBP at 15, 75 and 90 minutes with maximal effect at 75 minutes (93 ± 2 %). The dose of 3.0 g/kg significantly decreased SBP the whole 90 minutes of observation, while the highest dose (6 g/kg) significantly decreased SBP only at 30, 45, 75 and 90 minutes, with the maximal effect, 87 ± 2 and 91 ± 2 %, at 45 minute, respectively.

Table 8. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on systolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	198	204	187	186	194	158	175	184	191	188	154	172	168	169	191	139	175	155	167	183
2	184	185	185	204	180	156	181	156	179	184	143	180	147	183	171	138	187	188	180	157
3	181	197	186	201	182	151	177	165	184	145	144	194	162	206	176	167	197	187	157	170
4	210	182	188	190	181	177	158	187	174	173	155	204	217	182	171	156	200	186	167	173
5	190	182	186	181	181	171	168	171	178	166	172	183	165	175	165	162	164	171	181	182
6	197	198	211	209	206	127	169	207	207	213	120	184	150	200	165	127	191	220	171	185
7	201	202	182	199	193	164	151	176	175	149	156	158	182	174	162	132	171	187	182	158
8	180	182	184	198	180	147	181	166	158	177	154	170	206	148	178	151	180	173	163	157
9		201	209	186	204		173	217	166	187		185	193	161	190		202	153	167	184
10		192	202	207			170	193	192			157	186	194			196	166	174	
Mean	193	193	192	196	189	156^{aaa}	170^{aa}	$182^{a,c}$	$180^{aa,c}$	176	150^{aaa}	179^{cc}	178^{c}	$179^{aa,cc}$	$174^{a,c}$	147^{aaa}	186^{ccc}	179^{ccc}	$171^{aaa,cc}$	$172^{aa,cc}$
±SEM	4	3	3	3	4	6	3	6	4	7	5	5	7	6	4	5	4	6	3	4

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 8. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on systolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	144	197	165	197	190	145	197	178	184	200	150	193	160	176	195
2	142	189	178	195	193	147	191	166	221	174	133	181	187	190	168
3	148	168	171	170	185	143	159	176	186	172	136	182	166	185	185
4	152	172	201	171	147	148	183	197	179	163	148	173	184	167	163
5	143	164	170	189	172	151	153	167	177	167	141	158	174	180	154
6	117	194	230	200	199	124	213	195	192	191	131	185	211	192	189
7	154	177	172	172	136	164	166	165	192	149	137	160	185	180	157
8	122	178	182	143	157	143	206	183	168	157	138	191	181	172	167
9		187	151	170	203		178	173	174	191		186	168	185	201
10		170	180	171			146	181	180			154	193	167	
Mean	140^{aaa}	$180^{aa,ccc}$	180^{ccc}	$178^{a,cc}$	176^{cc}	146^{aaa}	179^{cc}	$178^{aa,cc}$	$185^{a,ccc}$	$174^{a,cc}$	139^{aaa}	$176^{aa,ccc}$	$181^{a,ccc}$	$179^{aa,ccc}$	$175^{a,ccc}$
±SEM	5	4	7	6	8	4	7	4	5	6	2	5	5	3	6

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

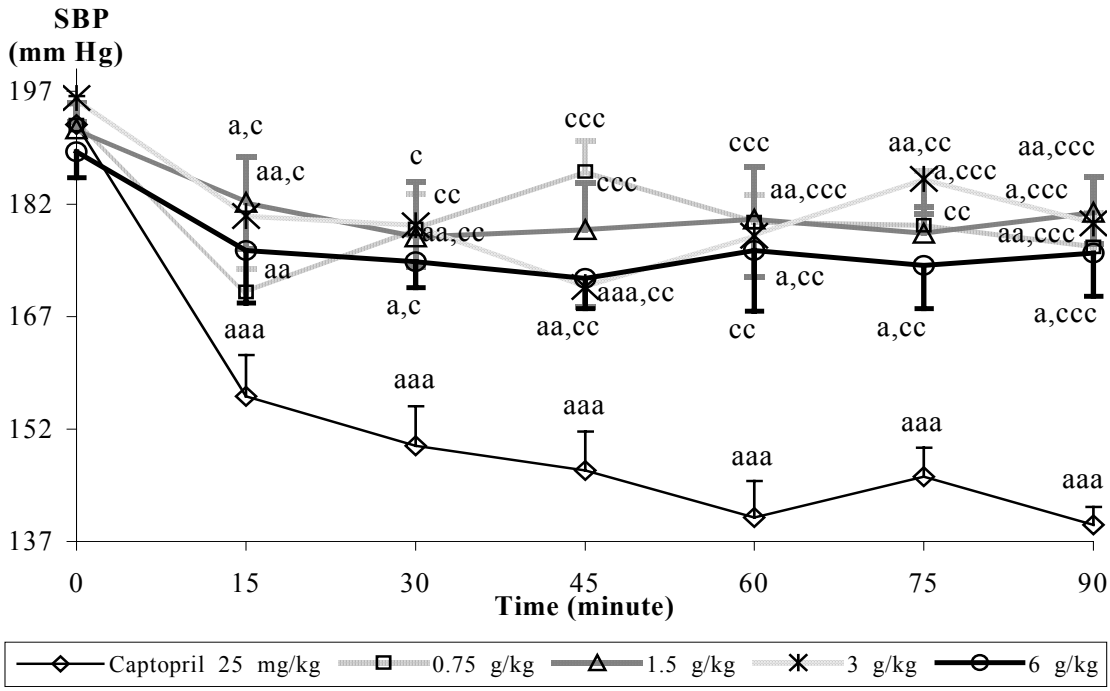

Figure 10. The effects of single oral dose of *A. scholaris* extract at 0.75, 1.5, 3, 6 g of dried bark/kg and captopril 25 mg/kg in hypertensive rats (n = 8-10) on systolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 9. The percentage of systolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	100	100	100	100	100	80	86	98	103	97	78	84	90	91	98	70	86	83	90	94
2	100	100	100	100	100	85	98	84	88	102	78	97	79	90	95	75	101	102	88	87
3	100	100	100	100	100	83	90	89	92	80	80	98	87	102	97	92	100	101	78	93
4	100	100	100	100	100	84	87	99	92	96	74	112	115	96	94	74	110	99	88	96
5	100	100	100	100	100	90	92	92	98	92	91	101	89	97	91	85	90	92	100	101
6	100	100	100	100	100	64	85	98	99	103	61	93	71	96	80	64	96	104	82	90
7	100	100	100	100	100	82	75	97	88	77	78	78	100	87	84	66	85	103	91	82
8	100	100	100	100	100	82	99	90	80	98	86	93	112	75	99	84	99	94	82	87
9		100	100	100	100		86	104	89	92		92	92	87	93		100	73	90	90
10		100	100	100			89	96	93			82	92	94			102	82	84	
Mean	100	100	100	100	100	81^{aaa}	89^{aa}	$95^{a,cc}$	$92^{aa,c}$	93^{c}	78^{aaa}	93^{c}	93^{c}	$91^{aa,c}$	$92^{aa,c}$	76^{aaa}	97^{ccc}	93^{cc}	87^{aaa}	$91^{aa,cc}$
±SEM	0	0	0	0	0	3	2	2	2	3	3	3	4	2	2	4	2	3	2	2

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 9. The percentage of systolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minute (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	73	97	88	106	98	73	97	95	99	103	76	95	86	95	101
2	77	102	96	96	107	80	103	90	108	97	72	98	101	93	93
3	82	85	92	85	102	79	81	95	93	95	75	92	89	92	102
4	72	95	107	90	81	70	101	105	94	90	70	95	98	88	90
5	75	90	91	104	95	79	84	90	98	92	74	87	94	99	85
6	59	98	109	96	97	63	108	92	92	93	66	93	100	92	92
7	77	88	95	86	70	82	82	91	96	77	68	79	102	90	81
8	68	98	99	72	87	79	113	99	85	87	77	105	98	87	93
9		93	72	91	100		89	83	94	94		93	80	99	99
10		89	89	83			76	90	87			80	96	81	
mean	73^{aaa}	$93^{aa,ccc}$	94^{ccc}	$91^{a,cc}$	93^{ccc}	76^{aaa}	93^{cc}	$93^{aa,cc}$	$95^{a,ccc}$	$92^{aa,cc}$	72^{aaa}	$92^{aa,ccc}$	$94^{a,ccc}$	$92^{aa,ccc}$	$93^{a,ccc}$
±SEM	2	2	3	3	4	2	4	2	2	2	1	2	2	2	2

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

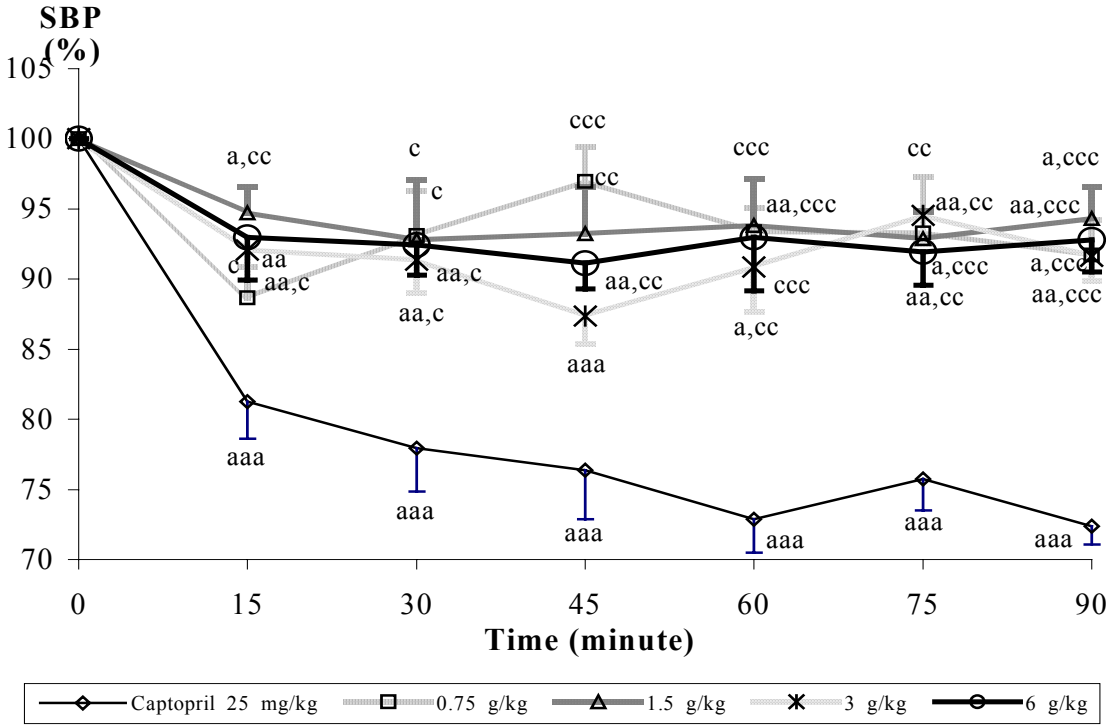

Figure 11. The percentage of systolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75, 1.5, 3, 6 g of dried bark/kg, and captopril 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding times.

- Diastolic blood pressure (DBP)

The effects of *A. scholaris* extract in hypertensive rats at the doses of 0.75, 1.5, 3, and 6 g of dried bark/kg and captopril at the dose of 25 mg/kg on DBP were shown in Table 10 and Figure 12. The percentage of DBP compared to pre-administration levels were also summarized in Table 11 and Figure 13.

All five groups of rats had comparable basal hypertensive level of DBP.

