Cardiovascular effects of aqueous leaf extract of Leonotis leonurus in anesthetized rats

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Thesis submitted in partial fulfillment of the requirements for the degree of Magister Pharmaceuticae, School of Pharmacy, University of the Western Cape.

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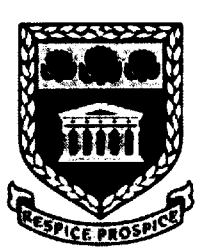

UNIVERSITY of the WESTERN CAPE

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KEYWORDS

Traditional medicines
Leonotis Leonurus
Cardiovascular
Anesthesized rats
Blood pressure
Heart rate
Hypotensive
Systolic pressure
Diastolic pressure
Aqueous leaf extract

ABSTRACT

Cardiovascular effects of aqueous leaf extract of Leonotis leonurus in anesthesized rats

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M.Pharm Thesis, School of Pharmacy, Department of Pharmacology, University of the Western Cape

The present study was designed to evaluate the hypotensive properties and the mechanisms of action of the aqueous leaf extract of *Leonotis leonurus* in anesthetized male Wistar rats, using computerized blood pressure recording system.

The effect of aqueous leaf extract of *L. leonurus* on the blood pressure was investigated by infusing intravenous doses of 200 mg/kg to 1g/kg into anesthetized rats. Its underlying blood pressure lowering mechanism was also studied by challenging the parasympathetic, sympathetic and muscarinic systems of anesthetized rats by pretreating with, dobutamine, atenolol, and atropine. Pretreating the animals with reserpine and *L. leonurus* was also employed in order to challenge the adrenal system of rats.

The results showed that the aqueous leaf extract of *Leonotis leonurus* produces a dose dependent fall in the systolic blood pressure, diastolic blood pressure and heart rate of the rats.This effect on heart rate may suggest the involvement of β-adrenergic receptors in the cardiac effect of *L. leonurus*.

Reserpine depletes adrenergic nerves of noradrenaline by blocking or destroying the storage mechanism within the nerve ending, so that there is

less transmitter available for release. Pre-treatment with reserpine 5 mg/kg produced supersensitivity to the chronotropic responses to adrenaline.

The same response was observed when the adrenergic system was challenged with *L. leonurus*. Pre-treatment with *L. leonurus* 800 mg/kg produced an exaggerated sympatho-mimetic response.

The present study also investigated the effects of the β-agonist dobutamine. Heart rate, diastolic and systolic pressures were recorded during *L. leonurus* 800 mg/g infusion alone and during the concurrent intravenous administration of dobutamine 20 μg/kg. The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate, diastolic and systolic pressure. The hypotensive effect of the aqueous leaf extract of *L. leonurus* was blocked by the concurrent treatment of rats with dobutamine 20 μg/kg. This antagonism of the hypotensive effect of *L. leonurus* extract by dobutamine suggests the involvement of β-adrenergic mechanism in the action of the leaf extract.

Also, the hyportensive effect of the aqueous leaf extract of *L. leonurus* was blocked by pre-treatment of rats with atenolol 12 mg/kg. This antagonism of the hypotensive effect of *L. leonurus* extract by atenolol pretreatment suggests the involvement of β-adrenergic mechanism in the action of the leaf extract.

Atropine is a muscarinic receptor antagonist that act by causing reversible blockade of the actions of cholinomimetics. When the hypotensive effect of *L. leonurus* was challenged with atropine (2.4 mg/kg), a partial blockade in heart rate with even a complete blockade in systolic and diastolic pressure was observed.
The antagonism of the hypotensive effect of the extract by atropine pretreatment suggested the involvement of cholinergic mechanism in the action of the leaf extract.

The results of the study was able to demonstrate dose dependent hypotensive effect of *Leonotis leonurus* and that effects may be through inhibition of sympathetic and cholinergic control of the arterial pressure and most significantly through muscarinic receptor blockade. However, the phytochemical studies of the active principles and additional drug-receptor response dynamics on the leaf extract of *L. leonurus* still needed to be investigated.

February 2008

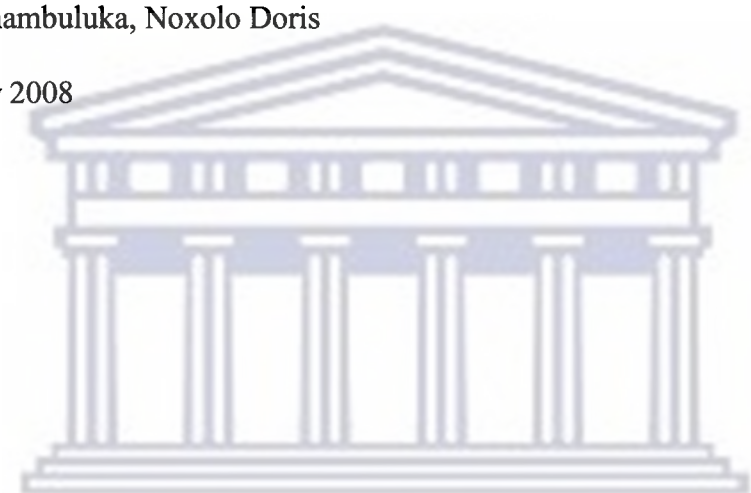
DECLARATION

I declare that *Cardiovascular effects of aqueous leaf extract of Leonotis leonurus in normotensive rats* is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged by complete references.

Full name: Tshambuluka, Noxolo Doris

Date: February 2008

Signed:



DEDICATION

I thank God for giving me the strength to make this dream a success. I also dedicate this work to my late grandfather, Mlelengwana, who believed in me and always encouraged me in my efforts, my mother, Nomvuselelo, for her continued support throughout my studies.

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LIST OF ABBREVIATIONS

ACE -	angiotensin-converting enzyme
α -	*alpha*
ANS -	Autonomic nervous system
AV node -	Atrio-ventricular node
ß -	*beta*
CNS -	Central nervous system
CO -	Cardiac output
CSIR -	Council for Scientific and Industrial Research
CV centre -	Cardiovascular centre
DMSO -	Dimethyl Sulfoxide
ECG -	Electrocardiogram
LDL -	Low-density lipoprotein
L. leonurus -	*Leonotis leonurus*
MABP -	Mean arterial blood pressure
MRC -	Medical Research Council
PVR -	Peripheral vascular resistance
R -	Resistance
SA Node –	sinoatrial node
SHR -	Spontaneously hypertensive rat
SNA -	sympathetic nerve activity
SVS -	Systemic vascular resistance
WHO -	Wold Health Organization

CHAPTER 1

1.1 Introduction

Medicinal plants have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies with healing properties is commonly found from traditional plants.

There is an increasing global interest in the field of traditional medicines. Most communities revert to the use of traditional medicine for self-medication. Some of the reasons for this perhaps being the fact that traditional medicines are easily accessible and affordable to our communities. The great concern is that there is little or no scientific evidence in the therapeutic effects of these herbal remedies. Also, there is little or no knowledge on efficacy, safety, contraindications, drug-interactions and finally the correct indications for these remedies. It is therefore of vital importance for researchers to conduct more concise research in this field in order to provide more information on these concerns.

According to World Health Organization (WHO), an estimated 80% of Africans use traditional medicines - compared to 60% of the world's population in general - and approximately 200 000 traditional healers practice in South Africa (WHO, 2002; Okello and Ssegawa 2007). In 2002 WHO stated that traditional medicines need to be evaluated for safety and effectiveness before they can be incorporated into national health policies.

In South Africa, National Reference Centre for African Traditional Medicines is an initiative spearheaded by the Department of Health, in partnership with the Council for Scientific and Industrial Research and the Medical Research Council. The center's remit is to gather, harness and synthesize information to promote, regulate and register African traditional medicines of plant origin. The following five focus areas have been identified: (1) establishing a research programme to filter claims of a cure and create information for the safe use of plants in public healthcare; (2) designing a database to collate all scientific

information on medicinal plants already available and ensure its accessibility; (3) identifying and addressing the educational needs of all stakeholders, especially traditional practitioners; (4) addressing all aspects of the legal issues related to medicinal plants, including intellectual property rights; (5) focussing on the development of new drugs and the standardization of medicines currently in use in terms of safety, quality and efficacy (Department of Health, MRC and CSIR 2004).

In addition, in South Africa, a National Research and Development platform for Novel Drug Development from Indigenous Medicinal plants in South Africa has been formed. Their project seeks to establish a scientific biotechnology infrastructure in South Africa for research and development of novel medicines and tonics from traditional medicinal plants used in southern Africa (Department of Health, MRC and CSIR 2004).

Little is known about the therapeutic effects of *Leonotis leonurus* (*L. leonurus*) in the cardiovascular system. Researchers like Njagi *et al.*, 2001; Mugabo *et al.*, 2002; Ojewole *et al.*, 2003; Obikeze *et al.*, 2004 have conducted research on the effects of *L. leonurus* on cardiovascular system.

Also, Bienvenu *et al* (2002) reported anticonvulsant activity and Desta (1994) reported anti-fertility activity of *L. leonurus*. Ojewole 2005 investigated the antinociceptive, antiinflammatory, and antidiabetic properties of the aqueous leaf extract of *L. leonurus* in mice and rats. More details on the studies they have conducted will be discussed under literature review.

1.2 Literature review

This section will be discussing traditional plants that possess cardiovascular effects, *L. leonurus* use in cardiovascular system. General description of *L. leonurus* and plant active compounds will also be covered.

In view of the lack of information in the literature evaluating the hypotensive property of *L. leonurus*, the present study was undertaken to investigate the effects of aqueous

extracts of leaves of *L. leonurus* on the blood pressure and heart rate in male Wister rats and to elucidate its mechanism of action.

This follows the fact that *L. leonurus* has been used in the field of traditional medicines as therapy for hypertension (Watt and Breyer-Brandwijk, 1962).

1.2.1 Plant description

The name *Leonotis* comes from the Greek word *leon* meaning "lion" and *otis* meaning "ear", alluding to the resemblance of the corolla to a lion's ear. *Leonurus* means lion coloured.

Leonotis leonurus is also known as "wild dagga". The "wild dagga" is a robust shrub that grows up to 2-3 m tall and 1.5 m wide. It is common and widespread throughout South Africa and grows among rocks in grassland areas.

The stem is velvety and woody at the base. The leaves are narrow, rough on the top and have velvety surface below, with serrate edges. *L. leonurus* has a characteristic odour; bright yellow-green colour and rough in texture. Occasionally, flowers and fruits are present. The "wild dagga" bloom profusely in autumn with characteristic bright orange flowers carried in compact cluster in whorls along the flower stalk. Apricot and creamy white flowered forms are also found. *L. leonurus* is an excellent plant for attracting wild life as the flowers profuse copious nectar that attracts birds, bees and butterflies. The wild dagga is fast growing and is frost hardy. It needs to be well watered in summer but does not need much water in winter. It is very easy to grow, especially in well-drained loamy soils with plenty of compost added (Turner, 2001).

Fig 1.1 *Leonotis leonurus* (Turner, 2001).

1.2.1.1 Vernacular names

L. leonurus is commonly known as "wild dagga" (Afrikaans), lion's ear, minaret flower (English), umfincafincane (Xhosa), lebake (Sotho) or umhlalampetu (Shangane).

1.2.1.2 Phytochemical tests on *Leonotis leonurus*

Diterpernoid labdane lactones, premarrubiin 0.00933-0.01567%, marrubiin (possibly an artifact derived from premarrubiin during extraction) have been found in purity tests of *L. leonurus* (Van Wyk *et al* 1997; Bruneton, 1995; McKenzie *et al* 2006). Assays done on *L. leonurus* indicated the presence of tannins, quinines, saponins, and alkaloids in preliminary tests; iridoids were not detected (Bienvenu *et al* 2002).

1.2.1.3 Traditional medicinal uses of *Leonotis leonurus*

L. leonurus is mainly used in the form of an aqueous decoction, taken orally, per rectum and as a topical application. It is used internally for the treatment of coughs, cold, influenza, headaches, dysentery chest infections, diabetes, hypertension, eczema,

epilepsy, delayed menstruation, intestinal worms, constipation, spider bites and scorpion stings and as an antidote for snake bite. It is used externally for relief of hemorrhoids, eczema, skin rashes and boils. (Codd, 1985; Forbes, 1986; Smith, 1966; Watt and Breyer-Brandwijk, 1962; Hutchings, 1966). *L. leonurus* is also used in epilepsy (Bienvenu *et al.*, 2002; Watt, 1967).

1.3 Description of active compounds found in medicinal plants

The active ingredients found in medicinal plants are chemical compounds that are responsible for the specific activity of the plant by acting directly or indirectly to prevent or treat disease and maintain health. It is therefore of vital importance that the active ingredients found in medicinal plants are discussed in this study in order to give a brief overview of their characteristics. Compounds that will be discussed in this section are: saponins, tannins, alkaloids, sugars and gums, and quinones. These active compounds can be extracted from plants in pure form after which they are identified and tested using various available methods.

1.3.1 Saponins

Saponins are glycosides with a distinctive foaming characteristic. Saponins consist of a sugar moiety, usually containing glucose, galactose, pentose or methylpentose, glycosidically linked to an aglycone (sapogenin) which may be triterpenoid or steroid in nature (Ohana *et al.*, 1998).

