EFFECT OF EXPRESSED JUICE OF FRESH *CENTELLA ASIATICA (L.) URBAN* LEAVES ON CARDIOVASCULAR FUNCTION IN DOCA –SALT HYPERTENSIVE RATS

SOMPONG MUANGNONGWA

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....

Miss. Sompong Muangnongwa
Candidate

....

Assoc.Prof. Suwan Thirawarapan,
Ph.D. (Physiology)
Major advisor

....

Asst.Prof. Wisuda Suvitayavat,
Ph.D. (Physiology)
Co-advisor

....

Dr. Amporn Jariyapongsakul,
Ph.D. (Physiology)
Co-advisor

....

Assoc.Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Faculty of Graduate studies

....

Asst.Prof. Supaporn Pongsakon
M.Sc.
Chair
Master of Science Programme
in Biopharmaceutical Sciences
Faculty of Pharmacy

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For the degree of Master of Science (Biopharmaceutical Sciences)

On
July 21, 2004

....

Miss. Sompong Muangnongwa
Candidate

....

Assoc. Prof.Suwan Thirawarapan,
Ph.D. (Physiology)
Chair

....

Dr. Amporn Jariyapongsakul,
Ph.D. (Physiology)
Thesis Defence Committee

....

Prof.. Nuntavan Bunyapraphatsara
Ph.D. (Phytochemistry)
Thesis Defence Committee

....

Assoc. Prof. Chongkol Thiangda
Dr.rer.nat.
Thesis Defence Committee

....

Assoc.Prof. Rassmidara Hoonsawat,
Ph.D.
Dean
Programme in Pharmaceutics

....

Assoc.Prof. Weena Jiratchariyakul, Dr.rer.nat.
Acting Dean
Faculty of Pharmacy
Mahidol University

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Sompong Muangnongwa

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SOMPONG MUANGNONGWA 4237861 PYBS / M

M.Sc. (BIOPHARMACEUTICAL SCIENCES)

THESIS ADVISORS : SUWAN THIRAWARAPAN, Ph. D. (PHYSIOLOGY), WISUDA SUVITAYAWAT, Ph. D. (PHYSIOLOGY), AMPORN JARIYAPONGSKUL, Ph. D. (PHYSIOLOGY).

ABSTRACT

Centella asiatica (L.) Urban, locally known as *bua-bok*, has several actions including vasodilation, the important mechanism in alleviating blood pressure as well as improving blood flow to various organs. Thus, this study is aimed to assess the effects of oral administration of the expressed leaf juice of *C. asiatica (L.)* on cardiovascular functions in DOCA-salt hypertensive rats and to clarify its usefulness for treating hypertensive conditions.

Systolic blood pressure and heart rate were measured by tail cuff method in hypertensive rats and normal rats two hours after a single oral administration of *C. asiatica* juice – at doses of 16, 24, and 32 g/kg – or 25 mg/kg captopril for the positive control group. For the group receiving the 32 g/kg dose, both the cutaneous and cerebral blood flows were measured by laser Doppler flowmetry under anesthetic condition two hours after the administration. During the regional cerebral blood flow determination, the systolic and diastolic blood pressures were simultaneously being measured via femoral artery.

C. asiatica juice at the doses of 24 and 32 g/kg significantly decreased systolic blood pressure only in hypertensive rats, while captopril decreased systolic blood pressure in both normal and hypertensive rats. *C. asiatica* juice lowered the heart rate in hypertensive rats, with increasing effects as the dose increased. In normal rats, only the 32 g/kg dose decreased the heart rate. In hypertensive rats, *C. asiatica* juice lowered systolic blood pressure less than did captopril and for a shorter length of time, and its maximum effect was more quickly achieved. *C. asiatica* also decreased the heart rate, while captopril did not.

The 32 g/kg dose of *C. asiatica* juice significantly increased cutaneous blood flow as well as regional cerebral blood flow in both normal and hypertensive rats, but the effect was greater in the latter. Both systolic and diastolic blood pressure decreased with the increase in blood flow.

This study reveals that increasingly higher oral doses of *C. asiatica* juice make the heart beat slower, lower blood pressure, and improve local blood flow in hypertensive rats, but only have a slight effect in normal rats.

KEY WORDS : *CENTELLA ASIATICA*/ CARDIOVASCULAR FUNCTIONS/ ORAL ADMINISTRATION/ DOCA-SALT HYPERTENSION

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ผลของน้ำคั้นใบบัวบกสดต่อระบบไหลเวียนเลือดของหนูขาวที่ชักนำให้เกิดความดันโลหิตสูง

EFFECT OF EXPRESSED JUICE OF FRESH *CENTELLA ASIATICA (L.) URBAN* LEAVES ON CARDIOVASCULAR FUNCTION IN DOCA-SALT HYPERTENSIVE RATS

สมปอง เมืองหนองหว้า 4237861 PYBS/M

วท.ม. (เภสัชศาสตร์ชีวภาพ)

คณะกรรมการควบคุมวิทยานิพนธ์ : สุวรรณ ธีระวรพันธ์ Ph. D. (Physiology), วิสุดา สุวิทยาวัฒน์ Ph. D. (Physiology), อัมพร จาริยะพงศ์สกุล Ph. D. (Physiology).

บทคัดย่อ

Centella asiatica (L.) Urban หรือบัวบก มีฤทธิ์หลายอย่างรวมทั้งขยายหลอดเลือดแดง ซึ่งเป็นกลไกสำคัญในการลดระดับความดันโลหิต และเพิ่มอัตราการไหลของเลือดไปยังอวัยวะต่างๆ การศึกษานี้จึงศึกษาฤทธิ์ของน้ำคั้นใบบัวบกโดยการให้ทางปาก ต่อการทำงานของระบบหัวใจและหลอดเลือดในหนูขาวที่ชักนำให้เกิดความดันโลหิตสูงด้วย DOCA-salt เพื่ออธิบายประโยชน์การใช้ในภาวะความดันโลหิตสูง

ศึกษาการเปลี่ยนแปลงความดันโลหิต systolic และอัตราการเต้นของหัวใจในหนูปกติและหนูความดันโลหิตสูงที่ไม่สลบ โดยวิธีการวัดความดันโลหิตหางหนูเป็นระยะเวลา 2 ชม.ของน้ำคั้นใบบัวบกขนาด 16, 24 และ 32 ก./กก. หลังการป้อนครั้งเดียว เปรียบเทียบกับ captopril 25 มก/กก เฉพาะน้ำคั้นใบบัวบกขนาด 32 ก./กก. ใช้ศึกษา ผลต่อการไหลเวียนเลือดเฉพาะที่ในหนูที่สลบ บริเวณผิวหนังและสมองส่วน cerebrum ซีกขวา 5 จุดโดย Laser Doppler Flowmetry เป็นระยะเวลา 2 ชม. ในระหว่างการวัดการไหลเวียนเลือดเฉพาะที่ของสมองทำการวัดความดันเลือด systolic และ diastolic ของหลอดเลือดแดง femoral ร่วมด้วย

น้ำคั้นใบบัวบกขนาด 24 และ32 ก/กก. ลดความดันโลหิต systolic อย่างมีนัยสำคัญทางสถิติในหนูความดันโลหิตสูงเท่านั้น ขณะที่ captopril ขนาด 25 มก/กก. ลดความดัน systolic ทั้งในหนูปกติและหนูความดันโลหิตสูง น้ำคั้นใบบัวบกลดอัตราการเต้นของหัวใจในหนูความดันโลหิตสูงอย่างมีนัยสำคัญทางสถิติตามขนาดที่เพิ่มขึ้น ในหนูปกติน้ำคั้นใบบัวบกขนาด 32 ก./กก. เท่านั้นที่ลดอัตราการเต้นของหัวใจ ในหนูความดันโลหิตสูงพบว่าน้ำคั้นใบบัวบกลดความดันเลือด systolic ได้น้อยกว่า ในระยะเวลาสั้นกว่าและให้ผลลดสูงสุดในเวลาเร็วกว่า captopril แต่มีผลลดอัตราการเต้นของหัวใจขณะที่ captopril ไม่มีผล

น้ำคั้นใบบัวบกขนาด 32 ก./กก. เพิ่มการไหลเวียนเลือดเฉพาะที่อย่างมีนัยสำคัญทางสถิติที่ผิวหนังและสมอง ทั้งในหนูปกติและหนูความดันโลหิตสูง แต่เพิ่มในหนูความดันโลหิตสูงมากกว่าหนูปกติความดัน ทั้ง systolic และ diastolic ลดลงหลังจากมีการเพิ่มของการไหลเวียนเลือดที่สมอง

ผลการศึกษานี้แสดงให้เห็นว่าการป้อนครั้งเดียวของน้ำคั้นใบบัวบก มีผลยับยั้งอัตราการเต้นของหัวใจ ลดความดันโลหิตและเพิ่มการไหลเวียนของเลือดเฉพาะที่ในหนูความดันโลหิตสูงขณะที่มีผลน้อยกว่าในหนูปกติ

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LIST OF ABBREVIATIONS

AV	=	atrioventricular
BF	=	blood flow
BP	=	blood pressure
BW	=	body weight
Ca^{2+}	=	calcium ion
CO	=	cardiac output
CO_2	=	gas carbondioxide
DBP	=	diastolic blood pressure
ET_A	=	endothelin A
ET_B	=	endothelin B
ECF	=	extracellular fluid
g.	=	gram
GMP	=	gaudenosine monophosphate
HR	=	heart rate
hr.	=	hour
kg	=	kilogram
mg	=	milligram
ml	=	milliliter
mmHg	=	millimeter mercury
mm	=	millimeter

LIST OF ABBREVIATIONS (continued)

min	=	minute
Na^{+}	=	sodium ion
No.	=	number
O_2	=	gas oxygen
PU	=	perfusion units
rCBF	=	regional cerebral blood flow
SA	=	sinoatrial
sec	=	second
SBP	=	systolic blood pressure
TPR	=	total peripheral resistant
ºC	=	degree celcious
%	=	percent

CHAPTER 1

INTRODUCTION

Herbs and other kinds of plants are used as an important therapeutic agents for a long time ago. Even nowadays, the knowledge of traditional medicine which inherited from ancestors is very useful for daily life. Some of them are more effective and valuable than those in modern medicine. Thailand is a tropical country with a diversity of plants. Many of them have been claimed to be used as drugs in traditional medicines. Presently, Thai medicinal plants have been widely used in therapy because these plants have fewer side effects and lower price comparing with modern drugs. However, most of their traditional use have been supported by few scientific data. So that the studies are required to gain more information in order to confirm and support the efficacy and safety of these plants in humans and to implement them in medicine.

Centella asiatica L.Urban belonged to the Umbelliferae family is a medicinal plant that has been used since prehistoric times, to treat a wide range of indications. It has been used for a long time by Thai people as fresh squeezed juice and fresh vegetable. It is popularly known as gotakola or Indian Penny wort, and used as a medicine in Indian Ayurvedic.

The pharmacological activities of *C.asiatica* include, wound healing, ulcer-protective anti-ulcer, effect on varicose veins, antimicrobial, antiviral, immunomodulatory, sedative, antidepressive, anticonvulsure and analgesic actions, and varicose vein improvement (1).

For the cardiovascular effect, several studies showed potential action of *C.asiatica* in improving venous insufficiency. Some studies indicated that *C.asiatica* had arterial vasorelaxant activity and hypotensive effect (2,3). In addition, *C.asiatica* showed its effect on learning and memory improvement, lipid peroxidation inhibition, and decrease the cholesterol (4,5,6). These results showed the potential effects of *C.asiatica* in decreasing blood pressure and improve the blood flow.

The present study was performed to assess the single oral administration effects of *C.asiatica* expressed juice on cardiovascular functions in normal and DOCA-salt hypertensive rats. The single oral administration of *C.asiatica* expressed juice on blood pressure, heart rate, local blood flow at 2 areas, cutaneous blood flow and regional cerebral blood flow were investigated.

CHAPTER 2
LITERATURE REVIEW

1. Regulation of cardiovascular functions

The vital role of the cardiovascular system in maintaining homeostasis depends on the control of continuous blood flow from the heart through the variable resistance of the vessels to the tissues and back to the heart. Numerous control mechanisms help to adjustment by altering the output of the heart pump, changing the diameter of the resistance vessels which primarily the arterioles and altering the amount of blood pooled in the capacitance of veins. The essential activity are the maintenance of the cardiac out put (CO), total peripheral resistance (TPR) and blood pressure (BP) on changeable situation. The regulatory system is complex which consists of three major types: local regulatory mechanisms, systemic regulation by hormones and systemic regulation by the nervous system (7, 8).

1.1 Local regulatory mechanisms

1.1.1 Autoregulation

The myogenic theory

This theory is based on stretching of the arterial as well as the blood pressure rises directly stimulates increased smooth muscle tone. It is probably due in part to the intrinsic contractile response of smooth muscle to stretch which could explain by the greater degree of contraction at higher pressure: the wall tension is proportionate to the distending pressure time the radius of the vessel and the maintenance of a given wall tension as the pressure rises would require a decrease in radius under law of Laplac. It helps to maintain relatively constant rates of blood flow and pressure within an organ despite changes in systemic arterial pressure. Therefore, it is believed that when high arterial pressure induced stretches on the blood vessel, this in turn causes vascular constriction and reduces the blood flow nearly back to normal. Conversely, at low pressures, the degree of stretch of vessels are less, so that the smooth muscle relaxes and allows increases flow. Otherwise, the myogenic mechanism protects the capillaries from excessively high blood pressure. That is, if the pressure in the small arteries and arterioles rises too high, these vessels would simply constrict and prevent this high pressure from being transmitted into the capillaries, which are so weak that excessive pressure could rupture them (7, 9, 10).

The Frank-Starling law

For the heart, cardiac muscle responses to stretching of its fibers by an increase in the strength of the subsequent contractions. The physiological of distension on ventricular as blood is returned more rapidly to the heart were influence to the increased volume stretches to the walls of the ventricles and causes a more forceful propulsion of blood out of the heart. This is one of the main factors balancing the amount of blood entering and leaving the heart. Thus, in each contraction amount of blood ejected by the heart is dependent on end diastolic volume. The increased distension of muscle fiber causes the greater contraction and the increase in stroke volume. However, the over distended muscle fiber resulted in the lesser contraction and the decreased stroke volume (11, 12).

The metabolic theory

Local changes in gas and metabolite concentrations act directly on vascular smooth muscle walls to produce vasodilator in the systemic circulation. Thereby, vasodilator substances tend to accumulate in active tissues and these metabolites also contribute to autoregulation. When the arterial pressure becomes too great, the excess flow provides too many nutrients to the tissues and also flushes vasodilator substances out of the tissues, both of these effects will then cause the blood vessels to constrict and the flow to return nearly to normal despite the increased pressure. On the other hand when blood flow decreases, the metabolic products that have accumulated cause vasodilation. The metabolic changes in most tissues that produce vasodilation include, decreases in O_2 tension and pH. These changes cause relaxation of the arteioles and precapillary sphincters. Increases in O_2 tension and osmolality also dilate the vessels. The direct dilator action of CO_2 is most pronounced in the skin and brain. Adenosine may play a vasodilator role in muscle, it also inhibits the release of norepinephrine. A rise in temperature exerts a direct vasodilator effect and the temperature rise in active tissues may contribute to the vasodilation. Injured arteries and arterioles constrict strongly. The constriction appears to be due to the local liberation of serotonin from platelets that stick to the vessel wall in the injured area (7, 9, 10).

1.1.2 Substances secreted by the endothelium

Endothelial cells, located between the circulating blood and the media and adventitia of the blood vessels, collectively constitute a large and important organ. They respond to flow changes, stretch, a variety of circulating substances and inflammatory mediators by secreting vasoactive substances. The vasoactive substances include nitric oxide, prostaglndins and endothelins.

Nitric Oxide (N0)

Important paracrine vasodilator released by endothelial cell is nitric oxide. This endothelium-derived relaxing factor (EDRF) is released continuously in significant amounts by endothelial cells in the arterioles and contributes to arteriolar vasodilation in basal state. NO is synthesized from arginine in a reaction catalyzed by nitric oxide synthase (NOS). Three isoforms of NOS have been identified: NOS 1, found in the nervous system: NOS 2, found in macrophages and other immune cells: and NOS 3, found in endothelial cells. NOS 1 and NOS 3 are activated by agents that increase intracellular Ca^{2+} concentration, including the vasodilators acetylcholine and bradykinin. The NO that is formed in the endothelium diffuses to smooth muscle cells, where it activates soluble guanylyl cyclase, producing cAMP, which in turn mediates the relaxation of vascular smooth muscle. NO is inactived by hemoglobin.

When flow to a tissue is suddenly increased by areriolar dilation, the large arteries to the tissue also dilate. This flow-induced dilation is probably due to local release of NO. The products of platelet aggregation also cause release of NO and the resulting vasodilation propably helps keep blood vessels with and intact endothelium patent. This is in contrast to injured blood vessels, where the endothelium is damaged at the site of injury and platelets therefore aggregate and produce vasoconstriction (10, 13).

Prostacyclin (PGI_2)

Prostacyclin produces vasodilation by stimulating adenylate cyclase, which increases cyclic adenosine monophosphate levels in vascular smooth muscle cells. Prostacyclin release in stimulated by either shear stress or endogenous mediators such as bradykinin, thrombin and serotonin. Prostacyclin also prevents platelets from aggregating on the endothelial surface and consequent clot and maintaining blood flow around it (10, 14).

Endothelins

Endothelial cells also produce the most potent vasoconstrictor agent yet isolated, a polypeptide that was initially called endothelin. Subsequent research demonstraed that there is a family of similar 21 amino-acid polypeptides now called endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). They are encoded by at least 3 different genes. ET-1 is produced by endothelial cells, and in humans its gene produces a 38-amino-acid proendothelin-1. Each is encoded by a different gene. Two different endothelin receptors have been cloned, both of which are coupled via G proteins to phospholipase C. The ET – A receptor, which is specific for ET-1 is found in many tissues and mediates the vasoconstriction produced by endothelin-1. The ET-B receptor responds to all three endothelins. It may mediate vasodilation and it appears to mediate the developmental effects of the endothelins. The biological actions of endothelins on hemodynamic are inducing contraction possibly veins more sensitive than arteries on vascular smooth muscle and causing initial depressor response followed by sustained pressor. Endothelins also have cardiac effects which are evoking positive inotropic and chronotropic effects on myocardium and stimulating intense vasoconstriction of coronary arteries (11, 14).

1.2 Systemic regulation by the nervous system

1.2.1 Autonomic Nervous System

Innervation of the heart

Reflex change in the heart rate are effected by central control of the parasympathetic (vagal) nerves and the sympathetic (accelerator) nerves to the heart. The right and left vagal were innervate to the atria, particularly the SA node and the AV node, with only a few parasympathetic fibers piercing the ventricles. The sympathetic nerves reach the heart by way of the inferior cervical ganglion and the sympathetic chain of ganglia. Sympathetic fibers are distributed mainly to the ventricles. Stimulation of the sympathetic nerves has the same effect as the application of the norepinephrine to the heart. Heart rate and contractility are increased, whereas the conduction time and refractory period are decreased. Stimulation of the parasympathetic nerves in general has the opposite effect, equivalent to the effects evoked by the application of acetlycholine to the heart, the heart slows down and beat is weaker with conduction time and refractory period are longer (10, 11).

Innervation of the blood vessels

The distribution of sympathetic nerve fibers to the blood vessels, that all the vessels except the capillaries, precapillary sphincters and most of the metarterioles are innervated. The innervation of the small arteries and arterioles allows sympathetic stimulation to increase the resistance and thereby to decrease the rate of blood flow through the tissues. The innervation of large vessels, particularly of the veins makes it possible for sympathetic stimulation to decrease the volume of these vessels and thereby to alter the volume of the peripheral circulatory system (10, 11).

1.2.2 Vasomotor Nerves

Sympathetic vasoconstrictor fibers

These nerves maintain a constant vascular tone in most tissues. Stimulation of the sympathetic nervous system by stress or exercise causes the release of norepinephrine, which rapidly enhances vasoconstriction and shunts blood from the vein and visceral organs to active skeletal and heart muscle (11).

Sympathetic vasodilator (cholinergic) fibers

Of lesser importance are the sympathetic fibers that cause vasodilation through the release of acetylcholine. These cholinergic sympathetic nerves are found mainly in the blood vessels of heart muscle. The resulting vasodilation at the onset of exercise speeds up the flow of blood through these active tissues (11).

1.2.3 Reflex Control of Heart and Blood Vessels

Chemoreceptor Reflexes

The chemoreceptor reflexes respond to changes in the carbon dioxide levels, oxygen levels, or pH in the blood and cerebrospinal fluid. The chemoreceptors involed are sensory neurons found in the carotid bodies, lacated in the neck near the carotid sinus, and the aortic bodies, situated near the arch of the aorta. These receptors monitor the composition of the arterial blood. Additional chemoreceptors on the medulla oblongata monitor the composition of the cerebrospinal fluid. The activation of chemoreceptors occurs through a drop in pH or in plasma O_2, or a rise in CO_2 levels. Any of these changes 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. A rise in blood flow and blood pressure is associated with an elevated respiratory rate. The 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 (10, 12).

Arterial Baroreceptors

The baroreceptors are stretch receptors in the walls of the heart and blood vessels. The carotid sinus and aortic arch receptors monitor the arterial circulation. Receptors are also located in the walls of the right and left atria at the entrance of the superior and inferior venae cavae and pulmonary veins, as well as in the pulmonary circulation. These receptors in the low-pressure part of the circulation are referred to collectively as the cardiopulmonary receptors. The baroreceptors are stimulated by distention of the structures in which they are located, and so they discharge at an increased rate when the pressure in these structures rises. Their afferent fibers pass via the glossopharyngeal and vagus nerves to the medulla.

Response of the baroreceptors to pressure, normally, the carotid sinus baroreceptors are not stimulated at all by pressure between 0-60 mmHg, but above 60 mmHg they response progressively more and more rapidly and reach a maximum at about 180 mmHg. The responses of the aortic barareceptors are similar to these of the carotid receptors except that they operate, in general at pressure levels about 30 mmHg higher. The baroreceptors respond extremely rapidly to changes in the arterial pressure, in fact, the rate of impulse firing even increases during systole and decreases again during diastole (10, 11).

Atrial Stretch Receptors

The stretch receptors in the atria are of 2 types: these that discharge primarily during atrial systole (type A), and those that discharge primarily late in diastole, at the time of peak atrial filling (type B). The discharge of type B baroreceptors is increased when venous return is increased and decreased by positive-pressure breathing, indicating that these baroreceptors respond primarily to distention of the arterial walls. The reflex circulatory adjustments initiated by increased discharge from most if not all of these receptors include vasodilation and a fall in blood pressure. However, the heart rate is increased rather than decreased. The atrial baroreceptors are an important part of a reflex mechanism that responds to increases in central venous pressure and regulates the ECF volume (10).

Pulmonary Receptors

Injections of serotonin, capsaicin, veratridine, and related drugs into the pulmonary artery activates C fiber endings close to capillaries in the lung and produces apnea followed by rapid breathing, hypotension, and bradycardia (pulmonary chemoreflex). This response, is blocked by vagotomy and is essentially the same as the coronary chemoreflex caused by injection of drug into the arterial supply of the left ventricle. However, the response is too rapid to be caused by the drugs reaching the left ventricular receptors (10).

Left Ventricular Receptors

When the left ventricle is distended in experimental animals, there is a fall in sysemic arterial pressure and heart rate. It takes considerable ventricular distention to produce this response, and its physiologic significance is uncertain. However, left ventricular stretch receptors may play a role in the maintenance of the vagal tone that keeps the heart rate low at rest (10).

Central Control of Circulatory Reflex

Bainbridge Reflex

Rapid infusion of blood or saline in anesthetized animals sometimes produce a rise in heart rate if the initial heart rate is low. This effect was described by Bainbridge in 1915, and since then it has been known as the Bainbridge reflex. It appears to be a true reflex rather than a response to local stretch, since it is abolished by bilateral vagotomy, and infusion of fluids in animals with transplanted hearts increases the rate of the recipient's atrial remnant but fails to affect the rate of the transplanted heart. The receptors may be the tachycardia-producing atrial receptors mentioned above. The reflex competes with the baroreceptor-mediated decrease in heart rate produced by volume expansion and is diminished or absent when the initial heart rate is high. There has been much debate about its significance, and its physiologic role remains unsettled (10).

Buffer Reflex

At normal blood pressure levels, the fibers of the buffer nerves discharge at a low rate. When the pressure in the sinus and aortic arch rises, the discharge rate increase and when the pressure fall, the rate declines. The compensatory response produced by increased discharge is a fall in blood pressure, because activity in the baroreceptor afferents inhibits the tonic discharge in the vasoconstrictor nerves. It is apparent that the baroreceptors on the arterial side of the circulation, their afferent connection to the vasomotor and cardioinhibitory areas and the efferent pathways from these areas constitute a reflex feedback mechanism that operates to stabilize the blood pressure and heart rate. Any drop in systemic artrial pressure decreases the inhibitory discharge in the buffer nerves and there is a compensatory rise in blood pressure and cardiac output. Any rise in pressure produces dilation of the arterioles and decreases cardiac output until the blood pressure returns to normal level (10).

Resetting the Baroreceptor Reflex

The suprabular center are responsible for modifying the excitablility of the vasomotor and cardiac centers in the medulla and pons, in other words the suprabular centers reset the baroreceptor reflex in response to the barrage of peripheral singnal received during increased activity. This resetting is essential for the maintenance of an adequately high blood pressure during exercise. If the baroreceptor reflex immediately lowered the elevated blood pressure, blood flow to the active tissue would decrease and muscle activity could not be sustained. It is believed that the hypothalamus, which exerts central control over the pool of sympathetic and parasympathetic motorneurons. The role of the cortex in these control mechanism still is not clear, apart form such common observations as increased heart rate and blushing caused by emotional stress but it appears that the various levels of control on central nervous system function as mutually interdependent loop (10, 11).

Bulbar Vasomotor and Cardiac Centers.

The vasomotor center is in the lower third pons and the upper part of the medulla. It consists of an excitatory portion that continuously fires sympathetic vasoconstrictor nerves to maintain vasomotor tone and an inhibitory portion that can inhibit sympathetic vasoconstriction, thereby allowing passive vasodilation. Also in the bulbar region is a cardiac center, which similarly consists of two functionally different regions. The cardiac acceleratory center causes an increased heart rate and cardiac contractility through stimulation of sympathetic nerves, whereas the cardiac inhibitory center slows the heart rate and decreases its contractility through parasympathetic activity (10, 11).

1.3 Systemic regulation by hormones

Humoral regulation of the circulation means regulation by substances secreated or absorbed into the body fluids, such as hormones and ions. Some of these substances are formed by special glands and then transported in the blood throughout the entire body. Others are formed in local tissue areas and cause only local circulatory effects. Among the most important of the humoral factors that affect circulatory function are the following.

Vasoconstrictor Agents

Norepinephrine and Epinephrine

Norepinephrine is an especially powerful vasoconstrictor hormone; epinephrine is less so and, in some vessels, even causes mild vasodilation. A special example of vasodilation occures to dilate the coronary arteries during increased heart activity. When the sympathetic nervous system is stimulated in most or all parts of the body during stress or exercise, the sympathetic nerve endings in the individual tissues release norepinephrine, which excites the heart, veins, and arterioles. In addition, the sympathetic nerves to the adrenal medullae cause these glands to secrete both norepinephrine and epinephrine into the blood. These hormones then circulate to all areas of the body and cause almost the same excitatory effects on the circulation as direct sympathetic stimulation, thus providing a dual system of control (8, 15).