Captopril at dose 25 mg/kg significantly decreased DBP throughout 90 minutes of study with maximal effect at 60 minutes (61 ± 4 %). These decreased DBP were significantly lower than all *A. scholaris* treated groups at the corresponding time from 45 to 90 minutes.

A. scholaris extract at dose 0.75 g/kg had no effect on DBP throughout 90 minutes of observation. The next dose, 1.5 g/kg, significantly decreased DBP at 45, 75 and 90 minutes with maximal effects at 75 minutes (87 ± 3 %). Similarly to the effect on SBP, the dose 3 g/kg of *A. scholaris* significantly decreased DBP at 30 to 90 minutes with maximal effects at 45 minutes (83 ± 4 %). The highest dose, 6 g/kg, significantly decreased DBP at 30 and 45 minutes with maximal effects at 30 minutes (85 ± 5%).

Table 10. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on diastolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	131	146	130	133	136	107	129	136	161	143	121	119	116	118	106	88	110	102	107	132
2	135	116	135	143	126	85	124	85	92	135	84	122	77	100	99	82	122	124	119	80
3	126	113	117	146	114	100	134	115	128		96	142	126	150	115	113	137	134	124	119
4	131	126	125	132	107	129	119	135	125	121	105	174	167	127	117	104	139	124	103	111
5	131	125	136	109	128	108	125	114	95	93	126	121	113	89	96	111	109	102	110	120
6	141	134	165	157	145	70	108	114	159	168		136	80	147	87	64	115	119	89	100
7	132	124	118	127	129	127	99	127	108	97	139	90	134	103	103	72	116	119	133	98
8	122	109	136	135	135	90	117	102	92	127	79	95	155	99	130	89	154	98	103	128
9		150	116	126	135		115	122	106	119		120	127	100	123		138	94	108	127
10		135	109	144			89	103	140			93	127	123			116	103	115	
Mean	131	128	129	135	128	102^{aa}	116	115	121	125	107^{a}	121	122	116^{aa}	108^{a}	90^{aa}	126^{ccc}	$112^{a,c}$	$111^{aa,c}$	$113^{a,c}$
±SEM	2	4	5	4	4	7	4	5	8	9	8	8	9	7	5	6	5	4	4	6

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 10. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on diastolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	84	155	119	145	144	87	149	129	118	155	96	146	113	92	118
2	74	121	113	133	137	82	138	105	126	123	70	126	126	115	115
3	90	110	117	103	156	80	100	124	137	116	81	123	113	120	126
4	102	102	141	121	81	92	126	106	115	104	98	116	117	105	116
5	77	95	105	127	124	91	97	107	111	109	78	98	104	106	80
6	62	141	170	132	136	66	165	141	104	134	73	105	151	141	149
7	83	119	105	115	77	101	113	87	124	87	72	82	127	100	100
8	70	113	124	86	94	72	133	135	86	97	84	130	122	112	116
9		128	90	114	146		118	98	111	116		129	90	124	142
10		89	126	108			90	92	116			95	102	109	
mean	80^{aaa}	117^{cc}	121^{cc}	$118^{a,cc}$	122^{cc}	84^{aaa}	123^{cc}	$112^{aa,c}$	$115^{aa,cc}$	116^{cc}	82^{aaa}	115^{cc}	$117^{aa,cc}$	$112^{aaa,cc}$	118^{cc}
±SEM	4	6	7	5	10	4	8	6	4	7	4	6	5	4	7

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$: significant difference from captopril at the corresponding time.

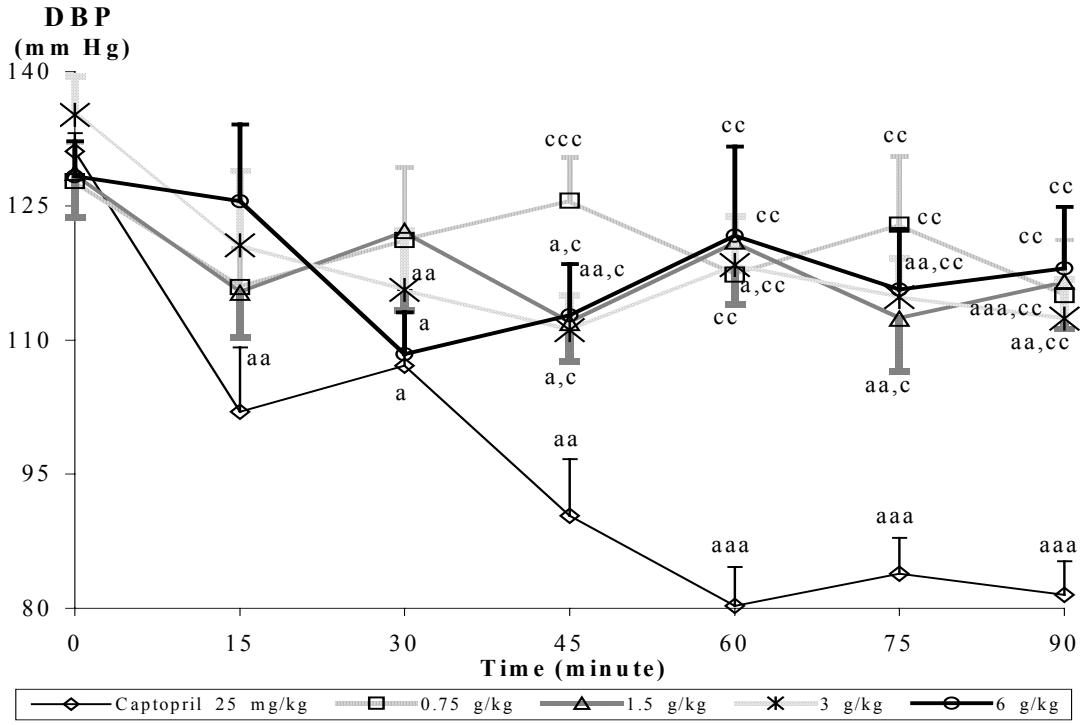

Figure 12. The effects of single oral dose of *A. scholaris* extract at 0.75, 1.5, 3, 6 g of dried bark/kg and captopril 25 mg/kg in hypertensive rats (n = 8-10) on diastolic blood pressure at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 11. The percentage of diastolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	100	100	100	100	100	82	88	105	121	105	92	82	89	89	78	67	75	78	80	97
2	100	100	100	100	100	63	107	63	64	107	62	105	57	70	79	61	105	92	83	63
3	100	100	100	100	100	79	119	98	88		76	126	108	103	101	90	121	115	85	104
4	100	100	100	100	100	98	94	108	95	113	80	138	134	96	109	79	110	99	78	104
5	100	100	100	100	100	82	100	84	87	73	96	97	83	82	75	85	87	75	101	94
6	100	100	100	100	100	50	81	69	101	116		101	48	94	60	45	86	72	57	69
7	100	100	100	100	100	96	80	108	85	75	105	73	114	81	80	55	94	101	105	76
8	100	100	100	100	100	74	107	75	68	94	65	87	114	73	96	73	141	72	76	95
9		100	100	100	100		77	105	84	88		80	109	79	91		92	81	86	94
10		100	100	100			66	94	97			69	117	85			86	95	80	
Mean	100	100	100	100	100	78^{aa}	92	91	89	96	82^{a}	96	97	85^{aa}	85^{a}	69^{aa}	100^{cc}	88^{a}	83^{aa}	88
±SEM	0	0	0	0	0	6	5	5	5	6	6	7	9	3	5	5	6	5	4	5

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 11. The percentage of diastolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	64	106	92	109	106	66	102	99	89	114	73	100	87	69	87
2	55	104	84	93	109	61	119	78	88	98	52	109	93	80	91
3	71	97	100	71	137	63	88	106	94	102	64	109	97	82	111
4	78	81	113	92	76	70	100	85	87	97	75	92	94	80	108
5	59	76	77	117	97	69	78	79	102	85	60	78	76	97	63
6	44	105	103	84	94	47	123	85	66	92	52	78	92	90	103
7	63	96	89	91	60	77	91	74	98	67	55	66	108	79	78
8	57	104	91	64	70	59	122	99	64	72	69	119	90	83	86
9		85	78	90	108		79	84	88	86		86	78	98	105
10		66	116	75			67	84	81			70	94	76	
Mean	61^{aaa}	92^{cc}	94^{cc}	88^{cc}	95^{cc}	64^{aaa}	97^{ccc}	$87^{aa,cc}$	$86^{aa,c}$	90^{cc}	62^{aaa}	91^{ccc}	$91^{a,ccc}$	$83^{aaa,c}$	92^{ccc}
±SEM	4	4	4	5	8	3	6	3	4	5	3	6	3	3	5

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

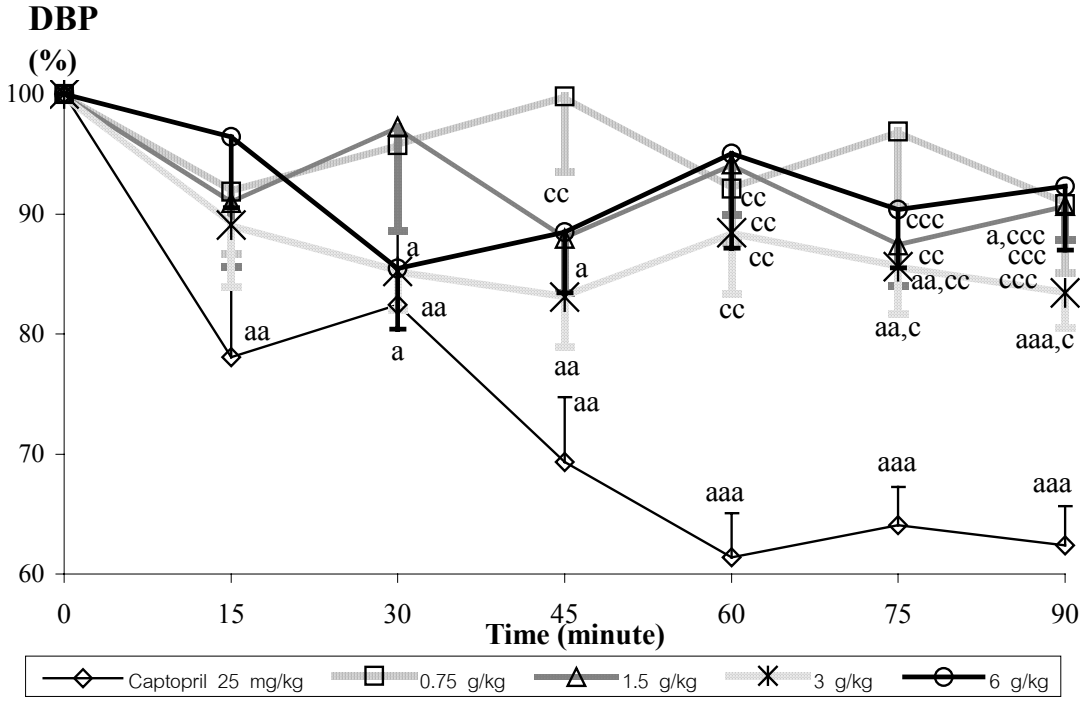

Figure 13. The percentage of diastolic blood pressure after single oral administration of *A. scholaris* extract at the doses of 0.75, 1.5, 3, 6 g of dried bark/kg, and captopril 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

- Mean arterial pressure (MAP)

The effects of *A. scholaris* extract at the doses of 0.75, 1.5, 3, and 6 g of dried bark/kg, and captopril at the dose of 25 mg/kg on MAP in hypertensive rats were shown in Table 12 and Figure 14. The percentage of MAP compared with pre-administration levels were also shown in Table 13 and Figure 15.