1.3.2 Tannins

Tannins are naturally occurring plant polyphenols. Their main characteristic is that they bind and precipitate proteins. They can have a large influence on the nutritive value of many foods eaten by humans and feedstuff eaten by animals. Tannins are found in most plants, especially most woody plants. The quantities vary. Often 1-5% is encountered. Tannins are common in fruits (grapes, persimmon, blueberry…), in tea, in chocolate, in

legume forages (trefoil), in legume trees (*Acacia spp., Sesbania spp.*), in grasses (sorghum, corn). There are two major types of tannins: condensed and hydrolyzable. Both have been used for tanning. The most important commercial tannins are condensed tannins (Reed, 1995).

1.3.3 Alkaloids

Alkaloids are most of the earliest isolated pure compounds with biological activity. This was due to the ease of isolation. The nitrogen generally makes the compound basic and the compound exists in the plant as a salt. Thus, alkaloids are often extracted with water or mild acid and then recovered as crystalline material by treatment with base.
Alkaloids have been defined in various ways, but one definition comes fairly close to actuality (Rajnikant, 2005).

1.3.4 Sugars and gums

The well known and the most common sugars are monosaccharides glucose and fructose, disaccharides sucrose. Sugar solutions are medicinally used in cough syrups and for intravenous feeding.
Mucilages and gums are formed when a number of sugar units are variously linked together to form long chain like structures (Van Wyk *et al.,* 1997).

1.3.5 Quinones

Quinones are similar to phenolic compounds but they are oxidized. They have oxygen in their ring structure rather than hydroxyl groups. Quinones have antibacterial and antifungal properties (Van Wyk *et al.,* 1997).

Saponins, tannins, alkaloids, and reducing sugars were found to be present in crude leaf powder of *L. leonurus.* The positive test of these compounds in *L. leonurus* can lead in explanation of the cardiovascular effects of *L. leonurus*. Further investigations are needed

in order to determine the mechanism by which *L. leonurus* exerts its cardiovascular effect and possible active compound involved in such activity.

1.4 Traditional plants with cardiovascular effects

A number of herbs used in cardiovascular system have been reviewed in recent years. Gold and Farnsworth, 2002; Lee, 1999 addressed the use of herbals, such as Hawthorn, Coenzyme Q10, Ginkgo biloba, Garlic, Soy, Motherworth, Butcher's broom and Ginseng for cardiovascular diseases and describes various herb-drug interactions that may influence standard therapy.

The first phyto-medicine discussed in this section is hawthorn (*Crataegus monogyna*). The Hawthorn is the badge of the Ogilvies and gets one of its most common popular names from blooming in May. Hawthorn is used in the form of fluid extract of berries and flowers. It has been approved for use in Germany for congestive heart failure. The mechanism of action of Hawthorn is blockade of Na+-K+ ATPase, possible inhibition of phosphodiesterase III, vasodilator action in the coronary circulation and peripheral vasculature through angiotensin-converting enzyme (ACE) inhibition, and perhaps beta blockade (Gundling and Ernest, 1999; Gold and Farnsworth, 2002). These activities mimic the pharmaceutical effects of ACE inhibitors, digoxin, and β-blockers.

Small controlled studies had shown consistently that hawthorn improves dyspnea, exercise tolerance and fatigue in patients with mild to moderate heart failure, and that it may be used as adjunct agent in the treatment of left ventricular dysfunction (Gundling and Ernest, 1999; Gold and Farnsworth, 2002; Lee, 1999).

Coenzyme Q10 or "ubiquinone" is essentially a vitamin or vitamin-like substance. Disagreements on nomenclature notwithstanding, vitamins are defined as organic compounds essential in minute amounts for normal body function acting as coenzymes or precursors to coenzymes. They are present naturally in foods and sometimes are also synthesized in the body. *Coenzyme Q10* likewise is found in small amounts in a wide variety of foods and is synthesized in all tissues. When used together with antihypertensive drugs in hypertensive patients, *Coenzyme Q10* had resulted in reduced

doses of antihypertensives Gold and Farnsworth, 2002). The antioxidant activity of *Coenzyme Q10* is thought to inhibit free radical-mediated myocardial damage in the injured myocardium during ischemia Gold and Farnsworth, 2002). The antioxidant activity also significantly inhibits the oxidation of LDL cholesterol (much more efficiently than vitamin E). This has great implications in the treatment of ischemia and reperfusion injury as well as the potential for slowing the development of atherosclerosis (Langsjoen *et al.*, 1988; Langsjoen *et al.*, 1994; Gold and Farnsworth, 2002; Gundling and Ernest, 1999).

Ginkgo biloba is a deciduous tree with large fan-shaped leaves from which the herb is derived. It has free-radical scavenging activity that may make it useful for the prevention and treatment of acute myocardial infarction (Gold and Farnsworth, 2002). *Ginkgo biloba* decreases platelet aggregation and causes vaso-relaxation by blocking nitric oxide metabolism, which may improve circulation in the systemic circulation and cerebral blood vessels (Gold and Farnsworth, 2002; Gundling and Ernest, 1999). There are few adverse effects associated with *Ginkgo biloba*, in combination with ticlopidine, *Ginkgo biloba's* anti-platelet effect is significantly enhanced, and with results of one study showing combined treatment prolonged bleeding times by 150% (Gold and Farnsworth, 2002; Gundling and Ernest, 1999).

Garlic (*Allium sativum L*) is a perennial plant in the family Alliaceae and genus Allium, closely related to the onion, shallot, and leek. Garlic cloves are commonly used as cardiovascular herb (Gold and Farnsworth, 2002). Garlic may inhibit platelet aggregation and enhance fibrinolytic potential, but the evidence to date does not support other therapeutic claims. Research has not supported garlic as an effective antihypertensive herb (Gold and Farnsworth, 2002). Garlic may cause minor reductions in total cholesterol, triglyceride levels, and low-density lipoprotein (LDL), or augment improvements from the combination of other treatments i.e. low-cholesterol diet or fish oil consumption (Koch and Lawson, 1996; Gold and Farnsworth, 2002).

Soy is another herb that has been demonstrated to reduce total cholesterol by 10% and decrease the risk of coronary heart disease by 20% (Gold and Farnsworth, 2002. A

reduction in blood lipids is critical to improve outcome in patients with cardiovascular disease. The main source of soy is soybeans, but soluble fibre from whole-oat products or *psyllium* seed husk also contain significant quantities of soy. Soy inhibits LDL oxidation resulting in a decrease in atherosclerotic plaque formation and thus the accompanying improvement in arterial compliance may contribute to the reduction in cardiovascular risk (Gold and Farnsworth, 2002).

Motherworth (*Leonurus cardiaca*) is another valuable cardio tonic herb. The leaves and flowers of this mint family plant are used as medicine. In Chinese herbal medicine, the seeds are also employed (Lee, 1999). Herbalists consider motherworth as a helpful diuretic and heart-strengthening herb. In particular, it is used to alleviate heart palpitations associated with anxiety attacks (Lee, 1999). Motherworth specifically targets alleviating racing heartbeats caused by anxiety and tension. Despite the name, men and women, alike, can benefit from the effect of this herb (Lee, 1999).

Butcher's broom is another cardio-active herb. Butcher's broom leaves are commonly used in treating discomfort and pain caused by poor circulation in the hands and legs (a heavy-leg feeling (Lee, 1999). This herb is particularly good for people who are on their feet most of the day and it also reduces edema. Butcher's broom's effect is enhanced when used in combination with Cayenne (Lee, 1999).

Ginseng (root) is another famous herb that has been introduced by the Chinese.
There are many types of Ginseng, the most common being *Panax* Ginseng, also called *Korean Red* Ginseng, and *American* Ginseng. The American Ginseng is said to be "cooler" in its effects, and therefore more appropriate for people with high blood pressure (Lee, 1999).
Ginseng means "root of man," and it has the property of increasing physical and mental endurance. It has the power to move people to their physical peak, and many athletes claim that it gives them a competitive edge.

Ginseng has "adaptogenic" properties, which means it has a unique ability to normalize body functions. That means for instance, if blood pressure goes too high, or if blood sugar falls too low, an adaptogen will return the body to normal levels (Lee, 1999).
Peucedanum Galbanum Herba consists of the fresh or dried leaves and smaller stems of *Peucedanum galbanum* (Lamiaceae) and drude (Apiaceae). A leaf decoction is used traditionally as a diuretic for the treatment of oedema, kidney and bladder ailments, and kidney stones (Campbell *et al.*, 1994; Finkelstein *et al.*, 1993).

Tridax procumbens Linn (compositae) is a common grass found in tropical southern part of Nigeria, growing primarily during raining season. Aqueous leaf extract of *Tridax procumbens* is used in traditional medicine to treat hypertension. This cardio active herb has been reported to significantly reduce mean arterial blood pressure and heart rate in normal rats at doses of 6 mg / kg and 9 mg / kg (Salahdeen *et al.*, 2004).

There is currently little or no scientific evidence in respect to the mechanism of action of L leonurus as it is currently used in the cardiovascular system. This study is therefore undertaken in order to confirm the effects of *L. leonurus* on the cardiovascular system and to elucidate the mechanism by which *L. leonurus* produces its effect.

1.5 *Leonotis Leonurus* and traditional medicines

Bienvenu *et al* (2002) discovered that, the aqueous leaf extract of *L. leonurus* has anticonvulsant activity in vivo in the mouse (dose: 200 mg / kg and 400 mg / kg).
Desta (1994) examined the anti-fertility activity of shade-dried roots of Ethiopian plants extracts in the rat, both in vitro (uterine stimulant activity) and *in vivo* (anti-implantation effects). Weak uterine stimulant activity of *L. leonurus* was shown for 95% ethanol leaf extracts but not for aqueous or n-butanol leaf extracts (conc. 2.0%). Anti-implantation activity was shown by both n-butanol and 95% ethanolic extracts but not by aqueous extracts (dose: 0. 93 g / kg intra-gastrically).

1.6 *Leonotis leonurus* in cardiovascular system

Ojewole, (2003) investigated the cardiovascular effects of an aqueous leaf extract of *L. leonurus* on arterial blood pressures and heart rates of normal and spontaneously hypertensive rats, using invasive and non-invasive techniques.

He discovered that the cardiovascular effects of *L. leonurus* leaf aqueous extract (25–800 mg / kg i.v) produced dose-related, significant decrease ($P < 0.05 - 0.001$) in the arterial blood pressure and heart rate of anaesthetized, normal and spontaneously hypertensive rats. The hypotensive effect of the leaf extract was more pronounced in the hypertensive rats than in the normal rats. He also discovered that the aqueous leaf extract of the plant (25– 800 mg / kg i.p) decreased the arterial blood pressure and heart rate of conscious, normal and spontaneously hypertensive rats in a dose-dependent fashion.

Obikeze *et al* (2004) discovered that, *L. leonurus* leaf aqueous extract at low doses ranging from 0.5 mg to 7.0 mg administered intravenously, significantly increased systolic blood pressure and mean arterial pressure. A significant increase in diastolic blood pressure and decreased in heart rate was noticed at dose ranges of 2.0 mg to 7.0 mg. In another study, Njagi *et al* (2001) has shown that, the infusion of a 1 mg/ml aqueous extract of leaves of *L. leonurus* has no significant effect on the systolic and diastolic blood pressure and on the heart rate of anaesthetized male Wistar rats. Mugabo *et al* (2002) discovered that, 1 mg/ml aqueous decoction of *L. leonurus* leaves had positive chronotropic and inotropic effects on isolated male Wistar rat heart.

The above studies prove that the aqueous leaf extract of *L. leonurus* possessess cardiovascular effects. Hypotensive effects were observed at high doses and hypertensive effects were observed at low doses. However, further studies are necessary to look closely at the blood lowering effect of *L. leonurus*. This study will therefore focus on determining the hypotensive dose dependent effect of aqueous leaf extract of *L. leonurus*, and the mechanism by which *L. leonurus* produces its effect will be determined.

1.7 Research question

This study raised a question that, by which mechanism does *L. leonurus* aqueous leaf extract decreases blood pressure and heart rate in male Wistar rats?

1.8 Aims and objectives of the study

The aim of this study was 1) to determine the cardiovascular effects of *L. leonurus* aqueous leaf extract in male Wistar rats *in vivo* and 2) to determine the mechanism by which the aqueous leaf extract of *L. leonurus* produces its hypotensive effect.

1.9 Hypothesis

It has been hypothesized that aqueous leaf extract of *L. leonurus* may decrease blood pressure and heart rate in male Wistar rats through inhibition of sympathetic system and muscarinic receptor blockade.

CHAPTER 2

Physiology of the cardiovascular system

2.1 Blood pressure

Blood pressure is the hydrostatic pressure exerted by blood on the walls of a blood vessel. Blood pressure is highest in the aorta and large systemic arteries and is generated by contraction of ventricles. Hypertension is the most common cardiovascular system disease generally defined as a resting systolic blood pressure >140mmHg or a diastolic pressure >90 mmHg, or both, in adults (Tortora and Grabowski, 1996).

The prevalence varies with age, race, education, and many other variables. Sustained arterial hypertension damages blood vessels in the kidney, heart, and brain and leads to an increased incidence of renal failure, coronary disease, cardiac failure and cerebral vascular accident. Effective pharmacological lowering of blood pressure has been shown to prevent damage to blood vessels and to substantially reduce morbidity and mortality rates (Katzung, 1998; Tortora *et al.*, 1996). Many effective drugs are available to control blood pressure. Some of them will be used in this study. They are discussed in chapter 3 below.