Angiotensin

Angiotensin is one of the most powerful vasocontrictor substances. It is a circulating polypeptide hormone formed in the plasma from substrates by the action of the enzyme renin which is rereased from the kidneys. Angiotensin II regulates aldosterone release from the adrenal cortex as part of the system for controlling body sodium balance. It is very important in blood volume regulation. Angiotensin II is also a very potent vasocontrictor agent. Although it should not be viewed as a normal regulator of arteriolar tone, direct vasoconstriction from angiotensin II seems to be an important compotent of the general cardiovascular response to severe blood loss. There is also strong evidence suggesting that direct vascular actions of angiotensin II may be involved in intrarenal mechanisms for controlling kidney function. In addition, angiotensin II may be partially responsible for the abnormal vasoconstriction that accompanies many forms of hypertension. Again it should be emphasized that our knowledge of many pathological situations-including hypertension is incomplete. These situations may well involve vascular influences which are not yet recognized. The effect of angiotensin is to powerfully constrict the small arterioles. If this occures in an isolated tissue area, the blood flow to that area can be severely depressed. However, the real importance of angiotensin is that it normally acts stimultaneously on all the arterioles of the body to increase the total peripheral resistance, thereby increasing the arterial pressure. Because of this, in addition to several renal and adrenocortical stimulatory effects of angiotensin, this hormone plays an integral role in the regulation of the arterial pressure (7).

Erythropoietin

Erythropoietin is released by the kidneys if the blood pressure declines or if the oxygen content of the blood becomes abnormally low. This hormone stimulates red blood cell production, elevating the blood volume and improving the oxygen-carrying capacity of the blood (10).

Vasopressin

This polypeptide hormone, also known as antidiuretic hormone (ADH), plays an important role in extracellular fluid homeostasis and is released into the bloodstream from the posterior pituitary gland in response to low extracellular volume and or high extracellular fluid osmolarity. Vasopressin acts on collecting ducts in the kidney to decrease renal excretion of water. Its role in body fluid balance has some very important indirect influences on cardiovascular functions. Vasopressin, however, is also a potent arteriolar vasoconstrictor. While it is not thought to be significantly involved in normal vascular control, direct vascular constriction from abnormally high levels of vasopressin may be important in the response to certain disturbances like severe blood loss through hemorrhage (10).

Vasodilator Agents

Bradykinin

Several substances called kinins that cause powerful vasodilation are formed in the blood and tissue fluids of some organs. The kinins are small polypeptides that are split away by proteolytic enzymes from alpha2-globulins in the plasma or tissue fluids. A proteolytic enzyme of particular importance is kallikrein, which is present in the blood and tissue fluids in an inactive form. This inactive kallikrein is activated by maceration of the blood, by tissue inflammation, or by other similar chemical or physical effects on the blood or tissues. As kallikrein becomes activated, it acts immediatedly on alpha2-globulin to release a kinin called kallidin that then is converted by tissue enzymes into bradykinin. Once formed, bradykinin persists for only a few minutes because it is inactivated by the enzyme carboxypeptidase or by converting enzyme, an enyme that also plays an essential role in activating angiotensin. Bradykinin causes both powerful arteriolar dilatation and increased capillary permeability. Kinins plays special roles in regulating blood flow and capillary leakage of fluids in inflamed tissues. It also is believed that bradykinin plays a normal role in regulating blood flow in the skin as well as in the salivary and gastrointestinal glands (10).

Histamine

Histamine is released in essentially every tissue of the body when the tissue becomes damaged or inflamed or is the subject of an allergic reaction. Most of the histamine is delivered from mast cells in the damaged tissues and from basophils in the blood. Its has a powerful vasodilator effect on the arterioles and, like bradykinin, has the ability to increase greatly capillary porosity, allowing leakage of both fluid and plasma protein into the tissues. In many pathological conditions, the intense arteriolar dilatation and increased capillary porosity produced by histamine cause tremendous quantities of fluid to leak out of the circulation into the tissues, inducing edema. The local vasodilatory and edema-producing effects of histamine are especially prominent in allergic reactions (10).

Effects of ions and other chemical factors on vascular control

Many different ions and other chemical factors can either dilate or constrict local blood vessels. Most of them have little function in the overall regulation of the circulation, but their specific effects can be listed as follows.

An increase in calcium ion concentration causes vasoconstriction. This results from the general effect of calcium to stimulate smooth muscle contraction.

An increase in potassium ion concentration cause vasodilation. This results from the ability of potassium ions to inhibit smooth muscle contraction.

An increase in magnesium ion concentration causes powerful vasodilation because magnesium ions inhibit smooth muscle generally.

The only anions to have significant effects on blood vessels are acetate and citrate, both of which cause mild degrees of vasodilation.

An increase in hydrogen ion concentration(decrease in pH) causes dilatation of the arterioles. A slight decrease in hydrogen ion concentration causes arteriolar constriction, but an intense decrease causes dilatation.

An increase in carbon dioxide concentration causes moderate vasodilation in most tissues but marked vasodilation in brain. Also, carbon dioxide, acting on the brain vasomotor center, has an extremely powerful indirect effect, transmitted through the sympathetic nervous vasoconstrictor system, to cause widespread vasoconstriction throughout the body (7, 9, 10).

Regulation of cerebral blood flow

1. Neural factors

The cerebral vessels receive innervation from the cervical sympathetic nerve fibers that accompany the internal carotid and vertebral arteries into the cranial cavity. The importance of neural regulation of the cerebral circulation is controversial, but the prevalent belief is that relative to other vascular beds sympathetic control of the cerebral vessels is weak and that the contractile state of the cerebral vascular smooth muscle depends mainly on local metabolic factors. There are no known sympathic vasodilator nerves to the cerebral vessels, but the vessels do receive parasympathetic fibers from the facial nerve, which produce a slight vasodilation on stimulation (10).

2. Local factors.

Generally total cerebral blood flow is constant. However, regional cortical blood flow is associated with regional neural activity. The magnitude of its uptake is determined from radioautographs of slices of the brain. The mediator of the like between cerebral activity and blood flow has not been established, but three possible candidates are pH, potassium, and adenosine.

The cerebral vessels are very sensitive to carbondioxide tension. Increases in arterial blood CO_2 tension ($PaCO_2$) elicit maked cerebral vasodilation; inhalation of 7% CO_2 results in a twofold increment in cerebral blood flow. By the same token decreases in $PaCO_2$, such as elicited by hypertention, produce a decrease in cerebral blood flow. CO_2 produces changes in arteriolar resistance by altering per vascular pH.

With respect to K^+, such stimuli as hypoxia, electrical stimulation of the brain, and seizures elicit rapid increases in cerebral blood flow and are associated with increases in perivascular K^+. The increments in K^+ are similar to those which produce pial arteriolar dilation which K^+ is applied topically to these vessels. However, the increase in K^+ is not sustained thoughout the period of stimulation. Hence, only the initial increment in cerebral blood flow can be attributed to the release of K^+.

Adenosine levels of the brain increase with ischemia, hypoxemia, hypotension, hypocapnia, electrical stimulation of the brain, or induced seizures. When it is applied topically, adenosine is a potent dilator of the pial arterioles. In short, any intervention that either reduces the O_2 supply to the brain or increases the O_2 need of the brain results in rapid formation of adenosine in the cerebral tissue. Unlike pH or K^+, the adenosine concentration of the brain increases with initiation of the stimulus and remains elevated throughout the period of O_2 imbalance. The adenosine released into the cerebrospinal fluid during conditions associated with inadequate brain O_2 supply is available to the brain tissue for reincorporation into cerebral tissue adenosine nucleotides.

All three factors, pH, K^+ and adenosine may act in concert to adjust the cerebral blood flow to the metabolic activity of the brain, but how these factors interact to accomplish this regulation of cerebral blood flow remains to be eluciduted.

The cerebral circulation shows reactive hyperemia and excellent autoregulation between pressure of about 60 and 160 mmHg. Mean arterial pressures below 60 mmHg result in reduced cerebral blood flow and syncope, whereas mean pressures above 160 mmHg may lead to increased permeability of the blood–brain barrier and cerebral edema. Autoregulation of cerebral blood flow is abolished by hypercapnia or any other potent vasodilator, and none of the candidates for metabolic for this phenomenon. Hence, autoregulation of cerebral blood flow is probably attributable to a myogenic mechanism, although experimental proof is still lacking (10).

2.Deoxycorticosterone acetate-salt (DOCA-salt) hypertension model.

Administration of some adrenal steroids in humans is usually accompanied by hypertension. In animal, too, it is possible to produce hypertension by giving steroid hormones. Deoxycorticosterone (DOC) and its acetate, were the first synthetic corticosteroids to become available in large quantities and were used in many studies in the late 1930s. To accelerate the development of DOC hypertension, it should be plus 0.9-1.0% NaCl in drinking fluid, called DOCA-salt hypertension model, is a popular model. The DOCA-salt hypertension can be induced by several methods, route of administration, different in salt of DOC, dose of DOCA and drinking fluid (16, 17).

The mechanisms in induction hypertension of DOCA-salt model is not simple but more complex. The proposed mechanisms are.

2.1 Neurogenic Mechanisms.

2.1.1 Sympathetic Role

Alteration in catecholamine metabolism has been best characterized in DOCA-salt model. Norepinephrine (NE) turnover in the heart was elevated in rats after treated with DOCA and 1% saline in the drinking water. The increased NE turnover was accompanied by the increase in rate of NE synthesis, as well as NE concentration in both plasma and urine. Penelope *et al* (18) reported that the factor which contributed with DOCA-salt hypertension in rats was supersensitivity of NE in caudal arteries and vascular bed. Moreover, Oparil S (19), reported that increased in renal efferent nerve activity contributed to the development of hypertension by causing increase in renal sodium retention. Renal denervation delayed the development and blunted the severity of hypertension.

2.1.2 **Central Nervous System.**

- Hypothalamus. Takeda *et al* (20) found that in DOCA-salt hypertensive rats, the depressor responses to intracisternal injections of adrenaline were augmented. So they suggested that adrenaline in the brain could contribute to the inhibitory mechanism of the sympathetic nerve system, and this mechanism may be attenuated in DOCA hypertensive rats.
- Medulla oblongata. The noradrenergic neurons in locus coeruleus of DOCA-salt treated rats had an increased responsiveness to the excitatory effect of vasopression in both prehypertensive and chronic stage of hypertension (21).

2.1.3 Gamma-aminobutyric acid (GABA).

The activity of central GABA ergic system was altered in the rat with established DOCA-salt hypertension and the alteration in central GABA ergic fuction may be related to the increased sympathoadrenal activity and the maintenance of hypertension in this model (22).

2.1.4 Baroreceptor Reflex.

In DOCA-salt model, the baroreceptor reflex cannot act against the development of hypertension and the reason was central component of the baroreceptor reflex mediated by the aortic depressor nerve was impaired before hypertension develops and that this impairment may contribute to the development of hypertension in DOCA-salt treated rats(23).

2.2 Humoral Control.

2.2.1 Renin-angiotensin.

DOCA-salt hypertensive rats had a reduction in PRA but an increase in the number of ANGII receptor binding sites in some area of brain, and also had the highest level of ACE in kidney, anterior pituitary, testis and choroid plexus compared with the other experimental hypertensive rats (24).

2.2.2 Vasopressin.

Pettinge WA *et al.* (25) measured the cAMP response to vasopressin in the glomerulus, proximal convoluted tubule, thin descending limb, medullary thick ascending limb and cortical thick ascending limb of Henle, medullary and cortical collecting tubule (CCT). The supersensitivity occurred only to vasopressin and only in the CCT and this effect to be exaggerated by sodium salt. DOCA-salt hypertension was vasopressin dependent. A specific exaggerated cAMP response to this hormone in the CCT would be expected to cause increased sodium retention. Whether increased sodium retention at this site contributed to hypertension in the DOCA-Na^+ rat is still unknown.

2.2.3 Atrial Natriuretic Peptide (ANP).

In DOCA-salt hypertensive rats plasma ANP were increased and may be the cause of volume expansion in this model. But the density of vascular receptors for ANP was reduced and by this reason may be the causes of decrease sensitivity of vascular responses to ANP in vitro and resistance to blood pressure lowering action of ANP in vivo (26).

2.3 Endothelial Cell.

Makynen *et al* (27) studied endothelical function in DOCA-salt model when given calcium supplementation. They found that increase calcium diet from 1.1% to 2.5% markedly opposed the development of DOC-NaCl hypertension, an effect associlated with improved arterial relaxation, while abnormalities of vascular contractile properties remained unaffected. In particular, the hyperpolarization-related component of endothelium-dependent arterial relaxation, mediated via opening of arterial K^+ channels, could be augmented by calcium supplementation in DOC-NaCl hypertension.

Matsumura *et al* (28) studied the contributions of endothelin-A and endothelin-B receptors in the pathogenesis of DOCA-salt induced hypertensive rats by giving ABT-672 (10[mg/Kg]/d), a selective ET_A receptor antagonist; A-192621 (30[mg/Kg]/d), a selective ET_B receptor antagonist; for 2 weeks after the start of hypertensive induction. In DOCA-salt rats, systolic blood pressure increased markedly after 3 to 4 weeks. Daily administration of ABT-627 abolished the further increases in blood pressure, whereas A-192621 did not affect the development hypertension. The development of vascular hypertrophy of aorta which observed in DOCA-salt hypertensive rats was markedly suppressed by ABT-627. In contrast, treatment with A-192621 significantly exaggerated these vascular changes. The DOCA-salt rats had renal dysfunctions with decreased renal blood flow and creatinine clearance, and increased urinary excretion of protein, blood urea nitrogen, fractional excretion of sodium, and urinary *N*-acetyl-β-glucosaminidase activity. Such damage was overcome by treatment with ABT-627 but worsen with A-192621 ABT-627 also reduced tubular dilatation and atrophy as well as thickening of small arteries of renal whereas more severe histopathologic changes were observed in A-192621-treated animals. These results strongly support that ET_A receptor-mediated action plays an important role in the pathogenesis of DOCA-induced hypertension. On the other hand. It seems likely that the ET_B receptor-mediated action protects against vascular and renal injuries in this model of hypertension.

Lange *et al* (29) found that endothelin peptide level was increased in the adrenal glands of DOCA-salt hypertensive rats. Endothelin stimulated adrenal medullary catecholamine release in normotensive and DOCA-salt hypertensive rats. However ET also inhibited adrenal medullary catecholamine release in DOCA-salt hypertensive rats. The venous smooth muscle tone was also increased in DOCA-salt hypertension through the independent actions of both endogenous endothelin-1 acting on subtype A receptors and sympathetically mediated venoconstrictor activity (30).
The overexpression of ET-1 occures in DOCA-salt hypertensive rats in blood vessels of different organs, including the heart, in which enhanced expression was found in the endothelium of epicardial and intramyocardial blood vessels and in the endocardium (31).

Integan *et al* (32) found that vasopressin play a critical role in modellating vascular structure and mechanisms, as well as blood pressure, in DOCA-salt induced hypertension. Moreover, these effects of vasopressin are in part mediated by enhancement of endothelin expression.

2.4 Other mechanisms.

2.4.1 Alteration of the Ion Transpost Across the Membrane.

In DOCA-salt hypertensive rats, the smooth muscle from aortas was more permeable to Na^+ than that from control rats because the aortic helically strips from DOCA-salt hypertensive rats were more sensitive and developed a greater maximal force than aortas from control normotensive rats (33).

Reduction of the Na^+-K^+ pumps activity was reported by Pamnani *et al* (34), in the tail artery of DOCA-salt treated rats. It was also observed that myocardial membrane Na^+-K^+ dependent ATPase was depressed in microsomes prepared from left ventricle of rats with DOCA-salt hypertension (35). From these findings, it was concluded that decreased Na^+-K^+ pump activity in vascular smooth muscle cells acts to partially depolarize the cell membrane, which contributed to increase reactivity to the pressor agents.

2.4.2 Calmodulin.

The intracellular calcium-dependent regulatory systems were genetically disrupted in spontaneously hypertensive rats, but this was probably not an important factor in the development of hypertension. Decreased calmoduim levels were demonstrated in the brain, heart, aorta and kidney of spontaneously hypertensive rats compared with those in Wistar-Kyoto rats. Calmodulin levels in the brain were also decreased in DOCA-salt rats, but not changed significantly in the heart, aorta and kidney compared with those in Wistar-Kyoto rats (36).

3. Centella asiatica L.Urban.

Centella asiatica is a medicinal plant that has been used since prehistoric times. *Centella asiatica L.Urban* has synonyms; *Centella coriacea Nannfd*, *Hydrocotyle asiatica L., Hydrocotyle lunata Lam*. And *Trisanthus cochinchinensis Lour*, *Centella coriacea Nannfd, Centella cordifolia, Centella dusenii Nannfd, Centella floridana (C.etR.) Nannfd., Centella repanda(PerS.) Small, Centella triflora(R. et P.) Nannfd*, It is in Eukaryota kingdom, Spermatophyta Division, Dicotyledoneae class, Apiaceae or Umbelliferae family, *Centella* genus and *Centella asiatica* species. It is a small creeping herb, rooting at nodes. The leaves are kidney-shaped about 1.3-6.3 cms diameter, leaf stalks 2-5 cms long , creeping plant that flowers between August and September. The flowers are of a light violet color. The gray to brownish-green plant has a small that is reminiscent of tobacco leaves, and a mildly bitter taste. The leaves have long petioles arising rosette-like from a common base, embracing the flowers;

inflorescence in single umbel, bearing 1-5 flowers and stalks are 3-6 inches long. It is widely distributed in wet place and warmer regions of both hemispheres throughout India, Malaysia, Thailand, Chaina, East and South Africa, Australia, Cambodia, Central America, Indonesia, lao, Madagascar, South America, The Pacific Islands, Southeast asia, Srilanka, Maxico, Venezuela, Columbia.

Centella asiatica was already being used for medicinal purposes in prehistoric times. It is possible that Centella is identical with the medicinal plant bearing the Sanskrit name "manduk-parni" mentioned by Susruta (approx. 1200 BC) (Madaus, 1938; Kartnig, 1988). By around 500 AD, *Centella asiatica* was already listed in the text books of codified Sanskrit medicine (Ayurveda) in use in India(1).

Chemical constituents

In addition to about 0.1% essential oils and other volatile constituents, *Centella asiatica* contains a wide range of other substances(see Table 1). These derive from the metabolism of phenylpropane and acetate, and belong to the flavonoids and terpenes. The substances of therapeutic interest are the saponin-containing triterpene acids and their suger esters, the most impotant being: asiatic acid, madecassic acid and the three asiaticosides, asiaticoside, asiaticoside A and asiaticoside B (1).

Main Groups	Constituents
Essential oil	
(0.1% of the plant)	Terpene acetate
	Germacrene
	Caryophyllene
	p-Cymol
	Pinene
Flavone derivatives	Quercetin glycoside
	Kaempferol, glycoside and in free form Astragalin
Sesquiterpenes	Caryophyllene
	Elemene and bicycloelemene
	Trans-farnesene
	Eermacrene D
Triterpenic steroids	Stigmasterol
	Sitosterol
Triterpenic acids	Asiatic acid
	6-hydroxy Asiatic acid
	Madecassic acid
	Madasiatic acid[1]
	Betulinic acid
	Thankunic acid[3]
	Isothankunic acid[3]
Triterpenic acid sugar esters	Asiaticoside (major component)
(= saponins or	Asiaticoside A
pseudosaponins)	Asiaticoside B
(1-8% depending	Asiaticoside A (Madecassoside) and B
on country or origin)	Braminoside[2]
	Brahmoside[2]
	Brahminoside[2]
	Thankuniside[3]
	Isothankuniside[3]

1 *Centella asiatica* from Mzdagascar
2 *Centella asiatica* from India
3 *Centella asiatica* from Northeast India

Table 1. *Centella asiatica*: Main constituents with differences depending on country of origin and their classification into main groups (1).

In animal experimental studies (in the mouse), acute toxicity was not shown for oral administration of 1 g/kg body weight of an ethanolic 50% extract of *Centella* (37).

The pharmacological activities of *C. asiatica* include, wound healing, Ulcer-protective and anti-ulcer effects, antimicrobial, antiviral, immunomodulatory effects, sedative, antidepressive, anticonvulsure, analgesic action and effect on varicose veins (1).

Sunilkuma (38) reported that when applied aqueous extract of *Centella asiatica* topically third for 24 days on the open wounds in rats increased cellular proliferation and collagen synthesis at the wound site, as evidenced by increase in collagen content and tensile strength.

Cesarone *et al* (39) found that when treat with total triterpenic fraction of C.asiatica (TTFCA) 60 mg twice daily in diabetic microangiopathy patients after 6 months there was significant improving microcirculation and decreasing capillary permeability. Also, Incandela L *et al* (40) reported that TTFCA tablets 60 mg twice daily for 12 months oral administration in severe diabetic microangiopathy there was significantly improving microcirculation.

Sangsirinavin (2) studied the cardiovascular action of various extract in cluded fresh juice, hot water extraction, 95 percent ethyl alcoholic extraction, alkaloid extraction and glycoside extraction of whole plant of *C. asiatica* in dog, rats and rabbit. The result showed that hot water extract (1:1) 1, 2, 3, 4 ml, fresh squeezed juice (1:1) dose 2, 3, 4 ml, glycoside extract dose 5, 10, 15, 20 mg/kg and alcoholic extract dose 300 and 400 mg/kg intravenous administration decreased arterial blood pressure and heart rate in dog. The oral administration of 50 ml fresh squeezed juice in anesthetized dogs showed no significant change in blood pressure and heart rate. While, fresh squeezed juice 1 ml, alcoholic at dose of 100, 200 mg and alkaloid at dose of 25, 50, 100, 200 mg intravenous administration had no effect on arterial blood pressure and heart rate in dogs.

The glycoside was a very potent hypotensive effect and bradycardia in dog. The blockade of autonomic ganglion by hexamethorium, blockage of muscarinic action by atropine, beta adrenergic blocking agent by propanolol, alpha blocking action by noradrenaline and histamine action by chlorpheniramine had no effect on hypotensive action of glycoside.

The diuretic action, when given glycoside solution 10 mg intraveneous administration in rat increased urine flow and also decreased systolic blood pressure, diastolic blood pressure and heart rate, Most of the antidiuretics are belive to act by influencing the liberation of antidiuretic hormone. The action was probably the result of an expansion of plasma volume or by increase in renal blood flow and it was possibly mediated by the reflex release of endogenous vasopressin from neurohypophysis in response to hypotension. The blood flow at rabbit ear was increased when perfused of *C. asiatica* glycoside solution at dose of 100 mg/ml. intraveneous administration which caused by vasodilatation. The glycoside also produced direct negative chronotropic and inotropic action on isolated rabbit heart when given glycoside solution at dose of 50 mg/ml. The cardiovascular effect of the glycoside is therefore not caused by the central action, but mainly direct on the cardiovascular system.

Sue Hay (3) studied effect of fractionated *C. asiatica* chloroform extracts on the isolated rat aortic strip preparations. The result showed that chloroform extracts inhibited the noradrenaline-induced contraction of the aorta with a non-parallel shift of the log dose response to the right and depressed maximum response at fixed concentrations of 0.13, 0.25 and 0.50 mg/ml.

Veerendra Kumar, Gupta (4) studied effect of aqueous, methanolic and chloroform extracts of whole plan of *C. asiatica* on cognition and markers of oxidative stress in rats. The result showed that, only the aqueous extract (200 mg/kg for 14 days) showed an improvement in learning and memory in both shuttle box and through paradigms. The aqueous extract, 200 and 300 mg/kg, showed a significant decrease in the brain levels of malondialdehyde (MDA) with simultaneous significant increase in levels of glutathione. There was a significant increase in the levels of catalase at the 300 mg/kg but no significant change in superoxide dismutase (SOD) levels were observed. Their studied demonstrated that the aqueous extract of whole plant of *C. asiatica* improved the learning and memory and had the antioxidant property by decreasing the lipid peroxidation and augmenting the endogenous antioxidant enzymes in brain.

Kumar and Muller (6) screened the inhibitory effect on lipid peroxidation of methanolic extracts of 28 different plant species by using bovine brain phosholipid liposomes. The result showed that *C. asiatica* methanolic extracts inhibited lipid peroxidation with IC_{50} values of 24 mg/ml.

Ratthanoo *et al.* (41), studied vasorelaxation effect of hexane extract from *C. asiatica* (L.) urban in the isolated rat aorta of 2K-1C hypertension. Vasorelaxant activities of fifteen fractions from hexane extract were tested in phenylephrine preconstricted aortic rings. The results demonstrated that the Ach-induced dilation in aortic rings from hypertensive rats was reduced compared with the controls, suggesting that endothelium was partially damaged in 2K-1C hypertensive rats. However, the contractile response to K^+ was not different from the control. Four fractions of hexane extract demonstrated aortic relaxation effect in a concentration-dependent manner.

Suhaila *et al.* (5) studied the efficacy of *C.asiatica* in alleviating cholesterol induced cardiovascular, hepatic and renal damages in rats. The result showed that *C.asiatica* did not significantly decrease the serum cholesterol and triglycerides in hypercholesterolaemic rats, but showed properties to reduce cholesterol injures to the heart and arteries. In addition the reduction in weight, fat deposition in the liver tissues, and degenerative change of the kidney tubules and glomeruli were observed. Epithelisation was also promoted in the kidney of hypercholesterolaemic rats.

CHAPTER 3
MATERIALS AND METHODS

Materials

1. Animals.

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

2. Chemicals

Captopril	(Sigma)
Centella asiatica.	
Calcium chloride dihydrate	(Merck)
Dextrose	(Mallinckrodt ®)
Dental Acryl.	(Temp Bond)
Deoxycorticosterone Acetate	(Sigma)
Ether anhydrous	(J.T. Beker Chem. Co.)
Heparin	(Leo Pharmaceutical Product)
Magnesium sulphate heptahydrate.	(May & Baker LTD.)
Normalsaline	(General Hospital Product Public. Co. LTD.)
Potassium chloride	(Merk)
Sodium dihydrophosphate dihydrate.	(Merck)
Sodium hydrogen carbonate	(Asia Pacific Specialty Chemicals Limited).
Sodium chloride	(Carlo Erba reagenti)
Sodium pentobarbital	(Nembutal, Abbot)
Urethane	(Merck)

Chemicals for preparation of artificial cerebrospinal fluid(mM/L)

- NaCl (118.0)
- KCL (4.0)
- $NaH2PO_4.2H_2O$ (1.2)
- $NaHCO_3$(25.0)
- Dextrose (5.0)
- $CaCl_22H_2O$(1.5)
- $MgSO_47H_2O$(1.2)

3. Equipments

Analytical balance (SATORIUS).
Automatic pipet (Pipetman).
Blood pressure recorder (Ugo Basile,Italy).
Cranial window.
Disposible syring 1,2.5,5,10,20 ml.
Esophageal tube feeding.
Elastic rubber.
Freeze dryer.
Guaze.
Jellco No.22 , 24.
Lamp 100 watt.
Laser Doppler Perfusion and Temperature Monitor. (Model DRT 4, Moor Instrument, England).
Magnetic stirrer (Ikamag).
Needle No. 18, 20, 21, 22, 23,24, 26.
Operating set.
Polygraph System Recorder (NIHON HOLDEN).
Pellet generator (Perkin-Elmer).
Pressure transducer for direct blood pressure measurement (Nihon RM 600,-NIHON KHODEN, Japan).
PE tube No. 10, 20.

