MECHANISMS OF THE CARDIOVASCULAR EFFECTS OF SOME MEDICINAL PLANTS: AN EXPERIMENTAL STUDY

By

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DECLARATION

I, Davie R.J. Kamadyaapa, Student Registration Number: **202050003** hereby declare that the thesis entitled:

"Mechanisms of the Cardiovascular Effects of Some Medicinal Plants: An Experimental Study"

is the result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use was made of the work of others, it is duly acknowledged in the text.

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My thanks to my parents in law who had taken care of our son Oscar whenever we needed it.

Above all, many, many thanks are due to my darling and wonderful wife, Shingirayi who shouldered the burden of nurturing me evening after evening, month after month and year after year. Thank you for all the love, patience and support. I love you. May the good Lord bless you my dear wife. You have been a source of my stamina to climb this rough hill.

ABSTRACT

With increasing numbers of patients suffering from hypertension and conventional therapy beyond the reach of a wide population in developing countries, medicinal herbs with antihypertensive properties are increasingly sought by patients as an alternative, cheap and accessible source of treatment. The drawback, however, is that, very little or no reliable data is available on the precise mechanism (s) of action, safety and efficacy of these medicinal herbs. We, therefore, investigated the mechanisms of the cardiovascular effects of some medicinal plants used in traditional treatment of hypertension in South Africa. It was envisaged that if the mechanism (s) of the cardiovascular effects of these plants are elucidated, these plants might provide a cheap and therefore, accessible source of novel treatment for hypertension in poor developing populations.

Based on ethnobotanical information, five plants namely *Ekebergia capensis* (Sparrm) [Maliaceae], *Persea Americana* (Mill) [Lauraceae], *Helichrysum ceres* (S. Moore) [Asteraceae], *Sclerocarya birrea* (A. Rich) [Anacardiaceae] and *Hypoxis hemerocallidea* (Fisch. & C.A. Mey) [Hypoxidaceae] were identified and authenticated by Prof. H. Baijnath of Botany discipline. Voucher specimens of the plants have been deposited at the Discipline of Botany herbarium, University of KwaZulu-Natal. Leaves from *E. capensis, P. americana, H. ceres,* and stem-bark from *S. birrea* were air-dried and ground into powder while fresh corms from *H. hemerocallidea* were peeled and crushed into smaller pieces before extracted using 95% ethanol, to yield five different crude ethanolic

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extracts of *E. capensis* (EKE), *P. americana* (PAE), *H. ceres* (HCE), *S. birrea* (SBE) and *H. hemerocallidea* (APE).

To evaluate the acute effects of the test extracts, six separate groups comprising of control and treated male normotensive Wistar rats (n=6) were anesthetized and placed on a continuous jugular infusion of hypotonic saline (0.077M NaCl) at 150μL/ min. The left carotid artery was cannulated with polythene tubing and then connected to a pressure transducer for blood pressure measurements. After a 3h equilibration period, consecutive 30 min blood pressure and heart rates measurements were recorded over the subsequent 4h of 1h 30 min control, 1h treatment and 1h 30 min recovery periods. To assess the long-term effects of the test extracts, six separate groups comprising of control and treated male Dahl salt sensitive (DSS) rats (n=8) were used. The rats were orally treated with the test extracts (80 mg/kg body weight) while the control animals were administered the vehicle daily for six weeks. Blood pressure and heart rate were measured using the indirect tail-cuff method twice a week. The *in vitro* cardiotonic effects of the test extracts were examined on rat isolated spontaneously-beating right and electrically-driven left atria, to evaluate their effects on the rate and force of myocardial contractility, respectively. Their vascular effects were evaluated in rat isolated endothelium-intact and endothelium-deprived aortic rings and in myogenic spontaneously-contracting portal veins. Responses of all the isolated preparations were measured using isometric force displacement transducer and Ugo-basile 'Gemini recorder'.

All the tested extracts demonstrated potent acute hypotensive effects in anaesthetized rats. However, only EKE and HCE exhibited potent bradycardiac effects. On the other hand, all the extracts showed antihypertensive and bradycardiac effects in DSS rats during a six week chronic experiment. All extracts except for EKE, exhibited concentration-dependent negative inotropic and chronotropic effects. The negative inotropic and chronotropic effects of HCE were mediated by activation of cholinergic receptors while the effects of the other extracts were mediated by non-specific mechanism (s). EKE, displayed a significant and concentration-dependent positive inotropic and chronotropic effects which involved activation of ß-adrenergic receptors and voltage-gated calcium channels. The test extracts also exhibited significant and concentration-dependent vasorelaxant effects, in both endothelium-intact and endothelium-deprived aortic rings. In some cases the vasorelaxant effects of the test extracts in intact aortic rings were shown to involve activation of endothelium-derived vasorelaxing factors, opening of potassium channels and blockade of calcium channels. In rat isolated portal veins, all test extracts except for EKE and HCE, caused an initial and transient increase in contraction followed by a long lasting venorelaxation. HCE caused significant and concentration dependent venorelaxation which was mediated by activation of cholinergic receptors. EKE induced tonic contractions at higher concentrations and this effect was shown to involve the voltage-gated calcium channels.

The present study provides some of the possible mechanism (s) of the cardiovascular effects of *E. capensis, P. americana, H. ceres, S. birrea* and *H. hemerocallidea*. Therefore, these plants would have the potential to provide a cheap and indeed, accessible source of novel treatment for hypertension in poor developing populations.

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ACE	angiotensin converting enzyme
ACh	acetylcholine
AVP	arginine vasopressin
cAMP	cyclic 3',5' -adenosine monophosphate
cGMP	cyclic 3',5' -guanosine monophosphate
CICR	Ca^{2+} -induced Ca^{2+} release
CO	cardiac output
CO_2	carbon dioxide
DAG	diacylglyceral
DOCA	deoxycorticosterone acetate
DSS	Dahl salt sensitive rats
ECF	extracellular fluid
ECG	electrocardiogram
EDRF	endothelium-derived relaxing factor
ET-1	endothelium-1
FAO	food agricultural organization
G	guanine
GMP	guanine monophosphate
GTP	guanosine triphosphate
Hz	hertz, or cycles per second
IP3	inositol trisphosphate

MAP	mean arterial pressure
MLCK	myosin light chain kinase
Mmol	millimol
mmHg	millimeters of mercury
ms	millisecond
min	minute
MB	methylene blue
NO	nitric oxide
PH	pulmonary hypertension
SHR	spontaneously hypertensive rats
SVR	systemic vascular resistance
TPR	total peripheral resistance
WKY	Wistar Kyoto rats
WHO	world health organization

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CHAPTER 1

1.0   INTRODUCTION/LITERATURE REVIEW

1.1   General

Hypertension, defined as an average diastolic pressure higher than 90 mmHg and a systolic pressure higher than 140 mmHg is a common health problem in developed countries. Its prevalence is reported to be on the increase in populations of the developing world (WHO Guidelines, 1999). Human hypertension is regarded usually as a slow-occurring disorder leading to cardiovascular complications that cause most of the morbidity and mortality in the elderly (Weindruch, 1995). Hypertension is a highly prevalent risk factor for cardiovascular disease (CVD), which affects approximately 1 billion individuals worldwide (Pearson, Jamison, and Tergo-Gauderies, 1993). Epidemiological studies have revealed that hypertension affected one quarter of the world population in the year of the millennium and it is predicted that this proportion will increase dramatically over the next two decades (Kearney, Whelton, Reynolds, Muntner, Whelton and He, 2005; Whelton, Brancati, Appel, and Clag, 1995).

Hypertension is important not only because of its high worldwide frequency, but also because it is a major modifiable risk factor for numerous cardiovascular diseases, such as stroke and coronary artery disease (Poulter, 2003). The most recent World Health Organization report highlighted the importance of blood pressure as a major

cardiovascular risk factor when it identified hypertension as a single most important preventable cause of premature death in developed countries (Ezzati, Vander and Lawes, 2005). Known as 'the silent killer' it may exist for a long period of time without manifesting symptoms such as impairing the patient's quality of life (QOL). Symptoms may only show after it has caused serious irreversible pathology and complications. It creates marked effects on patients and the society as a whole, either because of hypertension per se, or through its complications that can lead to the development of stroke, renal dysfunction, cardiovascular diseases such as heart attack, ischaemic heart diseases, coronary diseases and heart failure, which can cause death or irreversible disability (Murray and Lopez, 1994).

There are basically two types of hypertension classified based on their causes. It is known that more than 95% of hypertensive patients in the community are of essential hypertension (primary hypertension), sometimes called idiopathic hypertension (Sheila, Doggrell and Brown, 1998). This type of hypertension has no known specific cause though many cases are attributed to a genetic and hereditary origin. Many theories trying to determine a specific cause thought it might be multifactorial (Zicha and Kunes, 1999). This makes therapeutic intervention more difficult as it basically relies on symptomatic treatment. This can result in many abnormalities in the physiological regulatory systems for blood pressure including neurotransmitters and humoral factors with abnormalities of the cardiac and vascular smooth muscle and endothelium. There is a need, therefore, for improved integration of multidisciplinary research to clarify the pathophysiology of this complex and multifaceted disorder.

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The other type of hypertension known as secondary hypertension results from underlying and identifiable causes and this makes therapeutic intervention a little easier. This type of hypertension is only found in less than 5% of hypertensive patients (Zicha and Kunes, 1999).

1.2   Primary hypertension

Despite the fact that primary (essential) hypertension is one of the most prevalent diseases and an important risk factor for cardiovascular morbidity and mortality, the underlying pathophysiological abdormalities leading to the development of elevated arterial pressure in this disorder remain elusive (Zicha and Kunes, 1999). Indeed, there is no unifying hypothesis to account for the pathogenesis of primary hypertension (Zicha and Kunes, 1999). Studies indicate a natural progression of this disease, suggesting that early elevations in blood volume and cardiac output might initiate subsequent increases in systemic vascular resistance resulting in increased blood pressure (Sheila, Doggrell and Brown, 1998). Under normal conditions there is a consistent relationship that exists between the extracellular fluid volume and blood volume (Guyton and Hall, 1994). A chronic increase in sodium levels in the body due to excessive sodium retention leads to a chronic increase in the extracellular fluid volume, part of which is proportionately distributed to blood volume compartments. Increased blood volume will cause an increase in blood pressure either by increasing cardiac output or total peripheral resistance or both (Guyton and Hall, 1994). Thus, the mechanisms that regulate sodium balance are primarily responsible for control of blood pressure. Therefore, the long-term

regulation of arterial blood pressure may be intimately linked to the ability of the kidney to maintain normal sodium balance, extracellular fluid volume and blood volume at normal blood pressure. Guyton (1991) suggested that a basic underlying defect in many hypertensive patients is an inability of the kidneys to adequately handle sodium; thus, initiating the pathogenesis of most if not all cases of hypertension. On the other hand, when derangements in the renal function include increased levels of humoral or neural factors that directly induce vascular smooth muscle constriction, peripheral vascular resistance is increased (Navar, 1997). Therefore, decreased renal sodium excretion in the face of normal or increased sodium intake can lead to chronic increases in extracellular fluid volume which eventually leads to hypertension. In addition, activation of the renin-angiotensin-aldosterone system causes increased sodium retention which results in reduced water loss into the urine. The renin-angiotensin system serves as one of the most powerful regulators of blood pressure and sodium balance. Angiotensin II exerts its actions by activating its receptors (AT1), located on the membranes of the proximal and distal tubules of the nephron (Mitchell and Navar, 1995). Its vasoconstrictive actions in the kidney cause a decrease in blood flow and sodium excretion and this promotes tubular reabsorption of sodium and water. Angiotensin II also regulates secretion of aldosterone by the adrenal gland. Aldosterone increases sodium reabsorption in the distal tubules of the nephron by activating the cytoplasmic mineralocorticoid receptor (MR) (O'Neil, 1990). Therefore, both angiotensin and aldosterone although by different mechanisms stimulate distal tubular sodium reabsorption and decreases sodium and water loss by the kidney (Guyton, Coleman, Cowley, Scheel, Manning, and Norman, 1972; Cowley, 1992).

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There is recent evidence for increased vascular tone in essential hypertension (Tepel and Zidek, 1998). This could be mediated by increased sympathetic activity or by increased circulating levels of angiotensin II and endothelins which enhance vasoconstriction tone thereby increasing total peripheral vascular resistance (Mitchell and Navar, 1995). Furthermore, many mechanisms may operate to initiate and sustain hypertension. Therefore, it is clear that essential hypertension is a multifactorial dysfunctional process that can be caused by a myriad of different conditions ranging from stimulatory influences that inappropriately enhance tubular sodium reabsorption and systemic vascular resistance. Thus, the underlying cause of primary hypertension is not clear and this prevents the adoption of methods to more effectively manage or prevent its development.

Treatment of primary hypertension involves a pharmacologic intervention which antagonizes the effects of humoral substances like angiotensin II and calcium entry into cells (Mitchell and Navar, 1995). However, these treatments do not target the cause(s) of the underlying disease. An understanding of the normal mechanisms regulating sodium balance and how derangements lead to altered sodium homeostasis and hypertension may provide the basis for the rational approach to the treatment of essential hypertension.

1.3   Secondary hypertension

Secondary hypertension accounts for approximately 5-10% of all cases of hypertension (Zicha and Kunes, 1999). There are many known conditions that can cause secondary

hypertension, some of which are renal artery stenosis, chronic renal diseases, primary hyperaldosteronism, stress and phaeochromocytoma.

1.3.1   Renal artery stenosis

The narrowing of the vessel lumen as a result of renal artery diseases causes a reduction in pressure at the afferent arteriole in the kidney. Reduced arteriolar pressure and reduced renal perfusion stimulate renin release by the kidney (Tepel and Zidek, 1998). This increases circulating angiotensin II and aldosterone. Increased circulating levels of angiotensin II causes systemic vasoconstriction which increases total peripheral vascular resistance. Increased levels of aldosterone enhance renal reabsorption of sodium and water resulting in expansion of extracellular fluid volume. This causes an increase in blood volume with subsequent increases in cardiac output. Thus, hypertension due to renal artery stenosis results from both an increase in total peripheral resistance and an increase in cardiac output.

1.3.2   Chronic renal diseases.

Chronic renal diseases such as diabetic nephropathy, glomerulonephritis can damage nephrons in the kidney resulting in impairment of normal excretion of sodium and water; thus increasing the extracellular fluid volume, blood volume and hence workload of the heart (Tepel and Zidek, 1998). Chronic renal diseases may also cause increased release of renin resulting in renin-dependent form of hypertension (Mitchell and Navar, 1995).

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1.3.3 Primary hyperaldosteronism

Primary aldosteronism can be defined as overproduction of aldosterone independent of its normal chronic regulatory factors such as angiotensin II (Gordon, Stowasser, Tunny, Klemm, Rutherford, 1994). Hypertension, hypokalemia, suppressed plasma renin activity and increased secretion of aldosterone characterize this syndrome (Conn, 1955). It appears that with the current screening methods, primary aldosteronism may be the most common form of secondary hypertension, with its prevalence approaching 10% of all persons with hypertension worldwide (Stowasser, 2001; Fardella, Mosso, Gomez-Sanchez, 2000). The most common subtypes of primary aldosteronism are bilateral idiopathic hyperaldosteronism (IHA) and unilateral aldosterone producing adenoma (APA) (Young, 1999). Hyperaldosteronism also can result secondarily from any state of increased renin, such as renal artery stenosis, which results in increased circulating concentrations of angiotensin II and stimulation of aldosterone release (Lifton, 1996). Recently, it has been demonstrated that aldosterone accelerates hypertension in animal models of malignant hypertension (Rizzoni, Porteri, Castellano, 1996). Some studies have recently reported that aldosterone inhibits nitric oxide synthesis by increasing oxidative stress and thereby causing endothelial dysfunction with subsequent impairment in vasodilation (Rizzoni, Porteri, Castellano, 1996). Thus, increased secretion of aldosterone may cause an elevation of blood pressure by increasing both cardiac output as a result of sodium and water retention as well as total peripheral resistance due to vasoconstriction.

1.3.4   Stress

Emotional stress leads to activation of the sympathetic nervous system which causes increased release of noradrenaline from sympathetic nerves in the heart and blood vessels, leading to increased cardiac output and increased systemic vascular resistance (Pernini, Muller and Buhler, 1991). Activation of the sympathetic nervous system also increases circulating angiotensin II, aldosterone, and vasopressin, which can increase systemic vascular resistance contributing to a sustained increase in blood pressure (Mitchel and Navar, 1995). Urban hypertension is a good example of stress-induced hypertension.

1.3.5   Phaeochromocytoma

Phaeochromocytoma, a tumor usually found in the adrenal medulla releases a mixture of adrenaline and noradrenaline and this can lead to very high levels of circulating catecholamines (Bertram, 2004). High levels of circulating catecholamines may cause an increased sympathetic activation, which plays a role in short–term regulation of blood pressure (DiBona and Kopp, 1997). Catecholamines induce activation of both alpha-adrenoceptor mediated systemic vasoconstriction and beta-adrenoceptor mediated cardiac stimulation. The former effect of catecholamines results in increased total peripheral vascular resistance and the latter in increased heart rate and myocardial contraction causing an increased cardiac output (Bertram, 2004). On the other hand, the direct effects of increased activation of the sympathetic nervous system on the kidney function may result in renal sodium retention caused by decreases in glomerular filtration rate (GFR)

and increases in tubular reabsorption (Vari and Vavar, 1995). Taken together, these effects of catecholamines may contribute to significant elevations in blood pressure.

1.4   Regulation of blood pressure

Blood pressure is a product of the cardiac output (CO) and the total peripheral vascular resistance (TPR), mathematically represented by an equation:

BP   =   CO   x   TPR

=   (Heart rate x stroke volume) x TPR

(Vander, Sherman and Luciano, 2001)

An imbalance in this relationship is required for any change in blood pressure to occur. Thus, either an increase in CO or TPR, or both factors, results in an increase in blood pressure. This imbalance reflects a disruption of the normal pressure natriuresis relationship.

Therefore, two main concepts on the aetiology of hypertension have been posed to explain the key mechanisms that must become reset in order to sustain blood pressure changes in the long term. For one of them, an abnormally increased total peripheral resistance (TPR) is the key alteration in hypertension (Cowley, 1992). For the other, the kidney exerts a dominant control of blood pressure through the adaptation of blood volume by means of a pressure-sensitive, natriuresis-driven, diuretic mechanism (Guyton and Hall, 1994). Strong evidence supports the idea that total peripheral resistance (TPR) is increased in all forms of human and experimental hypertension (Somova, Channa and Khan, 1999). The aetiological participation of TPR in the origin

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and long-term maintenance of hypertension has been extensively debated (Guyton and Hall, 1994). It now seems clear that the renal, pressure-sensitive natriuresis and diuresis is the main mechanism of blood pressure control in the long term (Guyton, *et al.,* 1972 and Cowley, 1992). However, regardless of the origin of hypertension, the actual increase in arterial blood pressure is caused by either an increase in TPR or an increase in (CO) or both (Vander, Sherman and Luciano, 2001). The former is determined by the vascular tone of systemic resistance vessels, whereas the latter is determined by heart rate and stroke volume.

1.5 Regulation of cardiac and vascular smooth muscle contraction and relaxation

The process of smooth and cardiac muscle contraction is regulated principally by receptors and mechanical activation of the contractile proteins, myosin and actin. A change in membrane potential brought on by the firing of action potential or by activation of stretch-dependent ion channels in the plasma membrane, can also bring about contraction (Mcdolnad, Pelzer, Trautwein and Pelzer, 1994). For contraction to occur, myosin light chain kinase (MLCK) must phosphorylate the light chain of myosin to enable the molecular interaction of myosin and actin. The energy released from ATP by myosin ATPase activity results in the cycling of the myosin cross-bridges with actin for contraction. Contraction of smooth muscle is initiated by calcium-mediated change in the thick filaments. The intracellular concentration of calcium increases in response to specific stimuli through the voltage-dependent L-type Ca^{2+} channels (dihydropyridine

receptors) (Mcdolnad et *al.*, 1994). Agonists such as noradrenaline bind to their receptors which are coupled to G-protein which, activate adenylate cyclase to form cyclic adenosine monophosphate (cAMP) from ATP. Increased cAMP activates a cAMP-dependent protein kinase that phosphorylates the voltage-dependent L-type Ca^{2+} channels (dihydropyridine receptors), which cause increased calcium entry into the cells. The increased cytosolic calcium triggers a subsequent release of calcium from intracellular stores (sarcoplasmic reticulum) through calcium release channels (ryanodine receptors) by a process known as Ca^{2+}-induced Ca^{2+} release (CICR) (Fabiato, 1983). In cardiac muscle cells, increased cytosolic free calcium combines with an acidic protein, calmodulin. This complex activates myosin light chain kinase to phosphorylate the light chain of myosin thereby enhancing cardiac contraction (inotropy) (Mcdolnad *et al.*, 1994). In vascular smooth muscle cell, these receptors are also coupled to G-protein, which stimulates formation of cAMP. However, unlike in cardiac myocyte, an increase in cAMP in vascular smooth muscle cells leads to smooth muscle relaxation (Barany, 1996). The reason for this is that cAMP inhibits myosin light chain kinase responsible for phosphorylating smooth muscle myosin. In addition to calcium-dependent activation of myosin light chain kinase, the state of myosin light chain phosphorylation is further regulated by myosin light chain phosphatase also known as myosin phosphatase, which removes the high-energy phosphate from the light chain of myosin to promote smooth muscle relaxation (Barany, 1996; Fukata, Mutsuki and Kaibuchi, 2001 ; Ridrey, 1996). The myosin binding subunit, when phosphorylated, inhibits the enzymatic activity of myosin light chain phosphatase allowing the light chain of myosin to remain phosphorylated, thereby promoting contraction (Mcdolnad *et al.*, 1994). Smooth muscle

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relaxation occurs as a result of either removal of the contractile stimulus or by the direct action of a substance that stimulates inhibition of the contractile mechanism. However, whatever mechanism is involved, the process of relaxation requires a decreased intracellular calcium concentration and increased myosin light chain phosphatase activity (Morgan, 1990; Somlyo, Wu, Lalker and Somlyo, 1999). A decrease in the intracellular concentration of activator calcium elicits smooth muscle cell relaxation.

1.6 *In vitro* models for the study of cardiovascular system

1.6.1 Perfused organ system

Perfused organ preparations such as the modified Langendorff technique and the working heart preparation have been used to evaluate integrative myocardial functions *in vitro* upon exposure to different drugs and toxins. The isolated heart model such as the Langendorff heart allows for a broad range of physiological and pharmacological studies to be performed, while at the same time removing confounding effects of other organ systems (McFaul and McGrath, 1987). The working preparation is perfused through the left atrium to generate a left-sided working preparation. The perfusion fluid reaching the ventricle is ejected via the aorta into a chamber against hydrostatic pressure to mimic physiological resistance to flow. Potassium arrested-hearts can also be used to examine flow-dependent effects in the absence of myocardial function (McFaul and McGrath, 1987). The paced and perfused heart model led to the development of the Screenit system, which is a model that provides detailed information on drug-induced

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eletrophysiological effects. In brief, the Screenit system allows for a classification of drugs that is based on their effects on the action potential of the heart (Valentin, Hoffman, De Clerck, Hammond and Hondeghem, 2004).

Like wise, blood vessel segments from vascular beds can be isolated and processed for perfusion *in vitro*. Aortic preparations are most often preferred since tissue can be readily accessed for perfusion and superfusion (Crass, Hulsey and Bulkley, 1988). Use of perfused preparation in toxicological studies is advantageous because the level of structural organization is similar to that encountered *in vivo*, and changes in physiological or pharmacological sensitivity, excitability and contractility can be readily evaluated (Valentin *et al.*, 2004). In the case of vascular preparations, endothelial cells can be eliminated to directly assess interactions between luminal and medial cells. The most significant limitations of perfused preparations are the small number of replicate preparations that can be processed at any one time, and the short time available for isolation and placement of the tissue under physiological conditions. As with cardiac preparations, measurements of contractility, rate of tension development and stress development can be used to evaluate the vascular effects of drugs and chemicals.

1.6.2   Organ culture

Ingwall, DeLuca, Sybers and Wildenthal (1975) first described the culture of whole fetal hearts to study processes associated with myocardial cell injury. However, some studies reported variations of this technology (Tanaka, Kasuya, Saito and Shigenobu, 1987 and

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Gotlieb and Boden, 1984). Organ culture preparations offer long term stability, and allow the study of cell-cell and cell-substratum interactions, as well as structural functional relationships of the matrix (Koo and Gottlieb, 1992). More recently, isolated frog and mouse hearts have been used to evaluate the toxicity of *Ipomoea carnea,* a poisonous aqueous plant (Bachlav, Burande, Rangari and Mehta, 1999). Based on the combined effect of atropine, calcium channel blockers and several salt solutions it was demonstrated that *I. carnea* produced positive inotropic effects due to sodium extrusion or release of intracellular calcium.

1.6.3   Tissue slices.

The use of tissue slices as an *in vitro* model started gaining more acceptance as improved methods for obtaining reliable and consistent slices were made available. Thin slice preparations of cardiac tissue have been developed and characterized as models to evaluate toxicity of xenobiotics (Gandolfi, Brondel, Fisher and Michaudi, 1995). One of the advantages of this system is that assessment of the toxic potential of a chemical can be performed after a brief term or continuous exposure with this model. This model also allows study of the interactions between heterogeneous cell types comprising the tissue being studied there by providing a controlled system for the study of toxicity and cardiovascular injury *in vitro* (Gandolfi, Brondel, Fisher and Michaudi, 1995).

1.6.4   Isolated muscle preparations.

Strips of atrial, ventricles or papillary muscles (Foex, 1988), as well as strips from various vascular beds (Hester and Ramos, 1991), have been used to evaluate tension development *in vitro* in a bath containing oxygenated physiological solutions. Isolated preparations operate under constant conditions of carbon dioxide exchange, ionic gradients, and diffusion of by-products of cellular metabolism (Foex, 1988). In the case of vascular preparations, spiral strips or simple ring preparations are preferred over longitudinal strips to avoid alterations in the geometry of muscle fibres (Foex, 1988). After equilibration in a physiological solution, isolated preparations are subject to multiple stress/ relaxation cycles to define the length at which maximal contractility occurs in the response to a contractile agonist. Experiments can be conducted to evaluate isometric or isotonic force development, or a quick-release contraction in which the after load is varied during contraction (Foex, 1988). Because oxygenation of the tissue depends on diffusion, the thickness of the strips and the concentration of oxygen in the bath must be carefully monitored. Concentration-response relationships can be constructed for selected contractile agonists in the absence or presence of toxicant (Gibbs, Woolley, Kostanas and Gibson, 1984; Togna, Dolci and Caprino, 1984). These relationships are obtained by cumulative increases in the concentration of each agonist without intervening washout until attainment of maximal developed force. In the case of vascular preparations the effects of relaxing agents can also be evaluated. The vessel segment is pre-contracted to about 70-80% of the maximal contraction, and then

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challenged with the relaxing agent. Isolated preparations can be controlled with precision, but their stability is limited to brief periods of time (Foex, 1988).

Significant progress has been made in advancing the application of isolated vascular preparations to study vascular effects of plant extracts. Aortic rings from different strains of rats, guinea-pigs and rabbits are presently and widely used *in vitro* to evaluate the effects of different drugs (Hester and Ramos, 1991). The endothelium plays a greater role in the regulation of the vascular tone as such *in vitro* studies using isolated vascular tissues like arteries and veins has helped significantly in understanding the role of the endothelium both in normal and diseased conditions (Andriambeloson, Stoclet and Andriantsitohaina, 1999).

1.6.5 Cell culture system

Primary cultures can be established with relative ease from cell suspension of cardiac and vascular tissue. Vascular endothelial and smooth muscle cultures can also be established by the explant method in which pieces of tissue are placed in a culture vessel to allow for cellular migration and proliferation *in vitro*. Neonatal and embryonic cells of cardiac origin proliferate readily under appropriate conditions *in vitro* (Kasten, 1972). Vascular endothelial and smooth muscle cells derived from large and medium-sized vessels of embryonic, neonatal, or adult animals proliferate readily under appropriate conditions *in vitro* (Ramos, 1990). As such, cultures can be propagated to prepare cell strains that retain variable degrees of differentiation as a function of cultivation *in vitro*. Previous

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studies demonstrated the establishment of three mouse endothelial cell lines from aorta, brain capillaries, and heart capillaries (Bastaki, 1997). These cell lines exhibit endogenous expression of specific markers, as evidenced by angiotensin-converting enzyme, acetylated LDL receptor, constitutive endothelial nitric oxide synthase, and vascular cell adhesion molecule-1 and bind Griffonia simplicifolia-I lectin (Bastaki *et al.,* 1997). In other studies, an *in vitro* model of vascular injury by menadione-induced oxidative stress in bovine heart microvascular endothelial cells was developed (Kossenjans, Rymaszewski, Barankiewicz, Bobst and Ashraf, 1996). Evidence was obtained that menadione toxicity was mediated by poly (ADP-ribose) polymerase activation by hydrogen peroxide. Other important considerations related to the use of cultured cell systems in toxicology studies include recognition that the presence of serum modulates antioxidant capabilities *in vitro* (Bishop, Mirza, Crapo and Freeman, 1985), and that cardiovascular cells in culture undergo variable degrees of differentiation (Owens and Thompson, 1986).

1.7   Animal models of experimental hypertension.

It is generally believed that both genetic and environmental factors and their interactions play a critical role in the pathogenesis of hypertension and other cardiovascular diseases. Accordingly, there are substantial individual variations in these two triggering elements leading to many variations in the direct and indirect effects on the cardiovascular system which are difficult to differentiate, thereby rendering the study of essential hypertension difficult (Sheila, Doggrell and Brown, 1998). Since hypertension and associated

cardiovascular diseases are the leading causes of death of human beings, medical scientists are dedicated to elucidate the mechanism of and explore the treatment for hypertension. Animal models of human disease have been widely used to study aetiology and pathogenesis of human disease, to prevent disease or to find a therapy and identify risk factors contributing to the disease. Probably the most commonly and widely used animal is the rat, not only on account of its comparatively low maintenance cost and easy handling, but also because the pathophysiology developed in these animals is quite similar to that observed in humans and that many techniques have been developed to measure relevant functional parameters using a rat model (Sheila, Doggrell and Brown, 1998). For decades, rat models of hypertension have provided a powerful tool for the study of cardiovascular diseases in their early stages, as well as the investigation of the mechanisms of the pathogenesis of cardiovascular diseases and effects of drug intervention. It has been suggested that an ideal animal model for any cardiovascular disease in humans should at least possess five characteristics: (i) mimic the human disease, (ii) allow studies in chronic, stable disease, (iii) produce symptoms which are predictable and controllable, (iv) satisfy economical, technical and animal welfare considerations, and (v) allow measurement of relevant cardiac, biochemical and haemodynamic parameters (Sheila, Doggrell and Brown, 1998). However, the use of rat model as well as other animal models has some limitations. For example, it is argued that cardiovascular diseases such as hypertension and heart failure usually develop slowly in humans in contrast to the acute onset of symptoms in many surgical or drug-induced rat models of these diseases. Furthermore, these diseases are uncommon in young humans, but markedly increase with age whereas most models of hypertension and heart failure

only use young adult rats (Weindruch, 1995). Currently, the use of animal models is being limited by ethical concerns and legislation, as a reaction to the opposed opinions within the community on the necessity for the use of animals in research. Cell dispersion from adult hearts and the culture of neonatal or adult cardiac fibroblasts or neonatal cardiomyocytes have replaced some animal studies since these allow studies of a single cell type in the absence of homeostatic mechanisms over a wider range of experimental conditions than easily obtained *in vivo* (Sheila, Doggrell and Brown, 1998).

1.7.1   The major rat models of hypertension.

Basically, animal models of hypertension comprise primary and secondary hypertension. The primary hypertension includes genetically-induced and environmentally-induced hypertension, whereas the secondary hypertension includes pharmacologically-induced and renal-induced hypertension.

1.7.2   Genetically-induced hypertension.

Animals that have undergone artificial genetic manipulation are predestined to become hypertensive. It is believed that genetically hypertensive rats comprise the most popular models to study essential hypertension (Yagil and Yagil, 2005). Two good examples of genetically hypertensive rats are; spontaneously hypertensive rats (SHR) and the Dahl salt sensitive rats (DSS).

1.7.2.1   Spontaneously hypertensive (SHR)

The most commonly used model of cardiovascular disease is the spontaneously hypertensive rat (SHR), originally inbred from Wistar stock often with the Wistar Kyoto rat (WKY) as their normotensive control (Sheila, Doggrell and Brown, 1998). These rats develop hypertension at about 4-6 weeks of age, largely independent of dietary levels of either Na^+ or Cl^-. The various colonies of SHR are pre- hypertensive for the first 6–8 weeks of their lives with systolic blood pressure around 100–120 mmHg (Adams, Bobik and Korner, 1989) and then hypertension develops over the next 12–14 weeks (McGuire, 1985). Similar to human beings, hypertension develops more rapidly and becomes more severe in male than female in SHR (Adams, Bobik and Korner, 1989). *In vivo* studies have shown that, in the early stages of hypertension, SHRs have an increased cardiac output with normal total peripheral resistance (Smith and Hutchins, 1979). However, as the animals progress into the established hypertension state, the cardiac output returns to normal and the hypertrophied blood vessels produce an increase in the total peripheral resistance (Smith and Hutchins, 1979).

Studies in hypertension have commonly resorted to the use of SHRs which have, within each colony, uniform polygenetic disposition and excitatory factors (Lindpaintner, Kreutz and Ganten, 1992). These factors produce uniform changes in the indirect and direct effects on the cardiovascular system resulting in the reduction of individual variations. This lack of inter-individual variation is one of the major advantages of the SHR, but it means that the SHR can only model one of many possible causes of human hypertension

(Lindpaintner, Kreutz and Ganten, 1992). Another advantage of the SHR is that it follows the same progression of hypertension as human hypertension with pre-hypertensive, developing and sustained hypertensive phases with each phase lasting at least several weeks (Folkow, 1993). Because SHRs have a pre-hypertensive state, they have the important potential to be used in studies of the cause and development of hypertension. The SHR is a useful model as pharmacological agents which lower blood pressure in SHR are also effective in hypertensives. The SHR is a chronic stable model producing symptoms which are predictable and controllable and avoiding difficult or life-threatening technical interventions (Sheila, Doggrell and Brown, 1998). Thus, it is not surprising that SHRs have been used extensively and successfully for 30 years to test medicines for their effectiveness in lowering blood pressure, and to study the mechanisms of established hypertension. The common criticism of the SHR model is that it has been around for a long time yet we still know little about the cause of the onset of hypertension (Sheila, Doggrell and Brown, 1998).

1.7.2.2 Spontaneously hypertensive rats with heart failure (SHRs-F)

The SHR-F rat model has been long known to have many similarities to human essential hypertension-induced heart failure including the important feature that impaired myocardial performance is a late feature that precedes overt failure (Pfeffer, Pfeffer, Fishbein and Frohlich, 1979). The SHR-F model is a good model of human hypertension-induced heart failure as these conditions have many features in common. In the SHR, failure occurs around 2 years of age and may, therefore, be compromised by the effects of

ageing (Bing, Brooks and Robinson, 1995). This may make the interpretation of the rat data more difficult but it could also be argued that, since human heart failure is also commonly complicated by the effects of ageing, the aged SHR is the more realistic model (Sheila, Doggrell and Brown, 1998). This is a non-intervention model; as such there is no need for skilled technical assistance or mortality associated with surgery. The major disadvantage of the SHR-F model is the extended time frame and, therefore, increased costs of these experiments compared with other models of heart failure (Sheila, Doggrell and Brown, 1998).

1.7.2.3   Stroke-prone spontaneously hypertensive rats (SHR-SP)

The SHR stroke prone rat model (SHR-SP) is a further developed sub-strain of SHR, with even higher levels of blood pressure and a strong tendency to die from stroke (Yamori, 1984). For instance, the SHR- SP rats are hypertensive at 5 weeks and systolic blood pressure rises to at least 250 mmHg in males, in contrast to pressures of around 200 mmHg in SHR (Rubin, Miller, Rohrbacher and Walsh, 1984). Salt-loading accelerates the development of hypertension and the occurrence of stroke in this rat model (Bannister, 1992). However, development of stroke increases with age with 65% due to atheroma and thrombosis, 15 % due to haemorrhage and 15 % to embolism (Bannister, 1992). These lesions are similar to humans and as such, SHR-SP has been used to investigate preventive strategies for both stroke and cardiac hypertrophy due to severe hypertension (Rubin, Miller, Rohrbacher and Walsh, 1984). However, SHR-SP dies in

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early to mid-adulthood (52–64 weeks or around 14–20 weeks old following salt-loading) (Vacher, Richer, Fornes and Clozel, 1996). Therefore, this model does not mimic the most common age of onset of stroke in humans. Furthermore, there is insufficient evidence that effective therapeutic regimes in the SHR-SP can be translated into effective prevention of stroke in humans (Sheila, Doggrell and Brown, 1998).

1.7.2.4 Dahl salt-sensitive rats

Another model is the Dahl salt-sensitive rat, originally derived from Sprague-Dawley rat stock by Dahl on the basis of developing hypertension with high NaCl diet, with Dahl salt resistant rats as their corresponding control group (Sheila, Doggrell and Brown, 1998). When fed with normal salt diets, these rats become hypertensive, indicating that this is a genetic model of hypertension with the feature of salt sensitivity. However, Dahl salt sensitive rats become extremely hypertensive during a high sodium diet (Dahl, Heine and Tassinari, 1962). The introduction of this model has significantly helped researchers understand salt sensitive hypertension in humans. The development of hypertension and heart failure in this model can be controlled by titration of the amount of salt in their diet, and is reportedly to be more rapid and greater in male than female (Dahl, Heine and Tassinari, 1962). Addition of 8% NaCl to the diet at 6 weeks of age leads to concentric left ventricular hypertrophy at 11 weeks. Marked left ventricular dilation at 15–20 weeks leads to laboured respiration, left ventricular hypokinesis and sudden death (Inoko, Kihara, Morii, Fujiwara and Sasayama, 1994). There is also a reduction in response of the left papillary muscle to isoprenaline in this model (Inoko, *et al.*, 1994). These contractile

parameters appear to be very similar to those reported in end-stage human heart failure (Sheila, Doggrell and Brown, 1998). Recent studies have shown that modulation of nitric oxide (NO) production from L-arginine is integrally involved in the development of hypertension in these salt-sensitive rats (Greene, Yu, Roman and Crowley, 1990). Moreover, a large body of evidence suggests that the cardiovascular and renal deficits in Dahl salt sensitive rats are caused by a deficiency in nitric oxide-mediated vascular relaxation, particularly in the kidneys (Morgan, 1990). Salt is considered to be one of environmental triggers for human hypertension (Sheila, Doggrell and Brown, 1998). However, the appropriateness of this model may be questioned as the parallel group of humans who are as exquisitively salt-sensitive as the Dahl salt-sensitive rat may be a subset of African-Americans with inherited hypertension, salt sensitivity and a predisposition to kidney damage (Sanders, 1996) rather than a significant proportion of hypertensive humans. The symptoms of this model do, however, have many characteristics in common with the human disease (Sheila, Doggrell and Brown, 1998). Other advantages are that it is easy, non-invasive, relatively quick and consistent. This model is potentially useful in studies of the role of the L-arginine/ NO pathway as a mechanism by which a well-compensated hypertrophied heart eventually decompensates (Sheila, Doggrell and Brown, 1998).

1.7.2.5   Transgenic rats

Transgenic techniques are now increasingly used since they offer the possibility of analyzing responses by selected genes (Paul, Wagner, Hoffman, Urata and Ganten,

1994). The most commonly used species for transgenic experiments are mice (Sheila, Doggrell and Brown, 1998). However, their small size limits their usefulness in cardiovascular research as few techniques are available for functional studies. Since the renin–angiotensin system plays an important role in controlling the cardiovascular system, the murine Ren-2 gene was chosen to generate transgenic rats [TGR (mRen-2d) 27 rat] (Lee, Bohm, Kim, Bachmann, Bachmann, Bader and Ganten, 1995). The transgenic rat was produced by introduction of the murine Ren- 2 gene into the rat genome (Muller, Hilgers and Bohlender, 1995). The TGR (mRen-2d) 27 rat represents a well documented genetic model of hypertension. It provides a monogenic model of hypertension to study the role of the local RAS specifically in cardiovascular remodeling (Lee, *et al.,* 1995). Therefore, the major advantage of this model lies in the monogenic pathogenesis of hypertension, which allows investigation of precise phenotypic changes in various organs caused by a genetic perturbation of the RAS system. However, this model cannot be considered as genuine model of polygenic human hypertension, since this transgenic rat line is a model of hypertension with a precisely defined monogenetic defect (Lee *et al.,* 1995). Moreover, this rat model is characterized by an activation of tissue RAS and a depressed plasma and kidney renin activity (Muller, Hilgers and Bohlender, 1995), yet human hypertension is usually associated with normal or high plasma renin concentrations (Sheila, Doggrell and Brown, 1998). However, this model may provide a better understanding of the role of local renin-angiotensin systems in cardiovascular disease (Ohta, Kim, Wanibuchi, Ganten and Iwao, 1996). Transgenic rats expressing the human angiotensinogen gene have been used to test the functional importance of the local human renin- angiotensin system (Muller, Hilgers and Bohlender,

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1995), suggesting that they are becoming an increasingly important tool in understanding hypertension.

1.7.3   Pharmacologically-induced   hypertension   (mineralocorticoid hypertension)

The deoxycorticosterone acetate (DOCA)-salt-induced model of hypertension is a typical representative of pharmacologically-induced hypertension. Weekly subcutaneous injections of deoxycorticosterone acetate (30 mg kg^{-1} twice a week and salt loading as 1% NaCl in the drinking water is required to induce hypertension in rats (de Champlain, Krakoff and Axelrod, 1967; Schenk and McNeil, 1992). Saline is an important co-factor because it expedites the development of hypertension and makes it more severe (see 1.1). Nephrectomised rats given deoxycorticosterone or NaCl alone do not show any major changes in blood pressure, and it is only the combination of deoxycorticosterone and NaCl that produces a major increase in blood pressure with increases in cardiac and renal weight (Schenk and McNeil, 1992). Hypertension develops more quickly and becomes more severe in male than female DOCA–salt rats (Crofton and Share, 1997), suggesting the role of exogenous sex hormones, such as estrogen and progesterone. Whereas the direct cardiovascular effects of estrogen are poorly understood, its effects are believed to be mediated, at least in part, through the ability of this hormone to increase nitric oxide synthesis (Hishikawa, Nakaki, Marumo, Suzuki, Kato and Saruta, 1995). Nitric oxide plays a role in the endothelium-dependent vasodilation, thereby reducing the total peripheral resistance and hence, blood pressure. The combination of DOCA-salt and

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unilateral nephrectomy results in hypertension, cardiac and renal hypertrophy, and nephrosclerosis (Sellye, 1942). DOCA-salt hypertension is a low renin and volume overloaded form of hypertension (Crofton, and Share, 1997). There is evidence that arginine vasopressin (AVP) plays a role in both the development and maintenance of DOCA-salt hypertension (Sellye, 1942; Crofton and Share, 1978). The major limitations of the DOCA-salt model are: (1) the pharmacological large doses of drug required; (2) requirement for surgical reduction of renal mass; and (3) ingestion of a large amount of NaCl required (Sheila, Doggrell and Brown, 1998). Furthermore, hypersecretion of deoxycorticosterone is rarely observed in humans and is a result of a genetic defect (Crofton and Share, 1997). The major advantage of this model is that it allows investigating the role of sodium in the developmental stages of hypertension.

1.7.4 Diabetic hypertensive rats

Diabetes mellitus is an important risk factor in patients with hypertension and heart disease. Furthermore, diabetics have a high incidence of cardiovascular diseases, especially hypertension, atherosclerotic coronary disease, cardiomyopathy and microvascular damage (Kannel, Hjortland and Castelli, 1974 and Hsueh, 1992). Rapid injection of streptozotocin to adult rats produces many of the characteristics cardiovascular and renal pathophysiological features similar to those of humans with uncontrolled insulin-dependent diabetes (National diabetes Data Group, 1979). However, these rats can survive without exogenous insulin for at least 12 weeks and are usually normotensive (van Zwieten, Kam and Pijl, 1996). Streptozotocin-induced diabetics show

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characteristics similar to those of hypertensive-insulin-dependent diabetic humans (National diabetes Data Group, 1979). These rats show progressive cardiac deterioration (van Zwieten, Kam and Pijl, 1996) and have been used to measure responses of antihypertensive drugs on hypertrophy and vascular permeability (Hulthen, Cao, Rumble, Cooper and Johnston, 1996). However, this model may not be relevant since it is an insulin-dependent model, yet 85% of diabetics are non-insulin-dependent with many of these patients being hypertensive and obese (van Zwieten, Kam and Pijl, 1996). Therefore, models of non-insulin-dependent diabetic hypertension may be more relevant. The genetically- determined obese Zucker rat fulfils the criteria as a relevant model of non-insulin-dependent diabetes (van Zwieten, Kam and Pijl, 1996). The model exhibits moderately elevated blood pressure, progressive kidney damage and hypercholesterolaemia (Math, 1995). A newer genetic model of human non- insulin-dependent diabetes is the Otsuka Long-Evans Tokushima fatty rat which develops mild hypertension with typical cardiac and renal complications such as perivascular fibrosis and glomerulosclerosis (Yagi, Kim, Wanibuchi, Yamashita, Yamamura and Iwao, 1997).

1.7.5 Renal-induced hypertension (renovascular hypertension)

The physiological function of the kidney includes maintenance of electrolyte and fluid balance and secretion of renin, an important component of the RAS. Thus, its involvement in the regulation of blood pressure and its important role in the development of hypertension are well accepted. The first renovascular hypertension was induced by partial constriction of the renal artery of a dog in 1934 (Goldblatt, Lynch, Hanzal and Summerville, 1934). Since then, many renal-induced models of hypertension

(Renovascular hypertension) have been successfully established in rats, rabbits, sheep, and cats (Zandberg, 1984). Generally, renal-induced experimental hypertension includes two-kidney Goldblatt hypertension; where occlusion is done to one renal artery by a clip while the contralateral kidney is left intact; and one-kidney Goldblatt hypertension, where one renal artery is clipped and the contralateral kidney is removed. These models are volume overload forms of hypertension, as there is stimulation of the renin–angiotensin–aldosterone system in the absence of renal electrolyte and fluid loss. This would be an ideal model for studying the role of volume expansion in the development of hypertension. During the early developmental stage of these two renal-dependent models, when the clip is removed, arterial blood pressure returns to normal (Liard, Cowley, McCaan, McCaan and Guyton, 1974; Ten Berg, Lenen and De Jong, 1979). Thus, renal-induced hypertension is reversible and reproducible. Furthermore, these models provide a unique opportunity to investigate the changes which occur specifically at the level of the kidney, as well as the role of the kidney in the long-term blood pressure control. If the reversal of hypertension is time-dependent, it would suggest that relevant changes, perhaps structural, have developed.

1.7.6   Pulmonary hypertension

Pulmonary hypertension is uncommon disorder characterized by elevation of pulmonary vascular resistance and progressive right ventricular failure (Grossman, 1992). Pulmonary hypertension (PH) was historically classified in two categories based on its mode of occurrences: primary pulmonary hypertension, a rare disorder without a known

underlying cause, or pulmonary hypertension secondary to a variety of chronic lung and heart diseases (Mannino, 2002). The latter exhibits a much larger incidence and considerable morbidity and mortality (Grossman, 1992). The most common causes of secondary pulmonary hypertension is obstructive pulmonary disease, left ventricular systolic failure, pulmonary thromboembolism and hypoventilation (Mannino, 2002 and Grossman, 1992). Pulmonary hypertension is associated with functional and vascular changes that are modulated by endothelium-derived vasodilators and vasoconstrictors (Pietra, 2004). Pulmonary endothelium has an important modulatory role in maintaining a low basal pulmonary vascular tone by releasing a balanced amount of vasodilators, (including nitric oxide (NO) and prostacyclin) and vasoconstrictors (including endothelin-1 [ET-1]). Under physiological conditions, a precise and balanced release of vasodilators and vasoconstrictors contribute to appropriate organ perfusion. However, this balance is altered in disease states such as pulmonary hypertension (Chantal, 1999). In patients with pulmonary hypertension an imbalance in favour of vasoconstrictor release is observed (Chantal, 1999). The cause of endothelial dysfunction associated with pulmonary hypertension is not always clear although there is a hereditary component in patients with a familial history of primary pulmonary hypertension (Lane, 2000). In addition, many of the vasoconstrictors, including ET-1, also have a growth promoting effect on smooth muscle cells, ultimately leading to structural changes with increased muscularization and resistance to flow. Moreover, reduced production of prostacyclin and NO contributes to increased thrombogenicity (Pietra, 2004).

1.7.7   Portal hypertension

Portal hypertension is the most common complication of chronic liver diseases. This syndrome, defined by a pathological increase in the portal venous pressure, is characterized by an increase in the pressure gradient between portal vein and inferior vena cava, which is in normal condition within 1–5 mmHg (Ratti, Pozzi and Bosch, 2005). However, portal hypertension begins to manifest its complications when the portal pressure rises above the threshold value of 10–12 mmHg (Garcia-Tsao, Groszmann, Fisher, Conn, Atterbury and Glickman, 1985). Oesophageal variceal hemorrhage is a major complication of portal hypertension, therefore pharmacological treatment of portal hypertension aims to treat acute bleeding episodes or prevent variceal bleeding (Navasa, Bosch, Rodes, 1991). The two widely used vasoconstrictors in the treatment of acute variceal bleeding are vasopressin and somatostatin while propranolol is used as a prophylactic drug for prevention of variceal bleeding (Navasa, Bosch and Rodes., 1991). Because of the combined impact of these complications, portal hypertension represents the main cause of death in patients with cirrhosis (Ratti, Pozzi and Bosch, 2005).

The portal pressure gradient is the result of the interaction between portal blood flow and the vascular resistance that opposes the flow. Thus, portal pressure gradient can originate from an increase in intrahepatic vascular resistance, an increase in hepatic blood flow or from a combination of both. For many years, the increase in vascular resistance in portal venous system was thought to be determined by the disruption of liver architecture due to the progressive deposition of collagen in the Disse's space, scarring and nodule formation

(Ratti, Pozzi and Bosch, 2005). However, recent studies have demonstrated that vasoactive mediators may modulate intrahepatic vascular resistance. Thus, insufficient release of hepatic vasodilators, overproduction of vasoconstrictors, and a hyporeactivity to vasodilators are all responsible for the dynamic component of the increased intrahepatic resistance in liver cirrhosis (Groszmann and Abraldes, 2005; Garcia-Pagan and Bosch, 2004). An increased portal blood flow is generally observed in advanced stages of portal hypertension, and it is the result of an excessive arteriolar vasodilation in splanchnic organs draining in the portal venous system (Vorobioff, Bredfeldt and Groszmann, 1984).

The possibility of the pharmacological manipulation of the increased intrahepatic resistance of cirrhotic livers has challenged the paradigm that it was only possible to lower portal pressure by reducing the increased splanchnic blood flow with splanchnic vasoconstrictors (Garcia-Pagan and Bosch, 2004). However, reducing hepatic vascular resistance represents a novel strategy to treat portal hypertension, with the advantage of avoiding a further decrease in liver blood flow, but of improving liver perfusion (Loureiro-Silva, Cadelina, Iwakiri and Groszmann, 2003). Indeed, it has been demonstrated that increasing nitric oxide within the liver or reducing or blocking the effect of different vasoconstrictors can modulate hepatic vascular tone in cirrhotic livers (Garcia-Pagan and Bosch, 2004).