The diagnosis of blood pressure is based on repeated, reproducible measurements of elevated blood pressure. The diagnosis serves primarily as a prediction of consequences for the patient; it seldom includes a statement about the cause of hypertension. Epidemiological studies show that the risks of damage to kidney, heart, and the brain are directly related to the extent of blood pressure elevation (Tortora and Grabowski, 1996).

The risk of end organ damage at any level of blood pressure or age is greater in black people and relatively less in premenopausal women than in men (Katzung, 1998; Tortora and Grabowski, 1996).

Other positive risk factors include smoking, hyperlipidemia, diabetes, manifestations of end organ damage at the time of diagnosis, and a family history of cardiovascular disease (Katzung, 1998; Tortora and Grabowski, 1996).

2.2 Heart rate

Acting alone, the sinoatrial node produces a constant rhythmic heart rate. Regulating factors are reliant on the atrioventricular node to increase or decrease the heart rate to adjust cardiac output to meet the changing needs of the body. Most changes in the heart rate are mediated through the cardiac center in the medulla oblongata of the brain. The center has both sympathetic and parasympathetic components that adjust the heart rate to meet the changing needs of the body.

Peripheral factors such as emotions, ion concentrations, and body temperature may affect heart rate. These are usually mediated through the cardiac center (Tortora and Grabowski, 1996).

2.3 Conduction system

The function of the heart is to pump blood to the lungs through pulmonary circulation and to the rest of the body through systemic circulation. This is accomplished by systematic contraction and relaxation of the cardiac muscle in the myocardium.

The conduction system includes several components. The first part of the conduction system is the sinoatrial node, (also called the SA node or sinus node) nestled in the upper area of the right atrium. Without any neural stimulation, the sinoatrial node rhythmically initiates impulses that trigger each heart beat 70 to 80 times per minute. The impulse spreads troughout the atria, prompting the cardiac muscle tissue to contract in a wave-like manner. Because it establishes the basic rhythm of the heartbeat, it is called the pacemaker of the heart (Klabunde, 2005).

Other parts of the conduction system include the atrioventricular node, (also called AV node) which is situated in the lower portion of the right atrium. The atrioventricular node receives impulse from the SA node which in turn sends an impulse through the nerve network to the ventricles, thus initiate the same wave-like contraction of the ventricles. The electrical network serving the ventricles leaves the atrioventricular node through the

right and left bundle branches. These nerve fibers send impulses that cause the ventricles to contract (Klabunde, 2005).

2.4 Regulation of the blood pressure

The normal systolic blood pressure in rats and humans ranges between 100 and 139 mmHg during the systole and ranges between 60 and 90 mmHg during the diastole. The mean arterial blood pressure (MABP) is closer to diastolic than to systolic blood pressure. MABP is approximately one-third of the way between diastolic and systolic blood pressure i.e. MABP = diastolic BP + ⅓ (systolic BP – diastolic BP). Cardiac output is equal to the mean arterial blood pressure (MABP) divided by resistance (R) i.e. CO = MABP ÷ R (Tortora and Grabowski, 1996).

If cardiac output rises due to an increase in the stroke volume or the heart rate, then the blood pressure rises so long as the resistance remains steady. Alternatively, a decrease in cardiac output causes a decrease in blood pressure if resistance does not change (Tortora and Grabowski, 1996).

According to the hydraulic equation (Tortora and Grabowski, 1996), arterial blood pressure (BP) is directly proportional to the product of the cardiac output, CO and the resistance to passage of blood through precapillary arterioles (peripheral vascular resistance, PVR):

$$BP = CO \times PVR$$

As blood leaves the aorta and flows through the systemic circulation, its pressure falls progressively as the distance from the left ventricle increases. For instance, blood pressure decreases as blood passes into the arteriolar end of a capillary, through the venous end of a capillary and continues to drop as blood enters venules and then veins as these vessels are far from the pressure source, which is the left ventricle. Blood pressure drops even further as blood flows into the right ventricle (Tortora and Grabowski, 1996).

Blood pressure also depends on the total volume of blood in the cardiovascular system. The normal volume of blood in an adult is about five liters. Any decrease in this volume decreases the amount of blood circulating through the arteries each minute. This decrease can be counteracted by a homeostatic mechanism, but if this decrease is greater than 10% of the total blood volume, blood pressure drops.

Blood flow opposition resulting from friction between blood and the walls of blood vessels causes resistance to blood flow. The resistance to blood flow is dependent on blood viscosity, total blood vessel length, and the average blood vessel radius (Tortora and Grabowski, 1996).

2.4.1 Blood viscosity

The viscosity of blood depends greatly on the ratio of red blood cells to plasma volume and to a lesser extent on the concentration of proteins in plasma. Resistance to blood flow is directly proportional to the viscosity of blood. Any increase in blood viscosity as in dehydration, burns, increased red blood cells, increases resistance and thus blood pressure (Tortora and Grabowski, 1996).
Likewise, a decrease in blood cells or plasma proteins e.g. due to anemia, hemorrhage, decreases resistance and thus blood pressure.

2.4.2 Total blood vessel length

Resistance to blood flow through a vessel is directly proportional to the length of the blood vessel. The longer a blood vessel, the greater the resistance as blood flows through it (Tortora and Grabowski, 1996).

2.4.3 Average blood vessel radius

Resistance is inversely proportional to the fourth power of the radius of the blood vessel (R =1/r4). The smaller the radius of the blood vessel, the greater the resistance to blood flow (Tortora and Grabowski, 1996).

2.5 Systemic vascular resistance (SVR) or total peripheral resistance

SVR refers to all the vascular resistance offered by systemic blood vessels. Most resistance is in arterioles, capillaries, and venules. The diameter of arteries and veins is large and thus their resistance is very small because most of the blood does not come in physical contact with the walls of the blood vessel. The major function of arterioles is to control SVR and therefore blood pressure and blood flow to particular tissues, by changing their diameters. Arterioles need to slightly vasodilate or vasoconstrict to have a large effect on SVR. The principal center for regulation of SVR is the vasomotor center in the brain stem (Tortora and Grabowski, 1996).

2.6 Venous return

The volume of blood flowing back to the heart from the systemic veins depends on the pressure difference from venules to the right ventricles. Whenever the pressure increases in the right atrium, venous return decreases. The increase in pressure in the right atrium is caused by stenotic valve and/or an incompetent or leaking tricuspid valve that allows blood to flow backwards as the ventricles contract. This increased pressure in the right atrium therefore result in build-up of blood on the venous side of the systemic circulation (Tortora and Grabowski, 1996).

Respiratory and skeletal muscle pump are two other mechanisms that act as boost to venous return. Contraction of skeletal muscles in the lower limbs and the pressure changes in the throat and abdomen during respiration. The presence of valves in veins allows both of these pumps to contribute to venous return.

2.6.1 Skeletal muscle pump

When skeletal muscles contract, they tighten around the vein passing through them, which increases the venous blood pressure and the proximal valve opens. This pressure drives the blood towards the heart, and this action is called *milking*. When the muscles relax, this valve closes and prevents the backward flow of blood away from the heart (Tortora and Grabowski, 1996).

2.6.2 Respiratory pump

During inspiration, the diaphragm moves inferiorly. This causes a decrease in pressure in the thoracic cavity. As a result, a greater volume of blood moves from the compressed abdominal veins into the decompressed thoracic veins. When the pressure reverses during expiration, the valves in the veins prevent backflow of blood (Tortora and Grabowski, 1996).

2.7 Control of blood pressure and blood flow

From moment to moment several interconnected negative feedback systems control blood pressure by adjusting heart rate, stroke volume, systemic vascular resistance and blood volume. Some systems allow rapid adjustment of blood pressure to cope with sudden changes and others act more slowly to provide long term regulation of blood pressure. Even if blood pressure is steady, there may be a need to change the distribution of blood flow, which is accomplished mainly by altering the diameter of arterioles. Blood pressure and blood flow to specific tissues is regulated by neural, hormonal, and local negative feedback systems (Tortora and Grabowski, 1996).

2.7.1 Cardiovascular (CV) center

Group of neurons scattered within the medullar oblongata of the brain stem regulate heart rate, contractility of the ventricles, and blood vessel diameter. As a whole, this region is known as the cardiovascular center. Some of its neurons stimulate the heart (cardio-stimulatory) whereas others inhibit the heart (cardio-inhibitory center), either by causing constriction (vasoconstrictor center) or dilation (vasodilator center). These cluster neurons intercommunicate, function together, and are not clearly separated anatomically (Tortora and Grabowski, 1996).

2.7.2 Input to the cardiovascular center

The CV center receives input from both higher brain regions and from sensory receptors. Nerve impulses descend from higher brain regions including the cerebral cortex, limbic system, and hypothalamus to affect the CV center. For example, even before one starts a race, the heart rate may increase due to nerve impulses conveyed from the limbic system to the CV center. If the body temperature rises during race, the thermoregulatory center of the hypothalamus sends nerve impulses to the CV center of the medullar oblongata. This will result in vasodilation of the skin blood vessels, which allows heat to dissipate more rapidly. The two main types of sensory receptors that provide input to the cardiovascular center are baroreceptors and chemoreceptors. Baroreceptors are important pressure-sensitive sensory neurons that monitor stretching of the walls of blood vessels and the atria. Chemoreceptors monitor blood acidity, carbon dioxide level, and oxygen levels (Tortora and Grabowski, 1996).

2.7.3 Output from the cardiovascular center

Output from the CV center flows along sympathetic and parasympathetic fibers of the Autonomic nervous system (ANS). Sympathetic impulses reach the heart via the cardiac accelerator nerves. Sympathetic stimulation of the heart increases heart rate and contractility. Parasympathetic stimulation, conveyed along the vagus (X) nerves,

decreases heart rate. The CV center also continually sends impulses to smooth muscle in blood vessel walls via sympathetic fibers called vasomotor nerves. Thus autonomic control of the heart is the result of opposing sympathetic (stimulatory) and parasympathetic (inhibitory) influences.

On the other hand, autonomic control of blood vessel diameter is mostly controlled by the sympathetic division. Sympathetic vasomotor nerve fibers exit the spinal cord through all thoracic and the first one or two lumbar spinal nerves and pass into the sympathetic trunk ganglia. From here, impulses propagate along sympathetic nerves that innervate blood vessels in visceral and peripheral areas. Over these routes, the CV center (specifically, the vasomotor center) continually sends impulses to arterioles throughout the body but especially in the skin and abdominal viscera. The result is a moderate state of tonic contraction or vasoconstriction, called vasomotor tone, which sets the resting level of systemic vascular resistance (Tortora and Grabowski, 1996).

In the smooth muscle of most small arteries and arterioles, sympathetic stimulation causes vasoconstriction, which raises blood pressure and restricts blood flow to a tissue. This is due to activation of alpha-adrenergic receptors for norepinephrine and epinephrine in the vascular smooth muscle. In skeletal muscle and the heart, the smooth muscle of blood vessels displays beta-adrenergic receptors instead, and sympathetic stimulation causes vasodilation rather than vasoconstriction. In addition, some of the sympathetic fibers to blood vessels in skeletal muscle are cholinergic; they release acetylcholine, which causes vasodilation.

When sympathetic stimulation increases, for example, during exercise, both vasoconstriction and vasodilation occur, but in different tissues. As a result, systemic vascular resistance may increase, decrease, or stay the same (Tortora and Grabowski, 1996). The tissue that has dilated arterioles, however, will receive a larger share of the cardiac output. Sympathetic stimulation of most veins results in constriction that moves blood from reservoirs and increases blood pressure (Tortora and Grabowski, 1996).

2.7.4 Neural regulation of blood pressure

Regulation of blood pressure by the nervous system depends on the receptors in the periphery that monitor blood pressure (baroreceptors) and blood chemistry (chemoreceptors) and provide input to the cardiovascular center. Several neural reflexes contribute to blood pressure regulation by negative feedback systems (Tortora and Grabowski, 1996).

2.7.4.1 Baroreceptors

Baroreceptors are nerve cells capable of responding to changes in pressure or stretch. Baroreceptors in the walls of the arteries, veins and right atrium monitor blood pressure. The three most important negative feedback systems that baroreceptors participate in are the aortic reflex, carotid sinus reflex, and right heart reflex.

The carotid sinus reflex is responsible for maintaining normal blood pressure in the brain and is initiated by baroreceptors in the wall of the carotid sinus. The carotid sinus is a small widening of the internal carotid artery just above the point where it branches from the common carotid artery. When there is an increase in blood pressure, it stretches the wall of the aorta and carotid sinus, and stretching stimulates the baroreceptors. For the carotid sinus reflex, the impulses travel from the baroreceptors over sensory fibers in the glossopharyngeal (IX) nerves to the CV center of the medulla oblongata (Tortora and Grabowski, 1996).