Rat restrainer.
Stereotaxic frame.
Skull driller.
Tracheostomy tube.

Methods

1. Preparation of expressed juice of *C. asiatica* leaves

The 12 kgs *C. asiatica* was bought from Chokchai market, Ladprao Road, Bangkok, Thailand. The fresh leaves, 3044.03 grams, were washed and blended with distilled water at ratio of 1.6 g : 1ml by blender,then squeezed through cotton and muslin cloth. The juice was freeze dried by lyophilizer. One gram of *C. asiatica* leaves yieled 0.0158 gram of lyophilized powder. The lyophilized powder was kept in a tight container at temperature -20 °c until used. On the day of experiment, *C.asiatica* juice was freshly prepared by redissolving the lyophilized powder in distilled water.

2. Experimental Animal

2.1 Animal preparation.

Male Wistar rats weighing between 140-160 grams(g) (National Experimental Animal Center, Salaya, Mahidol University) were housed in the animal room of Faculty of Pharmacy, Mahidol University for at least one week before being used in the experiment. They were fed with regular rat chow and water *ad libitum*. The rats were divided randomly into normal and hypertensive groups.

2.2 DOCA-salt hypertension

DOCA-salt hypertension was induced by subcantaneous implantation of 100-150 mg Deoxycorticosterone acetate (DOCA) pellets at the dorsal area of neck under anesthetization with ether and aseptic technique. DOCA pellets were prepared by purching DOCA powder with pellet generator (Figure 1.). Following the surgery the rats were given 1% sodium chloride. Hypertensive condition defined as a SBP ≥ 180 mmHg.

3. Measurements

3.1 Blood Pressure

3.1.1 Indirect Method

Systolic blood pressure (SBP) measured via caudal artery in conscious rats by the tail-cuff method (Figure 2.). Rats were trained for 1 week to get used to the tail cuff method by placing them in rat restainers to restric 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 vasodilatation of caudal artery, then the rats were kept in restainers and waited for 3 minutes for reducing the stress from warming, before starting the measurement.

3.1.2 Direct Method

Direct arterial pressure (Systolic and diastolic blood pressure) were monitored via the femoral artery. Arterial pressure were measured with a pressure transducer connected to a polygraph system (Nihon Kohden, Japan) throughout the regional cerebral blood flow monitoring.

3.2 Hert rate.

Heart rate (HR) were determined from pulse rate obtained simultaneously in SBP measurement by tail cuff method

3.3 Local cutaneous blood flow.

The cutaneous blood flow were measured indirectly in anesthesized rats with urethane (0.8 g/kg intraperitoneal) by the laser doppler perfusion flowmeter and Temperature monitor. Rats were lie down in prone position and the fiber optic needle probe was fixed perpendicularly about 1 mm. above the plantar hindpaw skin (Figure 3). The blood flow at plantar hindpaw skin were measured continuously. The data were expressed in dimensionless perfesion units (PU).

3.4 Regional cerebral blood flow

To determine regional cerebral blood flow (rCBF), a laser doppler flowmeter were used. The rats were anesthetized by intraperitoneal administration of 60 mg/kg bw sodium pentobarbital. Additional dose of anesthetic were given as required to maintain anesthesia throughout the experiment based on testing of corneal reflex and response to tail pinch. The esophageal tube feeding was inserted, then tracheostomy was performed . The right femoral artery was canulated to record arterial pressure. Systolic (SBP) and diastolic blood pressure (DBP) were monitored continuously throughout the experiments with pressure transducer which recorded on polygraph.

After tracheostomy and femoral artery canulation had been performed, the rat was placed on surgical frame and the head was fixed with a stereotaxic frame. A small hole was drilled in the bone overlying the right parietal cortex to expose pial cerebral microvessels. The dura was opened by using microneedle. Stainless metallic frame was fixed to the cranial opening. An artificial cerebrospinal fluid was infused on top of the cranial space. The fiber optic needle probe was fixed perpendicularly about

1 mm. above the cranial space. Five different measurements were performed at each time and the mean was then determined for each animal (Figure 4).

Figure 1. Pellet generator

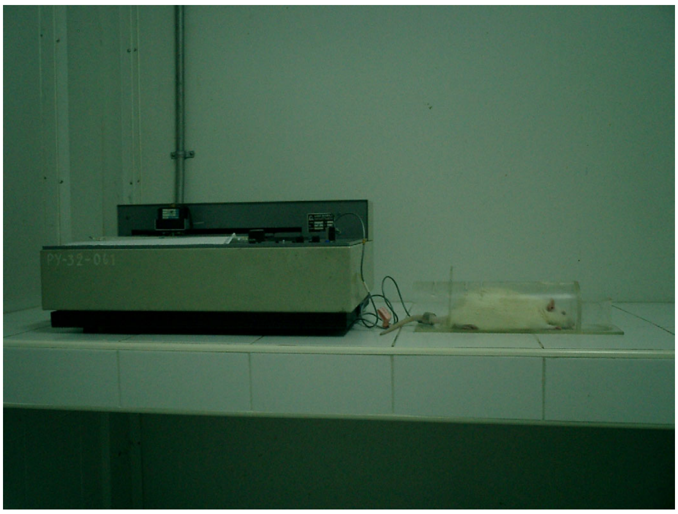

Figure 2. Indirect blood pressure measurement (tail cuff method)

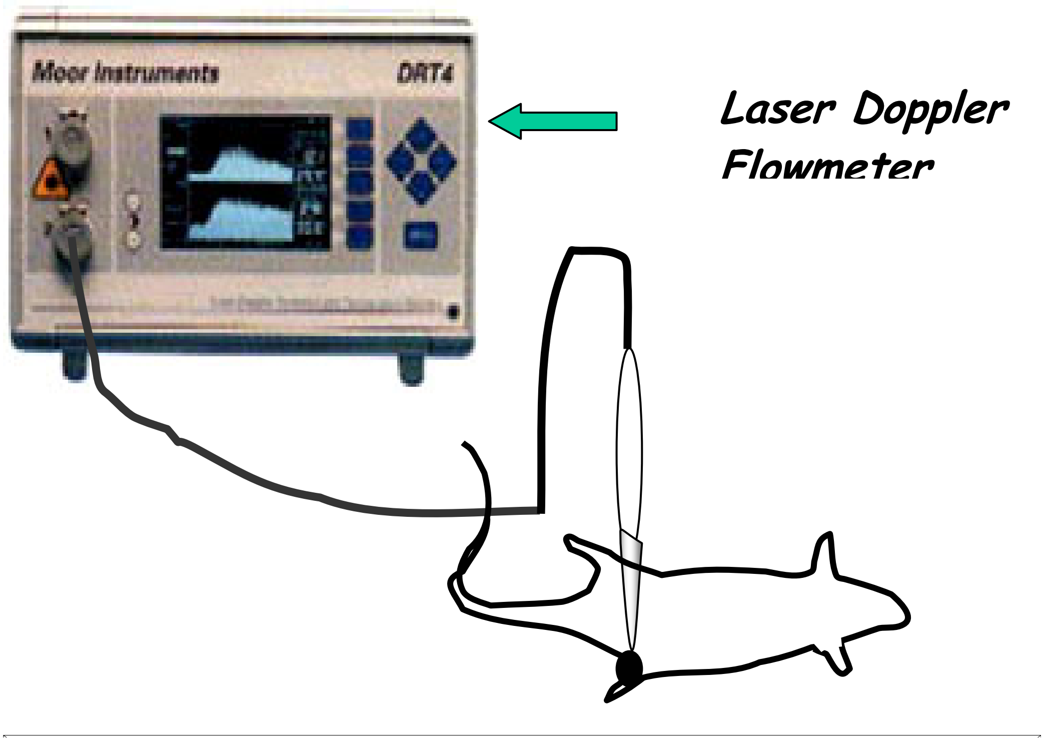

Figure 3. Local cutaneous blood flow measurement

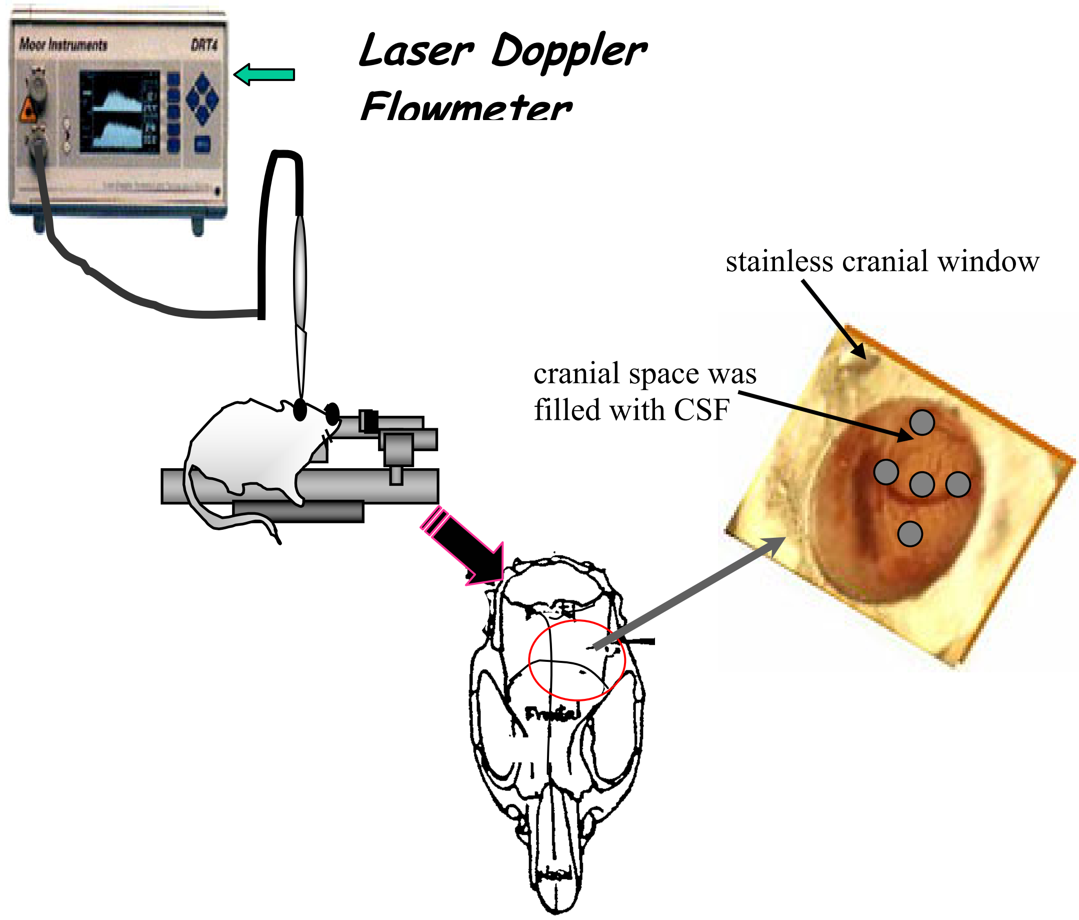

Figure 4. Schematic of a setup for monitoring regional cerebral blood flow in rat Grey dots (●) on the cranial space represent area for mornitoring regional cerebral blood flow

4. Experimental procedures.

Experiment 1. Effect of *C.asiatica* on blood pressure and heart rate.

The systolic blood pressure (SBP) and heart rate (HR) were detected in both normal and hypertensive rats (8 rats in each group) before (0 minute), and at 5, 15, 30, 45, 60, 90 and 120 minutes after esophageal feeding of captopril at the dose of 25 mg/kg The experiments were repeated in the same rat by giving *C.asiatica* juice at the doses of 16, 24 and 32 g/kg and 1 ml. distilled water as a normal control. Each administration was at least 2 days interval.

Experiment 2. Effect of *C.asiatica* on local cutaneous blood flow.

The rats from the experiment 1. were used for further study in this experiment with at least 1 week interval. The cutaneous blood flow in the plantar hindpaw skin were measured in rats anesthesized with urethane (0.8 g/kg intraperitoneal) before (0 minute) and at 5, 15, 30, 45, 60, 90 and 120 minutes after esophageal feeding of the *C.asiatica* juice at the dose of 32 g/kg.

Experiment 3. Effect of *C.asiatica* on regional cerebral blood flow.

The regional cerebral blood flow (rCBF) were measured in anesthesized rats, both normal (n=10) and hypertensive (n=8) rats before (0 minute) and at 5, 15, 30, 45, 60, 90 and 120 minutes after esophageal feeding of the *C.asiatica* juice at the dose of 32 g/kg. The systolic blood pressure and diastolic blood pressure were also monitored during regional cerebral blood flow measurement.

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). The paired Student' s t-test was used to compare the effects of *C.asiatica*, captopil and distilled water at various time points with their pre-administration. The comparisions between normal and hypertensive rats were made with an unpaired Student’ s t-test. One way analysis of varience (ANOVA) was used to compare the values for more than two groups. The p-values of less than 0.05 ($p<0.05$) were considered to be statistically significant difference.

CHAPTER 4
RESULTS

The normal and hypertensive rats had significant increased in weekly SBP from week 0 starting from week 3 (Table 2). The hypertensive rats had greater significant increase in weekly SBP than normal rats at week 2 to week 8 of study (Figure 5). The hypertensive rats had SBP greater than 180 mmHg at week 7 and week 8 with 63 and 66% increase, respectively (Table 3 and Figure 6).

There were significant increase in weekly heart rate at week 5, and 7 in hypertensive rats and at week 3 and week 7 in normal rats (Table 4 and Figure 7). However, there were no significant difference in percentage of weekly heart rate in both normal and hypertensive rats, and between normal and hypertensive rats at corresponding time (Table 5 and Figure 8).

The hypertensive rats had significant lower body weight than normal at the start of the experiment and at week 1, 6, 7 and 8. Both normal and hypertensive rats had significant increase in weekly body weight at week 1 to 8 (Table 6 and figure 9). Both groups had significant increase in percentage of weekly body weight, but significant lower in hypertensive than normal group at week1 and hypertensive higher than normal at week 5 (Table 7 and Figure 10).

Table 2. Weekly systolic blood pressure in normal rats (N) and hypertensive rats (H) (n=8 in each group).

n	Systolic blood pressure (mmHg)																	
	week 0		week 1		week 2		week 3		week 4		week 5		week 6		week 7		week 8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	100	125	110	120	110	125	120	130	130	150	125	145	120	160	130	185	130	195
2	120	130	110	120	120	130	125	145	130	165	130	170	125	190	135	190	140	200
3	95	115	100	125	120	140	120	150	120	150	125	155	130	160	130	180	130	190
4	100	110	120	110	115	120	120	130	130	150	125	160	125	180	140	195	135	210
5	120	125	120	120	120	125	125	135	125	155	130	160	130	185	135	190	130	190
6	100	120	115	125	110	130	120	140	135	160	125	165	135	170	130	200	140	195
7	120	95	115	100	120	120	130	130	120	150	120	145	130	160	145	195	135	195
8	120	125	120	120	110	125	120	135	125	160	130	175	125	190	130	200	140	190
mean	109	118	113	117	115	126^{a}	122^{+}	136^{+a}	126^{+}	155^{+a}	126^{+}	159^{+a}	127^{+}	174^{+a}	134^{+}	191^{+a}	135	195^{+a}
± sem	11	11	6	8	4	6	3	7	5	5	3	10	4	13	5	7	4	6

+ ($p < 0.01$) : significant difference from their starting values at week 0.

a ($p < 0.01$) : significant difference from the normal at the corresponding time.

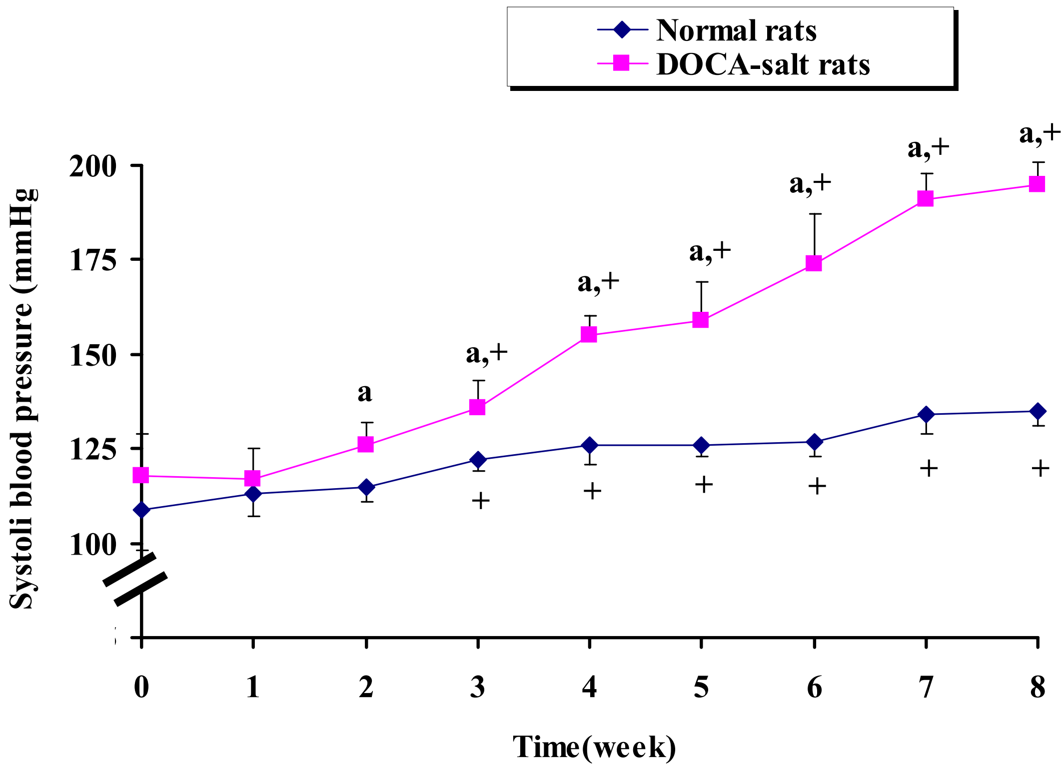

Figure 5. Weekly systolic blood pressure in normal and DOCA-salt rats (n=8 in each group).

All values were expressed as mean ± sem

\+ ($p < 0.01$) : significant difference from their starting values at week 0.

a ($p < 0.01$) : significant difference from the normal rats at the corresponding time.

Table 3. The percentage of weekly systolic blood pressure in normal (N), and hypertensive rats(H) (n=8 in each group).

n	The percentage of systolic blood pressure from week 0															
	1		2		3		4		5		6		7		8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	110	96	110	100	120	104	130	120	125	116	120	128	130	148	130	156
2	91	92	100	100	104	111	108	126	108	130	104	146	112	146	116	153
3	105	108	126	121	126	130	126	130	131	134	136	139	136	156	136	165
4	120	100	115	109	120	118	130	136	125	145	125	163	140	177	135	190
5	100	96	100	100	104	108	104	124	108	128	108	148	112	152	108	152
6	115	104	110	108	120	116	135	133	125	137	135	141	130	166	140	162.5
7	95	105	100	126	108	136	100	157	100	152	108	168	120	205	112	205
8	100	96	91	100	100	108	104	128	108	140	104	152	108	160	116	152
mean	104	99	106	108	112	116	117	131[a]	116	135[b]	117	148[b]	123	163[b]	124	166[b]
± sem	9	5	10	10	9	11	14	11	11	11	13	12	12	19	12	19

a ($p < 0.05$), b ($p < 0.01$) : significant difference from the normal at the corresponding time.

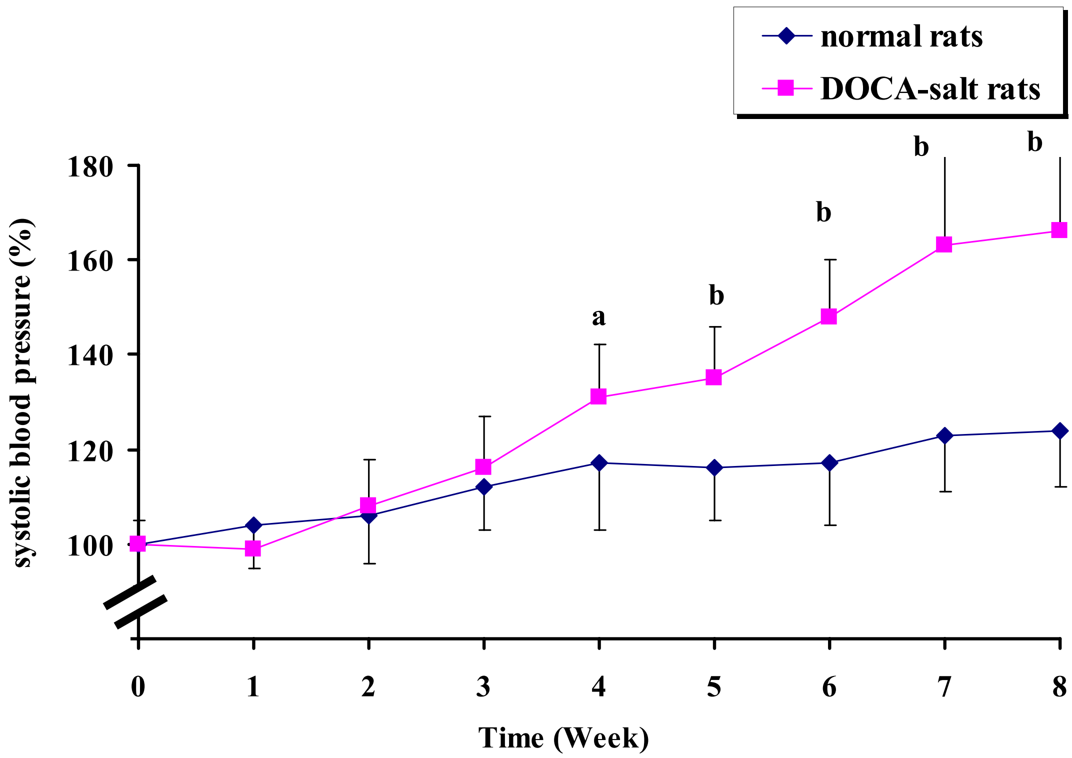

Figure 6. The percent change of weekly systolic blood pressure in normal and DOCA-salt rats (n=8 in each group).

All values were expressed as mean ± sem.

a ($p < 0.05$), b ($p < 0.01$), : significant difference from the normal rats at the corresponding time.

Table 4. Weekly heart rate in normal (N) and hypertensive rats (H) (n=8 in each group).

n	Heart rate (beats/min)																	
	week 0		week 1		week 2		week 3		week 4		week 5		week 6		week 7		week 8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	320	330	350	340	330	310	340	330	320	350	320	360	340	360	350	350	340	370
2	330	320	320	330	350	340	330	320	330	340	370	350	350	350	360	330	340	340
3	300	340	330	320	310	320	300	340	320	370	340	350	340	360	350	370	330	360
4	340	350	340	340	300	350	350	360	330	330	360	380	340	340	340	360	320	380
5	340	350	300	340	330	350	350	350	340	360	320	340	360	370	350	350	360	340
6	320	330	310	320	360	340	370	340	310	330	350	360	350	350	370	360	340	360
7	350	320	340	330	350	330	360	350	330	360	300	350	320	340	350	360	310	390
8	310	360	300	350	360	350	320	320	340	350	340	350	340	330	360	370	320	340
mean	326	337	323	333	336	336	340*	338	327	348[a]	337	355*	342	350	353[+]	356[+]	332	360[b]
± sem	16	14	19	10	22	15	22	14	10	14	23	11	11	13	9	13	15	19

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their starting values at week 0.

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the normal at the corresponding time.

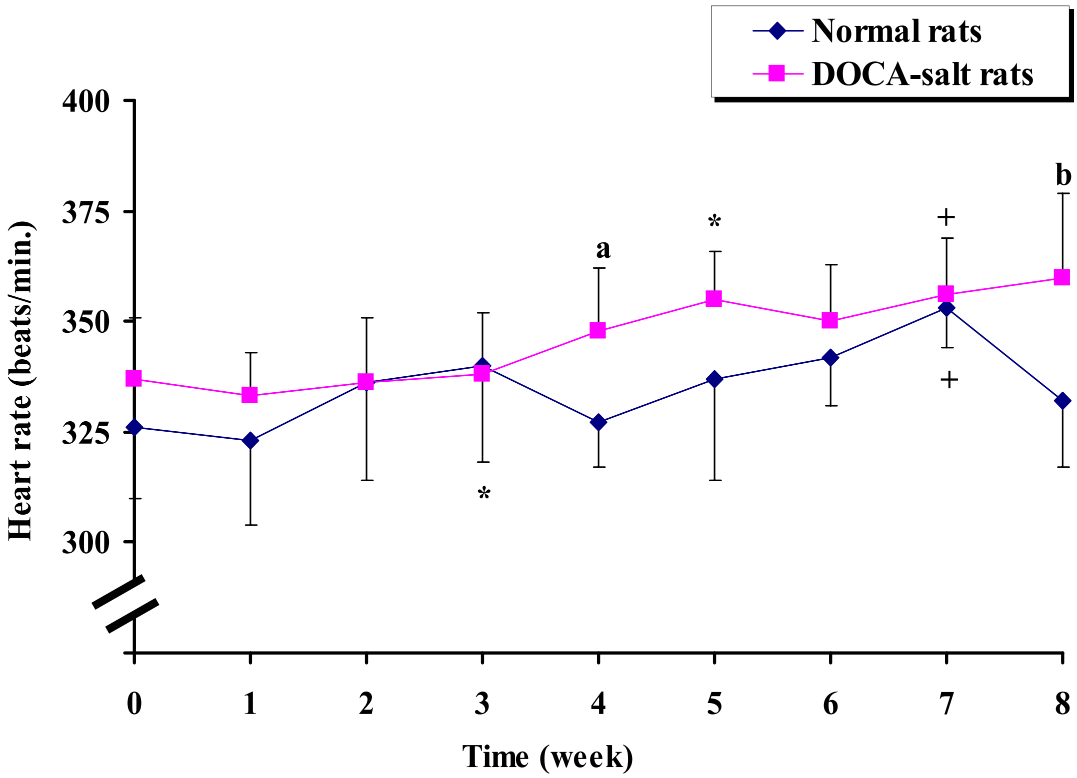

Figure 7. Weekly heart rate in normal and DOCA-salt rats (n=8 in each group).
All values were expressed as mean ± sem.
* ($p < 0.05$), + ($p < 0.01$) : significant difference from their starting values at week 0.
b ($p < 0.05$), a ($p < 0.01$) : significant difference from the normal at the corresponding time.

Table 5. The percentage of weekly heart rate in normal (N), and hypertensive rats (H) (n=8 in each group).

n	The percentage of weekly heart rate from week 0															
	1		2		3		4		5		6		7		8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	109	103	103	93	106	100	100	106	100	109	106	109	109	106	106	112
2	96	103	106	106	100	100	100	106	112	109	106	109	109	103	103	106
3	110	94	103	94	100	100	106	108	113	102	113	105	116	108	110	105
4	100	97	88	100	102	102	97	94	105	108	100	97	100	102	94	108
5	88	97	97	100	102	100	100	102	94	97	105	105	102	100	105	97
6	96	96	112	103	115	103	96	100	109	109	109	106	115	109	106	109
7	97	103	100	103	102	109	94	112	85	109	91	106	100	112	88	121
8	96	97	116	97	103	88	109	97	109	97	109	91	116	102	103	94
mean	99	98	103	99	103	100	100	103	103	105	104	103	108	105	101	106
± sem	7	3	8	4	4	5	5	5	9	5	6	6	6	4	7	8

No significant difference from their starting values at week 0 and between normal and hypertensive rats at the corresponding time.