All five groups of rats had comparable basal hypertensive level of MAP.

Captopril at dose 25 mg/kg significantly decreased MAP throughout 90 minutes of study with maximal effect at 60 minutes (66 ± 3 %). These decreased MAP were significantly lower than all *A. scholaris* treated groups at the corresponding time from 45 to 90 minutes.

A. scholaris extract at dose 0.75 g/kg significantly decreased MAP only at 15 and 60 minutes with maximal effects at 15 minutes (90 ± 4 %). The next dose, 1.5 g/kg, significantly decreased MAP at 45, 75 and 90 minutes with maximal effect at 45 minutes (90 ± 3 %). The dose 3 g/kg of *A. scholaris* has significant effect on MAP similar to SBP and DBP with maximal effects at 45 minutes (85 ± 3 %). The highest dose, 6 mg/kg, decreased MAP significantly only at 30 and 45 minutes with maximal effect at 30 minutes (89 ± 4 %).

The results indicated that oral administration of water extract of *A. scholaris* at doses 0.75-6 g of dried bark/kg decreased SBP, DBP and MAP in 2K1C hypertensive rats.

Table 12. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on mean arterial pressure at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	153	165	149	150	155	124	144	152	171	158	132	136	133	136	134	105	131	119	127	149
2	151	139	151	163	144	108	143	108	121	151	103	141	100	127	123	100	143	143	139	105
3	144	141	140	164	126	117	148	131	146		112	159	138	148	135	131	157	151	135	136
4	157	144	146	151	131	145	132	152	141	138	121	184	183	145	135	121	159	144	124	131
5	150	144	152	133	145	129	139	133	122	117	141	141	130	117	119	128	127	125	133	140
6	159	155	180	174	165	89	128	145	175	183		152	103	164	113	85	140	152	116	128
7	155	150	139	151	151	139	116	143	130	114	144	110	150	126	122	92	134	141	149	118
8	141	133	152	156	150	109	138	123	114	143	104	120	172	115	146	109	162	123	123	137
9		167	147	146	158		134	153	126	141		141	149	120	145		159	113	127	146
10		154	140	165			116	133	157			114	146	146			142	124	134	
Mean	151	149	150	155	147	120^{aa}	134^{a}	137	140^{a}	143	122^{aa}	140	140	134^{aaa}	130^{a}	109^{aa}	145^{ccc}	$134^{a,cc}$	$131^{aa,c}$	$132^{a,cc}$
±SEM	2	4	4	4	4	6	4	5	7	8	6	7	8	5	4	6	4	4	3	5

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 12. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on mean arterial pressure at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	104	169	134	162	159	106	165	145	140	170	114	161	128	120	143
2	96	143	134	153	155	103	155	125	157	140	91	144	146	140	132
3	109	129	135	125	165	101	119	141	153	134	99	142	130	141	145
4	118	125	161	137	103	110	145	136	136	123	114	135	139	125	131
5	99	111	126	147	140	111	115	127	130	129	99	118	127	130	104
6	80	158	190	154	157	85	181	159	133	153	92	131	171	158	162
7	106	138	127	134	96	122	130	113	146	107	93	108	146	126	119
8	87	134	143	105	115	95	157	151	113	117	102	150	141	132	133
9		147	110	132	165		137	123	132	141		148	116	144	161
10		116	144	129			108	121	137			114	132	128	
Mean	100^{aaa}	$137^{a,cc}$	140^{cc}	$138^{a,cc}$	139^{cc}	104^{aaa}	141^{ccc}	$134^{aa,cc}$	$138^{aa,cc}$	135^{cc}	101^{aaa}	$135^{a,ccc}$	$138^{aa,ccc}$	$134^{aaa,ccc}$	137^{ccc}
±SEM	4	6	7	5	9	4	7	5	4	6	3	5	5	4	6

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

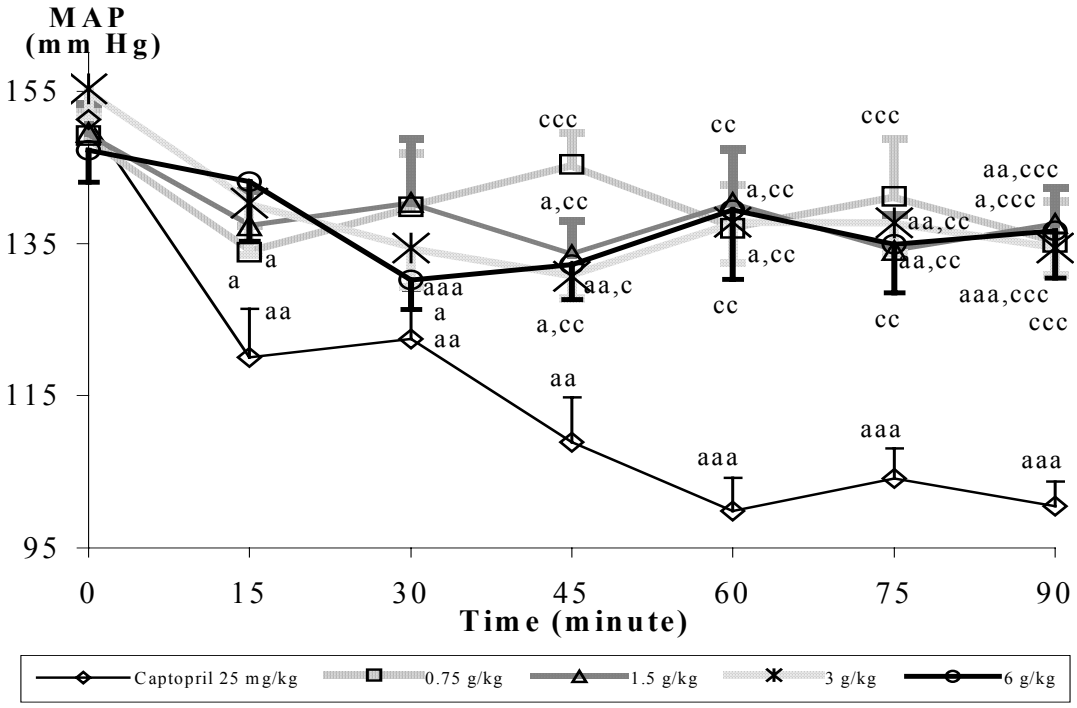

Figure 14. The effects of single oral dose of *A. scholaris* extract at 0.75, 1.5, 3, 6 g of dried bark/kg and captopril 25 mg/kg in hypertensive rats (n = 8-10) on mean arterial pressure at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 13. The percentage of mean arterial pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	100	100	100	100	100	81	87	102	114	102	86	82	89	91	86	69	79	80	85	96
2	100	100	100	100	100	72	103	72	74	105	68	101	66	78	85	66	103	95	85	73
3	100	100	100	100	100	81	105	94	89		78	113	99	90	107	91	111	108	82	108
4	100	100	100	100	100	92	92	104	93	105	77	128	125	96	103	77	110	99	82	100
5	100	100	100	100	100	86	97	88	92	81	94	98	86	88	82	85	88	82	100	97
6	100	100	100	100	100	56	83	81	101	111		98	57	94	68	53	90	84	67	78
7	100	100	100	100	100	90	77	103	86	75	93	73	108	83	81	59	89	101	99	78
8	100	100	100	100	100	77	104	81	73	95	74	90	113	74	97	77	122	81	79	91
9		100	100	100	100		80	104	86	89		84	101	82	92		95	77	87	92
10		100	100	100			75	95	95			74	104	88			92	89	81	
Mean	100	100	100	100	100	79^{aa}	90^{a}	92	90^{a}	95	81^{aa}	94	95	86^{aaa}	89^{a}	72^{aaa}	98^{ccc}	$90^{a,c}$	85^{aa}	$90^{a,c}$
±SEM	0	0	0	0	0	4	4	4	4	4	4	5	7	2	4	5	4	3	3	4

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

Table 13. The percentage of mean arterial pressure after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 (continued)

n	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	68	102	90	108	103	69	100	97	93	110	75	98	86	80	92
2	64	103	89	94	108	68	112	83	96	97	60	104	97	86	92
3	76	91	96	76	131	70	84	101	93	106	69	101	93	86	115
4	75	87	110	91	79	70	101	93	90	94	73	94	95	83	100
5	66	77	83	111	97	74	80	84	98	89	66	82	84	98	72
6	50	102	106	89	95	53	117	88	76	93	58	85	95	91	98
7	68	92	91	89	64	79	87	81	97	71	60	72	105	83	79
8	62	101	94	67	77	67	118	99	72	78	72	113	93	85	89
9		88	75	90	104		82	84	90	89		89	79	99	102
10		75	103	78			70	86	83			74	94	78	
mean	66^{aaa}	$92^{a,cc}$	94^{cc}	$89^{a,cc}$	95^{ccc}	69^{aaa}	95^{ccc}	$90^{aa,cc}$	$89^{aa,cc}$	92^{cc}	67^{aaa}	91^{ccc}	$92^{aa,ccc}$	$87^{aaa,cc}$	93^{ccc}
±SEM	3	3	3	4	7	3	5	2	3	4	2	4	2	2	4

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

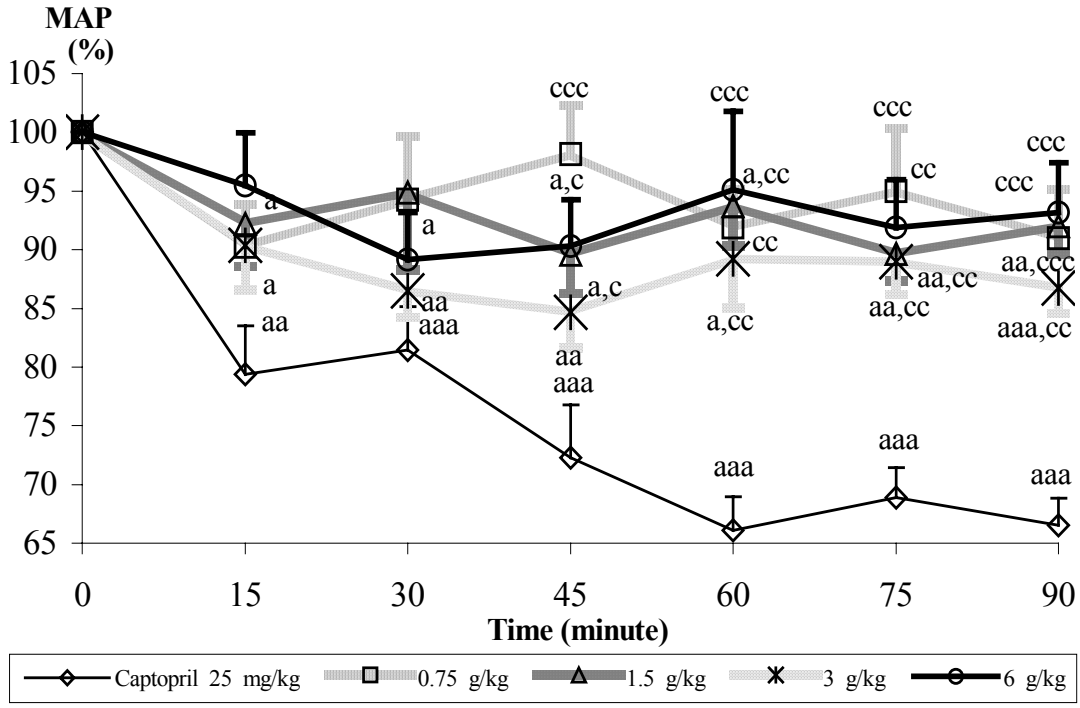

Figure 15. The percentage of mean arterial pressure after single oral administration of *A. scholaris* extract at the dose of 0.75, 1.5, 3, 6 g of dried bark/kg, and captopril 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$, $^{aaa}(p < 0.001)$: significant difference from their pre-administration (0 minute).