The aortic reflex controls general systemic blood pressure and is initiated by baroreceptors in the wall of the arch of the aorta. Impulses from baroreceptors in the arch of the aorta reach the CV center via sensory (afferent) fibers of the vagus (X) nerves.
When an increase in aortic and carotid artery pressure is detected in this manner, the CV center responds by putting out more parasympathetic impulses via motor (afferent) fibers of the vagus (X) nerves to the heart and fewer sympathetic impulses via cardiac accelerator nerves to the heart. The resulting decreases in heart rate and force of

contraction lower cardiac output. Also, the CV center sends out decreased sympathetic impulses along vasomotor fibers that normally cause vasoconstriction. The result is vasodilation, which lowers systemic vascular resistance (SVR). Decreased cardiac output and SVR both lower systemic arterial blood pressure (Tortora and Grabowski, 1996).

If blood pressure falls, on the other hand, baroreceptors are stretched less. They send nerve impulses at a slower rate to the cardiovascular center. In response, the CV center calls for increased sympathetic impulses, decreased parasympathetic impulses, and increased secretion of epinephrine and norepinephrine by the adrenal medullar. The effects on the heart and blood vessels are to accelerate heart rate, increase force of contraction, and promote vasoconstriction. As the heart beats faster and more forcefully and SVR increases, blood pressure increases and there is a return to homeostasis when blood pressure returns to normal level. This relationship between heart rate and blood pressure is called Marey's law of the heart (Tortora and Grabowski, 1996).

The ability of the aortic and carotid sinus reflex to correct a drop in blood pressure is very important when a person sits or stands from a lying position. Moving from a prone to an erect position decreases blood pressure in the head and upper part of the body. However, the reflexes quickly counteract the drop in pressure. If the pressure were to fall markedly, unconsciousness could occur.

The right heart (atria) reflex responds to increases in venous blood pressure. It is initiated by baroreceptors in the right atrium and venae cavae. When venous pressure increases, the baroreceptors send impulses through the vagus (X) nerves to the CV center. Returning impulses via sympathetic nerves increase heart rate and force of contraction. This mechanism is called the Bainbridge reflex (Tortora and Grabowski, 1996).

2.7.4.2 Chemoreceptors

Chemoreceptors are receptors that are sensitive to chemicals. Chemoreceptors that monitor blood chemicals are located close to the baroceptors of the carotid sinus and arch of the aorta in small structures called carotid bodies and aortic bodies, respectively. These chemoreceptors detect changes in blood level of oxygen, carbon dioxide and hydrogen

ions. If there is an excess of carbon dioxide (hypercapnia), or severe deficiency of oxygen (hypoxia), an increase in hydrogen ion concentration (increases acidity or acidosis), or the chemoreceptors are stimulated and send impulses to the CV center. In response, the CV center increases sympathetic stimulation to arterioles and veins. This brings about vasoconstriction and an increase in blood pressure (Tortora and Grabowski, 1996).

2.7.5 Hormonal regulation of blood pressure

Several hormones affect blood pressure and blood flow by three mechanisms: changing systemic vascular resistance, altering cardiac output, or adjusting the total blood volume.

2.7.5.1 Renin-angiotension-aldosterone (RAA) system

The kidney is primarily responsible for long-term blood pressure control by controlling blood volume. When blood volume falls or blood flow to the kidneys decreases, juxtaglomerular cells in the kidneys release increased amounts of an enzyme called renin into the bloodstream.

Renin acts on angiotensinogen to form angiotensin I. As this molecule passes through capillaries in the lungs, angiotensin-converting enzyme (ACE) changes it into angiotensin II. Angiotensin II helps to raise blood pressure in two ways.

It is a potent vasoconstrictor and thus raises total systemic resistance.

It stimulates secretion of aldosterone, which increases sodium ion and water reabsorption by the kidneys. This action increases total blood volume and thus increases blood pressure (Tortora and Grabowski, 1996).

2.7.5.2 Epinephrine and norepinephrine

Epinephrine and norepinephrine are produced by the adrenal medulla. These hormones increase cardiac output by increasing the rate and force of heart contractions and bring about vasoconstriction of arterioles and veins in the skin and in abdominal organs. They also dilate arterioles in cardiac and skeletal muscles (Tortora and Grabowski, 1996).

2.7.5.3 Antidiuretic hormone (ADH)

Antidiuretic hormone is produced by the hypothalamus and released from the posterior pituitary gland. One of the functions of Antidiuretic hormone is to cause vasoconstriction if there is a severe loss of blood due to hemorrhage. For this reason, Antidiuretic hormone is also called vasopressin (Tortora and Grabowski, 1996).

2.7.5.4 Atrial natriuretic peptide

Cells in the atria of the heart release atrial natriuretic peptide. Atrial natriuretic peptide lowers blood pressure by causing vasodilation and by promoting loss of salt and water in the urine, which reduces blood volume (Tortora and Grabowski, 1996).

2.7.5.5 Parathyroid hormone and calcitriol

Parathyroid hormone and calcitriol are hormones that regulate the circulating levels of calcium ions and phosphate ions in the blood. They also influence vascular smooth muscle. Parathyroid hormone causes vasodilation, which tends to decrease blood pressure. On the other hand, calcitriol, the active form of vitamin D, causes vasoconstriction, which increases blood pressure (Tortora and Grabowski, 1996).

2.7.6 Autoregulation

Autoregulation refers to a local, automatic adjustment of blood flow in a given region of the body to match the particular needs of the tissue. Oxygen in most body tissues is the principal, though not direct, stimulus for autoregulation.

Autoregulation is important in meeting the oxygen and nutritional demands of active tissues, such as heart and muscle tissue where the demand might increase as much as ten-fold (Tortora and Grabowski, 1996). Autoregulation is also the major regulator of the regional brain blood flow. Total blood flow to the brain remains almost constant, independent of level of exercise, but depending on mental and physical activities, distribution to various parts of the brain changes dramatically. For instance, blood flow increases to the motor speech areas when one talks, whereas it increases to the auditory areas when one is listening. Two types of stimuli, i.e. physical and chemical changes, generally cause autoregulation of blood flow (Tortora and Grabowski, 1996).

2.7.6.1 Physical changes

Warming promotes vasodilation while cooling causes vasconstriction. Smooth muscle in arteriole walls exhibits a myogenic response, i.e. smooth muscle contracts more forcefully when stretched and relaxes when stretching is less (Klabunde, 2005). In an arteriole, the amount that the smooth muscle stretches depends on blood flow. If blood flow decreases, stretch decrease the smooth muscle relaxes, and vasodilation occurs. When there is vasodilation, blood flow increases (Tortora and Grabowski, 1996).

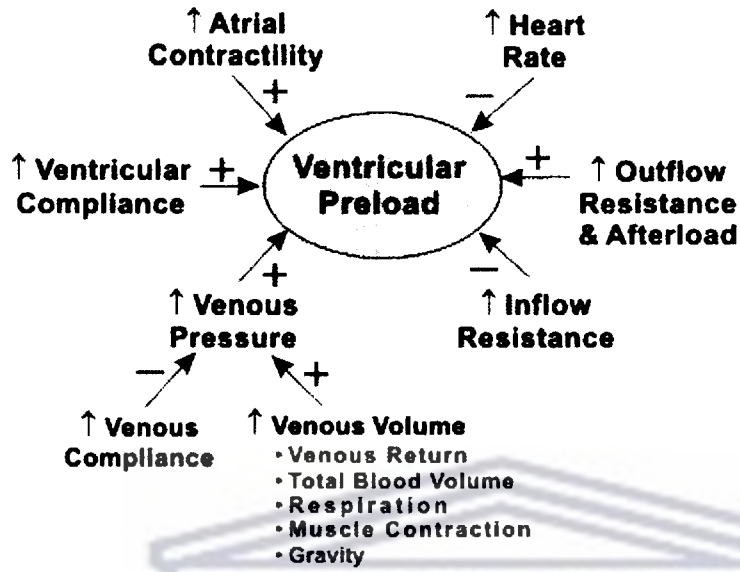
2.7.6.2 Chemical mediators

Cells in the blood, such as white blood cells, and platelets, and cells near blood vessels, including smooth muscle fibers, macrophages, and endothelial cells, synthesize and release a wide variety of vasoactive factors. These are chemicals that alter endothelium-derived relaxation factor (EDRF), now known to be nitric oxide. Other vasodilators, which widen blood vessels, include certain ions, Potassium and Hydrogen, certain

products such as lactic acids, and adenosine (Adenosine 5'-triphosphate). Vasoconstrictions, which narrow blood vessels, include certain eicosanoids such as thromboxane A_2 and prostaglandin F2α, superoxide radicals, angiotensins, and endothelins. Once released, vasodilators produce a local dilation of arterioles and relaxation of pre-capillary sphincters. The result is an increased flow of blood into tissue, which returns oxygen levels to normal. Vasoconstrictors have opposite effects. Stimuli that promote release of vasoactive factors include changes in tissue carbon dioxide and oxygen levels, mechanical stretch of the tissue, hormones in the blood, and local hormones (autocrines and paracrines) (Tortora and Grabowski, 1996).

2.7.7 Preload

Preload can be defined as the initial stretching of the cardiac myocytes prior to contraction. Preload, therefore, is related to the sarcomere length. Because sarcomere length cannot be determined in the intact heart, other indices of preload are used such as ventricular end-diastolic volume or pressure. For example, when venous return is increased, the end-diastolic pressure and volume of the ventricle are increased, which stretches the sarcomeres (and thus their preload increases) (Klabunde, 2005).



Factors determining ventricular preload. A "+" sign indicates that an increase in this particular variable increases ventricular end-diastolic volume, and therefore preload, while the "-" indicates that the variable decreases preload.

Fig 2.1 Factors determining ventricular preload (Klabunde, 2005)

As another example, hypovolemia resulting from a loss of blood due to hemorrhage leads to less ventricular filling and therefore shorter sacromere lengths (reduced preload). Changes in ventricular preload dramatically affect ventricular stroke volume by what is called the Frank-Starlings mechanism. Increased preload increases stroke volume, whereas decreased preload decreases stroke volume by altering the force of contraction of the cardiac muscle (Klabunde, 2005).

Ventricular preload is increased by:

- Increased venous blood pressure that can result from enhanced, increased blood volume, increased respiration activity, increased skeletal muscle pump activity, venous constriction (decreased venous compliance), gravity (e.g., head-down tilt).
- Increased atrial contractility resulting from sympathetic stimulation of the

heart.

- Reduced heart rate, which increases ventricular filling time.
- Ventricular systolic pressure, which causes blood to back up into the ventricle.
- Increased ventricular afterload, which decreases forward flow (i.e., ejection).
- Outflow valve (aortic and pulmonic valves) and inflow valve regurgitation (mitral and tricuspid valves).

Ventricular preload is decreased by:

- Decreased venous blood pressure, most commonly resulting from reduced blood volume (e.g., hemorrhage) or gravity (e.g., standing upright).
- Impaired atrial contraction that can result from atrial arrhythmias.
- Increased heart rate (e.g. atrial tachycardia), which reduces ventricular filling time.
- Decreased ventricular afterload, which enhances forward flow (i.e. ejection).
- Ventricular diastolic failure (decreased ventricular compliance) caused, for example, by ventricular hypertrophy.
- Inflow (mitral and tricuspid) valve stenosis.

2.7.8 Afterload

Afterload can be viewed as the "load" that the heart must eject blood against. In simple terms, the afterload is closely related to the aortic pressure. More precisely, afterload is related to ventricular wall stress (s), where

$S \alpha (P \times r) / h$ (P, ventricular pressure; r, ventricular radius; h, wall thickness) (Klabunde, 2005).

The pressure that the ventricle generates during systolic ejection is very close to aortic pressure, unless aortic stenosis is present. At a given pressure, wall stress and therefore afterload is increased by an increase in radius (ventricular dilation). A hypertopheid ventricle (thickened wall) reduces wall stress and afterload.

Afterload is increased when aortic pressure and systemic vascular resistance are increased, by aortic valve stenosis, and by ventricular dilation. When afterload increases,

there is an increase in end-systolic volume and a decrease in stroke volume. An increase in afterload decreases the velocity of fiber shortening. Because the period of time available for ejection is finite (~150-200 msec), a decrease in fiber shortening velocity reduces the rate of volume ejection so that more blood is left within the ventricle at the end of systole (increase end-diastolic volume).

Afterload per se does not alter preload; however, preload changes secondarily to changes in afterload (Klabunde, 2005). Increasing afterload not only reduces stroke volume, but it also increases left ventricular end-diastolic pressure (LVEDP) (i.e. increases preload). This occurs because the increase in end-systolic volume is added to the venous return into the ventricle and this increases end-diastolic volume. This increase in preload activates the Frank-Starling mechanism to partially compensate for the reduction in stroke volume caused by the increase in afterload.

The interaction between afterload and preload is utilized in the treatment of heart failure, in which vasodilator drugs are used to augment stroke volume by decreasing afterload, and at the same time, reduce ventricular preload (Klabunde, 2005).

From this chapter we learn that the main function of the cardiovascular system is to ensure adequate circulation of blood to all body tissues and capillary exchange between blood plasma, interstitial fluid and tissue cells. Several interconnected negative feedback systems control blood pressure by adjusting heart rate, stroke volume, systemic vascular resistance, and blood volume. This will help us in explaining the mechanism by which *L. leonurus* exert its blood pressure lowering effect.

CHAPTER 3

Drugs acting on the autonomic nervous system

3.1 Cholinergic and adrenergic agonists and antagonists

Two classes of drugs known with effect on the cardiovascular system functions will be briefly introduced in this chapter.
From each class, one drug used in experiments carried out in this study will be discussed.