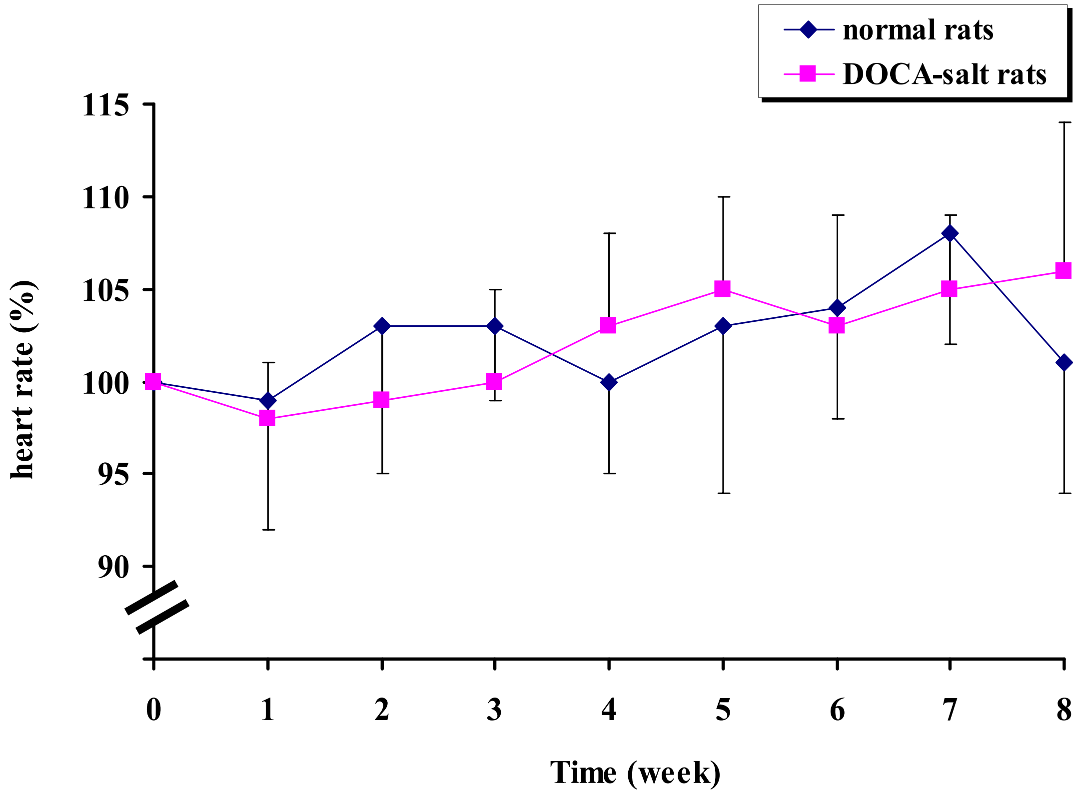

Figure 8. The percentage of weekly heart rate in normal and DOCA-salt rats (n=8 in each group).

All values were expressed as mean ± sem.

No significant difference from their starting values at week 0 and between normal and DOCA-salt rats at corresponding time.

Table 6. Weekly body weigh in normal (N), and hypertensive rats (H) (n=8 in each group).

n	Body weight (g)																	
	week 0		week 1		week 2		week 3		week 4		week 5		week 6		week 7		week 8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	158	130	198	176	211	220	232	249	247	277	232	293	261	257	279	247	283	250
2	150	142	194	174	223	227	240	237	269	269	240	289	280	264	269	261	294	256
3	150	133	193	150	199	210	226	209	247	213	236	289	275	272	275	251	280	262
4	157	145	231	193	267	238	272	257	283	298	280	298	283	264	269	256	279	253
5	162	144	227	170	223	214	252	211	242	224	252	291	265	241	280	264	291	254
6	156	145	224	174	255	238	280	264	294	289	280	238	292	251	275	263	285	260
7	168	137	195	175	261	217	287	219	296	298	281	263	279	244	270	259	286	262
8	156	146	210	162	254	203	288	206	266	259	299	315	291	273	284	235	274	253
mean	157	140^{a}	209^{+}	171^{+a}	236^{+}	220^{+}	259^{+}	231^{+}	267^{+}	265^{+}	262^{+}	284^{+}	278^{+}	258^{+a}	274^{+}	254^{+a}	284^{+}	256^{+a}
± sem	5	6	16	12	25	12	25	23	21	32	25	23	11	12	5	10	6	4

\+ ($p < 0.01$) : significant difference from their starting values at week 0.

a ($p < 0.01$) : significant difference from the normal at the corresponding time.

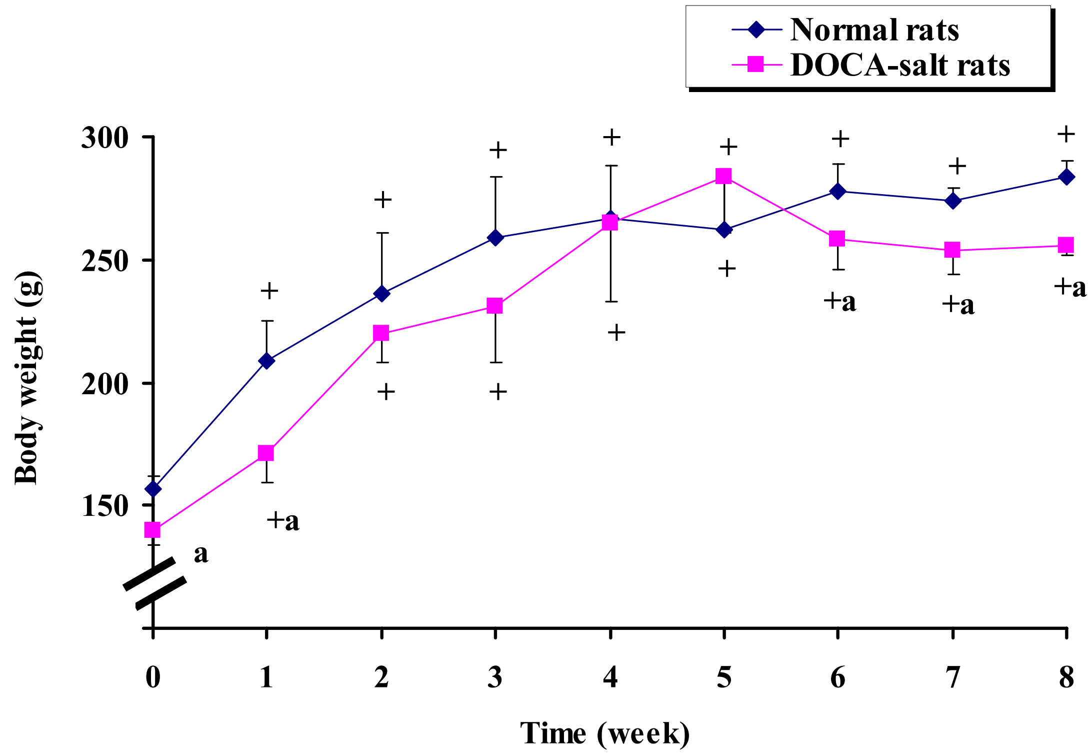

Figure 9. Weekly body weight in normal and DOCA-salt rats (n=8 in each group). All values were expressed as mean ± sem.

+ ($p < 0.01$) : significant difference from their starting values at week 0.

a ($p < 0.01$) : significant difference from the normal at the corresponding time.

Table 7. The percentage of weekly body weigh in normal (N) , and hypertensive rats(H) (n=8 in each group).

n	The percentage of weekly bodyweight from week 0															
	1		2		3		4		5		6		7		8	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	125	135	133	169	147	191	157	213	147	225	165	197	177	189	179	192
2	129	122	148	160	160	167	179	190	160	204	186	186	179	184	196	180
3	128	112	132	157	150	156	164	160	157	216	183	204	183	188	186	196
4	147	132	170	164	172	176	180	205	178	205	180	182	171	176	177	174
5	140	118	137	149	155	146	149	155	155	202	163	167	172	184	179	177
6	143	120	163	164	179	182	188	199	179	164	187	172	175	181	182	179
7	116	127	155	158	170	159	175	217	167	191	165	177	160	189	170	190
8	134	111	162	139	184	141	170	177	191	216	186	187	181	161	175	173
mean	132	122^{a}	150	157	164	164	170	189	166	202^{b}	176	184	174	181	180	182
± sem	10	8	14	9	13	17	12	23	14	18	10	12	7	9	7	8

a ($p < 0.05$), b ($p < 0.01$) : significant difference from the normal at the correspondind time.

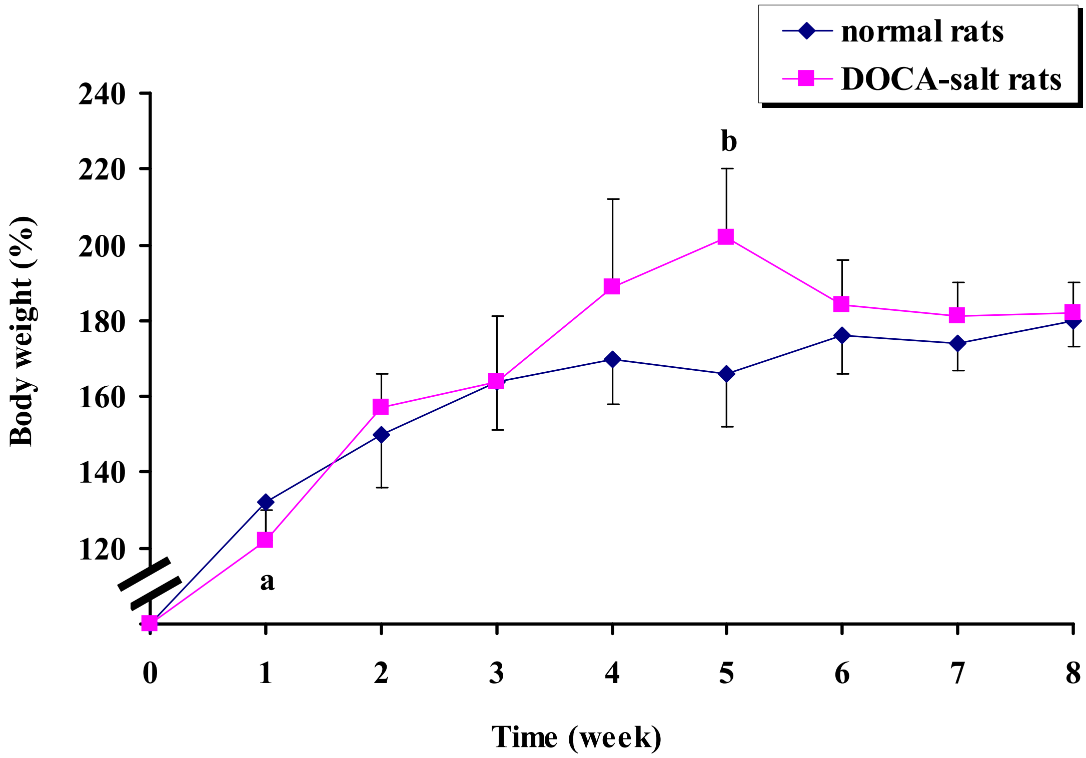

Figure 10. The percentage of weekly body weight in normal and DOCA-salt rats (n=8 in each group).

All values were expressed as mean ±sem.

a ($p < 0.05$), b ($p < 0.01$) : significant difference from the normal at the corresponding time.

Experiment 1. Effects on blood pressure and heart rate

1.1 Blood pressure

Normal rats

The effects of *C. asiatica* juice at doses of 16, 24, 32 g/kg, distilled water, and captopril at dose of 25 mg/kg on SBP in normal rats were shown in Table 8 and Figure 11. All five groups of rats had comparable basal normal levels of SBP. Captopril at dose of 25 mg/kg significantly decreased SBP from pre-administration at 30 to 120 minutes, with maximal effect at 60 minute, from 130 ± 3 to 104 ± 4. These decreased SBP were significantly lower than normal and *C.asiatica* treated groups at the corresponding times. The percentage decreased SBP of captopril were 87 %, 84 %, 79 %, 89 %, and 91 % at 30, 45, 60, 90, and 120 minutes respectively (Table 9 and Figure 12).

C. asiatica juice at dose of 16, 24, and 32 g/kg and distilled water had no effect on SBP throughout 120 minutes of observation (Figure 11 and 12).

Table 8. Effects of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril 25 mg/kg (D) on systolic blood pressure at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in normal rats (n=8 in each group).

n	Systolic blood pressure (mmHg)																			
	0 min					5 min					15 min					30 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	132	132	137	140	145	132	130	142	142	150	132	127	147	135	140	133	107	137	137	135
2	140	132	142	130	137	138	138	147	135	141	135	135	142	137	150	137	112	136	132	140
3	132	130	127	127	137	137	133	132	135	137	140	132	127	135	130	136	110	133	135	132
4	130	128	132	135	131	133	135	130	132	130	133	135	132	140	120	137	122	132	142	128
5	135	137	131	140	132	133	127	135	142	137	132	132	134	135	140	139	117	132	138	135
6	135	126	138	130	122	132	130	130	132	125	135	130	133	135	140	130	111	137	135	138
7	132	132	137	142	125	135	127	142	130	130	137	127	132	135	132	135	113	137	132	130
8	130	127	137	140	142	135	132	140	135	137	136	137	133	135	132	128	122	132	140	127
mean	133	130	135	135	134	134	131	137	135	136	135	132	135	135	135	134	114^{+a}	134	136	133
± sem	3	3	4	5	7	2	3	6	4	7	2	3	6	1	8	3	5	2	3	4

+ ($p < 0.01$) : significant difference from their pre-administration (0 minute)

a ($p < 0.01$) : significant difference from the control and *C.asiatica* 16, 24, 32 g/kg treated groups at the corresponding time.

Table 8. (continued) Effects of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril 25 mg/kg (D) on systolic blood pressure at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in normal rats (n=8 in each group).

n	Systolic blood pressure (mmHg)																			
	45 min					60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	137	117	132	137	132	134	100	135	135	141	136	115	137	135	160	145	122	130	145	145
2	140	97	132	130	132	137	107	135	132	142	137	132	137	140	152	130	122	140	135	152
3	130	112	122	134	137	134	105	137	132	125	132	110	132	130	130	135	117	132	132	130
4	130	107	137	136	133	135	110	135	127	127	128	115	132	138	125	127	117	135	138	135
5	135	122	135	137	132	130	110	142	127	125	133	112	137	137	135	127	127	132	137	130
6	132	114	137	132	131	130	105	132	135	127	132	110	137	132	125	135	114	132	137	135
7	130	105	139	137	137	132	100	130	140	135	135	120	132	135	127	135	122	146	141	135
8	135	110	137	135	137	135	100	137	132	117	135	120	137	135	130	135	117	142	137	137
mean	133	110^{+a}	134	135	134	133	104^{+a}	135	132	130	133	116^{+a}	135	135	135	133	120^{+a}	136	138	137
± sem	3	7	5	2	2	2	4	3	4	8	2	7	2	3	13	5	4	5	3	7

+ (p < 0.01) : significant difference from their pre-administration (0 minute)

a (p < 0.01) : significant difference from the control and *C.asiatica* 16, 24, 32 g/kg treated groups at the corresponding time.

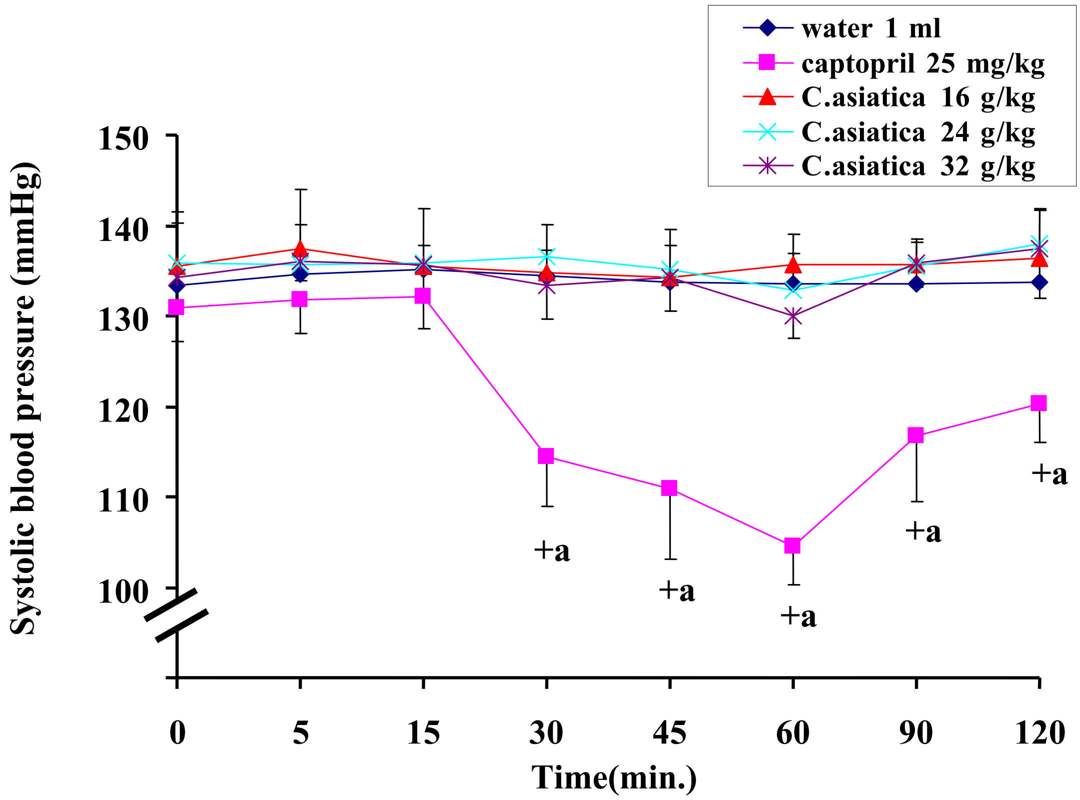

Figure 11. Effects of *C. asiatica* at dose of 16, 24, 32 g/kg, distilled water 1 ml, and captopril at the dose of 25 mg/kg on systolic blood pressure at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in normal rat (n=8 in each group).

All values were expressed as mean ±sem.

+ ($p < 0.01$) : significant difference from their pre-administration (0 minute)

a ($p < 0.01$) : significant difference from the control and *C. asiatica* 16, 24, 32 g/kg treated groups at the corresponding time.

Table 9. The percentage of systolic blood pressure after single oral administration of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril at dose of 25 mg/kg (D) in normal rats (n=8 in each group).

n	The percentage of systolic blood pressure from pre-administration																			
	5 min					15 min					30 min					45 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	100	98	103	101	103	100	96	107	96	96	100	81	100	97	93	103	88	96	97	91
2	98	104	103	103	102	96	102	100	105	109	97	84	95	101	102	100	73	92	100	96
3	103	102	103	106	100	106	101	100	106	94	103	84	104	106	96	98	86	96	105	100
4	102	105	98	97	99	102	105	100	103	91	105	95	100	105	97	100	83	103	100	101
5	98	92	103	101	103	97	96	102	96	106	102	85	100	98	102	100	89	103	97	100
6	97	103	94	101	102	100	103	96	103	114	96	88	99	103	113	97	90	99	101	107
7	102	96	103	91	104	103	96	96	95	105	102	85	100	92	104	98	79	101	96	109
8	103	103	102	96	96	104	107	97	96	92	98	96	96	100	89	103	86	100	96	96
mean	100	100	101	99	101	101	100	99	100	100	100b	87[a]	99	100	99	99	84[a]	98	99	100
± sem	2	4	3	4	2	3	4	3	4	8	3	5	2	4	7	2	5	3	3	5

a ($p < 0.01$) : significant difference from the control, *C.asiatica* 16, 24, and 32 g/kg treated groups at the corresponding time.

Table 9. (continued) The percentage of systolic blood pressure after single oral administration of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril at dose of 25 mg/kg (D) in normal rats (n=8 in each group).

n	The percentage of systolic blood pressure from pre-administration														
	60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	101	75	98	96	97	103	87	100	96	110	109	92	94	103	100
2	97	81	95	101	103	97	100	96	107	110	92	92	98	103	110
3	101	80	107	103	91	100	84	103	102	94	102	90	103	103	94
4	103	85	102	94	96	98	89	100	102	95	97	91	102	102	103
5	96	80	108	90	94	98	81	104	97	102	94	92	100	97	98
6	96	83	95	103	104	97	87	99	101	102	100	90	95	105	110
7	100	75	94	98	108	102	90	96	95	101	102	92	106	99	108
8	103	78	100	94	82	103	94	100	96	91	103	92	103	97	96
mean	99	79[a]	99	97	96	99	89[a]	99	99	100	99	91[a]	100	101	102
± sem	2	3	5	4	8	2	5	2	4	7	5	0	4	3	6

a ($p < 0.01$) : significant difference from the control, *C.asiatica* 16, 24, and 32 g/kg treated groups at the corresponding time.

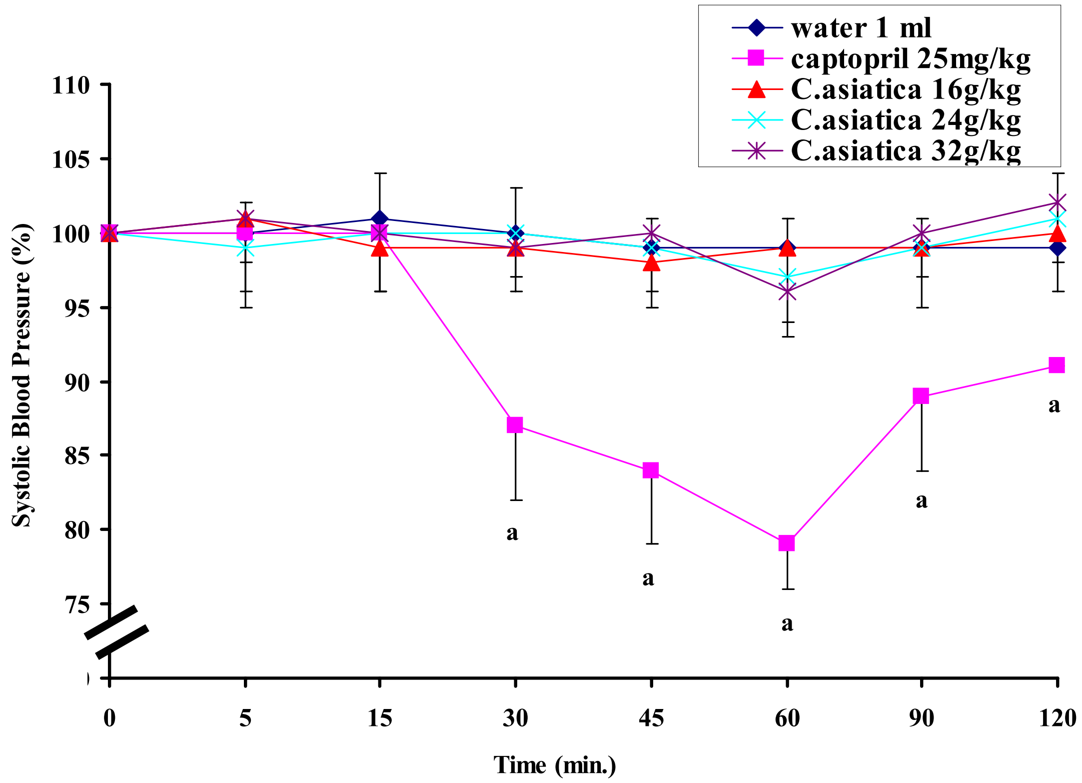

Figure 12. The percentage of systolic blood pressure (indirect) after single oral administration of *C. asiatica* 16, 24, 32 g/kg, distilled water 1 ml and captopril at the dose of 25 mg/kg (n=8 in each group).

All values were expressed as mean sem.

a ($p < 0.01$) : significant difference from the control, *C.asiatica* at dose of 16, 24, and 32 g/kg treated groups at the corresponding time.

Hypertensive rats

The effects of *C.asiatica* extract at the doses of 16, 24, and 32 g/kg, distilled water, and captopril at dose of 25 mg/kg on SBP in hypertensive rats were shown in Table 10 and Figure 13. All five groups of rats had comparable of basal hypertensive levels of SBP. In hypertensive rats, captopril at dose 25 mg/kg significantly decreased SBP from pre-administration similarly to the normal rats with maximal effect at 90 minute (from 201 ± 7 to 163 ± 9, 80 %), (Table 11 and Figure 14). *C. asiatica* juice at dose 16 g/kg and distilled water had no effect on SBP throughout 120 minutes of observation.

C. asiatica juice at dose 24 g/kg significantly decreased SBP in hypertensive rats at 30, 45, 60, and 90 minutes from 200 ± 10 to 186 ± 6, 180 ± 8, 180 ± 6 and 189 ± 6 mmHg with maximal effects at 45 minutes. The decreased SBP at 30, 45, and 60 minutes were significantly lower than normal control and *C. asiatica* 16 g/kg treated groups at the corresponding time. While *C. asiatica* juice at dose 32 g/kg significantly decreased SBP at 45 and 60 minute, from 191± 8 to 169 ± 6 and 175 ± 8 mmHg, with maximal effects at 45 minutes. The decreased SBP at 45 and 60 minute were significantly lower than normal control and *C. asiatica* juice at dose 16 g/kg treated group at corresponding time (Figure 13).

C. asiatica juice at dose of 24 and 32 g/kg had similar percentage decrease in SBP (Table 11 and Figure 14). They also had shorter duration and faster maximum of action than captopril (Figure 13 and 14).

The result indicated that oral administration of *C. asiatica* had no effect in normal rats but decreased SBP in hypertensive rats by dose-dependent, and the effect was greater in hypertension than normal.

Table 10. Effects of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32(C3) g/kg, distill water (W), and captopril 25mg/kg (D) on systolic blood pressure at 0, 5, 15, 30, 45, 60, 90, 120 minute in hypertensive rats (n=8 in each group).

n	Systolic blood pressure (mmHg)																			
	0 min					5 min					15 min					30 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	193	190	182	185	183	198	193	180	188	185	195	196	191	190	185	190	168	188	175	183
2	213	213	200	203	200	210	210	208	205	208	215	213	195	205	198	208	180	199	185	196
3	191	210	193	208	185	195	188	193	210	185	195	198	188	212	189	190	190	195	188	183
4	203	203	185	188	180	198	203	185	193	185	203	200	190	195	190	203	164	188	184	193
5	191	198	197	218	198	198	212	199	215	203	198	208	198	208	200	191	173	199	195	194
6	196	193	194	202	190	195	190	197	207	191	190	198	202	208	193	198	169	193	191	185
7	199	205	208	197	198	195	210	212	195	203	195	205	199	195	195	203	173	213	182	193
8	203	203	205	202	198	200	200	200	196	193	205	208	203	193	195	207	185	213	193	199
mean	198	201	195	200	191	198	200	196	201	194	199	203	195	200	193	198	175^{+b}	198	186^{+c}	190
± sem	7	7	9	10	8	4	9	10	9	9	7	6	5	8	4	7	8	9	6	6

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute)

b ($p < 0.05$) : significant difference from the control , *C.asiatica* at dose of 16, 24, 32 g/kg treated group at the corresponding time.

c ($p < 0.05$) : significant difference from the control and *C.asiatica* at dose of 16 g/kg at the corresponding time.

d ($p < 0.05$) : significant difference from the control, *C.asiatica* at dose of 16, and 24 g/kg at the corresponding time.