$^{c}(p < 0.05)$, $^{cc}(p < 0.01)$, $^{ccc}(p < 0.001)$: significant difference from captopril at the corresponding time.

- Heart rate

The effects of *A. scholaris* extract at the doses of 0.75, 1.5, 3, 6 g/kg and Captopril at the dose of 25 mg/kg on heart rate were shown in Table 14 and Figure 16. The percentage of heart rate compared to the pre-administration level were also shown in Table 15 and Figure 17.

All five groups of rats had comparable basal level of heart rate. There were no significant difference in heart rate from the pre-administration throughout 90 minutes of observation in captopril and 1.5 g/kg of *A. scholaris.* treated groups.

A. scholaris at the dose of 0.75 g/kg and 3 g/kg significantly decreased heart rate from pre-administration at 30 minutes (95 ± 2 %) and at 45 minutes (94 ± 3 %), respectively. The highest dose, 6 g/kg, significantly decreased heart rate at 75 and 90 minutes (95 ± 2 % and 94 ±1 %). There were no significant difference in heart rate between five groups.

This result indicated that water extract of *A. scholaris* at doses 0.75-6 g dried bark/kg decreased heart rate at a similar level.

Table 14. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on heart rate at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	380	385	371	359	392	401	360	387	345	373	392	364	366	329	355	383	377	386	335	351
2	367	351	361	320	357	305	318	323	322	391	321	330	320	339	369	369	355	344	320	354
3	381	363	347	343	345	383	377	354	336	351	385	336	354	358	325	377	387	366	338	330
4	331	334	286	349	348	356	318	399	335	343	338	321	332	330	334	329	325	339	347	335
5	339	370	375	336	315	359	397	321	333	320	372	342	320	324	316	383	367	306	302	315
6	399	347	399	403	361	403	399	436	336	386	341	371	437	322	387	348	362	488	328	387
7	312	347	362	299	368	341	337	352	368	341	328	331	329	330	329	336	342	316	318	336
8	340	306	376	391	382	372	308	335	374	380	358	309	346	342	360	385	339	335	335	345
9		350	345	364	357		340	360	386	341		329	350	393	347		329	336	365	340
10		380	358	366			348	357	363			320	355	332			316	334	316	
Mean	356	353	358	353	358	365	350	362	350	358	354	335[a]	351	340	347	364	350	355	330[a]	344
±SEM	11	7	9	10	7	12	10	11	7	8	9	6	11	7	8	8	7	16	6	7

[a]($p < 0.05$) : significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

Table 14. The effects of single oral dose of *A. scholaris* extract at 0.75 (A1), 1.5 (A2), 3 (A3), and 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats on heart rate at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	381	379	366	331	347	366	382	369	329	352	358	367	368	317	351
2	348	371	327	348	357	279	378	335	363	352	349	349	323	333	342
3	371	342	386	332	335	360	312	370	340	322	360	323	361	331	310
4	335	321	324	347	340	324	327	330	345	344	338	327	313	324	335
5	388	337	309	309	304	376	327	298	293	297	391	338	306	295	294
6	352	341	486	322	386	397	367	426	344	377	397	362	399	359	364
7	328	345	339	314	329	334	345	338	287	343	341	337	329	353	361
8	349	312	305	345	336	350	333	335	341	331	341	320	308	335	335
9		332	341	349	341		328	365	361	333		339	386	365	335
10		336	341	318			340	334	315			324	327	320	
Mean	357	342	352	332	342	348	344	350	332	339[a]	359	339	342	333	336[aa]
±SEM	8	6	17	5	7	13	8	11	8	7	8	5	11	7	8

[a]($p < 0.05$), [aa]($p < 0.01$) : significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

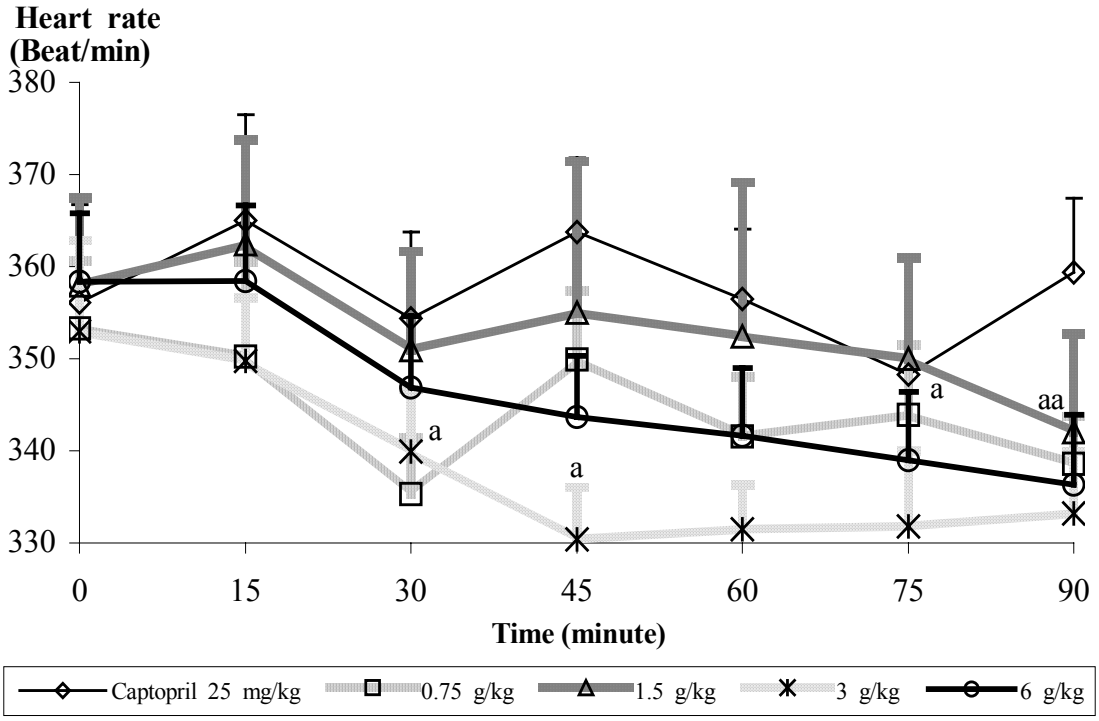

Figure 16. The effects of single oral dose of *A. scholaris* extract at 0.75, 1.5, 3, 6 g of dried bark/kg and captopril 25 mg/kg in hypertensive rats (n = 8-10) on heart rate at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

[a]($p < 0.05$), [aa]($p < 0.01$) : significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

Table 15. The percentage of heart rate after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg, and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes

No	0 min					15 min					30 min					45 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	100	100	100	100	100	106	94	104	96	95	103	95	99	92	91	101	98	104	93	90
2	100	100	100	100	100	83	91	89	101	110	87	94	89	106	103	101	101	95	100	99
3	100	100	100	100	100	101	104	102	98	102	101	93	102	104	94	99	107	105	99	96
4	100	100	100	100	100	108	95	140	96	99	102	96	116	95	96	99	97	119	99	96
5	100	100	100	100	100	106	107	86	99	102	110	92	85	96	100	113	99	82	90	100
6	100	100	100	100	100	101	115	109	83	107	85	107	110	80	107	87	104	122	81	107
7	100	100	100	100	100	109	97	97	123	93	105	95	91	110	89	108	99	87	106	91
8	100	100	100	100	100	109	101	89	96	99	105	101	92	87	94	113	111	89	86	90
9		100	100	100	100		97	104	106	96		94	101	108	97		94	97	100	95
10		100	100	100			92	100	99			84	99	91			83	93	86	
mean	100	100	100	100	100	103	99	102	100	100	100	95[a]	98	97	97	103	99	99	94[a]	96
±SEM	0	0	0	0	0	3	2	5	3	2	3	2	3	3	2	3	2	4	3	2

[a]($p < 0.05$) : significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

Table 15. The percentage of heart rate after single oral administration of *A. scholaris* extract at the doses of 0.75 (A1), 1.5 (A2), 3 (A3), 6 (A4) g of dried bark/kg and captopril (C) 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes (continued)

No	60 min					75 min					90 min				
	C	A1	A2	A3	A4	C	A1	A2	A3	A4	C	A1	A2	A3	A4
1	100	98	99	92	89	96	99	99	92	90	94	95	99	88	90
2	95	106	91	109	100	76	108	93	113	99	95	99	89	104	96
3	97	94	111	97	97	94	86	107	99	93	94	89	104	97	90
4	101	96	113	99	98	98	98	115	99	99	102	98	109	93	96
5	114	91	82	92	97	111	88	79	87	94	115	91	82	88	93
6	88	98	122	80	107	99	106	107	85	104	99	104	100	89	101
7	105	99	94	105	89	107	99	93	96	93	109	97	91	118	98
8	103	102	81	88	88	103	109	89	87	87	100	105	82	86	88
9		95	99	96	96		94	106	99	93		97	112	100	94
10		88	95	87			89	93	86			85	91	87	
mean	101	97	99	95	96	98	98	98	94	95^{a}	101	96	96	95	94^{aa}
±SEM	3	2	4	3	2	4	3	3	3	2	3	2	3	3	1

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$: significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

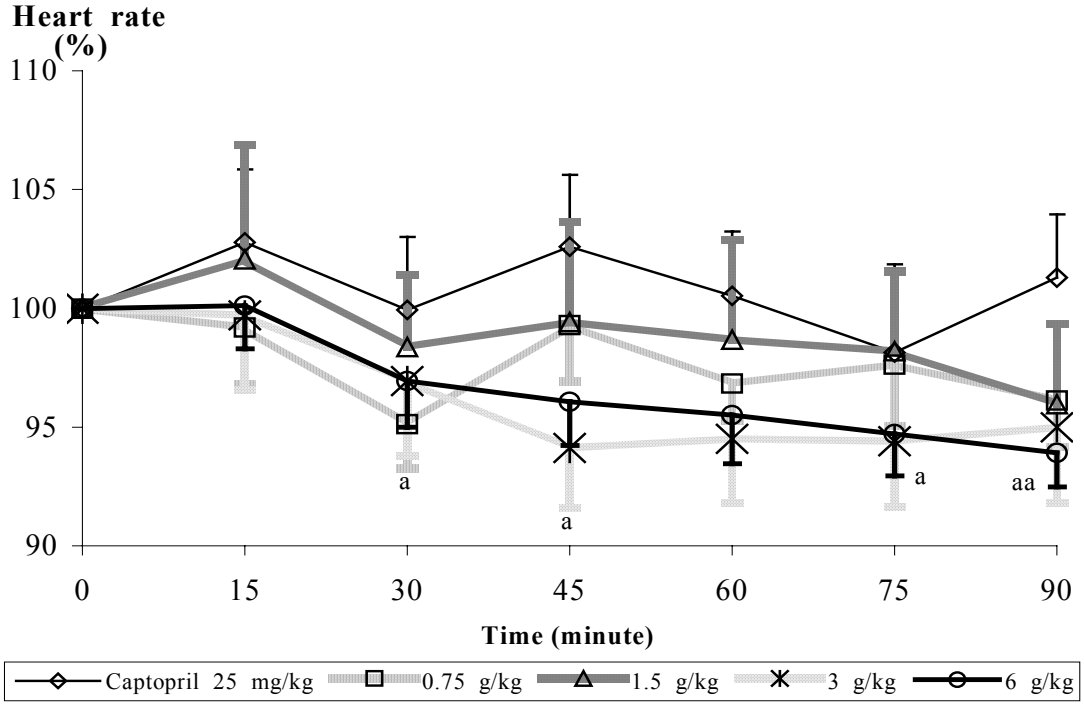

Figure 17. The percentage of heart rate after single oral administration of *A. scholaris* extract at the doses of 0.75, 1.5, 3, 6 g of dried bark/kg, and captopril 25 mg/kg in hypertensive rats at 0, 15, 30, 45, 60, 75 and 90 minutes.