3.1.1 Cholinergic / muscarinic receptor antagonists

Atropine and scopolamine are the prototype of drugs that block acetylcholine effect at the muscarinic receptors. These are parasympathetic postganglionic receptors for acetylcholine in the heart, smooth muscle, and exocrine glands. Atropine is an alkaloid originally derived from the leaves of the deadly nightshade, or *Atropa belladonna*, which belongs to the potato family.

3.1.1.1 Mode of action of atropine in the cardiovascular system

Atropine acts at muscarinic receptors and cause reversible blockade of the actions of cholinomimetics. The atria of the heart are richly innervated by parasympathetic (vagal) nerve fibres, and the sinoatrial node is therefore sensitive to muscarinic receptor blockade. The effect of moderate to high therapeutic doses of atropine in the innervated and spontaneously beating heart is a clear blockade of vagal slowing and a relative tachycardia. Low doses of atropine cause parasympathetic stimulation and often result in initial bradycardia before the effects of peripheral vagal block become manifest. This effect has been ascribed to central stimulation of the vagal nucleus, although other evidence suggests that it may be due to block of presynaptic muscarinic receptors on vagal postganglionic fibres that normally limit acetylcholine release in the sinal node. The same mechanism oparate in the control of atrioventricular node function, in the

presence of high vagal tone. Administration of atropine can significantly reduce the PR interval of the ECG by blocking muscarinic receptors in the AV node. Muscarinic effects on atrial muscle are similarly blocked, but except in atrial flutter and fibrillation these effects are of no clinical significance. The ventricles, at therapeutic drug levels, are less affected by antimuscarinic drugs because of a lesser degree of muscarinic control (Sommers, 2002; Brunton *et al.*, 2006).

3.1.1.2 Effects of atropine in the cardiovascular system

Atropine reduces the effect of vagal nerve stimulation, primarily on the SA node. Since stimulation of the vagus nerve slows heart rate, atropine increases heart rate by blocking this effect. Atropine also speeds-up conduction through the AV node, and thus lessening heart block in certain cases (Katzung, 1998; Sommers, 2002).

3.1.2 Adrenergic drugs (sympathomimetics)

In 1895, a physician called Dr Oliver discovered the hypertensive effects of adrenaline (epinephrine). The effect was later confirmed in animals and that led to isolation and synthesis of adrenaline in the early 1900s. In 1910, Barger and Dale invented the word "sympathomimetic", meaning, compounds that simulate the effects of sympathetic nerves not only in varying intensity but with varying precision. They also pointed out that noradrenaline mimicked the action of the sympathetic nervous system more closely than did adrenaline. In 1948, Ahlquist proposed that two different adrenoceptors exist (*alpha* and *beta*). Antagonists were discovered to be selectively and competitively blocking these receptors (Katzung, 1998).

3.1.2.1 Mode of action

Catecholamines (adrenaline, noradrenaline, dobutamine, dopamine) act on β-adrenoceptors as first messenger transmitters, combining with receptors on the outside of the cell membrane of the end organ, thus activating the enzyme adenylyl cyclase on the inside of the cell membrane, which causes an increase in intracellular cyclic AMP, the second messenger, which is destroyed by intracellular phosphodiesterase. It is this second messenger that initiates a sequence of changes that differ among tissues. These include contraction of cardiac muscle, relaxation of vascular and bronchial smooth muscles, and release of glucose or potassium from liver cells (Brunton *et al.*, 2006).

Sympathomimetics may be classified by their mode of action:

Directly, i.e. adrenoceptor agonists that act on the adrenergic receptors (noradrenaline, isoprenaline, dopamine, dobutamine and phenylepherine etc.
Indirectly, i.e. by causing a release of noradrenaline from stores at nerve endings (amphetamines, tyramine, and ephedrine).
Sympathomimetics can also act through the combination of both mechanisms, though often with a preponderance of one or other synthetic agents (Brunton *et a.l*, 2006; Katzung, 1998; Klabunde, 2007).

3.1.2.2 Effects of sympathomimetics

The overall effect of a sympathomimetic depends on the site of action, receptor specificity and on the dose; for instance adrenaline usually dilates muscle blood vessels (β_2) mainly arterioles, but vein also in very large doses, it constrict them (α) (Brunton *et al.*, 2006).
The end results are often complex and unpredictable, partly because of the variability of homeostatic reflex responses and partly because what is observed, e.g. a change in blood pressure, is the result of many factors, e.g. vasodilation (β) in some areas, vasoconstriction in others (α), and cardiac stimulation (β) (Katzung, 1998).

Dobutamine resembles dopamine structurally, but possesses a bulky aromatic subtituents on the amino group. The pharmacological effects of dobutamine due to the direct interactions with alpha and beta receptors do not appear to result from release of norepinephrine from sympathetic nerve endings nor are exerted via dopaminegic receptors. Dopamine possesses a center of asymmetry, both enatiomeric forms are present in the racemic mixture used clinically. The (-) isomer of dobutamine is a potent agonist at *alpha*-1-receptors and is capable of causing marked pressor response. In contrast, (+) isomer is a potent *alpha*-1-receptor antagonist which can block the effect of dobutamine. The effects of these two isomers are mediated via *beta* receptors. Dobutamine has a relatively more prominent inotropic than chronotropic effects on the heart. Although not completely understood, this useful selectivity may arise because peripheral resistance is relatively unchanged. Alternatively, cardiac alpha receptors may contribute to the inotropic effect. The relatively constant peripheral resistance presumably reflects counterbalancing of alpha receptor-mediated vasoconstriction and beta receptor mediated vasodilation Brunton; *et al* 2006).

In this study, the effect of the plant is compared to the effect of existing drugs that have an effect in blood pressure and heart rate. It is therefore important to understand the mechanism by which these drugs act.

3.2 Adrenoceptor blockers

Adrenoceptor blocking drugs occupy the adrenoceptor receptors in competition with adrenaline and noradrenaline. There are two main classes of adrenoceptors, namely, *Alpha* adrenoceptors and *Beta* adrenoceptors.

3.2.1 *Alpha*-adrenoceptor blockers

Vascular smooth muscle has two primary types of alpha-adrenoceptors: alpha$_1$ (α_1) and alpha$_2$ (α_2). The a_1-adrenoceptors are located on the vascular smooth muscle. In contrast, α_2-adrenoceptors are located on the sympathetic nerve terminals as well as on vascular smooth muscle. Smooth muscle (postjunctional) α_1 and α_2-adrenoceptors are linked to a Gq-protein, which activates smooth muscle contraction through the IP3 signal transduction pathway. Prejunctional α_2-adrenoceptors located on the sympathetic nerve terminals serve as a negative feedback mechanism for norepinephrine release (Brunton *et al.*, 2006).

3.2.1.1 Mode of action

Alpha-adrenoceptor drugs blockers inhibit the effect of sympathetic nerves on blood vessels by binding to α -adrenoceptors located on the vascular smooth muscle. Most of these drugs act as competitive antagonists to the binding of norepinephrine that is released by sympathetic nerves synapsing on smooth muscle. Therefore, sometimes these drugs are referred to as sympatholytics because they antagonize sympathetic activity (Katzung, 1998).

Non-selective α_1 and α_2-adrenoceptor antagonists block postjunctional α_1 and α_2-adrenoceptors, which causes vasodilation; however, the blocking of prejunctional α_2-adrenoceptors leads to increased release of norepinephrine, which attenuates the effectiveness of the α_1 and α_2-postjunctional adrenoceptor blockade. Furthermore, blocking α_2-prejunctional adrenoceptors in the heart can lead to increases in heart rate and contractility due to the enhanced release of norepinephrine that binds to β_1-adrenoceptors (Brunton *et al.*, 2006; Katzung, 1998; Klabunde, 2007).

3.2.1.2 Effects of *alpha*-adrenoceptor blockers

α -blockers dilate both arteries and veins because both vessel types are innervated by sympathetic adrenergic nerves; however, the vasodilator effect is more pronounced in the arterial resistance vessels (Brunton *et al.*, 2006; Katzung, 1998; Klabunde, 2007).

3.2.2 *Beta*-adrenoceptor blocker

3.2.2.1 Mode of action

β-adrenoceptor blockers inhibit the β-receptor effect of noradrenaline and adrenaline. β-adrenoceptor blockage affects the regulation of circulation through a number of mechanisms. These include a reduction in myocardial contractility, heart rate, and cardiac output. β-adrenoceptor blockage in the juxtaglomerular complex reduces renin secretion and diminishing production of circulating angiotensin II, and thus this action likely contributes to the hypertensive effect of this class. β-adrenoceptor antagonist may lower blood pressure by other mechanisms. These include alteration of the control of the sympathetic nervours system at the level of the cental nervous system (CNS), altered baroreceptor activity, altered peripheral adrenergic neuron function, and increased prostacyclin biosynthesis. β-adrenoceptor blockers may be pure antagonist or may have some agonist activity, known as partial agonist. The β-adrenoceptor cardiovascular effects are dependent on the amount of sympathetic tone present (Brunton *et al.*, 2006).

3.2.2.2 Effects of β-adrenergic blockers

The main cardiac effects resulting from the reduction of sympathetic drive are reduced myocardial contractility (rate of rise of pressure in the ventricles) and reduced automaticity (heart rate) (Katzung, 1998).

A decrease in rate causes a decrease in the cardiac output/min and thus a decrease in overall cardiac oxygen consumption.

Some beta adrenoceptor blockers have higher affinity for cardiac β_1 receptors than for cardiac and peripheral β_2 receptors. β_1-receptors are called β_1 selective or cardio selective, e.g. atenolol used in this study is cardio selective (Brunton *et al.*, 2006; Melmon, 1992; Katzung, 1998; Sommers, 2002).

3.3 Synaptic neurotransmission blocker (Reserpine)

Reserpine is an adrenergic blocking agent used to treat mild to moderate hypertension via the disruption of norepinephrine vesicular storage.

3.3.1 Mode of action

Reserpine, binds tightly to adrenergic storage vesicles in central and peripheral adrenergic neurons and remains bound for prolonged periods of time. The interaction inhibits the vesicular catecholamine transporter that facilitates vesicular storage. Thus, nerve endings lose their capacity to concentrate and store norepinephrine and dopamine. Catecholamines leak into the cytoplasm, where they are metabolized by intraneuronal monoamine oxidase, and little or no active transmitter is discharged from nerve endings when they are depolarized. The overall result is a pharmacological sympathectomy. A similar process occurs at storage sites for 5-hydroxytryptamine. Reserpine-induced depletion of biogenic amines correlates with evidence of sympathetic dysfunction and antihypertensive effects. Recovery of sympathetic function requires synthesis of new storage vesicles, which takes days to weeks after discontinuation of the drug. Since reserpine depletes amines in the CNS as well as in the peripheral adrenergic neuron, it is probable that its antihypertensive effects are related to both central and peripheral actions (Brunton *et al.*, 2006)

3.3.2 Effects of reserpine on blood pressure

The antihypertensive actions of reserpine are a result of its ability to deplete catecholamines from peripheral sympathetic nerve endings. These substances are

normally involved in controlling heart rate, force of cardiac contraction and peripheral resistance (Brunton *et al.*, 2006; Katzung, 1998).

A selective beta adrenoceptor blocker, atenolol; ganglionic blocker, resepine; cholinergic receptor antagonist, atropine; and adrenergic agonist, adrenaline discussed above are used in this study in order to try to establish the mechanism by which *L. leonurus* acts.

The next chapter discusses materials used in plant preparation, chemical tests and animal experiments conducted in investigating this project's hypothesis.

CHAPTER 4

4.1 Materials and methods

4.1.1 Equipment and materials used in recording blood pressure and heart rate *in-vivo* experiments

BP amplifier (AD instruments)
PowerLab 4/20T (AD Instruments) used for BP and heat rate recording.
Computer monitor LG 52X max
Temperature probe (AD Instruments)
Chart 4.0 for windows computer software (Lasec)
Ascor AP22 syringe pump (Poland)
A heated rat-operating table with holders (BioScience)
An overhead lamp (to lighten the operating field)
Cannula made from 1.5 mm plastic tubing drawn out to 0.5 mm (to connect the animal artery to the pressure transducer)
Blood pressure transducer with a three-way tap
A venous cannula made from 0.5 mm plastic tubing, with a tip little less than 0.5 mm
0.5 mm plastic tubing connected to syringe
Thread (to keep the cannula in place)
Oxygen mask
Cotton wool

4.1.2 Chemicals and drugs used in *in-vivo* experiments

Sodium Chloride 0.9 % used as solvent (Adcock Ingram, South Africa)
Dimethyl Sulfoxide used as solvent (DMSO) (Sigma-Aldrich, Germany)
Heparin Sodium 0.1% used as an anticoagulant (Intramed, South Africa)
Oxygen (Afrox, South Africa)
Adrenaline used as an adrenoceptor agonist (Fluka Chemie, USA)

Atropine Sulphate used as a muscarinic receptor blocker (Merck-Generics, South Africa)

Atenolol used as a selective *beta*-1-adrenoceptor blocker (Sigma-Aldrich, Germany)

Dobutamine used as *beta*-agonist (Eli Lilly, SA)

Reserpine used as a ganglionic blocker (Sigma-Aldrich, Germany)

L. leonurus leaves (Montague Museum, South Africa)

Sodium pentobarbitone 6% used as an anesthetic (Kyron Labolatries, South Africa)

4.1.3 Materials and equipment used in extraction and chemical tests

Blender (Kenwood CG100 PK032/AD)

Soxhlet extractor

Freeze-drier (labconco, Missouri)

Filter paper 0. 45 μm (Schleicher & Schuell MicroSciece)

4.1.4 Chemicals used in phytochemical screening

Dragendorff's reagent used to test for alkaloids (Prepared from tartaric acid (Techno PharmChem Bahadurgarh, India) bismuth oxide (BDH Lab Reagents) 40% KI (Holpro Analytics (Pty) Ltd.