There are mainly two types of pharmacological approaches towards the treatment of portal hypertension: by reducing portal blood flow or portal vascular resistance. The

former is the basis for use of vasoconstrictors, the latter, vasodilators (Navasa, Bosch and Rodes, 1991). However, current therapeutic drugs for portal hypertension are quite limited due to their side effects or low efficacy. In this context, it is well justified to search for some potential alternatives in the treatment of portal hypertension.

1.7.8 Insulin resistance and hypertension.

Hypertension is approximately twice as common in diabetic subjects as in the general population (Tauscher, Egger and Herman, 1989). The frequent association of impaired glucose tolerance, diabetes mellitus, obesity and hypertension was first reported in 1929 (Major, 1929). The presence of insulin resistance with compensatory hyperinsulinemia is considered as the common underlying metabolic disorder that links these conditions.

Studies have also documented an independent association of hypertension with insulin resistance (Ferrannini, Buzzigoli, Bonadonna and Giorico, 1987). These authors showed a severe impairment of insulin-mediated glucose uptake in a group of lean hypertensive subjects with normal glucose tolerance. Since this first report, the relationship between hypertension and insulin resistance in human essential hypertension has stimulated great interest and debate (Rossetti and Frontoni, 1993).

Insulin resistance with compensatory hyperinsulinemia is commonly described in non obese patients with essential hypertension (Major, 1929; Bonora, Zavaroni, Alpi, Pezzarossa, Bruschi and Dall'Aglio, 1987). However, the relationship between

hypertension and insulin resistance is still not completely understood, since the impact of high blood pressure per se on insulin-mediated glucose metabolism is not easily distinguishable.

The past few years, animal models of genetic and acquired hypertension have been studied in order to address this question. One would envisage an impaired insulin-mediated glucose metabolism in animal models of hypertension if the decreased insulin sensitivity described in hypertensive individuals is the consequence of metabolic or hemodynamic alterations due to elevated blood pressure. Indeed, several researchers have reported impairment in insulin-mediated glucose metabolism in genetic animal models of hypertension. For example, Mondon and Reaven, (1988) reported impairment in insulin-mediated glucose metabolism in genetic animal model of hypertension, demonstrating a decreased whole body insulin clearance and insulin mediated glucose metabolism. In a separate study, Somova, Channa and Khan, (1999), demonstrated an impairment in insulin mediated glucose metabolism in Dahl salt sensitive rats, a genetically non-diabetic and non-obese insulin resistant rat model of hypertension.

Among the various mechanisms postulated to link insulin resistance and hyperinsulinemia to hypertension, sympathetic activation seems, to be the most important. This is suggested by the finding that insulin infusion in most of the clinical conditions, like in the case of obesity-related hypertension, increases muscle nerve sympathetic activity in healthy subjects (Berne, Fagius, Pollare, Hjelmdahl. 1992) and in borderline hypertensive patients (Anderson, Balon, Hoffmann, Sinkey and Mark, 1992).

Additionally, both insulin resistance and hypertension have been associated with impaired endothelial function and insulin-mediated vasodilation (Panza, Quyyumi, Brush and Epstein, 1990 and Petrie, Ueda, Webb, Elliott and Connell, 1996). A large body of evidence indicates that insulin causes limb vasodilatation, decreases adrenergic-mediated vasoconstriction, and potentiates acetylcholine-mediated vasodilatation (Baron, 1996). All of these actions appear to protect against the development of arterial hypertension. It could be anticipated, therefore, that if the vasculature is resistant to insulin vasomodulation, just as skeletal muscle is resistant to insulin's metabolic action; this same defect, insulin resistance might be responsible for the development or maintenance of arterial hypertension. Alternatively, insulin could enhance vascular responsiveness and sustain arterial hypertension directly by activating the sympathetic nervous system, which acts on the vasculature, heart, and kidneys (Anderson *et al.*, 1992). Importantly, because insulin is a direct vasodilator, activation of other physiological mechanisms is probably required if insulin is to have a causal role in the pathogenesis of hypertension. Therefore, interventions to improve insulin sensitivity and hypertension should be initiated early.

1.8   Incidence and prevalence of hypertension in developing nations

It is predicted that almost three-quarters of the world-wide population with hypertension will be in developing countries by the year 2025 (Kearney *et al.*, 2005), with this occurrence fuelled by urbanization. Urbanization in Africa has played a significant role in modification of lifestyle patterns. For example, with urbanization, black South Africans

undergo a nutritional transition from traditional, rural, carbohydrate food with a low glycaemic index to a diet high in fat and poor-quality carbohydrate fast foods (Bourne, Lambert and Steyn, 2002). This has resulted in 58.5% of South African black women becoming over weight or obese, with some women's shapes augmented by the culturally desirable value of obesity (Bourne, Lambert and Steyn, 2002).

Prevalence of hypertension in developing nations has been difficult to quantify possibly because the majority of the developing countries do not have national estimates of prevalence of hypertension (Ibrahim, 1995). A World Health Organisation analysis showed that the prevalence of hypertension in developing countries varied from 1% in some African countries to more than 30% in Brazil (Nissinen, Bothig, Granroth and Lopez, 1988). The prevalence of hypertension in black people in the West Indies and United States of America has been found to be higher than those in any part of sub-Saharan Africa (Seedat, 2000).

Epidemiological studies in Egypt indicate that hypertension is extremely common among Egyptians, and it is believed that this situation exists in many other developing countries (CAPMSA, 1990). Cardiovascular diseases in Egypt are now the main cause of death being responsible for 42.5% of all deaths, while 20 years earlier they accounted for only 12.4% of mortality (CAPMSA, 1990). Comparing the prevalence of hypertension in three countries, China, Egypt and United States of America, it has been shown that Egypt has the highest prevalence rate of hypertension according to the Egyptian National Hypertension Project data (Ibrahim, 1995). Mortality statistics have

shown a downward trend in mortality from hypertension and cardiovascular diseases in most developing countries (Seedat, 2000). According to the recent editorial on heart diseases in Africa it is concluded that coronary heart diseases account for a small but progressively growing proportion of heart diseases (Walker and Sareli, 1997). However, the low trend in the prevalence of hypertension and cardiovascular disease will probably change due to urbanization and acculturation just as was the case with black Americans and in western populations (Morris, 1951; Perkoff and Strand, 1973; Seedat, 1983; Kromhout, 1996 and Gandhi, 1997).

The prevalence of hypertension among South African Blacks is higher (34% among women and 27% among men) and as is the case with all urban populations, it rises with age (Edwards, 1995). Furthermore, in a study conducted in the adult population of Durban in South Africa, hypertension was demonstrated to be highest in urban Zulus (25%), intermediate in whites (17.2%) and lowest in ethnically Indian people (14.2%) (Seedat, 1983). Several theories have attributed the cause of this high prevalence to diet, change of exercise patterns, breakdown of culture, and stressful aspects of urban life (Edwards, 1995). Reviews have indicated that in South Africa just as it is the case with other countries, the prevalence in the rural communities is much lower than in the cities (Seedat, 1983; Fraser, 1986). For instance, it was shown to be 4.1% in Ghana (Pobee, 1977), 5.9% in Nigeria (Oviasu, 1978), 7% in Lesotho (Mokhobo, 1976) and 7.37% in the rural Zulu (Seedat, 1983). It is suggested that exposure to stressors, such as natural disasters, industrial noise, overcrowding, unemployment and environments posing constant threats are among other factors that have been linked to the development of

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hypertension (Edwards, 1995). There are a number of factors that could be attributed to the projected rise in persistent hypertension and cardiovascular disease mortality rate in future like adverse life style changes that are accompanying industrialization and urbanization, salt consumption in Africa is increasing, (Whelton, He, Appel, 2002). Cigarette smoking has increased in Africa as tobacco companies lose their holds in the developed world, they are turning to developing nations as their target markets, alcohol consumption is on the increase in Africa, physical inactivity and many more (Diabetes Prevention Program Research Group, 2002).

Additionally, due to economic constraints facing developing countries, diseases such as HIV/AIDS along with the ravages of persistent infectious diseases, famine, drought and civil strife, take priorities for restricted health budgets over apparently non-urgent health priorities such as hypertension. Thus, these factors will continue to dominate over hypertension and, therefore, its prevalence and incidence will continue to increase in developing countries. Under this situation, global approaches focusing on lifestyle changes should be initiated as preventive measures, whereas approaches for individuals should focus on extensive search for cheaper and easily accessible antihypertensive therapy such as utilization of medicinal plants traditionally used as antihypertensives.

1.9 Evaluation and diagnosis of hypertension

It is recommended that accurate measurements of blood pressure and verification of elevated blood pressure be made on multiple occasions over time (Phyllis, 2003). White

coat hypertension present in 20% of patients with elevated blood pressure is said to be associated with a low cardiovascular risk (Phyllis, 2003). However, studies indicate that it may be a precursor of sustained hypertension and therefore warrants monitoring (Phyllis, 2003). When diagnosing and evaluating a person for hypertension, apart from history taking and physical examination, it is required that several tests that include urinalysis, complete blood count, blood chemical tests (i.e., potassium, sodium, creatine, fasting glucose, total cholesterol and high density and low density lipoprotein) and a 12 lead electrocardiography are routinely conducted (Phyllis, 2003). The evaluation should identify signs of cardiovascular, cerebrovascular or peripheral vascular diseases and other risk factors that are frequently present in patients with hypertension (Materson, Reda and Cushman, 1994). It is also recommended to investigate further, severe hypertension or clinical or laboratory results that could suggest the possibility of renal disease, adrenal hypertension or renovascular hypertension (Phyllis, 2003). According to the Joint National Committee on Prevention, Detection, Evaluation and Treatment of Blood Pressure, blood pressure is classified as in table below:

1.9.1   Classification of blood pressure in adults

Category	Blood pressure (mmHg)
Optimal	<120/80
Normal	<130/<85
High normal	130-139 systolic pressure or 85-89 diastolic pressure
Hypertension	
Stage 1 (mild)	140-159 systolic pressure or 90-99 diastolic pressure
Stage 2 (moderate)	160-179 systolic pressure or100-109 diastolic pressure
Stage 3 (severe)	≥180 systolic pressure or ≥110 diastolic pressure
Stage 4 (malignant)	≥210 systolic pressure or ≥ 120 diastolic
Isolated systolic hypertension	≥140 systolic pressure or <90 diastolic

Adapted from Staessen, Wang, Bianchi and Birkenhager, (2003).

1.10 Blood pressure measurement in experimental animals.

Animal models of hypertension play an essential role in exploring the mechanisms of blood pressure control and the actions of the cardiovascular drugs. Arterial blood pressure is often measured to assess the response of the cardiovascular system to treatment (e.g., to drug or stress) or as an endpoint (e.g., studies of hypertension). The manner in which blood pressure is measured varies from one laboratory to another, and the specific values obtained, their reliability and interpretation, are strongly influenced by the method selected. The measurement of blood pressure in conscious rats has been restricted to two methods: The direct and indirect measurements.

1.10.1 Indirect methods.

Indirect methods refer to the noninvasive methods of blood pressure measurement. The most common indirect method used in rodent studies has been the use of a tail-cuff device which comes in combination with blood flow sensor. There are a number of tail-cuff devices available, including one intended for use in small animals, such as rats (e.g., IITC Life Sciences 31, USA). Indirect methods have some advantages in that they are less demanding technically and they are suitable for chronic studies since serious risks to the animal health are minimal. However, the indirect methods suffer from the limitations of being indirect, discontinuous, and requiring restraint of animals. Additionally, in most cases, some degree of heating of the animal is usually used to ensure sufficient tail blood flow for easy measurement to be made (Bunag, 1991). The issue of restraint stress, heat

stress, and environmental influences on physiological parameters in the most commonly used rat models of hypertensions has been raised (Sponer, Muller-Beckmann and Martin, 1973). It has been suggested that, even when minimal external warming is used, the combination of restraint and warming may lead to significant increase in the core body temperature (Sponer, Muller-Beckmann and Martin, 1973). Since both restraint and warming constitutes stresses that may affect blood pressure, the values of blood pressure obtained with tail-cuff method may, not only reflect the animals' general blood pressure levels but also the reactivity of blood pressure to the stress of the procedure. Because of these limitations, it is often recommended that results obtained by tail-cuff method be verified by direct blood pressure measurement (Bunag and Butterfield, 1982).

1.10.2 Direct methods.

Direct methods refer to techniques by which arterial blood pressure is measured directly with the aid of a sensor device (catheter) implanted invasively within a suitable artery. The sensor is connected to a calibrated pressure transducer for blood pressure measurement (Sponer, Muller-Beckmann and Martin, 1973). This is the most accurate method of blood pressure measurement.

The major advantages of this method are firstly, that the materials needed for the procedure are inexpensive. Secondly, the precise calibration is easier and the whole procedure can be performed under normal physiological conditions without involving the

warming, restraining of the animals and this allows continuous long-term blood pressure recordings under conditions of relatively low stress.

Disadvantages of the direct methods include: (1) the disturbance to the animal, its blood pressure and heart rate, caused by catheter implantation surgery and anaesthesia; (2) the method from the technical point of view is critical, such that if not properly done, it creates the potential for infection, and the potential for loss of catheter patency (Sponer, Muller-Beckmann and Martin, 1973). This may lead to a degradation or loss of blood pressure signal and limited dynamic response, which makes detection of the true systolic and diastolic pressures challenging in small animals with high heart rates (Sponer, Muller-Beckmann and Martin, 1973).

1.11 Management and treatment of hypertension

The primary goal of the treatment or management of hypertension is to prevent cardiovascular diseases and death (Phyllis, 2003). Successful treatment of hypertension has been associated with reductions in the risk of cardiovascular mortality and morbidity, cardiovascular events, left ventricular hypertrophy, stroke, myocardial infarction, alzheimer's, dementia, renal complications, deterioration of renal function, renal failure and other complications (Guidelines Part I: Detection and Diagnosis of hypertension, 2003 and Collins, 1990). Hypertension can be managed, controlled and treated either by using the available conventional antihypertensive drugs or through non-pharmacological approach (e.g., lifestyle modification).

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1.11.1 Treatment of hypertension using drugs.

Available antihypertensive drugs classified according to their principle regulatory site or mechanism (s) on which they act are described below.

1.11.1.1 Calcium channel blockers

Calcium channel blockers preferentially bind to the L-type high voltage calcium channels and this inhibits influx of calcium ions across the cell membrane (Bertram, 2004). Blockade of calcium channels in vascular endothelial cells inhibits vasoconstriction and enhances vasodilation. This results in reduced total peripheral vascular resistance and subsequent blood pressure. On the other hand, blockade of calcium channels in myocardial cells, results in inhibition of force and rate of myocardial contractions. The result is a decrease in cardiac output leading to a decrease in blood pressure (Bertram, 2004). Dihydropyridines, class of calcium channel blockers are more selective as vasodilators. Drugs in this class such as nifedipine exert their effects more on the blood vessels and have less cardiac depressant effect (Bertram, 2004). Their common side effects include headache, excessive hypotension, oedema and reflex tachycardia (Bertram, 2004). Non-dihydropyridines such as verapamil have greater cardiodepressant effect and may decrease heart rate and cardiac output. These cardiac selective non-dihydropyridine calcium channel blockers can result in excessive bradycardia and impaired electrical conduction (Bertram, 2004).

1.11.1.2   Beta-adrenergic blockers

The beta adrenergic blockers appear to lower blood pressure and provide target organ protection by several different mechanisms. Beta-adrenergic blockers inhibit the renin-angiotensin system by decreasing renin release by the juxtaglomerular cells of the kidney (Lopez-Sendon, Swedberg, Mcmurray, Tamargo, maggion, Dargie, Tendera, Waagstein, Kjekshus, Lechat and Torp-Pedersen, 2004). These drugs also inhibit the sympathetic nervous system outflow, and decreasing force and rate of cardiac contractility with a decrease in cardiac output (Lopez-Sendon *et al.*, 2004). Thus, the drugs act by reducing adrenergic nerve stimulation, the excitatory nerve stimulation that causes vasoconstriction, tachycardia and positive inotropy, thereby resulting in reduced vascular resistance and cardiac output.

The beta blockers are classified into three generations of agents; the first generation ß-blockers, the second generation ß-blockers and the third generation ß-blockers (Michael, 2005). The first generation ß-blockers are also known as non-selective ß-blockers. These ß-blockers exert equal blockade of $ß_1$ and $ß_2$ adrenergic receptors. A typical example in this class is propranolol (Michael, 2005). The second generation agents are called selective ß-blockers, since they exhibit higher binding affinity to $ß_1$ than to $ß_2$ receptors. These drugs include metoprolol and atenolol (Brixius, Bundkirchen, Bolck, Mehlhorn and Schwinger, 2001). The third generation ß-blockers appear to have a high binding affinity for $α_1$-adrenergic receptors and exhibit vasodilating activity through their blockade of $α_1$-adrenergic receptors. A good example is labetalol. Nebivolol, in contrast,

is a third generation ß-blocker that has a higher affinity for $ß_1$ and provides endothelium-dependent vasodilation associated with activation of the L-arginine/nitric oxide pathway (Bowman, Chen and Ford, 1994; Ritter, 2001). Despite the protective potential offered by these drugs, concerns regarding their safety and tolerability, including potential adverse metabolic effects limit their use in clinical practice (Michael, 2005).

1.11.1.3   Angiotensin-converting enzyme inhibitors (ACE-inhibitors)

These drugs lower blood pressure by reducing plasma concentrations of angiotensin II and aldosterone by blocking ACE-effects of converting angiotensin I to angiotensin II, thereby, disrupting the renin-angiotensin-aldosterone system (Collins, 1990). They also block the break down of bradykinin, a vasodilator substance (Bertram, 2004).,By blocking the break down of bradykinin, ACE- inhibitors increase the bradykinin levels, which contribute to vasodilation. Therefore, ACE- inhibitors lower blood pressure by: dilating arteries and veins by blocking angiotensin II formation and inhibiting bradykinin metabolism. They also lower blood pressure by down regulating sympathetic adrenergic activity and by promoting renal excretion of sodium and water by blocking the effects of angiotensin II in the kidney and by blocking angiotensin II stimulation of aldosterone sectretion. Captopril and enalapril are examples of antihypertensive drugs in this class (Bertram, 2004).

The common annoying side effect of angiotensin converting enzyme inhibitors is dry cough which is attributed to the effect of bradykinin (Bertram, 2004). Hypotension can also be a problem especially in heart failure patients (Bertram, 2004).

1.11.1.4   Angiotensin II Receptor Blockers (ARBs).

Angiotensin receptor blockers (ARBs) are receptor antagonists that block the type 1 angiotensin II receptors (AT1) on blood vessels (Dzau, 1993). They block the effects of angiotensin II thereby preventing angiotensin II induced vasoconstriction. Therefore, these drugs appear to lower blood pressure by lowering the total peripheral vascular resistance. These drugs also decrease aldosterone synthesis and therefore, sodium excretion is increased. ARBs are appearing as the most effective antihypertensive therapy, which interferes with the renin-angiotensin system compared with ACE-inhibitors. This is because, in spite of their therapeutic efficiency, ACE- inhibitors are not highly effective in lowering angiotensin II levels, since other enzymes, such as chymase, are able to synthesize angiotensin II (Reilly, Tewksbury, Schechter and Travis, 1982). In fact, chymase-dependent angiotensin II production is up regulated in human diabetic kidney and diseased blood vessels (Doggrell and Wanstall, 2004). Therefore, the most logical and effective way of inhibiting the effects of angiotensin II is by blocking its targeted receptors.

1.11.1.5   Diuretics.

As expected, in most forms of hypertension, the hypertensive state is maintained by an elevation in blood volume, which in turn increases the cardiac output. The sodium retention resulting from increased RAAS activity is associated with increased extracellular fluid volume. Diuretic drugs appear to lower blood pressure by increasing renal sodium and water loss thereby, decreasing extracellular fluid volume, blood volume and cardiac output (Bertram, 1990). Thiazide diuretics are more appropriate for most patients with mild or moderate hypertension. The primary site of action of thiazide diuretics is on the distal tubules where they produce increased urinary excretion of sodium and potassium (Bertram, 2004). More powerful diuretics such as furosemide are more appropriate in severe hypertension. These diuretics act on the loop of Henle where they promote sodium, potassium and water excretion. The common side effects of thiazides and furosemide include hypokalaemia, hyperuricaemia, hyperglycaemia, hypovolaemia and glycosuria (Parry, 1984).

Potassium sparing diuretics are useful to prevent excessive potassium depletion and to enhance the natriuretic effects of other diuretics (Bertram, 2004). However, these diuretics are said to be less potent when they are used alone, but become more useful when they are used in combination with other diuretics. A good example in this class of diuretics is spironolactone, an aldosterone antagonistic agent. This drug antagonizes the effect of aldosterone on the distal tubule thereby, increasing sodium excretion and promoting the retention of potassium (Parry, 1984).

1.12   Medicinal plants.

Economic problems facing the developing populations and poor health services resulted in the search for cheap and easily accessible alternative therapies from natural sources for many human ailments including hypertension. The most important cheap source of treatment is medicinal plants. Among all known natural drugs, those originating from plant tissues have been celebrated since antiquity as apparently limitless source of novel drugs (De Pasquale, 1984). A main fraction of population in developing countries remains dependant on ancestral plant knowledge for health care (Balick, 1990). This ratio keeps increasing with the state of poverty of these countries. In addition, WHO encourages the inclusion of medicinal plants in programmes of developing countries because of the great potential these plants represent in combating various diseases (WHO, 1992). It is estimated that more than 80% of the population in developing countries depend on plants for their medical needs (Balick, 1990).

According to the Herbal Medicinal Industry, a medicinal plant is any plant used in order to alter physiological and pathological process. A phytopharmaceutical preparation or herbal medicine is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins, oils and fruits) (Arias, 1999).

Plants can be used as therapeutical resources in different ways (Rates, 2000). They can be used as herbal teas, or as other home remedies, when they are considered as medicinal plants. They can be used as crude extracts or "standard enriched fractions" in

pharmaceutical preparations such as tinctures, fluid extracts, powder, pills and capsules, when they are considered as phytopharmaceutical preparations or herbal medicines. Plants can be subjected to successive extraction and purification procedures to isolate the compounds of interest which can be active and used directly as a drug (Rates, 2000). Several plant extracts with potential therapeutic properties for treatment of hypertension and its associated complications, such as coronary heart diseases, angina, arrhythmias and congestive heart failure have been identified (Miller, 1998; Gelfand, Mavi, Ndemera and Drummon, 1985; Somova, Nadar, Rammanan and Shode, 2003; Osim, Mbajiorgu, Mukarati, Makufa, Munjeri and Musabayane, 1996). Thus, the economic view of health services and the recognition that the research on medicinal plants used in folk medicine represent a suitable approach for the development of new drugs (Elisabetsky and Posey, 1986), have led to an increased interest in the search for plants with hypotensive properties. The following section describes some of the medicinal plants that have been studied and demonstrated hypotensive properties.

1.12.1 *Dorstenia scabra* [Moraceae]

Dorstenia scabra also known as *Dorstenia psilurus*, is a small herb occurring in Central Africa and reaching 80 cm in height and 7 cm in girth (Hutchings, 1989). The plant usually grows on the borders of rivers and lakes. The leaf extract of *Dorstenia scabra* is widely used in ethnomedical practice in the grassland region of Cameroon for the treatment of arthralgia and cardiovascular disorders, mainly hypertension (Dimo, Rakotonirina, Tan, Dongo, Dongmo, Kamtchouing, Azay, Abegaz, Cros, and Ngadjui,

2001). A decoction of the leaves and roots is used to treat rheumatism, snakebites, and headache and stomach disorders (Ngadjui, Dongo, Happi, Bezabih, and Abegaz, 1998). Elsewhere, the plant extract is used as a diuretic, tonic, stimulant and analgesic (Ruppelt, Pereira, Goncalves and Pereira, 1991). The chemical constituents of *D.scabra* include phenolic compounds (6,8-diphenyl-3'[O], 4'-(2,2-dimethylpyrano)-3,5,7-trihydroflavone,3,6-di-phenyl-(2-hydroxy-3-methylbut-3-enyl)-5,7,2',4,-tetrahydroxyflavone, as well as the common sterol 2-sitosterol (Ngadjui *et al.,* 1998). Recently, methanolic extract of this plant has been shown to exhibit hypotensive effects in fructose-induced hypertensive rats, an insulin resistant model of hypertension (Dimo, *et al.,* 2001).

1.12.2   *Ajuga iva* L. [Labiatae]

Ajuga iva is one of the most commonly used medicinal plant in Morocco (Bellakhdar, Claisse, Fleurentin, Younos, 1991). Various parts of this plant have been used for a variety of human ailments such as hypertension, diabetes mellitus, kidney disorders fever, toothache and dysentery (Ziyyat, Legssyer, Mekhfi, Dassouli, Serhrouchni, Benjelloun, 1997). Furthermore, it has been reported that some species of the genus Ajuga are especially used as diuretic agents (Aliotta and Pollio, 1994) and as a remedy for hypertension (Kokwaro, 1976). Recently, studies have reported the hypoglycaemic effect of the aqueous extract of this plant in streptozotocin-induced diabetic rats (Hilaly and Lyoussi, 2002). In trying to establish the scientific basis for the traditional use of this plant as an anti-hypertensive, Hilaly, Lyoussi, Wibo and Morel (2004) demonstrated the

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hypotensive and vasorelaxant effects of aqueous extracts of this plant *in vivo* and *in vitro*, respectively in experimental animal paradigms. Furthermore, compounds isolated from the genus Ajuga, are reported to exert cardiotonic activities (Kuria and Muriuki, 1984),

1.12.3   *Coscinium fenestratum* Colebr. [Menispermaceae]

Coscinium fenestratum, is a woody climber found in South East Asia. The plant is widely used in the region for medicinal purposes (Siwon, Verpoorte, Van Essen and Svendsen, 1980). Various parts of this plant have been used for a variety of human ailments such as fever, muscle pain, stomach pain, malaria, diarrhoea, ulcers and infection of the eyes (Siwon, *et al.*, 1980). In the North-Eastern part of Thailand, aqueous extracts of the dried wood is widely used for traditional treatment of high blood cholesterol, hyperglycaemia as well as hypertension (Singh, Singh, Bani and Malhotra, 1990). Laboratory studies have reported the hypotensive effects of ethanolic wood extract of the plant in anaesthetized rats, dogs and guinea pigs (Singh *et al.*, 1990). Chemical investigations on the wood of *Coscinium fenestratum* have resulted in isolation of berberine as the main alkaloidal constituent and a smaller amount of protoberberine (Rojsanga, Gritsanapan, Suntornsuk, 2006). Recently, *Coscinium fenestratum* has been reported to possess various pharmacological actions such as antioxidant (Venukumar, and Latha, 2002) antiproliferative (Ueda, Tezuka, Banskota, Le Tran, Tran, Harimaya, Saiki and Kadota, 2002), antidiabetic, antihypertensive (Punitha, Rajendran, Shirwaikar and Shirwaikar, 2005 and antibacterial (Nair, Narasimhan, Shiburaj and Abraham, 2005) activities.

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1.12.4 *Costus afer* Ker. [Zingiberaceae]

Costus afer is a tall perennial herbaceous, unbranched medicinal plant with creeping rhizome. Commonly known as gingerlily, the plant is found in moist or shady forests and riverbanks of tropical West Africa including Nigeria, Ghana and Cameroon (Iwu, 1983). This plant and other species of the genus are used as remedy for cough, inflammation, arthritis, as laxative, and purgative,, diuretics, rheumatism and treatment of several diseases including hypertension (Oliver, 1960). In Nigeria the decoction of the stem or powdered fruits is used as cough medicine. Whole boiled root is applied to cuts and sores, and soothing fomentation for rheumatic pains prepared with boiled leaves (Oliver, 1986; Iwu and Anyanwu 1982). Studies on animals demonstrated that decoctions of the stem ameliorated all signs associated with adjuvant-induced polyarthritis in rats (Awouters, Niemeeger, Lanaert, Jaseen, 1978). In Cameroon and Ivory Coast, the decoction of the plant is given to diabetic patients to alleviate the clinical signs associated with diabetes mellitus (Iwu, 1993). Phytochemical screening of the extract revealed the presence of saponins, cyanogenic glycosides, tannins, flavonoids and carbohydrates (Oliver, 1986).

1.12.5 *Berberis vulgaris* L. var. [Berberidaceae]

Berberis vulgaris is a bush with yellow to brown coloured bark. The plant has obviate leaves, bearing pendulous yellow flowers in spring (Aynehchi, 1986).Various parts of this plant, such as, its root, bark, leaf and fruit have been used as folk medicine for long in Iran (Zargari, 1983). In Iranian traditional medicine the plant is reported to possess

several properties, such as antibacterial, antiarrhythmic, antihypertensive and antipyretic activities (Zargari, 1983; Aynehchi, 1986). Furthermore, the crude extract of this plant has been shown to exert anticholinergic activities (Shamsa, Ahmadiani and Khosrokhavar, 1999). In an attempt to establish the scientific basis for the traditional use of this plant as an anti-hypertensive, Fatehi, Saleh, Hassanabad, Farrokhfal, Jafarzadeh, Davodi, (2005) demonstrated the hypotensive effects of aqueous extracts of this plant in DOCA-salt induced hypertensive rats. In addition, they demonstrated the vasodilatory effect of the aqueous extract of this plant *in vitro*. Pharmacological studies on an alkaloid isolated from this plant, demonstrated that it possesses potent vasodilatory and antiarrhythmic activity, and prolongs the action potential duration in Purkinje fibres and ventricular muscles (Chiou, Yen and Chen, 1991; Ricciopo, 1993 and Kathleen, 2000). There is some evidence that alkaloids isolated from *Berberis vulgaris* have anti-inflammatory and antinociceptive effects (Kupeli, Kosar, Yesilada, Husnu and Baser, 2002).

1.12.6 *Croton cajucara* Benth. [Euphorbiaceae]

Croton cajucara is a large tree that grows abundantly in Amazon region of Brazil and other parts in the Southern region of Africa (Berg, 1982). In Brazil, it is locally called as sacaca and extracts prepared from its stem bark are used in folk medicine to treat hepatic and kidney disorders, obesity, and hypertension (Berg, 1982). Chemical investigations on the bark have led to the isolation of several diterpenes, which include trans-dehydrocrotonin (t-DCTN), trans-crotonin (t-CTN), cis-cajucarin B, trans-cajucarin B,

cajucarin A, cajucarinolide and sacacarin, and a triterpene acetyl aleuritolic acid (Maciel,

Pinto, Brabo and Silva, 1998). Studies carried out with the most active compound, t-

DCTN, revealed its wide pharmacological profile that includes anti-inflammatory,

gastroprotective, hypoglycemic, hypolipidemic, anti-estrogenic, vasorelaxant,

cytotoxicity and anti-tumour actions (Brito, Rodrý´guez, Hiruma-Lima, Haun and Nunes,

1998; Ichihara, Takeya, Hitotsuyanagi and Morita, 1992).

1.12.7   *Cecropia pachystachya* **Mart. [Moraceae]**

Cecropia pachystachya also known as *Cecropia adenopus* is a plant that grows in

paranaense phytogeographical province, in Northeast Argentina, Paraguay and southern

Brazil (Cabrera and Willink, 1980). The plant reaches a height of about 10 m and has

large wide palm-shaped leaves that are dark-green in colour in the upper and silver-grey

below. The plant is widely employed in herbal medicine as a dietary supplement, used for

treating cough and asthma and also as cardiotonic, antihypertensive and diuretic (Gupta,

1995). More recently, an *in vitro* study demonstrated the antioxidant properties of the

extracts of this popular plant (Velazquez, Tournier, Mordujovich de Buschiazzo,

Saavedra and Schinella, 2003). Some laboratory studies have reported the

antihypertensive (Salas, Brenes and Morales, 1987), diuretic (Vargas Howell and Ulate

Montero, 1996), hypoglycemic (Roman-Ramos, Flores-Saenz, Partida-Hernandez, Lara-

Lemus, Alarcon-Aguilar, 1991), analgesic and central depressor effect (Perez-Guerrero,

Herrera, Ortiz, Alvarez de Sotomayor, Fernandez, 2001) of this plant. The

phytochemical analysis of the plant revealed the presence of alkaloids, cardiotonic

glycosides, flavonoids, tannins, triterpenoids and saponin glycosides (Morton, 1981). It is likely that the antihypertensive properties of this plant are attributed to the presence of these compounds.

1.12.8 *Zingiber officinale* Roscoe. [Zingiberaceae]

Zingiber officinale is a large biennial herb that grows abundantly in South Asia. The rhizome of the plant, commonly known as ginger, is widely used for its medicinal properties (Ghayur, Gilani, Maria, Afridi and Houghton, 2005). Ginger is well known all over the world especially for its use in disorders of the gastrointestinal tract such as constipation, dyspepsia, diarrhoea, nausea and vomiting (Tyler, 1993). Ginger is also commonly used by the traditional healers in South Asia for treatment of cardiopathy, high blood pressure, palpitations and to improve the circulation for its use as a vasodilator (Kapoor, 1990; Duke, 2002). Recently, the hypotensive, endothelium-independent vasodilator and cardio-suppressant properties of this plant have been reported (Ghayur and Gilani, 2005). Current observations suggests that the aqueous ginger extract lowers blood pressure through a dual inhibitory effect mediated via stimulation of muscarinic receptors and blockade of Ca^{2+} channels (Ghayur, Gilani, Maria, Afridi and Houghton, 2005). Phytochemical studies have shown that the major active compounds of ginger are the gingerols, shogaols, zingerone, phytosterols and paradol (Langner, Greifenberg and Gruenwald, 1998). 6-gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome (Connell and McLachlan, 1972).

1.12.9   *Eugenia uniflora* L. [Myrtaceae]

Eugenia uniflora is one of the common plant species growing in subtropical North and North Eastern Argentina, Brazil, Uruguay and Paraguay (Rotman, 1995). The plant grows from 3 to10m in height. It bears red fruits which are edible. Its ovate leaves are used in folk medicine for the treatment of hypertension and digestive disorders in Argentina (Amat and Yagia, 1991). In Paraguay, leaves are reported to have diuretic and anti-inflammatory properties, both of which, offer cardioprotection in humans (Amat and Yagia, 1991). Recent laboratory studies in rats reported the hypotensive, vasodilatory and diuretic activities of aqueous leaf extract of this plant (Consolini, Baldini and Anibal, 1999). This provides the pharmacological basis for the empirical use of this plant as antihypertensive.

The phytochemical profile of this plant revealed the presence of phenolic compounds, flavonoids, leucoanthocyanidis, steroids and triterpenoids, essential oils, limonene, cineol, pulegone, camphor and sesquiterpenes (Bandon, Mendiondo, Rondina and Coussio 1972; Retamar, 1982; Adebajo, Oloke and Aladesanmi 1989). Essential oils present in the leaf of the plant have been shown to possess antibacterial and antifungal effects, while flavonoids have antioxidant properties (Consolini, Baldini and Anibal, 1999).

1.12.10 *Urtica dioica* L. [Urticaceae]

Urtica dioica is a perennial herb, which grows up to 1800 m in deserted field (Pignatti, 1982). The stem is erect and green; the leaves are opposite, cordate at the base, oblong or ovate, finely toothed, dark green above and paler beneath. The small, green, dioecious flowers occur as racemes in the axils of the upper leaves. Usually, the plant has either male or female flowers, in separate inflorescences. In many ethnobotanical reports, the aerial parts of *Urtica dioica* are recognized as a natural remedy for hypertension (Ziyyat, Legssyer, Mekhfi, Dassouli, Serhrouchni and Benjelloun, 1997). The aqueous extracts of aerial parts have been reported to exhibit hypotensive and diuretic effects (Tahri, Yamani, Legssyer, Aziz, Mekhfi, Bonouham and Ziyyat, 2000) and prostatic antihyperplasic activity *in vitro* (Lichius and Muth, 1997).

1.12.11 Botany of plants used in the study

The plants used in the present study were chosen based on the following criteria:

i)   information for their use in traditional medicine in the treatment of hypertension and its associated diseases, such as diabetes mellitus;

ii) the presence of natural compounds with structural similarities to the already known bioactive compounds with cardiovascular properties.

1.12.11.1 *Ekebergia capensis* **Sparrm [Maliaceae]**

Ekebergia is a small genus of African trees belonging to the family of the Maliaceae. (Mabina, 1995). This is a fairly large tree, widespread in Eastern Africa from Sudan to the Cape (See Figure 1). *Ekebergia capensis*, or the Cape Ash as it is known locally, is used medicinally and magically by the Zulu people. Decoctions made from the chopped bark are traditionally taken as an emetic for heartburn, for coughs and other respiratory complaints while leaves are used in an infusion as a purgative parasiticide (Hutchings, Scott, Lewis, Cunningham, 1996; Bryant, 1966). The use of the plant in curing tuberculosis-related symptoms such as cough, fever, blood in the sputum, etc. has been reported (Hutchings, Scott, Lewis, Cunningham, 1996). In the KwaZulu-Natal province of South Africa, the roots of *Ekebergia capensis* are used in a decoction to treat gastritis, hyperacidity and coughing (Pujol, 1990). An extract from the bark is used to treat coughs and root extracts are used in the treatment of dysentery (Pooley, 1993). The roots have been used for the treatment of diarrhoea by the Kikuyu tribe (Gachathi, 1989). Extracts from *Ekebergia capensis* have been reported to inhibit drug-resistant and drug sensitive strain of mycobacterium tuberculosis. For instance, at a concentration of 1.0 mg/ml, the plant extract effectively inhibited a drug resistant strain (CCKO28469V) (Lall, and Meyer, 1999). Furthermore, decoctions made from the wood of this plant are used by the local Zulu community to either provide supplements that promote foetal growth or to act as uterotonic agents that induce or facilitate labour (Sewram, Raynor, Mulholland, and Raidoo., 2000).

Previous phytochemical studies have resulted in isolation of several compounds including b-sitosterol ; oleanonic acid ;3-epioleanolic acid; 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetra-cosatetraene and 7-hydroxy-6-methoxycoumarin, 3a-hydroxy-3-deoxyangolensate, limonoids, ekebergin and beta-amyrin (Serge,1998; Nashiyama, Moriyasu, Ichimaru , Tachibana, Kato, Mathenge. 1996). A major class of bioactive compounds present in this plant are triterpenes, which have been reported to exhibit a large variety of cardiovascular actions of medicinal herbs. For example, oleanolic acid, a major triterpene present in this plant (Sewram *et al.*, 2000) has been reported to possess antihypertensive, antidysrhythmic, cardiotonic, diuretic, hypoglycaemic, antihyperlipidemic and antioxidant properties (Somova, Nadar, Rammanan and Shode, 2003 and Somova, Mipando and Shode, 2004). Thus, *Ekebergia capensis* was chosen based on the presence of triterpenes which, have been demonstrated to exhibit cardioprotective actions.
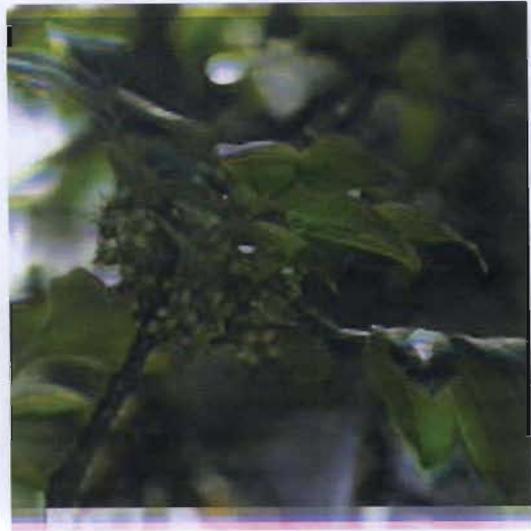
Figure 1. **Flowers (left) and leaves (right) of *Ekerbegia capensis* plant.**

1.12.11.2 *Sclerocarya birrea* **(A. Rich). Hochst. [Anacardiaceae]**

The Marula (*Sclerocarya birrea*) is one of the most highly valued indigenous trees in the Southern Africa. It is reported that the Tonga people celebrate the feast of the first fruits by pouring a drink offering of the fresh juice of the fruit over the tombs of the dead chiefs (Palgrave, 1983). The pulp of the fruit is delicious and the nut is also edible. Some tribes such as the Pedi make a relish from the leaves (Fox and Young, 1982). *Sclerocarya birrea* commonly known as 'Marula' is a medium sized tree that grows up to approximately 15 meters in height (See Figure 2). It bears flaky rough stem bark and small flowers with red sepals and yellow petals. The plant produces large rounded fruits which contain edible nuts with high content of vitamin C, a naturally occurring antioxidant (Balick, 1990).

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An ethnopharmacological survey identified a number of medicinal uses of this plant in Southern, Eastern and tropical West Africa (Watt and Breyer-Brandwijk 1962; Oliver-Bever 1986 and Hutchings, Scott, Lewis and Cunningham, 1996). Zulu people use bark decoctions for diarrhoea. Bark decoctions are used for dysentery and diarrhoea in some parts of Southern Africa (Watt and Breyer-Brandwijk, 1962). The Venda use bark for treating fevers, stomach ailments and ulcers (Mobogo, 1990). The bark decoction is used as anticough; the leaves, the pulp of fruit and mistletoe are used for hypertension (Guinko 1984). The bark and leaves are also used as antihyperglycemic (Nacoulma-Ouédraogo, 1996). Roots are used for many purposes including sore eyes in Zimbabwe (Gelfand, Mavi, Drummond and Ndemera, 1985). In addition, the presence of a large amount of ascorbic acid, a powerful antioxidant, could be of relevance in the counteraction of some of the clinical features associated with hypertension (Eromosele, Eromosele and Kuzhkuzha, 1991). Recently, Ojewole, (2003) reported the anti-inflammatory activity of aqueous and methanolic extracts of this plant in an experimental mammalian animal model.

Chemical analysis of this plant reveals the presence of (-)-Epicatechin-3-galloyl ester (Galvez, Crespo, Zarzuelo, Dde Witte and Spiessens, 1993), crude oil carbohydrate, crude protein, fibre and saponins (Ogbobe, 1992), minerals (Smith, Clegg, Keen and Grivetti. 1996) and ascorbic acid (Eromosele, Eromosele and Kuzhkuzha, 1991), alkaloids, anthocyans, flavonoids, tannins and saponosides (Galvez *et al.,* 1993). Saponins have been reported to stimulate nitric oxide release from vascular endothelial cells to induce vascular smooth muscle relaxation (Rice-Evans, Miller and Paganga,

1996). Similarly, plant derived polyphenols, including flavonoids have been demonstrated to relax blood vessels in an endothelium-dependent, nitric oxide-mediated manner (Fitzpatrick, Hirschfield, Ricci, Jantzen and Coffey, 1995; Rice-Evans, Miller and Paganga, 1996; Andriambeloson, Stoclet and Andriantsitohaina, 1999; Huang, Chan, Lau, Yao, Chan and Chen 1999). Flavonoids, saponins and tannins have also been demonstrated to possess angiotensin converting enzyme inhibitory activity (Lacaille-Dubois, Franck and Wagner, 2001). Therefore, the presence of these pharmacologically active compounds with cardiovascular activities prompted us to include this plant in the present study.

Figure 2. **Stem, leaves flowers and fruits of *Sclerocarya birrea***

1.12.11.3   *Persea americana* Mill. [Lauraceae]

Persea americana Mill. Lauraceae, a native of tropical America Mexico, is now widely

cultivated throughout the tropics and subtropics of the world. Commonly known as

Avocado pear, Alligator pear or Mexican avocado, it is an evergreen tree, 14-20 m high

with large spreading and flat-topped crown and deeply fissured brow corky bole (See

Figure 3). The branches are grey and also fissured but the twigs are green and smooth

(FAO. 1986). The plant bears fruits of various shapes and colours. The fruits are spherical, ovoid, ellipsoid or pyriform of about 8-20 cm long depending on the variety (Ross, 1999). The fruits are green, red or purple with a creamy or whitish pulp which is highly nutritious. In addition to its nutritional values, *Persea americana* is widely used in the traditional medicine of many countries. Ethnopharmacy of the Aztec culture used decocts of avocado seeds as a potent agent to treat mycotic and parasitic infections. Also local anaesthetic effects of avocado seeds preparations are known to decrease muscle pain (Argueta, Cano and Rodarte, 1994; Cabrera, 1996). Morphological parts of this plant are extensively used in traditional medicine for the treatment of various ailments. In Congo Brazzaville, a decoction of the stem bark is taken to relieve cough; while in Mexico, it is used as an aphrodisiac, emmenagogue, to prevent miscarriage, and in the treatment of haemorrhage between menstrual periods, insomnia, anaemia, diabetes, diarrhoea, dysentery and so forth (Watt and Breyer-Brandwijk, 1962). The leaves are used in Brazil and Jamaica for the treatment of high blood pressure (Watt and Breyer-Brandwijk, 1962). The hot water extract is orally taken as a diuretic and for hypertension in West Africa (Ross, 1999). Previous studies on this plant have shown that the leaf extracts of *Persea americana* possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, antidiabetic, hypoglycaemic, hypotensive and antihypertensive properties (Adeyemi, Okpo and Ogunti, 2002; Antia, Okonon and Okon, 2005; Adeboye, Fajonyomi, Makinde, and Taiwo, 1999). However, the mechanisms of the antihypertensive action of the extracts from this plant have not been established. Thus, *Persea americana* was included in the present study in order to elucidate the mechanisms of its reported antihypertensive activity.

Phytochemical screening of the *Persea americana* reveals the presence of alkaloids, coumarins and triterpene glycosides and tannins, (Valeri and Gimeno, 1953), saponins and flavonoids (Geissman and Dittmar, 1965), phytosterols, triterpenes (Werman, Mokady and Neeman, 1990; Lozano, Dhuique Mayer, Bannon and Gaydou, 1993) fatty acids, furanoic acids (Farines, Soulier, Rancurel, Montaudoin and Leborgne, 1995), some carotenoids, vitamin B, vitamins C and E (Slater, Shankman, Shepherd and Alfin-Slater. 1975).

Figure 3. **Shows a picture of a branch of *Persea americana* with some fruits.**

1.12.11.4 *Helichrysum ceres* S. Moore [Asteraceae]

The genus Helichrysum, belonging to the family Asteraceae is represented by approximately 500 species in the world. This genus is represented in South African flora by 245 species (Hilliard, 1983). The plant Helichrysum is a perennial, aromatic shrub which bears a large number of bracts located in several rows and which are yellow and shiny (Hutchings, 1989) (See Figure 4). The plant is traditionally used for treatment of various human ailments (Litvinenko, Popova, Popova, and Bubenchikova 1992). Some members of this genus have stimulatory activity on the secretion of gastric juice. Moreover, some species from this genus are used in folk medicine for their anti-inflammatory and anti-allergic properties (Carini, Aldini, Furlanetto, Stefania nd Facino., 2001). Other species from this genus are reported to be used in the treatment of tuberculosis, (Watt and Breyer-Brandwijk, 1962). *Helichrysum ceres* is reported to be used in the treatment of hypertension, infectious diseases and respiratory diseases in most parts of the Southern Africa (Hutchings, 1989; Watt and Breyer-Brandwijk, 1962). For instance, Zimbabwean traditional healers use extracts of *Helichrysum ceres* for management of heart and kidney disorders (Musabayane, Munjeri and Mdege, 2003). Recent laboratory studies have reported the hypotensive, natriuretic and diuretic effects of aqueous leaf and root extracts in rats (Musabayane, Munjeri and Mdege, 2003).

The medicinal properties of this genus are mainly attributed to the presence of flavonoids, but they may be also influenced by other organic and inorganic components, such as coumarins, phenolic acids and antioxidant micronutrients, e.g. Cu, Mn, Zn (Dombrowicz,

Swiatek, and Kopycki, 1994).   In the present study the plant was chosen with the aim of

elucidating the mechanisms of its reported cardiovascular actions.

Figure 4.   *Helichrysum ceres* **plant**

1.12.11.5 *Hypoxis hemerocallidea* **Fisch. & Mey [Hypoxidaceae].**

Hypoxis hemerocallidea, formerly known as *Hypoxis rooperi*, is a plant that belongs to a family known as hypoxidaceae. The plant is commonly referred to as 'African potato' even though the underground stem does not look like a potato but it's a corm bearing many nodes and nodules (See Figure 5). The plant is widely used in traditional medicine for different ailments. For instance, the plant is used by the Zulu community for hypertension, arthritis, diabetes, urinary tract infections (Hutchings, 1989). The plant is also used as a stimulant of human immune system and therefore indirectly relieving HIV-AIDS infections, as an agent for slowing down the growth of cancer cells, as having positive effect in combating "yuppie flu" and as being effective in alleviating arthritis (Hutchings, 1989; Drewes and Horn., 1999). Other pharmacological studies have reported anti-inflammatory activity, anti-microbial and anti-cancer activities of the Hypoxis plant (Ojewole 2005; Albrecht, 1996). Others have reported antioxidant property of extracts from this plant (Laporta, Perez-Fons, Mallavia, Cuturla and Michol, 2007). Ojewole and Zibula, (2000) reported the potent hypoglycaemic effects of *Hypoxis hemerocallidea* corm methanolic extract in both normal and diabetic Wistar rats. Diabetes mellitus and hypertension are commonly associated conditions. Moreover, epidemiological studies indicate that the prevalence of hypertension in type 2 diabetes is higher than in the general population (UK Prospective Diabetes Study Group, 1998). Thus, based on its reported hypoglycaemic effects and its use in traditional medicine for treatment of hypertension, we selected this plant to investigate its pharmacological basis for its use in the treatment of hypertension.

The phytochemical studies have reported the presence of hypoxoside as a major compound which is biologically inactive, but once formed it is rapidly converted to a more biologically active aglycone form, called rooperol (Drewes, Hall, Learmonth and Upfold, 1984; Albrecht, 1996). Other compounds present in Hypoxis plant include β-sitosterol, stigmasterol, β-sitosterol glycoside and sitostanol (Mills, Cooper, Seely and Kanfer, 2005).

Figure 5. *Hypoxis hemerocallidea* **plant**

1.13 Basis of the project.

The available conventional treatments of hypertension are far too costly and inaccessible to the poor majority in the developing nations. Thus, in spite of the effective conventional drug therapy available for hypertensive patients in general, economic problems in the developing nations continue to influence the lower rate of control of hypertension. Moreover, the cost analysis of possible antihypertensive drug treatment indicates that the developing countries cannot afford the same treatment as developed countries (Nissine, Bothig Granroth and Lopez, 1988). It is evident enough, therefore, that with increasing numbers of patients suffering from hypertension and conventional therapy beyond the reach of a wide population in developing countries, medicinal herbs with antihypertensive properties are increasingly sought by patients as well as health care professionals as an alternative, cheap and accessible source of treatment. However, although a lot of medicinal plants are used in the treatment of cardiovascular disorders or display antihypertensive activities, there is little or no reliable information regarding their safety and mechanisms of actions. Screening of anti-hypertensive effects in traditional medicines has been performed over many years by utilizing several animal models (Villar, Paya and Terencio, 1986). Our research team in the Departments of Physiology and Pharmacology at the University of KwaZulu Natal, Westville campus embarked on a huge project of screening the biopharmacological effects of some African medicinal plants on cardiovascular functions, in an effort to identify cheap and accessible treatment of hypertension and its complications. The present study forms part of this project and is aimed at establishing the mechanism(s) of the cardiovascular effects of some medicinal plants used in the traditional treatment of hypertension and other cardiac disorders by

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traditional health practitioners. It is envisaged that treatment of hypertension in poor African nations would become cheaper and therefore accessible by using scientifically proven phytotherapy. Thus, it would be necessary to establish the mechanism (s) of the cardiovascular effects and efficacy of the traditional herbs and develop them into therapeutic formulations that are readily affordable and accessible to the community. In Western medicine, drug development has become increasingly more mechanistic in focus with the aim of excluding unwanted side-effects (Hansen, Nyman, Smitt, Adsersen, Gudiksen, Rajasekharan and Pushpangadan, 1995). Similarly, the focus of the present study is on the establishment of the mechanisms of the cardiovascular effects of some medicinal plants used in traditional management of hypertension and other cardiovascular disorders in the Southern region of Africa.