Table 10. (continued) Effects of *C.asiatica* juice at the dose of 16 (C1), 24 (C2), 32(C3) g/kg, distill water (W), and captopril 25mg/kg (D) on systolic blood pressure at 0, 5, 15, 30, 45, 60, 90, 120 minute in hypertensive rats. (n=8 in each group)

n	Systolic blood pressure (mmHg)																			
	45 min					60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	190	161	188	175	174	193	160	190	190	172	190	158	198	188	185	193	177	193	188	182
2	210	163	195	180	180	215	170	195	190	165	210	165	208	198	198	208	183	195	199	192
3	188	171	193	192	164	190	168	193	178	168	190	168	204	193	192	190	188	192	198	184
4	198	166	183	171	160	200	165	186	180	168	205	163	190	180	183	206	195	186	193	188
5	206	168	203	195	171	203	167	213	178	180	195	160	193	180	193	198	192	193	212	200
6	201	157	198	172	163	190	162	191	177	175	193	150	185	193	192	193	190	192	196	190
7	199	174	194	178	172	190	172	200	173	188	190	183	203	193	196	195	193	208	204	198
8	208	170	198	178	175	203	173	195	175	185	200	160	211	193	203	213	197	210	208	203
mean	200	166^{+d}	194	180^{+c}	169^{+c}	198	167^{+d}	195	180^{+c}	175^{+c}	196	163^{+b}	199	189*	192	199	189^{+}	196	199	192
± sem	8	5	6	8	6	8	4	8	6	8	7	9	9	6	6	8	6	8	7	7

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute)

b ($p < 0.05$) : significant difference from the control , *C.asiatica* at dose of 16, 24, 32 g/kg treated group at the corresponding time.

c ($p < 0.05$) : significant difference from the control and *C.asiatica* at dose of 16 g/kg at the corresponding time.

d ($p < 0.05$) : significant difference from the control, *C.asiatica* at dose of 16, and 24 g/kg at the corresponding time.

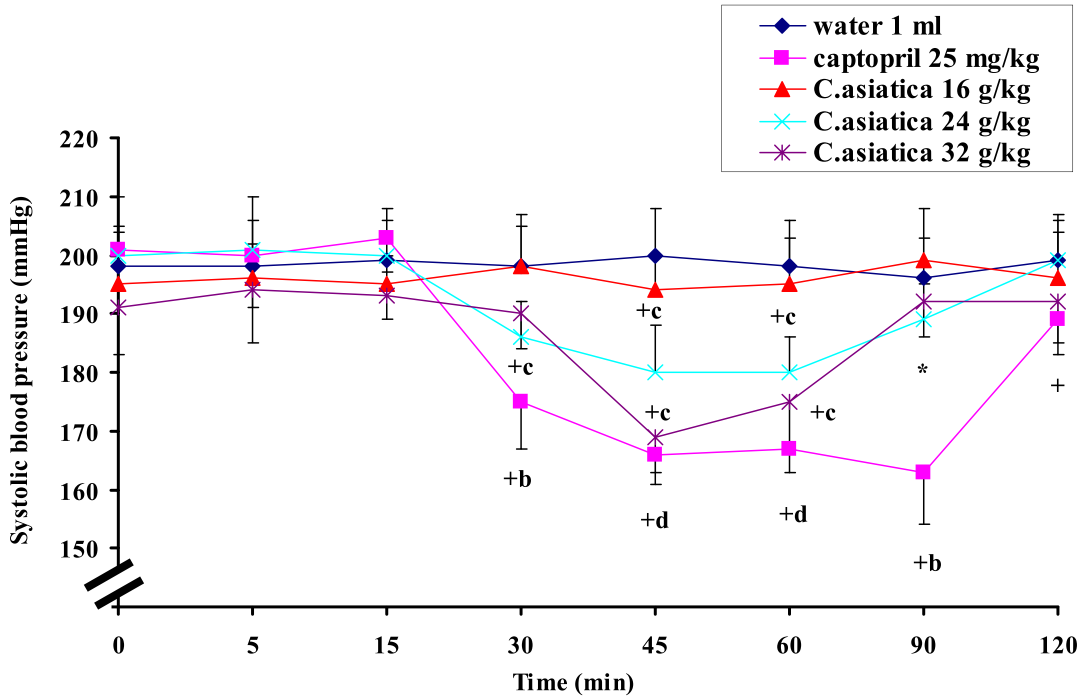

Figure 13. Effects of *C. asiatica* at the doses of 16, 24, 32 g/kg, distilled water 1 ml, and captopril at dose of 25 mg/kg on systolic blood pressure in hypertensive rats at 0, 5, 15, 30, 45, 60, 90, and 120 minutes (n=8 in each group).

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute)

b ($p < 0.05$) : significant difference from the control, *C.asiatica* at dose of 16, 24, and 32 g/kg at the corresponding time

c ($p < 0.05$) : significant difference from the control, *C. asiatica* at dose of 16 g/kg at the corresponding time.

d ($p < 0.05$) : significant difference from the control, *C. asiatica* at dose of 16, and 24 g/kg at the corresponding time.

Table11. The percentage of systolic blood pressure after single oral administration of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg , distill water (W), and captopril 25mg/kg (D) in hypertensive rats (n=8 in each group).

n	The percentage of systolic blood pressure from pre-administration																			
	5 min					15 min					30 min					45 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	102	101	98	101	101	101	107	104	102	101	98	88	103	94	100	98	84	103	94	95
2	98	98	104	100	104	100	106	97	100	99	97	84	99	91	98	98	76	97	88	90
3	102	89	100	100	100	102	102	97	101	102	99	90	101	90	98	98	81	100	92	88
4	97	100	100	102	102	100	108	102	103	105	100	80	101	97	107	97	81	98	90	88
5	103	107	101	98	102	103	105	100	95	101	100	87	101	89	97	107	84	103	89	86
6	99	98	101	102	100	96	102	104	102	101	101	87	99	94	97	102	81	102	85	85
7	97	102	101	98	102	97	98	95	98	98	102	84	102	92	97	100	84	93	90	86
8	98	98	97	97	97	100	101	99	95	98	101	91	103	95	100	102	83	96	88	88
mean	99	99	100	99	101	99	103	99	99	100	99	86^a	101	92^b	99	100	81^a	99	89^c	88^c
± sem	2	5	2	1	2	2	3	3	3	2	1	3	1	2	3	3	2	3	2	3

a ($p < 0.01$) : significant difference from the control, C.asiatica at dose of 16, 24, and 32 g/kg at the corresponding time.

e ($p < 0.05$), b ($p < 0.01$) : significant difference from the control, C. asiatica at dose of 16, and 32 g/kg at the corresponding time.

d ($p < 0.05$), c ($p < 0.01$) : significant difference from the control, C.asiatica at dose of 16 g/kg, and captopril at dose of 25 mg/kg at the corresponding time.

Table11. (continued) The percentage of systolic blood pressure after single oral administration of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distill water (W), and captopril 25mg/kg (D) in hypertensive rats (n=8 in each group).

n	The percentage of systolic blood pressure from pre-administration														
	60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	100	84	104	102	93	98	83	108	101	101	100	93	106	101	99
2	100	79	97	93	82	98	77	104	97	99	97	85	97	98	96
3	99	80	100	85	90	99	80	105	92	103	99	89	99	95	99
4	98	81	100	95	93	100	80	102	95	101	101	96	100	102	104
5	106	84	108	81	90	102	80	97	82	97	103	96	97	97	101
6	96	83	98	87	92	98	77	95	95	101	98	98	98	97	100
7	95	83	96	87	94	95	89	97	97	98	97	94	100	103	100
8	100	85	95	86	93	98	78	102	95	102	104	97	102	102	102
mean	99	82[a]	99	89[d]	90[c]	98	80[a]	101	94[e]	100	99	93[a]	99	99	100
± sem	3	2	4	6	3	2	3	4	5	2	2	4	2	2	2

a ($p < 0.01$) : significant difference from the control, *C.asiatica* at dose of 16, 24, and 32 g/kg at the corresponding time.

e ($p < 0.05$), b ($p < 0.01$) : significant difference from the control, C. asiatica at dose of 16, and 32 g/kg at the corresponding time.

d ($p < 0.05$), c ($p < 0.01$) : significant difference from the control, *C.asiatica* at dose of 16 g/kg, and captopril at dose of 25 mg/kg at the corresponding time.

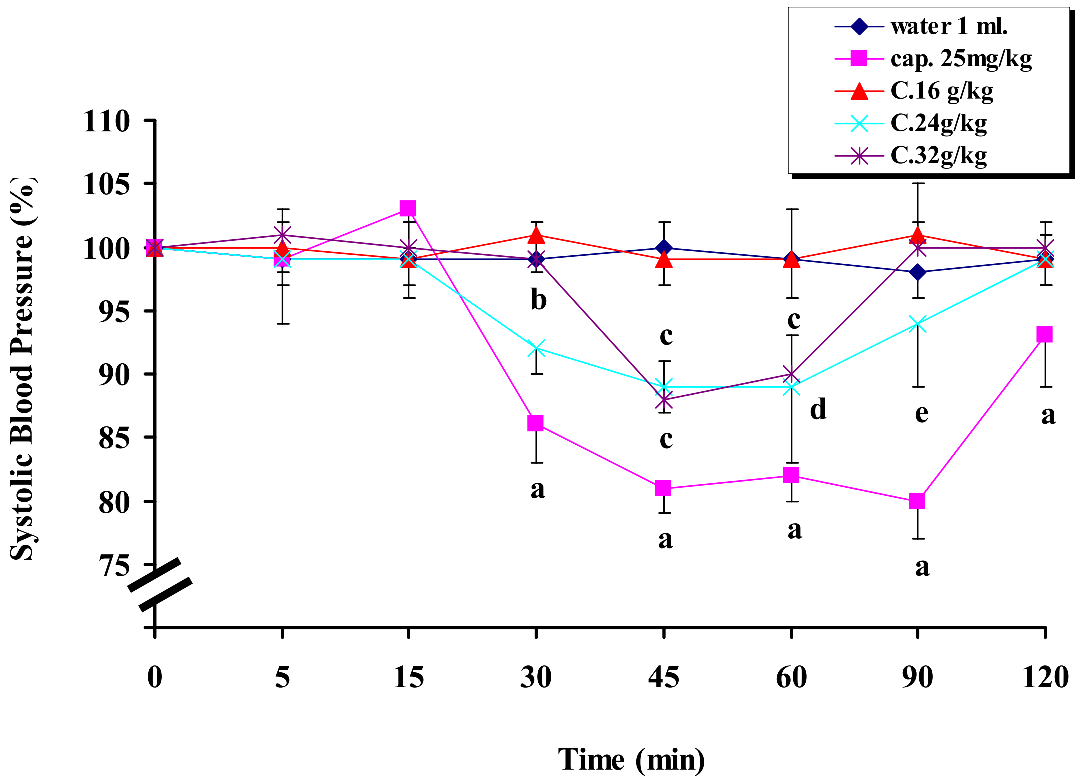

Figure 14. The percentage of systolic blood pressure after single oral administration of *C. asiatica* at the doses of 16, 24, and 32 g/kg distilled water 1 ml, and captopril at dose of 25 mg/kg (n=8 in each group).

All values were expressed as mean ± sem.

a ($p < 0.01$) : significant difference from the control, *C.asiatica* at dose of 16, 24, and 32 g/kg at the correspong time.

e ($p < 0.05$), b (p < 0.01) : significant difference from the control, *C. asiatica* at dose of 16, and 32 g/kg at the corresponding time.

d ($p < 0.05$), c (p < 0.01) : significant difference from the control, *C.asiatica* at dose of 16 g/kg, and captopril at dose of 25 mg/kg at the corresponding time.

1.2 Heart rats

Normal rats

The effects of *C. asiatica* juice at the dose of 16, 24, and 32 g/kg, distilled water, and captopril at the dose of 25 mg/kg on heart rate in normal rats were shown in Table 12 and Figure 15. The heart rate in treated groups with captopril at the dose of 25 mg/kg, *C. asiatica* at the doses of 24 g/kg, and 32 g/kg were significant the greater than the control at the start of experiment (week 0). There were no significant difference in heart rate from the pre-administration throughout 120 min of observation in captopril treated group, but their heart rate were significant greater than control at 15, 30, 60 and 90 min.

C. asiatica juice at the doses of 16 and 24 g/kg had no effect on HR in normal rats throughout 120 minutes of observation. *C.asiatica* juice at the dose of 32 g/kg significantly decreased heart rate (HR) from pre-admistration at 45 minutes from 358 ± 8 to 348 ± 4. The *C. asiatica* at the doses of 24 and 32 g/kg had significant greater heart rate than control at 15, 30, 60 and 90 min. However, there were no significant difference in percentage of heart rate from their pre-administration and between five groups (Table 13 and Figure 16).

Table 12. Effects of *C. asiatica* at the dose of 16(C1), 24(C2), 32(C3) g/kg, distill water (W) and captopril 25 mg/kg(D) on heart rate at 0, 5, 15, 30, 45, 60, 90,and 120 minute in normal rats (n=8 in each).

n	Heart rate (beats/min)																			
	0 min					5 min					15					30				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	340	350	350	355	355	360	355	335	355	365	350	360	355	360	360	335	350	345	355	360
2	345	365	350	345	370	355	360	350	355	360	340	360	345	345	360	340	360	350	350	375
3	335	355	335	350	365	340	360	345	350	365	340	355	340	350	355	345	360	340	360	360
4	335	345	345	345	360	345	360	340	355	360	340	360	350	355	350	335	360	350	355	365
5	340	350	345	355	355	330	355	350	345	355	340	345	340	365	360	335	355	355	375	345
6	345	370	355	365	350	340	360	355	360	355	325	355	360	350	350	350	345	360	345	355
7	340	355	355	350	345	350	350	350	345	350	335	365	345	350	360	350	355	345	350	355
8	325	360	345	365	365	345	365	360	370	360	345	355	355	355	375	350	355	350	365	370
mean	338	356^{a}	347	356^{a}	358^{a}	345	358	348	354	358	339	356^{a}	348	353^{a}	358^{a}	342	355^{b}	349	356^{a}	$360^{a,c}$
± sem	6	8	6	7	8	9	4	7	8	5	7	5	7	6	7	7	5	6	9	9

+ ($p < 0.01$) : significant difference from their pre-administration. (0 minute).

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the control at the corresponding time.

c ($p < 0.05$) : significant difference from *C.asiatica* at dose of 16 g/kg at the corresponding time.

Table 12. (continued) Effects of *C. asiatica* at the dose of 16(C1), 24(C2), 32(C3) g/kg, distill water (W) and captopril 25 mg/kg(D) on heart rate at 0, 5, 15, 30, 45, 60, 90,and 120 minute in normal rats (n=8 in each).

n	Heart rate (beats/min)																			
	45					60					90					120				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	335	360	350	345	350	345	350	345	345	345	350	350	345	355	350	350	355	340	350	360
2	360	370	350	355	355	340	360	360	350	345	335	360	355	355	355	355	380	355	360	365
3	345	350	335	355	345	335	350	345	335	335	325	360	335	365	360	335	355	340	345	370
4	335	350	355	365	350	330	350	335	350	345	335	350	345	345	355	330	355	345	355	355
5	330	340	345	360	345	325	360	350	345	345	345	355	355	365	355	340	360	345	355	365
6	335	350	360	345	345	345	360	345	350	360	350	365	355	350	375	350	380	355	355	345
7	340	350	335	345	345	335	360	350	355	360	345	355	340	350	370	345	360	355	350	360
8	345	345	350	365	355	350	340	340	355	360	345	355	350	355	355	330	360	360	365	350
mean	340	351	347	354 b	348[+]	338	353[a]	346	348	349	341	356[a]	347	355[a]	359[a,c]	341	363	349	354	358
± sem	9	9	8	8	4	8	7	7	6	9	8	5	7	7	8	9	10	7	6	8

+ ($p < 0.01$) : significant difference from their pre-administration. (0 minute).

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the control at the corresponding time.

c ($p < 0.05$) : significant difference from *C.asiatica* at dose of 16 g/kg at the corresponding time.

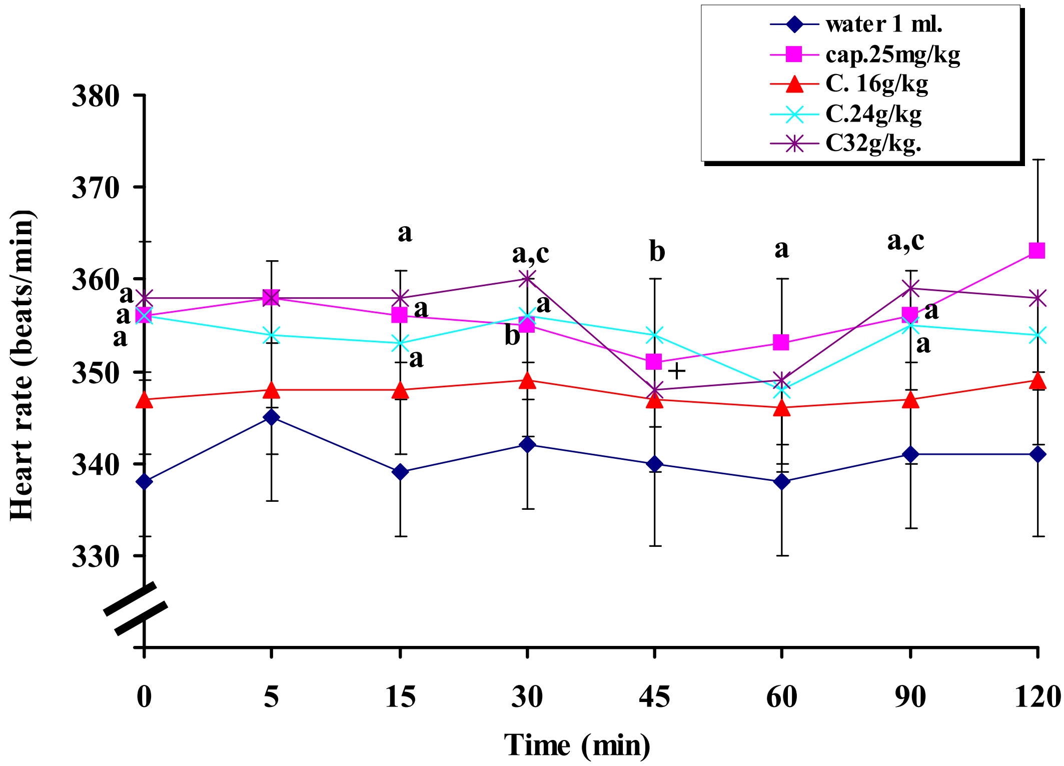

Figure 15. Effects of *C. asiatica* at the doses of 16, 24, 32 g/kg, and distilled water 1 ml., and captopril at dose of 25 mg/kg on heart rate (indirect) in normal rats at 0, 5, 15, 30, 45, 60, 90, and 120 minutes (n=8 in each group).

All values were expressed as mean ± sem.

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the control at the corresponding time.

c ($p < 0.05$) : significant difference from *C.asiatica* at dose of 16 g/kg at the corresponding time.

Table 13. The percentage of heart rate after single oral administration of *C. asiatica* at the doses of 16(C1), 24(C2), 32(C3) g/kg, distill water (W) and captopril 25 mg/kg(D) in normal rats (n=8 in each).

n	The percentage of heart rate from pre-administration																			
	5 min					15					30					45				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	105	101	95	100	102	102	102	101	101	101	98	100	98	100	101	98	102	100	97	98
2	102	98	100	102	97	98	98	98	100	97	98	98	100	101	101	104	101	100	102	95
3	101	101	102	100	100	101	100	101	100	97	102	101	101	102	98	102	98	100	101	94
4	102	104	98	102	100	101	104	101	102	97	100	104	101	102	101	100	101	102	105	97
5	97	101	101	97	100	100	98	98	102	101	98	101	102	105	97	97	97	100	101	97
6	98	97	100	98	101	94	95	101	95	100	101	93	101	94	101	97	94	101	94	98
7	102	98	98	98	101	98	102	97	100	104	102	100	97	100	102	100	98	94	98	100
8	106	101	104	101	98	106	98	102	97	102	107	98	101	100	101	106	95	101	100	97
mean	101	100	99	99	99	100	99	99	99	99	100	99	100	100	100	100	98	99	99	97
± sem	3	2	2	1	1	3	2	1	2	2	3	3	1	3	1	3	2	2	3	1

No significant difference from their pre-administration and between groups at the corresponding time.

Table 13. (continued) The percentage of heart rate after single oral administration of *C. asiatica* at the doses of 16(C1), 24(C2), 32(C3) g/kg, distill water (W) and captopril 25 mg/kg(D) in normal rats (n=8 in each).

n	The percentage of heart rate from pre-administration														
	60					90					120				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	101	100	98	97	97	102	100	98	100	98	102	101	97	98	101
2	98	98	102	101	93	97	98	101	102	95	102	104	101	104	98
3	100	98	102	95	91	97	101	100	104	98	100	100	101	98	101
4	98	101	97	101	95	100	101	100	100	98	98	102	100	102	98
5	95	102	101	97	97	101	101	102	102	100	100	102	100	100	102
6	100	97	97	95	102	101	98	100	95	107	101	102	100	97	98
7	98	101	98	101	104	101	100	95	100	107	101	101	100	100	104
8	107	94	98	97	98	106	98	101	97	97	101	100	104	100	95
mean	99	98	99	98	97	100	99	99	100	100	100	101	100	99	99
± sem	3	2	2	2	4	2	1	2	2	4	1	1	1	2	2

No significant difference from their pre-administration and between groups at the corresponding time.

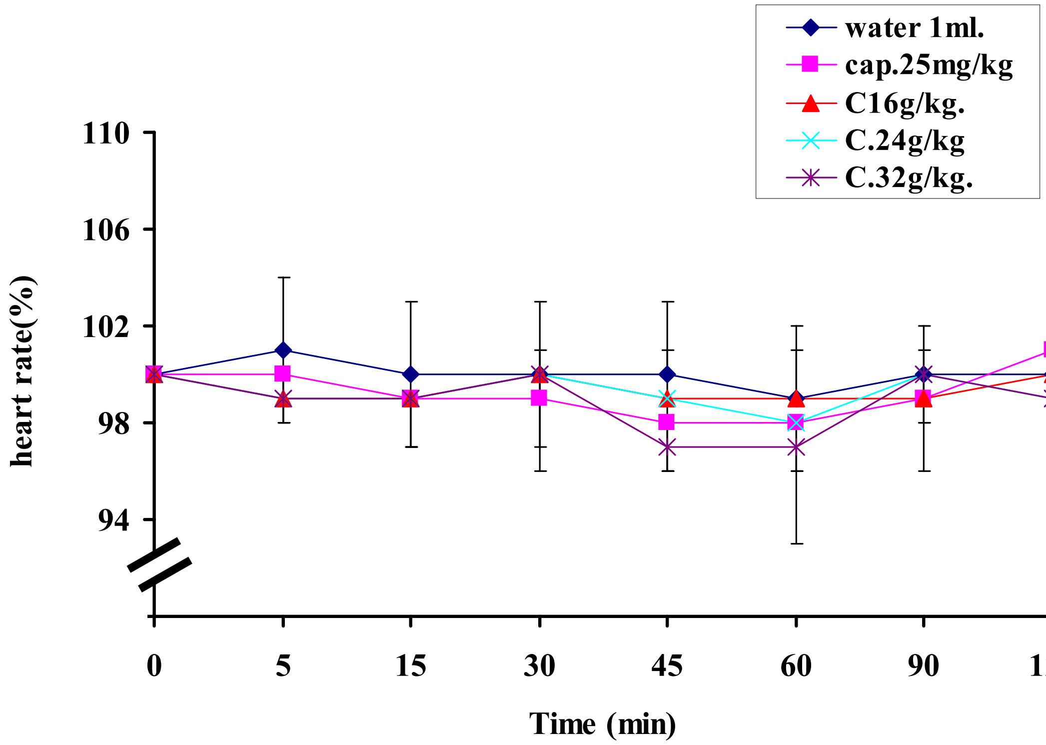

Figure 16. The percentage of heart rate as compared to the pretreatment value after single oral administration of *C. asiatica* at the dose of 16, 24, 32 g/kg, distill water 1 ml., and captopril 25 mg/kg in normal rats (n=8 in each group).

All values were expressed as mean± sem.

No significant difference from their pre-administration and between groups at the corresponding time.

Hypertensive rats

The effects of *C. asiatica* at the doses of 16, 24, and 32 g/kg, distilled water, and captopril at the dose of 25 mg/kg on HR in hypertensive rats were shown in Table 13 and Figure 17. The heart rate in captopril and *C. asiatica* at the dose of 32 g/kg treated groups were significant greater than the control at the start of experiment. There were no significant difference in heart rate from the pre-administration throughout 120 min in captopril treated group, but their heart rate were significant greater than control at 45 and 120 min.

C. asiatica juice at the doses of 16 and 24 g/kg significantly decreased HR from pre-administration at 60 minute from 361 ± 8 to 356 ± 7 and 360 ± 5 to 340 ± 8 respectively. *C. asiatica* juice at the dose of 32 g/kg significantly decreased HR from pre-administration at 45, and 60 minute from 363 ± 6 to 338 ± 8 and 355 ± 8 respectively. At 60 min, *C. asiatica* at the dose of 24 g/kg had significant lower heart rate than control, captopril and *C. asiatica* the dose of 16 g/kg treated groups (Table 14). However, only *C. asiatica* the doses of 32 g/kg at 45 min and *C. asiatica* the dose of 24 g/kg at 60 min, had lower significant percentage in heart rate compared to the other groups (Table 15 and Figure 18). The result indicated that oral administration of *C. asiatica* juice decreased the HR by dose dependent.

Table14. Effects of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril 25 g/kg (D) on heart rate at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in hypertensive rats (n=8 in each group).

n	Heart rate (beats/min)																			
	0min					5 min					15 min					30 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	365	365	345	355	370	355	365	360	355	365	350	365	350	365	370	360	370	365	355	355
2	335	360	370	355	360	335	365	355	360	355	345	365	365	360	360	340	360	365	365	355
3	350	355	365	365	365	345	360	375	365	365	345	355	370	355	365	340	350	365	355	370
4	360	355	360	360	360	350	360	380	365	360	350	360	345	345	350	350	355	370	365	360
5	355	370	360	355	355	350	365	360	365	360	360	380	355	355	355	350	370	355	360	360
6	345	370	365	370	375	350	370	355	360	365	350	380	355	360	370	345	365	370	370	365
7	350	375	355	360	360	355	370	355	370	370	355	370	360	365	350	345	370	355	360	360
8	345	375	370	365	365	360	380	360	355	370	355	375	350	365	365	350	370	365	360	370
mean	350	365[a]	361	360	363[a]	350	366	362	361	363	351	368	356	358	360	347	363	363[a]	361[b]	361
± sem	9	8	8	5	6	7	6	9	5	5	5	9	8	6	8	6	7	5	5	5

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration. (0 minute)

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the control at the corresponding time.

c ($p < 0.01$) : significant difference from captopril at the dose of 25 mg/kg at the corresponding time.

d ($p < 0.01$) : significant difference from *C.asiatica* at the dose of 16 g/kg at the corresponding time.

e ($p < 0.05$) : significant difference from *C.asiatica* at the dose of 24 g/kg at the corresponding time.