Meyer's reagent used to test for alkaloids (Prepared from mercury (II) chloride (NT Lab. Supplies) and Pottassium Iodide (Holpro Analytics (Pty) Ltd).

Chloroform used to test for alkaloids (Kimix Chemical & Lab Supplies)

Sulphuric acid 1% used to test for alkaloids (min. 95% Supplied by Sigma-Aldrich)

Carbon tetrachloride used to test for anthraquinones (B & M Scientific)

Hydrochloric acid used to test for anthraquinones (32 % HCl Merck Chemicals)

Dilute ammonia solution used to test for antraquinones (9.5 – 10.5 % (w/w) ammonia (BDH Ltd Poole England) made up with freshly boiled & cooled distilled water.

Ferric chloride used to test for anthraquinones and tannins (Merck Chemicals) and made up to either 5 % or 15 % solution.

Fehling's A solution used to test for reducing sugars (Prepared from copper (II) sulphate pentahydrate in a mixture of 0.5ml conc. sulphuric acid (min. 95%; Sigma-Aldrich)
Fehling's B solution used to test for reducing sugars (Prepared from potassium sodium (+) tartrate tetrahydrate (BDH Ltd Poole England) and Sodium hydroxide (Merck Chemicals).

4.2 Preparation of plant material

4.2.1 Plant collection

Fresh leaves of *L. leonurus* were collected at Montague Museum in Cape Town, during the month of March and April 2004 and were identified and authenticated by the Botany Department of the University of the Western Cape. Following identification, a voucher specimen of the plant was deposited in the herbarium of the Botany Department of University of the Western Cape.

4.2.2 Drying of plant material

The leaves of the plant were removed from the stems dried in an oven at 30°C for 72 hours. 680g of dried leaves was ground into powder with warring commercial laboratory blender (Kenwood CG100 PK032/AD).

4.2.3 Extraction

The aqueous extract of *L. leonurus* leaves powder was prepared by means of a soxhlet extractor. An amount of 30g of the powder was extracted with distilled water for each preparation. A total of 300g of the powder was used. The extracts were cooled and combined in round bottom flasks. These were frozen at -18°C. The frozen extracts were then freeze-dried under reduced pressure over 3-4 days. 57g of the freeze-dried extract was obtained and kept in a brown bottle and stored in a refrigerator at -4°C.

4.2.8 Detection of chemical constituents

The active ingredients found in medicinal plants are chemical compounds that are responsible for the specific activity of the plant by acting directly or indirectly to prevent or treat disease and maintain health. These active compounds can be identified and tested using various available methods. In this study, a method of phytochemical screening of active ingredients will be followed as described by Sofowora, 1993). Following the research question of this study, the plant will be tested for the presence of saponins, tannins, alkaloids, anthraquinones, and reducing sugars.

4.2.8.1 Test for anthraquinones

0, 1 g of the powdered *L. leonurus* leaves was shaken with 10 ml of 15 % Ferric Chloride solution and 5 ml of HCl and this was immersed in a water bath for about 10 minutes. It was filtered immediately. The filtrate was cooled down and extracted with 10 ml of carbon tetrachloride. The carbon tetrachloride layer was separated and washed with 5 ml of water and shaken with 5 ml of diluted ammonia solution (Sofowora, 1993).

4.2.8.2 Test for alkaloids

0, 5 g of powdered *L. leonurus* leaves was boiled with 10 ml of diluted HCl (alcoholic) in a boiling tube for 5 min and the debris was allowed to cool down and settle. Supernatant was filtered using glass wool into another tube, followed by the addition of 3 drops of Mayor's reagent (potassium mercuric iodide solution). Precipitation occurred.

The remainder of the extract was further extracted with 5 ml of chloroform. The chloroform layer was separated and washed with a little water. The chloroform layer was evaporated to dryness.

The residue was dissolved in 1 ml 1% sulphuric acid and in aliquots of 0, 5 ml was added 3 drops of mayor's reagent (Sofowora, 1993).

4.2.8.3 Test for reducing sugars

0.2 g of the *L. leonurus* leaves powder was boiled with 5 ml of water. The extract was cooled and filtered. An equal quantity of Fehling's A and B solutions was added to the filtrate and heated on a water bath (Sofowora, 1993).

4.2.8.4 Test for saponins

0.2 g of the powdered *L. leonurus* leaves was shaken together with water (Sofowora, 1993).

4.2.8.5 Test for tannins

0.2 g of the powdered *L. leonurus* leaves was boiled with 5 ml of water. The extract was cooled and filtered. A few drops of 5 % of ferric chloride solution was added to the filtrate. (Sofowora, 1993).

The phytochemical screening of *L. leonurus* indicated the presence of alkaloids, tannins, saponins and reducing sugars. The next step would therefore be to identify which of the above chemical compounds is responsible for the hypotensive effects of *L. leonurus*.

4.3 Preparation of animal for *in vivo* experiments

4.3.1 Handling of an animal

Proper handling of animal is important as this can affect the experimental results. Good results are unlikely to be obtained from animals in poor conditions, e.g. a sick or frightened animal may die during anesthesia. A rat was lifted by the tail without alarming it and put on a flat surface as soon as possible. As this allows its feet a grip, it was then pulled away from where it was held and the scruff of the neck was then grasped in the other hand. It was then held securely while the injection was made.

4.3.2 Anaesthetized rats

Rats (250 to 300 g) were anaesthetized with a dose of 40 mg per kg of 6 % Sodium pentobarbitone intraperitonially.

4.3.3 Cannulation of the trachea

When the anesthesia was induced and the animal was unconscious and flaccid the animal was placed on its back on the operating table, the legs were attached the table with thread. The front of the neck was cut open with scissors. This exposed the longitudinal muscle in front of the trachea. The muscle was divided with blunt dissection along the middle line. The trachea was thus exposed and freed from the connective tissue with curved iris forceps. Two strong threads were passed around the trachea but not tied. A cut was made between the two threads big enough to allow the insertion of the cannula. The top thread was tied and pulled towards the head. The cut end of the trachea was kept open with the forceps while this was being done. The second thread was then tied around the cannula; the free ends of the thread furthest from the lungs were also tired loosely round the cannula to keep it properly aligned.

Mucus accumulating in the trachea was removed by sucking using a cannula attached to the syringe. Care was taken to make sure that bronchioles were not damaged, as this would cause edema and thus respiratory problems.

4.3.4 Cannulation of the jugular vein

The choice of the vein depended upon its size and the convenience of its position. In this experiment, the external jugular vein was chosen. When the vein had been located a length of about 10mm was freed from connective tissue by blunt dissection with curved forceps, but fine pointed forceps were sometimes needed to ensure that the entire connective tissue sheath around the vein was removed. This avoided the possibility of the cannula being inserted between the connective tissue and the vein instead of into the vein.

Whenever the vein collapsed or went into spasm while it was being stripped, it was swabbed with saline and made to distend.

As suitable length of vein had been prepared, outward flow of blood was occluded by placing a bulldog clip on the end nearest to the heart. Two threads were carefully passed around the vein and half-tied. The vein was then fully distended by using a finger to push the blood towards the bulldog clip. The thread furthest from the heart then tied firmly and held taut so as to stretch the vein slightly. A small cut was made, not more than halfway through the vein, as near to the tied thread as possible, i.e. as far from the bulldog clip as possible so that, if the cannulation was unsuccessful there was a room for a second attempt. The cut was made at an angle so that there was a small flap to make it easier for the insertion of the cannula. The second thread was then tied around the cannula and the bulldog removed. The first thread was tied around the cannula to keep it aligned correctly. The cannula and tubing connected to it were filled with saline or drug to be tested before they were inserted to the vein. The tubing connected to the cannula was further connected into the syringe, the syringe was then placed on the two-way injection pump which allowed injection of the drug followed by saline to wash it all in.

4.3.5 Cannulation of the femoral artery

The usual reason to cannulate an artery is to record blood pressure (McLeod *et al*; 1976) and in this case the femoral artery was chosen. The procedure for inserting the cannula is very similar to the cannulation of the vein. Two threads are passed around the cleaned length of artery. The thread furthest from the heart was tied and then when the artery was distended with blood, the bulldog clip was placed at the end nearest to the heart. The arterial cannula was then prepared and connected to the blood pressure transducer through a three-way tap with a syringe full of heparinized saline attached. The connection between the three-way tap and the transducer was closed and all the air bubbles in the arterial cannula were flushed out with heparinized saline and the tap set as to close off the arterial cannula. A slanting cut was made in the artery between the tied thread and the bulldog clip. The cannula was inserted and tied into place. The pressure in the transducer

was raised, by means of the syringe, to the expected blood pressure of the animal. The tap was set so that the syringe was closed off and the arterial cannula was connected to the recording device. Care was taken to make sure that there were no leaks in the system as to keep the pressure steady and kept closed except when injections were made. The bulldog clip was then removed. There was a little flow of blood out of the animal into the cannula if the arterial pressure had been correctly guessed.

The need for heparin to prevent clots forming is greater with arterial cannula than with venous cannula. This is because a clot in a venous cannula can easily be dislodged by pressure but the direction of the flow forces any clot back into an arterial cannula (McLeod *et al*; 1976). The injections were made against the arterial pressure, which was enough to displace the plunger of a syringe, so it was important to make sure that there was no chance of the blood forcing its way into the injection system at any time.

4.3.6 Oxygen supply

The animals were supplied with oxygen as the animal's normal breathing becomes inadequate. Oxygen was supplied through a conical mask with a cotton wool plugged at its apex. The recommended rate and volume of respiration for rats is 50 strokes /min and 8ml/kg.

4.3.7 Temperature maintenance during anesthesia

The temperature of the animals was maintained by using a heat adjustable operating table and the overhead lamp. The rectal temperature of the animal was recorded by using the rectal temperature probe and kept within limits i.e. 37.3° C ± 0.5° C. This was done by adjusting the position of the lamp or adjusting temperature on the knob of the disserting table.

4.3.8 Recording of blood pressure and heart rate

The arterial cannula was connected to the blood pressure transducer (Power lab T20) through a three-way tap with a syringe full of heparinized saline attached. The connection between the three-way tap and the transducer was closed and all air bubbles in the arterial cannula were flushed out with a heparinized saline (1 in 10 solution). The tap was set in order to close off the arterial cannula.

4.3.9 Parameters assessed

Parameters that were assessed in this experiment were; heart rate, systolic pressure and diastolic pressure. These parameters were recorded using the PowerLab software system. The baseline pressures were recorded before drug infusion and drugs were infused over 3 minutes. The highest value obtained within 3 minutes was recorded as the response to the drug. The difference between the baseline value and the response value was taken as the change in pressure and / or heart rate.

4.3.10 Infusion of drugs

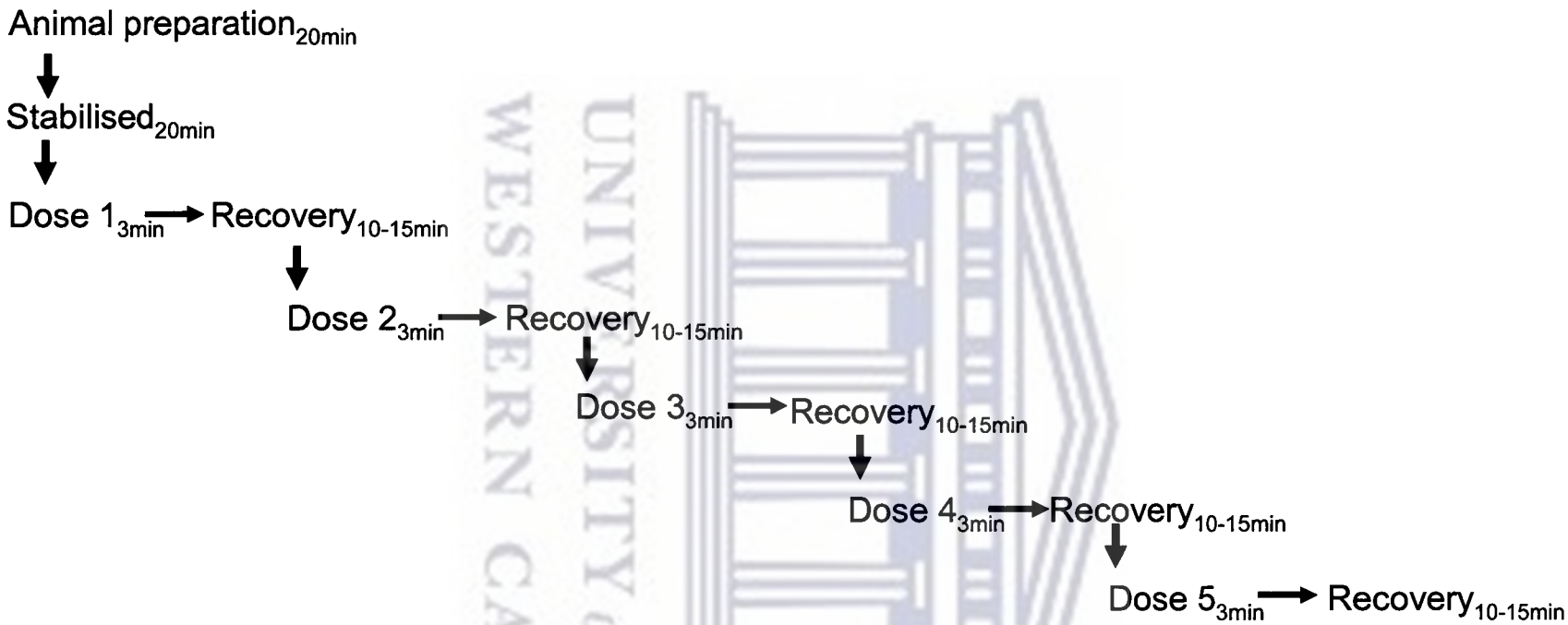
All the drugs were dissolved in 0.9% Sodium Chloride, except for atropine. Atropine was received in an injectable form of 0.6mg/ml. It was used undiluted i.e. at doses of 2.4 mg / kg. The plant extract was first ground in a mortar with a pestle and filtered with 0, 5μm filter. Drugs were infused at a rate of 0.16 ml / min over 3 min using Ascor AP22 syringe pump. Results were recorded within 3 minutes and thereafter flushed with normal saline. The animal pressures were allowed to stabilize for 10 to 15 min and the animal was used for further dosages. Six rats were used for each set of experiments conducted.