We hypothesized that if the mechanisms of the cardiovascular effects of *Hypoxis hemerocallidea* corm ('African Potato'), *Ekebergia capensis, Persea americana* (Avocado) and *Helichrysum ceres* leaf and *Sclerocarya birrea* stem-bark ethanolic extracts could be established, then these plants would have the potential to provide a cheap and accessible source of treatment of hypertension and other cardiovascular disorders.

There are no reports based on scientific observations in the literature on the mechanisms of the cardiovascular effects of these plants. Therefore, in the present study, the effects of ethanolic extracts of these plants on heart rate and blood pressure were evaluated in rats. Contractility of rat isolated atria, aortic rings and portal veins were studied *in vitro*.

The *in vivo* cardiovascular studies were performed on both normotensive and hypertensive rats to verify whether the extracts had the ability to modify cardiovascular functions under pathological conditions. To establish possible mechanisms of the cardiovascular activities of the extracts, use was made of the positive standard control drugs used in conventional medicine, whose mechanisms of action are well established.

1.14   Aims

The main aim of the present study was to establish the mechanisms of cardiovascular effects of some medicinal plants used in traditional treatment of hypertension and other cardiac disorders in South Africa.

1.14.1   Objectives:

i)   To evaluate the *in vitro* and *in vivo* cardiovascular effects of various extracts of some medicinal plants.

ii)   To establish the mechanism (s) of the cardiovascular effects of various extracts of some medicinal plants.

CHAPTER 2

2.0   **MATERIALS AND METHODS**

2.1   **Ethical consideration.**

All the procedures followed were approved by the Ethics Committee of the University of KwaZulu-Natal (Ethical clearance number: HSS/05014A, See Appendix 1) and conformed to the "Principles of laboratory animal care" (WIH publication 85-123, revised in 1985).

2.2   **Materials**

2.2.1   **Drugs**

Methoxamine hydrochloride, acetylcholine chloride, indomethacin, NG-nitro-L-arginine-methyl-ester (L-NAME), methylene blue, atropine sulphate, glibenclamide, propranolol, noradrenaline, prazosin, reserpine and nifedipine were obtained from Sigma (St. Louis, MO 63178, USA), Heparin Novo (Nordisk (Pty) Ltd, Johannesburg, RSA) All other chemicals and reagents were of the analytical grade and supplied by Merck chemicals (Pty) Ltd, 259 Davidson Rd, Wadenville, RSA). Indomethacin was dissolved in 0.5% sodium bicarbonate. Glibenclamide was dissolved in dimethyl sulfoxide (DMSO). All other drugs, including Krebs' solution were freshly prepared in deionized water.

2.3 Identification of plants and collection of plant materials.

Five plants namely *Ekebergia capensis* (Sparrm) [Maliaceae], *Persea americana* (Mill) [Lauraceae], *Helichrysum ceres* (S. Moore) [Asteraceae], *Sclerocarya birrea* (A. Rich) [Anacardiaceae] and *Hypoxis hemerocallidea* (Fisch. & C.A. Mey) [Hypoxidaceae] were identified and authenticated by Prof. H. Baijnath of Botany Discipline. The plant materials were collected in different areas in KwaZulu-Natal Province between July and December 2004. Voucher specimens of the plants have been deposited at the Discipline of Botany Herbarium, University of KwaZulu-Natal.

2.4 Plant extraction.

2.4.1 Leaf and corm extraction.

Leaves from *E. capensis*, *H. ceres* and *P. americana* were air-dried under room temperature. The air-dried plant leaves were then ground separately into powder using a commercial blender (Waring blender) and then weighed. The ground material was soaked in 99% ethanol for 48 hours after which, the soaked material was filtered using 30 cm filter paper (Whatman, England). The filtrate was concentrated under reduced pressure in a vacuo using a rotary evaporator at 55 °C (Buchi, Lotavapor, Essen, Germany). The concentrated crude extract was recovered and allowed to air-dry under room temperature and thereafter, the percent yield was determined. The recovered extracts from *E. capensis*, *H. ceres* and *P. americana* were represented as EKE, HCE and PAE,

respectively. For *H. hemerocallidea* the extraction method was the same except that fresh corms were crushed into smaller pieces after peeling. The concentrated crude extract was recovered and freeze dried to yield a brown powdery material which was represented as APE.

2.4.2   Stem-bark extraction.

Stem-bark from *Sclerocarya birrea* was air-dried and ground into powder using a Thomas-Wiley Laboratory mill model 4 (Arthur H Thomas company Philadelphia, PA., USA). Following this, the extraction procedure was as described in 2.4.1. The final crude extract was denoted SBE.

All extracts were freshly prepared on each day of experiment by dissolving in DMSO using the method previously described by Musabayane, Bwititi and Ojewole, (2006).

2.5   Animals

Male Wistar (250-300 g) and weanling Dahl salt-sensitive (DSS) rats (100-150 g) bred and housed at Biomedical Research Unit of the University of KwaZulu-Natal were used for the *in vivo* acute and chronic studies, respectively. Male Wistar rats weighing 350-400 g were used for *in vitro* studies. The animals were maintained under the conditions of constant temperature (22±2 °C), carbon dioxide content of <5000 p.p.m., relative humidity of 55 ± 5%, and illumination (12h light/dark cycles) and the noise levels of <65

decibels. The animals were fed with standard animal chow (Epol-diet 4700, Epol, RSA) and had free access to drinking water.

2.6   Blood pressure measurement

2.6.1   Acute studies.

The effects of the test extracts on the mean arterial blood pressure and heart rate were evaluated in anaesthetized male Wistar normotensive rats using a method previously described by Musabayane, Munjeri and Mdege, (2003). On each day of experimentation, the animals were anaesthetized by an intraperitoneal injection of trapanal (sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate, Byk Gulden, Konstanz, Federal Republic of Germany) at 0.11g./ kg. body weight. The animals were tracheotomized to maintain clear airway entry. The rats were placed on a warming table (CF Palmer, London, England) to maintain their body temperature at $37 \pm 1°C$. The left carotid artery was catheterized with heparinized saline (50 units/ml saline) polythene tubing (internal diameter, 0.58mm and external diameter 0.96mm, Clay Adams, New Jersey, USA). The catheter was then connected to a pressure transducer (Statham MLT0380, Ad instruments, compatible with PowerLab system ML410/W, Inc, Bella Vista, Australia) for continuous measurement of blood pressure and heart rate. The right external jugular vein was cannulated with the same type of tubing to allow continuous infusion of 0.077 M NaCl (Merk Ltd) at 150µL/ min (Harvard Apparatus, Model 2400003, South Natick, Massachusetts, USA). The perfusion of this solution is used in order to maintain a correct plasmatic oncotic pressure

and to avoid fluid shifts during surgery (Maddox, Price and Floyd, 1977). The urinary bladder of each rat was also cannulated with similar caliber polythene tubing via an incision in the abdominal wall just for urine voidance.

Following a 3h equilibration period, blood pressure and heart rate measurements were recorded over the 4h post-equilibration period of 1h control, 1h 30 min treatment and 1h 30 min recovery periods. Thus, after a 3h equilibration period, two control measurement periods of 30 minutes each were allowed. At the end of this control period, the plant extracts were added to the infusate and delivered at 0.06 µg/ min for 90 minutes, giving a total dose of 120 mg/ kg body weight. During this treatment period, three experimental measurements of 30 minutes each were taken. After the treatment period, the infusion of the plant extracts was discontinued and replaced again by the 0.077 M NaCl at 150 µL/ min and three recovery measurement periods of 30 minutes each were allowed. The control animals (n=6) were continuously infused with 0.077 M NaCl at 150 µL/ min throughout the experimental period. Therewith, urine was voided continuously via the cannulated urinary bladder. Data from the control group was used as baseline.

2.6.2   Chronic studies.

In order to evaluate the long-term effects of the test extracts on blood pressure and heart rate, six separate groups comprising of one control group and five treated groups of weanling male Dahl salt sensitive rats (n=8, per group) were used. The Dahl salt sensitive rats develop hypertension as they progress with age.

Before the chronic experiment started the rats were subjected to a training programme for one week so as to allow them to become accustomed to the whole procedure involved in blood pressure measurement. This training programme involved: the handling of the rats, placing them in restrainers and holders, preheating and the measurement of blood pressure on the tail. The values obtained during the training programme were not included in the analysis at the end of the experiment. After the training period, systolic blood pressure, diastolic blood pressure, mean arterial pressure and heart rate were measured using an indirect tail cuff computerised blood pressure monitor (IITC Life Sciences Model 31, Life sciences, Woodland Hills, California, USA). This unit works with IITC hardware blood pressure system that determines both blood pressure and heart rate. The system employs an automatic scanner and pump, sensing cuff and amplifier to measure and count the pulse rate in the animal tail. The results are displayed as data plots and summary data of systolic, diastolic and mean blood pressure and heart rate on the computer screen. Small, medium and large restraining devices and 2 different tail cuffs (10mm and 15mm) were used to compensate for the increase in body weight during the 6- week programme. Three readings were observed for each rat per session. Owing to the sensitivity of the method, no prolonged preliminary warming of the animals was necessary, but a constant room temperature of 26°C was maintained.

2.6.2.1   Treatment

The extracts were prepared fresh on each day of the experiment by using the method previously described by Musabayane, Bwititi and Ojewole, (2006). The extracts were

dissolved in dimethyl sulfoxide (DMSO, 2 ml) and normal saline (19 ml). The animals were treated with specific extracts orally by gavage at a dose of 80 mg/ kg. body weight daily at 09h00 for six weeks. The animals in the control group were given an equal volume of DMSO-saline dissolution medium (1 ml/ kg. body weight) free of crude extract.

2.7   *In vitro* pharmacological studies.

2.7.1   Cardiotonic experiments.

The experimental procedure used for rat isolated atria was adopted from that described by Ojewole (2006). Briefly, male Wistar rats were sacrificed by stunning and exsanguination. An incision was made on the skin of the thorax and the underlying muscle layer. The ribs and the diaphragm were cut open to expose the heart. The entire heart was excised rapidly and placed in a petri dish containing Krebs Hensenlet physiological solution (of the following composition : NaCl, 118mM; KCl, 4.7mM; $NaH_2PO_4.2H_2O$, 1.28 mM; $NaHCO_3$, 25.0 mM; $CaCl_2.2H_2O$, 2.52 mM; $MgCl_2$, 1.2 mM; glucose, 5.55 mM at pH adjusted to 7.4) aerated continuously with carbogen (a mixture of 95% oxygen and 5% carbon dioxide). The right and left atria were isolated and transferred to another petri dish also containing Krebs-Hensenlet physiological solution which was aerated continuously at $34\pm1°C$. In order to evaluate the effects of the plant extracts on the rate of myocardial contraction, the rat isolated spontaneously-beating right atria were used. The rat isolated electrically-driven left atria were used to evaluate the

effects of the various extracts on the force of myocardial contraction. The ends of the contraction axis of the right atrial tissue were tied with cotton threads. The isolated left atrium was impaled on a thin platinum wire electrode, while in tying up the cotton thread, a round loop was made at one end to hook the right atrial tissue to a fixed position in the organ bath. The atrial tissues were then suspended inside the 30-ml Ugo Basile organ bath (Ugo-Basile, Comerio, Italy) containing Krebs-Hensenlet physiological solution under an applied resting tension of 1.0g. The left atrium was electrically driven with square wave pulses of 5msec. duration at a frequency of 3 Hz and a supramaximal voltage of 5-10 volts, delivered by an SRI stimulator (Preamplifier, Bioscience, United Kingdom). The physiological solution was continuously aerated with carbogen at $34\pm1^{\circ}C$. The other end of the cotton thread was connected to isometric force displacement transducer (model 7200, Ugo-Basile, Comerio, Italy). The atrial tissues were allowed to equilibrate for a period of 60 minutes, during which time the bathing physiological solution was changed every 15 minutes.

2.7.1.1 Experimental protocol

After an equilibration period, graded concentrations of EKE, HCE, PAE, APE, SBE and/or reference drugs were added to the organ bath cumulatively to generate cumulative concentration-response curves for each extract or standard positive drugs. Isometric contractions were recorded by means of Ugo-Basile force-displacement transducers

(model 7200, Ugo Basile, Comerio, Italy), and pen-writing 'Gemini' recorders (Gemini 7070, Ugo-Basile, Comerio, Italy).

2.7.1.2   Cholinergic mechanisms

To find out whether the inotropic and chronotropic effects exhibited by the test extracts were mediated through cholinergic receptors, the atrial preparations were pretreated with atropine sulphate ($1\mu M$), a muscarinic receptor antagonist (Arunlakhshana and Schild, 1959). Atropine sulphate was added to the organ bath ten minutes before re-determination of the effects of the test extracts/drugs. The cumulative concentration-response curves generated for each extract/drug in the presence of atropine were then compared to those generated in the absence of atropine. The effects of the extracts/drugs were expressed as percentage of the baseline values (n= 8 preparations for each concentration).

2.7.1.3   Adrenergic mechanisms.

In order to examine the involvement of beta-adrenergic receptors in the inotropic and chronotropic effects of the test extracts, the atrial preparations were pretreated with propranolol ($1\mu M$), a non-selective beta adrenergic receptor blocking agent (Hoffman and Lefkowitz, 1990). Propranolol was added 5 minutes before re-determination of the

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effects of the test extracts. The cumulative concentration-response curves generated for each extract/drugs in the presence of propranolol were then compared to those generated in the absence of propranolol (n= 8 preparations for each concentration).

2.7.1.4   Involvement of catecholamines

To examine whether the cardiotonic effects of the test extracts were mediated through modulation of noradrenaline release from junctional stores, the atrial preparations were pretreated with reserpine ($5\mu M$), to deplete junctional noradrenaline stores (Salzmann, Bormann, Herzig, Markstein and Scholtysik, 1985). The cumulative concentration-response curves were then generated for each extract and compared to those generated in the absence of reserpine (n= 8 preparations for each concentration).

2.7.1.5   Effects of various extracts on positive inotropic effects of noradrenaline and calcium.

The effects of various extracts on noradrenaline-induced positive inotropic effects were investigated in rat isolated electrically-driven left atrial muscle preparations. After an equilibration period, cumulative concentration-response curve ($0.1-1.6\mu M$) for noradrenaline was generated in the absence of the test extracts (n= 8 preparations for each concentration). Then the left atrial muscle preparations were pretreated with a known

concentration of the test extract for 10 minutes, followed by cumulative addition of noradrenaline (0.1-1.6μM) to re-establish the cumulative concentration-response curves. The cumulative concentration-response curves generated in the presence of the test extracts were compared to those generated in the absence of the extracts. The similar procedure was followed to determine antagonistic effects of the extracts on calcium-induced positive inotropic effects in the rat isolated left atria. The concentrations of calcium used were 0.5-8 μM (in each case n= 8 preparations).

2.7.2   Vascular experiments

2.7.2.1   Preparation of aortic rings.

The experimental procedure used for rat isolated aorta was adopted from that described in detail by Ojewole (2006). Briefly, male Wistar rats were sacrificed by stunning and exsanguination. The descending thoracic aorta was quickly removed and placed in a petri dish containing Krebs-Hensenlet physiological solution (of composition as described in 2.7.1). The aorta was freed of extraneous and excess connective tissue and cut into rings of about 4-5 mm in width. The dissecting procedures were carefully done to protect the functional endothelium from inadvertent damage. When required by the experimental protocol, mechanical removal of endothelium was achieved by gently rubbing the luminal surface of the aortic ring six times back and forth with a distilled water- moistened cotton wool. A pair of isolated aortic rings, one with intact functional endothelium and the other one without a functional endothelium, were always set up in parallel for comparison. The

rings were suspended under the resting tension of 1 g, in a 30-ml Ugo-Basile organ-bath (Ugo-Basile, Comerio, Italy) containing Krebs Hensenlet physiological solution maintained at 36 ±1°C and aerated continuously with carbogen (a mixture of 95% oxygen and 5% carbon dioxide). The aortic preparations were left to equilibrate for 60 minutes during which, the physiological bathing solution was changed every 15 minutes before they were challenged with graded concentrations of various plant extracts.

2.7.2.2   Experimental protocol.

After an equilibration period of 60 minutes, the aortic rings were contracted with methoxamine hydrochloride (ME, 10 µM). After a sub-maximal and stable contraction was achieved, acetylcholine (Ach, 10 µM) was added to the organ bath to induce vasorelaxation so as to confirm the integrity of the endothelium. A relaxation $\geq 70\%$ induced by acetylcholine on the methoxamine-induced contraction was considered representative of an acceptable presence of a functional endothelial layer, while a relaxation $\leq 10\%$, indicated a satisfactory effectiveness of the endothelium removal.

2.7.2.3   First protocol-aortic rings with functional endothelium.

Forty minutes after the confirmation of endothelial integrity, the endothelium-intact aortic rings were pre-contracted with methoxamine hydrochloride (10 µM) or low potassium concentration, K^+ (20mM) or high potassium concentration, K^+ (80mM). After a sustained stable tonic contraction was achieved, graded concentrations of various plant

extracts were added to the organ bath cumulatively to obtain concentration-dependent relaxant responses. The vasorelaxant effects of the extracts were calculated from the decrease of tonic contraction and expressed as the percentage of maximal contraction induced by the agonist (n= 8 preparations for each concentration).

2.7.2.4　　Second protocol- aortic rings without functional endothelium.

Forty minutes after the confirmation of endothelial removal, the aortic rings were pre-contracted with methoxamine hydrochloride. After a sustained stable tonic contraction was achieved, graded concentrations of various plant extracts were added cumulatively to the organ bath containing the endothelium-deprived aortic rings, to obtain cumulative-concentration-dependent relaxant responses. The responses obtained in the endothelium-deprived aortic rings were compared with those obtained in endothelium-intact aortic rings (2.7.2.3).

2.7.2.5　　Third protocol- involvement of endothelium-derived relaxing factors.

To verify the involvement of the endothelium-derived vasodilators, 40 minutes after the confirmation of endothelial integrity, specific inhibitors were added to the organ bath, 30 minutes before the aortic rings were pre-contracted with methoxamine hydrochloride. Thus, to investigate the role of nitric oxide, prostacyclin and cyclic guanosine monophosphate (cGMP), the endothelium-intact aortic rings were pretreated with L-NAME (100 μM) a nitric oxide synthase inhibitor, indomethacin (10 μM), a

cyclooxygenase inhibitor and methylene blue (10 μM), a guanylate cyclase inhibitor, respectively, 30 minutes before the aortic rings were pre-contracted with methoxamine hydrochloride. After a sustained stable tonic contraction was achieved, graded concentrations of various plant extracts were added cumulatively to the organ bath containing the endothelium-intact aortic rings to obtain cumulative-concentration-dependent relaxant responses. The responses obtained in the presence of specific inhibitors were compared with those obtained in the absence of the inhibitors (2.7.2.3).

2.7.2.6   Fourth protocol- involvement of potassium and calcium channels.

To investigate whether the vascular effects of the test extracts involved potassium channels, 40 minutes after the confirmation of endothelial integrity, the endothelium-intact aortic rings were precontracted with low potassium concentration (K^+ 20mM). After a stable tonic contraction was achieved, graded concentrations of various extracts were added to the organ bath to generate cumulative-concentration response curves. To verify the involvement of modulation of ATP-sensitive potassium channels, the aortic rings were pretreated with glibenclamide (3μM), an ATP-sensitive potassium channel blocker, 10 minutes before precontracted with (K^+ 20mM). After a stable tonic contraction was achieved, cumulative-concentration response curves for the extracts were re-established. The cumulative-concentration response curves generated in the presence of glibenclamide were compared to those generated in the absence of glibenclamide.

To investigate whether the vasorelaxant effects of various extracts involved modulation of calcium channels, 40 minutes after the confirmation of endothelial integrity, the endothelium-intact aortic rings were precontracted with high potassium concentration (K^+ 80mM). After a stable tonic contraction was achieved, graded concentrations of various extracts were added cumulatively to the organ bath to establish cumulative-concentration response curves.

2.7.3   Studies on portal veins

2.7.3.1   Preparation of portal veins.

Healthy male Wistar rats were sacrificed by stunning and exsanguination. The abdomen of the rat was quickly opened by midline incision, and the intestines were pulled aside. The portal veins were carefully cleaned free of associated connective, extraneous and fatty tissues. The portal veins were tied with a cotton thread at each end (between the hepatic bifurcation of the vein and the anterior mesenteric vein) and then excised. From the time of the dissection, the portal veins were maintained in Krebs-Henseleit physiological solution (of composition described in 2.7.1). Each isolated portal vein was suspended vertically under an applied resting tension of 0.5 g in a 30-ml Ugo-Basile organ-bath (Ugo-Basile, Comerio, Italy) containing Krebs-Henseleit physiological solution which was maintained at 36±1°C, pH 7.4 and continuously bubbled with carbogen (95% oxygen and 5% carbon dioxide gas mixture). Two isolated venous tissue preparations (control and treatment) were always set-up for comparison. The venous

preparations were allowed to equilibrate for 60 minutes during which time the bathing physiological solution was changed every 15 minutes before they were challenged with graded concentrations of various test extracts. The tissue responses were recorded isometrically by means of Ugo-Basile force displacement transducers (model 7200 Ugo-Basile, Comerio, Italy) and pen-writing "Gemini" recorders (model 7070 Ugo Basile, Comerio, Italy).

2.7.3.2 Experimental protocol.

After an equilibration period of 60 minutes, graded concentrations of various test extracts were added to the organ bath cumulatively to establish cumulative-concentration dependent responses. To investigate whether the effects of the extracts involved modulation of cholinergic receptors, alpha-adrenergic receptors and calcium channels, the portal vein preparations were pretreated with atropine sulphate (a muscarinic receptor blocker) prazosin (alpha 1-adrenergic receptor blocker) and nifedipine (L-type voltage-operated calcium channel blocker), respectively, 5 minutes before re-establishing the cumulative-concentration dependent responses. The responses obtained in the presence of specific blockers were compared with those obtained in the absence of the blockers. The effects of the extracts were calculated as percentage of the baseline values (n=8 preparations for each concentration).

2.7.4   Data analysis.

Values obtained are presented as means (± SEM). The data obtained were treated and presented separately for normotensive Wistar and hypertensive DSS-treated rats to determine the effects of various plant extracts on the parameters measured. Data from the control normotensive Wistar and hypertensive DSS rats were used as baseline. Duration of the acute effects of the test extracts on mean arterial blood pressure was used as a basis to classify the extracts. Extracts of *E. capensis*, *H. hemerocallidea* and *P. americana* caused a transient fall in mean arterial blood pressure and were classified as group A extracts. Extracts from *H. ceres* and *S. birrea* caused a persistent reduction in mean arterial blood pressure and were classified as group B extracts. *In vitro* cardiotonic experiments, data from vehicle-treated preparations were used as baseline. Therefore, the inotropic and chronotropic effects of the extracts were calculated as percentage of the baseline values. In experiments involving aortic rings, the effects of the extracts were calculated as percentage of the maximum contractile response of the aortic preparations to an agonist (methoxamine or potassium). Statistical significance was determined through one-way analysis of variances (ANOVA) followed by Dunnett multiple comparison test to compare the means. A value of $p < 0.05$ was considered statistically significant. Graphs were plotted and statistical analysis was performed using GraphPad Prism version 2.00 for windows (Graphpad software, San Diego, CA, USA).

CHAPTER 3

3.0   **RESULTS**

The results in this chapter will describe the following ;(1) plant extraction, (2) acute and long-term effects of plant extracts on blood pressure and heart rate, (3) *in vivo* inotropic effects of the plant extracts.

3.1   Plant extraction results.

Five plants namely *Ekebergia capensis* (Sparrm) [Maliaceae], *Persea Americana* (Mill) [Lauraceae], *Helichrysum ceres* (S. Moore) [Asteraceae], *Sclerocarya birrea* (A. Rich) [Anacardiaceae] and *Hypoxis hemerocallidea* (Fisch. & C.A. Mey) [Hypoxidaceae] were extracted using 95% ethanol to yield five different ethanolic extracts denoted EKE, PAE, HCE, SBE and APE, respectively. The percent yield of these crude extracts ranged from 8% to 15 % (Table 1).

Table 1.   % yield of crude ethanolic extracts.

Plant		%
Hypoxis hemerocallidea	(APE)	10
Persea americana	(PAE)	8
Sclerocarya birrea	(SBE)	13
Helichrysum ceres	(HCE)	10
Ekebergia capensis	(EKE)	15

3.2   Blood pressure.

3.2.1   Acute effects of extracts on mean arterial pressure (MAP) and heart rate

Plant extracts were divided into groups of A and B based on the duration of their acute effects on MAP as previously described in section 2.8.4. In this study, the acute effects of group A test extracts, namely, EKE, APE, PAE and group B extracts, namely, HCE, SBE on MAP and heart rate were evaluated in male anaesthetized Wistar normotensive rats.

Group A extracts, namely, EKE, APE, and PAE at a dose of 120 mg/kg. body weight, caused significant reductions in the mean arterial pressure (MAP) during treatment period. However, this effect was so transient, such that the MAP started to increase progressively before the end of treatment period (Figure 6). During recovery period, the MAP increased progressively to values that were comparable to those recorded during control period. On the other hand, group A extracts, did not exhibit any significant effect on the heart rate (Table 2).

In contrast, group B extracts, namely, HCE and SBE at the same dose of 120 mg/kg. body weight, induced significant and progressive, long-lasting reductions in the MAP, with no significant effect on the heart rate during treatment period (Figure 6). This fall in the MAP persisted during recovery period, such that the MAP values at the end of recovery period were significantly lower as compared to those recorded during pretreatment period (Figure 6).
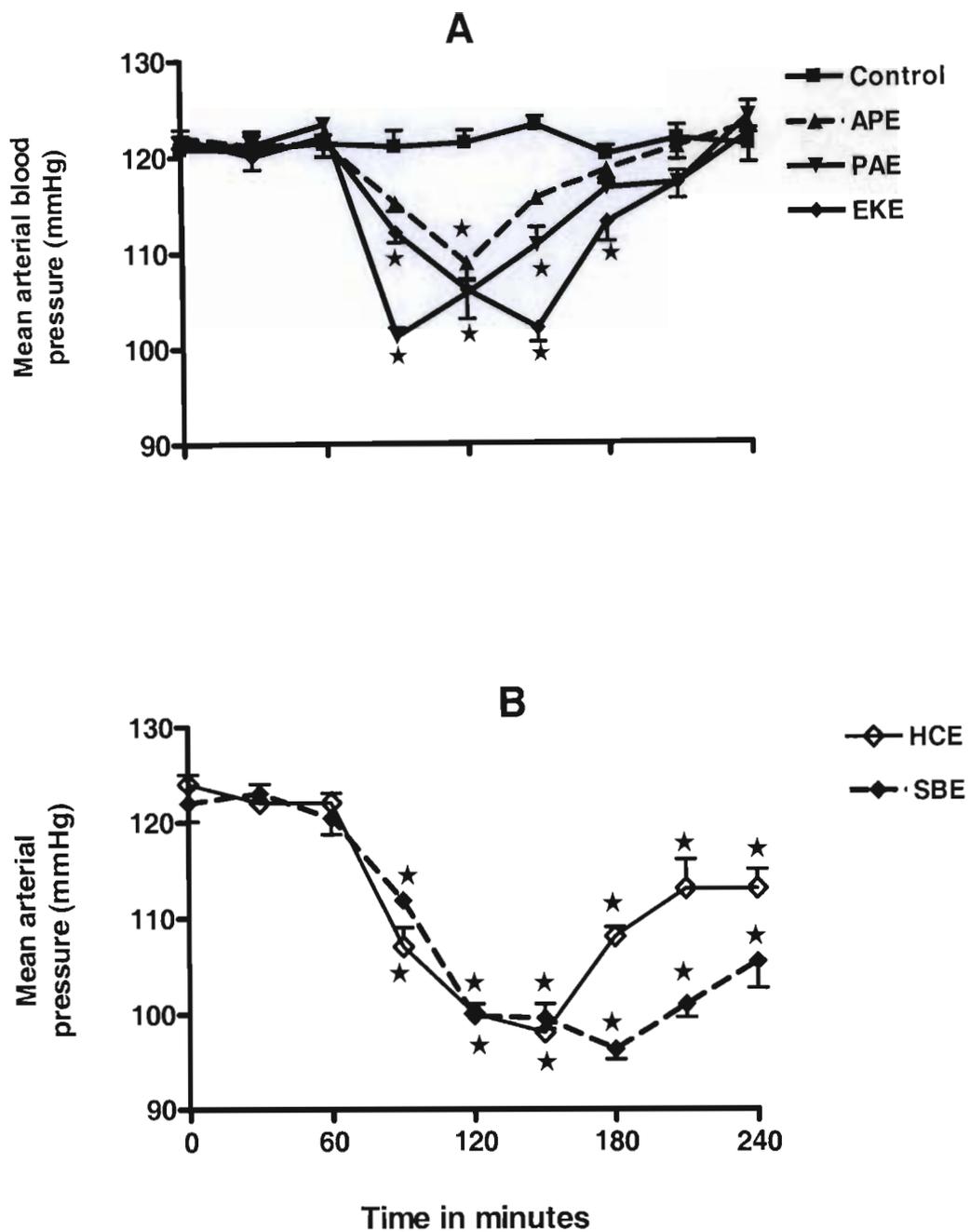
Figure 6.

Effects of various plant extracts infusion on the mean arterial blood pressure in normotensive male Wistar rats. Values are means ± SEM; (n=6 animals per group).

$P^\star < 0.05$ by comparison with control period.

3.2.2 Long-term effects of various extracts on blood pressure and heart rate.

To evaluate the long-term effects of the various test extracts on mean arterial blood pressure (MAP), systolic and diastolic blood pressures and heart rate, separate groups of Dahl salt sensitive-rats (DSS) were treated with extracts at a dose of 80 mg/ kg. body weight, daily for six weeks at 09h00.

In this study, the MAP, systolic and diastolic blood pressures in DSS-untreated rats increased progressively and significantly to develop hypertension in week five. At the end of six weeks, the DSS-untreated rats displayed extremely higher blood pressure (MAP, systolic, diastolic) values while blood pressure values in the treated groups were within the normal range by comparison with values in week one (Figures 7, 8). In addition, in the DSS-untreated rats, the heart rate increased following a similar pattern (Table 3).

The extracts from both groups (A and B), prevented the increase in the MAP, systolic and diastolic blood pressures in the DSS-treated groups during the entire period of study (Figures 7, 8). In addition, group A extracts, APE and PAE substantially but transiently reduced the MAP and systolic blood pressures during week 4 and 5 compared to week 1. While HCE from group B caused a potent decrease in systolic blood pressure during week 5 compared to week1. Therefore, each test extract demonstrated antihypertensive potential by preventing increases in the MAP, systolic and diastolic blood pressures in Dahl salt sensitive rats, a genetically hypertensive rat model.

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In Group A extracts, EKE exhibited no significant effect on the heart rate, whereas, APE and PAE exhibited significant decreases in heart rates from week 4. Group B extracts, HCE and SBE, decreased the heart rates in the Dahl salt sensitive rats, from week 4 (Table 3).
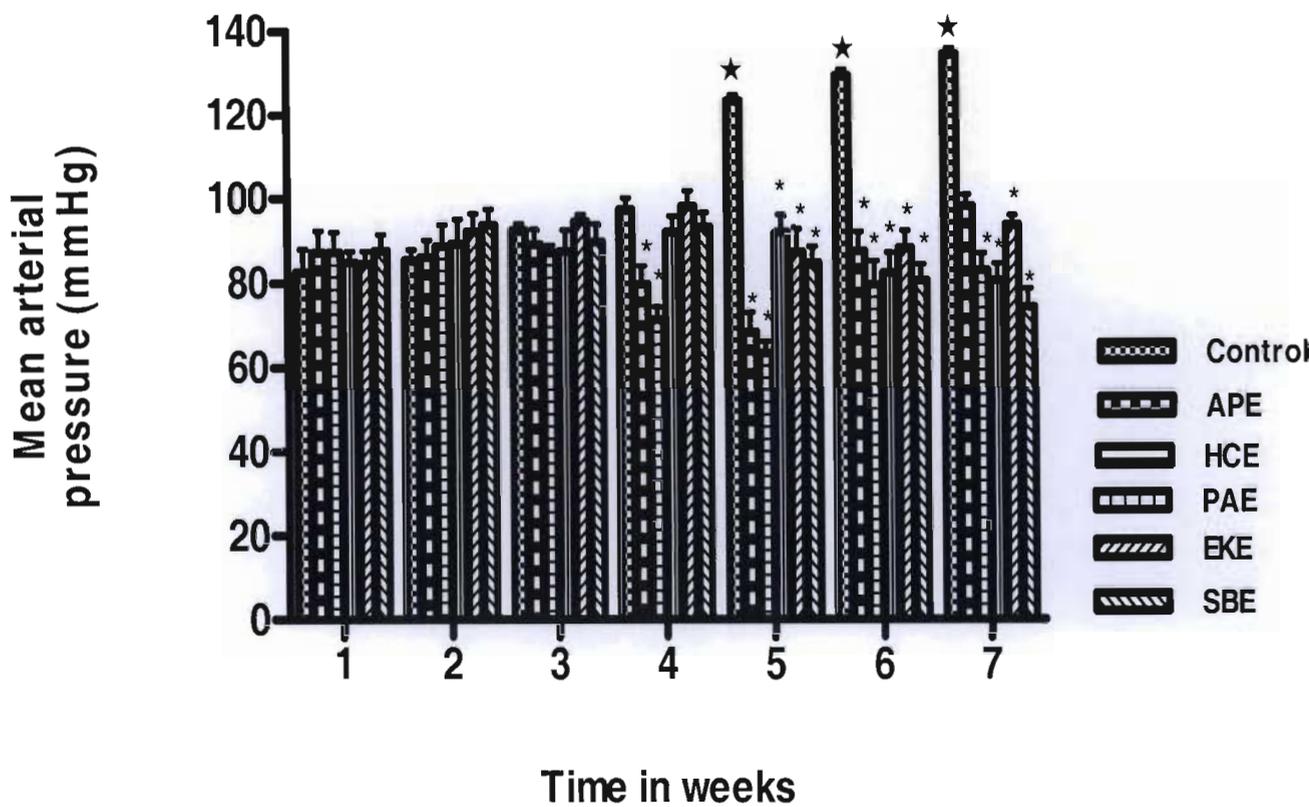
Figure 7.

Effects of long-term treatment of various plant extracts on the mean arterial blood pressure in DSS rats. Values are means ±SEM; (n=8 animals per group).

$P*$ <0.05 by comparison with control animals and P^*< 0.05 by comparison with week 1.
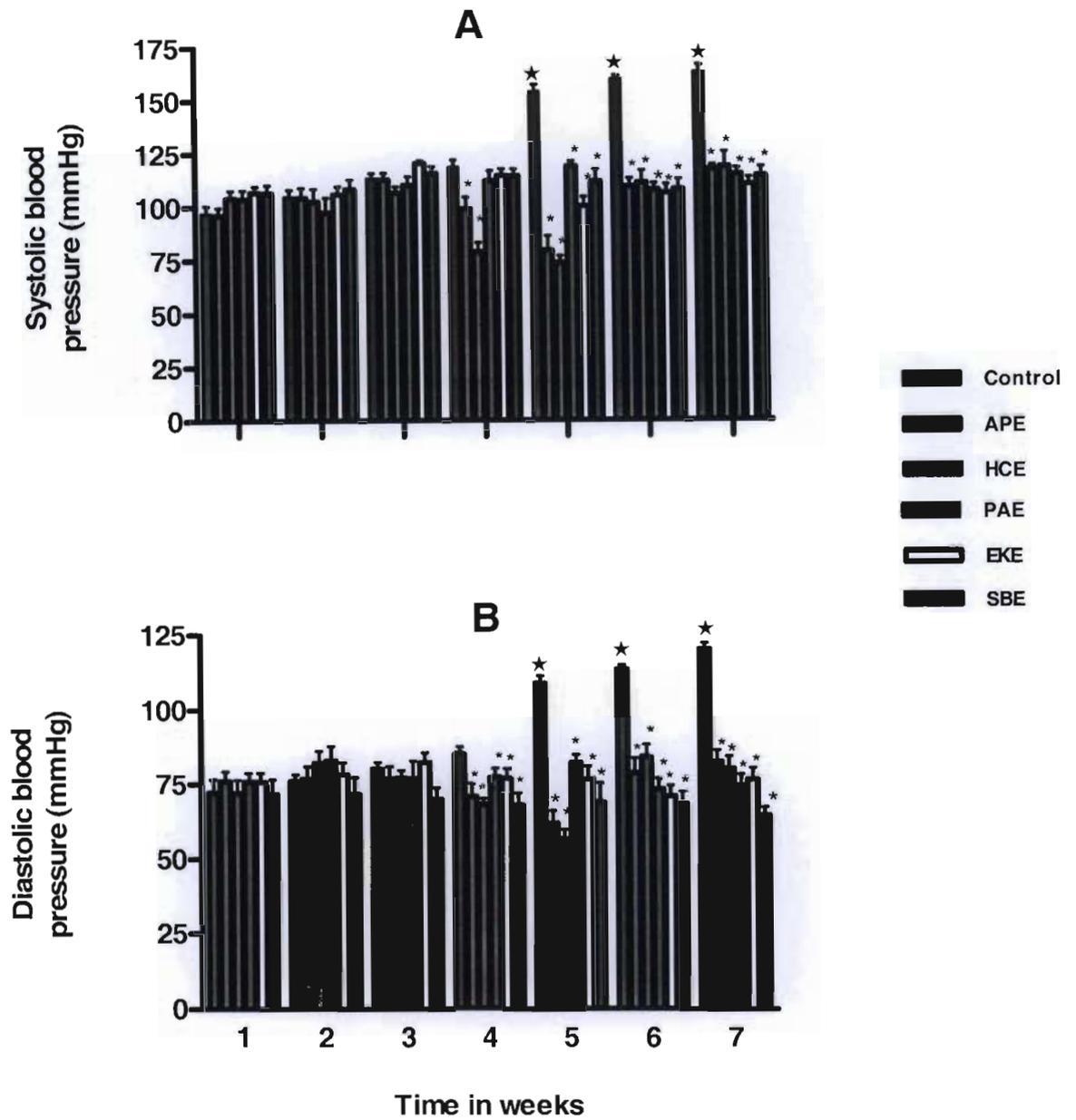
Figure 8.

Effects of long-term treatment of various plant extracts on systolic **(A)** and diastolic **(B)** blood pressure in DSS rats. Values are means ±SEM; (n=8 animals per group).

$P*$ <0.05 by comparison with control animals and $P^\star$< 0.05 by comparison with week 1.

Table 2. Acute effects of various plant extracts on heart rate. Values are means ± SEM, (n=6 animals per group). $P^* < 0.05$ by comparison with control period.

Parameter	Treatment	Time in minutes							
		30	60	90	120	150	180	210	240
Heart rate (Beats/min) Group A	**Control**	410±6	410±6	409±6	408±6	411±4	410±6	410±4	411±4
	APE	373±12	377±14	386±8	365±6	370±9	382±7	381±3	382±5
	PAE	392±14	395±17	394±16	392±12	395±11	390±21	400±11	402±13
	EKE	410±5	409±7	408±5	411±10	409±7	410±10	410±9	408±11
Group B	**HCE**	400±4	396±6	400±10	401±4	400±10	400±12	403±10	398±10
	SBE	386±11	388±11	388±13	390±10	391±13	387±11	384±18	386±13

Table 3. Effects of long-term oral treatment of various plant extracts on heart rate in DSS rats. Animals were administered 80 mg /kg. body weight daily for 6 weeks. Values are means ± SEM, (n=8).

P * < 0.05 by comparison with values in week 1.

Parameter	Treatment	Time in weeks						
		1	2	3	4	5	6	7
Heart rate (Beats/min)								
	Control	380±12	380±10	401±7	380±9	379±12	381±18	396±9
	EKE	354±15	353±19	358±15	365±16	359±12	352±12	345±8
Group A	**APE**	381±12	376±9	356±14*	331±11*	306±12*	409 ±8*	399±6*
	PAE	381±14	371±16	378±12	365±15*	354±9*	365±15*	354±13*
Group B	**HCE**	381±12	369±18	387±17	360±11*	364±6*	348±10*	341±6*
	SBE	381±13	376±12	370±17	367±14*	361± 9*	359±11*	356±7*

CHAPTER 4

4.0 *IN VITRO* CARDIOTONIC RESULTS.

This chapter will describe the effects of the positive standard drugs (noradrenaline, acetylcholine and calcium) and various extracts, EKE, APE, PAE, HCE and SBE on the rate (chronotropy) and force (inotropy) of myocardial contraction. In addition, the antagonistic effects of the extracts on the positive inotropic effects of noradrenaline and calcium will also be described.

4.1 Cardiotonic effects of noradrenaline.

The effects of noradrenaline on the rate of myocardial contractions (chronotropy) were evaluated on the rat isolated spontaneously-beating right atria. Noradrenaline caused a significant and concentration-dependent (0.1-1.6 µM) increase in the rate of spontaneous contractions (positive chronotropy) of the right atrial muscles. This effect was completely abolished by prior treatment of the atrial muscle preparations with propranolol (1 µM), a non-selective beta adrenergic receptor blocker (Figure 9A). Additionally, the effects of noradrenaline on the force of myocardial contraction (inotropy) were evaluated on the rat isolated electrically-driven left atria. Similarly, noradrenaline induced a significant and concentration-dependent (0.1-1.6 µM) increase in the force of myocardial contraction (positive inotropy) in the electrically-driven left atrial muscle. Pretreatment of the left

atrial muscle preparations with propranolol resulted in a complete inhibition of the positive inotropic effects of noradrenaline (Figure 9B).
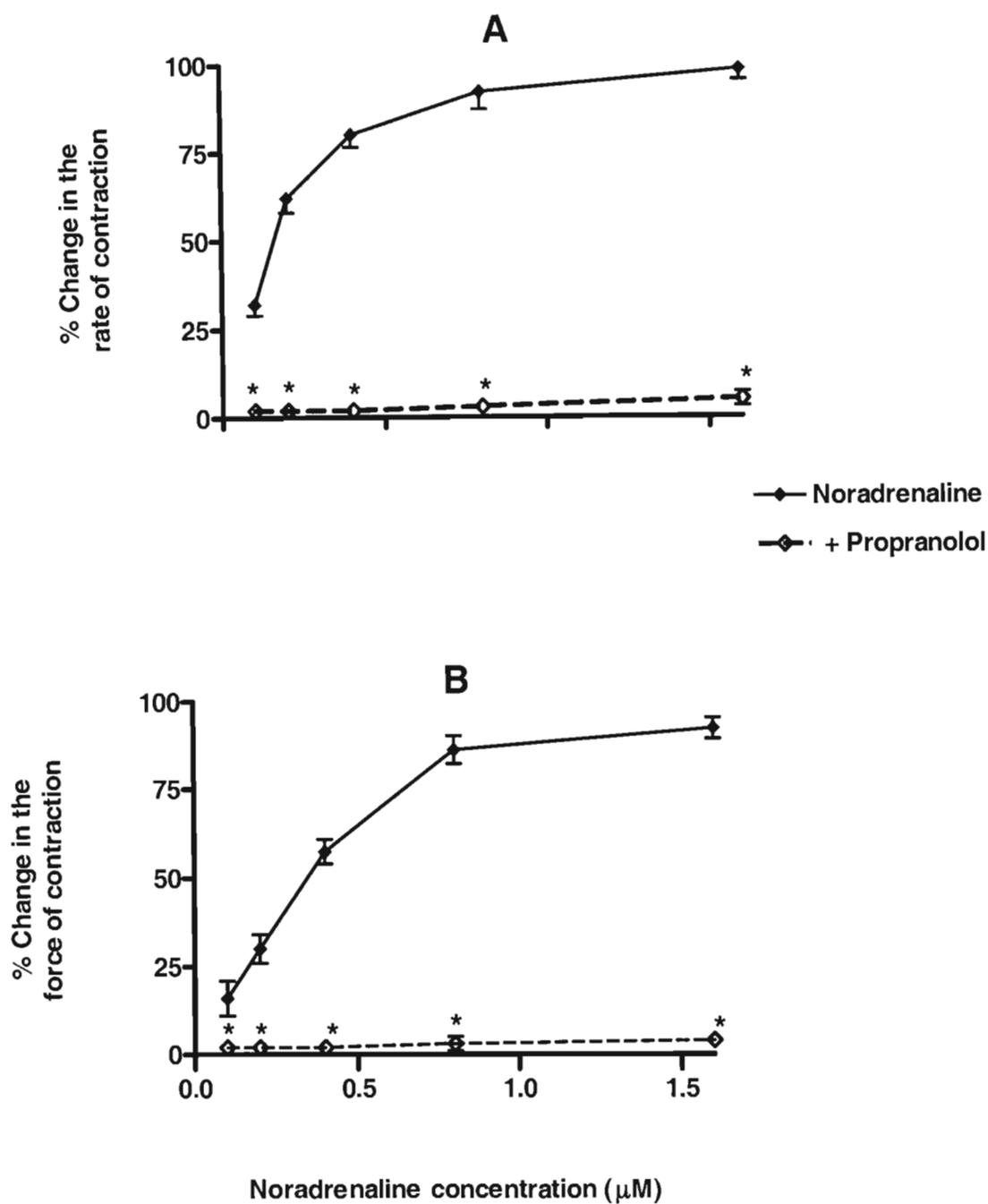
Figure 9.

Concentration-dependent response curves showing the effects of noradrenaline (0.1-1.6µM) on the rate (**A**) and force (**B**) of myocardial contraction in the absence and presence of propranolol (1µM). Values are means ± SEM, (n=8; for each concentration). P* <0.05 by comparison with noradrenaline alone.

4.2   Cardiotonic effects of acetylcholine (Ach).

The effects of acetylcholine on the rate of myocardial contraction were evaluated in the rat isolated spontaneously-beating right atria. Acetylcholine induced significant and concentration-dependent (0.2-1.0 μM) decreases in the rate of spontaneous contractions (negative chronotropy) of the right atrial muscles. This cardio-inhibitory effect of acetylcholine on the rate of myocardial contractions was completely abolished by prior treatment of the right atria with atropine sulphate (1 μM), a cholinergic receptor antagonist (Figure 10A). The effects of acetylcholine on the force of myocardial contraction were examined in the rat isolated electrically-driven left atria. Acetylcholine significantly reduced the force of myocardial contraction (negative inotropy) in a concentration-dependent manner (0.2-1.0 μM). Similarly, pretreatment of the left atrial muscle preparations with atropine sulphate (1μM) completely abolished the cardio-inhibitory effects of acetylcholine on the force of myocardial contraction (Figure 10B). Thus acetylcholine exhibited negative chronotropic and inotropic effects in rat isolated right and left atria respectively. These cardio-inhibitory effects of acetylcholine were completely abolished in the presence of atropine sulphate (Figure 10).
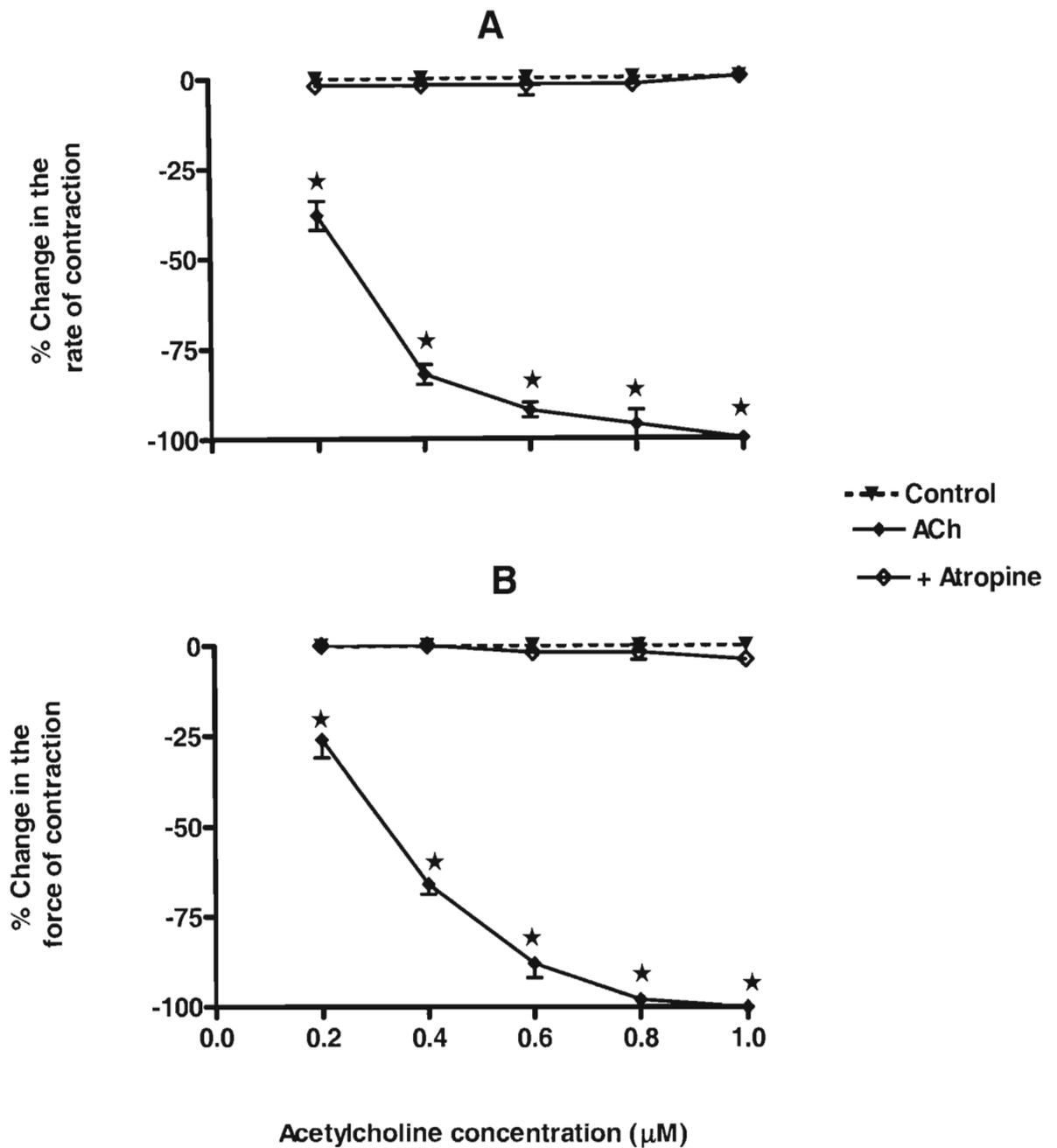
Figure 10.