Table14. (continued) Effects of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distilled water (W), and captopril 25 g/kg (D) on heart rate at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in hypertensive rats (n=8 in each group).

n	Heart rate (beats/min)																			
	45 min					60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	355	360	355	355	330	355	370	345	340	355	355	370	350	365	365	360	365	355	355	355
2	335	355	360	355	345	365	360	360	325	355	335	365	375	355	360	345	360	365	365	370
3	340	360	370	340	350	355	355	355	340	340	355	350	355	370	355	355	360	360	350	360
4	355	360	350	345	340	355	350	355	335	345	360	360	360	360	365	350	360	365	365	360
5	360	370	355	355	330	345	375	365	355	360	345	370	360	355	365	350	365	360	355	355
6	355	370	365	340	350	345	375	360	345	360	340	370	370	355	360	345	380	370	365	365
7	340	370	360	360	330	340	370	345	340	360	355	375	360	355	375	350	380	365	365	370
8	355	380	365	355	335	345	380	365	345	365	350	370	365	370	375	350	375	370	360	360
Mean	349	365[a]	360	350[c]	338[+]	350	366	356*	340 [+b,c,d]	355*[e]	349	366	361	360	365	350	368[b]	363	360	361
± sem	9	8	6	7	8	8	10	7	8	8	8	7	7	6	7	4	8	5	5	5

* ($p < 0.05$), + ($p < 0.01$): significant difference from their pre-administration. (0 minute)

b ($p < 0.05$), a ($p < 0.01$): significant difference from the control at the corresponding time.

c ($p < 0.01$) : significant difference from captopril at the dose of 25 mg/kg at the corresponding time.

d ($p < 0.01$) : significant difference from *C.asiatica* at the dose of 16 g/kg at the corresponding time.

e ($p < 0.05$) : significant difference from *C.asiatica* at the dose of 24 g/kg at the corresponding time.

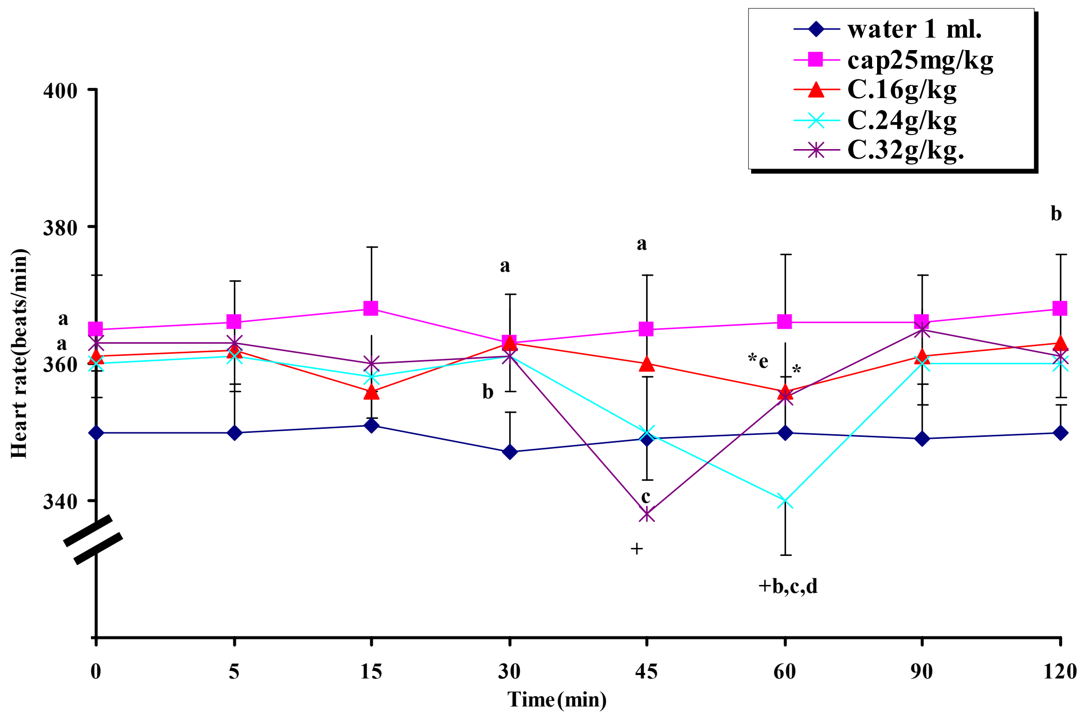

Figure 17. Effects of *C. asiatica* at the dose of 16, 24, 32 g/kg, distilled water 1 ml, and captopril at dose of 25 mg/kg on heart rate in hypertensive rats at 0, 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the control at the corresponding time.

c ($p < 0.01$) : significant difference from captopril at the dose of 25 mg/kg at the corresponding time.

d ($p < 0.01$) : significant difference from *C.asiatica* at the dose of 16 g/kg at the corresponding time.

e ($p < 0.05$) : significant difference from *C.asiatica* at the dose of 24 g/kg at the corresponding time.

Table15. The percentage of heart rate after single oral administration of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distill water (W), and captopril 25 mg/kg (D) in hypertensive rats (n=8 in each group).

n	The percentage of heart rate from pre-administration																			
	5 min					15 min					30 min					45 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	97	100	104	100	98	95	100	101	102	100	98	101	105	100	95	97	98	102	100	89
2	100	101	95	101	98	102	101	98	101	100	101	100	98	102	98	100	98	97	100	95
3	98	101	102	100	100	98	100	101	97	100	97	98	100	97	101	97	101	101	93	95
4	97	101	105	101	100	97	101	95	95	97	97	100	102	101	100	98	101	97	95	94
5	98	98	100	102	101	101	102	98	100	100	98	100	98	101	101	101	100	98	100	92
6	101	100	97	97	97	101	102	97	97	98	100	98	101	100	97	102	100	100	91	93
7	101	98	100	102	102	101	98	101	101	97	98	98	100	100	100	97	98	101	100	91
8	104	101	97	97	101	102	100	94	100	100	101	98	98	98	101	102	101	98	97	91
mean	99	100	100	100	99	99	100	98	99	99	98	99	100	99	99	99	99	99	97	92[a]
± sem	2	1	3	2	1	2	1	2	2	1	1	1	2	1	2	2	1	1	3	2

a ($p < 0.01$) : significant difference from the control, captropril at dose of 25 mg/kg, *C.asiatica* at the dose of 16,and 24 g/kg at the corresponding time.

b ($p < 0.05$) : significant difference from control, captopril at dose of 25 mg/kg, and *C.asiatica* at the dose of 16 g/kg at the corresponding time.

Table15. (continued) The percentage of heart rate after single oral administration of *C. asiatica* juice at the dose of 16 (C1), 24 (C2), 32 (C3) g/kg, distill water (W),and captopril 25 mg/kg (D) in hypertensive rats (n=8 in each group).

n	The percentage of heart rate from pre-administration														
	60 min					90 min					120 min				
	W	D	C1	C2	C3	W	D	C1	C2	C3	W	D	C1	C2	C3
1	97	101	100	95	95	97	101	101	102	98	98	100	102	100	95
2	108	100	97	91	98	100	101	101	100	100	102	100	98	102	102
3	101	100	97	93	93	101	98	97	101	97	101	101	98	95	98
4	98	98	98	93	95	100	101	100	100	101	97	101	101	101	100
5	97	101	101	100	101	97	100	100	100	102	98	98	100	100	100
6	100	101	98	93	96	98	100	101	95	96	100	102	101	98	97
7	97	98	97	94	100	101	100	101	98	104	100	101	102	101	102
8	100	101	98	94	100	101	98	98	101	102	101	100	100	98	98
mean	99	100	98	94^{b}	97	99	99	99	99	100	99	100	100	99	99
± sem	3	1	1	2	2	1	1	1	2	2	1	1	1	2	2

a ($p < 0.01$) : significant difference from the control, captropril at dose of 25 mg/kg, *C.asiatica* at the dose of 16,and 24 g/kg at the corresponding time.

b ($p < 0.05$) : significant difference from control, captopril at dose of 25 mg/kg, and *C.asiatica* at the dose of 16 g/kg at the corresponding time.

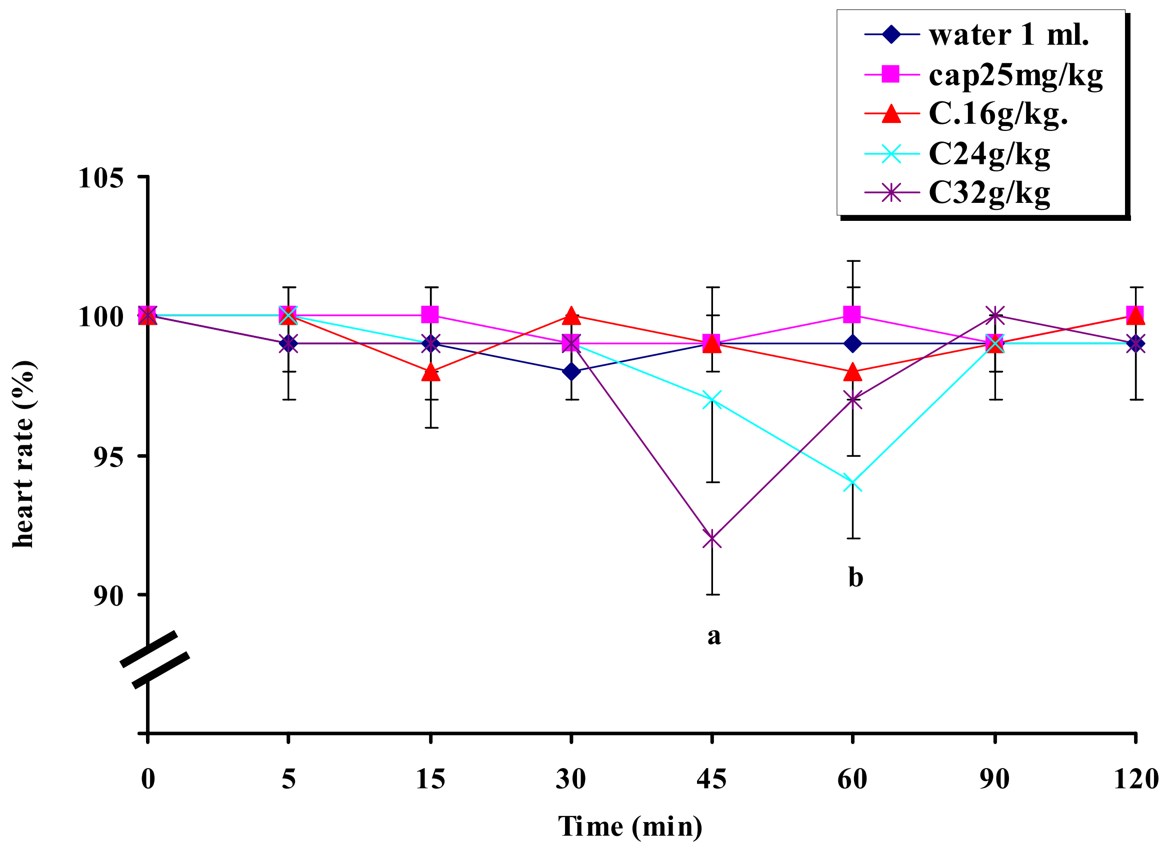

Figure 18. The percentage of heart rate after single oral administration of distilled water 1 ml., captopril 25 mg/kg, *C.asiatica* 16,24, and 32 g/kg in hypertensive rats at 5, 15, 30, 45, 60, 90 and 120 minutes (n=8 in each group).

All values were expressed as mean ± sem.

a ($p < 0.01$) : significant from the control, captopril at the dose of 25 mg/kg, *C.asiatica* at the dose of 16 and 24 g/kg at the corresponding time.

b ($p < 0.05$) : significant difference from the control, captopril at the dose of 25 mg/kg, *C.asiatica* at the dose of 16 g/kg at the corresponding time.

Experiment 2. Effects on local cutaneous blood flow.

The effects of *C. asiatica* at the dose of 32 g/kg on cutaneous blood flow in normal and hypertensive rats were shown in Table 15 and 19.

The normal and hypertensive rats had a similar level of cutaneous blood flow at pre-experimentation. *C. asiatica* juice at the dose of 32 g/kg significantly increased cutaneous blood flow from pre-administration at 30 to 120 minute in normal rats, and at 30 to 90 minutes in hypertensive rats (Table 15 and figure 19). The elevation of cutaneous blood flow at 30 to 90 minutes were significantly greater in hypertensive than normal rats. The maximal elevation of cutaneous blood flow were observed at 60 minutes in normal rats, and at 45 minutes in hypertensive rats. The percentage increase in cutaneous blood flow was significant greater in hypertensive than normal rats at 45 min, 202.2 ± 41.5 and 143 ± 23.2 respectively (Table 16 and Figure 20).

The result indicated that *C. asiatica* juice significantly increased cutaneous blood flow in both normal and hypertensive rats, with the greater effect in hypertensive rats.

Table16. Effects of *C. asiatica* at the dose of 32 g/kg on cutaneous blood flow at 0, 5, 15, 30, 45, 60, 90, and 120 minute in normal (N) and hypertensive rats (H) (n=8 in each group).

n	Cutaneous blood flow (PU)															
	0min		5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	41.3	50.1	34.1	56.1	43.1	45.3	41.6	71.6	47.5	91.3	63.2	87.2	51.7	85	37.2	68.1
2	32.5	46.4	33.4	49.7	34.6	47.6	51.8	81.3	48.5	97.4	53.6	98.7	52.4	86.3	38.7	49.9
3	40.7	49.8	36	56.3	39.5	40.2	56	79.7	68.4	96.2	74.6	102	72.8	94.5	64.1	65.1
4	36.6	56.6	36.5	49.4	35.8	40.2	42.7	77.5	65.3	94.6	75.8	91.6	60.6	83.9	53.1	65.2
5	40.4	35.7	45.9	38.1	41.5	60.7	56	59.7	49.3	98	68.6	89.6	67.6	69.1	55.4	46
6	42.8	35.4	43.5	30.4	40.4	49.2	64.4	50.6	63.8	87.5	79.6	79.6	69.9	81.6	43.7	46.3
7	41.4	46	42.3	41.1	47	38.6	56.9	67.4	48.9	67.6	58.4	93.9	75.8	65.6	55.6	49.5
8	38.2	49.7	39	40.9	39.7	36.4	45.4	64.5	55.2	98.1	77.3	68.2	69.2	76.3	37.8	37.5
mean	39.2	46.2^{b}	38.8	45.2	40.2	44.7	51.8^{+}	69^{+a}	55.8*	91.3^{+a}	68.8^{+}	88.8^{+a}	65^{+}	80.2^{+a}	48.2*	53.4
± sem	3.3	7.3	4.6	9.1	3.9	7.8	7.9	10.6	8.6	10.2	9.5	10.8	9.1	9.5	10.1	11.1

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the normal at the corresponding time.

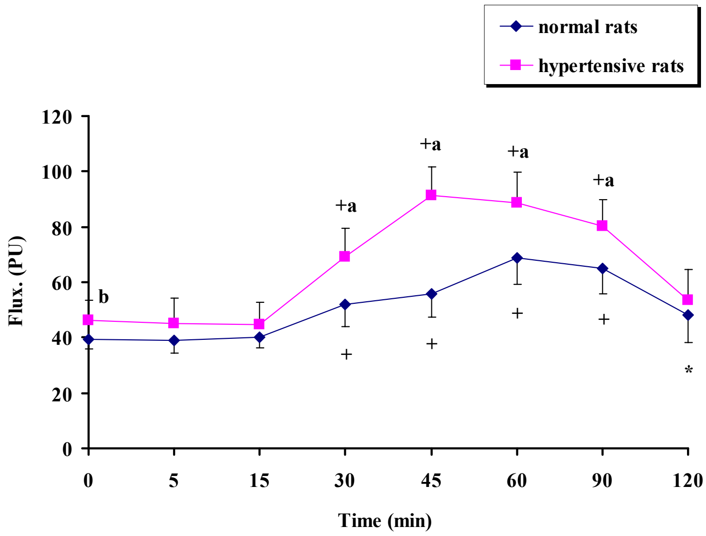

Figure 19. Effects of *C. asiatica* at the dose of 32 g/kg on cutaneou blood flow at 0, 5, 15, 30, 45, 60, 90 and 120 minutes in normal and hypertensive rats (n=8 in each group).

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

b ($p < 0.05$), a ($p < 0.01$) : significant difference from normal rats at the corresponding time.

Table17. The percentage of cutaneous blood flow after oral administration of *C. asiatica* at the dose of 32 g/kg in normal (N) and hypertensive rats (H) (n=8 in each group).

n	The percentage of cutaneous blood flow from pre-administration													
	5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	82.5	111.9	104.3	90.4	100.7	142.9	115	182.2	153	174	125.1	169.6	90	135.9
2	102.7	107.1	106.4	102.5	159.3	175.2	149	209.9	164.9	212.7	161.2	185.9	119	107.5
3	88.4	113	97	80.7	137.5	160	168	193.1	183.2	204.8	178.8	189.7	157.4	130.7
4	99.7	87.2	97.8	71	116.6	136.9	178.4	167.1	207.1	161.8	165.5	148.2	145	115.1
5	113.6	106.7	102.7	170	138.6	167.2	122	274.5	169.8	250.9	167.3	193.5	137.1	128.8
6	101.6	85.8	94.3	138.9	150.4	142.9	149	247.1	185.9	224.8	163.3	230.5	102.1	130.7
7	102.1	89.3	113.5	83.9	137.4	146.5	118.1	146.9	141	204.1	183	142.6	134.3	107.6
8	102	82.2	103.9	73.2	118.8	129.7	144.5	197.3	202.3	137.2	181.1	153.5	98.9	75.4
mean	99	97.9	102.4	101.3	132.4^{+}	150.1^{+}	143^{+}	202.2^{+a}	175.9^{+}	196.2^{+}	165.6^{+}	176.6^{+}	122.9*	116.4
± sem	9.5	12	6.1	35.2	19.1	15.7	23.2	41.5	23	36.5	18.4	29.2	24.2	19.9

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage at 0 minute.

a ($p < 0.01$) : significant difference from the normal at the corresponding time.

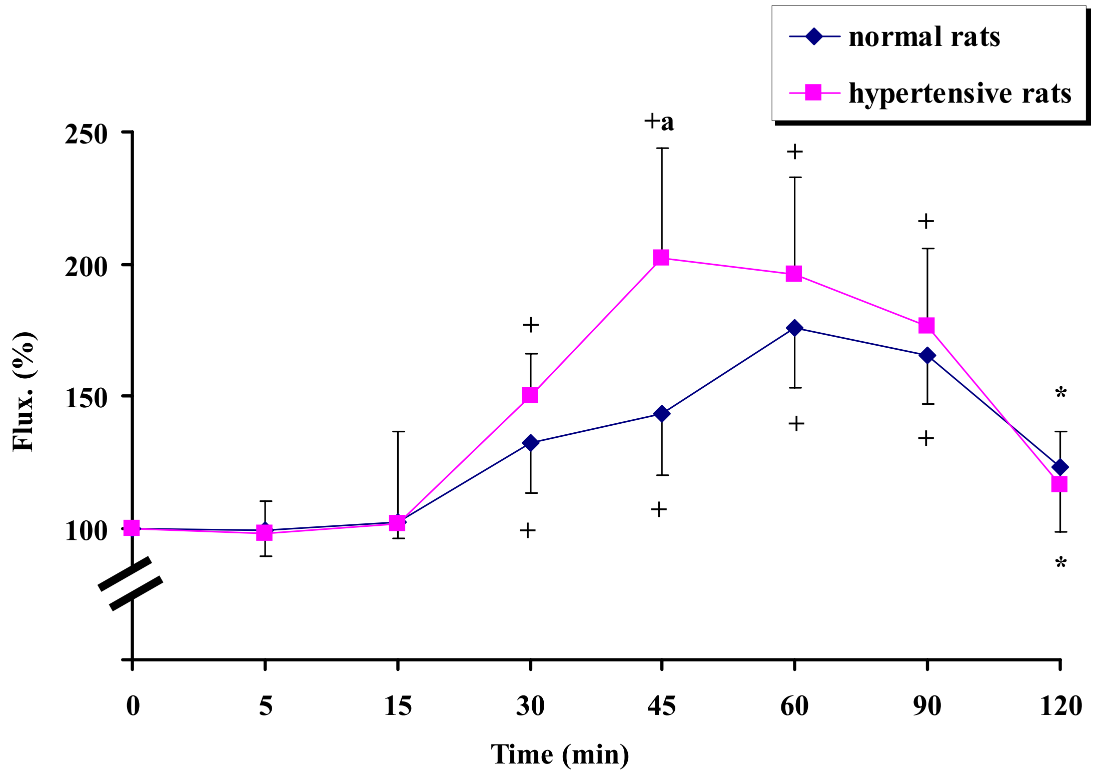

Figure 20. The percentage change of cutaneous blood flowafter single oral administration of *C. asiatica* at the dose of 32 g/kg in normal and hypertensive rats (n=8 in each group).

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

a ($p < 0.01$) : significant difference from the normal at the corresponding time.

Experiment 3. Effects on regional cerebral blood flow.

The effects of *C.asiatica* at the dose of 32 g/kg at five points of rCBF in normal and hypertensive rats were shown in Table 17 and Figure 21 and Table 18 and Figure 22. There were significant increase in rCBF from pre-administration in normal rats at point 1 from 15 to 60 min, point 2 from 5 to 120 min, point 3 and 4 from 15 to 90 min, and point 5 from 15 to 60 min. In hypertensive rats, the rCBF were significant increase, at point 1 from 15 to 90 min, point 2 from 15 to 120 min, point 3 from 5 to 90 min, point 4 from 5 to 120 min and point 5 from 30 to 120 min. The percentage increase rCBF from pre-administration at 5 points in normal and hypertensive rats were shown in Table 19 and Figure 23, and Table 20 and figure 24, respectively. There were no difference in percentage of regional cerebral blood flow at all five points in both normal and hypertensive rats.

The average of five points rCBF of both group were summarized in Table 21 and Figure 25. The normal rats had significantly greater average rCBF than hypertensive rats.*C.asiatica* juice significantly increased average rCBF from 5 minute to 90 minutes in normal rats, and from 5 minute to 120 minutes in hypertensive rats, with the maximal effects at 45 minutes in both groups. The average rCBF of hypertensive rats were significantly lower than normal rats only at 5 and 15 minutes after administration. The percentage elevation of average rCBF is greater in hypertensive rats than normal rats at 45 to 120 minutes (Table 22 and Figure 26).

The change in SBP and DBP during the measurement of rCBF were shown in Table 23, Figure 27, and Table 24, Figure 28, respectively. *C.asiatica* juice 32 g/kg significantly decreased SBP only in hypertensive rats from 15 to 120 minutes. While in experiment 1, the significant decreased SBP were observed at 45 and 60 minutes. Moreover, there were no alteration in DBP in normal rats, but significantly decreased in hypertensive rats at 45 to 120 minutes. The maximal decreased in SBP and DBP were observed at 60 minutes (Table 25, Figure 29, and Table 26, Figure 30, respectively.

The result indicated that *C. asiatica* significantly increased rCBF in both normal and hypertensive rats with greater response in hypertensive group, but decreased both SBP and DBP only in hypertensive group.

Table18. Effects of *C. asiatica* juice at the dose of 32 g/kg on regional cerebral blood flow 5 point in normal rats (n=10) at 0, 5, 15, 30, 45, 60, 90, and 120 minute.

n	Regional cerebral blood flow (PU)																			
	0 min					5 min					15 min					30 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	105.5	111.5	89.4	111.5	95.5	99.6	117	83.2	109.5	81.6	99.1	127.5	80.2	127.5	93.7	116.5	127.5	105.9	141	117.5
2	125.5	109	84.3	98.3	95.3	144	112.5	86.3	110	110	136	108	96.2	118	105	146.5	76.2	98.9	127.5	123
3	72.8	71.3	85.6	124.5	113	70.8	73.5	85.8	113.5	115.5	118	87.2	91	147	140.5	147	78.8	98.8	162	149.5
4	110.5	98.8	98.1	98	104	121	120.5	113.5	117	124	117	124.5	130.5	125	122	149.5	151.5	136.5	129.5	119
5	94.3	101.4	94.5	103.5	103.5	96.8	117	121	108.4	110.5	121	108.5	113.5	100.5	115.5	147	135	142.5	141	137.5
6	101.7	92.8	79.9	117.5	117.5	98	93.3	87	112	112	106	96.8	111.5	128.5	128.5	120.5	138.5	131.5	147.5	147.5
7	96.2	106.5	86.6	98.2	112	115	123.5	95.4	103.5	119	127	108	110	116	126	140	143.5	120	126	166
8	116.5	100.3	131.5	104.5	109	110	105	130.5	115.5	122.5	128	119	141	124.5	113	169	122.5	150	161.5	140.5
9	95.5	98.3	121	94.9	90.9	91.8	103	111.5	114.5	99.8	115.5	128.5	121	112.5	115.5	126	147	115	145	138
10	88	69.8	96.5	94.1	64.6	96.9	80.8	109	94.8	58.3	104	82.9	101.5	87.2	90.4	125	92.2	116.5	111.5	104.5
mean	100.6	95.9	96.7	104.5	100.5	104.3	104.6*	102.3	109.8	105.3	117.6+	109*	109.6*	118.6*	115+	138.7+	121.2*	121.5*	139.2*	134.3+
±sem	14	14	16	10	15	19	17	16	6	20	11	16	18	16	15	16	28	18	15	18

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute)

No significant difference between 5 points at corresponding time.

Table18. (continued) Effects of *C. asiatica* juice at the dose of 32 g/kg on regional cerebral blood flow 5 point in normal rats (n=10) at 0, 5, 15, 30, 45, 60, 90, and 120 minute.

n	Regional cerebral blood flow (PU)																			
	45 min					60 min					90 min					120 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	103.6	117	134.5	132	134	135.5	136	132	136	129.5	139.5	143	117	123.5	116.5	108.5	116.5	93.7	115	100.1
2	138.5	134.5	132.5	138.5	127	125.5	126.5	123	81.7	120.5	110	103	81.7	84.3	82.4	102.3	104	81.4	99.8	82.6
3	105	99.5	116	132.5	174.5	120	80.6	104.5	135	131.5	108	91.4	106.5	125	140	76.9	83.5	106	145.5	119.5
4	133.5	145.5	124.5	140	124.5	132.5	135.5	118	91.2	134.5	115.5	122.5	102	95.2	114.5	114.5	113.5	96.6	87.5	93.8
5	147	137.5	162	146	136	130.5	140	152.5	137.5	121.5	100.1	108	126.5	121.5	112.5	94.4	106.5	115	99.3	100.5
6	145	141	144.5	154.5	154.5	134.5	143	125	114.5	114.5	117	127	113	121.5	121.5	106.5	109	91.3	119	119
7	143.5	123.5	127.5	156	167	149.5	123	114.5	131.5	127.5	117.5	103.5	98.3	115	110.5	111	110	87.7	90.5	97.2
8	152	127	157.5	145.5	153.5	175	135.5	153.5	141	146.5	137.5	122.5	136	127.5	106.5	122.5	98.8	121	120.5	103
9	127.5	141.5	137.5	135.5	135	102.9	148.5	137.5	106.5	117.5	80.5	117	127	116	82.8	84.1	112	120.5	105.5	95.5
10	128	112.5	130	134	110	124.5	115.5	108.5	118	115	100.7	95.1	95.5	107.5	78.9	95.6	85.5	106.5	79.3	72.2
mean	132.3^{+}	127.9*	136.6*	141.5*	141.6^{+}	133^{+}	128.4*	126.9*	119.2*	125.8^{+}	112.6	113.3*	110.3*	113.7*	106.6	101.6	103.9*	101.9	106.1	98.3
±sem	16	14	14	8	20	19	19	16	20	10	17	15	16	14	19	14	11	13	19	14

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute)

No significant difference between 5 points at corresponding time.