The experimental protocols were followed as shown in Fig 4.1, 4.2, 4.3, and 4.4.

Fig 4.1

Experimental Protocol 1

Dose Response Curves (Adrenaline, Atenolol, L. leonurus)

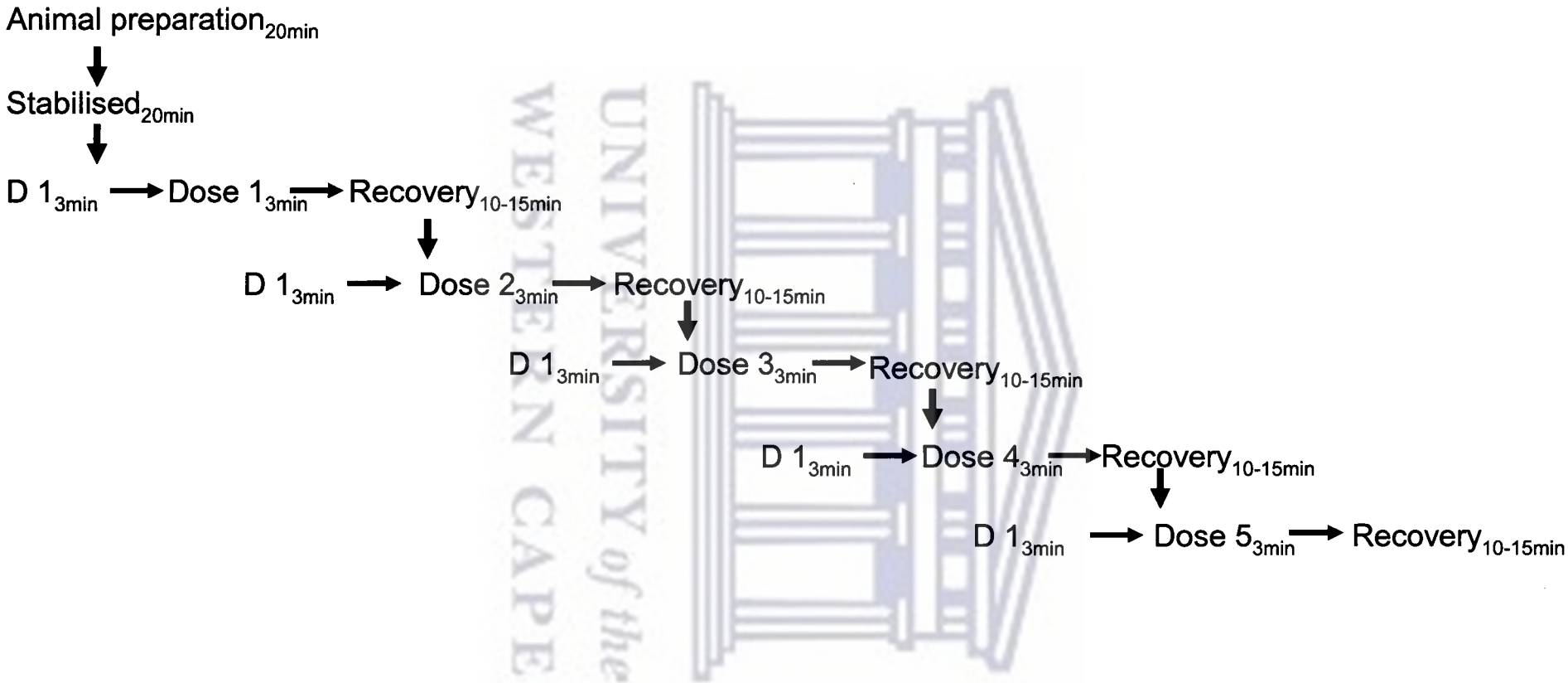


Dose1= Test drug concentration (e.g. adrenaline 0.04mg)
Recover= Flushing with normal saline.

Fig 4.2

Experimental Protocol 2

Dose Response Curves (Adrenaline, *L. leonurus*) after pretreating with D1



Dose1= Drug concentration in a series (e.g. Adrenaline 0.04mg/kg , *L. leonurus* 200 mg/kg)

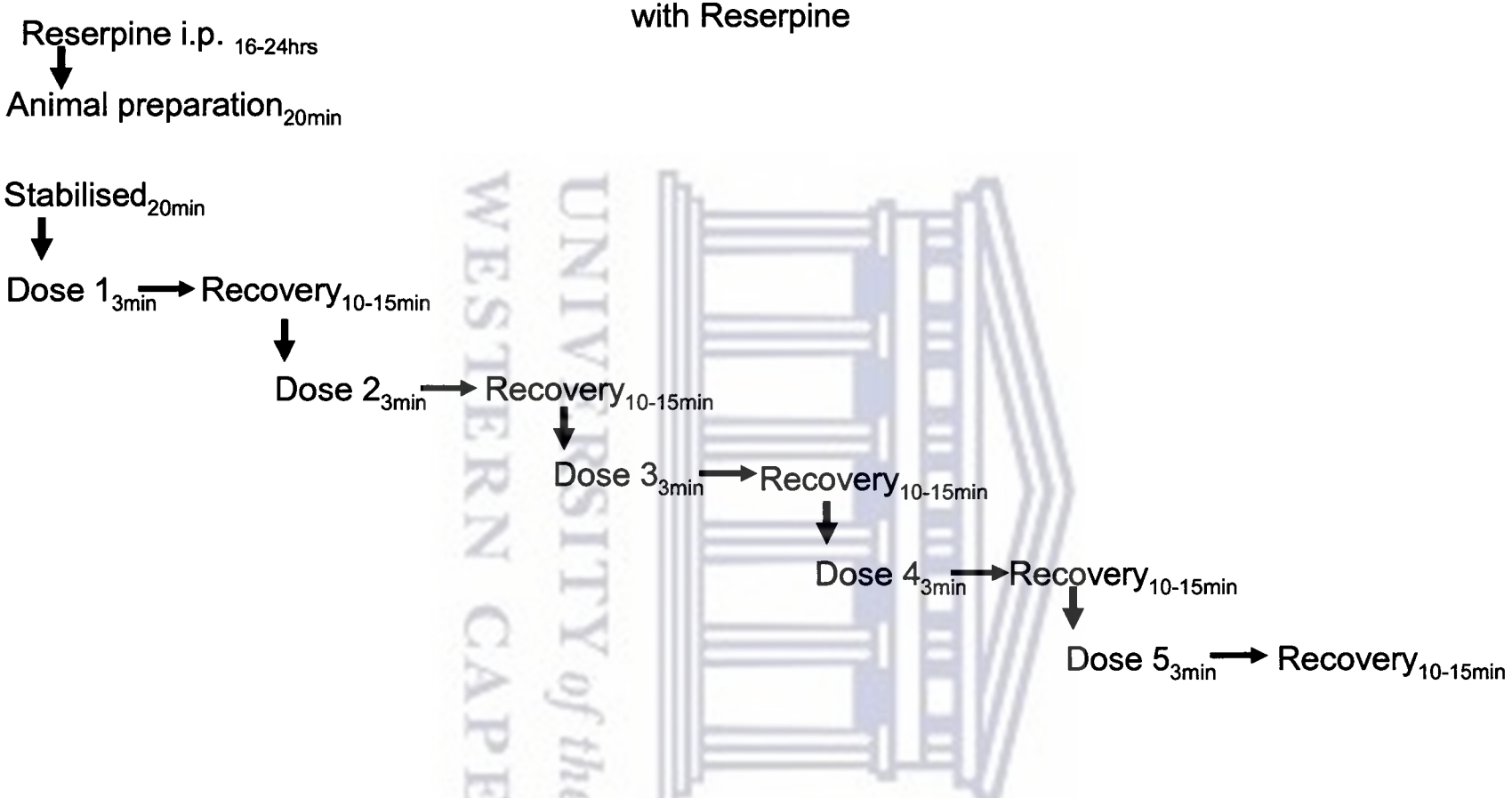
D1= Pretreating drug (Atenolol 12 mg/kg, *L leonurus* 800mg/kg, atropine 2.4 mg/kg)

Recovery= Flushing with normal saline.

Fig 4.3

Experimental Protocol 3

Dose Response Curves (Adrenaline) after pretreating with Reserpine

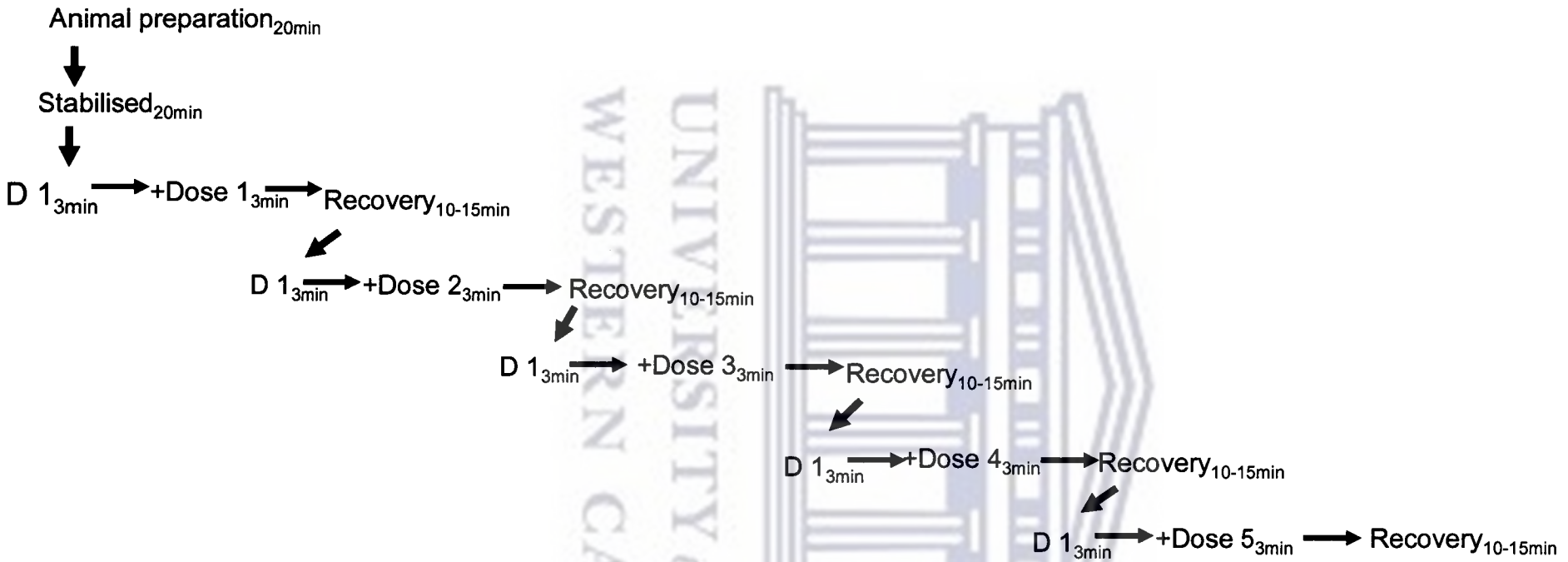


Dose1= Test drug concentration (e.g. adrenaline 0.01mg)
Recovery= Flushing with normal saline.
Reserpine i.p.= Pretreatment 16 to 24hrs prior experiment.