Concentration-dependent response curves showing the effects of acetylcholine (0.2-1μM) on the rate **(A)** and force **(B)** of myocardial contraction in the absence and presence of atropine (1μM). Values are means ± SEM, (n=8; for each concentration). $P^\star$ <0.05 by comparison with control.

4.3   Cardiotonic effects of EKE

The effects of EKE on the rate of myocardial contraction were evaluated in rat isolated spontaneously-beating right atria.   EKE induced a significant and concentration-dependent (2.5-40 mg/ml) increase in the rate of spontaneous contractions (positive chronotropy) of the right atrial muscle preparations. In order to assess whether the positive chronotropic effect of EKE was mediated through stimulation of beta adrenergic receptors, the right atrial muscle preparations were pretreated with propranolol (1 μM), a non-selective beta-adrenergic receptor blocker. This treatment caused an almost complete abolition of the positive chronotropic effect of EKE (Figure 11A).

In addition, to investigate if the cardio-stimulatory effect of EKE involved activation of the cholinergic receptors, the right atrial muscle preparations were pretreated with atropine sulphate (1 μM). This pretreatment did not modify the effect of EKE on the rate of myocardial contraction (Figure 11A). In order to assess whether the positive chronotropic effect of EKE was mediated through modulation of noradrenaline release from junctional stores, the right atria were pretreated with reserpine (5 μM) to deplete junctional noradrenaline stores. However, this treatment did not significantly alter the positive chronotropic effects of EKE (Figure 11A). Involvement of the voltage-operated calcium channels in the chronotropic effect of EKE was also investigated. The right atrial muscle preparations were pretreated with nifedipine (1 μM), a voltage-operated calcium channel blocker. Nifedipine caused a significant reduction but not abolition of the positive chronotropic effect of EKE (Figure 11A).

The effects of EKE on the force of myocardial contraction were evaluated in rat isolated electrically-driven left atria. EKE significantly and concentration-dependently increased the force of myocardial contraction (positive inotropy) in a rat isolated left atria. Pretreatment of the left atrial muscle preparations with propranolol caused almost a complete inhibition of the positive inotropic effect of EKE (Figure 11B). In addition, pretreatment of the left atrial muscle preparations with nifedipine, caused a significant decrease but not an abolition of the positive inotropic effect of EKE (Figure 11B). However, pretreatment of the left atrial preparations with reserpine and atropine did not change the positive inotropic effect of EKE (Figure 11B).
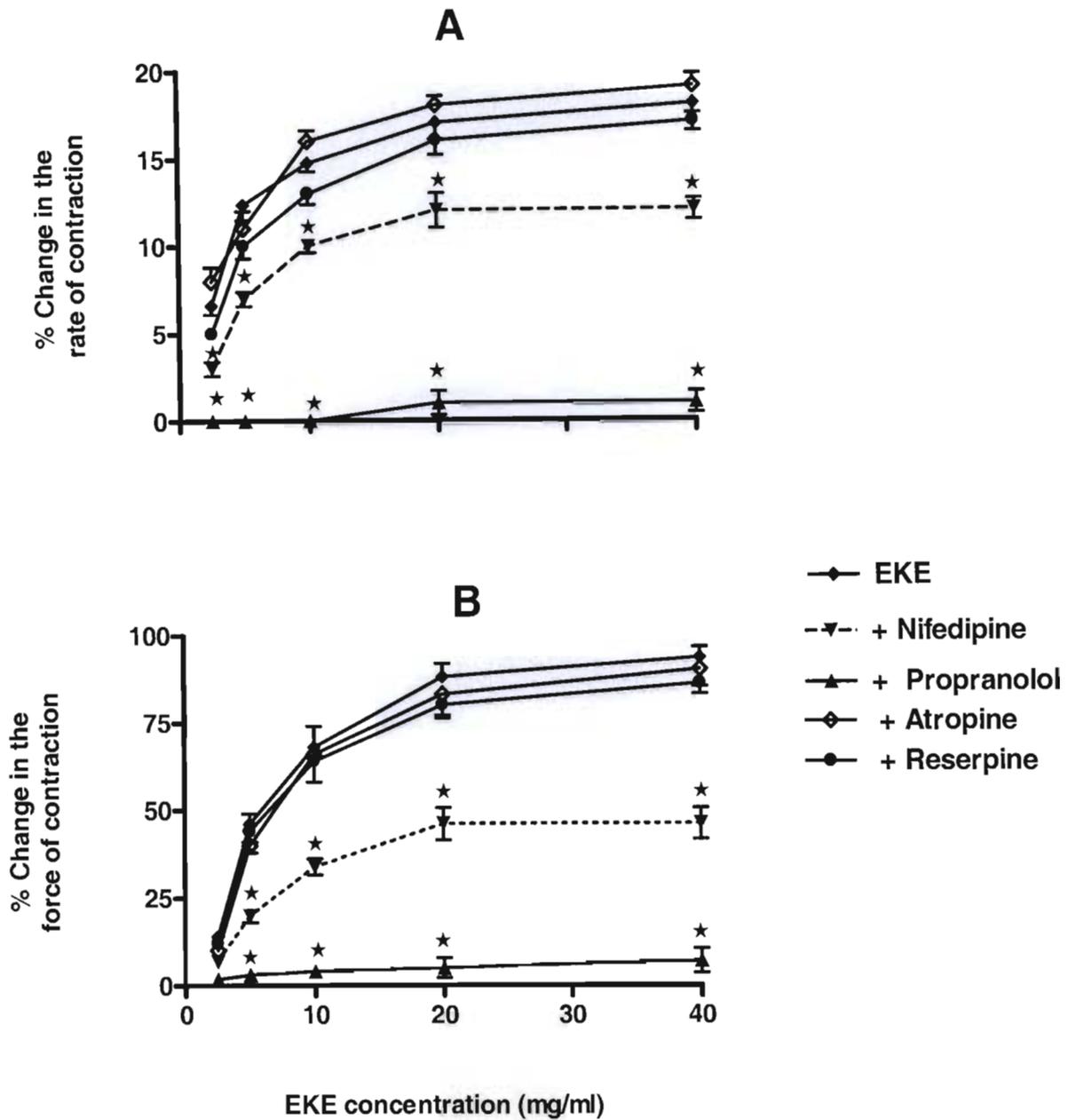
Figure 11.

Concentration-dependent response curves showing the cardio-stimulatory effects of EKE (2.5-40 mg/ml) on the rate **(A)** and force **(B)** of myocardial contraction in the absence and presence of atropine (1 μM), nifedipine (1 μM), propranolol (1 μM) and reserpine (5 μM). Values are means $\pm$ SEM, (n=8; for each concentration). $P^{\star}$ <0.05 by comparison with EKE alone.

4.4   Cardiotonic effects of APE

The effects of APE on the rate of myocardial contraction were evaluated in rat isolated spontaneously-beating right atria. APE induced a significant and concentration-dependent (12.5-400 mg/ml) decrease in the rate of spontaneous contractions (negative chronotropy) of the right atrial muscle preparations. In order to assess whether the negative chronotropic effect of APE was mediated through stimulation of cholinergic receptors, the right atrial muscle preparations were pretreated with atropine sulphate (1 μM), a cholinergic receptor antagonist. Pretreatment with atropine sulphate did not modify the cardio-inhibitory effect of APE on the rate of myocardial contraction (Figure 12A).

The effects of APE on the force of myocardial contraction were evaluated in rat isolated electrically-driven left atria. APE significantly and concentration-dependently decreased the force of myocardial contraction (negative inotropy) in a rat isolated left atria. Pretreatment of the left atrial muscle preparations with atropine sulphate did not alter the negative inotropic effect of APE (Figure 12B).

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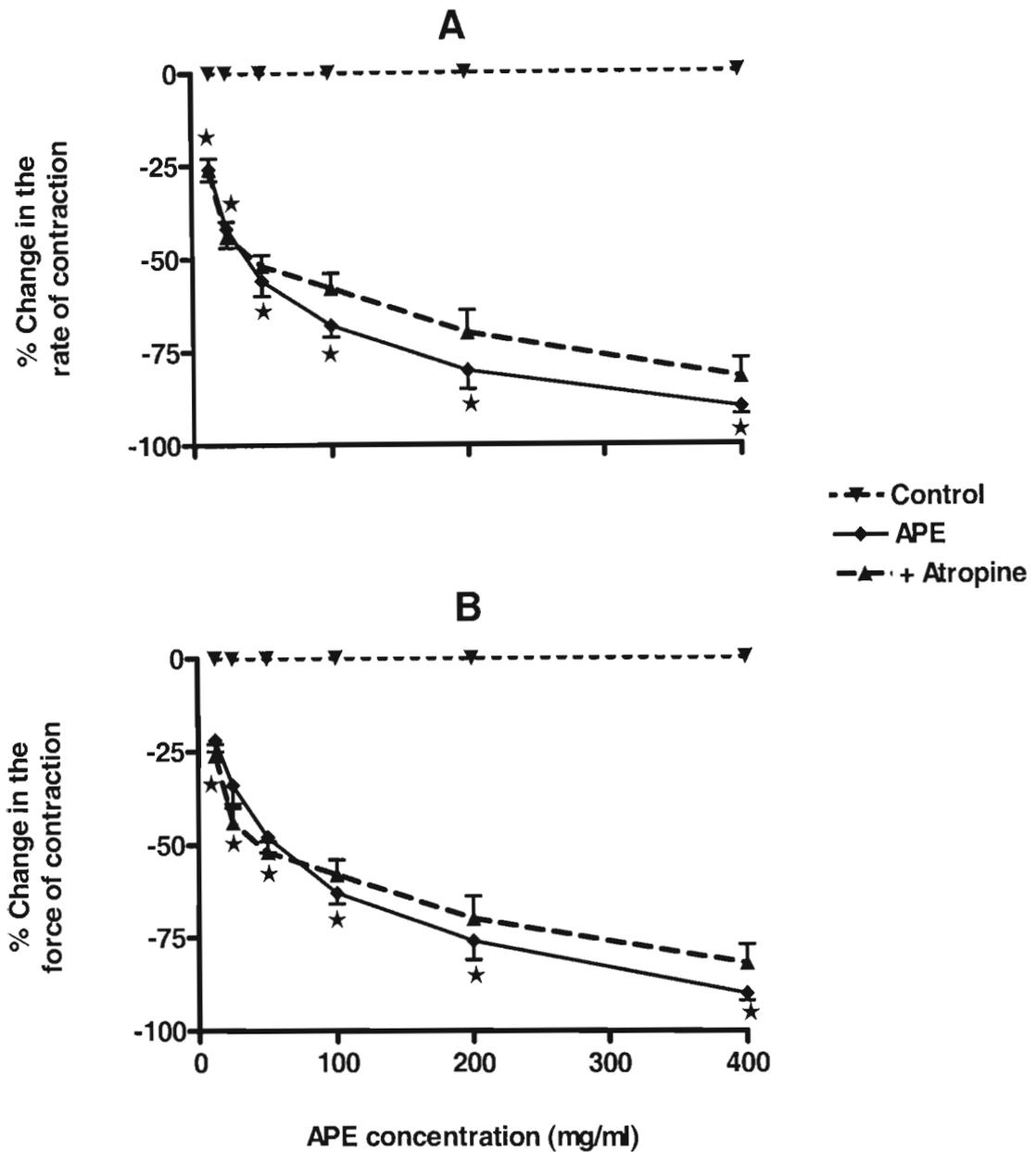
Figure 12.

Concentration-dependent response curves showing the cardio-inhibitory effects of APE (12.5-400 mg/ml) on the rate (**A**) and force (**B**) of myocardial contraction in the absence and presence of atropine (1μM). Values are means ± SEM, (n=8; for each concentration). P^* <0.05 by comparison with control

4.5 **Effects of APE on the positive inotropic effects of noradrenaline and calcium.**

The effect of APE on the concentration-dependent positive inotropic effect of noradrenaline was evaluated in rat isolated electrically-driven left atria. Firstly, a concentration-response curve for noradrenaline was established in the absence of APE (Figure 13A). The left atrial muscle preparations were then pretreated with APE. APE significantly antagonized the noradrenaline-induced positive inotropic effect in a concentration dependent manner (100 and 400 mg/ml) (Figure 13A). Similarly, APE antagonized the calcium-induced inotropic effect also in a concentration-dependent manner (100 and 400 mg/ml) (Figure 13B).
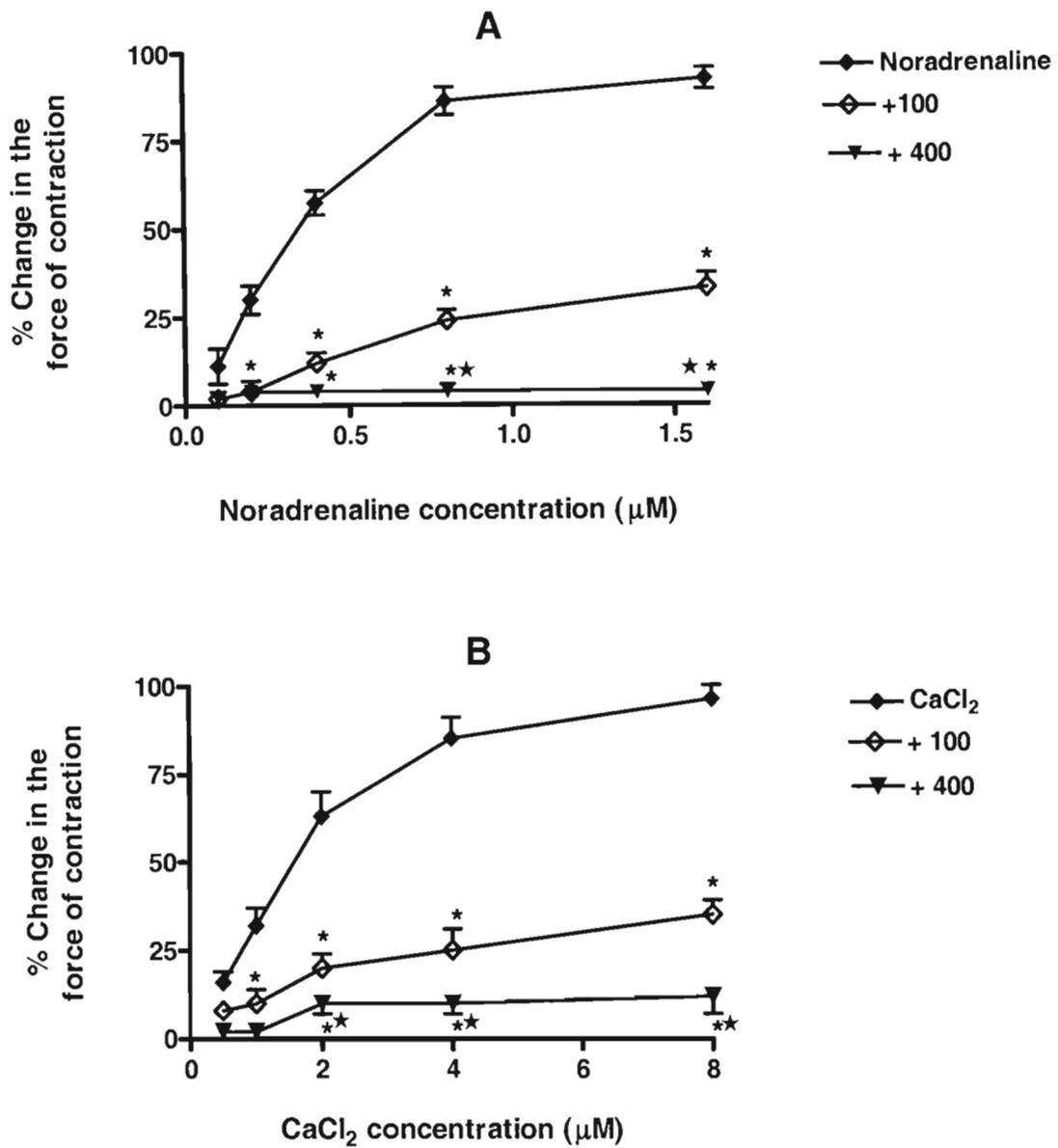
Figure 13.

Effects of APE (100 and 400 mg/ml) on the positive inotropic effects induced by noradrenaline **(A)** and $CaCl_2$ **(B)**. Values are means ± SEM, (n=8; for each concentration). $P* < 0.05$ by comparison with noradrenaline **(A)** or $CaCl_2$ **(B)** and $P^{\star} < 0.05$ by comparison with 100 mg/ml APE.

4.6   Cardiotonic effects of PAE

The effects of PAE on the rate of myocardial contraction were evaluated in rat isolated spontaneously-beating right atria.   PAE induced a significant and concentration-dependent (25-400 mg/ml) decrease in the rate of spontaneous contractions (negative chronotropy) of the right atrial muscle preparations. In order to investigate whether the negative chronotropic effect of PAE was mediated through activation of cholinergic receptors, the right atrial muscle preparations were pretreated with atropine sulphate (1 μM), a cholinergic receptor antagonist. Pretreatment with atropine sulphate did not affect the cardio-inhibitory effect of PAE on the rate of myocardial contraction (Figure 14A).

The effects of PAE on the force of myocardial contraction were evaluated in rat isolated electrically-driven left atria. PAE significantly and concentration-dependently decreased the force of myocardial contraction (negative inotropy) in a rat isolated left atria. Pretreatment of the left atrial muscle preparations with atropine sulphate did not change the negative inotropic effect of PAE (Figure 14B).
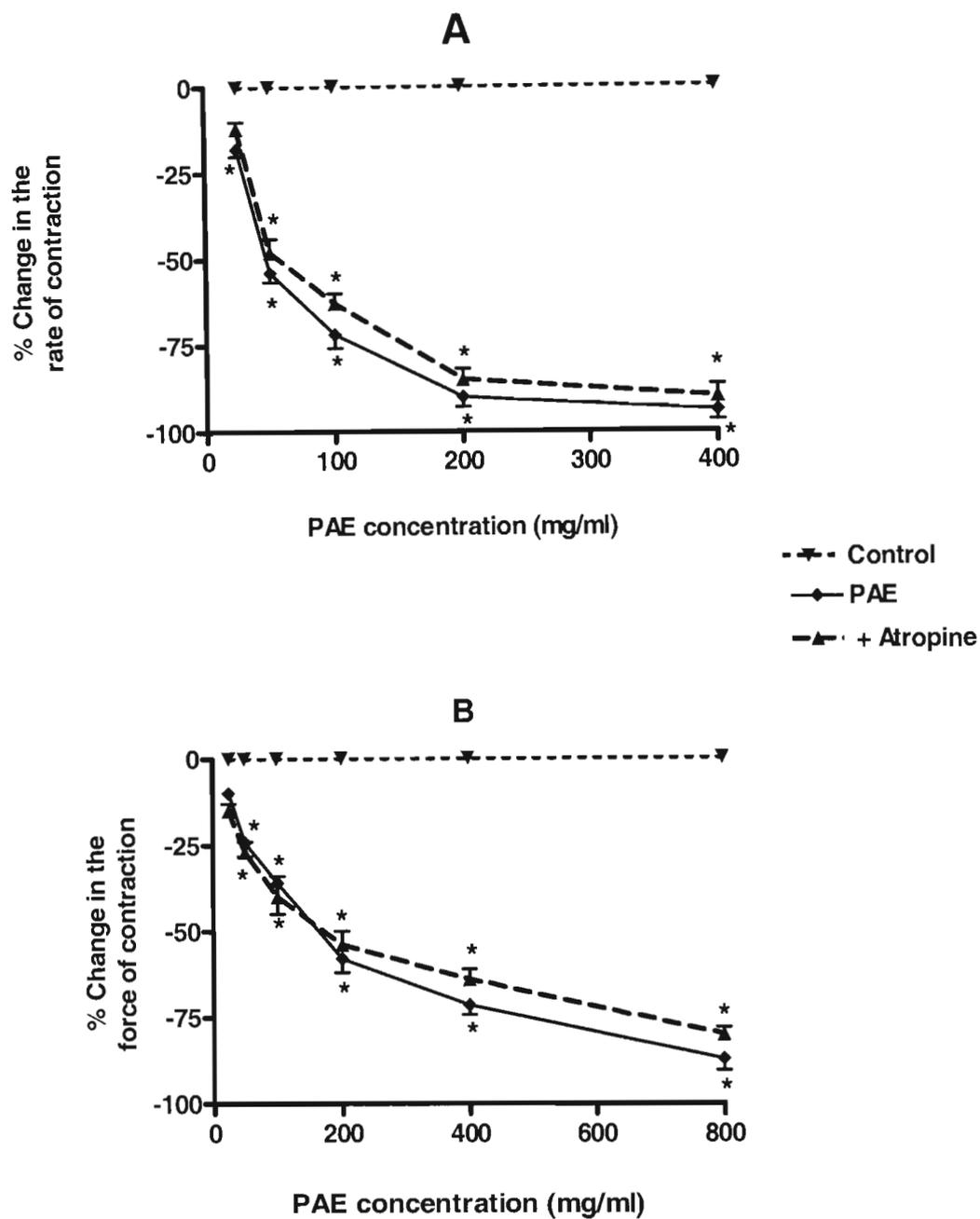
Figure 14.

Concentration-dependent response curves showing the cardio-inhibitory effects of PAE (25-800 mg/ml) on the rate **(A)** and force **(B)** of myocardial contraction in the absence and presence of atropine (1 μM). Values are means ± SEM, (n=8; for each concentration).

P^* <0.05 by comparison with control.

4.7   Effects of PAE on the positive inotropic effects of noradrenaline and calcium.

The effect of PAE on the concentration-dependent positive inotropic effect of noradrenaline was evaluated in rat isolated electrically-driven left atria. Firstly, a concentration-response curve for noradrenaline was established in the absence of PAE (Figure 15A). The left atrial muscle preparations were then pretreated with PAE. PAE significantly reversed the noradrenaline-induced positive inotropic effect in a concentration dependent manner (200 and 800 mg/ml) (Figure 15A). Similarly, PAE inhibited the calcium-induced positive inotropic effect also in a concentration-dependent manner (200 and 800 mg/ml) (Figure 15B).
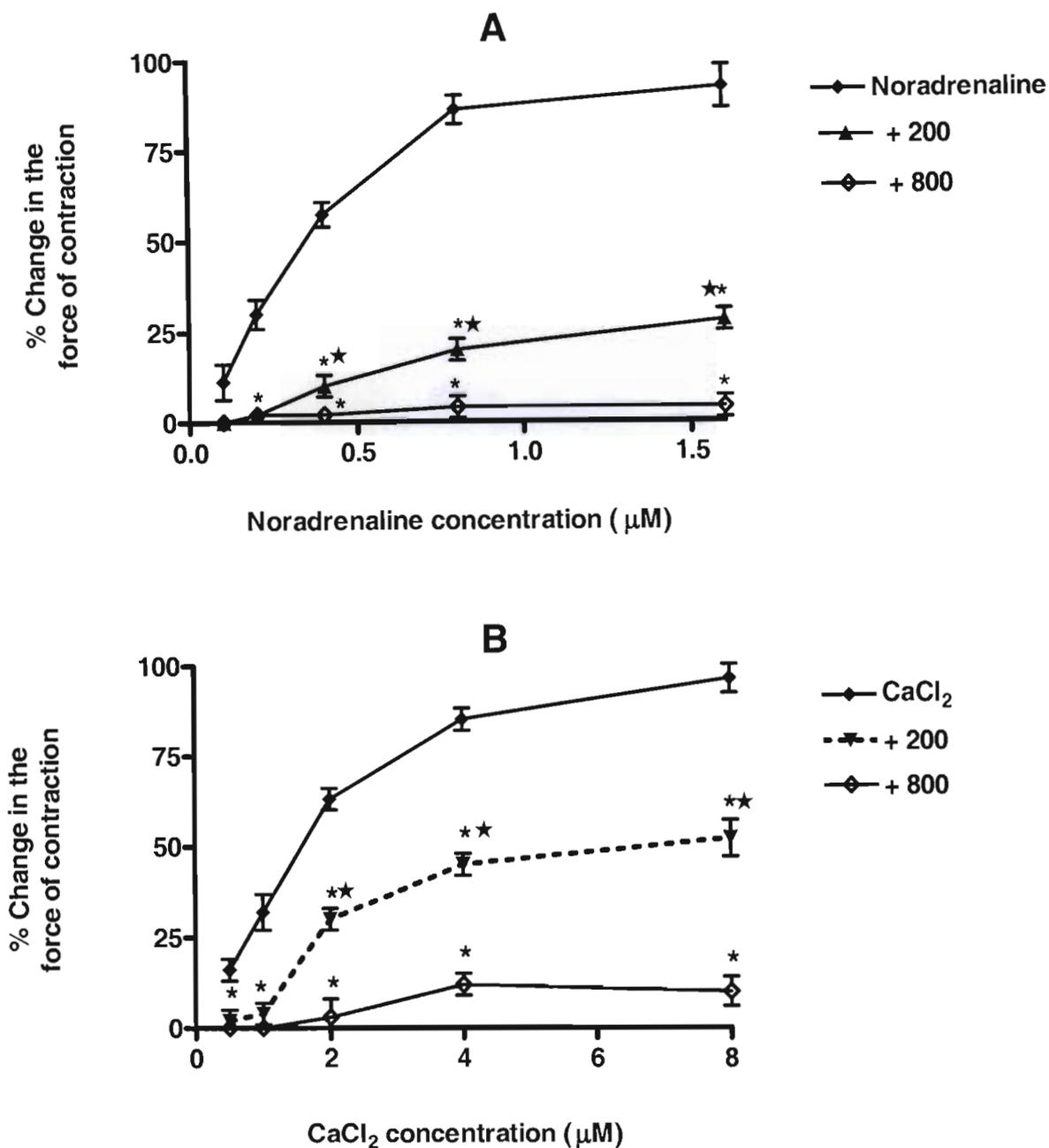
Figure 15.

Effects of PAE (200 and 800 mg/ml) on the positive inotropic effects of noradrenaline
(**A**) and $CaCl_2$ (**B**). Values are means ± SEM, (n=8; for each concentration).

$P^*< 0.05$ by comparison with noradrenaline (**A**) or $CaCl_2$ (**B**)

and $P^\star <0.05$ by comparison with 800 mg/ml PAE.

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4.8   Cardiotonic effects of HCE

The effects of HCE on the rate of myocardial contraction were evaluated in rat isolated spontaneously-beating right atria.   HCE induced a significant and concentration-dependent (10-160 mg/ml) decrease in the rate of spontaneous contractions (negative chronotropy) of the right atrial muscle preparations. In order to assess whether the negative chronotropic effect of HCE was mediated through activation of cholinergic receptors, the right atrial muscle preparations were pretreated with atropine sulphate (1 μM), a cholinergic receptor antagonist. Pretreatment of the atrial preparations with atropine sulphate elicited a complete abolition of the negative chronotropic effect of HCE (Figure 16A).

The effects of HCE on the force of myocardial contraction were evaluated in rat isolated electrically-driven left atria. HCE significantly and concentration-dependently decreased the force of myocardial contraction (negative inotropy) in a rat isolated left atria. However, pretreatment of the atrial preparations with atropine sulphate exhibited partial blockade of the negative inotropic effects of HCE, with complete inhibition of the effects of the three lower concentrations and significant inhibition but not complete abolition of the last two higher concentrations (Figure 16B).
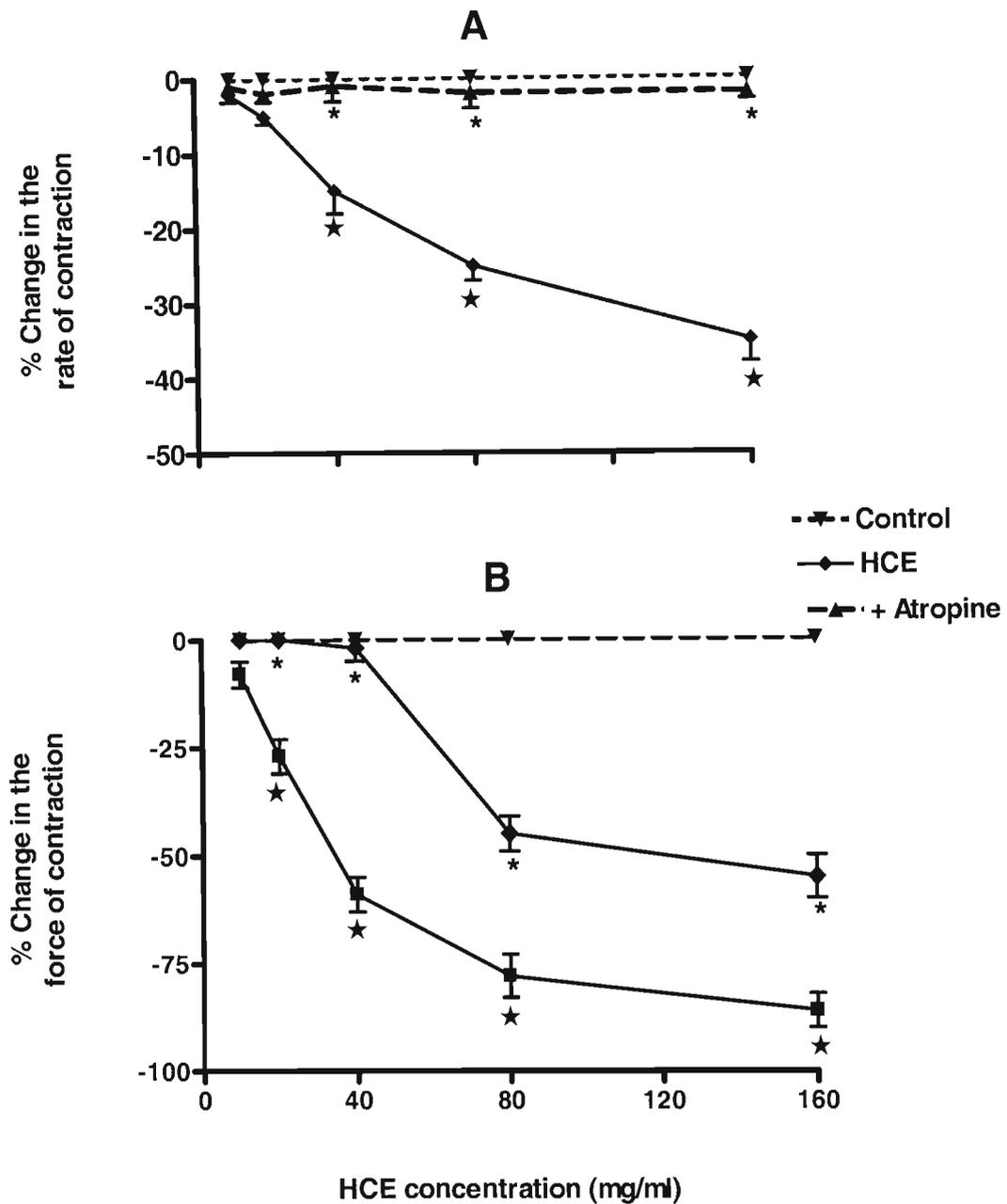
Figure 16.

Concentration-dependent response curves showing the cardio-inhibitory effects of HCE (10-160 mg/ml) on the rate **(A)** and force **(B)** of myocardial contraction in the absence and presence of atropine (1μM). Values are means ± SEM, (n=8; for each concentration). P^* <0.05 by comparison with control and P^* < 0.05 by comparison with HCE alone.

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4.9   **Effects of HCE on the positive inotropic effects of noradrenaline and calcium.**

The antagonistic effect of HCE was evaluated on the concentration-dependent positive inotropic effect of noradrenaline in rat isolated electrically-driven left atria. Firstly, a concentration-response curve for noradrenaline was established in the absence of HCE (Figure 17A). The left atrial muscle preparations were then pretreated with HCE. HCE significantly antagonized the noradrenaline-induced positive inotropic effect in a concentration dependent manner (40 and 80 mg/.ml) (Figure 17A). Similarly, HCE antagonized the calcium-induced inotropic effect also in a concentration-dependent manner (40 and 80 mg/.ml) (Figure 17B).
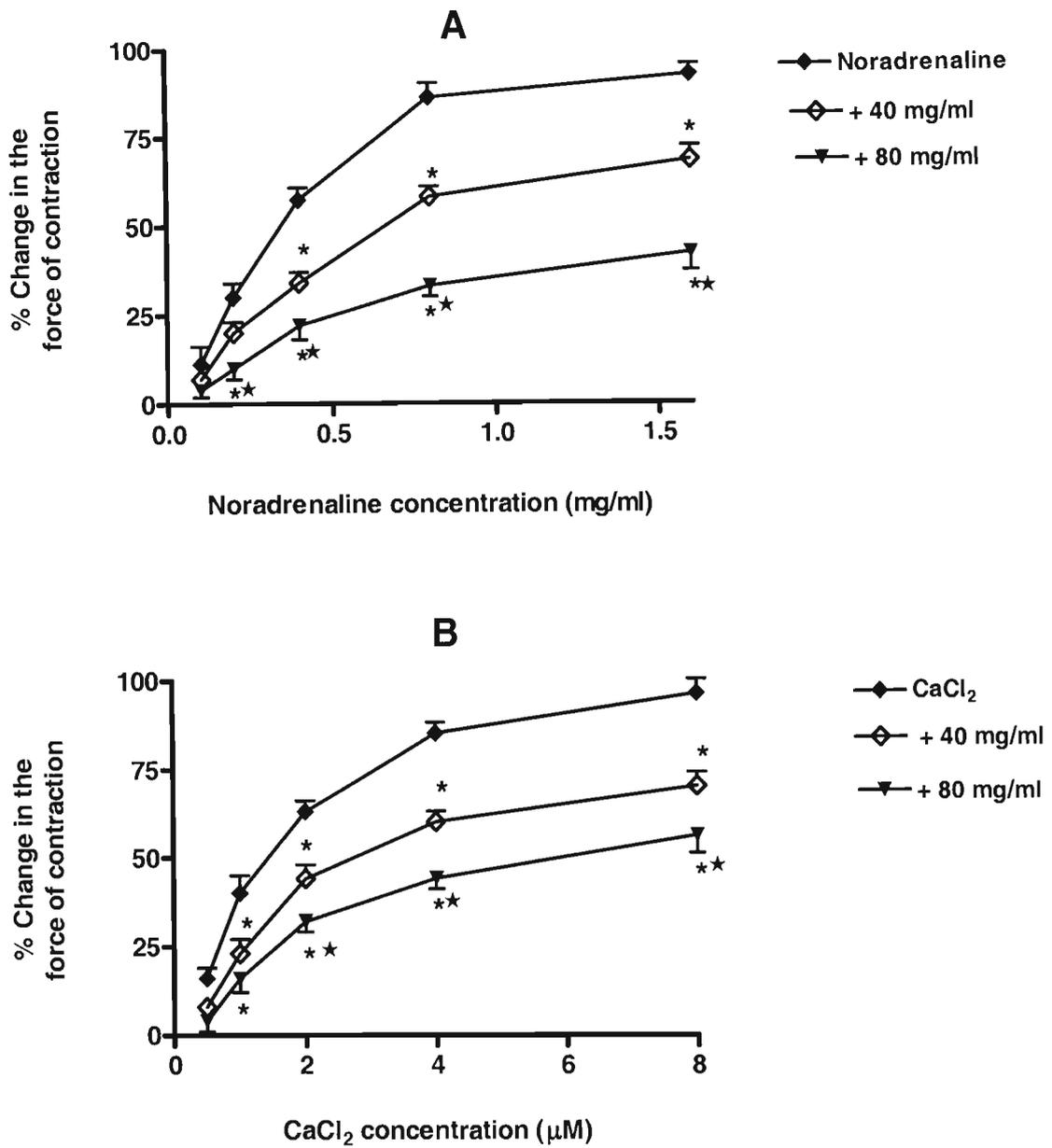
Figure 17

Antagonistic effects of HCE (40 and 80 mg/ml) on the positive inotropic effects of noradrenaline (**A**) and $CaCl_2$ (**B**). Values are means ± SEM, (n=8 for each concentration). $P*$ < 0.05 by comparison with noradrenaline (**A**) or $CaCl_2$ (**B**) and P^* <0.05 by comparison with 40 mg/.ml HCE.

4.10   Cardiotonic effects of SBE

The effects of SBE on the rate of myocardial contraction were evaluated in rat isolated spontaneously-beating right atria. SBE induced a significant and concentration-dependent (25-400 mg/.ml) decrease in the rate of spontaneous contractions (negative chronotropy) of the right atrial muscle preparations. In order to investigate whether the negative chronotropic effect of SBE was mediated through activation of cholinergic receptors, the right atrial muscle preparations were pretreated with atropine sulphate (1 μM), a cholinergic receptor antagonist. Pretreatment with atropine sulphate did not affect the cardio-inhibitory effect of SBE on the rate of myocardial contraction (Figure 18A).

The effects of SBE on the force of myocardial contraction were evaluated in rat isolated electrically-driven left atria. SBE significantly and concentration-dependently decreased the force of myocardial contraction (negative inotropy) in a rat isolated left atria (Figure 18B). Pretreatment of the left atrial muscle preparations with atropine sulphate did not affect the negative inotropic effect of SBE.
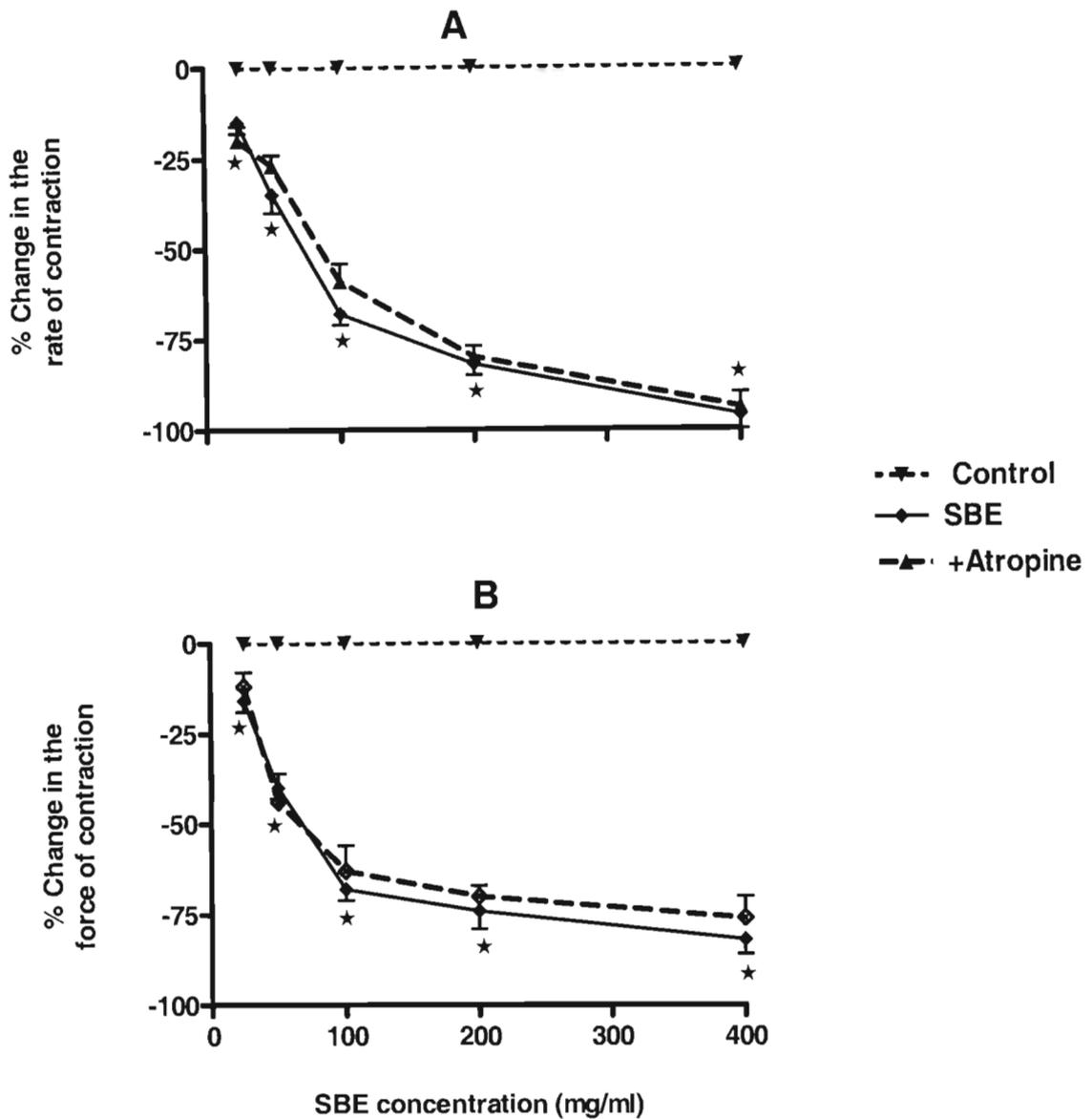
Figure 18

Concentration-dependent response curves showing the cardio-inhibitory effects of SBE (25-400 mg/ml) on the rate (**A**) and force (**B**) of myocardial contraction in the absence and presence of atropine (1μM). Values are means ± SEM, (n=8; for each concentration). P^* <0.05 by comparison with control.

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4.11   Effects of SBE on the positive inotropic effects of noradrenaline and calcium.

The effect of SBE on the concentration-dependent positive inotropic effect of noradrenaline was evaluated in rat isolated electrically-driven left atria. Firstly, a concentration-response curve for noradrenaline was established in the absence of SBE (Figure 19A). The left atrial muscle preparations were then pretreated with SBE. SBE significantly inhibited the noradrenaline-induced positive inotropic effect in a concentration dependent manner (100 and 400 mg/.ml) (Figure 19A). SBE also inhibited the calcium-induced inotropic effect in a concentration-dependent manner (100 and 400 mg/.ml) (Figure 19B).
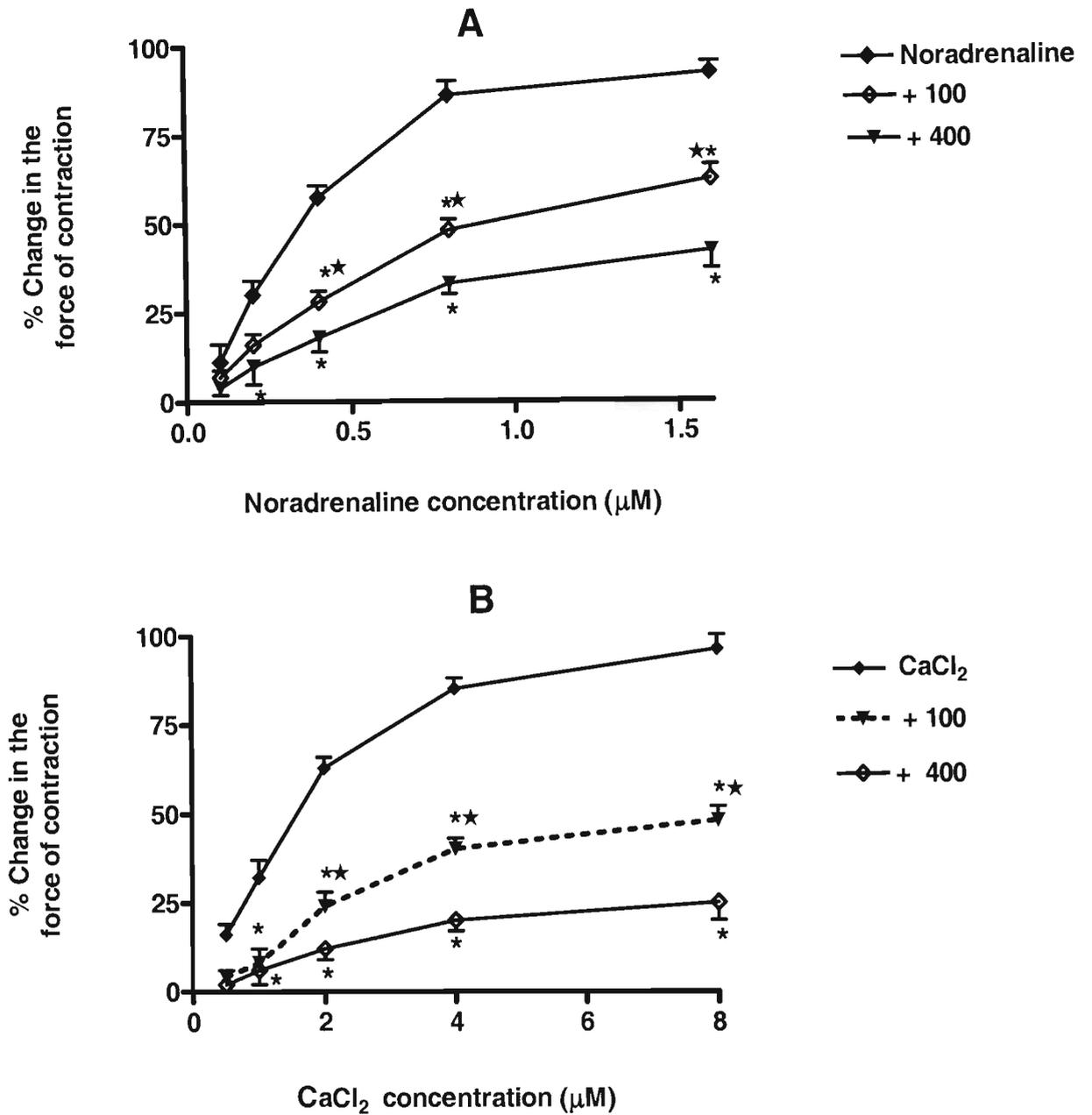
Figure 19.

Effects of SBE (100 and 400 mg/.ml) on the positive inotropic effects of noradrenaline
(A) and $CaCl_2$ (B). Values are means ± SEM, (n=8; for each concentration).

$P^* < 0.05$ by comparison with noradrenaline (A) or $CaCl_2$ (B) and $P^{\star} < 0.05$ by comparison with 400 mg/.ml SBE.

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CHAPTER 5

5.0 *IN VITRO* VASCULAR EFFECTS OF EXTRACTS.

This chapter will describe the following; (1) vasorelaxant effects of various extracts evaluated on endothelium-intact and endothelium-deprived aortic rings; (2) the role of endothelial-derived relaxing factors in the vasorelaxant effects of the various extracts; (3) the role of calcium and potassium channels in the vasorelaxant effects of various extracts and (4) the effects of various extracts on the spontaneous-myogenic contractions of the portal vein.

5.1 Vasorelaxant effects of EKE.

The vasorelaxant effects of EKE were evaluated in endothelium-intact aortic rings precontracted with methoxamine hydrochloride ($10\mu M$), an alpha-1 adrenergic receptor agonist to establish a cumulative concentration-response curve for EKE. EKE induced an endothelium and concentration-dependent (10-160 mg/ml) vasorelaxation in intact aortic rings precontracted with methoxamine hydrochloride (Figure 20A).

5.1.1. Role of the endothelium

In order to assess the role of the functional endothelium in the vasorelaxant effects of EKE, tests were conducted in denuded-aortic rings precontracted with methoxamine hydrochloride. When contraction reached a stable plateau, a cumulative concentration-

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response curve for EKE was re-established and compared to that of intact aortic rings. The removal of endothelium significantly inhibited the vasorelaxation induced by EKE (Figure 20A).

5.1.2   Role of endothelial vasodilators.

To examine the role of nitric oxide in the vasorelaxant effects of EKE, endothelium-intact aortic rings were pretreated with L-NAME (100 μM), a nitric oxide synthase inhibitor before the contractions were evoked by methoxamine hydrochloride. L-NAME significantly inhibited the vasorelaxation induced by EKE (Figure 20B).

To examine the role of prostacyclin in the vasorelaxant effects of EKE, endothelium-intact aortic rings were pretreated with indomethacin (10 μM), a cyclooxygenase inhibitor before the contractions were evoked by methoxamine hydrochloride. Indomethacin significantly inhibited the vasorelaxant effect induced by EKE (Figure 20B).

To assess the involvement of cGMP in the vasorelaxant effects of EKE, endothelium-intact aortic rings were pretreated with methylene blue (20 μM), a guanylate cyclase inhibitor before the contractions were induced by methoxamine hydrochloride. Methylene blue significantly inhibited the vasorelaxant effect of EKE (Figure 20B).
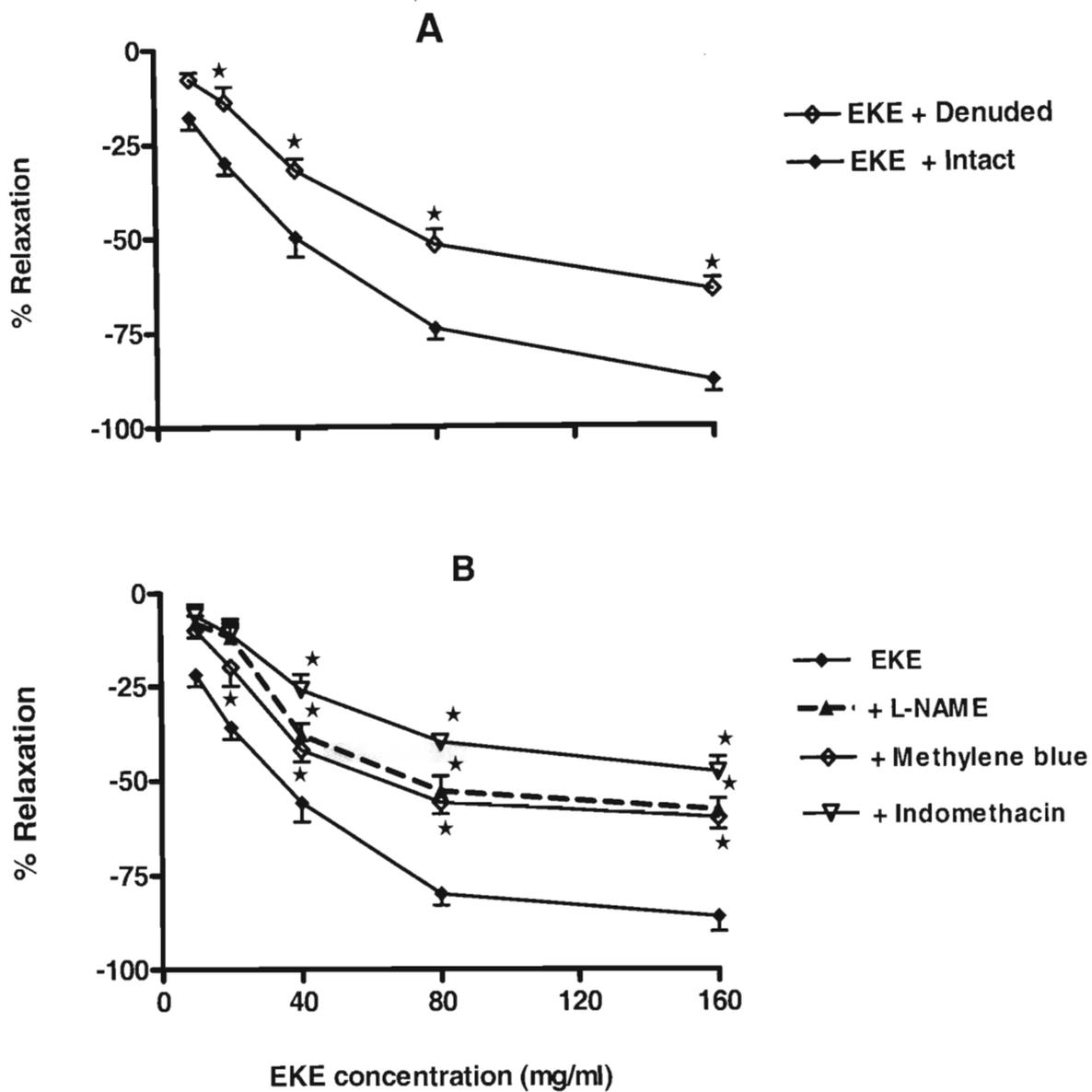
Figure 20.