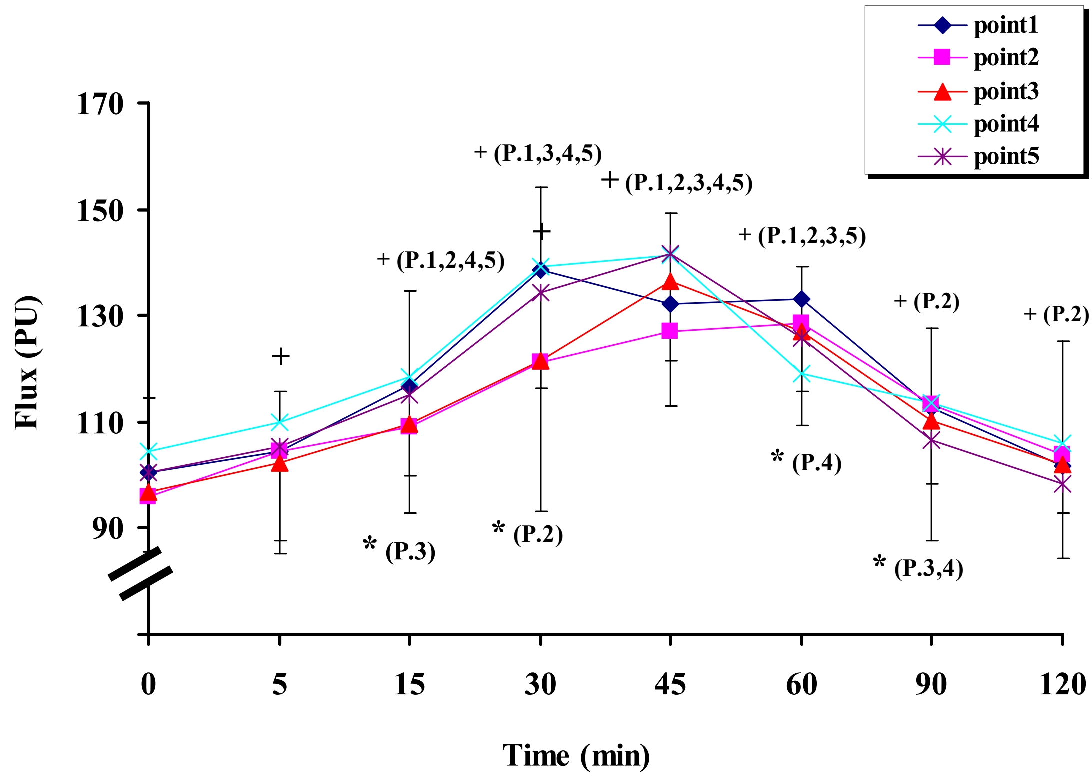

Figure 21. Effects of *C. asiatica* at the dose of 32 g/kg on regional cerebral blood flow 5 point in normal rats (n=10) at 0, 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

No significant difference between 5 points at corresponding time.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute)

P = point

Table 19. Effects of *C. asiatica* juice at the dose of 32 g/kg on regional cerebral blood flow 5 point in hypertensive rats (n=8) at 0, 5, 15, 30, 45, 60, 90, and 120 minute.

n	Regional cerebral blood flow (PU)																			
	0 min					5 min					15 min					30 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	67.3	63.5	80.6	62.9	54.3	74.8	72.3	79.9	82.8	69.2	103.5	103	83.9	114	126.5	95.8	121	139.5	119	144.5
2	88.9	90.5	94.8	67.7	88.6	77.9	89.6	110.5	86.2	96.5	104.5	122	98.8	71.1	87.7	127.5	118	107	114	115
3	80.2	76.2	64.5	80.8	100.5	75.3	84.7	76.3	91	106.5	83.2	93.9	96.5	104	112.5	119	111.5	109.5	117	124.5
4	97.8	96	107.5	110	85.9	106.5	95.1	122	120.5	81	110.5	93	109	108.5	97.8	120	107.2	150.5	133.5	122
5	78.9	89.4	90.9	75	107	81.5	87.3	94.8	84.9	106.5	94.1	99.9	96.7	94.9	114	119	119	138	119.5	144.5
6	95.5	107.5	103.5	81.2	116	90.7	105.4	118	91.4	115.5	114.5	117.5	121	100.8	118	120.5	130.5	129.5	96.5	130
7	95	105	100	66.9	110	87.9	98.2	103.5	75.8	110.5	110.5	118.5	116.5	104.3	126.5	119.5	121	122.5	112	147.5
8	81.1	97.3	73.4	108	108	87.2	88.8	86.9	111.5	111.5	101.6	115.5	113.5	109	109	131.5	133.5	120.5	136.5	136.5
mean	85.5	90.6	89.4	81.5	96.2	85.2	90.1	98.9*	93*	99.6	102.8^{+}	107.9*	104.4*	100.8*	111.5	119.1^{+}	120.2*	127.1*	118.5*	133*
±sem	10	14	15	18	19	10	9	17	15	16	10	11	12	13	13.4	10	8	15	12	12

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

No significant difference between 5 points at corresponding time.

Table 19. (continued) Effects of *C. asiatica* juice at the dose of 32 g/kg on regional cerebral blood flow 5 point in hypertensive rats (n=8) at 0, 5, 15, 30, 45, 60, 90, and 120 minute.

n	Regional cerebral blood flow (PU)																			
	45 min					60 min					90 min					120 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	107.5	199	105	115	106	92.4	129	125	95.5	137	86.7	115.5	96.5	83.2	98.4	78.7	107.9	86.1	82	65.6
2	121	132.5	119.5	121	124	125.5	109.5	122.5	135	128.5	106.5	80.1	149.5	121	126.5	77.5	93.6	79.9	93.4	90.2
3	167.5	145.5	123	127	149	166	139.5	134	131.5	142.5	114	121	94.1	110	120	131.5	101.4	101.9	103.9	110.5
4	154	131	144.5	146.5	136.5	132	147	157.5	137	132.5	126.5	120.5	137.5	132.5	94.7	107.5	105.3	119	122.5	99.5
5	127	122.5	134	121	143	120	138.5	146.5	124	145.5	109.5	129	140.5	117	135	93.1	112.5	110.5	93.3	116.5
6	167	129.5	161.5	113	150	142.5	144.5	137.5	132.5	168	139.5	139.5	126.5	118.5	135	111	118	117.5	93.1	125.5
7	131	133.5	129.5	123.5	133.5	126.5	135.5	130.5	121.5	129	94.6	119	117	110	112	84.3	97.8	94	86.8	100.4
8	141.5	136	129.5	148.5	148.5	126	116.5	122	126.5	126.5	105.8	109	115.5	116.5	116.5	96.8	106	91.5	121	121
mean	139.5^{+}	141.1*	130.8*	126.9*	136.3*	128.8^{+}	132.5*	134.4*	125.4*	138.6*	110.3^{+}	116.7*	122.1*	113.5*	117.2*	97.5	105.3*	100	99.5*	103.6*
±sem	21	24	16	13	15	20.7	13	12	13	13	16	17	20	14	15	18	7	14	15	19

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute)

No significant difference between 5 points at corresponding time.

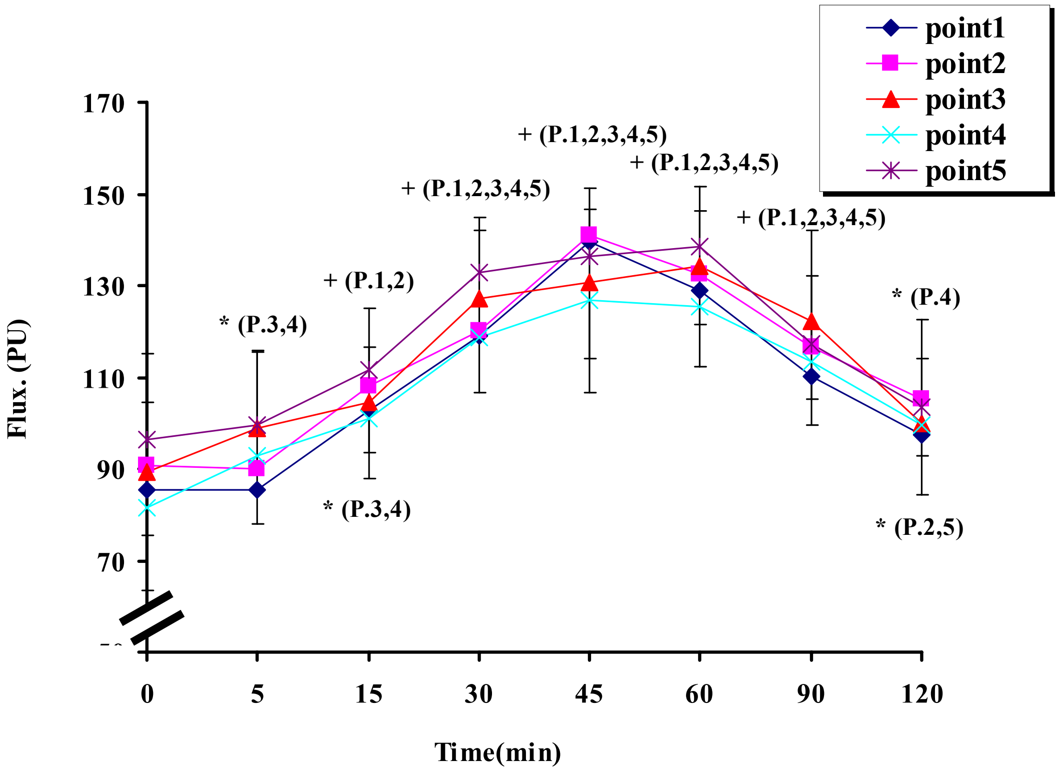

Figure 22. Effects of *C. asiatica* at the dose of 32 g/kg on regional cerebral blood flow (5 points) in hypertensive rats (n=8) at 0, 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute).

No significant difference between 5 points at coresponding time.

P = point

Table 20. The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose 32 g/kg in normal rats (n=10).

n	The percentage of regional certebral blood flow from pre-administration																			
	5 min					15 min					30 min					45 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	94	104	93	98	85	93	114	89	114	98	110	114	118	126	123	98	104	150	118	140
2	114	103	102	111	115	108	99	114	120	110	116	69	117	129	129	110	123	157	140	133
3	97	103	100	91	102	162	122	106	118	124	201	110	115	130	132	144	139	135	106	154
4	109	121	115	119	119	105	126	133	127	117	135	153	139	132	114	120	147	126	142	119
5	102	115	128	104	106	128	107	120	97	111	155	133	150	136	132	155	135	171	141	131
6	96	100	108	95	95	104	104	139	109	109	118	149	164	125	125	142	151	180	131	131
7	119	115	110	105	106	132	101	127	118	112	145	134	138	128	148	149	115	147	128	149
8	94	104	99	110	112	109	118	107	119	103	145	122	114	154	128	130	126	119	139	140
9	96	104	92	120	109	120	130	100	118	127	131	149	95	152	151	133	143	113	142	148
10	110	115	112	100	90	118	118	105	92	139	142	132	120	118	161	145	161	134	142	170
mean	103	108	106	105	104	118	114	114	113	115	140	126	127	133	134	132	134	143	132	141
±sem	9	7	11	9	11	19	10	15	10	12	26	24	20	11	14	18	17	21	12	14

a ($p < 0.05$) : significant difference from point 2.

Table 20. (continued) The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose 32 g/kg in normal rats (n=10).

n	The percentage of regional certebral blood flow from pre-administration														
	60 min					90 min					120 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	128	121	147	121	135	132	128	130	110	121	102	104	104	103	104
2	100	116	145	83	126	87	94	96	85	86	81	95	96	101	86
3	164	113	122	108	116	148	128	124	100	123	105	117	123	116	105
4	119	137	120	93	129	104	123	103	97	110	103	114	98	89	90
5	138	138	161	132	117	106	106	133	117	108	100	105	121	95	97
6	132	154	156	97	97	115	136	141	103	103	104	117	114	101	101
7	155	115	132	133	113	122	97	113	117	98	115	103	101	92	86
8	150	135	116	134	134	118	122	103	122	97	105	98	92	115	94
9	107	151	113	112	129	84	119	104	122	91	88	113	99	111	105
10	141	165	112	125	178	114	136	98	114	122	108	122	110	84	111
mean	133	134	132	113	127	113	118	114	108	105	101	108	105	100	97[a]
±sem	20	18	18	18	21	19	15	16	12	13	9	9	10	10	8

a ($p < 0.05$) : significant difference from point 2.

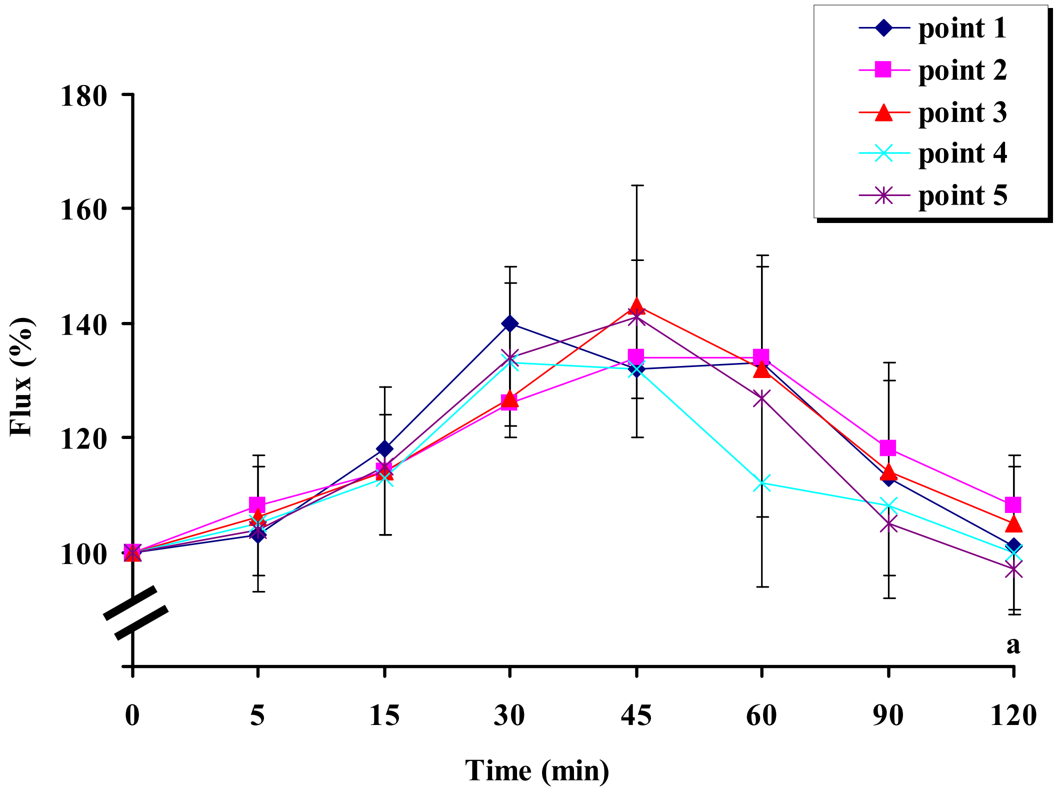

Figure 23. The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose of 32 g/kg in normal rats (n=10) at 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

a ($p < 0.05$) : significant difference from point 2.

Table 21. The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose of 32 g/kg in hypertensive rats (n=8).

n	The percentage of regional cerebral blood flow from pre-administration																			
	5 min					15 min					30 min					45 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	111	113	99	131	127	153	162	104	181	232	142	190	173	189	266	159	313	130	182	195
2	87	99	116	127	108	117	134	104	105	98	143	130	112	168	129	136	146	126	178	139
3	93	111	118	112	105	103	123	149	128	111	148	146	169	144	123	208	190	190	157	148
4	108	99	113	109	94	112	96	101	98	113	122	111	140	121	142	157	136	134	133	158
5	103	97	104	113	99	119	111	106	126	106	150	133	151	159	135	160	137	147	161	133
6	94	98	114	112	99	119	109	116	124	101	126	121	125	118	112	174	120	156	139	129
7	92	93	103	113	100	116	112	116	155	115	125	115	122	167	134	137	127	129	184	121
8	107	91	118	103	103	125	118	154	100	100	162	137	164	126	126	174	139	176	137	137
mean	99	100	110	115 [a,b]	104	120	120	118	127	122	139	135	144	149	145	163	163	148	158	145
±sem	8	7	7	9	10	14	20	20	28	44	14	24	23	25	49	23	63	23	20	23

a ($p < 0.01$) : significant difference from point 1.

b ($p < 0.05$) : significant difference from point 2.

Table 21. (continued) The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose of 32 g/kg in hypertensive rats (n=8).

n	The percentage of regional cerebral blood flow from pre-administration														
	60 min					90 min					120 min				
	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5	point 1	point 2	point 3	point 4	point 5
1	137	203	155	151	252	128	181	119	132	181	116	169	106	130	120
2	141	120	129	199	145	119	88	157	178	142	87	103	84	137	101
3	206	183	207	162	141	142	158	145	136	119	163	133	157	128	109
4	134	153	146	124	154	129	125	127	120	110	109	109	110	111	115
5	152	154	161	165	135	138	144	154	156	126	117	125	121	124	108
6	149	134	132	163	144	146	129	122	145	116	116	109	113	114	108
7	133	129	130	181	117	99	113	117	164	101	88	93	94	129	91
8	155	119	166	117	117	130	112	157	107	107	119	108	124	112	112
mean	150	149	153	157	150	128	131	137	142	125	114	118	113	123	108
±sem	23	30	26	27	43	14	29	17	23	25	23	23	21	9	8

a ($p < 0.01$) : significant difference from point 1.

b ($p < 0.05$) : significant difference from point 2.

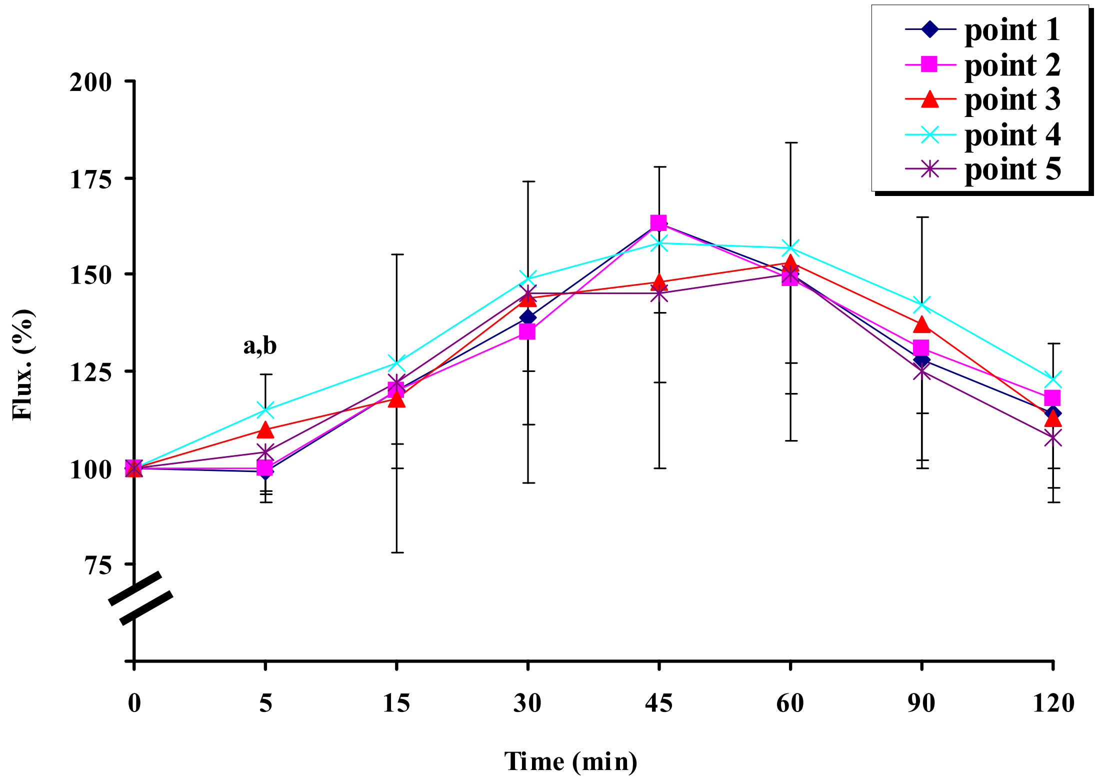

Figure 24. The percentage of regional cerebral blood flow 5 point after single oral administration of *C. asiatica* at the dose of 32 g/kg in hypertensive rats n=8.

All values were expressed as mean ± sem.

a ($p < 0.01$) : significant difference from point 1 .

b ($p < 0.05$) : significant difference from point 2.

Table 22. Effects of *C. asiatica* at the dose of 32 g/kg on average regional cerebral blood flow from 5 point determinations at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in normal (n=10) and hypertensive rats (n=8).

N	Average regional cerebral blood flow (PU)															
	0 min		5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	102	65	98	75	105	106	121	123	124	126	133	113	127	96	106	84
2	102	86	112	92	112	96	114	116	134	123	115	124	92	116	94	86
3	93	80	91	86	116	98	127	116	125	142	114	142	114	111	106	109
4	101	99	119	105	123	103	137	126	133	142	122	141	109	122	101	110
5	99	88	110	91	118	99	140	128	145	129	136	134	113	126	103	105
6	101	100	100	104	114	114	137	121	147	144	126	145	102	131	108	113
7	99	95	111	95	117	115	139	124	143	130	129	128	108	110	99	92
8	112	93	116	97	125	109	148	131	147	138	150	121	126	112	113	107
9	100		104		118		134		135		122		104		103	
10	82		88		93		109		129		116		95		87	
mean	99	88^{a}	105*	93^{+a}	114^{+}	105^{+a}	131^{+}	123^{+}	136^{+}	134^{+}	126^{+}	131^{+}	109*	116^{+}	102	101*
±sem	7	11	10	9	8	7	11	5	8	8	10	11	11	11	6	11

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute)

a ($p < 0.05$) : significant difference from the normal at the corresponding time.

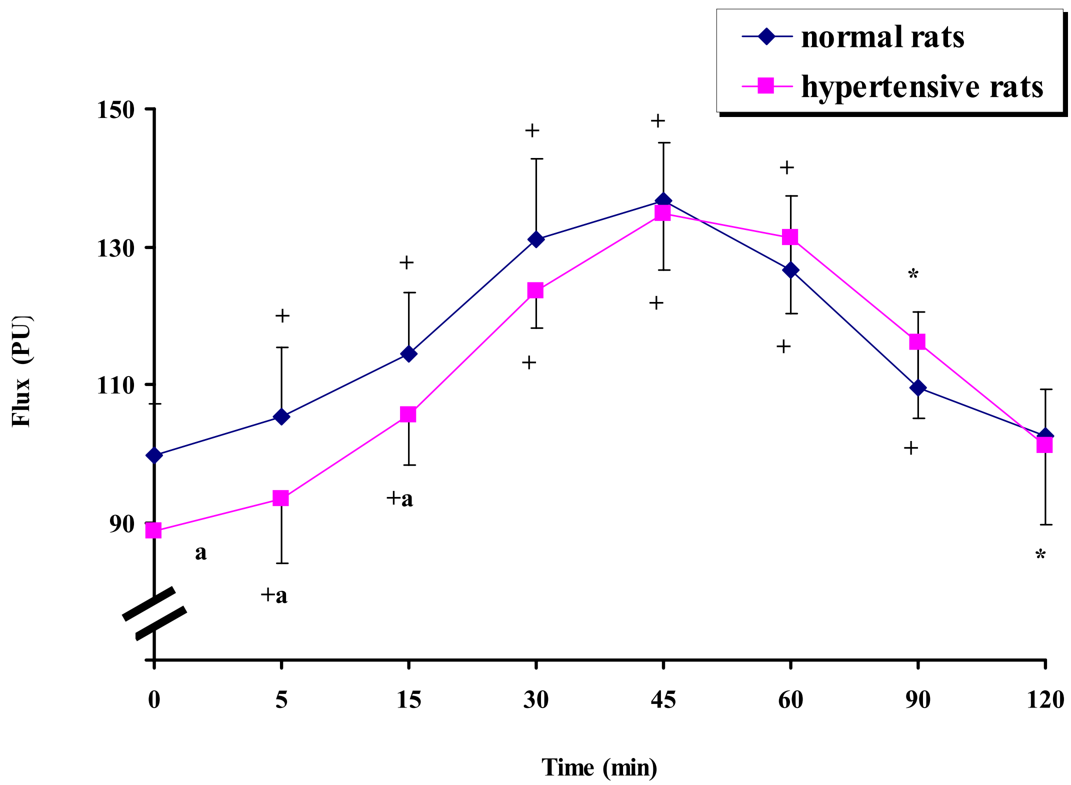

Figure 25. Effects of *C. asiatica* at the dose of 32 g/kg on average regional cerebral blood flow from 5 point determinations at 0, 5, 15, 30, 45, 60, 90, and 120 minutes in normal rats (n=10) and hypertensive rats (n=8).

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute).

a ($p < 0.05$) : significant difference from the normal at the corresponding time.

Table 23. The percentage of regional cerebral blood flow after single oral administration of *C. asiatica* at the dose of 32 g/kg in normal (N, n=10) and hypertensive rats (H, n=8).

n	The percentage of average regional cerebral blood flow from pre-administration													
	5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	96	115	102	163	118	189	121	193	130	173	124	147	103	129
2	109	106	109	111	111	134	131	143	112	144	90	134	92	100
3	97	107	124	122	136	145	134	177	122	177	122	138	113	136
4	117	106	121	104	135	127	131	143	120	142	107	123	100	111
5	111	103	119	112	141	145	146	146	137	152	114	143	104	119
6	99	104	112	114	135	121	145	144	124	145	100	131	106	113
7	112	100	118	121	140	130	144	136	130	134	109	115	100	96
8	103	104	111	117	132	140	131	148	133	130	112	120	100	115
9	104		118		134		135		122		104		103	
10	107		113		132		157		141		115		106	
mean	105*	105^{+}	114^{+}	120*	131^{+}	141^{+}	137^{+}	154^{+a}	127^{+}	150^{+a}	109*	131^{+a}	102	115^{*a}
±sem	6	4	6	18	9	21	10	20	8	17	10	11	5	13

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

a ($p < 0.05$) : significant difference from the normal at the corresponding time.