All values were expressed as mean ± sem.

[a]($p < 0.05$), [aa]($p < 0.01$) : significant difference from their pre-administration (0 minute).

No significant difference among five groups at the corresponding time.

Experiment 2.2 Effect on the contraction of isolated aortic ring

2.2.1 Responses to norepinephrine (NE)

The response to NE (10^{-9}-10^{-5} M) in cumulative doses of isolated aortic ring from normal and hypertensive rats were shown in Table 16 and Figure 18. The increased force of isolated aortic rings to NE was dose-dependent manner. It was noted that the isolated aortic rings of hypertensive rat demonstrated lesser response to NE, especially at high dose, than those from normal rats. The NE 10^{-6} M was used to examine the effect of *A. scholaris* extract in the next study.

Table 16. Effects of norepinephrine $10^{-9} - 10^{-5}$ M in cumulative doses on the aortic ring contraction of normal and hypertensive rat

No	Force of contraction of aortic ring (mg)									
	Normal rat					Hypertensive rat				
	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
1	80	500	560	710	910	70	100	110	130	130
2	120	390	480	670	920	110	150	170	180	180
3	30	130	150	200	240	100	190	200	230	230
4	525	1450	1525	1650	1675	90	170	180	200	200
5	0	75	1050	1425	1450	70	90	100	110	110
mean ±sem	151 ± 96	509 ± 248	753 ± 241	931 ± 266	1039 ±249	88 ± 8	140 ± 19	152 ± 20	170 ± 22[a]	170 ± 22[a]

[a]($p < 0.05$) : significant difference from normal rats at corresponding concentration.

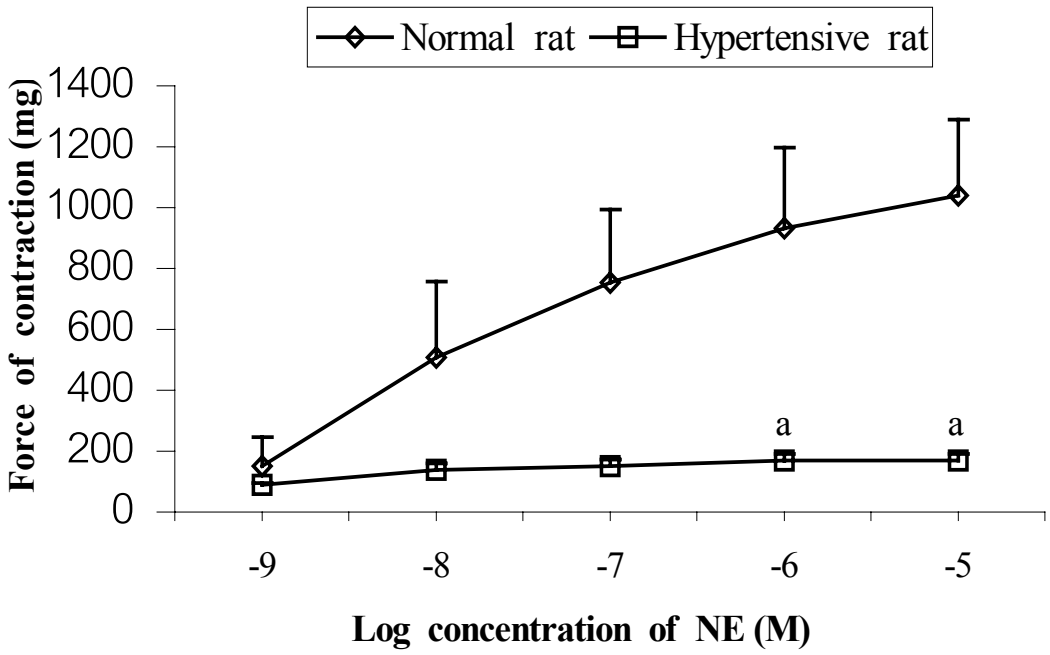

Figure 18. Effect of norepinephrine 10^{-9}-10^{-5} M in cumulative doses on the aortic ring contraction of normal and hypertensive rats (n = 5).

All values were expressed as mean ± sem.

[a]($p < 0.05$) : significant difference from normal rats at corresponding concentration.

2.2.2 Response to *A. scholaris* extract

The effect of captopril at dose of 0.42 mg/ml and *A. scholaris* extract at doses of 12.7, 25, 51 and 102 mg of dried bark/ml on 10^{-6} M NE induced isolated aortic ring contraction from normal and hypertensive rats were shown in Table 17, 18 and Figure 19, and Table 19, 20 and Figure 20, respectively. Captopril and *A. scholaris* extract, except the lowest dose, decreased the contractile effect of NE 10^{-6} M. Comparison between normal and hypertensive rats, both captopril and *A. scholaris* extract exhibited slightly greater effect on normal than hypertensive rats (Figure 20). *A. scholaris* extract showed the inhibiting effect on NE-induced contraction in a dose-independent manner in both normal and hypertensive rats.

The result indicated that water extract of *A. scholaris* at the doses 12.7-102 mg of dried bark/ml had vasodilating effect on arterial vasculature.

Table 17. Effects of 10^{-6} M norepinephrine (NE) in the absence and presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml on the isolated aortic rings contraction of normal rat

No	Force of contraction of aortic ring (mg)									
	control group		*A. scholaris* treated group							
	NE	NE + C	NE	NE + A1	NE	NE + A2	NE	NE + A3	NE	NE + A4
1	500	300	400	250	550	330	620	230	530	130
2	920	310	500	770	880	410	190	140	250	120
3	250	70	140	430	320	200	190	140	440	150
4	1150	1100	1250	1025	925	725	750	650	500	325
5	1350	450	1575	1275	1275	850	1350	1000	1000	750
mean ±sem	834±203	446±174	773±272	750±187	790±164	503±122[a]	620±214	432±171	544±124	295±120[aa]

[a]($p<0.05$), [aa]($p<0.01$) : significant difference from the effect of NE 10^{-6} M alone

Table 18. The percentage of aortic ring contraction of 10^{-6} M norepinephrine (NE) in the presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml of normal rat

N	Force of contraction of aortic ring (%)									
	captopril		*A. scholaris*							
	NE	C + NE	NE	A1 + NE	NE	A2 + NE	NE	A3 + NE	NE	A4 + NE
1	100	60	100	63	100	60	100	37	100	25
2	100	34	100	154	100	47	100	74	100	48
3	100	28	100	307	100	63	100	74	100	34
4	100	96	100	82	100	78	100	87	100	65
5	100	33	100	81	100	67	100	74	100	75
Mean ± sem	100 ± 0	50 ± 13[a]	100 ± 0	137 ± 45	100 ± 0	63 ± 5[aa]	100 ± 0	69 ± 8[a]	100 ± 0	49 ± 9[aa,b]

[a](p<0.05), [aa](p<0.01) : significant difference from the effect of NE 10^{-6} M alone

[b]($p < 0.05$) : significant difference from treated group with *A. scholaris* extract 12.7 mg of dried bark/ml

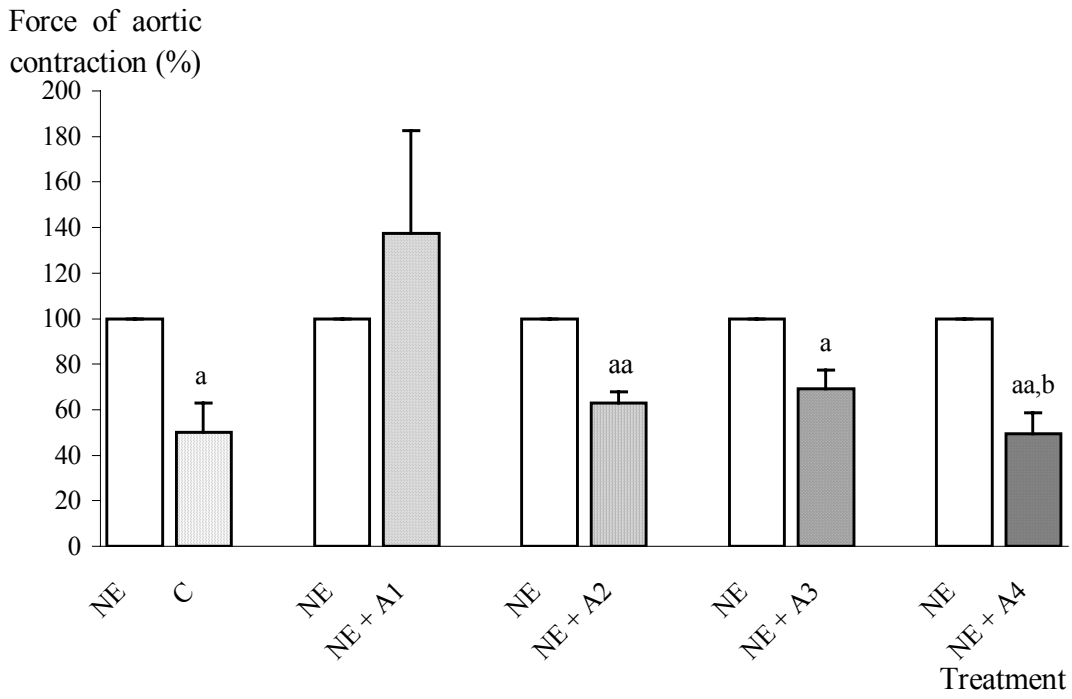

Figure 19. The percentage of aortic ring contraction of 10^{-6} M norepinephrine (NE) in the presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml in normal rat.

All values were expressed as mean ± sem.

[a]($p < 0.05$) : significant difference from the effect of NE 10^{-6} M alone.

[b]($p < 0.05$) : significant difference from treated group with *A. scholaris* extract 12.7 mg of dried bark/ml.