Concentration-dependent vasorelaxant effects of EKE (10-160 mg/.ml) on rat endothelium-intact and denuded aortic rings, precontracted with methoxamine in the absence (**A**) and presence (**B**) of L-NAME (100µM), indomethacin (10µM) and methylene blue (20µM). Values are means ± SEM, (n=8; for each concentration).

$P^\star$ <0.05 by comparison with EKE+ intact (**A**) or EKE alone (**B**)

5.1.3 Role of calcium and potassium channels.

To evaluate the role of calcium channels in the vasorelaxant effects of EKE, tests were conducted in intact-aortic rings precontracted with high (K^+ 80mM). EKE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with high (K^+80mM), (Figure 21).

To evaluate the role of potassium channels in the vasorelaxant effects of EKE, the test was conducted in intact-aortic rings precontracted with low (K^+ 20mM). EKE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low (K^+ 20mM). Pretreatment of the aortic rings with glibenclamide, before precontracted with low (K^+20mM), did not modify the vasorelaxant effect of EKE (Figure 21).
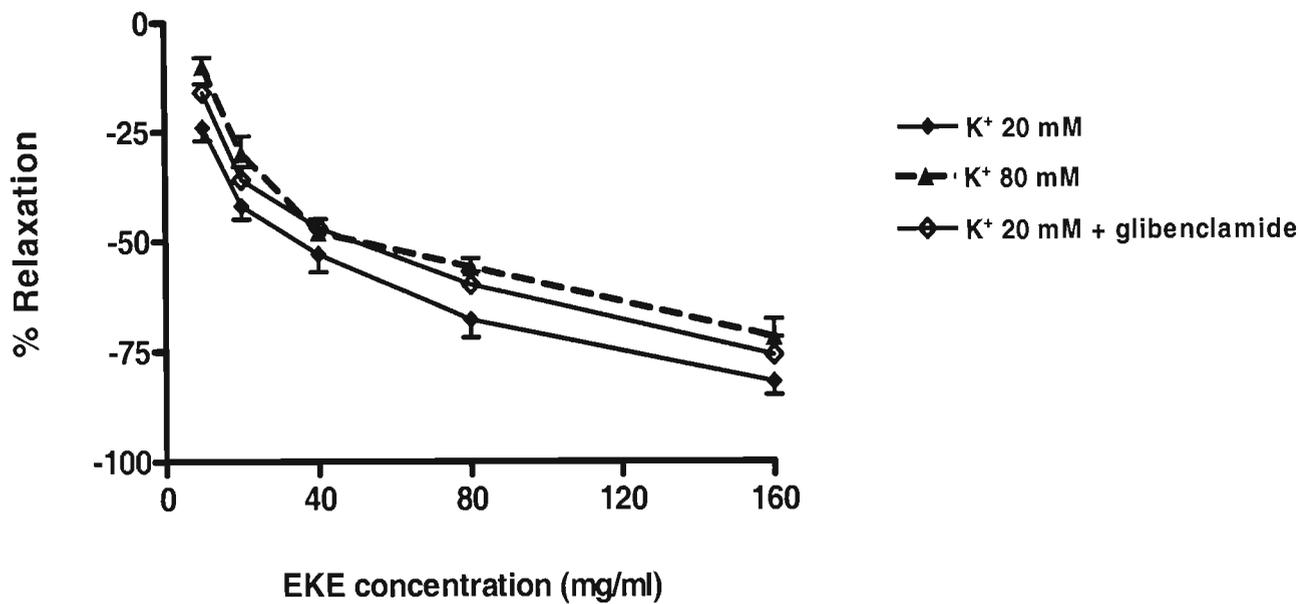
Figure 21.

Concentration-dependent vasorelaxant effects of EKE (10-160 mg/.ml) on rat isolated endothelium-intact aortic rings precontracted with high K^+ (80 mM) or low K^+ (20 mM) concentrations in the absence and presence of glibenclamide (3μM). Values are means ± SEM, (n=8; for each concentration).

5.2 Vasorelaxant effects of APE.

The vasorelaxant effects of APE were evaluated in endothelium-intact aortic rings precontracted with methoxamine hydrochloride (10μM), an alpha-1 adrenergic receptor agonist to establish a cumulative concentration-response curve for APE. APE induced an endothelium and concentration-dependent (5-160 mg/.ml) vasorelaxation in intact aortic rings precontracted with methoxamine hydrochloride (Figure 22A).

5.2.1 Role of endothelium.

In order to assess the role of the functional endothelium in the vasorelaxant effects of APE, studies were conducted in denuded-aortic rings precontracted with methoxamine hydrochloride. When contraction reached a stable plateau, a cumulative concentration-response curve for APE was established and compared to that of intact aortic rings. The removal of endothelium completely abolished the vasorelaxation induced by APE (Figure 22A).

5.2.2 Role of endothelial vasodilators.

To examine the role of nitric oxide in the vasorelaxant effects of APE, endothelium-intact aortic rings were pretreated with L-NAME (100 μM), a nitric oxide synthase inhibitor before the contractions were evoked by methoxamine hydrochloride. L-NAME significantly reduced the vasorelaxant effect of APE (Figure 22B).

To examine the role of prostacyclin in the vasorelaxant effects of APE, endothelium-intact aortic rings were pretreated with indomethacin (10 µM), a cyclooxygenase inhibitor before the contractions were evoked by methoxamine hydrochloride. Indomethacin significantly inhibited the vasorelaxation induced by APE (Figure 22B).

To assess the involvement of cGMP in the vasorelaxant effects of APE, endothelium-intact aortic rings were pretreated with methylene blue (20 µM) a guanylate cyclase inhibitor before the contractions were induced by methoxamine hydrochloride. Methylene blue significantly inhibited the vasorelaxation induced by APE (Figure 22B).
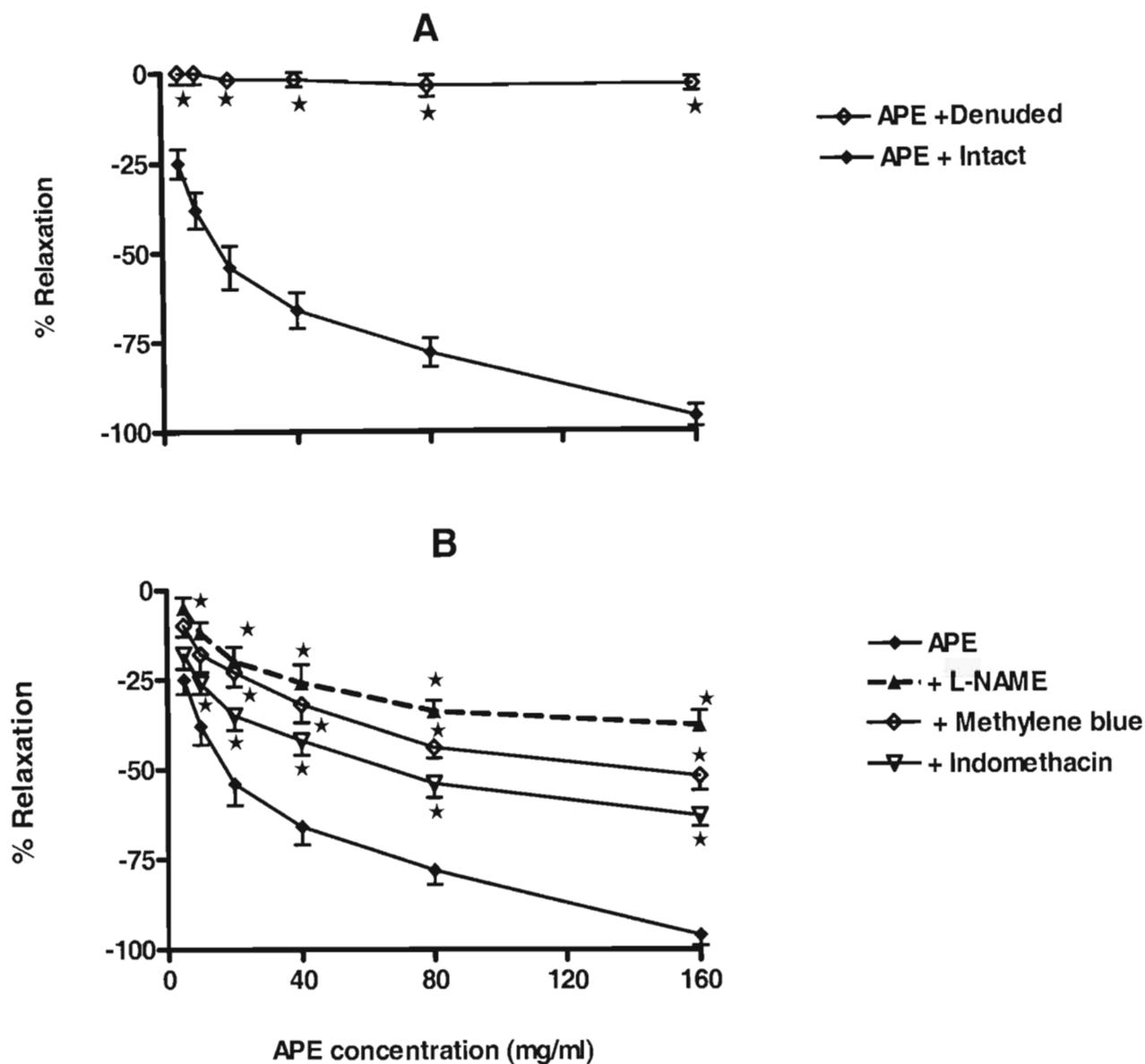
**Figure 22.**

Concentration-dependent vasorelaxant effects of APE (5-160 mg/.ml) on rat endothelium-intact and denuded aortic rings, precontracted with methoxamine in the absence (**A**) and presence (**B**) of L-NAME (100μM), indomethacin (10μM) and methylene blue (20μM). Values are means ± SEM, (n=8; for each concentration).

P^* <0.05 by comparison with APE+ intact (**A**) or APE alone (**B**).

5.2.3   Role of calcium and potassium channels.

To evaluate the role of calcium channels in the vasorelaxant effects of APE, studies were conducted in intact-aortic rings precontracted with high (K^+ 80mM). APE induced a weak but significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with high (K^+80mM) (Figure 23).

To evaluate the role of potassium channels in the vasorelaxant effects of APE, investigations were conducted in intact-aortic rings precontracted with low (K^+ 20mM). APE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low (K^+ 20mM). Pretreatment of the aortic rings with glibenclamide, before precontracted with low (K^+20mM), caused a significant reduction in the vasorelaxant effects of APE (Figure 23).
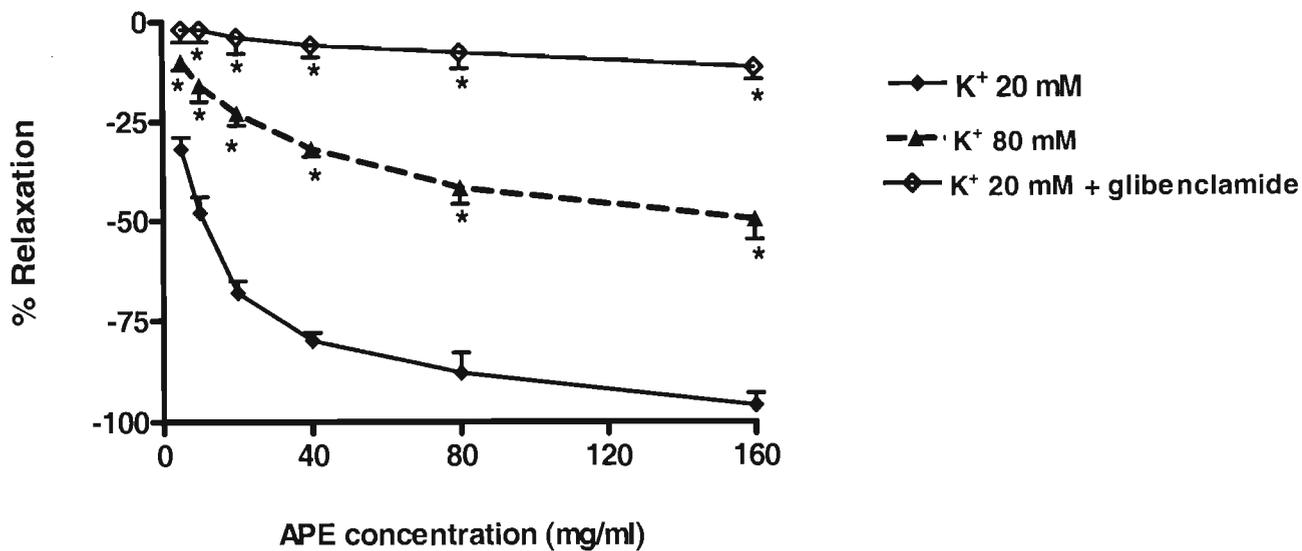
Figure 23.

Concentration-dependent vasorelaxant effects of APE (5-160 mg/.ml) on rat isolated

endothelium-intact aortic rings precontracted with high K^+ (80 mM) or low K^+ (20 mM)

concentrations in the absence and presence of glibenclamide (3µM). Values are means ±

SEM, (n=8; for each concentration).

$P^* < 0.05$ by comparison with K^+ (20 mM).

5.3   Vasorelaxant effects of PAE.

The vasorelaxant effects of PAE were evaluated in endothelium-intact aortic rings precontracted with methoxamine hydrochloride (10µM), an alpha-1 adrenergic receptor agonist to establish a cumulative concentration-response curve for PAE. PAE induced an endothelium and concentration-dependent (50-800 mg/.ml) vasorelaxation in intact aortic rings precontracted with methoxamine hydrochloride (Figure 24A).

5.3.1   Role of endothelium.

In order to assess the role of the functional endothelium in the vasorelaxant effects of PAE, tests were conducted in denuded-aortic rings precontracted with methoxamine hydrochloride. When contraction reached a stable plateau, a cumulative concentration-response curve for PAE was established and compared to that of intact aortic rings. The removal of endothelium significantly inhibited the vasorelaxation induced by PAE (Figure 24A).

5.3.2   Role of endothelial vasodilators.

To examine the role of nitric oxide in the vasorelaxant effects of PAE, endothelium-intact aortic rings were pretreated with L-NAME (100 µM), a nitric oxide synthase inhibitor before the contractions were evoked by methoxamine hydrochloride. L-NAME significantly inhibited the vasorelaxation induced by PAE (Figure 24B).

To examine the role of prostacyclin in the vasorelaxant effects of PAE, endothelium-intact aortic rings were pretreated with indomethacin (10 μM), a cyclooxygenase inhibitor before the contractions were evoked by methoxamine hydrochloride. The vasorelaxant effect of PAE was not affected in the presence of indomethacin (Figure 24B).

To assess the involvement of cGMP in the vasorelaxant effects of PAE, endothelium-intact aortic rings were pretreated with methylene blue (20 μM) a guanylate cyclase inhibitor before the contractions were induced by methoxamine hydrochloride. Methylene blue significantly inhibited the vasorelaxation induced by PAE (Figure 24B).
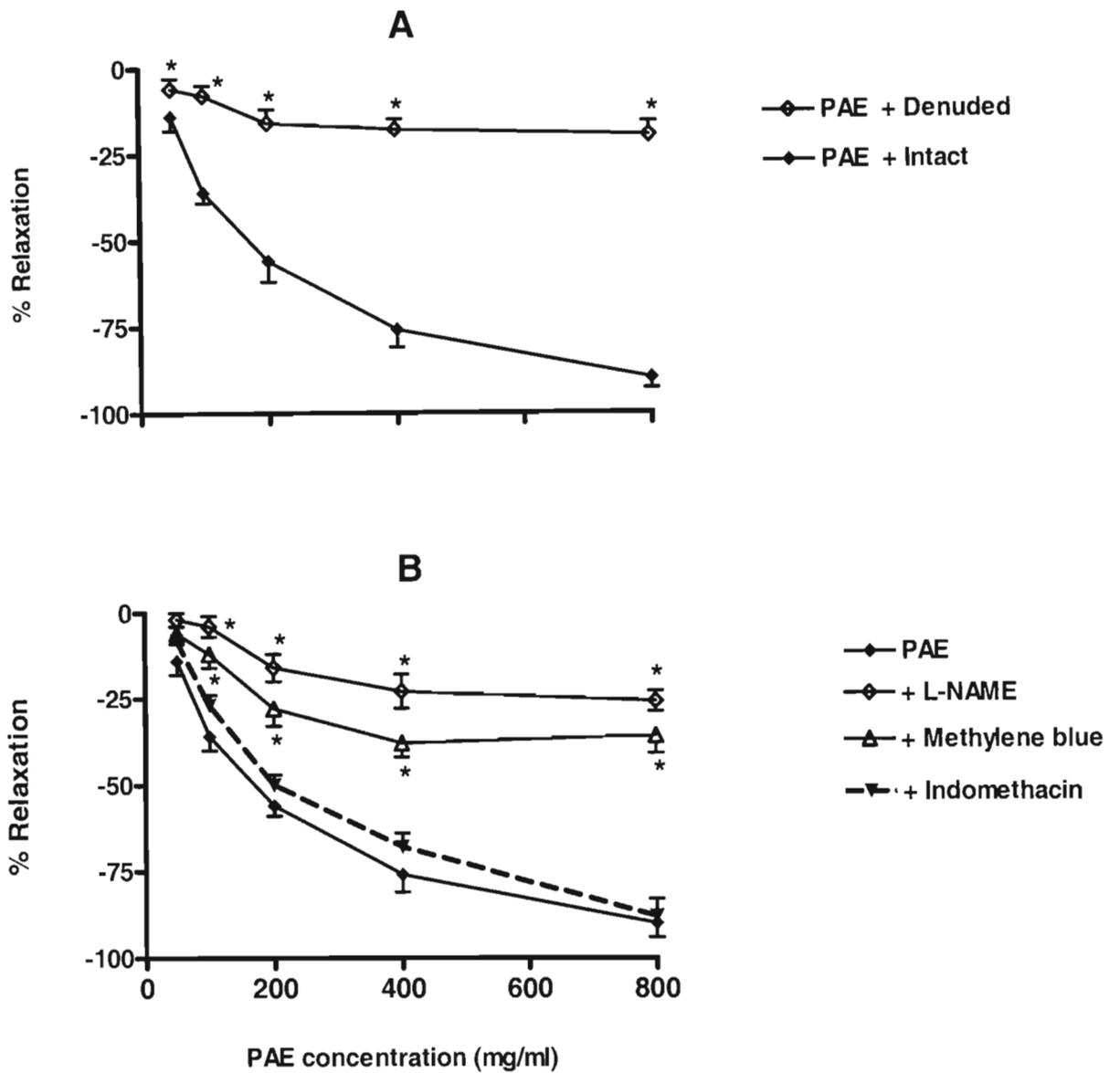
Figure 24.

Concentration-dependent vasorelaxant effects of PAE (50-800 mg/.ml) on rat endothelium-intact and denuded aortic rings, precontracted with methoxamine in the absence (**A**) and presence (**B**) of L-NAME (100μM), indomethacin (10μM) and methylene blue (20μM). Values are means ± SEM, (n=8; for each concentration). P^* <0.05 by comparison with PAE + intact (**A**) or PAE alone (**B**).

5.3.3   Role of calcium and potassium channels.

To evaluate the role of calcium channels in the vasorelaxant effects of PAE, studies were conducted in intact-aortic rings precontracted with high (K^+ 80mM). PAE induced weak vasorelaxation in the endothelium-intact aortic rings precontracted with high (K^+80mM) (Figure 25).

To evaluate the role of potassium channels in the vasorelaxant effects of PAE, investigations were conducted in intact-aortic rings precontracted with low (K^+ 20mM). PAE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low (K^+ 20mM). Pretreatment of the aortic rings with glibenclamide, before precontracted with low (K^+20mM), significantly inhibited the vasorelaxant effects of PAE (Figure 25).
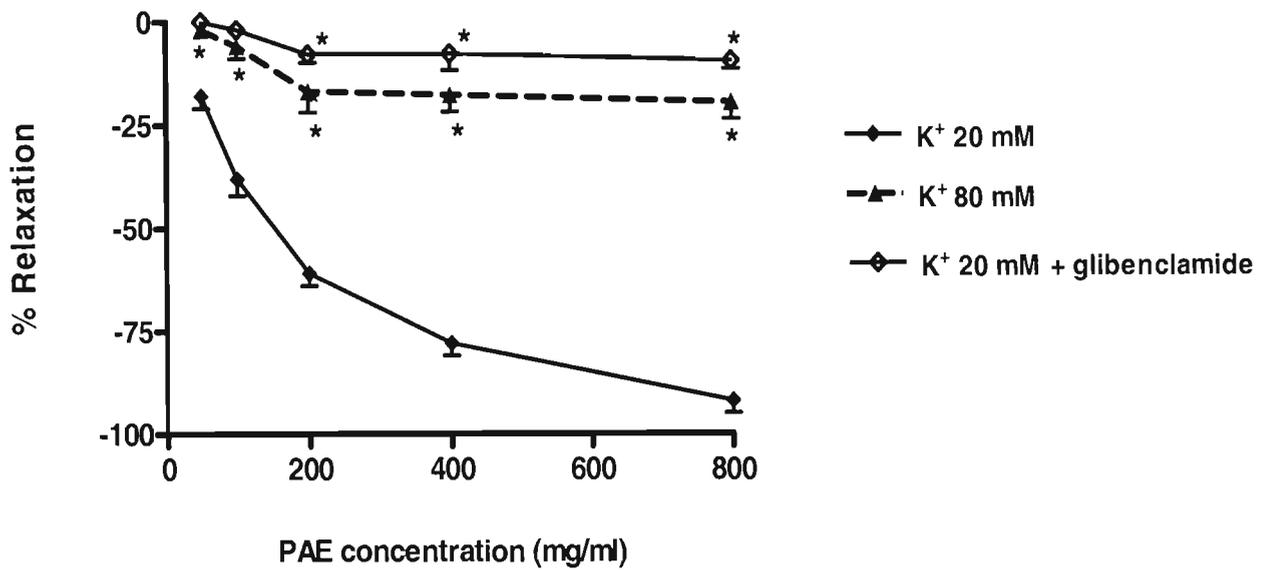
Figure 25.

Concentration-dependent vasorelaxant effects of PAE (50-800 mg/.ml) on rat isolated endothelium-intact aortic rings precontracted with high K^+ (80 mM) or low K^+ (20 mM) concentrations in the absence and presence of glibenclamide (3μM). Values are means ± SEM, (n=8; for each concentration). $P* < 0.05$ by comparison with K^+ (20 mM).

5.4 Vasorelaxant effects of HCE.

The vasorelaxant effects of HCE were evaluated in endothelium-intact aortic rings precontracted with methoxamine hydrochloride ($10\mu M$), an alpha-1 adrenergic receptor agonist to establish a cumulative concentration-response curve for the extract. HCE induced an endothelium and concentration-dependent (10-160 mg.ml^{-1}) vasorelaxation in intact aortic rings precontracted with methoxamine hydrochloride (Figure 26A).

5.4.1 Role of endothelium.

To investigate the role of the functional endothelium in the vasorelaxant effects of HCE, the test was conducted in denuded-aortic rings precontracted with methoxamine hydrochloride. When contraction reached a stable plateau, a cumulative concentration-response curve for HCE was established and compared to that of intact aortic rings. The removal of endothelium significantly inhibited the vasorelaxation induced by HCE (Figure 26A).

5.4.2 Role of endothelial vasodilators.

To examine the role of nitric oxide in the vasorelaxant effects of HCE, endothelium-intact aortic rings were pretreated with L-NAME (100 μM), a nitric oxide synthase inhibitor before the contractions were evoked by methoxamine hydrochloride. L-NAME significantly inhibited the vasorelaxation induced by HCE (Figure 26B).

To examine the role of prostacyclin in the vasorelaxant effects of HCE, endothelium-intact aortic rings were pretreated with indomethacin (10 µM), a cyclooxygenase inhibitor before the contractions were evoked by methoxamine hydrochloride. Indomethacin significantly inhibited the vasorelaxation induced by HCE (Figure 26B).

To assess the involvement of cGMP in the vasorelaxant effects of HCE, endothelium-intact aortic rings were pretreated with methylene blue (20 µM) a guanylate cyclase inhibitor before the contractions were induced by methoxamine hydrochloride. Methylene blue significantly inhibited the vasorelaxation induced by HCE (Figure 26B).

A

B

HCE concentration (mg/ml)
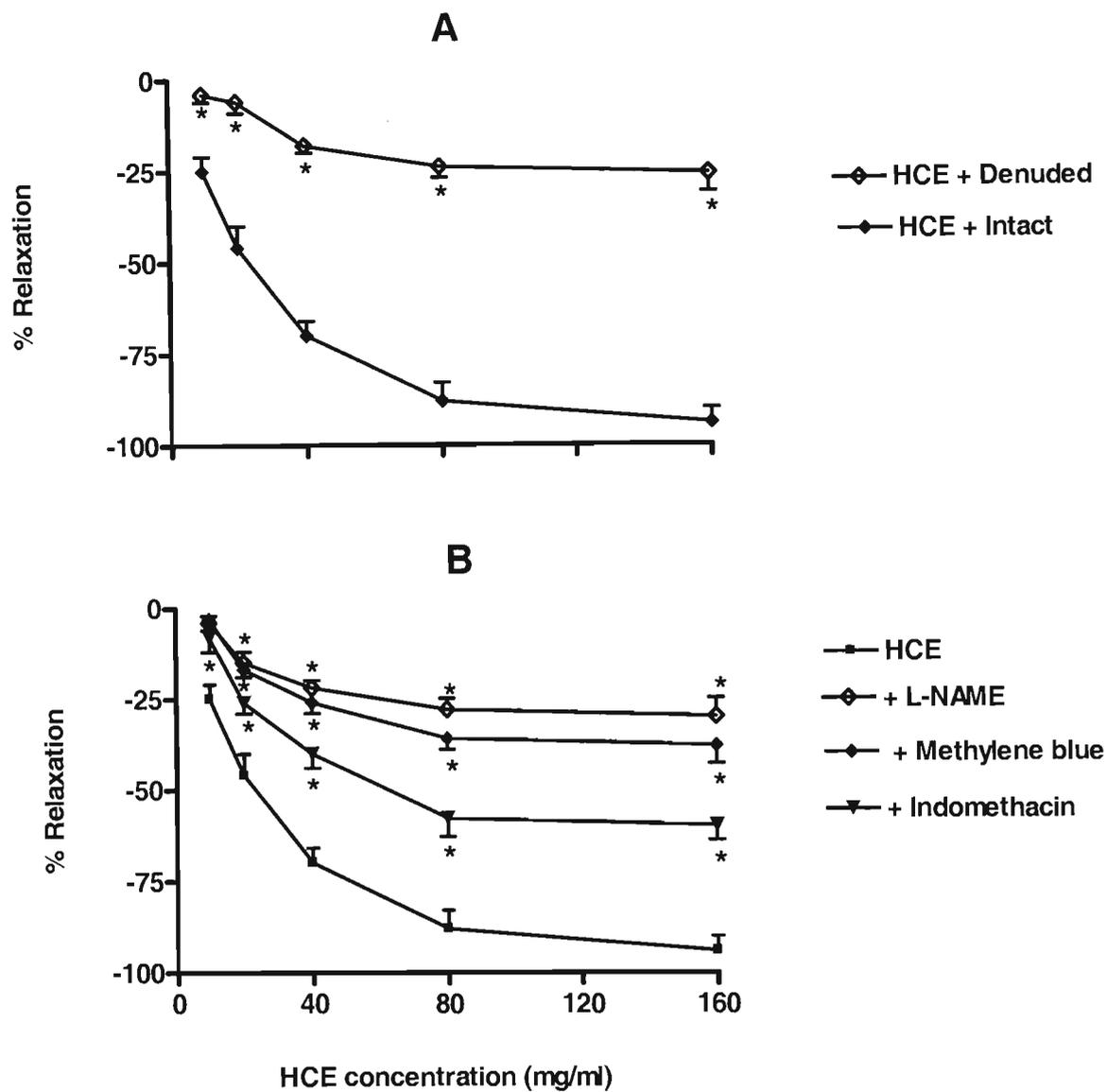
Figure 26.

Concentration-dependent vasorelaxant effects of HCE (10-160 mg/.ml) on rat endothelium-intact and denuded aortic rings, precontracted with methoxamine in the absence (**A**) and presence (**B**) of L-NAME (100μM), indomethacin (10μM) and methylene blue (20μM). Values are means ± SEM, (n=8; for each concentration).

P^* <0.05 by comparison with HCE + intact (**A**) or HCE alone (**B**).

5.4.3   Role of calcium and potassium channels.

To evaluate the role of calcium channels in the vasorelaxant effects of HCE, studies were conducted in intact-aortic rings precontracted with high (K^+ 80mM). HCE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with high (K^+80mM) (Figure 27).

To evaluate the role of potassium channels in the vasorelaxant effects of HCE, studies were conducted in intact-aortic rings precontracted with low (K^+ 20mM). HCE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low (K^+ 20mM). Pretreatment of the aortic rings with glibenclamide, before precontracted with low (K^+20mM), did not cause any change in the vasorelaxant effects of HCE (Figure 27).
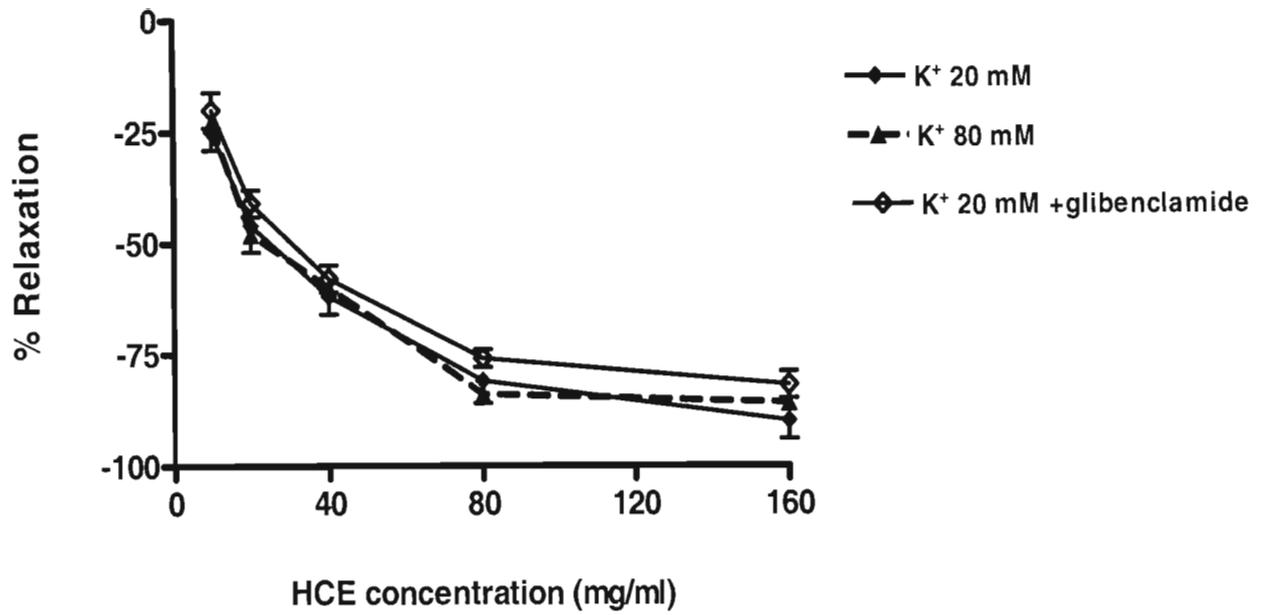
Figure 27.

Concentration-dependent vasorelaxant effects of HCE (10-160 mg/.ml) on rat isolated endothelium-intact aortic rings precontracted with high K^+ (80 mM) or low K^+ (20 mM) concentrations in the absence and presence of glibenclamide (3μM). Values are means ± SEM, (n=8; for each concentration).

5.5 Vasorelaxant effects of SBE.

The vasorelaxant effects of SBE were evaluated in endothelium-intact aortic rings precontracted with methoxamine hydrochloride ($10\mu M$), an alpha-1 receptor agonist to establish a cumulative concentration-response curve for the extract. SBE caused an endothelium and concentration-dependent (10-160 mg/.ml) vasorelaxation in intact aortic rings precontracted with methoxamine hydrochloride (Figure 28A).

5.5.1 Role of endothelium.

In order to assess the role of the functional endothelium in the vasorelaxant effects of SBE, the test was conducted in denuded-aortic rings precontracted with methoxamine hydrochloride. When contraction reached a stable plateau, a cumulative concentration-response curve for SBE was established and compared to that of intact aortic rings. The removal of endothelium significantly inhibited the vasorelaxation induced by SBE (Figure 28A).

5.5.2 Role of endothelial vasodilators.

To examine the role of nitric oxide in the vasorelaxant effects of SBE, endothelium-intact aortic rings were pretreated with L-NAME (100 μM), a nitric oxide synthase inhibitor before the contractions were evoked by methoxamine hydrochloride. L-NAME significantly inhibited the vasorelaxation induced by SBE (Figure 28B).

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To examine the role of prostacyclin in the vasorelaxant effects of SBE, endothelium-intact aortic rings were pretreated with indomethacin (10 μM), a cyclooxygenase inhibitor before the contractions were evoked by methoxamine hydrochloride. Indomethacin significantly reduced but not abolished the vasorelaxation induced by SBE (Figure 28B).

To assess the involvement of cGMP in the vasorelaxant effects of SBE, endothelium-intact aortic rings were pretreated with methylene blue (20 μM) a guanylate cyclase inhibitor before the contractions were induced by methoxamine hydrochloride. Methylene blue significantly inhibited the vasorelaxation induced by SBE (Figure 28B).
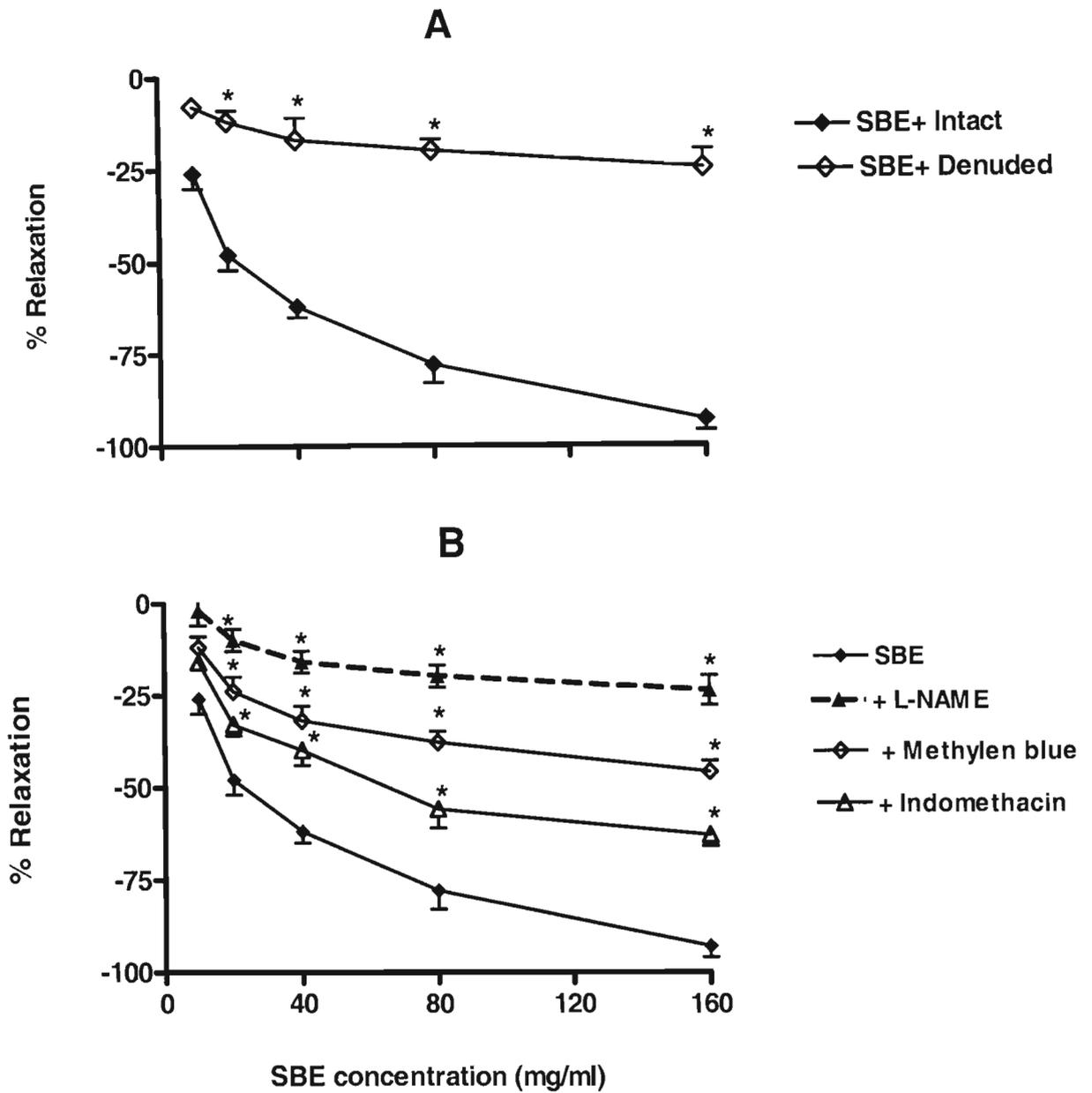
Figure 28.

Concentration-dependent vasorelaxant effects of SBE (10-160 mg/.ml) on rat endothelium-intact and denuded aortic rings, precontracted with methoxamine in the absence **(A)** and presence **(B)** of L-NAME (100μM), indomethacin (10μM) and methylene blue (20μM). Values are means ± SEM, (n=8; for each concentration).

P^* <0.05 by comparison with SBE + intact **(A)** or SBE alone **(B).**

5.5.3   Role of calcium and potassium channels.

To evaluate the role of calcium channels in the vasorelaxant effects of SBE, the test was conducted in intact-aortic rings precontracted with high (K^+ 80mM). SBE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with high (K^+80mM) (Figure 29).

To evaluate the role of potassium channels in the vasorelaxant effects of SBE, the test was conducted in intact-aortic rings precontracted with low (K^+ 20mM). SBE induced a significant and concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low (K^+ 20mM). Pretreatment of the aortic rings with glibenclamide, before precontracted with low (K^+20mM), did not have any effect on the vasorelaxant effects of SBE (Figure 29).
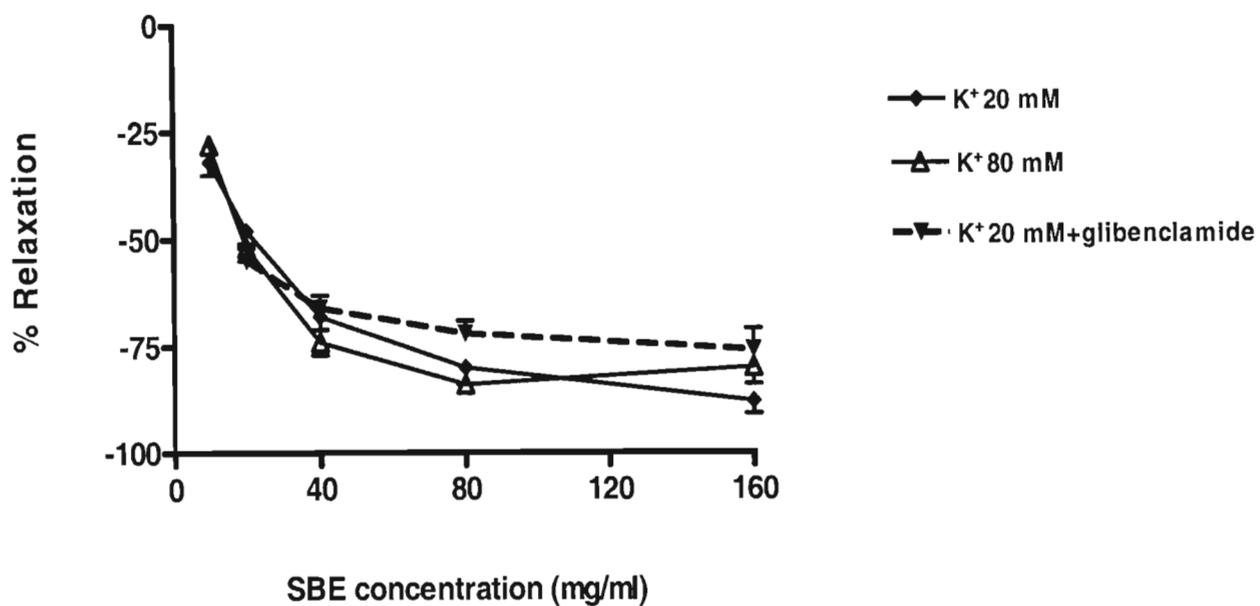
Figure 29.

Concentration-dependent vasorelaxant effects of SBE (10-160 mg/.ml) on rat isolated endothelium-intact aortic rings precontracted with high K^+ (80 mM) or low K^+ (20 mM) concentrations in the absence and presence of glibenclamide (3μM).

Values are means ± SEM, (n=8; for each concentration).

5.6   **Effects of various extracts on the contractile activity of rat isolated portal vein preparations.**

The effects of various extracts, EKE, APE, PAE, HCE and SBE on the rhythmic, myogenic-spontaneous contractions were evaluated in rat isolated spontaneously contracting portal veins. The relationships between the extracts and adrenergic, cholinergic receptors and calcium channels were also tested.

5.6.1   **Effects of EKE on myogenic-spontaneous contractions of portal vein.**

The effects of EKE on the myogenic spontaneous contractions were investigated in rat isolated portal veins. EKE concentration-dependently increased the myogenic spontaneous contractions in rat portal vein preparations. EKE at a concentration of 40 mg/.ml, caused tonic contractions (contracture) in the rat portal vein preparations (Figure 30A).

To investigate whether the effects of EKE on the myogenic spontaneous contractions were mediated through activation of alpha-1 adrenergic receptors, the portal vein preparations were pretreated with prazosin (1μM), an alpha-1 adrenergic receptor blocker. The effect of EKE on the myogenic activity was not affected by this prior treatment with prazosin (Figure 30B).

To test the involvement of voltage-operated calcium channels in the effects of EKE on myogenic activity, the portal vein preparations were pretreated with nifedipine ($1\mu M$). Nifedipine significantly reduced the stimulatory contractile effect induced by the higher concentration of EKE, (40 mg/.ml) (Figure 30B).
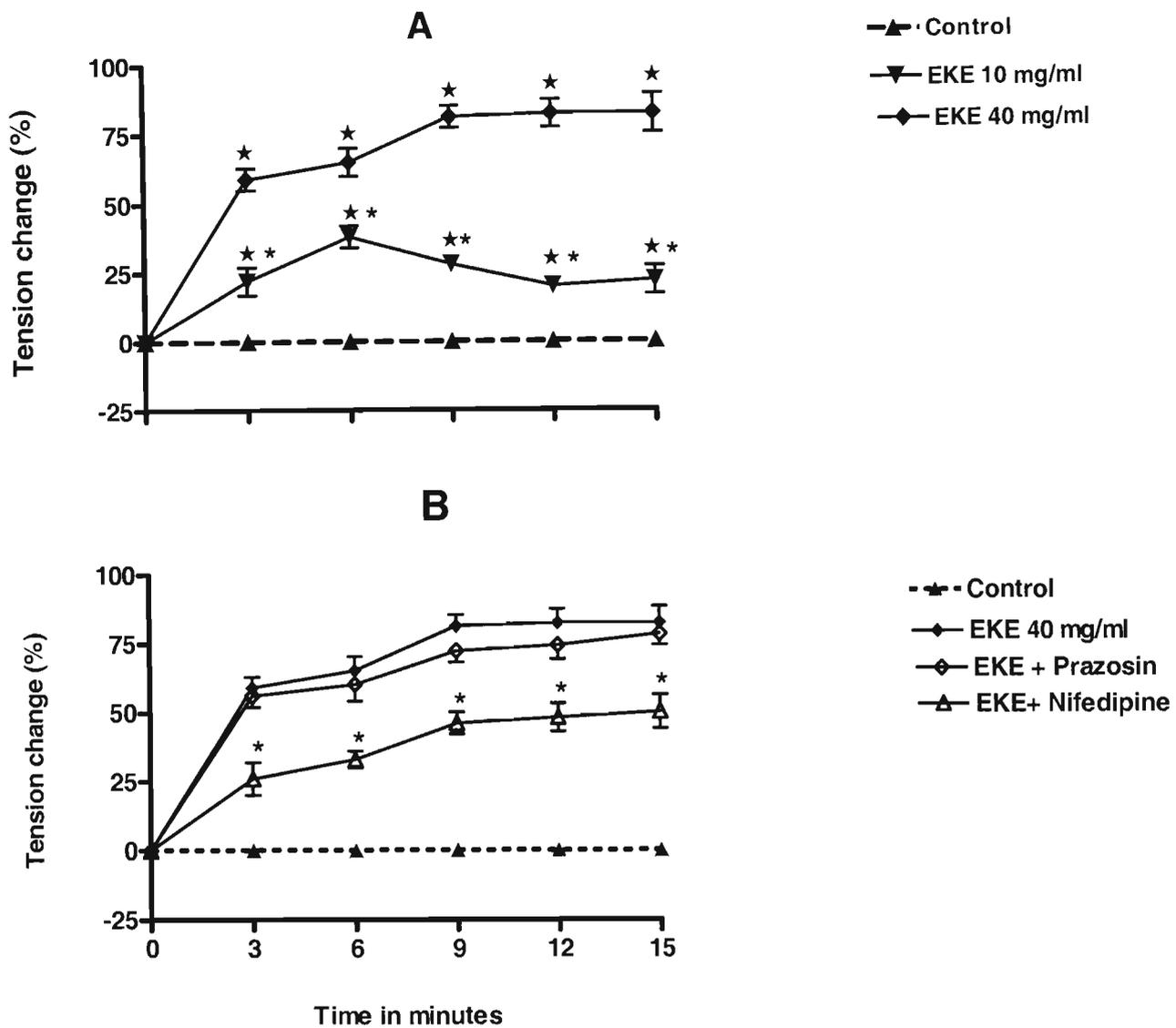
Figure 30.

Contractile effects of EKE (10 and 40 mg/ml) on rhythmic, myogenic spontaneous contractions of rat portal veins in the absence (**A**) and presence (**B**) of prazosin (1μM) and nifedipine (3μM). Values are means ± SEM, (n=8; for each concentration).

$P^{\star} < 0.05$ by comparison with control and $P^* < 0.05$ by comparison with EKE, 40 mg/ml.

5.6.2   Effects of APE on myogenic-spontaneous contractions of portal vein.

The effects of APE on the myogenic spontaneous contractions were investigated in rat isolated portal veins. APE induced concentration-dependent biphasic effect on the myogenic-spontaneous contractions of the rat portal vein preparations. The biphasic effect of APE consisted of an initial slight and transient increased contraction of short duration, followed by a secondary, longer-lasting relaxation of the rat portal vein preparations (Figure 31A).

In order to investigate whether the initial stimulatory effect of APE involved activation of voltage-operated calcium channels, portal vein preparations were pretreated with nifedipine ($1\mu M$), a calcium channel blocker. Pretreatment of the rat isolated portal vein with nifedipine ($1\mu M$) completely inhibited the contractile phase induced by APE. (Figure 31B).

In addition, to investigate whether secondary, longer-lasting venorelaxation were mediated through activation of cholinergic receptors, the portal vein preparations were pretreated with atropine sulphate ($1\mu M$), a cholinergic receptor antagonist. However, pretreatment of the isolated portal veins with atropine sulphate, did not alter the sustained, long lasting venorelaxation induced by APE (Figure 31B).
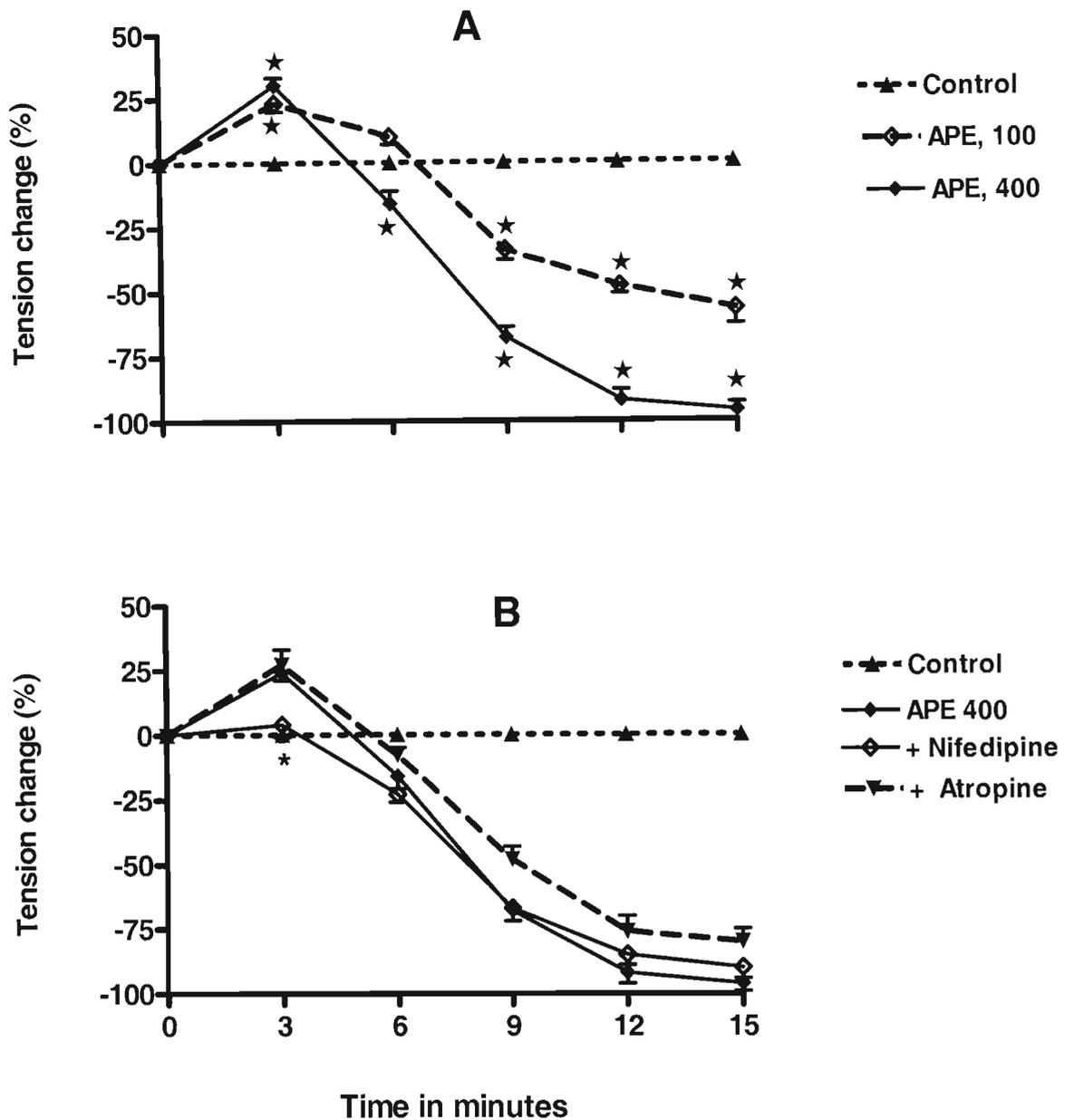
Figure 31.