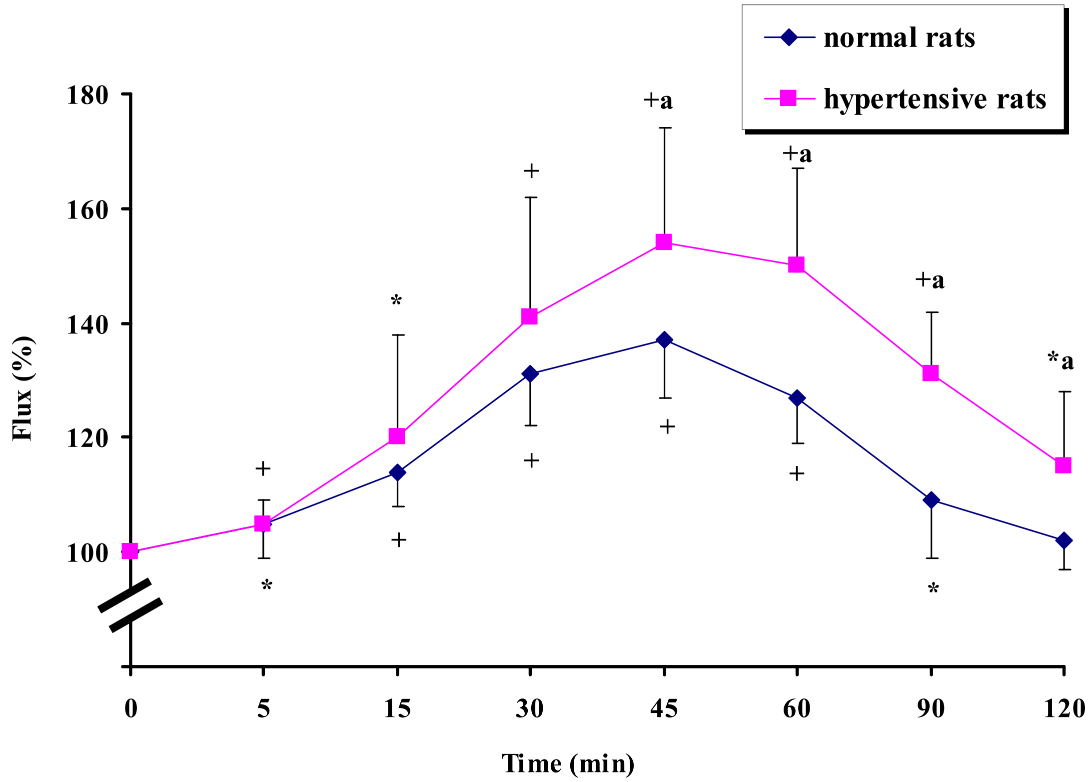

Figure 26. The percentage change of average regional cerebral blood flow after single oral administration of *C. asiatica* at the dose of 32 g/kg on in normal rats (n=10) and hypertensive rats (n=8).

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

a ($p < 0.05$) : significant difference from the normal.

Table 24. Effects of *C. asiatica* at the dose of 32 g/kg on systolic blood pressure of femoral artery at 0, 5, 15, 30, 45, 60, 90 and 120 minute in normal rats (N, n=10) and hypertensive rats (H, n=8).

n	Systolic blood pressure (mmHg)															
	0 min		5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	110	192	110	187	110	180	115	160	115	160	115	162	115	180	115	185
2	120	195	120	162	120	162	120	160	120	160	120	167	120	170	120	167
3	160	202	155	200	155	200	157	200	160	160	160	165	150	190	150	200
4	160	200	160	202	160	190	140	190	142	175	140	170	150	180	160	190
5	140	230	140	230	140	230	150	222	150	200	147	200	145	195	140	195
6	150	182	155	180	142	180	142	180	160	180	150	162	155	160	150	162
7	152	220	150	220	147	205	140	195	137	190	140	180	150	180	150	192
8	157	200	152	195	140	170	147	180	152	190	152	190	157	190	160	200
9	150		150		150		147		150		150		152		152	
10	152		150		150		150		152		152		150		147	
mean	145	202	144	197	141	189*	141	185^{+}	144	176^{+}	142	174^{+}	144	180^{+}	144	186^{+}
±sem	5	5	5	7	5	7	4	7	4	5	4	4	4	4	5	5

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

Hypertensive rats had significant higher systolic blood pressure than normal at all corresponding times.

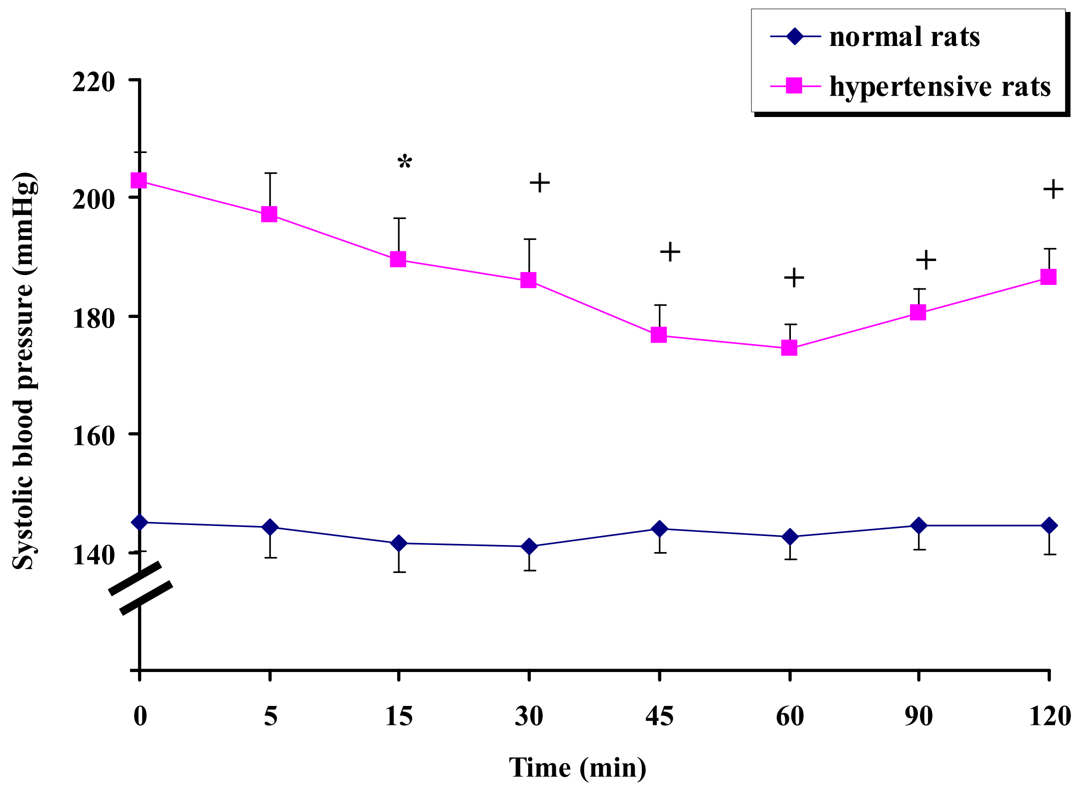

Figure 27. Effects of *C. asiatica* at the dose of 32 g/kg on systolic blood pressure of femoral artery in normal rats (n=10) and hypertensive rats (n=8) at 0, 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from pre-administration (0 minute).

Table 25. Effects of *C. asiatica* at the dose of 32 g/kg on diastolic blood pressure of femoral artery at 0, 5, 15, 30, 45, 60, 90 and 120 minute in normal (N, n=10) and hypertensive rats (H, n=8) in each group.

n	Diastolic blood pressure (mmHg)															
	0 min		5 min		15 min		30 min		45 min		60 min		90 min		120 min	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	90	127	80	125	85	125	80	100	80	100	80	105	80	120	80	120
2	100	90	100	80	100	77	100	90	100	100	100	100	100	95	100	92
3	105	170	110	170	110	170	120	170	122	140	110	142	110	150	110	167
4	120	170	120	177	120	170	110	170	110	160	112	150	120	160	120	170
5	110	155	100	160	100	160	110	160	110	130	110	130	110	130	110	130
6	120	105	120	110	110	110	115	110	120	110	120	92	120	90	120	92
7	115	200	120	200	120	190	117	185	115	180	120	170	120	170	125	190
8	125	140	120	140	110	120	115	120	120	117	120	120	127	120	127	125
9	105		110		120		117		120		120		130		130	
10	120		120		120		122		122		122		122		122	
mean	115	156	115	159	113	153	115	152	117	139*	116	134^{+}	120	136^{+}	120	145*
±sem	3	12	4	13	3	13	3	13	4	10	4	9	4	10	5	12

* ($p < 0.05$), + ($p < 0.01$) : significant difference from their pre-administration (0 minute).

Hypertensive rats had significant higher diastolic blood pressure than normal at all corresponding times.

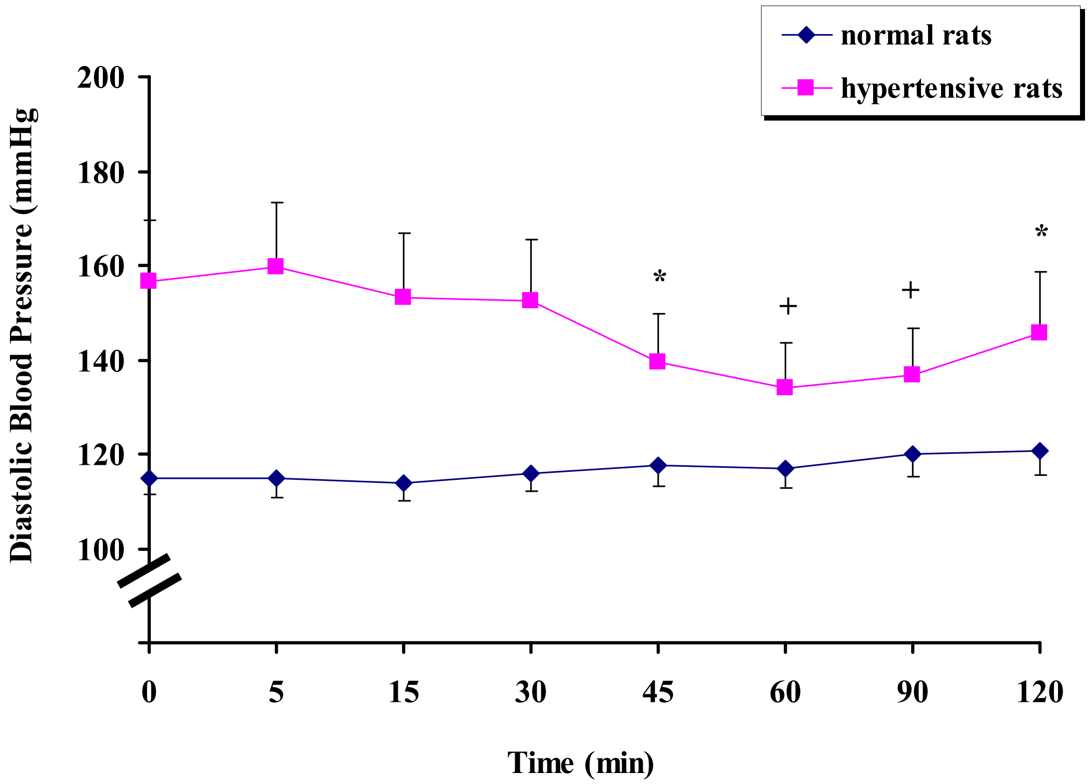

Figure 28. Effects of *C. asiatica* at the dose of 32 g/kg on diastolic blood pressure of femoral artery in normal rats (N, n=10) and hypertensive rats (H, n=8) at 0, 5, 15, 30, 45, 60, 90, and 120 minutes.

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the normal at the corresponding time.

Table 26. The percentage of systolic blood pressure at femoral artery after single oral administration of *C. asiatica* at the dose of 32 g/kg in normal (N, n=10) and hypertensive rats (H, n=8).

n	The percentage of systolic blood pressure from pre-administration													
	5		15		30		45		60		90		120	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	100	97	100	93	104	83	104	83	104	84	104	93	104	96
2	100	83	100	83	100	82	100	82	100	85	100	87	100	85
3	96	99	96	99	98	99	100	79	100	81	93	94	93	99
4	100	101	100	95	87	95	88	87	87	85	93	90	100	95
5	100	100	100	100	107	96	107	86	105	86	103	84	100	84
6	103	98	94	98	94	98	106	98	100	89	103	87	100	89
7	98	100	96	93	92	88	90	86	92	81	98	81	98	87
8	96	97	89	85	93	90	96	95	96	95	100	95	101	100
9	100		100		98		100		100		101		101	
10	98		98		98		100		100		98		96	
mean	99	96	97	93	97	91*	99	87^{+a}	98	85^{+a}	99	88^{+a}	99	91^{+a}
±sem	2	5	3	6	5	6	6	6	5	4	3	4	3	6

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

a ($p < 0.01$) : significant from the normal at the corresponding time.

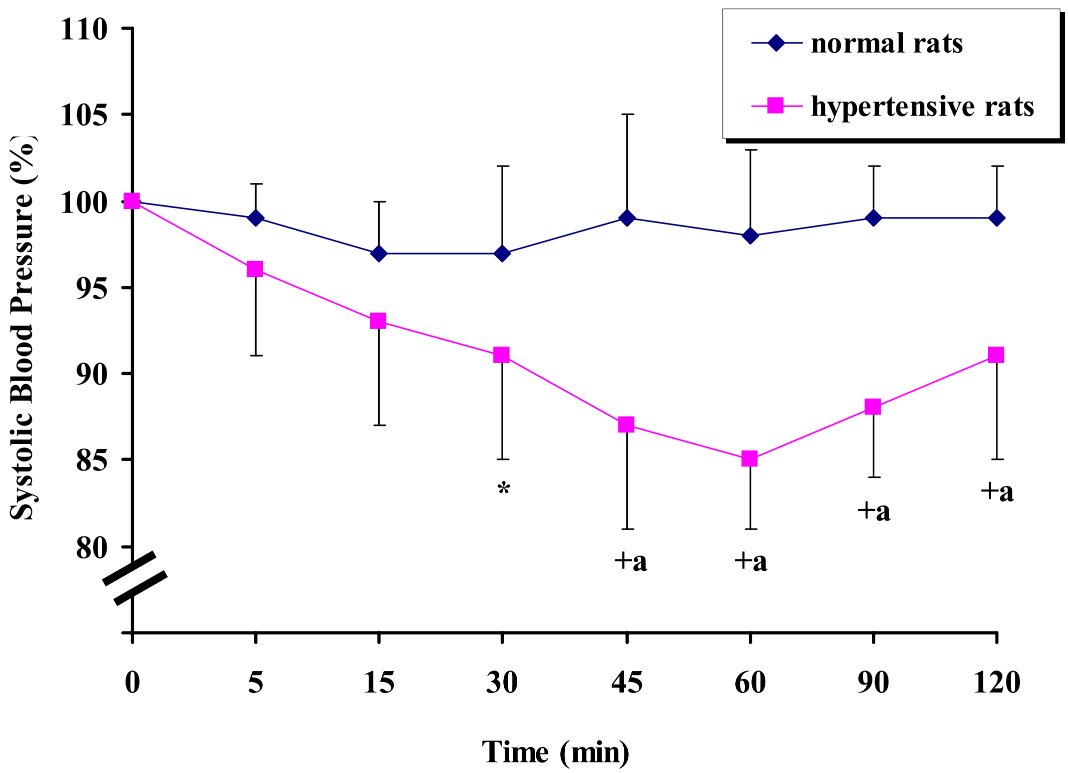

Figure 29. The percentage of systolic blood pressure of femoral artary after single oral administration of *C. asiatica* at the dose of 32 g/kg in normal rats (N, n=10) and hypertensive rats (H, n=8).

All values were expressed as mean ± sem

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

a ($p < 0.01$) : sign in normal rats (n=10) and hypertensive rats (n=8).

Table 27. The percentage diastolic blood pressure after single oral administration of *C. asiatica* at the dose of 32 g/kg in normal (N, n=10) and hypertensive rats (H, n=8).

n	The percentage of diastolic blood pressure from pre-administration													
	5		15		30		45		60		90		120	
	N	H	N	H	N	H	N	H	N	H	N	H	N	H
1	88	98	94	98	88	78	88	78	88	82	88	94	88	94
2	100	88	100	85	100	100	100	111	100	111	100	105	100	102
3	104	100	104	100	114	100	116	82	104	83	104	88	104	98
4	100	104	100	100	91	100	91	94	93	88	100	94	100	83
5	90	103	90	103	100	103	100	83	100	83	100	83	100	83
6	104	104	91	104	95	104	100	104	100	87	100	85	100	87
7	104	100	104	95	101	92	100	90	104	85	104	85	108	95
8	96	100	88	85	92	85	96	83	96	85	101	85	101	89
9	104		114		111		114		114		123		123	
10	100		100		101		101		101		101		101	
mean	99	99	98	96	99	95	100	91	100	88^{+a}	102	90^{+a}	102	93^{*b}
±sem	5	5	7	7	8	9	8	11	6	9	8	7	8	6

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the normal at the corresponding time.

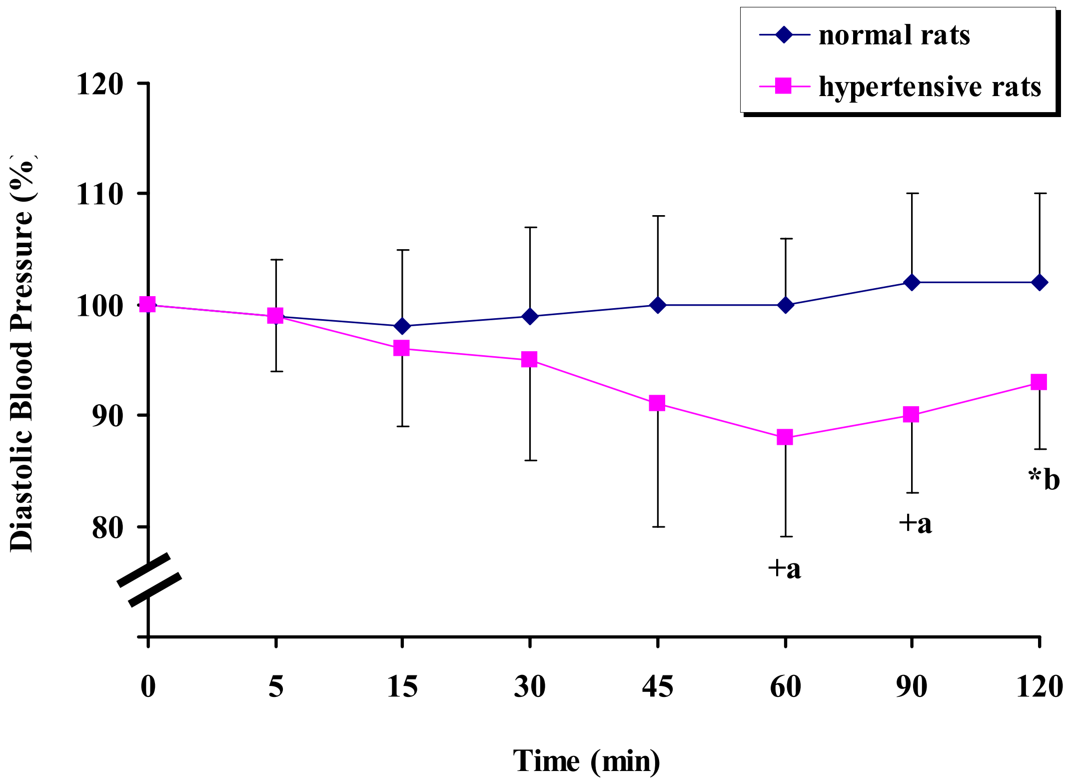

Figure 30. The percentage of femoral artery after single oral administration of C.asiatica at the dose of 32 g/kg diastolic blood pressure in normal (N, n=10) and hypertensive rats (H, n=8).

All values were expressed as mean ± sem.

* ($p < 0.05$), + ($p < 0.01$) : significant difference from the percentage 0 minute.

b ($p < 0.05$), a ($p < 0.01$) : significant difference from the normal at the corresponding time.

CHAPTER V
DISCUSSION

The present study assessed the effects of expressed juice of fresh *C. asiatica* leaves by oral administration on cardiovascular functions in normal and hypertensive rats. Specifically, the effect on blood pressure, heart rate, and cutaneous and cerebral regional blood flow were examined after single oral administration. The *C.asiatica* expressed juice was prepared and kept in the form of lyophilized powder to get the homogeneous component of *C.asiatica* juice.

The weekly SBP were increased gradually both normal and DOCA-salt treated rats throughout the 8 weeks of study. However, the DOCA-salt treated rats had greater increase weekly SBP than normal rats. At the week 8, the percentage increase of SBP from the start of the study were 24% in normal and 66% in DOCA-salt treated rats. The half of DOCA-salt treated rats had increased SBP to 180 mmHg or greater at week 6, and all had SBP over 180 mmHg at week 7. During 8 weeks, DOCA-salt treated rats had similar growth rate and no change in heart rate compared to normal rats.

C. asiatica expressed juice decreased SBP in dose dependent maner when given orally in hypertensive rats, while, they had no effect in normal rats. On contrary, captopril, angiotensin converting enzyme inhibitor (ACEI), commonly used in treatment of hypertension, decreased SBP in DOCA-salt hypertensive rats as well as normal rats. Captopril decreased SBP in DOCA-salt hypertensive and normal rats in the same level, 80 and 79% from the pre-administration, respectively. While, captopril had shorter duration and delayed maximum effect in DOCA-salt hypertensive rats when compared to normal rats. Comparision the decreasing effect on SBP in hypertensive rats, *C.asiatica* juice had lower potency (about 10%), shorter duration and faster maximal effect than captopril.

C. asiatica juice at the doses of 24 and 32 g/kg had similar percentage decrease in SBP and maximal action at 45 min. Though all three doses of *C.asiatica* juice had no effect on SBP in normal rats, the highest dose used, 32 g/kg, decreased heart rate to

97% of pre-administration. In hypertensive rats, all three doses of *C.asiatica* juice decrease heart rate in dose-dependent maner. The higher dose had greater potency, faster onset and longer duration than the lower dose. Captopril showed no alteration in heart rate both in normal and hypertensive rats.

The present study showed that *C.asiatica* juice had antihypertensive and negative inotropic effect in hypertensive rats. The previous work of Sangsirinavin (2), using the various extracts included fresh juice, hot water extract, 95% alcohol extract, alkaloids and glycosides were given to anesthesized normal dogs by intravenous administration. The alkaloids had no effect on blood pressure and heart rate, while glycosides decreased both blood pressure and heart rate. The alcoholic extract exhibited slightly decreased blood pressure but slightly increased heart rate. Glycoside also decreased force and rate of isolated rabbit heart contraction with greater response on force than rate. The 2-4 ml of fresh juice (1:1 in water) decreased blood pressure and heart rate in dose-dependent maner. The use of heated fresh juice or hot water extract gave the same effects as fresh juice, suggested that the active component is heat – stable. The triterpenic acids and their sugar esters included asiatic acid, madecassic acid and asiaticosides, were the main compositions of *C.asiatica* which had the biological activities (1). The alcoholic and water extract of *C.asiatica* contained the mixture of triterpenic acids and triterpenic sugar esters. While the triterpinic acids were found in the extract of polar organic solvents, chloroform and hexane. However, the asiaticosides were transformed *in vivo* to asiatic acid after oral administration (43).

There was report that fresh juice of *C.asiatica* given by oral administration were not observed in anesthesized normal dog. Similarly, our results showed that *C.asiatica* juice had no effect on SBP and heart rate in normal animals. Moreover, the result from the direct blood pressure measurement via femoral artery in regional cerebral blood flow determination showed that *C.asiatica* juice given orally had no effect on both SBP and DBP in anesthesized normal rats. In addition, the result also supported that *C.asiatica* juice given orally decreased both SBP and DBP in anesthesized hypertensive rats.

Several studies demonstrated that the extract of *C.asiatica* had vasorelaxing effect. The chloroform extract of whole plant decreased the maximal response in noradrenaline-induced contraction of aortic strip from normal rats (3). The vasodilation action also demonstrated using hexane extract in phenylephrine-induced contraction of aortic strips from hypertensive rats (41). Since blood pressure was determined by the product of cardiac output and total peripheral resistance, the decreased blood pressure can resulted from vasodilation and/or decrease of heart function. The results from the present study and the studies mentioned above suggest that *C.asiatica* possessed antihypertensive action by causing arterial vasodilation and depression of heart function.

C. asiatica juice at the dose of 32 g/kg was used for exploring its effect on cutaneous blood flow and regional cerebral blood flow (rCBF) in normal and DOCA-salt hypertensive rats. Compared with age-and sex-matched normal control rats, cutaneous blood flow was not altered and rCBF was decreased in DOCA-salt hypertensive rats. Interestingly, our results demonstrated that *C.asiatica* juice increased cutaneous blood flow in both normal and hypertensive rats, but the effect was greater and longer in hypertensive rats. The maximal increase were 102% at 45 minute in hypertensive and 76% at 60 minute in normal rats. Similarly, *C.asiatica* increased cerebral blood flow at all 5 points of determinations in normal and hypertensive rats. The increased cerebral blood flow at 5 points in hypertensive rats were greater than 50%.(50-63%), while in normal rats were less than 44% (33-44%). Although, the pre-adminstration average regional cerebral blood flow of hypertensive rats were 11% lower than normal rats, these flow increased in hypertensive greater than normal rats at 45-120 minute. At this point, it could be implied that *C.asiatica* had a vasodilatory effect and resulted in increasing blood flow to the various organs. Hypertension is an important risk factor for cerebrovascular disease including stroke and has a role in the development of vascular cognitive impairment, vascular dementia and finally brain damage is developed (42). In the present study, acute *C.asiatica* treatment increased regional cerebral blood flow in DOCA-salt hypertensive rats. In addition *C.asiatica* demonstrated antioxidant property in the brain (4, 6). The whole plant aqueous extract 300 mg/kg decreased the brain level of malonaldehyde with simultaneous increased endogenous antioxidant enzymes, glutathione and catalase, after daily treatment for 14 days in rats. It also showed the improvement of learning and memory in these animals (4). According to these data, *C.asiatica* is useful in hypertension and may improve brain damage from cerebral hypoperfusion.

Several studies showed the wound healing effect of *C.asiatica* extract given orally or topically by increasing cellular proliferation and collagen synthesis (1, 38). The increased blood flow at wound area was also important for the process of wound healing. In diabetic patients with microangiopathy, the triterpene fractions of *C.asiatica* significantly improved microcirculation and decreased capillary permeability after oral administration for 14 days (39, 40). These might result from the vasodilation action and the protective effect on the vascular wall of *C.asiatica*. The action of *C.asiatica* in improved microcirculalation and accerelated wound healing suggested the potential use in diabetic patients.

This study demonstrated the actions of *C.asiatica* on cardiovascular functions, especially in hypertensive conditions as antihypertensive, negative chronotropic to the heart, and increased blood flow to the organs.

CHAPTER 6

CONCLUSION

Oral administration of *C. asiatica* juiced at the doses of 16, 24 and 32 g/kg possesses the inhibition action on cardiovascular functions by decreasing blood pressure only in hypertensive rats, and decreasing heart rate in hypertensive and normal rats. In hypertensive rats, *C. asiatica* juice lowered systolic blood pressure with a lower potency, shorter duration and faster maximal action, than captopril at dose of 25 mg/kg. *C. asiatica* juice also increased blood perfusion on cutaneous blood flow as well as regional cerebral blood flow in both normal and hypertensive rats. These effects were greater in hypertensive than normal rats.

This study reveals the potential role of *C. asiatica* in using as antihypertensive and improvement of cerebral blood flow.

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BIOGRAPHY

NAME	Miss Sompong Muangnongwa
DATE OF BIRTH	19 May 1971
PLACE OF BIRTH	Loi-Et , Thailand
INSTITUTE ATTENDED	Mahidol University, 1989-1993 Bachelor of Nursing Mahidol University, 1999-2004 Master of Science in Biopharmaceutical Sciences (Physiology)
GRADUATION GRANT	Research grant partially support by Faculty Graduate Studies, Mahidol University
POSITION OFFICE	Sirikit Medical Center, Nursing Department, Ramathibodi, Hospital, Thailand Position : Nurse instuctor