Table 19. Effects of 10^{-6} M norepinephrine (NE) in the absence and presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml on the isolated aortic rings contraction of hypertensive rat

N	Force of contraction of aortic ring (mg)									
	Captopril		*A. scholaris*							
	NE	C + NE	NE	A1 + NE	NE	A2 + NE	NE	A3 + NE	NE	A4 + NE
1	140	80	120	150	140	120	120	120	120	80
2	220	140	190	220	210	160	180	120	80	30
3	250	90	170	220	200	130	150	100	230	160
4	210	140	210	300	290	200	200	100	110	50
5	90	90	170	150	130	100	130	120	130	80
mean ± sem	182 ± 29	108 ±13[a]	172 ± 15	208 ± 28	194 ± 29	142 ± 17[a]	156 ± 15	112 ± 5	134 ± 25	80 ± 22[aaa]

[a](p<0.05), [aaa](p<0.001) : significant difference from the effect of NE 10^{-6} M alone

Table 20. The percentage of aortic ring contraction of 10^{-6} M norepinephrine (NE) in the presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml of hypertensive rat

n	Force of contraction of aortic ring (%)									
	Captopril		*A. scholaris*							
	NE	C + NE	NE	A1 + NE	NE	A2 + NE	NE	A3 + NE	NE	A4 + NE
1	100	57	100	125	100	86	100	100	100	67
2	100	64	100	116	100	76	100	67	100	38
3	100	36	100	129	100	65	100	67	100	70
4	100	67	100	143	100	69	100	50	100	45
5	100	100	100	88	100	77	100	92	100	62
mean ± sem	100 ± 0	$65 \pm 10^{a,bbb}$	100 ± 0	120 ± 9	100 ± 0	$75 \pm 4^{aa,bb}$	100 ± 0	75 ± 9^{bb}	100 ± 0	$56 \pm 6^{aa,bbb}$

$^{a}(p<0.05)$, $^{aa}(p<0.01)$: significant difference from the effect of NE 10^{-6} M alone

$^{b}(p < 0.05)$, $^{bbb}(p<0.001)$: significant difference from treated group with *A. scholaris* extract 12.7 mg of dried bark/ml

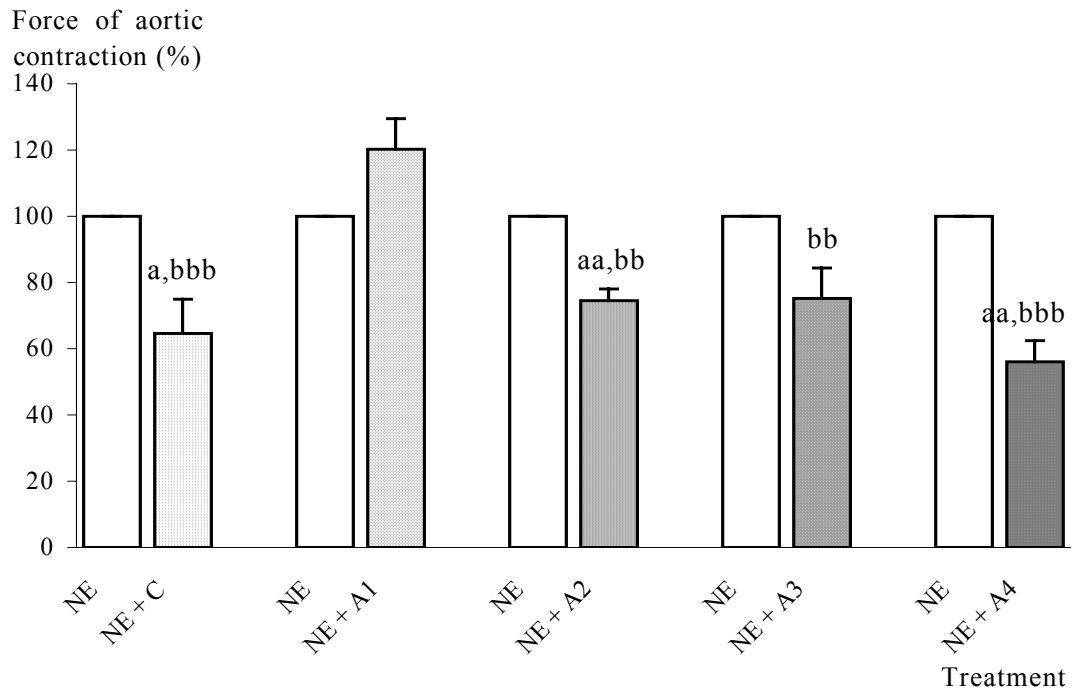

Figure 20. The percentage of aortic ring contraction of 10^{-6} M norepinephrine (NE) in the presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml in hypertensive rat.

All values were expressed as mean ± sem.

$^{a}(p < 0.05)$, $^{aa}(p < 0.01)$: significant difference from the effect of NE 10^{-6} M alone.

$^{bb}(p < 0.01)$, $^{bbb}(p < 0.001)$: significant difference from treated group the with *A. scholaris* extract 12.7 mg of dried bark/ml.

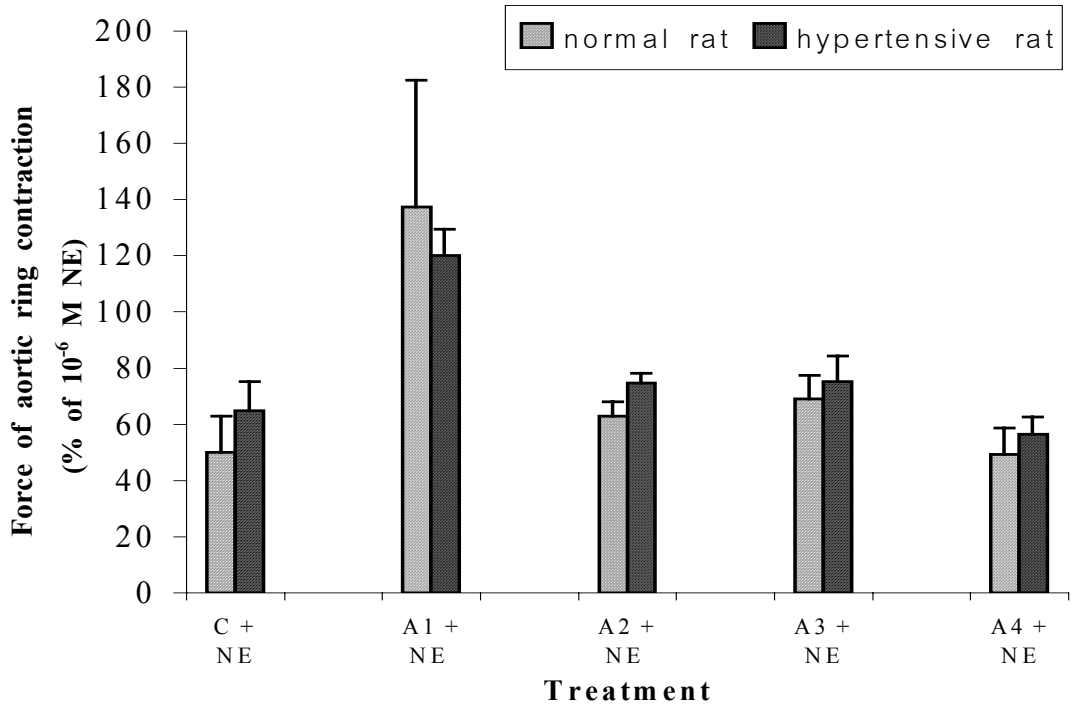

Figure 21. The percentage of aortic ring contraction of 10^{-6} M norepinephrine (NE) in the presence of *A. scholaris* 12.7 (A1), 25 (A2), 51 (A3), 102 (A4) mg of dried bark/ml and captopril (C) 0.42 mg/ml in normal and hypertensive rat.

All values were expressed as mean ± sem.

There were no significant difference between normal and hypertensive group with the same treatment.

CHAPTER V
DISCUSSION

Within the enzyme cascade of the RAS, the ACE plays an important role in the regulation of blood pressure. ACE inhibitors were developed as therapeutic agents targeted for the treatment of hypertension (37).

Starting from the model substances, captopril, a large number of synthetic ACE inhibitors has been developed in two decades. In contrast, the number of natural products so far identified as inhibitors of ACE is relatively small. Screening for anti-hypertensive effects in traditional medicines has been performed over many years (11-18,60) and several animal models have been utilized (133). A number of screening studies have been made on the ACE inhibitory activities of higher plants with the traditional use for lowering blood pressure. The *in vitro* assays for the determination of ACEI activity in crude plant extracts have been published using the same principle by measuring the amount of cleaved product from ACE activity, but using the different method in determination, spectrophotometry (60), fluorometry (17,18) and HPLC (11-16).

In this study, the spectrofluorometry was used as a method for screening for ACEI activity, though the HPLC assay was claimed to be more sensitive and less interference from plant constituents (63). The spectrofluorometric method was used in a serial experiments to screen for ACEI activity of crude extracts from *Areca cateshu* which led to isolation of active fractions (17,18). The active fractions consisted of several condensed tannins which ACEI activity is proportional to their degree of polymerization (19). The active fraction with this *in vitro* ACEI activity also demonstrated blood pressure lowering effect in hypertensive rats (19). There was also evidence showed good correlation of the ACEI assay by spectrometry and HPLC assay of medicinal plants (67). In addition, the fluorometric assay is less complicated procedures and lower cost than the HPLC. This assay can also run on large number of the sample.

Therefore, the spectrofluorometry can be used as an assay for ACEI screening of plant extracts.

In the present study, *in vitro* screening for ACEI activity of Thai medicinal plants have been carried out in 15 plant materials from 12 species which were extracted with water because the antihypertensive plants are most active in water extract (12). Only 4 specimens from 3 species of the water extract of Thai medicinal plants showed ACEI activity more than 50 % (*A. galanga*, *A. heterophyllus* and *L. speciosa*). Several of medicinal plants lacked of ACEI activity in this study had been previously reported this activity from various countries. These are *C. asiatica* (11), *J. sambac* (14), *O. tenuiflorum* (13,14) and *P. betle* (14). The same plant specimens collected from different localities also resulted in different ACEI activity (13). In addition, *O. sanctum* showed different ACEI activity using the same method (13,14). The phytoconstituents were known to vary depending not only on ecological factors such as time of collection, climate, habitat, but also the screened part and type of extraction. These might cause the different ACEI activity obtained from these studies. Several plants reported to have antihypertensive effect from the pharmacological studies, showed no ACEI activity. These were *A. heterophyllus*, *H. rosa-sinensis* (109), *H. sabdariffa* (113-116), and *N. nucifera* (108). These medicinal plants may decrease blood pressure by the other mechanisms that not involved the pathway of Ang II generation of the renin-angiotensin system.

There was a report that non-medicinal plants also had ACEI activity (133). In this study, the 34 plant materials from 18 species of mangrove plants which had antioxidant activity (134) were also screened for ACEI activity because antioxidant substances protection against oxidative cardiovascular injury and may have the effect on vascular functions (135,136). Approximately one-third of these species exhibited ACEI activity greater than 50%. The mangrove plants with high antioxidant activity (such as *A. alba* flower ED_{50} was 7.67 μg/ml, *A. marina* flower ED_{50} was 8.67 μg/ml) showed low ACEI activity (0% and 15.63 %, respectively), while the others whose antioxidant activity were low (such as *B. parviflora* leave ED_{50} was 105 μg/ml) had high ACEI activity (73.65 %). The

result from this study showed no correlation between the ACEI activity and the antioxidant activity.

In the present studies, the 50 % inhibition of *in vitro* ACE activity was equivalent to the activity of captopril, a synthetic ACEI used in hypertensive treatment, at the concentration of 0.0029 mg/ml. Among the screened plants, the highest ACEI activity obtained from the water extract of medicinal plants and ethyl acetate of mangrove plants were *A. scholaris* bark and *A. alba* fruit, which were equal to captopril 0.0031 and 0.0056 mg, respectively.

Cuphea carthagenesis which contained β-sitosterol, betulinic acid showed *in vitro* ACEI activity (16,137). Since, the bark of *A. scholaris* are reported to contain betulinic acid (90), β-sitosterol (91), it is possible that the observed activity for *A. scholaris* is due to these compounds.

The water extract of medicinal plant, *A. scholaris* bark, which exhibited highest ACEI was selected to examine the antihypertensive action. To test for the antihypertensive activity of *A scholaris* water extract, the renovascular hypertensive model was used. Two-kidney, one-clip hypertension is the form that is most often encountered in man. It has been extensively studied in various experimental animals, especially the dog and the rat. In rats in which a constricting clip had been placed on one renal artery, blood pressure was elevated as early as the first and second days after operation, in parallel with a rise in the peripheral plasma concentrations of active renin and Ang II. After that, although the blood pressure remains elevated, there is less marked elevation of renin and Ang II. Occasionally, in experimental animals with a very severe unilateral renal artery stenosis, there is progressive and severe elevation of renin and Ang II accompanied by increasing hypertension (44).