Contractile effects of APE (100 and 400 mg/ml) on rhythmic, myogenic spontaneous contractions of rat portal veins in the absence (**A**) and presence (**B**) of atropine (1μM) and nifedipine (3μM). Values are means ± SEM, (n=8; for each concentration).

P^*< 0.05 by comparison with control and P* <0.05 by comparison with APE 400 mg/ml.

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5.6.3   Effects of PAE on myogenic-spontaneous contractions of portal vein.

The effects of PAE on the myogenic spontaneous contractions were investigated in rat isolated portal veins. PAE induced concentration-dependent biphasic effect on the myogenic-spontaneous contractions of the rat portal vein preparations. Similar to APE, the biphasic effect of PAE consisted of a significant, initial slight and transient increased contraction of short duration, followed by a secondary, longer-lasting relaxation of the rat portal vein preparations (Figure 32A). Pretreatment of the rat isolated portal vein preparations with nifedipine ($1\mu M$), or atropine sulphate ($1\mu M$), did not show any significant effect on the biphasic effects induced by PAE (Figure 32B).
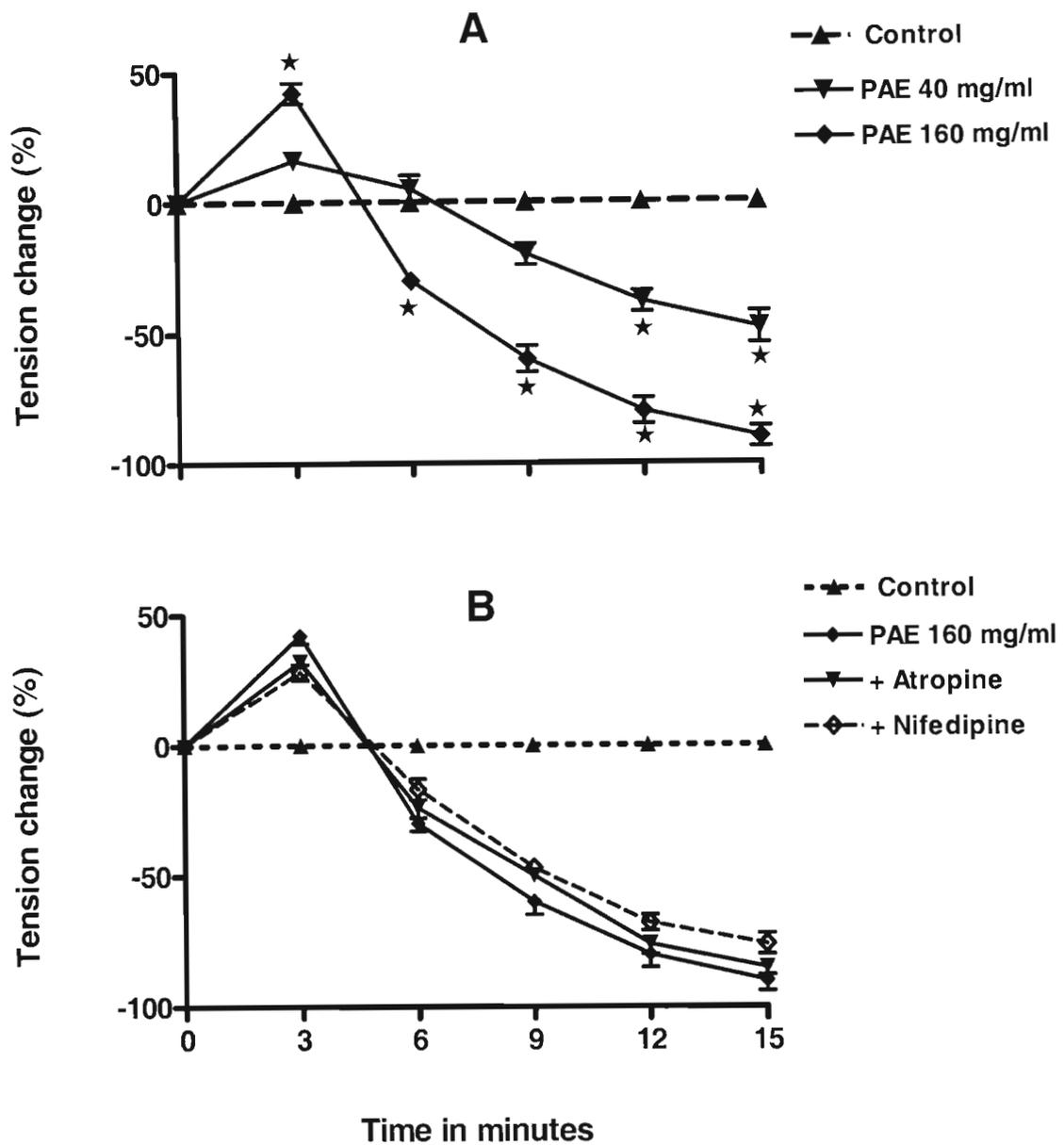
Figure 32.

Contractile effects of PAE (40 and 160 mg/ml) on rhythmic, myogenic spontaneous contractions of rat portal veins in the absence (**A**) and presence (**B**) of atropine (1µM) and nifedipine (3µM). Values are means ± SEM, (n=8; for each concentration).

P^*< 0.05 by comparison with control.

5.6.4   Effects of HCE on myogenic-spontaneous contractions of portal vein.

The effects of HCE on the myogenic spontaneous contractions were investigated in rat isolated portal veins. In contrast to APE, PAE and SBE, HCE did not stimulate, rather, it induced a significant and concentration-dependent longer lasting venorelaxation in the rat isolated portal vein preparations (Figure 33A).

To investigate if this effect was mediated through cholinergic receptors, the portal vein preparations were pretreated with atropine. Pretreatment of the rat isolated portal vein preparations with atropine sulphate ($1\mu M$) significantly inhibited the venorelaxation induced by HCE (Figure 33B).
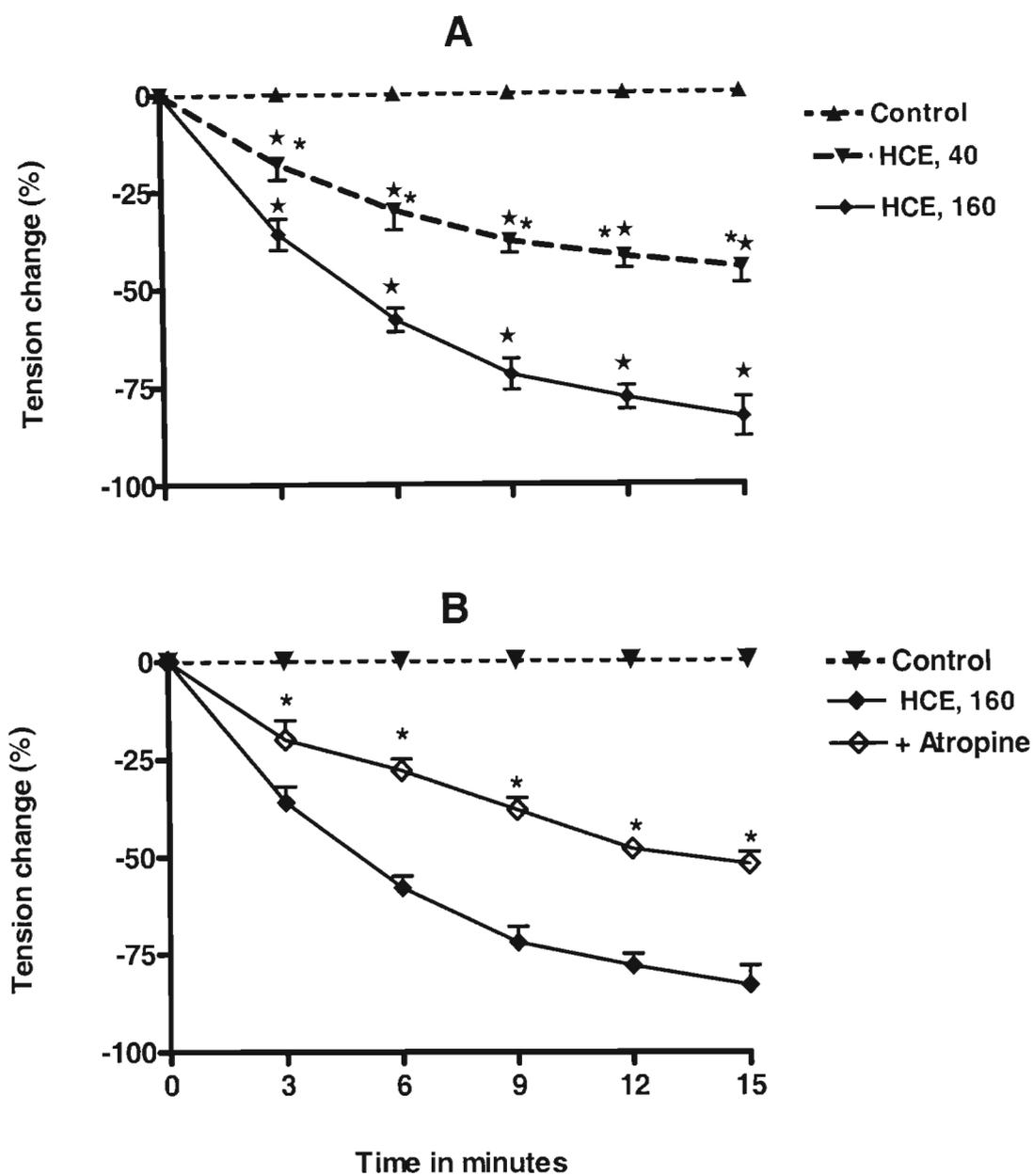
Figure 33.

Contractile effects of HCE (40 and 160 mg/ml) on rhythmic, myogenic spontaneous contractions of rat portal veins in the absence **(A)** and presence **(B)** of atropine (1μM) Values are means ± SEM, (n=8; for each concentration).

$P^{\star} < 0.05$ by comparison with control and $P^{\ast} < 0.05$ by comparison with HCE, 160mg/ml.

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5.6.5 Effects of SBE on myogenic-spontaneous contractions of portal vein.

The effects of SBE on the myogenic spontaneous contractions were investigated in rat isolated portal veins. SBE induced concentration-dependent biphasic effect on the myogenic-spontaneous contractions of the rat portal vein preparations. Similar to APE and PAE, the biphasic effect of SBE consisted of a significant, initial slight and transient increased contraction of short duration, followed by a secondary, longer-lasting relaxation of the rat portal vein preparations (Figure 34A). Pretreatment of the isolated portal veins with atropine or nifedipine did not significantly change the biphasic effect induced by SBE (Figure 34B).
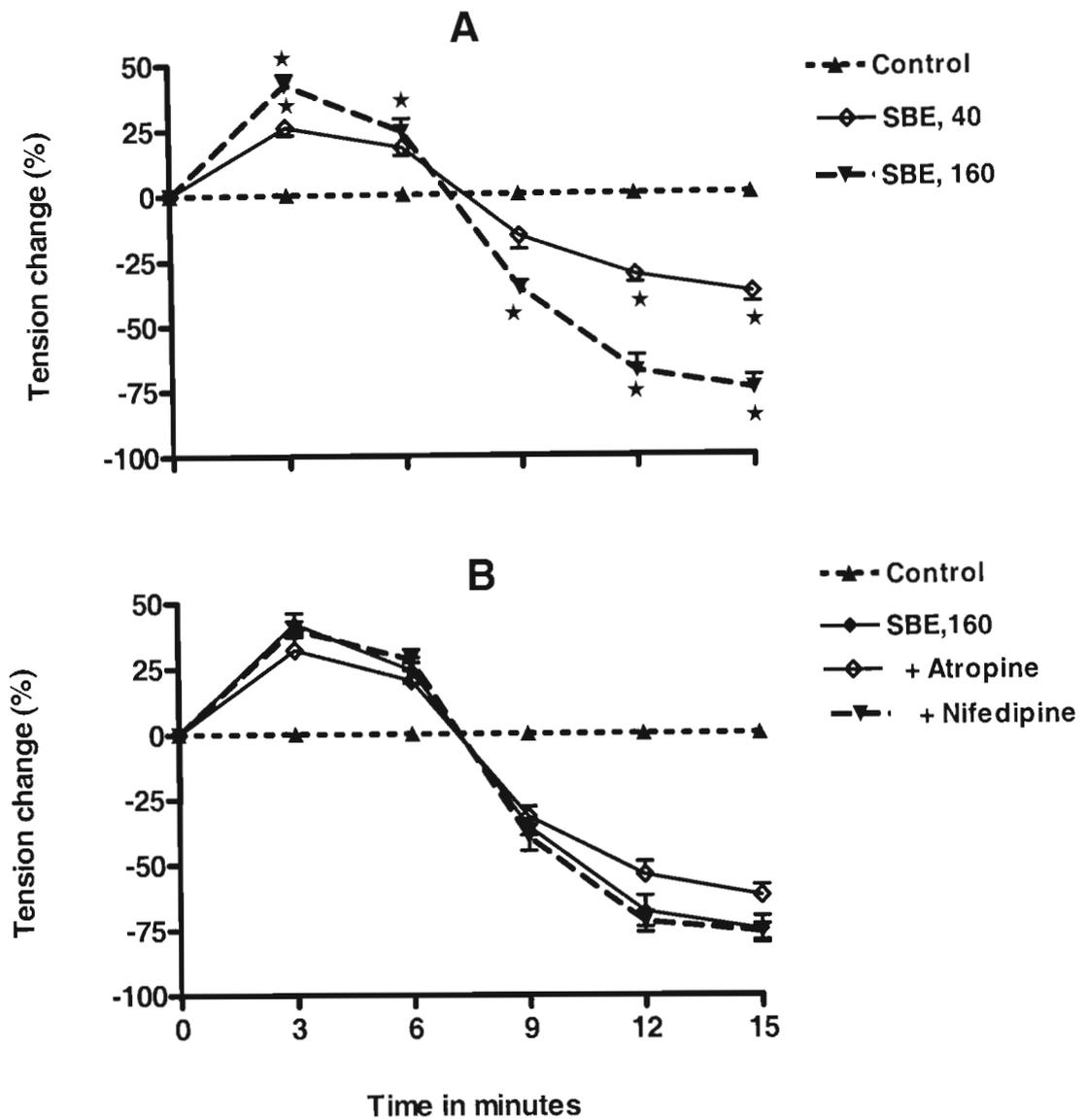
Figure 34.

Contractile effects of SBE (40 and 160mg/.ml) on rhythmic, myogenic spontaneous contractions of rat portal veins in the absence **(A)** and presence **(B)** of atropine (1μM) and nifedipine (3μM). Values are means ± SEM, (n=8; for each concentration).

$P^* < 0.05$ by comparison with control

5.7   Summary of Results.

- All crude ethanolic extracts exhibited hypotensive effects both in acute and chronic studies.

- All extracts demonstrated potent bradycardiac effects in chronic studies. However, these extracts did not exhibit significant effect on heart rate in acute experiments.

- Apart from EKE, all the other extracts exhibited negative inotropic and chronotropic effects on rat isolated left and right atria. On the other hand, these extracts antagonized the positive inotropic effects induced by noradrenaline and calcium.

- EKE, induced positive inotropic and chronotropic effects on rat isolated left and right atria, respectively.

- The extracts also demonstrated potent vasorelaxant effects involving both endothelium-dependent and independent mechanisms in rat isolated aortic rings.

- Apart from HCE, all extracts induced biphasic contractile effects in rat isolated portal veins. However, at higher concentration, EKE induced a tonic contraction in a portal vein.

- HCE significantly induced venorelaxation of the spontaneously contracting portal veins.

CHAPTER 6

6.0   DISCUSSION

6.1   General.

The aims of the present study were to investigate the cardiovascular effects of some medicinal plants, namely *Ekebergia capensis, Persea americana, Helichrysum ceres, Sclerocarya birrea* and *Hypoxis hemerocallidea in vivo* and establish their possible mechanisms of actions *in vitro*. To establish the mechanisms of the antihypertensive actions of these plants, we evaluated the direct effects of the various test extracts on the rat isolated cardiac and vascular preparations.. The extracts exhibited hypotensive effects *in vivo*, cardio-inhibitory and vasorelaxant effects *in vitro*. We suggest that these plants exhibit their antihypertensive actions via the reduction of the total peripheral vascular resistance by directly dilating the blood vessels through both endothelium-dependent and independent mechanisms and through the reduction of cardiac output by directly inhibiting the rate and force of myocardial contractions. Thus, observations of the study suggest that crude extracts from *Ekebergia capensis, Persea americana, Helichrysum ceres, Sclerocarya birrea* and *Hypoxis hemerocallidea*, would have the potential to provide cheap and accessible source of traditional medicine for the treatment of hypertension and other associated cardiovascular disorders.

6.2   Hypotensive effects

The acute effects of various plant extracts on the MAP, heart rate and myocardial contractility were evaluated in male anaesthetized Wistar normotensive rats. Group A extracts, namely, EKE, APE and PAE caused significant but transient reduction in MAP, with no significant effect on the heart rate (See Figure 6). The transient effects may suggest that the active principles in group A extracts are rapidly biotransformed or metabolized or may be acting on some systems with a mode of action rapidly reversible. In contrast, group B extracts, namely HCE and SBE induced significant and progressive, long-lasting reduction in the MAP with no significant effect on the heart rate. This may suggest that the active principles in group B extracts could act on some systems with a mode of action slowly reversible or that the active components are slowly metabolized or the extracts could have some toxic effects. The present findings have also been observed with other plant extracts (Tahri, Yamani, Legssyer, Aziz, Mekhfi, Bnouham, and Ziyyat, 2000). The acute hypotension elicited by these test extracts may be attributed, in part, to their vasorelaxant and cardio-depressant effects (described in detail later), which might have resulted in decreased total peripheral vascular resistance and cardiac output respectively. As described already in 1.4, maintenance of the normal blood pressure is dependent on the balance between the cardiac output (CO) and the total peripheral resistance (TPR). The cardiac output and the total peripheral resistance set the mean arterial pressure (MAP) since they determine the average blood volume in the systemic arteries over time and it is this blood volume that causes pressure (Vari and Navar, 1995). In hypertensives, therefore, the antihypertensive drugs work by altering the balance between these two factors to bring down blood pressure towards normal. It is important

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to recognize that when heart rate or stroke volume increases, cardiac output increases as well and so does systolic and the corresponding mean arterial pressure (Bertram, 2004). The observed acute hypotensive effects of the test extracts, therefore, could be mediated by their direct effect on the blood vessels and cardiac muscles resulting in decreased total peripheral resistance and cardiac output, respectively and hence hypotension. This suggestion is corroborated by the observations that these extracts except EKE, elicited potent negative inotropic effects *in vivo* (See Table 2), which could have resulted in significant reductions in cardiac output. On the other hand, all extracts exhibited vasorelaxant effects in rat isolated aortic rings *in vitro* (described in details later), which might have caused an acute reduction in the total peripheral resistance, resulting in overall reduction in mean arterial pressure. Furthermore, the long term effects of the test extracts were evaluated in Dahl salt sensitive-rats (DSS, a genetically hypertensive rat model) for six weeks. The results demonstrated that each of the test extracts prevented the development of hypertension., DSS-untreated rats progressively and significantly developed hypertension and increased heart rate during week five (See Figure 7). The long-term antihypertensive effects of the test extracts may be attributed to the presence of bioactive compounds in the test extracts which may be directly acting on the cardiovascular system or indirectly influencing other systems that regulate the functions of the cardiovascular system.

Several chemical constituents have been identified and isolated from extracts of *Sclerocarya birrea, Persea americana* and *Helichrysum ceres*. These compounds include a wide spectrum of polyphenols, tannins, triterpenes and saponins with a large variety of

pharmacological actions (Ogbode, 1992; Eromosele, Eromosele and Kuzhkuzha, 1991; Galvez et al., 1993). Moreover, saponins have been reported to stimulate nitric oxide release from vascular endothelial cells to cause smooth muscle cell relaxation (Fitzpatrick, Hirschfield, Ricci, Jantzen and Coffey, 1995). Similarly, plant-derived polyphenols including flavonoids which are abundant in these plants have been demonstrated to relax blood vessels in an endothelium-dependent, nitric oxide-mediated manner (Fitzpatrick, Hirschfield, Ricci, Jantzen and Coffey, 1995; Rice-Evans, Miller and Paganga, 1996; Andriambeloson, Stoclet and Andriantsitohaina, 1999; Huang et al., 1999). The hypotensive effects displayed by SBE, PAE, and HCE can, therefore, be attributed to the presence and bioactivities of these compounds.

Furthermore, flavonoids, saponins and tannins, which are also present in SBE, PAE and HCE, have been demonstrated to possess angiotensin converting enzyme inhibitory activity (Lacaille-Dubois, Franck and Wagner, 2001). Inhibition of angiotensin converting enzyme is currently considered to be a useful therapeutic approach in the treatment of hypertension (Lacaille-Dubois, Franck and Wagner, 2001). Angiotensin converting enzyme (ACE) inhibitors block the conversion of the inactive angiotensin I (Ang I) to the active pressor molecule angiotensin II (Ang II) (Lacaille-Dubois, Franck and Wagner, 2001). Angiotensin II is the most important humoral factor in the renin angiotensin system (RAS), which mediates inositol triphosphate (IP3) production and calcium mobilization which cause vasoconstriction, aldosterone stimulation, and sympathetic nervous system activation (Burrell and Johnston, 1997). Therefore, the presence of flavonoids, saponins and tannins in these extracts, with angiotensin

converting enzyme inhibitory activities might have offered a long-term cardioprotection in Dahl salt sensitive rat model. Methanolic and aqueous leaf extract of *Persea americana,* containing tannins and flavonoids have been demonstrated to elicit inhibitory effects on the binding of angiotensin II to AT1 receptors (Liu, Hsu, Tsai, Chan, Liu, Thomas, Tomliso, Lo and Lin, 2003). The AT1 receptor is the major physiological target of angiotensin II (AII) in the cardiovascular system (Lacaille-Dubois, Franck and Wagner, 2001). Inhibition of the binding of angiotensin II to AT1 receptors is presently considered as a novel strategy in the search for new antihypertensive therapy (Liu *et al.,* 2003). This is because, in spite of their therapeutic efficiency, angiotensin converting enzyme inhibitors are not highly effective in lowering angiotensin II levels since other enzymes, such as chymase, are able to synthesize angiotensin II (Reilly, Tewksbury, Schechter and Travis, 1982). In fact, chymase-dependent angiotensin II production is upregulated in human diabetic kidney and diseased blood vessels (Huang *et al.,* 2003; Doggrell and Wanstall, 2004).

Since the presence of these major compounds in *Sclerocarya birrea, Persea americana* and *Helichrysum ceres,* has been reported (Liu, Hsu, Tsai, Chan, Liu, Thomas, Tomliso, Lo and Lin, 2003; Ogbode, 1992; Eromosele, Eromosele and Kuzhkuzha, 1991; Galvez *et al.,* 1993), it is possible that these compounds contributed significantly in the long-term cardioprotective effects of these extracts.

On the other hand, the hypotensive effects of *Ekebergia capensis* may be attributed to the presence of triterpenes and other active compounds. The major triterpene reported in this

plant is oleanolic acid (Sewram *et al.,* 2000). Moreover, Somova *et al.,* (2003), reported the beneficial effects of oleanolic acid and other triterpenes in preventing the development of salt-sensitive, insulin resistant hypertension in genetic Dahl rat model of hypertension. In addition, oleanolic acid and other triterpenes have been reported to possess antioxidant, antihyperlipidemic and hypoglycaemic properties, all of which play an important role in the prevention and control of hypertension (Somova *et al.,* 2001).

Similarly, the hypotensive effects of *Hypoxis hemerocallidea* may be attributed to the presence of some bioactive compounds such as phytosterols (Mills, Cooper, Seely and Kanfer, 2005). Phytosterols exist in several forms but the most abundant ones are b-sitosterol and stigmasterol which are present in *Hypoxis hemerocallidea* (Mills, Cooper, Seely and Kanfer, 2005). Phytosterols have been reported to possess some beneficial cardiovascular properties (Jones, MacDougall, Ntanios and Vanstone, 1977). Recently, it has been reported that b-sitosterol and stigmasterol induce the synthesis of prostacyclin *in vitro* (Awad, Smith and Fink, 2001). Prostacyclin is a potent inhibitor of platelet aggregation and causes vasodilation of blood vessels, suggesting the possibility that the phytosterols present in APE may have offered protection against the development of hypertension in experimental animals. Thus, it may be suggested that the hypotensive effect of APE could be related to the presence and bioactivities of the b-sitosterol and stigmasterol which might have lead to the increased synthesis of prostacyclin thereby resulting in vasodilation and hence hypotension.

Therefore, the observed antihypertensive effects elicited by various ethanolic extracts from the five studied plants may be attributed to the potential of the major active compounds present in these extracts to induce vasorelaxation, thereby lowering total peripheral vascular resistance and to suppress myocardial contractility, resulting in reductions in cardiac output and hence, hypotension. The long-term antihypertensive effects, on the other hand, may be related to the inhibitory activities of the active compounds contained in the extracts on the renin-angiotensin system or/and other systems that may be directly or indirectly influencing the cardiovascular systems.

6.3 Positive inotropism and chronotropism

The present study has demonstrated that ethanolic leaf extract of *Ekebergia capensis* (EKE) exhibits a concentration-dependent, selective positive inotropic effect with little, but significant effect on spontaneous heart rate (See Figure 11). The study has also shown that the positive inotropic and chronotropic effects of EKE are not affected by pretreatment of the atrial preparations with reserpine (See Figure 11). Reserpine depletes junctional noradrenaline stores, thereby discarding the ability of noradrenaline release in response to stimulation (Salzmann, Bormann, Herzig, Markstein and Scholtysik, 1985). Therefore, the study has shown that positive inotropic and chronotropic effects of EKE are not mediated through modulation of noradrenaline release from junctional stores since its effects were not affected by prior treatment of the atrial preparations with reserpine, suggesting that activation of the sympathetic nervous system does not play an important role in the inotropic and chronotropic effects of EKE.

The study has also shown that the positive inotropic and chronotropic effects of EKE is not affected by pretreatment of the atrial preparations with atropine sulphate, a cholinergic receptor antagonist (Arunlakhshana and Schild, 1959) (See Figure 11). It is generally known that activation of cholinergic receptors in the myocardium induces negative inotropic and chronotropic responses (Landzberg, Parker, Gauthier and Colucci, 1994). However, in some specific cases positive inotropic responses have been reported (Caulfied, 1993; Bohm, Giersschik, Schwinger, Uhlmann and Erdmann, 1994; Landzberg *et al.,* 1994). In addition, studies have demonstrated that acetylcholine elicits a positive inotropic response in isolated cardiac tissue of some species (Endoh and Blinks, 1984; Tajima, Tsuji, Sorota and Pappano, 1987 and Eglen, Montgomery and Whiting, 1988). Acetylcholine mediates its effects through stimulation of cholinergic receptors, meaning that the myocardium consists of two cholinergic components. One, which mediates stimulatory and the other, which mediates inhibitory effects. (Landzberg *et al.,* 1994). However, the present study has provided evidence that the positive inotropic and chronotropic effects of EKE are not mediated through activation of cholinergic receptors, since its effects were not affected in the presence of atropine sulphate.

In contrast, the study has demonstrated that pretreatment of the atrial preparations with propranolol almost completely abolished the inotropic and chronotropic effects of EKE (See Figure 11). Propranolol is a non-selective ß-adrenergic receptor blocker (Hoffman and Lefkowitz, 1990). In the presence of propranolol, the positive inotropic and chronotropic effects of noradrenaline are inhibited (Hoffman and Lefkowitz, 1990) (See Figure 9). In this respect, since the positive inotropic and chronotropic effects of EKE

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were completely abolished after blockade of myocardial ß-adrenergic receptors, it may be suggested that the positive inotropic and chronotropic effects of EKE are mediated through a noradrenaline-like mechanism. Therefore, the study has demonstrated that the positive inotropic and chronotropic effects of EKE are mediated through ß-adrenergic-dependent mechanisms.

In addition, pretreatment of the atrial preparations with nifedipine (an L-type Ca^{2+} channel blocker), partially, but significantly reduced the positive inotropic and chronotropic effects of EKE, suggesting the important role of the voltage-operated calcium channels in the cardio-stimulatory effects of EKE. It is widely accepted that cardiac contraction is to a great extent regulated by the concentration of cytosolic Ca^{2+} in cardiac muscle cells (Fabiato, 1883). The influx of Ca^{2+} into the cardiac cells during depolarization initiates the process of myocardial contraction. Thus, the present observation suggests that the cardio-stimulatory activities of EKE, besides ß-adrenergic mechanism, are also mediated at least, in part, through activation of L-type Ca^{2+} channels causing an influx of calcium and subsequent positive inotropy and chronotropy. However, involvement of other mechanism (s) in the cardio-stimulatory effects of EKE cannot be ruled out.

Considering that the positive inotropic effect of EKE was accompanied by positive chronotropism, it may be suggested that other mechanisms are partially involved in its effects. One of the mechanisms may include non-adrenergic mechanism like phosphodiesterase III inhibition, which would probably result in increased cAMP levels

and subsequent positive inotropy (Sys, Boels and Brutsaert, 1987). Moreover, it is reported that all inotropic mechanisms involving cAMP go along with a positive chronotropic effect (Gilani, Janbaz, Aziz, Herzig, Kazmi, Choudhary and Herzig, 1999). This speculation is supported by the fact that EKE, besides inducing positive inotropic effects, it also caused some chronotropic effects.

In the present study, we have demonstrated that ethanolic leaf extract of *Ekebergia capensis* causes a concentration-dependent inotropic action with little chronotropic effect in isolated rat electrically-driven left and spontaneously-beating right atria respectively. We have also demonstrated that these effects are mediated through ß-adrenergic receptor stimulation and calcium channel activation. We have also shown that its inotropic and chronotropic effects are not mediated through the release of noradrenaline from junctional stores. These observations may suggest that EKE may posses bioactive components which interact with the ß-adrenergic receptors and calcium channels and that these two components may act synergistically to bring about the overall cardio-stimulatory effects of EKE.

The present study has demonstrated that *Ekebergia capensis* may posses a potential cardioinotropic principle, which may provide beneficial effects in conditions of cardiac heart failure or ventricular dysfunction. Positive inotropic drugs have therapeutic value in congestive heart failure (Erdmann, 1989), however, the current available cardiotonic drugs have major disadvantages that limit their clinical use. For example, cardiac glycosides like digitoxin have narrow margin of therapeutic safety, mainly due to

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potential arrhythmogenesis (Paker, 1988), while inotropes involving increases in cyclic adenosine monophosphate (cAMP) levels, like sympathetic stimulants and phosphodiestrase (PDE) inhibitors (Siegl, 1986), induce tachycardia, which is unwanted side effect in this mechanism of action. Therefore, EKE may provide a cheap source of safe treatment of hypertension complicated with heart failure. The presence of a triterpene, oleanolic acid and perhaps other triterpenes, may contribute to the positive inotropic and chronotropic effects of EKE. Moreover, oleanolic acid has been recently reported to possess positive inotropic and chronotropic activities in guinea-pig isolated atrial muscle preparations (Somova, Mipando and Shode, 2004).

6.4   Negative inotropism and chronotropism

In the present study, the negative inotropic and chronotropic activities of ethanolic extracts prepared from the leaves of *Persea americana* (PAE), *Helichrysum ceres* (HCE) and stem-barks of *Sclerocarya birrea* (SBE) and corm of *Hypoxis hemerocallidea,* 'African Potato' (APE), respectively. The study has demonstrated that all extracts (PAE, HCE, SBE and APE) elicited significant and concentration-dependent negative inotropic and chronotropic activities (See Figures 12, 14, 16 and 18). The study has also shown that pretreatment of the atrial preparations with atropine sulphate did not affect the negative inotropic and chronotropic effects induced by PAE, APE and SBE (See Figures 12, 14 and 18). Atropine sulphate is a cholinergic muscarinic receptor antagonist (Arunlakhshana and Schild, 1959). In its presence, the negative inotropic effect of

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acetylcholine is inhibited (Brown and Taylor, 1996) (See Figure 10). Acetylcholine is a neurotransmitter released by the parasympathetic nervous system and mediates its action by stimulation of the cholinergic muscarinic receptors (Brown and Taylor, 1996). Therefore, the fact that the negative inotropic and chronotropic effects of PAE, APE and SBE persist even after blockade of the cholinergic receptors, suggests that the negative inotropic and chronotropic effects of these test extracts are not mediated through activation of cholinergic receptors. This finding suggests that these extracts exhibit their cardio-inhibitory effects through some different mechanisms.

There is a possibility that these test extracts may exert their effects directly on the myocardium or there could be other non-specific mechanisms through which they induce their cardio-inhibitory effects. Moreover, these test extracts were found to antagonize beta-adrenergic receptor agonist noradrenaline-induced positive inotropic actions in rat isolated left atria in a concentration dependent manner (See Figures 13, 15 and 19). This finding may provide evidence for beta-adrenergic receptor inhibitory potential of these test extracts. Thus, the test extracts may have a direct $beta_1$-adrenergic receptor blocking action, since $beta_1$-adrenergic receptors mediate the positive inotropic effects of the catecholamines (Hoffman and Lefkowitz, 1990).

On the other hand, these extracts significantly antagonized the concentration-dependent positive inotropic effects induced by calcium in a concentration-dependent manner (See Figures 13, 15 and 19). Contraction of the cardiac muscle is to a large extent regulated by cytosolic Ca^{2+} levels in the cardiac cells. The influx of Ca^{2+} into the cardiac cells during

depolarization marks the process of excitation-contraction coupling. Therefore, by virtue of their antagonistic effects on the positive inotropic effects of Ca^{2+}, these extracts appear to possess calcium antagonistic component (s) which might interfere with calcium influx at the cell membrane of the myocardium thereby, inhibiting myocardial contraction. Thus, the cardio-inhibitory effects of these extracts might well be related to inhibition of voltage-dependent calcium channels.

In contrast, pretreatment of the atrial preparations with atropine sulphate significantly reduced the negative inotropic and chronotropic effects of HCE with a complete abolition of the effects of the three lower concentrations, suggesting that HCE mediates its cardio-inhibitory effects partly, through activation of cholinergic receptors (See Figure16). However, the negative inotropic effect of the highest concentration persisted in the presence of atropine sulphate. This partial inhibition would suggest that besides activation of cholinergic receptors, HCE involves other mechanism (s) through which it mediates its cardio-inhibitory effects. However, the possibility that at this concentration, HCE competed with atropine sulphate for the cholinergic receptors cannot be ruled out.

Furthermore, in separate experiments, HCE significantly reduced the concentration-dependent positive inotropic effects induced by noradrenaline and calcium chloride (See Figure 17). We can, therefore, speculate that HCE may interact with adrenergic receptors and calcium channels to elicit its cardio-inhibitory effects. Therefore, it is likely that the negative inotropic and chronotropic effects of HCE are mediated by muscarinic receptors most likely M_2 receptors (Caulfield, 1993). This is because it has been reported that the

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negative phase of muscarinic receptor stimulation is mediated by M_2 (Caulfield, 1993; Landzberg *et al.,* 1994). The other possible mechanisms may involve its antagonistic actions on beta$_1$-adrenergic receptors and calcium channels.

6.5   Vasorelaxant effects of various plant extracts.

In the present study, we investigated the vasorelaxant effects of group A extracts (EKE, APE and PAE) and group B extracts (HCE, SBE) in rat isolated endothelium-intact and endothelium-deprived aortic rings precontracted with methoxamine hydrochloride (α_1-adrenoceptor agonist). All the test extracts from each group significantly induced vasorelaxant effects in a concentration-dependent manner in endothelium-intact aortic rings precontracted with methoxamine hydrochloride (See Figure 20, 22, 24, 26 and 28).

Removal of the endothelium significantly reduced, but not completely abolished the vasorelaxant effects of EKE and PAE from group A and HCE, and SBE from group B extracts, suggesting that the vasorelaxations induced by EKE, PAE, HCE and SBE are mediated by both endothelium-dependent and endothelium-independent mechanisms (See Figure 20, 24, 26 and 28). In contrast, the removal of the endothelium completely abolished the vasorelaxant effect of APE, from group A extracts (See Figure 22), suggesting that the vasorelaxant effect of APE involves predominantly the functional endothelium-dependent mechanisms.

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6.5.1 Role of endothelium-derived relaxing factors.

Since the extracts induced both endothelium-dependent and endothelium independent relaxations in the rat isolated-aortic rings precontracted with methoxamine, an attempt was made to investigate what endothelium-derived vasorelaxing factors contribute to the vasorelaxations induced by the test extracts. Pretreatment of the endothelium-intact aortic rings with L-NAME, a non-selective nitric oxide synthase inhibitor (Rees, palmer, Hodson and Moncada, 1989), significantly reduced but not abolished the vasorelaxations induced by the test extracts from each group (See Figure 20, 22, 24, 26 and 28), suggesting the important role of nitric oxide in the vasorelaxant effects of these extracts. The endothelium is a dynamic organ that plays an important role in the regulation of the vascular tone, structure, and function (Vanhoutte, 1989). The endothelium regulates the vascular tone by sensing various physiological stimuli and triggering release of multiple vasoactive substances, including nitric oxide (Behrendt and Ganz, 2002; Gibbons, 1997). Nitric oxide, enzymatically synthesized from the amino acid L-arginine, was first discovered in the vascular endothelium (Furchgott and Zawadzki, 1980). Endothelial nitric oxide plays an essential role in the control of vascular tone and structure (Luscher and Vanhoutte, 1986; Vanhoutte and Mombouli, 1996). The vascular tone plays an important role in the regulation of arterial blood pressure. A reduced production of nitric oxide by vascular endothelial cells is closely associated with endothelial dysfunction, which is proposed to be an important factor in cardiovascular diseases, especially in development of atherosclerosis and hypertension (Busse and Fleming, 1996). Moreover, endothelium-dependent vasorelaxation is reported to be impaired in human and

experimental hypertension (Luscher and Vanhoutte, 1986). Decreased bioavailability of nitric oxide, results in decreased vasodilation, thus causing an increase in vascular resistance and hence increased blood pressure (Luscher and Vanhoutte, 1986; Behrendt and Ganz, 2002; Panza, 1997). Moreover, endothelial dysfunction is closely associated with reduced bioavailability of nitric oxide. Owing to the importance of blood vessels for the function and regulation of the cardiovascular system, blood vessels are the major target for pharmacotherapy in patients with cardiovascular risk factors including hypertension (Luscher, Spieker, Noll and Cosentino, 2001). Therefore, the development of vasodilators with the ability to restore the bioavailability of nitric oxide in the vascular system can present a novel strategy in the search for drugs of hypertension and its associated cardiovascular diseases (Yin, Kang, Choi, Kwao and Lee, 2005).

The present study has demonstrated that the vasorelaxant effects of various extracts (EKE, APE, PAE, HCE and SBE) are dependent on the synthesis or release of the endothelium-derived nitric oxide, since the vasorelaxant effects of these extracts were significantly reduced in the presence of L-NAME, an inhibitor of the enzyme which catalyses synthesis of nitric oxide (nitric oxide synthase).

Pretreatment of the endothelium-intact aortic rings with methylene blue, a non-specific guanylate cyclase inhibitor (Mayer, Brunner, Schmidt, 1993; Kawada, Ishibashi, Sasage, Kato and Imai, 1994) caused a significant reduction in the vasorelaxations induced by each of the test extracts (See Figure 20, 22, 24, 26 and 28), confirming a predominant role of endothelium-derived nitric oxide in the vasorelaxations induced by these extracts.

The relaxation of vascular smooth muscle mediated by nitric oxide involves a sequence of steps. Nitric oxide is formed in the endothelium by activation of nitric oxide synthase. Once formed, the nitric oxide diffuses out of the endothelium with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylate cyclase (Furchgott and Vanhoutte, 1989). Soluble guanylate cyclase is an intracellular second messenger for nitric oxide in the target tissue, where it catalyzes the formation of cGMP (Arnold, Mittal, Katsuki and Murad; Lucas, Pitari, Kazerounian, Ruiz-Stewart, Park, Schulz, Chepenik and Waldman, 2000). Increases in cGMP subsequently lead to protein kinase C phosphorylation and smooth muscle relaxation (Furchgott and Vanhoutte, 1989; Nathan, 1992). In the present study, the role of cGMP on the vasorelaxant effects of various extracts was investigated. The endothelium-intact aortic rings were pretreated with methylene blue, an inhibitor of guanylate cyclase. Methylene blue significantly reduced the vasorelaxations induced by each test extract, suggesting that cGMP signal pathway plays an important role in the nitric oxide-dependent vasorelaxant effects of the tested extracts. Therefore, the fact that both L-NAME and methylene blue significantly reduced the vasorelaxations induced by the extracts clearly indicates that the active components contained in these test extracts act on the vascular endothelium via nitric oxide synthase/cGMP pathway.

However, besides blocking guanylate cyclase, methylene blue also inhibits nitric oxide synthase at even higher potency than guanylate cyclase (Mayer, Brunner and Schmidt, 1993). Furthermore, methylene blue stimulates the production of superoxide anion, which inactivates nitric oxide (Furchgott and Vanhoutte, 1989). Both these actions can decrease

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the tissue nitric oxide levels, so that the observed effect of methylene blue on the vasorelaxatons induced by these test extracts could also be due to changes in nitric oxide rather than cyclic GMP levels. However, its overall effects on the vasorelaxations induced by the test extracts generally suggest a predominant role of endothelium derived nitric oxide.

The possibility that endothelial vasorelaxing factors derived from cyclooxygenase pathway ; Prostacyclin (PGI_2) released from endothelial cells (Jaffe, Levin, Weksler and Marcus, 1982), participated in the vasorelaxant effects of the test extracts was also evaluated. In this respect, pretreatment of the endothelium-intact aortic rings with indomethacin, an inhibitor of the cycloooxygenase, significantly reduced but not abolished the vasorelaxant effects of HCE, APE, EKE and SBE (See Figure 20, 22, 26 and 28), suggesting that these extracts, in addition to nitric oxide synthase/cGMP pathway, mediate their vasorelaxant effects via cyclooxygenase pathway probably, through the release of prostacyclin. In contrast, cyclooxygenase pathway does not seem to play an important role on the vasorelaxant effect of PAE, since in the presence of indomethacin the vasorelaxant effect of this test extract was not affected (See Figure 24), thus excluding possible involvement of prostacyclin, another endothelial vasodilator, in the vasorelaxant effect of PAE.

6.5.2 Involvement of calcium and potassium channels.

Involvement of calcium channels in the vasorelaxant effects induced by the test extracts was evaluated in the endothelium-intact aortic rings precontracted with high K^+ (80mM) concentrations. In this study, HCE, EKE and SBE induced concentration-dependent vasorelaxations in the intact aortic rings precontracted with high K^+ (80mM) concentration (See Figure 21, 27, and 29), suggesting the participation of calcium channels in the vasorelaxant effects of these extracts.

The contractile responses induced by high K^+ (80 mM) or increases of $CaCl_2$ in KCl-depolarized muscles are due to the influx of extracellular Ca^{2+} through L-type voltage-sensitive channels (VOCs), (Godfraind, Miller and Wibo, 1986) and have been used to provide a simple means of studying drugs with possible Ca^{2+}-entry blocking properties (Cauvin, Loutzenhiser and Van Breemen, 1983; Godfraind, Miller and Wibo, 1986). Calcium channel blockers inhibit noncompetitively the contractions which are induced by K^+ depolarizations (Godfraind and Kaba, 1969). In this study, HCE, EKE and SBE induced concentration-dependent vasorelaxations in the intact aortic rings precontracted with high K^+ (80mM) concentration. Since high K^+ produces smooth muscle contraction by promoting Ca^{2+} entry through the voltage-sensitive Ca^{2+} channels, which are readily activated by membrane depolarization (Godfraind and Kaba, 1969), the vasorelaxant effects of HCE, EKE and SBE against high potassium-induced contractions can be visualized as blockade of Ca^{2+} channels. Therefore, the vasorelaxant effects of these extracts on the high K^+-induced contraction indicate that these extracts may interfere with

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Ca^{2+} influx on the membranes of the vascular smooth muscle cells. In contrast, PAE and APE induced weak or mild vasorelaxations in the aortic rings precontracted with high K^+ (80mM) concentration, thereby, (See Figure 23, and 25), excluding the involvement of calcium channels in their vasorelaxant effects.

In the present study, the role of potassium channels in the vasorelaxant effects of the various test extracts was investigated in the endothelium-intact aortic rings precontracted with low K^+ (20mM) concentration. In this study, each of the test extracts induced a concentration-dependent vasorelaxation in the endothelium-intact aortic rings precontracted with low K^+ (20 mM) (See Figure 21, 23, 25, 27 and 29). However, PAE and APE induced the vasorelaxations more effectively in aortic rings precontracted with low K^+ (20mM) than those precontracted with high K^+ (80 mM) concentrations. This observation is similar to those found with potassium channel openers such as those of benzopyrane group (Edwards and Weston, 1993; Hamilton and Weston, 1989).

In the vascular smooth muscle cells, Ca^{2+} is required to activate nitric oxide synthase in endothelial cells in order to transform L-arginine to L-citrulline and produce nitric oxide in a reaction dependent on a Ca^{2+}-calmodulin interaction (Bredt and Snyder, 1990).
In addition, activation of K^+-channels results in hyperpolarization of the endothelial cell membranes, providing a driving force for entry of Ca^{2+} which in turn activates nitric oxide synthesis and subsequent vasorelaxation. Therefore, besides activation of the nitric oxide synthesis, the opening of potassium channels also appears to be a possible mechanism for the vasorelaxation induced by the test extracts. It is reported that any

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substance that selectively relaxes the contractions induced by low K^+ (<30 mM) is considered as a potassium channel opener and such experiments allow to distinguish potassium channel openers from calcium channel blockers (Hamilton, Weir and Weston, 1986; Kishil, Morimoto, Nakajima, Yamazaki, Tsujitama and Takayanagi, 1992). In the present study, APE and PAE exhibited weak vasorelaxations against high K^+-induced contractions, but selectively and significantly relaxed the low K^+-induced contractions, suggesting that potassium channels play an important role in their vasorelaxant effects in rat aortic rings.

It is conceivable that nitric oxide and prostacyclin are not the only endothelial mediators released from the endothelium in response to the various tested extracts in the present study, considering the fact that vasorelaxant effects of these test extracts were not completely abolished in the presence of L-NAME and indomethacin. Recently, it has been suggested that the remaining part of vasorelaxation induced by acetylcholine that is resistant to nitric oxide synthase inhibition is mediated through the release of endothelium-derived hyperpolarizing factor (EDHF) that acts ultimately by opening potassium channels (Hamilton and Weston, 1989; Edwards and Weston, 1993). Potassium channel openers are relatively a new class of drugs comprised of a diverse group of molecules with a wide range of potential therapeutic uses (Quest, 1992). The compounds open K^+ channels, causing membrane potential hyperpolarization through the increase in K^+ efflux, thus causing a decrease in the cellular free Ca^{2+} and smooth muscle relaxation (Quest and Cook, 1989; Nelson and Quayle, 1995). Different types of K^+ channels are expressed in vascular smooth muscle cells (Kuriyama, Kitamura and

Nabata, 1995) and agents that block these channels are useful tools for examining the role of a particular K^+ channel. In the present study, pretreatment of the endothelium-intact aortic rings with glibenclamide, a selective blocker of ATP-sensitive K^+ channels (Buckingham, Halmiton, Howlett, Mootoo and Wilson, 1989; Frank, Puschman, Schusdziarra and Allescher, 1994), did not affect the vasorelaxant effects of EKE, HCE and SBE (See Figure 21, 27 and 29), suggesting that ATP-dependent K^+ channels do not play an important role in their vasorelaxant effects. However, glibenclamide significantly reduced the vasorelaxations induced by PAE and APE (See Figure 23 and 25), suggesting an important role of ATP-dependent K^+ channels in the vasorelaxant effects of these two extracts.

It is reported that the vasorelaxant actions of K^+ channel opening agents in smooth muscle tissues are lower at high K^+ (>35mM) than low K^+-induced contractions (Ito, Kanno, Suzuki, Masuzawa-Ito, Takewaki, Ohashi, Asano and Suzuki, 1992). In the present study, the highly significant vasorelaxant effects of APE and PAE demonstrated in endothelium-intact aortic rings precontracted with low K^+ (20mM) than of high K^+ (80mM) also supports the possible involvement of K_{ATP} channel activation in the vasorelaxations induced by these test extracts.

6.6   Effects of various plant extracts on portal vein.

In this study, the effects of the test extracts on the contractility of the rat isolated-myogenic- spontaneously contracting portal vein were evaluated. EKE displayed biphasic

effects on the myogenic-spontaneous contractions of the portal vein, with low to moderate concentrations causing an initial significant increase in contractions, followed by venorelaxations. However, the higher concentration (40 mg/.ml) induced a significant and sustained tonic contraction, which was not accompanied by venorelaxation (See Figure 30). Studies have reported that the contractile activity in a portal vein is essentially due to alpha$_1$-adrenergic receptor activation (Han, Abel and Minneman, 1987; Maramatsu, Ohmura, Kigosh, Hashimoto and Oshita, 1990). However, pretreatment of the rat isolated portal vein with prazosin, an alpha$_1$-adrenergic receptor antagonist (Han, Abel and Minneman, 1987 and Maramatsu *et al.,* 1990) did not affect the tonic contractile effect of EKE (See Figure 30), suggesting that its tonic contractile effect in a rat portal vein is not mediated by activation of alpha$_1$-adrenergic receptors. In contrast, pretreatment of the portal veins with nifedipine (a voltage-gated Ca^{2+}channel blocker), significantly reduced the tonic effect induced by EKE (See Figure 30), suggesting that EKE induces its tonic contractile effect in a rat portal vein partly, through activation of the voltage-gated Ca^{2+} channels.

The structural and pharmacological features of the portal veins offer convenient preparation of venous smooth muscle, which possibly models the pharmacological reactivity of blood vessels (Bourreau, Lambert and Steyn, 1988). Spontaneous phasic contractions in a portal vein are thought to result from depolarizations stimulated by spontaneous transient inward currents (STIC) and influx of Ca^{2+} through voltage-gated calcium channels (Burt, 2003). Influx of Ca^{2+} through voltage-gated Ca^{2+} channels following depolarization may then stimulate Ca^{2+} induced Ca^{2+} release from intracellular

stores of the portal vein causing a contractile response (Burt, 2003). Therefore, the fact that nifedipine significantly reduced the tonic contractile effect of EKE in the portal veins, suggests that the test extract induced the tonic contractions, in part, by activation of the voltage-gated calcium channels thereby causing an influx of Ca^{2+} and the subsequent contractile effect.

Additionally, it is suggested that inhibition of the Ca^{2+}–dependent ATPase results in prolongation of the time required to restore the resting cytosolic Ca^{2+} level after contraction and this slows the rate of relaxation resulting in sustained tonic contraction (Choi and Eisner, 1995). It may be possible, therefore, that EKE exerts some inhibitory action on the Ca^{2+} –dependent ATPase, thereby resulting in prolongation of cytosolic Ca^{2+} level, and thus, maintaining the tonic contraction. On the other hand, APE, PAE and SBE exhibited biphasic contractile responses in rat isolated, myogenic-spontaneously contracting portal veins, with an initial, transient increase in contraction followed by a sustained and long lasting venorelaxation (See Figures 31, 32 and 34). Pretreatment of the rat isolated portal vein with nifedipine (1µM) completely inhibited the contractile phase induced by the highest used concentration of APE (400 mg/.ml), but did not affect the contractile phase induced by PAE and SBE (See Figures 31, 32 and 34). These findings suggest that the initial contractile effect of APE could be mediated through activation of the voltage-gated calcium channels while the effects of PAE and SBE do not involve these channels to induce contractions in this tissue. In addition, pretreatment of the isolated portal veins with atropine sulphate, did not alter the sustained, long lasting venorelaxations induced by the highest concentrations of APE, PAE and SBE, (See

Figures 31, 32 and 34), thus discarding involvement of cholinergic receptors in their mechanisms of venorelaxation.

In contrast, without causing any initial contraction, HCE induced a significant and concentration-dependent venorelaxation in the rat isolated portal veins (See Figure 33). Pretreatment of the rat isolated portal vein preparations with atropine sulphate, significantly inhibited, but did not abolish the venorelaxation induced by the highest concentration of the test extract (160 mg/.ml) (See Figure 33). This observation indicates that HCE may induce venorelaxation in portal vein via stimulation of cholinergic receptors.

The overall effects of these test extracts in a portal vein suggest that these plants could provide a source of novel drugs for the treatment of portal hypertension. Portal hypertension is the most common complication of chronic liver diseases. This syndrome, defined by a pathological increase in the portal venous pressure, is characterized by an increase in the pressure gradient between portal vein and inferior vena cava (Ratti, Pozzi, Bosch, 2005). The portal pressure gradient is the result of the interaction between portal blood flow and the vascular resistance that opposes the flow (Ratti, Pozzi, Bosch, 2005). Thus, portal pressure gradient can originate from an increase in intrahepatic vascular resistance, an increase in hepatic blood flow or from a combination of (Ratti, Pozzi, Bosch, 2005). There are mainly two types of pharmacological approaches towards the treatment of portal hypertension: by reducing portal blood flow by using vasoconstrictors or portal vascular resistance by using vasodilators (Navasa, Bosch, Rodes, 1991).

On the basis of the findings demonstrated by the present study, it is clear that these plants may provide a cheap source of treatment for portal hypertension. Thus, EKE due to its potent contractile effects demonstrated in isolated portal veins, may be used as a source of vasoconstrictor agents to reduce portal blood flow, one of the factors that increases portal pressure. On the other hand, due to their potential to induce long lasting venorelaxations, the other extracts (APE, PAE, SBE and HCE), may provide a source of vasodilator agents, which would be used to reduce portal vascular resistance, another causal factor for portal hypertension. In addition, these extracts with potent venorelaxant effects, may represent a novel strategy to treat portal hypertension, by reducing hepatic vascular resistance with the advantage of avoiding a further decrease in liver blood flow, but of improving liver perfusion (Loureiro-Silva, Cadelina, Iwakiri and Groszmann, 2003).

6.7   Conclusion.

The present study has demonstrated the potential of the ethanolic extracts from five different selected plants to reduce blood pressure. All the tested extracts exhibited antihypertensive effects both in acute and chronic studies. However, of the five extracts only HCE demonstrated potent heart rate lowering effect (bradycardia) in the acute experiments while in chronic experiments, all tested extracts demonstrated the potential to lower heart rate.

The present study has also demonstrated that apart from EKE, all extracts exhibit negative inotropic and chronotropic effects on the rat isolated electrically-driven left and spontaneously-beating right atria respectively. The negative inotropic and chronotropic effects of HCE were shown to be mediated through activation of the cholinergic receptors. We speculate that the extracts might exert some inhibitory effects on the beta-adrenergic receptors and the voltage-gated calcium channels, since they antagonized the positive inotropic effects of noradrenaline and calcium. In contrast, EKE was shown to exhibit positive inotropic and chronotropic effects which have been shown to be mediated by activation of the beta-adrenergic receptors and voltage-operated calcium channels.

This study has also demonstrated that all tested extracts have vasorelaxant effects on the vascular smooth muscles of the rat isolated aorta. Apart from APE, all extracts exhibited both endothelium-dependent and endothelium-independent vasorelaxations. APE was shown to exhibit an endothelium-dependent vasorelaxation. Vasorelaxant effects of these test extracts were demonstrated to involve the activation of endothelial vasodilators, opening of potassium channels and blockade of calcium channels.

The study has also demonstrated the biphasic contractile effects of some of the tested extracts in rat isolated portal veins, except for EKE and HCE. These extracts induced an initial and transient contractile effect followed by a sustained and long lasting venorelaxation. On the other hand, HCE induced venorelaxation in the spontaneously

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contracting portal vein, the effect of which was mediated by activation of cholinergic receptors. EKE, on the contrast, induced tonic contraction at higher concentration in the rat isolated spontaneously contracting portal veins. This effect was shown to involve activation of voltage-gated calcium channels.

Taken together, these findings suggest that, these plant extracts exert their hypotensive effects through their cardio-suppressant actions causing a reduction in both stroke volume and cardiac output and through their potent vasorelaxant effects causing a reduction in the total peripheral vascular resistance. Thus, a combined decrease in cardiac output and total peripheral vascular resistance results in hypotension.

The present study has provided the mechanistic basis for the use of *Ekebergia capensis, Persea americana, Helichrysum ceres, Sclerocarya birrea* and *Hypoxis hemerocallidea* in the management of hypertension and other cardiovascular disorders. Therefore, crude extracts from these plants can be recommended for an additional and rational therapy of hypertension, as a cheap and accessible source of treatment of hypertension and other associated cardiovascular disorders.

6.8   Short Falls

The following are some of the short falls of the present study;
The doses used in the present study were determined based on the preliminary work in our laboratory. There was no work on the toxicity of the plant extracts. Therefore, there

may be a possibility that the observed effects of the extracts were partly due to the toxic effects of the extracts.

During acute and chronic studies in rats only a single dose was used. Use of a series of doses would be ideal to asses whether the effects of the extracts may be dose-dependent or not.

Pharmacological studies were only done *in vitro*. Pharmacological studies should have been conducted in whole animals in order to confirm the *in vitro* results, since there are possibilities that the *in vitro* studies may provide false results when applied to the whole animal studies.

The study does not justify use of ethanolic extracts, since traditional healers usually administer aqueous extracts to their patients.

6.9   Future work and recommendations

1.   Further work will be devoted to isolate and structurally identify the active ingredients in these extracts.

2.   Since none of the active compounds reported to be present in these plants were tested for their cardiovascular effects in this work, future work will be devoted to

screening the isolated compounds of these extracts and probably compare their effects with the available synthetic compounds.

3. Since it is a common practice that traditional healers combine more than one plant to formulate a herbal medicine, in future we will investigate the combined effects of these extracts in the treatment of hypertension. Furthermore, efforts will be made to compare the cardiovascular effects of these ethanolic extracts to those of aqueous extracts considering that traditional healers normally administer aqueous extracts to their patients.

4. Many factors may contribute to the long-term beneficial effects of the test extracts on the cardiovascular system. For instance vascular smooth muscle cell proliferation is essential factor involving the formation of atherosclerotic plaques. Therefore, since nitric oxide plays a major role in vascular smooth muscle cell proliferation the effects of these extracts and their isolated active ingredients on vascular smooth muscle proliferation will be carried out *in vitro*. Future work will also investigate whether the extracts may induce actual production of nitric oxide and cGMP *in vitro*.

5. It is generally accepted that the kidney exerts a dominant role in controlling blood pressure. Taking this into account, future work will investigate the effects of these extracts on the renal function.

6. At present, nothing is known regarding the pharmacodynamics of these test extracts. Therefore, future studies on the possible degradation or chemical modification of the test extracts in the body will be necessary to confirm the relevance of the *in vitro* studies described by the present study.

7. Toxicological studies of these extracts will be conducted by a dye-reduction colorimetric (MTT) assay on cardiovascular cell lines to determine optimal doses.

Chapter 7

7.0   **REFERENCES**

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8.0 APPENDICES

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16 AUGUST 2005

MR. DR KAMADYAAPA (202050003)
HUMAN PHYSIOLOGY

Dear Mr. Kamadyaapa

ETHICAL CLEARANCE NUMBER : HSS/05014A

I wish to confirm that ethical clearance has been granted for the following project:

"Mechanisms of the cardiovascular effects of some medicinal plants: An experimental study""

Yours faithfully
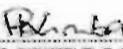
MS. PHUMELELE XIMBA
RESEARCH OFFICE

PS: The following general condition is applicable to all projects that have been granted ethical clearance:

THE RELEVANT AUTHORITIES SHOULD BE CONTACTED IN ORDER TO OBTAIN THE NECESSARY APPROVAL SHOULD THE RESEARCH INVOLVE UTILIZATION OF SPACE AND/OR FACILITIES AT OTHER INSTITUTIONS/ORGANISATIONS. WHERE QUESTIONNAIRES ARE USED IN THE PROJECT, THE RESEARCHER SHOULD ENSURE THAT THE QUESTIONNAIRE INCLUDES A SECTION AT THE END WHICH SHOULD BE COMPLETED BY THE PARTICIPANT (PRIOR TO THE COMPLETION OF THE QUESTIONNAIRE) INDICATING THAT HE/SHE WAS INFORMED OF THE NATURE AND PURPOSE OF THE PROJECT AND THAT THE INFORMATION GIVEN WILL BE KEPT CONFIDENTIAL.

cc. Faculty Officer
cc. Supervisor (Prof. CT Musabayane)
cc   Co. Supervisors (Prof. J Ojewole and Prof. F Shode)

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Cardiovascular Topics

Some *in vitro* and *in vivo* cardiovascular effects of *Hypoxis hemerocallidea* Fisch & CA Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal models

JAO OJEWOLE, DR KAMADYAAPA, CT MUSABAYANE

Summary

This study was undertaken to investigate some cardiovascular effects of *Hypoxis hemerocallidea* Fisch & CA Mey (Hypoxidaceae) corm (African potato) aqueous extract in experimental animal paradigms. The effect of the corm aqueous extract (APE) on myocardial contractile performance was evaluated on guinea-pig isolated atrial muscle strips *in vitro*; whereas the antihypertensive (hypotensive) effect of the plant extract was examined in hypertensive Dahl salt-sensitive rats *in vivo*. APE (25–400 mg/ml) produced concentration-dependent, significant ($p < 0.05$–0.001) negative inotropic and negative chronotropic effects on guinea-pig isolated electrically driven left, and spontaneously beating right atrial muscle preparations, respectively. Moreover, APE reduced or abolished, in a concentration-dependent manner, the positive inotropic and chronotropic responses of guinea-pig isolated atrial muscle strips induced by noradrenaline (NA, 1–100 μM) and calcium (Ca^{2+}, 5–40 mM). The negative inotropic and chronotropic effects of APE on guinea-pig atrial muscle strips were not modified by exogenous administration of atropine (ATR, 7.5×10^{-7}–2.5×10^{-6} M) to the bath fluid. APE also significantly reduced ($p < 0.05$–0.001) or abolished in a concentration-dependent manner, the rhythmic, spontaneous, myogenic contractions of portal veins isolated from rats. Furthermore, APE caused dose-related transient but significant ($p < 0.05$–0.001) reduc-

tions in the systemic arterial blood pressure and heart rates of the hypertensive rats used.

Although the exact mechanisms of the cardiodepressant and the transient hypotensive (antihypertensive) actions of APE could not be established in the present study, we exclude the involvement of the cholinergic system; since the extract's cardiovascular effects were resistant to atropine pretreatment. However, the results of this laboratory animal study indicated that APE caused bradycardia and brief hypotension in the mammalian experimental models used. These observations tend to suggest that the herb may be used as a natural supplementary remedy in some cases of cardiac dysfunctions and in essential hypertension. The findings of this experimental animal study lend pharmacological support to the folkloric, anecdotal uses of the African potato in the management and/or control of certain cardiac dysfunctions and essential hypertension in some rural communities of southern Africa.

The floral biodiversity of South Africa has provided our traditional health practitioners with an impressive pool of 'natural pharmacy' from which plants are selected as remedies, and/or as ingredients to prepare herbal medicines (phytomedicines) for a plethora of human disorders. We have recently examined some of the commonly used South African medicinal plants for their chemical constituents and pharmacological activities.[1-7] In an effort to establish a scientific basis for their folkloric, ethnomedical uses. One such example is *Hypoxis hemerocallidea* (Fisch & CA Mey) [family: Hypoxidaceae] – previously known as *Hypoxis rooperi*.

This southern African 'miracle medicinal plant' is a tuberous, perennial herb with long, strap-shaped leaves and yellow, star-shaped flowers. The broad and slightly hairy leaves of *H hemerocallidea* are arranged one above the other to form three distinct groups of leaves spreading outwards from the centre of the plant, while the bright yellow, star-shaped flowers are borne on long, slender stalks.[8] The tuberous rootstock (corm) of the

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herb, popularly known as 'African potato', is widely used in southern African traditional medicine as a remedy for an array of human ailments. Traditional healers have used the corm as *muthi* (isiZulu word, meaning 'medicine') for centuries, and now, the humble African potato has been claimed to be an 'amazing plant medicine' in the fight against various modern human ailments such as HIV/AIDS-related diseases, arthritis, yuppie flu, hypertension, diabetes mellitus, cancer, psoriasis, gastric and duodenal ulcers, tuberculosis, urinary tract infections, asthma, and some central nervous system disorders, especially epilepsy and childhood convulsions.[4]

Previous studies in our laboratories have indicated that the corm of *H. hemerocallidea* possesses anti-inflammatory, hypoglycaemic and other pharmacological properties.[?] The present study was prompted by the claim of some traditional health practitioners in KwaZulu-Natal that decoctions and infusions of African potato are effective remedies for the management and/or control of hypertension and some cardiac disorders. The core aim of this study was, therefore, to investigate the cardiac and antihypertensive (hypotensive) effects of *H. hemerocallidea* corm aqueous extract in laboratory, experimental animal paradigms.

Materials and methods

The experimental protocol used in this study was approved by the Ethics Committee of the University of Durban-Westville and conforms to the *Guide to the Care and Use of Animals in Research and Teaching.*[?]

Plant material

Fresh corms of *H. hemerocallidea* were purchased from a fruit kiosk along West Street in Durban, KwaZulu-Natal, between June and November 2003. The corms were identified by Prof H Baijnath (former chief taxonomist/curator of the University of Durban-Westville's Department of Botany) as those of *Hypoxis hemerocallidea* Fisch & CA Mey [family: Hypoxidaceae]. A voucher specimen of the plant has been deposited in the University's Botany Departmental Herbarium.

Preparation of corm aqueous extract

One kilogram of *H. hemerocallidea* fresh corms was washed with distilled water, cut into smaller pieces and ground in a Waring commercial blender. The milled corm was soaked in hot distilled water and extracted twice, on each occasion with 2.5 litres of hot distilled water (at 90–100°C) for 12 hours. The combined extract solubles were concentrated to dryness under reduced pressure in a rotary evaporator at $70 \pm 1°C$. The resulting crude aqueous extract was freeze dried, finally giving 78 g (7.8% yield) of a dark brown, powdery, aqueous extract residue (APE). Without any further purification, aliquot portions of the APE were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

Animal material

Healthy, male Dunkin-Hartley guinea-pigs (*Cavia porcellus*) weighing 300 to 450 g and healthy young adult, male Wistar rats (*Rattus norvegicus*) weighing 250 to 300 g were used.

The animals were kept and maintained under laboratory conditions of temperature, humidity and light, and were allowed free access to food (standard pellet diet) and water *ad libitum*. All the animals were fasted for 16 hours, but still allowed free access to water before the commencement of our experiments. Guinea-pig isolated atrial muscles were used for the *in vitro* evaluation of the effects of APE on myocardial contractility; whereas rat isolated portal veins were used to examine the vasodilatory effects of the extract. Dahl salt-sensitive rats were used for the *in vivo* investigation of the hypotensive (antihypertensive) effect of APE.

Isolated muscle experiments

Guinea-pig isolated electrically driven left, and spontaneously beating right, atrial muscle preparations

The guinea-pigs were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as previously described by Ojewole.[?] The isolated left atrium of each guinea-pig was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution (of composition, in g/litre: NaCl, 6.92; KCl, 0.34; NaH_2PO_4, 0.15; $NaHCO_3$, 2.10; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00 – pH adjusted to 7.4) maintained at $34 \pm 1°C$ and continuously aerated with carbogen (95% O_2 + 5% CO_2 gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5-msec duration at a frequency of 3 Hz and a supramaximal voltage of five to 10 volts, delivered by an SRI stimulator.

The spontaneously beating right atrium of each animal was also set up under the same physiological experimental conditions and allowed to beat spontaneously. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously beating right atrial muscle preparations were always set up at a time (one used as the test, and the other the control preparation) to allow for changes in the atrial tissue sensitivity. The atrial muscle preparations were left to equilibrate for 45 to 60 min (during which time the bathing physiological solution was changed every 15 min) before they were challenged with APE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied graded concentrations of APE and/or the reference agonist drugs used, whereas the control atrial muscle strips were treated with distilled water (0.1–0.4 ml) only. The electrically provoked and spontaneous contractions of the atrial muscles, as well as the APE- and reference agonist drug-induced responses of the atrial muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat isolated portal vein preparations

The rats were sacrificed by stunning and exsanguination. The abdomen of each rat was quickly opened by midline incision and the intestines were pulled aside. The portal vein of each rat, with an *in situ* length of approximately 20 mm, was carefully cleaned free of extraneous connective and fatty tissues, and then removed from the animal. Each isolated portal vein was suspended under an applied resting tension of 0.5 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiologi-

cal solution (pH adjusted to 7.4) maintained at 34 ± 1°C and continuously aerated with carbogen. Two isolated venous tissue preparations (one control and the other APE- or reference drug-treated test) were always set up in order to make allowance for changes in tissue sensitivity. Control venous muscle strips were treated with distilled water only (the vehicle in which APE and reference drugs were dissolved). The venous tissue preparations were allowed to equilibrate for 45 to 60 min (during which time the bathing physiological solution was changed every 15 min) before they were challenged with APE or any of the reference drugs used. The APE- and reference drug-induced responses of the venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Whole animal experiments

Systemic arterial blood pressures and heart rates of anaesthetised rats

Male hypertensive, young adult Dahl salt-sensitive rats weighing 250 to 300 g were used. Before the commencement of our experiments, the animals were placed on 4% saline water and normal food (standard pellet diet) for six to eight weeks (during which time the arterial blood pressure of the animals rose to between 170/125 and 186/130 mmHg). Rats with an arterial blood pressure of 170/120 mmHg and above were considered to be hypertensive and used in this study.

Each of the animals was anaesthetised with intraperitoneal injection of 0.11 g/kg of Trapanal [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate]. The right femoral vein of each rat was cannulated (with a small polythene cannula) for the administration of the plant extract and reference drugs. In order to minimise the problem of blood coagulation, heparin (500 units/kg) was intravenously administered to the animal and flushed in with 0.2 ml of 0.9% w/v sodium chloride solution. The left carotid artery of each rat was also cannulated and connected to a four-channel Grass polygraph for systemic arterial blood pressure recording. The trachea of each rat was cannulated for artificial respiration, but the animal was allowed to breathe spontaneously. The rat's body temperature was maintained at 36 ± 1°C with an incandescent lamp placed over its abdomen.

After a 20-min stabilisation period, the systemic arterial blood pressure (systolic, diastolic and mean arterial pressures) and heart rates of each rat were measured and recorded. The effects of APE and other drugs [acetylcholine (0.5–4.0 μg/kg iv) and noradrenaline (0.5–4.0 μg/kg iv)] on systemic arterial blood pressure and heart rates (calculated from the ECG limb lead II recording at a fast paper speed of 25 mm/sec) were recorded by means of a four-channel Grass polygraph recorder (model 79D). In some of the rats, the hypotensive (depressor) effect of APE (25–400 mg/kg iv) was examined after atropinisation [pretreatment of the rats with atropine sulphate (1.5 mg/kg ip) 18 to 24 hours before use]. Because APE and other drugs used in this study were dissolved in distilled water, rats treated with distilled water (3 ml/kg iv) alone were used as control animals under the same experimental conditions.

Drugs used

The following compounds and drugs were used: *H hemerocallidea* corm aqueous extract (APE), acetylcholine chloride,

atropine sulphate, (-)-noradrenaline hydrochloride, (+)-propranolol hydrochloride, calcium chloride, potassium chloride and Trapanal [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate]. All drugs were dissolved in distilled water each day at the beginning of our experiments. Drug concentrations and doses quoted in the text refer to the salts, except APE, and denote final organ-bath concentrations in the *in vitro* experiments.

Data analysis

Experimental data obtained from test guinea-pig isolated atria, rat isolated portal vein and anaesthetised hypertensive rats treated with APE alone, as well as those obtained from distilled water-treated control isolated atria, portal vein and anaesthetised rats were pooled and expressed as means (± SEM). Statistical comparison of the differences between the APE- and reference drug-treated test means, and distilled water-treated control means, was performed with GraphPad InStat software (version 3.00, GraphPad software, San Diego, California, USA) using one-way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer multiple comparison test. Values of $p < 0.05$ were taken to imply statistical significance.

Results

Isolated muscle experiments

Guinea-pig isolated, electrically driven left, and spontaneously beating right atrial muscle preparations

Sequential administrations to the bath fluid of relatively low to high concentrations of APE (25–400 mg/ml) significantly reduced ($p < 0.05$–0.001) or abolished the force of contractions of guinea-pig isolated electrically driven left atrial muscle preparations in a concentration-related manner (Fig. 1). The negative inotropic effect of APE on these muscle strips was not affected by prior exogenous administration of atropine (ATR, 7.5×10^{-7}–2.5×10^{-6} M) to the bath fluid.

At the same concentration range, APE also significantly reduced ($p < 0.05$–0.001) or abolished the rate of contractions of guinea-pig isolated, spontaneously beating right atrial muscle preparations in a concentration-dependent manner (Fig. 2). Sequential administrations to the bath fluid of high concentrations of APE (> 400 mg/ml) always produced car-
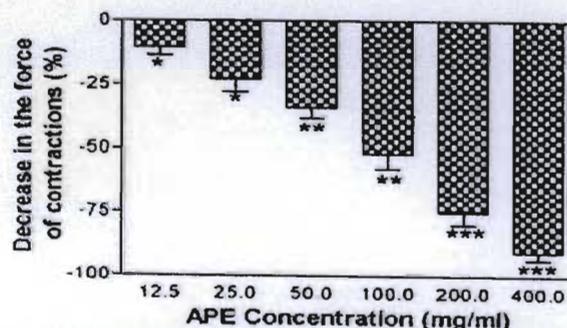

Fig. 1. Effects of graded concentrations of APE on guinea-pig isolated, electrically driven left atrial muscle strips. Each value represents the mean (± SEM) of eight to 10 observations, while the vertical bars denote standard errors of the means (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control).

diac arrhythmias in these muscle strips. However, the negative chronotropic effect of APE was not antagonised by atropine, which reduced or abolished the negative chronotropic effect of acetylcholine (ACh, $7.5 \times 10^{-}-3.5 \times 10^{-}$ M) on six other such muscle preparations examined. APE significantly reduced ($p < 0.05-0.001$) or abolished, like propranolol ($10^{-}-10^{-}$ M), the positive inotropic and chronotropic effects of noradrenaline (NA, 1–100 μM) on all the other eight isolated atrial muscle strips tested. The extract also significantly ($p < 0.05-0.001$) inhibited or abolished calcium-induced (Ca^{2+}, 5–40 mM) positive inotropic and chronotropic responses on all the other nine atrial muscle strips examined.
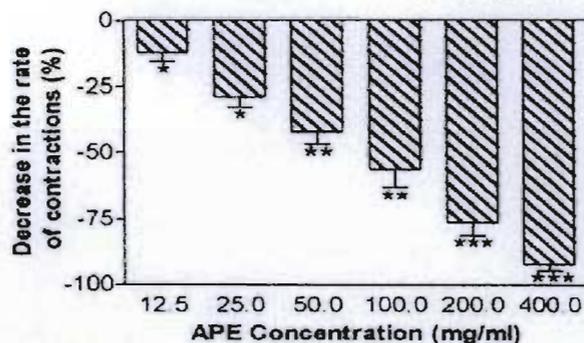

Fig. 2. Effects of graded concentrations of APE on guinea-pig isolated, spontaneously beating right atrial muscle strips. Each value represents the mean (± SEM) of eight to 10 observations, while the vertical bars denote standard errors of the means (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control).
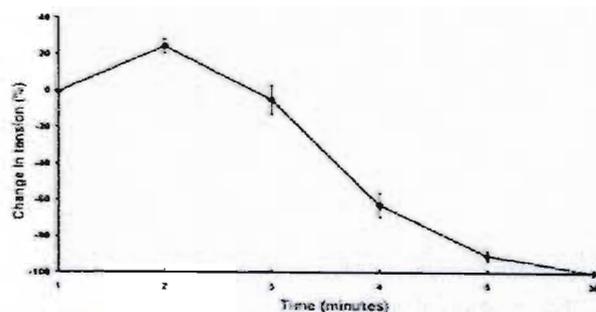

Fig. 3. Effects of APE (400 mg/ml) on rhythmic, myogenic, spontaneous contractions of rat isolated portal veins. Each point represents the mean (± SEM) of eight to 10 preparations, while the vertical bars denote standard errors of the means.

Rat isolated portal vein

Sequential administrations to the bath fluid of relatively low to high concentrations of APE (25–400 mg/ml) always induced concentration-dependent, biphasic effects on the amplitude and frequency of the rhythmic, myogenic contractions of the rat isolated portal veins. The biphasic effect produced by APE always consisted of an initial slight but significant ($p < 0.05$) contraction (stimulation) of short duration, followed by a secondary, longer-lasting and significant ($p < 0.05-0.001$) relaxation (inhibition) of the vascular muscle preparation (Fig. 3). At the same concentration range, APE also inhibited or abolished in a concentration-dependent manner, contractions of the venous muscle preparations induced by noradrenaline (1–100 μM) or potassium (5–40 mM).

Whole animal experiments
Systemic arterial blood pressures and heart rates of rats

Acute intravenous administrations of APE (25–400 mg/kg iv) into anaesthetised, hypertensive Dahl salt-sensitive rats produced dose-related, significant reductions ($p < 0.05-0.001$) in the systemic arterial blood pressure and heart rates of the rats (Table 1). The transient hypotensive (antihypertensive) effect of the extract persisted for seven to 45 min, depending on the APE dose administered. Furthermore, the extract dose-dependently inhibited or abolished the pressor effects of noradrenaline (0.5–4.0 ug/kg iv) on systemic arterial blood pressure and heart rates of the animals. Pretreatment of the anaesthetised, hypertensive rats with atropine sulphate (1.5 mg/kg ip, 18 to 24 hours before use) abolished or markedly reduced the depressor effects of acetylcholine (0.5–4.0 μg/kg iv) on the systemic arterial blood pressure and heart rates. However, the depressor effects of APE on systemic arterial blood pressure and heart rates of the rats were not affected by pretreatment of the animals with atropine sulphate.

Discussion

The results obtained in this study show that relatively low to high concentrations of *H hemerocallidea* corm extract produced dose-related, significant reductions ($p < 0.05-0.001$) in systemic arterial blood pressure and heart rates of the anaesthetised, hypertensive rats used. While biomedical literature abounds with information on the chemistry and some pharmacological properties of African potato extracts,[ ] there is relatively little information on the cardiovascular effects of the herb in humans and other mammals.[ ]

Although the precise mechanism of the cardiovascular effects of African potato *in vitro* and *in vivo* is still obscure, it is clear from the results of the present study that the cardiode-

TABLE 1. EFFECTS OF APE (25–400 MG/ KG IV) ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF HYPERTENSIVE, DAHL SALT-SENSITIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	Before treatment (control values)	After treatment with APE (mg/kg iv)				
		25	50	100	200	400
Systolic BP (mmHg)	182.4 ± 6.3	168.5 ± 6.2	156.4 ± 5.6*	140.4 ± 5.4**	122.6 ± 4.3***	102.6 ± 4.4***
Mean BP (mmHg)	146.8 ± 5.6	136.6 ± 5.5	124.7 ± 5.4*	110.5 ± 4.7**	96.3 ± 4.4***	88.3 ± 4.6***
Diastolic (mmHg)	120.5 ± 4.8	118.1 ± 4.6	110.6 ± 4.5*	96.4 ± 4.2**	84.7 ± 4.6***	71.4 ± 4.9***
Heart rate (beats/min)	476.4 ± 20.1	440.7 ± 18.4	396.3 ± 16.4*	336.1 ± 14.9**	280.4 ± 12.5***	182.5 ± 10.4**

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control

pressant and hypotensive effects of APE are highly unlikely to be mediated via the cholinergic mechanism. Our observation in the *in vitro* isolated atrial muscle preparations indicating that the negative inotropic and chronotropic effects of APE were not modified by bath-applied atropine further buttresses the hypothesis of non-cholinergic mediation.

The antagonism of the positive inotropic and chronotropic effects of noradrenaline by APE on isolated atria of guinea-pigs could, in part at least, be taken to suggest β_1-adrenoceptor blockade. However, the inhibitory effects of APE on Ca^{2+}-induced positive inotropic and chronotropic responses of the guinea-pig isolated atrial muscle strips would tend to suggest a non-specific spasmolytic action of the plant extract on the myocardium. Inhibition of noradrenaline- and potassium-induced contractions of the rat isolated portal veins by APE would also appear to support the non-specific, spasmolytic hypothesis.

The observation that relatively high concentrations of APE (> 400 mg/ml) usually induced cardiac arrhythmias in isolated, spontaneously beating right atrial muscles of guinea-pigs is in consonance with the finding of Ker who reported ventricular tachycardia as an adverse effect of chronic ingestion of African potato tea. In the isolated portal vein vascular smooth muscle used, APE would appear to change the physical state functions of the muscle cells' membrane potentials, probably by hyperpolarising the cells of the isolated blood vessel strips. The cardiodepressant effects of the herb extract are, therefore, likely to be partly mediated through (1) β_1-adrenoceptor blockade, (2) direct action on contractile machinery of the myocardium; (3) inhibition of Ca^{2+} flux; and/or (4) interference with membrane mobilisation of Ca^{2+} needed to maintain normal contractions of the myocardium.

Coetzee *et al.* observed, in their studies on pharmacokinetic and cardiovascular effects of hypoxoside and rooperol in Chaema baboons, that pure hypoxoside produced no cardiovascular effect, whereas pure rooperol caused moderate, transient increases in cardiac output, stroke volume and vascular pressure without an increase in heart rate or filling pressure. The investigators, therefore, suggested that rooperol increased myocardial contractility. The discrepancies between our results and the findings of Coetzee *et al.* may be due to (1) species variation (rats and guinea-pigs vs baboons), and (2) the fact that we used a crude aqueous extract of African potato in our study, whereas Coetzee *et al.* used pure hypoxoside and rooperol.

The exact mechanism through which the plant extract acts to depress systemic arterial blood pressure in the experimental animal model used in this study is unknown at present. In physiological parlance, however, the hypotensive effect of APE is likely to be due, in part at least, to its vasodilatory action (its main, secondary effect on rat isolated portal veins), through which route it probably acts to decrease vascular resistance. In this way, APE may be reducing total peripheral resistance (TPR) in the anaesthetised rats. Similarly, the direct cardiodepressant effect of the APE may lead to a reduction in cardiac output (CO) via a marked reduction in the heart rate. The combined reductions in the TPR and CO would subsequently lead to a fall in systemic arterial blood pressure — since arterial blood pressure is a product of cardiac output and total peripheral resistance.

The *H hemerocallidea* corm has been reported to contain phytosterols (mainly β-sitosterol) and some sterolins. However, the chemical constituent/s of the corm that is/are responsible for the observed cardiodepressant and hypotensive effects of APE in our study is/are still unknown. Although the exact chemical compound/s responsible for the cardiodepressant and hypotensive effects of African potato aqueous extract still remain/s speculative, experimental evidence obtained in the present laboratory animal study indicates that the herb possesses cardiodepressant (bradycardic) and hypotensive properties.

In summary, the findings of this experimental animal study lend pharmacological support to folkloric, anecdotal, ethnomedical uses of African potato as a natural supplementary remedy in the management and/or control of certain cardiac dysfunctions and essential hypertension in some rural communities of southern Africa.

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Cardiovascular Topics

Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals

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Summary

The cardiovascular effects of *Persea americana* Mill (Lauraceae) aqueous leaf extract (PAE) have been investigated in some experimental animal paradigms. The effects of PAE on myocardial contractile performance was evaluated on guinea pig isolated atrial muscle strips, while the vasodilatory effects of the plant extract were examined on isolated portal veins and thoracic aortic rings of healthy normal Wistar rats *in vitro*. The hypotensive (antihypertensive) effect of the plant extract was examined in healthy normotensive and hypertensive Dahl salt-sensitive rats *in vivo*.

P americana aqueous leaf extract (25–800 mg/ml) produced concentration-dependent, significant ($p < 0.05$–0.001), negative inotropic and negative chronotropic effects on guinea pig isolated electrically driven left and spontaneously beating right atrial muscle preparations, respectively. Moreover, PAE reduced or abolished, in a concentration-dependent manner, the positive inotropic and chronotropic responses of guinea pig isolated atrial muscle strips induced by noradrenaline (NA, 10^{-9}–10^{-5} M), and calcium (Ca^{2+}, 5–40 mM). PAE (50–800 mg/ml) also significantly reduced ($p < 0.05$–0.001) or abolished, in a concentration-dependent manner, the rhythmic, spontaneous, myogenic contractions of portal veins isolated from healthy normal Wistar rats. Like acetylcholine (ACh, 10^{-8}–10^{-4} M), the plant extract (25–800 mg/ml) produced concentration-related relaxations of isolated endothelium-containing thoracic aortic rings pre-contracted with noradrenaline. The vasorelaxant effects of PAE in the isolated, endothelium-intact aortic rings were markedly inhibited or annulled by N^ω-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), a nitric oxide synthase inhibitor. Furthermore, PAE (25–400 mg/kg iv) caused dose-related, transient but significant reductions ($p < 0.05$–0.001) in the systemic arterial blood pressure and heart rates of the anaesthetised normotensive and hypertensive rats used.

In conclusion, the results of this laboratory animal study indicate that PAE caused bradycardia, vasorelaxation and hypotension in the mammalian experimental models used. The vasorelaxant action of PAE was endothelium dependent, and was therefore possibly dependent on the synthesis and release of nitric oxide (NO). The vasorelaxant effects of PAE appeared to contribute significantly to the hypotensive (antihypertensive) effects of the plant extract. However, the findings of this study tended to suggest that *P americana* leaf could be used as a natural supplementary remedy in essential hypertension and certain cases of cardiac dysfunctions in some rural Africa communities.

In our current pharmaco-chemical exploration of African medicinal plants, we have examined in our laboratories some of the frequently used South African medicinal plants for their chemical constituents and pharmacological actions,[1-10] in an attempt to establish a scientific basis for their folkloric, ethnomedical uses. One of such commonly used African medicinal plants is *Persea americana* Mill (family: Lauraceae).

P americana, otherwise known as the avocado pear, Mexican avocado and so on, is a medium-sized, single-stemmed, terrestrial, erect, perennial, deciduous, evergreen tree 15–20 m in height. Although a native of Central America (Mexico), *P americana* is now found in most tropical and

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subtropical countries of the world. The branches are fissured and grey, but the twigs are green and smooth. The 15–25-cm long and 10–20-cm broad leaves with well-developed petioles are spirally arranged, often clustered near the branch ends, narrowly to broadly elliptical or obovate, and are usually pointed at the tip.[11]

The greenish-yellow flowers are borne on branched, compact panicles, which are shorter than the leaves. The often pear-shaped, one-seeded fruits are variable in size and shape according to the variety, up to 18 cm long and usually shiny and green, or brownish when ripe. The flesh is soft, oily, greenish or yellow surrounding one large, loose round seed.[11] The avocado is now cultivated commercially as a fruit crop in many countries of the world. In many parts of Africa, the fruits of the avocado are much sought after by humans and some animals as valuable foodstuff. Besides the oil, avocado fruit pulp contains carbohydrates and more protein than any other fruit, while its contents of vitamins A and B are high.[11,12]

In addition to the nutritional value of its fruit, the leaves and other morphological parts of P americana possess medicinal properties and are widely used in traditional medicines of many African countries. For example, the fruit pulp is eaten as an aphrodisiac and as an emmenagogue in South Africa,[13] while a hot-water extract of the leaves is taken orally as a diuretic and for hypertension in many West African countries.[14] In some other parts of the world, various morphological parts of P americana have been employed for a wide range of human ailments. Products of the plant have been effectively used for the management, control and/or treatment of amenorrhoea, anaemia, insomnia, hyperlipidaemia, hypertension, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis, and so forth.[11,12]

Previous studies on the avocado have shown that leaf extracts of P americana possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, anti-diabetic, hypoglycaemic, hypotensive and antihypertensive properties.[15,16] The present study was prompted by the claim of some traditional health practitioners in KwaZulu-Natal that decoctions and infusions of avocado leaves are effective remedies for the management and/or control of hypertension and certain cardiac disorders.

The aim of the present study was, therefore, to investigate the cardiac, vascular and antihypertensive (hypotensive) effects of P americana aqueous leaf extract in experimental animal paradigms, with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant leaf in the management, control and/or treatment of essential hypertension and certain cardiac dysfunctions in some rural African communities.

Materials and methods

The experimental protocol used in this study was approved by the ethics committee of the University of Durban-Westville and conforms to the *Guide to the Care and Use of Animals in Research and Teaching*.[17]

Plant material and preparation

Fresh leaves of P americana were collected from a playground behind Willowpark Centre along Umbilo Road in Durban, between January and June 2003. The leaves were identified by Prof H Baijnath, the former chief taxonomist/curator of the Department of Botany, University of Durban-Westville, as those of P americana Mill (family: Lauraceae). A voucher specimen of the plant has been deposited in the Botany Departmental Herbarium.

Room air-dried leaves (1 kg) of P americana were milled in a Waring commercial blender. The powdered leaf was macerated in distilled water and extracted twice, on each occasion with 2.5 l of distilled water at room temperature for 48 hours, with occasional shaking. The combined distilled water extracts were concentrated to dryness at 60 ± 1°C in a rotary evaporator. Freeze drying and solvent elimination under reduced pressure finally gave 21.50 g (2.15% yield) of a light-brown, powdery aqueous leaf extract. This crude extract was used in our study without further purification. Aliquot portions of the residue from the aqueous extract were weighed and dissolved in distilled water for use on each day of our experiments.

Animal material

Healthy male Dunkin-Hartley guinea pigs (*Cavia porcellus*) weighing 300–450 g, and healthy young adult male Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept under laboratory conditions of temperature, humidity and light and were allowed free access to food (standard pellet diet) and water *ad libitum*. All the animals were fasted for 16 hours, but allowed free access to water before the commencement of our experiments. Guinea pig isolated atrial muscles were used for the *in vitro* evaluation of the effects of the aqueous extract on myocardial contractility, whereas rat isolated portal veins and thoracic aortic rings were used to examine the vasorelaxant effects of the extract. Normotensive (normal) Wistar, and hypertensive Dahl salt-sensitive rats were used for the *in vivo* investigation of the hypotensive (antihypertensive) effect of the aqueous extract.

Isolated muscle experiments

Guinea pig muscle strips

The guinea pigs were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as previously described by Ojewole.[18]

The isolated left atrium of each guinea pig was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution (composition in mmol/l, pH adjusted to 7.4: NaCl, 118; KCl, 4.7; NaH$_2$PO$_4$, 1.28; NaHCO$_3$, 25.0; MgCl$_2$, 1.2; CaCl$_2$, 2.52; glucose, 5.55) maintained at 34 ± 1°C and continuously aerated with carbogen (95% O$_2$ + 5% CO$_2$ gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5-ms duration at a frequency of 3 Hz and a supramaximal voltage of 5–10 V, delivered by

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an SRI stimulator. The spontaneously beating right atrium of the animal was also set up under the same physiological experimental conditions and allowed to beat spontaneously. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously beating right atrial muscle preparations were always set up at a time (one as the test, and the other as the control) to allow for changes in the atrial muscle sensitivity.

The atrial muscle preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied graded concentrations of PAE and/or reference agonist drugs used, whereas the control atrial muscle strips were treated with volumes of distilled water (0.1–0.6 ml) equivalent to the volumes of bath-applied PAE solution used. The electrically provoked and spontaneous contractions of the atrial muscles, as well as the PAE- and reference agonist drug-induced responses of the atrial muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat portal veins

The rats were sacrificed by stunning and exsanguination. The abdomen of each rat was quickly opened by midline incision, and the intestines were pulled aside. The portal vein of each rat, with an *in situ* length of approximately 2 cm, was cleaned of extraneous connective and fatty tissues and then removed from the animal. Each isolated portal vein was suspended under an applied resting tension of 0.5 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution. Two isolated venous tissue preparations (one control and the other PAE- or reference drug-treated test) were always set up in order to make allowances for changes in the venous tissue sensitivity. Control venous muscle strips were treated with distilled water only (the vehicle in which PAE and reference drugs were dissolved). The venous tissue preparations were allowed to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The plant extract- (50–800 mg/ml) and reference drug-induced responses of the venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat thoracic aorta rings

The rats were sacrificed by decapitation. The descending thoracic aorta of each normotensive rat was quickly and carefully excised and placed in a Petri dish filled with ice-cold Krebs-Henseleit physiological solution. The aorta was cleaned of extraneous fat and connective tissues and cut into rings approximately 3–4 mm in width. All dissecting procedures were carefully done to protect the functional endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by

gently rubbing the luminal surface three times with distilled water-moistened cotton wool, followed by six times with a small, plastic tubing. A pair of rat isolated aortic rings, one with intact functional endothelium, and the other one with endothelium denuded, were always set up in parallel for appropriate comparison.

Each of the isolated endothelium-containing and endothelium-denuded aortic rings was suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution maintained at $36 \pm 1^{\circ}C$ and continuously aerated with carbogen (95% O_2 + 5% CO_2). The aortic tissue preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with graded concentrations of PAE or any of the reference drugs used. At the end of the equilibration period, the aortic ring preparations were initially contracted with bath-applied noradrenaline (10^{-5} M).

Endothelial integrity and successful removal of the functional endothelium was assessed by the presence or absence, respectively, of relaxant response to acetylcholine (10^{-5} M). ACh-induced relaxation $\leq$ 5% was taken as satisfactory removal of the functional endothelial layer. Such endothelium-denuded aortic muscle preparations were used in this study. After the subsequent wash-out and equilibration period of 30 min, cumulative dose-response curves were obtained with noradrenaline in aortic rings with and without endothelium.

Subsequently, 20-min pretreatment of the aortic muscle preparations with graded concentrations of the plant extract (25–800 mg/ml) was carried out before the next cumulative additions of noradrenaline (10^{-9}–10^{-5} M) to the bath fluid. After the addition of each NA concentration, a plateau response was obtained before the addition of the next higher dose in all cases of cumulatively applied noradrenaline concentrations. Consecutive dose-response curves were taken at 30-min intervals, during which time the physiological bath solution was changed three to five times until the tension developed returned to basal level.

Following 20-min incubation of the aortic ring preparations with the plant extract (25–800 mg/ml), the arterial relaxant effect of PAE was examined on endothelium-containing and endothelium-denuded aortic ring preparations pre-contracted with sequentially applied or cumulatively administered noradrenaline (10^{-9}–10^{-5} M). The effect of the vehicle in which PAE and the reference drugs used were dissolved (distilled water), was also tested. After each challenge, the aortic rings were washed three to five times with fresh physiological solution and allowed to equilibrate for 30 min before they were challenged again with any of the reference drugs or PAE. The contractile and/or relaxant effects of all the reference drugs, as well as PAE-induced relaxations of the isolated aortic ring preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Whole-animal experiments

Normotensive Wistar and hypertensive Dahl salt-sensitive rats weighing 250–300 g were used. Before the commence-

ment of our experiments, the salt-sensitive rats were placed on 4% saline water and normal food (standard pellet diet) for six to eight weeks (during which time the arterial blood pressure of the animals rose to between 170/130 and 190/140 mmHg). Salt-sensitive rats with arterial blood pressure ≥ 170/120 mmHg were considered to be hypertensive and used in this study.

Each of the normotensive and hypertensive rats was anaesthetised with intraperitoneal injection of 0.11 g/kg of Trapanal* [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate]. The right femoral vein was cannulated with a small polythene cannula for the administration of the plant extract and reference drugs. In order to minimise blood coagulation, heparin (500 units/kg) was intravenously administered to the animal, and flushed in with 0.2 ml of 0.9% w/v sodium chloride solution. The left carotid artery of each rat was also cannulated and connected to a four-channel Grass polygraph for systemic arterial blood pressure recording. The trachea of each rat was cannulated for artificial respiration, but the animal was allowed to breathe spontaneously. The rat's body temperature was maintained at 36 ± 1°C with an incandescent lamp placed over the abdomen.

After 20 min stabilisation period, systemic arterial blood pressure (systolic, diastolic and mean arterial pressures) and heart rate of each rat were measured and recorded. The effects of PAE and the reference drugs [acetylcholine (0.5–4.0 µg/kg iv) and noradrenaline (0.5–4.0 µg/kg iv)] on systemic arterial blood pressure and heart rates (calculated from the ECG limb lead II recording at a fast paper speed of 25 mm/sec.) were recorded by means of a four-channel Grass polygraph recorder (model 79D). In some of the rats, the hypotensive (depressor) effect of PAE (25–400 mg/kg iv) was examined after atropinisation [pretreatment of the rats with atropine sulphate (1.5 mg/kg ip) 18–24 hours before use]. Because PAE and other drugs used in this study were dissolved in distilled water, rats treated with distilled water (2 ml/kg iv) alone were used as control animals under the same experimental conditions.

Compounds and drugs used

The following compounds and drugs were used: P americana aqueous leaf extract, acetylcholine chloride (Sigma, England); (-)-noradrenaline hydrochloride (Sigma, England); atropine sulphate (Sigma, England); Nᵒ-nitro-L-arginine methyl ester (L-NAME) (Sigma, England); Trapanal* [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate] (Byk Gulden, Konstanz, Germany); (±)-propranolol hydrochloride (Sigma, England); calcium chloride and potassium chloride (Sigma, England). The drugs were dissolved in distilled water each day at the beginning of our experiments. Drug concentrations and doses quoted in the text refer to the salts, except PAE, and denote final organ bath concentrations in the in vitro experiments.

Data analysis

Data obtained from test guinea pig isolated atria, rat isolated portal vein, aortic ring strips, and anaesthetised normotensive and hypertensive rats treated with PAE alone, as well as

those obtained from distilled water-treated control isolated atria, portal veins, aortic rings and anaesthetised rats, were pooled and expressed as means (± SEM). Statistical comparison of the differences between PAE- and reference drug-treated test means, and distilled water-treated control means, was performed with GraphPad InStat Software (version 3.00, GraphPad Software, San Diego, California, USA) using one-way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer multiple-comparison tests. Values of $p ≤ 0.05$ were taken to imply statistical significance.

Results

Isolated muscle experiments

Guinea pig muscle preparations

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE (25–800 mg/ml) significantly reduced ($p < 0.05–0.001$) or abolished the force of contractions of guinea pig isolated electrically driven left atrial muscle preparations in a concentration-related manner (Fig. 1). The negative inotropic effect of PAE on these muscle strips was not affected by prior exogenous administration of atropine to the bath fluid.
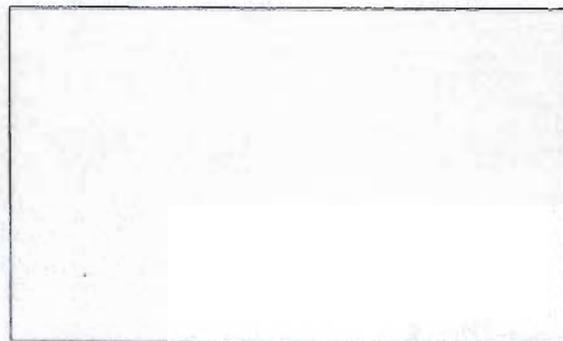

Fig. 1. Effects of graded concentrations of PAE (25–800 mg/ml) on guinea pig isolated electrically driven left atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

At the same concentration range, the plant extract also significantly reduced ($p < 0.05–0.001$) or abolished the rate of contractions of guinea pig isolated spontaneously beating right atrial muscle preparations in a concentration-dependent manner (Fig. 2). However, the negative chronotropic effect of PAE on these muscle strips was not antagonised by atropine which reduced or abolished the negative chronotropic effect of acetylcholine on six other spontaneously beating right atrial muscle preparations examined. PAE significantly reduced ($p < 0.05–0.001$) or abolished, like propranolol, the

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Fig. 2. Effects of graded concentrations of PAE (25–400 mg/ml) on guinea pig isolated spontaneously beating right atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs vehicle-treated control.


Fig. 3. Effects of PAE (800 mg/ml) on rhythmic myogenic spontaneous contractions of rat isolated portal veins. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight to 10 preparations, while the vertical bars denote standard errors of the means. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs vehicle-treated control.

positive inotropic and chronotropic effects of noradrenaline on all eight other isolated atrial muscle strips tested. The plant extract also significantly ($p < 0.05$–0.001) inhibited or abolished calcium-induced positive inotropic and chronotropic responses on all other nine atrial muscle strips examined.

Rat portal veins

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE always induced concentration-dependent, biphasic effects on the amplitude and frequency of the rhythmic myogenic contractions of the rat isolated portal veins. The biphasic effect produced by PAE always consisted of an initial slight but significant ($p < 0.05$) contraction (stimulation) of short duration, followed by a secondary longer-lasting and significant ($p < 0.05$–0.001) relaxation (inhibition) of the venous muscle preparations (Fig. 3). At the same concentration range, the plant extract also inhibited or abolished in a concentration-dependent manner, contractions of the venous muscle preparations induced by noradrenaline or potassium.

Rat aortic ring strips

Cumulative additions of graded concentrations of noradrenaline to the bath fluid provoked concentration-dependent contractions of both endothelium-containing and endothelium-denuded normotensive rat isolated aortic ring strips, with a maximum of 3.76 ± 0.30 g tension developed. Acetylcholine provoked concentration-related, significant relaxations ($p < 0.05$–0.001) of endothelium-containing aortic ring preparations pre-contracted with bath-applied noradrenaline, but did not significantly relax ($p > 0.05$) endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline.