In this studies, the SBP of 10 % of rat was increased over 180 mm.Hg since second week of surgery. At the fourth week , 70 % of rats had SBP greater than 180 mm.Hg. The rat became hypertensive usually within 4 to 6 weeks of surgical induction.

The single oral effect of water extract of *A. scholaris* bark was studied in hypertensive rats using tail cuff method. This method required the pre-warm period of animal to cause the vasodilation before measuring the blood pressure.

The blood pressure can be changed quickly by external stimuli and the mood of the animals. The pre-warm period interval required for repeated measurements can cause the stress condition to the animal. From our preliminary study, the maximal effect of plant extract on lowering blood pressure was attained during 15 to 75 min. Then, the blood pressure was slowly rised. In this study, the effect of the plant extract on blood pressure was determined at 15 min-interval for 90 min after oral administration to avoid the stress condition to the animal.

The water extract of *A. scholaris* decreases SBP, DBP and MAP in a dose-independent manner. All 4 doses of the extract lowered the blood pressure and heart rate at the similar levels. The decreased SBP is faster in onset and longer in duration than DBP. Although the percent of maximal effect among 4 doses of the extract were similar, the higher dose tended to have faster time to maximal effect. The highest dose of the extract (6 g/kg) decreased the systolic blood presence about one-third of the effect of captopril 25 mg/kg. The extract had slight effect on decreasing heart rate, while captopril had no effect. This suggested that the water extract of *A. scholaris* decreased blood pressure, partly by depression heart function.

The antihypertensive effect of *A scholaris* was supported by the screening study for the biological action of Indian plants which showed that 50 % alcoholic extract of *A. scholaris* had hypotensive effect in normotensive dogs (74). The blood pressure lowering effect of the extract were also supported by the result from the study of isolated aortic rings. In this study, it was noted that the isolated aortic rings of hypertensive rats had lesser response to the same dose to NE, especially at high dose, than those from normal rats. In several experimental models of hypertension including spontaneously hypertensive rats, renovascular hypertension and mineralocorticoid-salt hypertension, the increased wall thickness and intimal thickening of arterial wall occurred in association with increase in blood pressure and this structural changes affected the changes in vascular reactivity to vasoactive agents (138). There were several reports that isolated aortic ring from experimental hypertension had similar, increased or decreased response when compared to

those from normotensive one (139-141). In aorta derived from 2K1C renal hypertensive rats, the reactivity to NE was only found to be enhanced when endothelium was present (142). It was suggested that the difference in vascular reactivity may reflect the difference in endothelial function. The artery from hypertensive state with the loss of endothelium-dependent vasorelaxation property, in association with the increase in wall thickness enhanced its vasoconstrictive state. Moreover, the artery tend to have reduced force production capabilities possibly due to the decrease in myosin content (143). These may result in decrease response to NE of isolated aortic rings from hypertensive rat in this study.

Captopril decreased the contractile response of isolated aortic rings to NE about 50 % and 35 % from normal and hypertensive rats, respectively. The water extract of *A. scholaris* diminished the contractile effect of isolated aortic rings to norepinephrine in a dose-independent manner from both normal and hypertensive rats at the lower level than captopril. Similarly with the captopril, the water extracts of *A. scholaris* bark had slightly greater effect in isolated aortic ring from normal than hypertensive rats, but it was not significant differences.

The results from this study indicated that Thai medicinal and mangrove plants possess the inhibitory activity on ACE. *A. scholaris* with approximated 50 % of *in vitro* ACEI activity also exhibited antihypertensive and vasodilating action to experimental renal hypertensive rats. These suggest the potential role of Thai medicinal and mangrove plants as a source of ACEI. Further studies are needed to examine the specific actions of the plant extract as ACE inhibitor and to explore for the active constituents.

CHAPTER VI
CONCLUSION

The data from this present study can be summarized as follow.

From 49 specimens of 31 plant species, the ACEI activity of greater than 50 % were determined, by using fluorometric assay, in 9 specimens of 6 species of ethyl acetate extract of mangrove plants and 4 specimens from 3 species of water extract of medicinal plant.

The water extract of *Alstonia scholaris* (L.) which had 52.89 % of ACEI activity, had significant antihypertensive effect in a dose-independent manner with lesser effect compared to captopril. The extract slightly decreased heart rate, while captopril had no effect. In addition, the extract also decreased contractile response of isolated aortic ring to NE with the greater effect in normal than hypertensive rats, but in a lower level than captopril.

The results from this study indicated that Thai medicinal plants and mangrove plants had ACEI activity, and *A. scholaris*'s bark water extract with ACEI activity can decreased blood pressure and caused vasodilatation in isolated aortic ring obtained from hypertensive rats.

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APPENDIX

Appendix A

Yield of lyophilized extract compared with dried plant powder (%).

Plant species	part screened[a]	Local name	yield (%)
I. Medicinal plant (water extract)			
Alpinia galanga (L.) Willd.	Rh	Kha daeng	17.91
	Rh	Kha lueang	20.96
Alstonia scholaris (L.) R.Br.	L	Phaya sattaban	14.28
Artocarpus heterophyllus Lam.	L	Khanun	9.71
Centella asiatica (L.) Urb.	L	Bua bok	1.58
Hibiscus rosa-sinensis L.	L	Chaba	17.73
Hibiscus sabdariffa L.	C	Krachiep	45.00
Jasminum sanbac (L.) Aiton	L	Mali	25.88
Lagerstroemia speciosa (L.) Pers.	Bk	Inthanin	8.06
	L		11.67
Nelumbo nucifera Gaertn.	R	Bua khao	12.32
	R	Bua daeng	3.77
Ocimum tenuiflorum L.	Ar	Ka phrao	13.95
Pandanus amaryllifolius Roxb.	L	Toei hom	10.48
Piper betle L.	L	Phlu	10.32

Yield of lyophilized extract compared with dried plant powder (%). (continued)

Plant species	part screened[a]	Local name	yield (%)
II. Mangrove plant (ethyl acetate extract)			
Acanthus ebracteatus Vahl.	L	Ngueak plamo dok khao	7.44
Avicennia alba Blume.	B	Samae khao	2.69
	Fl		12.50
	Fr		12.50
	L		14.35
Avicennia marina (Forsk.) Vierth	B	Samae thale	2.24
	Fl		3.12
	L		6.76
Barringtonia maunwongyathiae	Bk	Chik dong	3.08
	L		5.92
	S		0.66
Bruguiera gymorrhiza (L.) Savigny	B	Phangka hua sum dok daeng	3.00
	Fl		7.54
	L		6.52
Bruguiera parviflora Wight &Am.ex Griff.	L	Thua dam	8.87
Flagellaria indica L.	Fr	Wai ling	7.58
Lumnitizera racemosa Willd.	Fr	Fat dok khao	4.00
Molineria latifolia Herb.	L	Phrao nok nhum	4.02
	R		4.77
Nypa fruticans Wurmb.	Sm	Chak	6.60
Phoenix paludosa Roxb.	Py	Peng	2.64
Rhizophora apiculata Blume.	B	Kong kang bai lek	1.44
	L		6.44
Rhizophora mucronata Poir.	L	Kong kang bai yai	8.12

Yield of lyophilized extract compared with dried plant powder (%). (continued)

Plant species	**part screened**[a]	**Local name**	**yield (%)**
Sonneratia caseolaris (L.) Engl.	L	Lam phu	13.89
Suaeda maritina (L.) Dum.	P	Cha khram (green leaves)	2.72
	P	Cha khram (red leaves)	1.71
Xylocarpus granatum Koen.	L	Tabun khao	12.47
	Sb		5.80
Xylocarpus rumphii (Kostel.) Mabberley	B	Taban	8.67
	E		6.36
	L		9.38
	Sd		12.97
Wedelia biflora (L..) DC	Fl	Ben chamat thale	10.72

[a]Ar=Aerial part, Bk=Bark, B=Branches, C=Calyx, E=Exocarp, Fl=Flowers, Fr=Fruits, L=Leaves, P=Pods, Py=Young pods, Rh=Rhizome, R=Roots, Sm=Stamen, Sd=Seeds, S=Stem, Sb=Stem bark.

Appendix B

Preparation of reagents for ACEI activity test

All reagents were prepared by using distilled and deionized water.

The phosphosaline buffer (pH 8.3) was prepared by dissolving 87.09 g.(0.5 mole) K_2HPO_4 and 87.67 g (1.5 mole) NaCl in 900 ml H_2O, adjusting the pH to 8.3 with 1 N HCl, adding water to a total volume of 1 l and readjusting the pH if necessary.

The substrate solution was prepared by dissolving 46.6 mg of Hippuryl – Histidine – Leucine (Hippuryl – His – Leu) in 4 ml of 25 mM NaOH. The substrate – buffer solution was prepared by mixing buffer, substrate and water at a ratio of 1 : 1 : 2.8 (final concentration in the assay mixture : 0.1 M K_2HPO_4, pH 8.3, 0.3 M NaOH ; 5 mM Hippuryl – His – Leu)

The enzyme solution was prepared by dissolving purified ACE in the phosphosaline buffer.

The inhibitor (sample) solution was prepared by dissolving 59.4 mg of medicinal plant extract in 0.8 ml water and the mangrove plant extract in 0.8 ml dimethylsulphoxide (DMSO). (final concentration in the assay volume : 0.33 mg/ml).

Two percent of *O* – phthaldialdehyde (OPA) was prepared by dissolving 200 mg in 10 ml purified methanol immediately prior to addition of enzyme to a series of assay mixture.

Enzyme incubation

The enzyme reaction was initiated by the addition of substrate in the enzyme and sample solution. The assay mixture, consisting of 240 µl of enzyme solution (0.8 milliunits of ACE), 10 µl of sample solution and 250 µl of substrate solution, was incubated at 37°C. After 1 hour of incubation, 1.45 ml of 0.3 M NaOH was added to the assay mixture to stop the enzymic reaction, then 100 µl of OPA reagent was added to form the fluorescence adduct of the aldehyde and the histidyl moiety of his – leu released from the substrate. Exactly 10 mins later, the reaction was terminated by the addition of 200 µl of 3 M HCl. After adding solution, the assay mixture was mixed by using vortex mixer. After 25 minutes of termination reaction, the assay mixture is centrifuged for 10 mins and the supernatant was collected and submitted for analysis using spectrofluorometry at excitation wavelength at 360 nm and emission wavelength at 485 nm. The fluorescence was read at 45 minutes after addition of HCl.(See Diagram)