Like acetylcholine, PAE produced concentration-depend-

ent, significant relaxations ($p < 0.05$–0.001) of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline (Fig. 4), but did not relax endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline. Moreover, the plant extract shifted cumulatively administered noradrenaline concentration-response curves to the right in a non-parallel and non-competitive fashion, and suppressed NA-induced maximal contractions of endothelium-containing aortic ring muscle preparations. Ten minutes' prior incubation of the endothelium-intact aortic ring tissues with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished PAE- or ACh-induced
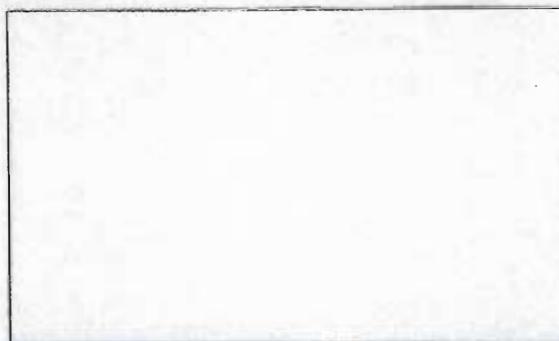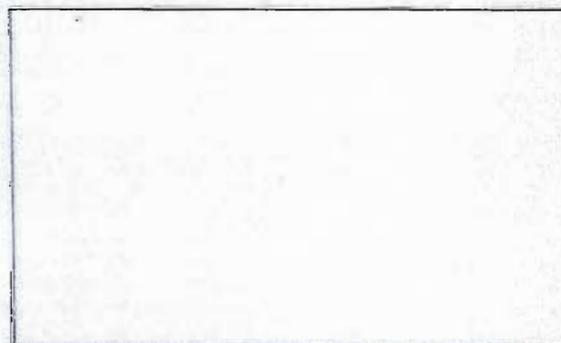
Fig. 4. Arterial relaxant effects of graded concentrations of PAE (50–800 mg/ml) on noradrenaline (10^{-5} M)-induced contractile responses of endothelium-intact aortic rings from normal rats. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. $^*p < 0.05$; $^{**}p < 0.01$, $^{***}p < 0.001$ vs vehicle-treated control.

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TABLE 1. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF NORMOTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	Before treatment: control values	After treatment: PAE (25–400 mg/kg iv)				
		25	50	100	200	400
Systolic BP (mm Hg)	124.5 ± 4.6	112.5 ± 4.4	91.6 ± 4.6*	73.4 ± 4.1**	58.5 ± 4.0***	42.6 ± 3.4***
Mean BP (mm Hg)	111.4 ± 4.1	98.8 ± 4.7	84.3 ± 4.8*	66.5 ± 4.0**	51.4 ± 4.1***	38.4 ± 3.1***
Diastolic (mm Hg)	94.3 ± 4.0	80.4 ± 4.2	68.5 ± 4.0*	56.4 ± 4.3**	44.3 ± 4.3***	31.4 ± 3.0***
Heart rate (beats/min)	396.6 ± 18.6	364.8 ± 18.2	332.3 ± 15.4*	302.8 ± 14.4**	283.5 ± 12.3***	242.7 ± 11.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control.

TABLE 2. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF HYPERTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	Before treatment: control values	After treatment: PAE (25–400 mg/kg iv)				
		25	50	100	200	400
Systolic BP (mmHg)	188.2 ± 6.4	173.6 ± 6.6	156.4 ± 6.3*	140.6 ± 6.0**	124.4 ± 4.8***	101.3 ± 4.8***
Mean BP (mmHg)	146.8 ± 6.1	132.4 ± 6.4	120.6 ± 5.8*	106.4 ± 5.2**	92.5 ± 4.0***	76.7 ± 4.4***
Diastolic (mmHg)	120.4 ± 6.3	106.4 ± 4.0	91.3 ± 5.1*	78.5 ± 4.4**	64.3 ± 4.2***	50.5 ± 4.0***
Heart rate (beats/min)	424.6 ± 20.4	391.4 ± 18.6	356.5 ± 16.0*	318.2 ± 15.2**	286.4 ± 14.6***	236.4 ± 12.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control.

relaxations of the endothelium-containing aortic rings pre-contracted with noradrenaline. Ten minutes' prior incubation of the aortic ring tissues with atropine sulphate also inhibited or abolished acetylcholine-induced relaxations of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline.

Whole animal experiments

Acute intravenous administrations of PAE into anaesthetised normotensive and hypertensive rats produced transient, dose-related, significant reductions ($p < 0.05$–0.001) in the systemic arterial blood pressure and heart rates of the rats (Tables 1, 2). The transient hypotensive (antihypertensive) effect of the plant extract persisted for 12–85 min, depending on the PAE dose administered. Furthermore, the plant extract dose-dependently inhibited or abolished the pressor effects of noradrenaline on systemic arterial blood pressure and heart rates of the animals. Pre-treatment of the normotensive and hypertensive rats with atropine sulphate abolished or markedly reduced the depressor effects of acetylcholine on systemic arterial blood pressure and heart rates of the animals. However, the depressor effects of PAE on blood pressure and heart rates were not affected by pre-treatment with atropine sulphate.

Discussion

The results of this study indicated that the aqueous leaf extract of *P americana* possessed cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the experimental animal paradigms used. This evidence was in agreement with the findings of some of the earlier investigators who have reported vasorelaxant[16] and hypotensive[17] effects of the leaf extract in experimental animal models.

Furchgott and Zawadzki[18] first described the involvement of the endothelium-derived relaxing factor (EDRF),

which was subsequently determined to be nitric oxide or NO derivatives synthesised from guanidine groups of L-arginine.[20,21] Endothelium-dependent relaxation, which has been demonstrated in many vascular preparations, including some veins, arteries and microvascular vessels, occurs in response to stimulation by a variety of substances, such as acetylcholine, adenine nucleotides,[22] thrombin, substance P,[23] calcium ionophore A23187, bradykinin and histamine.[24] The vasodilatation effects of endothelium-dependent substances can be inhibited by several L-arginine analogues, such as N-monomethyl-L-arginine (L-NMMA) and N$^\omega$-nitro-L-arginine methyl ester (L-NAME).[25,26,27]

Endothelial nitric oxide plays a vital role in the control of vasomotor tone and structure.[22,28] On the other hand, vascular tone plays an important role in the regulation of arterial blood pressure. The development and maintenance of hypertension has been suggested to involve a reduced endothelium-dependent vasodilator influence on the vascular tissue.[28] Impairment of endothelium-dependent vascular relaxation in human and experimental hypertension has been observed by Luscher and Vanhoutte,[29] and the ability of nitric oxide to maintain vascular tone has been shown to be deficient in this condition.[30,31] Because NO is a potent vasodilator, a deficient production and/or release of endothelium-derived NO will result in diminished vasodilator tone, thus allowing vascular resistance to rise, and this, in turn, will lead to elevated blood pressure.[32,33]

Relaxation of vascular smooth muscle by NO involves a series of steps. Nitric oxide is formed in functional endothelium by the activation of nitric oxide synthase (NOS), which uses L-arginine as a substrate. Once formed, NO diffuses out of the endothelium, with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylate cyclase.[34] This enzyme catalyses the conversion of guanidine triphosphate (GTP) to cyclic guanidine monophosphate (cGMP), which in turn, causes relaxation of

the vascular smooth muscle cells.[20,26,30,31]

In pathological conditions of the cardiovascular system, there is a dysfunction in the integrity of the vascular endothelium with a subsequent reduction in the release, bioavailability and/or action of nitric oxide.[28] NO release and function have been shown to decrease in cardiovascular diseases, such as hypertension,[22] atherosclerosis[32] and congestive heart failure.[33] Therefore the development of vasodilators which can restore the level and integrity of NO in the vascular system would potentially contribute to the treatment of these cardiovascular diseases.[28]

In the present study, the plant extract, like acetylcholine, caused concentration-dependent relaxation of the normotensive rat isolated endothelium-containing aortic ring preparations pre-contracted with noradrenaline. This vasorelaxant property would appear to have contributed, at least in part, to the antihypertensive (hypotensive) effect of the plant extract. The arterial muscle relaxant effect of the extract disappeared by removal of the functional endothelium.

Furthermore, pre-treatment of the endothelium-containing aortic ring preparations with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished the vasorelaxant effect of PAE. Taken together, these observations would appear to suggest that the vasorelaxant effect of the extract, like that of acetylcholine, was dependent on the formation and/or synthesis and release of endothelium-derived nitric oxide, since removal of the functional endothelial cells led to the absence of relaxant response to PAE in the endothelium-denuded aortic ring preparations. These observations are in agreement with the findings of Martin et al.,[34] Ignarro et al.,[35] Kang et al.,[36-38] Baisch et al.[39] and Yin et al.[24]

The present study also suggests that the endothelium-dependent vasorelaxant effect of PAE could be mediated via endothelial NO signaling in the aortic tissue preparations. However, the release of endothelial NO and the opening of potassium channels have also been implicated in the vasorelaxant effects of extracts from some other medicinal plants.[36-40]

Noradrenaline-induced contractions of blood vessels have been shown to be partly due to calcium release from intracellular storage sites and partly due to the influx of extracellular calcium into the cell via receptor-gated channels following alpha$_1$ (α_1)-adrenoceptor activation.[42] In the present study, endothelium-containing aortic rings pre-contracted with NA in Krebs-Henseleit solution with and without normal calcium concentrations were relaxed by exogenous additions of PAE or acetylcholine. Moreover, the non-parallel shift of the noradrenaline concentration-response curves to the right by the plant extract seems to suggest a mechanism of non-competitive α_1-adrenoceptor blockade. This hypothesis is in consonance with the work of Abreu et al.[43] on ethanolic extract of Jatropha gossypiifolia Linn in rats.

The findings of the present study indicated that PAE induced vasorelaxation in normotensive rat isolated portal veins and endothelium-containing aortic rings, and caused hypotension in anaesthetised, normotensive and hypertensive rats. Although α_1-adrenoceptor blockade may have partially contributed to the hypotensive effect of the plant extract, the experimental evidence obtained in the present study tends to suggest that vasorelaxation might largely have been responsible for the hypotensive action of the plant extract. This vasorelaxant effect of the extract was probably mediated through endothelium-dependent NO production and cGMP release, and not related to activation of vascular endothelial muscarinic receptors.

Although the precise mechanism of the hypotensive action of PAE could not be established in the present study, we excluded involvement of cholinergic mechanisms. However, a complicating factor in the interpretation of the data obtained in the hypotensive experiments was the bradycardia associated with the reduction in systemic arterial blood pressure of the rats. Firstly, the reduction in heart rate could, on its own, have been the cause of the hypotension. However, based on the results obtained from the rat isolated aortic rings, it would seem unlikely that the fall in arterial blood pressure produced by PAE was solely dependent on reduction in heart rate. Secondly, the observed transient, secondary reflex tachycardia accompanying the fall in arterial blood pressure would probably suggest that the plant extract did not affect central cardiovascular centres and/or brain cardiovascular receptors. The plant extract may, therefore, also have had a direct effect on the sinus node of the heart, or on the central nervous system control machinery of arterial blood pressure.

P americana has been reported to contain many bioactive chemical compounds, including polyphenolics, tannins, coumarins, flavonoids, triterpenoids, phytosterols (especially β-sitosterol), biotin, α-tocopherol, carotene, ascorbic acid, scopoletin, quercetin, oils, organic acids and inorganic substances such as calcium, magnesium, zinc and phosphorus.[11,12] However, our present state of knowledge of the chemical constituents of the leaf extract is limited. It is therefore impossible for us at this stage to identify with certainty the vasorelaxant and antihypertensive constituents of PAE. Although we speculate that one or more of the major chemical constituents of the plant [namely flavonoids, polyphenols, tannins, coumarins (especially scopoletin and other coumarins), triterpenoids and phytosterols] may possibly have accounted for the observed cardiodepressant, vasorelaxant and antihypertensive properties of the plant extract, there are no sufficient scientific data at present to justify this speculation. However, the experimental evidence obtained in the present study showed that P americana aqueous leaf extract produced significant cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the laboratory animal paradigms used.

In conclusion, the findings of the present laboratory animal study lend pharmacological support to the suggested anecdotal ethnomedical uses of P americana aqueous leaf extract as a natural supplementary remedy in the management, control and/or treatment of hypertension and certain cardiac disorders in some rural Africa communities.

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ARTICLE

Effects of *Ficus thonningii* (Blume) [Morarceae] Stem-Bark Ethanolic Extract on Blood Glucose, Cardiovascular and Kidney Functions of Rats, and on Kidney Cell Lines of the Proximal (LLC-PK1) and Distal Tubules (MDBK)

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Previous observations indicate that *Ficus thonningii* (Blume) [Moraceae] stem-bark extracts may be useful in the control of diabetes mellitus. Accordingly, we investigated in some experimental animal paradigms the effects of *F. thonningii* stem-bark ethanolic extract (FTE) on renal and cardiovascular functions as complications of diabetes. Oral glucose tolerance tests were conducted in separate groups of non-diabetic and STZ-treated diabetic rats given glucose load (0.86 g.kg^{-1}, p.o.) after 18-h fast, followed by various FTE doses (60, 120, and 240 mg.kg^{-1}). Rats treated with deionized water (3 mL.kg^{-1} p.o.), or metformin (500 mg.kg^{-1} p.o.) acted as untreated and treated positive controls, respectively. Blood glucose was monitored at 15-min intervals for the first hour, and hourly thereafter for 3 h. Acute effects of FTE on kidney function and mean arterial blood pressure (MAP) were investigated in anaesthetized rats challenged with hypotonic saline after a 3.5-h equilibration for 4 h of 1 h control, 1.5 h treatment, and 1.5 h recovery periods. FTE was added to the infusate during the treatment period. Chronic effects of FTE were studied in individually caged rats treated daily with FTE (120 mg.kg^{-1}, p.o.) for five weeks. Cytotoxicity of FTE was assessed by dye-reduction colorimetric (MTT) assay on MDBK and LLCPK1 kidney cell lines exposed for 24 h, 48 h, and 72 h to graded concentrations of the extract. Myocardial contractile performance was evaluated on rat isolated atrial muscle strips. FTE,

like metformin, decreased blood glucose levels in non-diabetic 35 and STZ-diabetic rats. Both acute and chronic FTE treatments did not affect renal function. In vitro studies demonstrated that FTE increased MDBK cell metabolic activity by an average of 15% (72 h), and LLCPK1 mirrored the controls. Acute intravenous infusion of FTE reduced the MAP from 119 ± 1 mmHg to 40 98 ± 4 mmHg. The MAP also was reduced throughout the five-week experimental study period. FTE also produced concentration-dependent, negative inotropic and chronotropic effects on rat isolated, electrically driven left-, and spontaneously beating right-, atrial muscle preparations. Our experimental findings sug- 45 gest that FTE possesses reno- and cardio-protective effects in diabetes mellitus.

Keywords   *Ficus thonningii*, ethanolic extract, diabetes mellitus, renal and cardiovascular effects

INTRODUCTION

We have previously reported that *Ficus thonningii* (Blume) [Moraceae] stem-bark ethanolic extract reduces blood glucose in non-diabetic and diabetic rats after five weeks exposure.[1] Because diabetes is often associated with impaired kidney function and cardiovascular disor- 55 ders, appropriate goals in the control of diabetes mellitus using *F. thonningii* should include not only regulating blood glucose, but also the prevention or alleviation of these complications. Accordingly, the current study was designed to establish in some experimental animal para- 60 digms the effects of *F. thonningii* bark ethanolic extracts

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(FTE) not only on blood glucose, but also renal and cardiovascular functions. Thus, the main purpose of the present study was to investigate the effects of short-term (acute) and long-term (chronic) administration of FTE on renal fluid and electrolyte handling, as well as on blood pressure in male Wistar rats. Available evidence suggests that some herbal extracts may interfere with the concentrating and diluting mechanisms of tubular transport processes in the proximal tubule and distal tubule cells and/or on other protein components of tubular cell membranes.[2,3] Indeed, previous studies in our laboratories show that some crude plant extracts impair the renal handling of fluid and electrolytes.[4] Thus, we further speculated that FTE influences tubular reabsorption and secretion by altering tubular epithelial cells' viability. Therefore, the second objective was to assess the effects of FTE on cell viability on previously validated porcine proximal tubule and bovine distal tubule cell lines. Cell culture systems provide a good model for the evaluation of cytotoxicity of various compounds. We, therefore, employed the extensively used in vitro cell culture techniques of the proximal (LLC-PK1) and distal tubule (MDBK) cells[5-7] to study FTE-induced renal effects. The technique mimics the in vivo state, as these cell lines maintain similar biochemical function of levels of marker enzymes exhibited by freshly isolated cells.[8] We also assessed the effects of FTE on glomerular filtration rate (GFR), an indicator of renal tubular function.[9,10] Furthermore, the study investigated the influence of FTE on blood pressure, a parameter associated with the deterioration of kidney function in diabetic patients.[11] In an effort to shed more light on the plausible mechanism(s) through which FTE may affect blood pressure, we have also investigated the effects of FTE on myocardial contractility in vitro.

MATERIALS AND METHODS

Preparation of Plant Extract

Pieces of *Ficus thonningii* stem-barks were identified by Prof. H. Baijnath, the former Chief Taxonomist/Curator of the University of Durban-Westville's Department of Botany. A voucher specimen of the plant has been deposited in the University's Botany Departmental Herbarium. The stem-barks were air-dried at room temperature and milled into fine powder with a commercial blender. The powdered stem-bark was macerated in 95% ethanol for 24 h (with occasional shaking) and filtered. The filtrate was concentrated under reduced pressure in a rotary evaporator at $60 \pm 1°C$. The crude, powdery *Ficus thonningii* stem-bark ethanolic extract (FTE) was used throughout this study without further purification.

Animals

Male Wistar rats (250–300 g body weight) maintained under laboratory conditions of temperature, humidity, and 12 h light/12 h dark regime at the Biomedical Resource Unit, University of KwaZulu-Natal, were used. The rats were exposed to both food (Epol-diet 4700, Epol, South Africa) and water ad libitum. Ethical clearance was obtained for this study from the University of KwaZulu-Natal's Ethics committee.

Experimental Design

Oral glucose tolerance test (OGTT) studies were carried out in male, non-diabetic, and streptozotocin (STZ)-treated diabetic Wistar rats (250–300 g body weight). In vivo studies on renal function and blood pressure were carried out in non-diabetic and streptozotocin (STZ)-induced diabetic rats, while the in vitro effects of the plant's extract were conducted on kidney cell lines LLC-PK1 and MDBK. Effects of FTE on myocardial contractile performance were evaluated on rat isolated atrial muscle strips.

Induction of Diabetes Mellitus

Diabetes mellitus was induced in the diabetic group of rats by intraperitoneal injections of STZ (60 mg.kg^{-1}) in citrate buffer, pH 6.3. Vehicle (citrate buffer)-treated animals acted as controls. Animals that exhibited glucosuria after 24 h, tested by urine test strips (Rapidmed Diagnostics, Sandton, South Africa), were considered diabetic. Plasma glucose concentration of 25 mmol.L^{-1} measured after one week was considered as a stable diabetic state before our experimental procedures.

Series 1: OGTT

The rats used were divided into the following groups for OGTT: non-diabetic control, treated non-diabetic, control STZ-treated diabetic, and treated STZ-diabetic rats (n = 6 in each group). Rats treated with deionized water served (3 mL.kg^{-1}, p.o.) served as control animals. All of the animals were starved for 18 h before being orally treated with glucose (0.86 g.kg^{-1}, body weight, p.o.), followed by FTE at various doses (60, 120, and 240 mg.kg^{-1}, p.o.). To establish whether FTE possesses pharmacological activities comparable to synthetic hypoglycemic drugs already in use, studies were conducted in separate groups of non-diabetic and STZ-diabetic rats orally treated with

metformin (500 mg.kg^{-1}, p.o.). Blood samples were collected from the tail veins of the animals at 15-min intervals for the first hour, and hourly thereafter for the subsequent 3 h for glucose measurements using Bayer's glucometer Elite® (Elite (Pty) Ltd, Health Care Division, South Africa).

Series 2: Renal Function

Acute Studies

Male Wistar rats were divided into groups of untreated control and treated rats (n = 6 in each group). Rats were anaesthetized by an intraperitonial injection of Trapanal (sodium 5- ethyl-(1-methylbutyl)-2-thiobarbiturate, Byk Gulden, Konstanz, Germany) at a dose of 0.11 g.kg^{-1} and tracheotomized to maintain clear airway entry. The right jugular vein was cannulated with polyethylene tubing (i.d. 0.86 mm; o.d 1.27 mm, Portex, Hythe, Kent, UK) to allow intravenous infusion of 0.077 M NaCl. The urinary bladder of each rat was also cannulated with similar calibre polythene tubing via an incision in the abdominal wall. The body temperature of each animal was maintained at 37 ± 1°C with a heated table.

The control group of animals (n = 6) was placed on a continuous infusion of 0.077 M NaCl at 9 mL-h^{-1} (Harvard syringe infusion Pump 22). Following an initial equilibration period of 3.5 h, eight consecutive urine collections were made into pre-weighed plastic vials at 30-min intervals over the subsequent 4 h for measurements of urine flow and Na$^+$ and K$^+$ excretion rates. The control group of rats was designed to check the stability of renal function.

Treated Group

Renal effects of the crude plant's extract (FTE) were studied in a group of rats following a 3.5 h equilibration period. FTE solution was prepared by using a modified method that has been previously described. [12,13] The extract was freshly dissolved in dimethyl sulfoxide (DMSO, 2 ml) and normal saline (19 ml) before use in each case. Urine samples were collected for 1 h (control period) for measurements of urine flow and Na$^+$ and K$^+$ excretion rates, following which the extract solution was infused at 0.06 µg-min^{-1} for 1.5 h (treatment period), resulting in a total dose of 18 g.kg^{-1} (for a 300-g rat). The animals were then switched back to the infusate alone for the last 1.5 h (recovery period).

Blood Pressure Measurements

Test groups of rats were surgically prepared as described for the renal studies, except that a heparinized cannula (Portex, i.d. 0.86 mm; o.d. 1.27 mm) was also inserted into the left common carotid artery to permit the recording of mean arterial blood pressure at 30-min intervals (Statham MLT 0380, Ad Instruments, compatible with the PowerLab System ML410/W, Australia).

Chronic Studies

Wistar rats (250–300 g body weight) were housed individually at the Biomedical Resource Unit, University of KwaZulu-Natal, in Makrolon polycarbonate metabolic cages (Techniplats, South Africa) that were cleaned daily. All animals were maintained on a 12 h dark/light cycle and allowed free access to water and food (Epol-diet 4700, Epol, South Africa). In those animals in which the effects of FTE were investigated, the rats were treated with FTE (120 mg.kg^{-1}, p.o.) daily for five weeks at 09h00. Control rats were similarly treated with distilled water (3 mL.kg^{-1}). Urine volume and total urinary outputs of Na$^+$ and K$^+$ were determined from 24 h samples for all groups.

Blood Pressure Measurements

Mean arterial blood pressure (MAP) was monitored every third consecutive day for five weeks at 09h00 using non-invasive tail cuff method with photoelectric sensors (IITC Model 31 Computerized Blood Pressure Monitor, Life Sciences, Woodland Hills, California, USA). The unit works with IITC hardware system to measure blood pressure and heart rate in conscious rats. The animals had been warmed in an enclosed chamber (IITC Model 303sc Animal Test Chamber, IITC Life Sciences, Woodland Hills, California, USA) for 30 min at ± 30°C before taking BP readings.

Terminal Studies

At the end of the five-week plants' extracts treatment period, blood glucose was measured from the tail veins of all groups of non-fasted animals using Bayer's glucometer Elite® (Elite (Pty) Ltd, Health Care Division, South Africa). Blood samples were also collected from all groups of animals by cardiac puncture into individual pre-cooled heparinized containers. Separated plasma was analyzed for Na$^+$, K$^+$, creatinine, and urea concentrations. Blood for insulin was collected into plain tubes, and the separated serum was stored in a Bio Ultra freezer (Mallkinckrodt, Ohio, USA) at −70°C until assayed.

Analytical Methods

Measurement of Electrolytes, Insulin, and Glomerular Filtration Rate

Urine volume was determined gravimetrically. Na⁺ and K⁺ concentrations were determined by ion activity using the Beckman Coulter (Synchron LX20 Clinical Systems, USA). Urea and creatinine analyses were performed using the Beckman Coulter instrument. Creatinine estimation employed the reaction of creatinine and sodium picrate to form creatinine picrate. Urea estimation employed the hydrolytic degradation of urea in the presence of urease. The methods used reagent kits from Beckman Coulter, Ireland, Inc., and measured using Beckman Coulter (Synchron LX20 Clinical Systems, USA). Glomerular filtration rate (GFR), as assessed by creatinine clearance, was calculated from measurements of urinary and plasma concentrations of creatinine and urine flow rate in the fifth week.

Plasma insulin concentrations were measured by Coat-A-Count procedure using a kit from Diagnostic Products Corporation, Los Angeles, USA. This is a solid-phase radioimmunoassay procedure based on insulin-specific antibody immobilized to the wall of a polypropylene tube. The lower limit of detection was 55 pg.mL⁻¹. Inter- and intra-assay coefficients of variation were 8.1% (n = 20) and 8.3% (n = 20), respectively.

Series 3: Cell Culture Studies

LLC-PK1 and MDBK cells were grown and maintained at 37°C in Eagle's Minimum Essential Medium (EMEM) (containing 0.1 mM Hepes buffer) supplemented with 5% heat inactivated foetal calf serum, 1% L-glutamine, and 1% penstrep-fungizone [complete culture medium (CCM)] (Delta Bioproducts, South Africa). Once the cells reached confluence, they were detached from the culture flask (75 cm²) with 0.025% (w/v) trypsin and resuspended in CCM. Cell viability was determined in the presence of 0.2% (w/v) trypan blue in a haemocytometer. A 200-µl aliquot of the cell suspension (1.5 × 10⁶ cells) was transferred into separate 96-well microtiter plates (Greiner Bio-one GmbH, Germany). Thereafter, the viability of cells incubated at 37°C for 24, 36, and 72 hours, containing various concentrations of FTE in separate wells, was assessed (0, 100, 200, 400, 600, 800, and 1000 µl mL⁻¹, n = 6 for each dilution). The wells were aspirated after each incubation and washed with Hank's balanced salt solution (HBSS). All supernatants were discarded. The cells were resuspended in 100 µl CCM containing 10 µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (i.e., 5mg.ml⁻¹ MTT salt in HBSS, Calbiochem, Darmstadt, Germany) and incubated for four hours at 37°C. After 4 h, the plates were centrifuged (20 min, 2000 rpm at room temperature). The supernatant was removed, and any resulting formazan crystals were then solubilized with 100 µl dimethylsulphoxide (DMSO). After one hour, the optical density was determined spectrophotometrically using an ELISA plate reader (Bio-Tek Instruments) at 595 nm and a reference wavelength of 655 nm. Absorbance was expressed as percentage cleavage activity. Percentage cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells.

Series 4: Isolated Atrial Muscle Strips

In order to throw some light on the plausible mechanism(s) by which FTE may influence blood pressure, we studied the effects of the extract on rat isolated atrial muscle strips. The Wistar rats used were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as described by Ojewole.[14] The isolated left atrium of each rat was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution (by composition, in mmol/L NaCl, 118; KCl, 4.7; NaH₂PO₄, 1.28; NaHCO₃, 25.0; MgCl₂, 1.2; CaCl₂, 2.52; and glucose, 5.55 pH adjusted to 7.4) maintained at 34 ± 1°C and continuously aerated with carbogen (95% O₂ + 5% CO₂ gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5 msec duration at a frequency of 3 Hz and supramaximal voltages of 5–10 volts, delivered by an SRI stimulator. The spontaneously beating right atrium of the animal was also set up under the same physiological, experimental conditions. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously-beating right atrial muscle preparations, were always set up at a time (one used as the test and the other as the control) to allow for changes in the atrial muscle sensitivity. The atrial muscle preparations were left to equilibrate for 45–60 min (during which time the bathing physiological solution was changed every 15 min) before they were challenged with FTE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied, graded concentrations of the extract and/or reference drugs used, while the control atrial muscle strips were treated with volumes of distilled water equivalent to the volumes of bath-applied FTE (5–80 g.mL⁻¹). The electrically provoked and spontaneous contractions of the atrial muscles, as well as the FTE- (and reference drug)-induced

responses of the atrial muscle preparations, were recorded
isometrically by means of Ugo Basile force-displacement
transducers and pen-writing 'Gemini' recorders (model
7070).

DATA PRESENTATION

All data are expressed as means ±standard error of
means (SEM). Cell viability was expressed as a percent-
age relative to control cells not exposed to any of the test
compounds. Data obtained from test rat isolated atria
treated with FTE alone, as well as those obtained from
deionized water-treated control atrial strips, were pooled
and compared with those of reference drugs. A statistical
comparison of the differences between FTE and respective
controls was performed with GraphPad InStat Software
(version 3.00, GraphPad Software, San Diego, California,
USA) using one-way analysis of variance (ANOVA; 95%
confidence interval), followed by Tukey-Kramer multiple
comparison test. A value of $p < 0.05$ was considered
significant.

RESULTS

OGTT

Figure 1A compares the OGTT responses in acutely
treated non-diabetic rats with respective control animals.
Oral administration of various doses of FTE (60, 120, and
240 mg.kg^{-1}) decreased the blood glucose concentrations
in a dose-dependent manner, with all doses exerting maxi-
mum effects after 60 min. The hypoglycemic effect of
FTE was still significant by the end of the 4 h experimen-
tal period. A similar pattern of hypoglycemic effects was
observed with metformin. Untreated control non-diabetic
rats exhibited significantly high plasma glucose concentra-
tions by comparison with treated animals. The plasma glu-
cose concentrations of the control, non-diabetic rats
increased to 6.8 ± 0.3 mmol.L^{-1} by 30 min from a baseline
value of 4.2 ± 0.1 mmol.L^{-1} before slowly declining to $4.3 \pm$
0.1 mmol.L^{-1} (n = 6) after 4h, a value that was signifi-
cantly elevated when compared to animals administered
the highest dose of FTE at the corresponding period.

Similarly, the oral administration of various doses of
FTE (60, 120, and 240 mg.kg^{-1}) decreased the blood glu-
cose concentrations of STZ-treated diabetic rats in a dose-
dependent manner by 45 min until the end of the 4 h
experimental period (see Figure 1B). The glucose concen-
trations of STZ-treated diabetic control animals orally
loaded with glucose did not significantly decline by the
end of the 4-h experimental period. Metformin induced
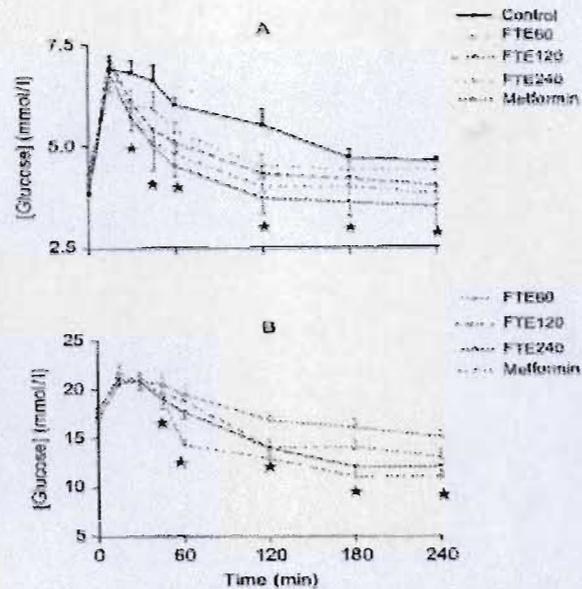
Figure 1. Comparison of OGTT responses in separate groups
of (A) non-diabetic and (B) streptozotocin (STZ)-treated diabetic
rats treated with graded doses of FTE with control animals
treated with deionized water or positive controls treated with
metformin. Values are presented as means, and vertical bars
indicate SE of means (n = 6 in each groups). *$p < 0.01$ by
comparison with control animals.

marked reductions in blood glucose concentrations by
45 min until the end of the 4-h experimental period.

In those animals in which FTE was chronically
administered (120 mg.kg^{-1}, p.o.) daily for five weeks at
09h00, the mean plasma concentration of glucose was sig-
nificantly decreased in non-diabetic and STZ-induced dia-
betic rats by the end of the experimental period in
comparison with respective control animals at the corre-
sponding time (see Table 1).

Renal Function Tests

Urine flow and Na$^+$ excretion rates ranged from 9 to
10 ml.h^{-1} and 619 to 660 μmol.h^{-1}, respectively, in vehicle-
infused control animals during the 4 h post-equilibration
period, values that compared with the infusion rate
(9 mL.h^{-1} and 693 μmol.h^{-1}, respectively). K$^+$ excretion
rate was also stable throughout the post-equilibration
period, ranging from 226 to 256 μmol.h^{-1}. No significant
changes in renal fluid flow and electrolyte excretion rates

Table 1

Plasma glucose, Na⁺, K⁺, urea and creatinine concentrations and GFR in non-diabetic and STZ-diabetic control and rats administered FTE every third consecutive day for five weeks (n = 6 in all groups)

Measure	Non-diabetic control	Non-diabetic FTE treated	STZ-diabetic control	STZ-diabetic FTE-treated
Na⁺ (mmol)	142 ± 2	143 ± 2	142 ± 1	143 ± 2
K⁺ (mmol)	3.62 ± 0.34	3.21 ± 0.13	3.76 ± 0.21	3.10 ± 0.01
Urea (mmol)	15 ± 1	14 ± 2	33 ± 4	38 ± 5
Creatinine (µmol)	97 ± 4	$85 \pm 2^*$	102 ± 4	$94 \pm 7^*$
Glucose (mmol)	7.7 ± 0.2	$5.5 \pm 0.1^*$	36.6 ± 0.3	$28.0 \pm 0.5^*$
GFR (ml.min⁻¹)	2.64 ± 0.02	2.98 ± 0.03	0.7 ± 0.2	$1.4 \pm 0.3^*$

$^*p < 0.01$ by comparison with respective control animals.

were observed in animals that were acutely treated with FTE. Compared with the control rats, the FTE-treated animals attained stable Na⁺ excretion and urine flow rate, which approximated the infusion rates throughout the experimental period. Similarly, the mean weekly urine volume and the urinary Na⁺ and K⁺ outputs were not significantly different between animals chronically treated with FTE (120 mg.kg⁻¹, p.o.), and untreated rats. Urinary creatinine and urea outputs were not significantly different between the control and treated rats throughout the five-week experimental period. However, FTE treatment significantly reduced ($p < 0.01$) plasma creatinine concentration, unlike plasma urea concentration that was not altered (see Table 1). By the end of the five-week period, FTE administration significantly elevated ($p < 0.01$) the GFR value in STZ-treated diabetic rats, but the increase in non-diabetic rats did not achieve statistical significance.

Hemodynamic Studies

The acute infusion of hypotonic saline to control animals did not show any significant variations in the mean arterial blood pressure throughout the 4-h post-equilibration period. However, acute intravenous infusion of FTE at 120 µg.h⁻¹ for 1.5 h reduced the mean arterial blood pressure from a mean pre-treatment value of 119 ± 1 mmHg to 98 ± 4 mmHg (n = 6) by the end treatment. The hypotensive effect of FTE persisted during the post-treatment period to a mean value of 110 ± 4 mmHg at the end of the experiment (see Figure 2A). The mean arterial blood pressure (MAP) changes due long-term (chronic) FTE treatments are shown in Figure 2B. Chronic treatment of the rats with FTE (120 mg.kg⁻¹ daily for five weeks, p.o.), caused significant decreases in MAP in non-diabetic and STZ-treated diabetic rats throughout the study period in
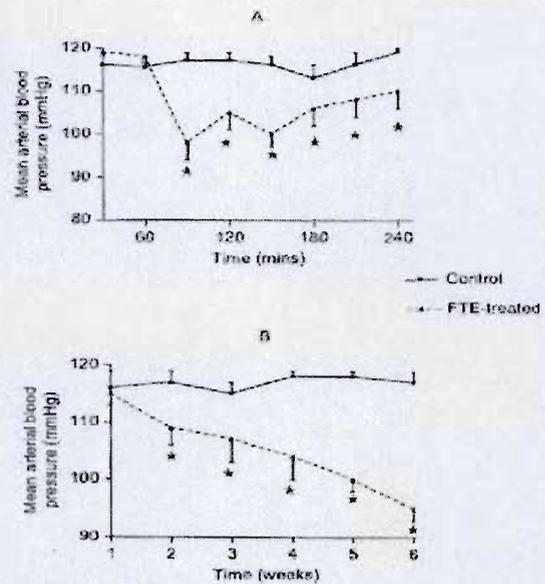

Figure 2. Effects of chronic FTE treatment on mean arterial blood pressures in (A) non-diabetic or (B) STZ-induced diabetic rats. Values are presented as means and vertical bars indicate SE of means (n = 6 in each group). $^*p < 0.01$ by comparison with control animals.

comparison with control rats at the corresponding period (Figure 2).

CELL CULTURE STUDIES

The MTT assay is a quantitative colorimetric method based on the reduced cleavage of the water soluble

monotetrazolium salt MTT to a purple formazan in metabolically active cells. The MTT salt is actively transported into metabolically viable cells. Both Figures 3A and 3B show the viability of LLC-PK1 and MDBK cells treated with various concentrations of FTE after 24, 48, and 72 hours. There was no toxicity noted in both cell lines after treatment with FTE. In Figure 3A, LLC-PK1 cell metabolism was increased with increased doses of FTE (600–1000 µg.mL⁻¹) for all incubation time periods. In contrast, the MDBK cells (Figure 3B) show increased metabolism for all concentrations of FTE. There was a significant increase in metabolism and cell viability after 72-h incubation, with significance being more pronounced at the highest concentrations (800 and 1000 µg.mL⁻¹).

Rat Isolated Atrial Muscle Strips

The cardiac effects of FTE are shown in Figure 4. This figure shows that FTE (5–80 mg.mL⁻¹) produced concentration-related, negative chronotropic (Figure 4A) and inotropic (Figure 4B) effects on rat isolated spontaneously beating right- and electrically driven left- atrial muscle strips, respectively. The cardio-depressant effects of FTE were not modified by bath-applied atropine (10^{-8}–10^{-6} M), suggesting that the cardio-inhibitory effects of the plant's extract are unlikely to be mediated through cholinergic mechanisms.

DISCUSSION

In the present study, we have evaluated the effects of short-term (acute) and long-term (chronic) oral treatments of *Ficus thonningii* stem-bark ethanolic extract (FTE) on cardiovascular systems and kidney functions of rats. The major findings of this study, apart from confirming our previous observations of the hypoglycaemic effects of oral administrations of the plant's extract,[1] show that FTE decreases blood pressure without significant influences on

Figure 3. Viability of (A) LLC-PK1 and (B) MDBK cell lines treated with FTE. The cells were treated with 100, 200, 400, 600, 800, or 1000 µg FTE. Cell viability was determined by the Tryphan blue exclusion assay. Values for untreated control were taken as 100%. Each dose represents the mean of six treatments, while the vertical bars denote standard errors of the means.
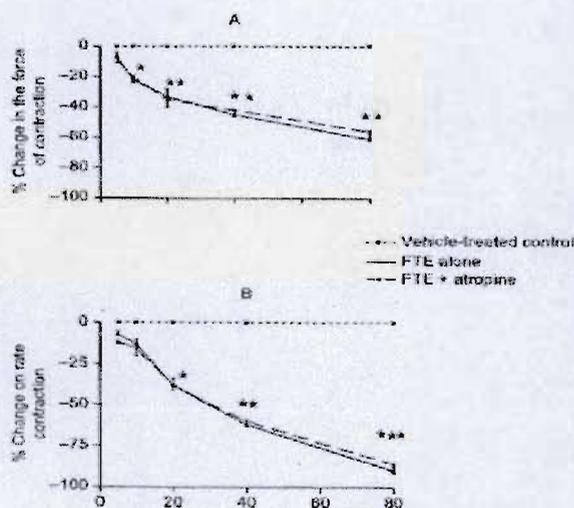
Figure 4. Effects of sequentially applied graded concentrations of FTE (5–80 mg.mL⁻¹) on the (A) rate and (B) force of contractions of rat isolated spontaneously beating right- and electrically driven left-atrial muscle strips, respectively. Each point represents the mean of 8–10 observations, while the vertical bars denote standard errors of the means. *p < 0.05, **p < 0.01, ***p < 0.001 versus control.

renal function in normotensive rats. The management of diabetes mellitus without side effects is a global challenge, thus increasing the demand for natural products with antidiabetic activity.[15] Cardiovascular and renal complications are the major causes of mortality in diabetes mellitus.[16] We suggest that the use of FTE in the control of diabetes mellitus may be beneficial when hypertension and compromised renal function co-exist. This is significant considering the fact that diabetes mellitus is associated with cardiovascular complications and deterioration of kidney dysfunction in diabetic patients[17] and experimental animals.[18–20] Therefore, the management of diabetes with FTE has the potential to address both renal and cardiovascular protection. Conventionally, renoprotection is achieved through a reduction in blood pressure with antihypertensive regimens.[17, 21–23] Of note in the present study is the hypotensive effect of FTE without altering kidney function, in contrast to previous reports of impaired renal function following the administration of some hypoglycaemic plant extracts.[4,24] A significant increase in GFR as assessed by creatinine clearance and a concomitant decrease in plasma creatinine concentration was observed for the STZ-induced diabetic group treated with FTE over the five-week period. This finding is significant, given the fact that some antihypertensive agents (e.g., thiazide diuretics) and ß-blockers influence glycemic control in a deleterious manner.[25] Evidence from biomedical literature suggests that some herbal extracts have protective effects against cardiovascular disease in diabetes.[26] We and several other authors have previously used creatinine clearance in rats to monitor GFR. [9–10, 29–31] It is likely that chronic FTE treatment increased creatinine secretion as evidenced by increased MDBK cell metabolic activity. However, further studies are required to establish the mechanism(s) through which FTE reduces plasma creatinine levels. The cell culture studies provide an experimental model to assess cytotoxicity and metabolic activity of FTE and the two renal cell lines. Initially, the MTT assay was used as a measure of cell viability and proliferation, with the mitochondrial reduction by succinate reductase system being the major contributor to MTT reduction.[29] However, recent evidence shows that most MTT reduction occurs extra-mitochondrially with MTT salt crossing the intact plasma membranes to be reduced intracellularly.[30] Other investigators have shown that most of the cellular reductions of MTT are dependent on microsomal enzymes and not only on succinate dehydrogenase.[31–33] This microsomal reduction requires NADH and NADPH and is not affected by respiratory inhibitors.[34] This clearly indicates that cellular reduction of MTT is related more to the glycolytic rate, and thus NADH production, than to respiration, and is therefore primarily a measure of the rate of glycolytic NADH

production.[28] Our cell culture data clearly support this finding, as MTT reduction is increased in the presence of FTE, especially at the higher concentrations. Our data also confirm the lack of toxicity of FTE on renal cells derived from two species, viz., pig, and bovine. The findings of the present study suggest that FTE is a useful agent in increasing glucose uptake by renal cells and glucose uptake studies are needed to verify this phenomenon. Our data indicate that FTE has the potential to reduce plasma levels of creatinine in patients with diminished renal function and reduce cardiovascular and renal complications as well. The elevation of plasma creatinine is a risk factor for the development of cardiovascular[33] and end stage renal[34] diseases. FTE reduced mean arterial pressure in normotensive rats without significant effects on renal fluid and electrolyte handling, suggesting that the cardiovascular effects of FTE are mediated through influences on components of the cardiovascular system. We suggest that the cardio-inhibitory effects of FTE may contribute, in part at least, to the hypotensive of the plant's extract. This hypothesis is supported by our findings from experiments on isolated guinea-pig atrial muscle strips which demonstrated significant negative chronotropic and inotropic, cardiodepressant effects of FTE. In conclusion, our experimental findings suggest that FTE possesses reno- and cardioprotective effects in diabetes mellitus.

ACKNOWLEDGMENT

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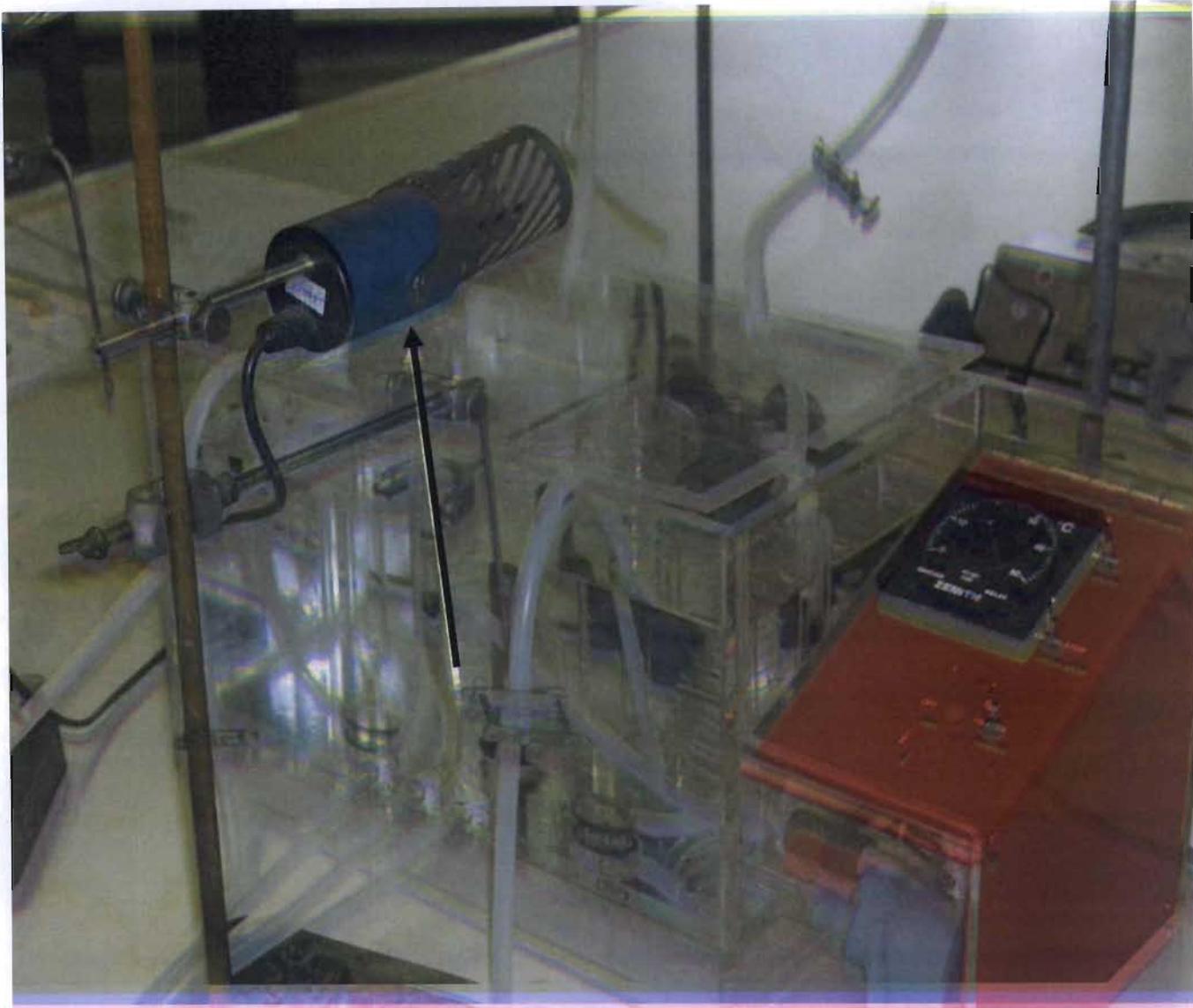
Figure 35

A picture of the experimental apparatus used for *in vitro* studies. The picture shows the water bath containing organ baths, a network of tubings for perfusing isolated tissues and the force displacement transducer indicated by the arrow on the picture.
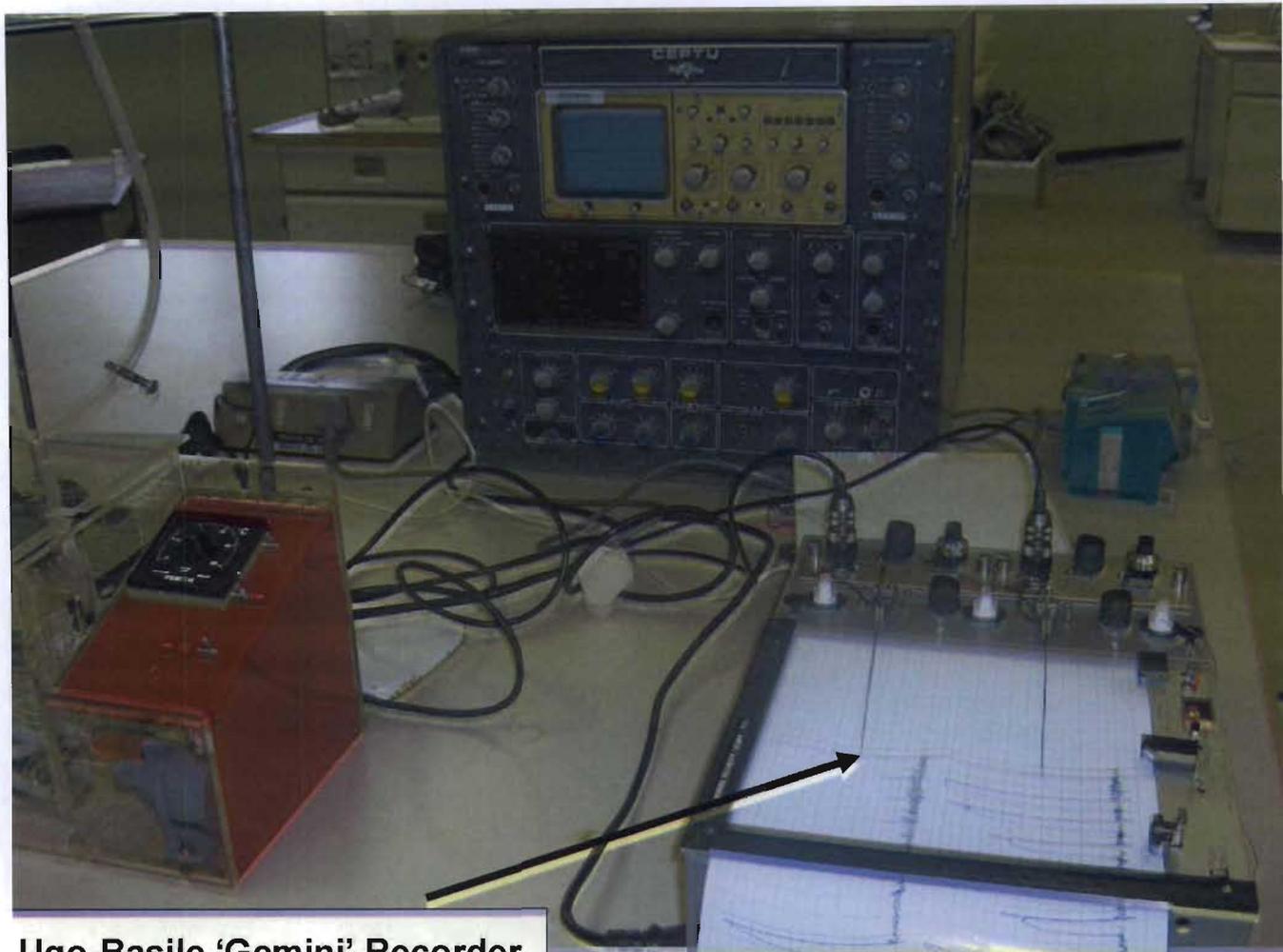
Ugo-Basile 'Gemini' Recorder

Figure 36

Picture showing other components of the experimental apparatus used for in vitro studies. It shows stimulator and the Ugo-Basile 'Gemini' recorder.