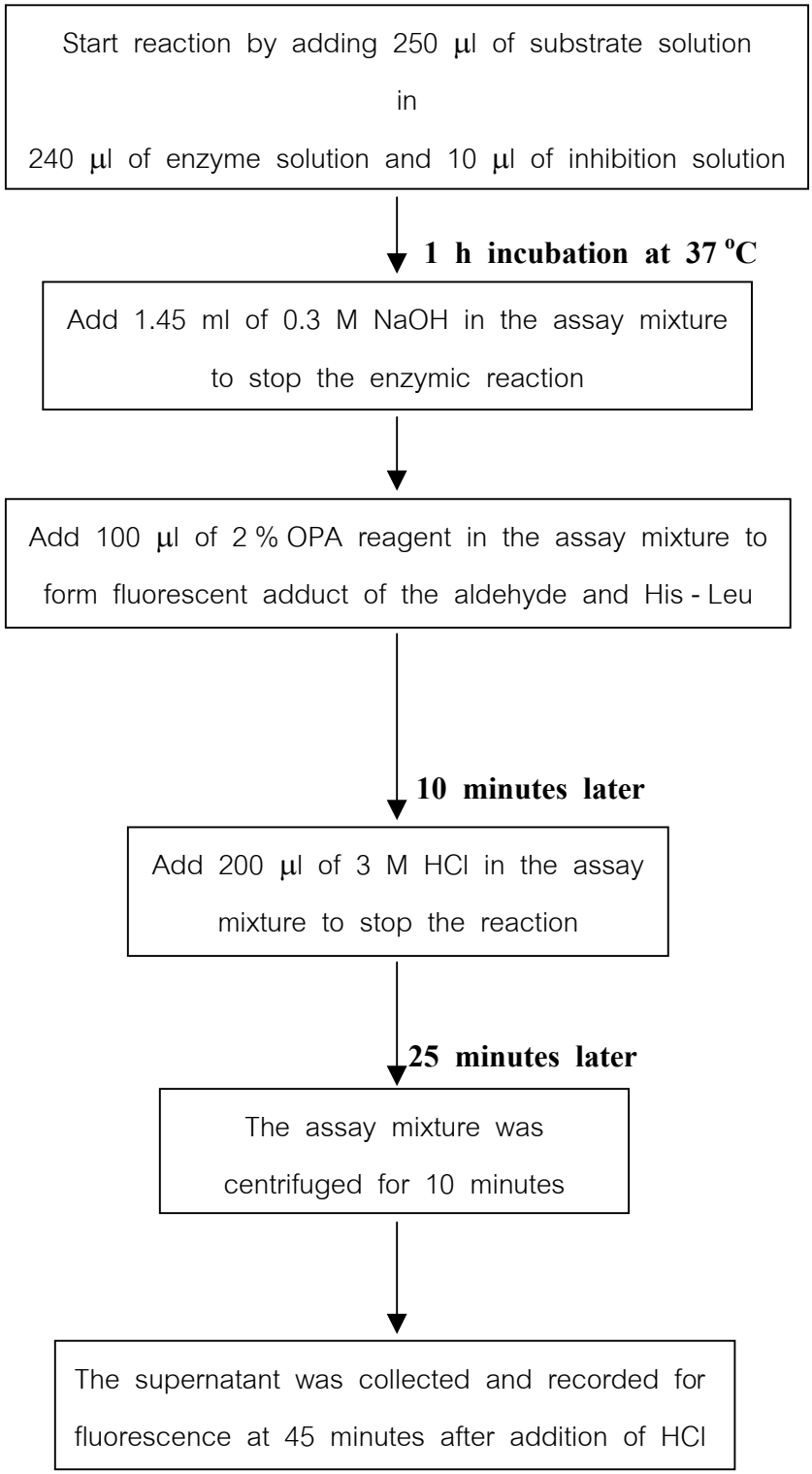
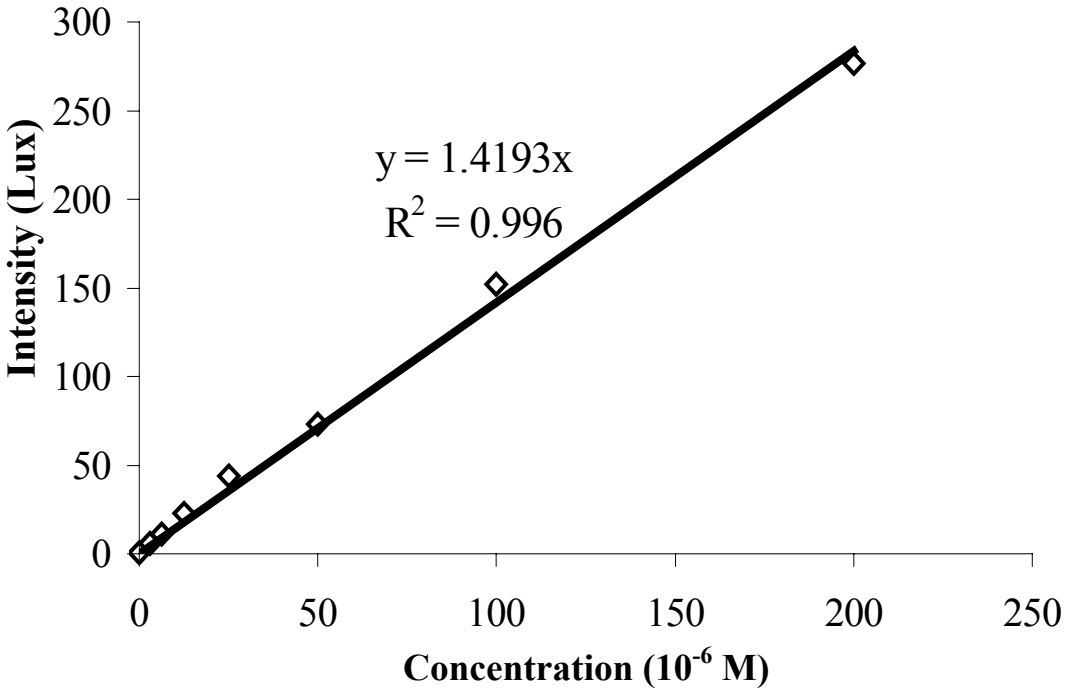
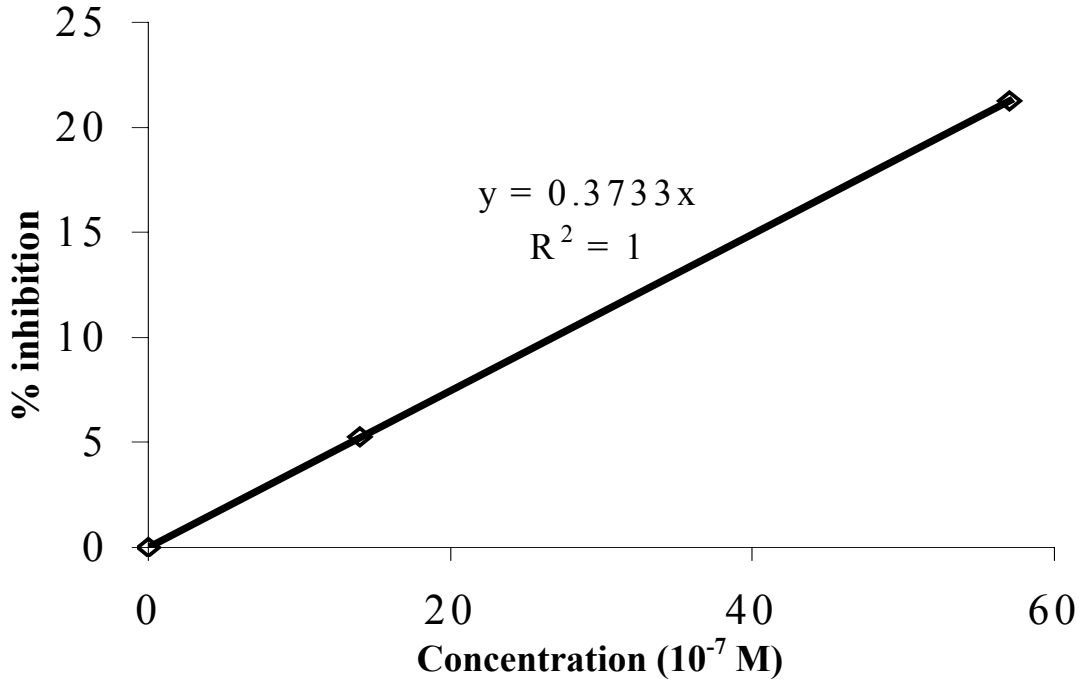


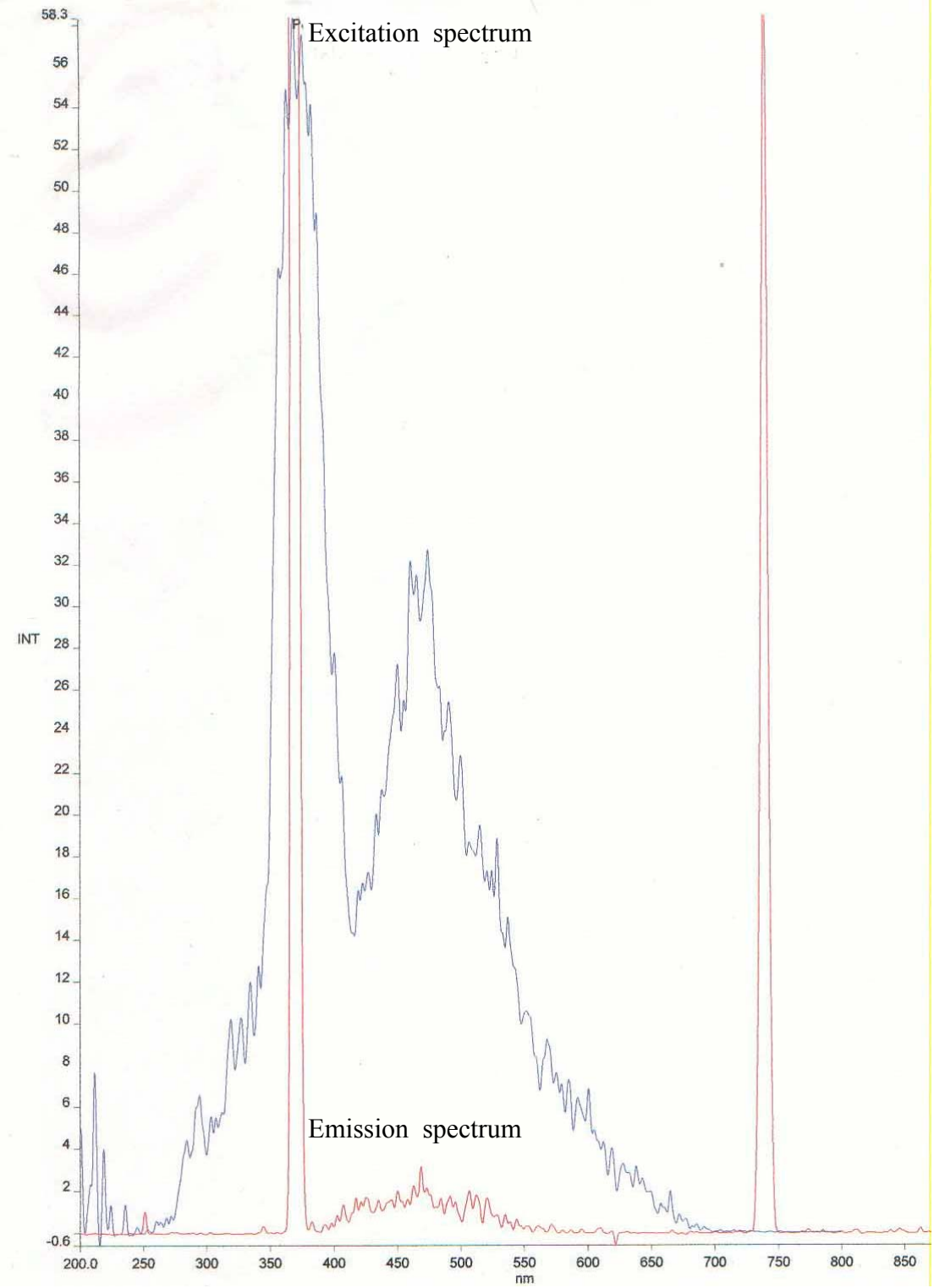
Diagram of enzyme incubation



Standard curve of His-Leu at the doses of 3.125, 6.5, 12.5, 25, 50, 100 and 200 x 10^{-6} M



Standard curve of % inhibition of captopril at the doses of 0, 14 and 57 x 10^{-7} M



Spectrofluorometry spectra of the control incubation without inhibitor.

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