Experimental Protocol 4

Fig 4.4

Dose Response Curves (*L. leonurus* with Dobutamine)



Dose 1= Test drug concentration (e.g. *L. leonurus* 200 mg / kg)
D 1 = Pretreating drug (Dobutamine 20 mcg/kg)
+ = given concurrently with Dose 1
Recovery = Flushing with normal saline

4.4 Data analysis

Data obtained from animal experiments was analyzed by means of Graph Pad Prism version 4 (2003) and SAS (SAS Institute Inc., Cary, NC, USA). Changes in blood pressure and heart rate were calculated by getting a difference between the basal values and response values.

In this analysis, single drugs were evaluated in order to establish whether there was evidence of a significant difference in the response at different doses. A second design of this study was a two-factor study with repeated measures on one factor. The first factor was the experimental treatment administered. There were two levels of this factor. This was called a between subject factor as different animals were in each treatment group. The second factor was the dose. There were five levels of this factor. This was called a within-subjects factor as the same animal was observed at each dose level. Alternatively the analysis was done by considering the animal to be a random effect and dose to be a fixed effect (with 5 to 7 levels) and the analysis was done using a Mixed Model Approach.

Even though this model would appear to be appropriate for our designs, there were still some underlying assumptions that were made about the distribution of the random errors, namely that those errors are normally distributed. In checking the assumption of normal error terms it was found that in several cases that assumption does not appear to be valid. With that in mind, results were either needed to be interpreted as 'approximate' or we needed to try an alternate approach to the analysis. An alternate approach was to use nonparametric methods. As a first step Friedman's test was used to establish whether there were any differences across the doses. If there was evidence of a difference, then pair-wise comparisons of responses at the different dose levels was done by using the Wilcoxon Signed Rank test. While this test is valid with a small sample size of n=6, one of the limitations is that the smallest possible p-value for this test is 0.03125 i.e. it is not mathematically possible to have a p-value of 0.01 or smaller. Consequently we used this only as a guide when interpreting the results from the mixed model analysis of variance. Specifically we proceeded as follows. If a statistical test for normality of the residuals from the model (namely the Shapiro-Wilk test) had a p-value that is not under 0.01, we

based conclusions on the results of the mixed model analysis. However if the test for normality had a p-value less than 0.01, then we considered a result significant only if it shown significance in the mixed model analysis and had a p-value of 0.03125 when considering the Signed Rank test for the corresponding pair of doses. Since we were testing at five to seven doses, and we were testing three outcomes (Systolic Pressure, Diastolic Pressure, and Heart Rate) and we were doing this for 3 different drugs and 5 different combinations of drugs, there were a large number of different tests. Whenever we had a large number of tests, we needed to be more conservative in the choice of significance level. Typically a level of 0.05 was used but in this case a 0.01 level instead was used.

The data plots were shown with bar graphs using Graph Pad Prism. These plots allow us to see how much consistency or variability there was in the responses at different doses or we could see a complete separation of the responses for the two groups.

4.5 Ethical considerations

This study was approved by the University of the Western Cape Ethics Committee and animals were treated according to the University of the Western Cape animal regulations act.

CHAPTER 5

Results

Introduction

Chapter 5 is divided into three parts. Part one shows the results of the chemical tests performed on the aqueous extract in order to qualitatively describe the types of chemical compounds present in *L. leonurus*. Part two shows the effects of adrenaline, atenolol and the aqueous extract of *L. leonurus* on the blood pressure and heart rate through dose response curve. Part three describes the effects of adrenaline on the blood pressure and heart rate obtained in rats pretreated with *L. leonurus* and reserpine. Part three also describes the effects of *L. leonurus* on blood pressure and heart rate in rats pretreated with dobutamine, atenolol and atropine.

Presentation of results

5.1 Chemical tests

When the powdered *L. leonurus* leaves were subjected to test for the presence of anthraquinones,the absence of a rose pink to cherry red colour in the ammonic layer indicated the absence of anthraquinones in *L. leonurus*.

When the powdered *L. leonurus* leaves were subjected to test for the presence of alkaloids, a white to buff precipitate produced when mayor's reagent was added, and an orange-red precipitate produced when Dragendorff's reagent was added, suggested the presence of alkaloids in *L. leonurus*.

When the powdered *L. leonurus* leaves were subjected to test for the presence of reducing sugars, a red-brown precipitate was formed, indicating the presence of reducing sugars.

When the powdered *L. leonurus* leaves were subjected to test for saponins, persistent (compared to water) froth observed suggested the presence of saponins.

When the powdered *L. leonurus* leaves were subjected to test for tannins, a blue-black precipitate occurred. This suggested the presence of tannins in *L. leonurus*.

Dose response curve experiments

5.2.1 Adrenaline

The minimum and maximum effective doses of adrenaline were determined by conducting a series of serial dilutions. The minimum effective dose was found to be 0.04 mg/kg and the maximum was 0.2 mg/kg. Adrenaline was used as indicated in the following figure at the dosage of 0.04, 0.08, 0.12, 0.16, and 0.2 mg/kg. Adrenaline was dissolved in normal saline and carefully covered with foil throughout the experiments as it degrades quickly on exposure to light. Adrenaline was infused at a rate of 0.16 ml / min over 3 minute and the catheter was flushed with normal saline (0.5 ml). The animal was given 10 to 15 min to recover from the effect of adrenaline. The next high dose was given after the animal has reached the baseline blood pressure and heart rate (i.e. the blood pressure and heart rate recorded before the administration of the first dose). In total, one rat was given five different doses of adrenaline and six rats were used for this set of experiments.

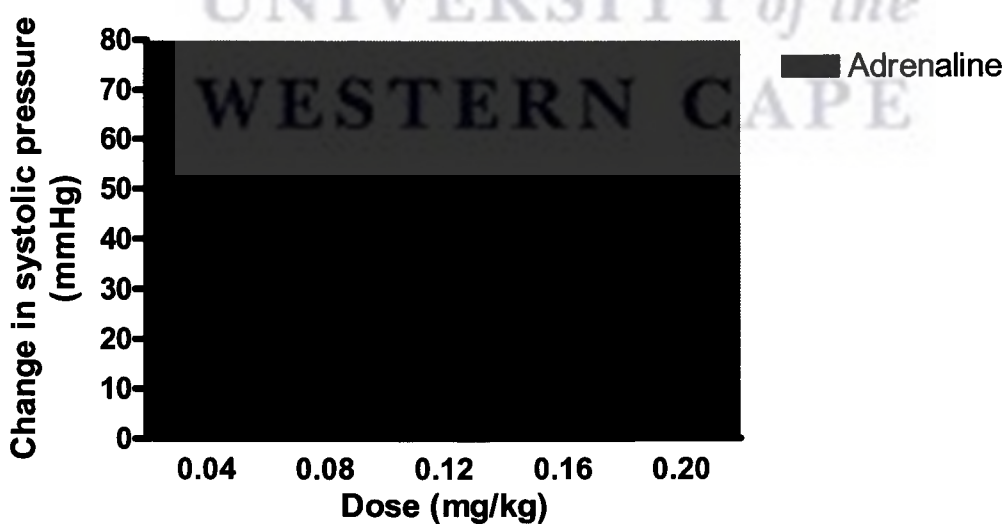


Fig 5.1 Effects of adrenaline on systolic blood pressure

The systolic blood pressure increased with increasing doses of adrenaline.

The minimum effect recorded was an increase in blood pressure of 41.2 mmHg and the maximum effect was 73.57 mmHg. At the dose higher than 0.12 mg the animal died. Therefore 0.12 mg was considered as the maximum effective dose. A significant ($P < 0.05$) difference in increased systolic blood pressure was observed between doses 0.04 mg and 0.12 mg (13.500 ± 3.441 mmHg) ($P = 0.003$) and between doses 0.04 mg and 0.2 mg (32.366 ± 5.675) ($P = 0.0005$).

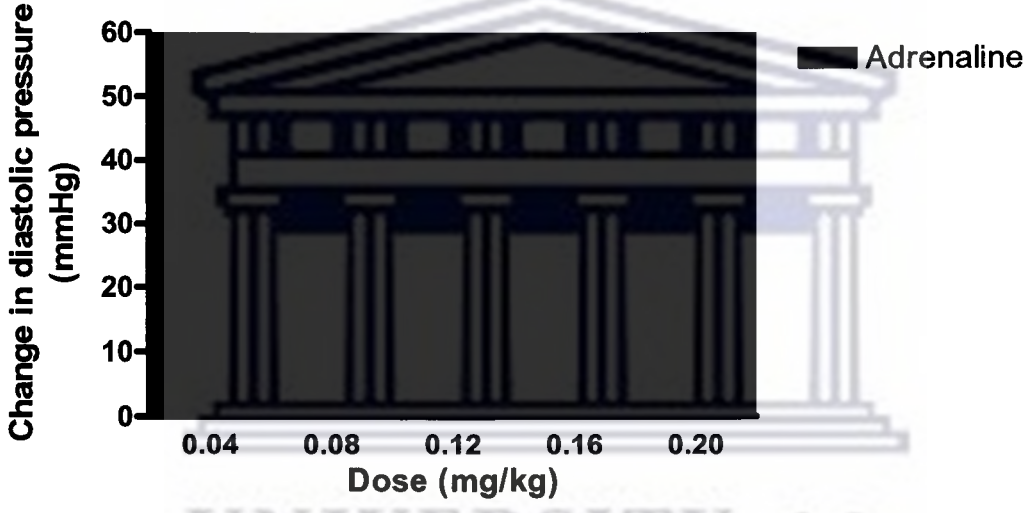


Fig 5.2 Effects of adrenaline on diastolic blood pressure

The diastolic blood pressure increased with increasing doses of adrenaline. The minimum effect recorded was an increase in blood pressure of 16.45 mmHg and the maximum effect was 45.07 mmHg. A significant ($P < 0.05$) difference in increased diastolic blood pressure was observed between doses 0.04 and 0.16 mg (14.866 ± 5.323 mmHg) ($P = 0.05$) and between doses 0.08 and 0.2 mg (25.933 ± 11.813) ($P = 0.05$).

Dose (mg)	Δ systolic pressure (mmHg)	Δ diastolic pressure (mmHg)
0.04	41.200 ± 2.693	16.450 ± 2.135
0.08	47.250 ± 1.150	19.133 ± 1.675
0.12	54.700 ± 2.140	22.666 ± 2.056
0.16	64.866 ± 4.050	31.316 ± 4.871
0.2	73.566 ± 4.995	45.066 ± 11.709

Table 5.1 Means and SEM of change in systolic and diastolic pressure for adrenaline.

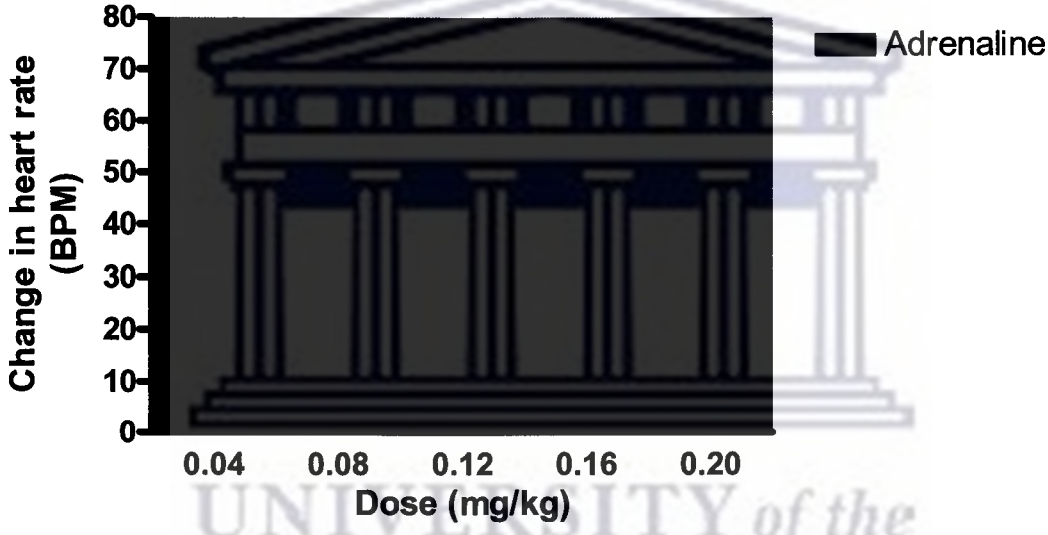


Fig 5.3 Effects of adrenaline on heart rate

The heart rate increased with increasing doses of adrenaline. The minimum effect recorded was an increase in heart rate of 40.83 beats/minute and the maximum effect was 69.67 beats/minute. A significant (P = 0.0063) difference was observed between the effects obtained with the doses of 0.04 mg and 0.2 mg (26.833 ± 8.760).

Dose (mg)	Δ heart rate (beats/minute)
0.04	40.833 ± 6.620
0.08	49.166 ± 5.746
0.12	53.333 ± 7.172
0.16	61.000 ± 8.173
0.2	69.666 ± 5.736

Table 5.2 Means and SEM of change in heart rate for adrenaline.

5.2.2. Atenolol

The minimum and maximum effective doses of atenolol were determined by conducting a series of serial dilutions. The minimum effective dose was found to be 0.4 mg/kg and the maximum was 60 mg/kg. Atenolol was used as indicated in figure 5.4 to 5.6 below at dosages of 0.4, 4.0, 12, 20, 30, 40, and 60 mg/kg. Atenolol powder was dissolved in normal saline and it was infused at a rate of 0.16 ml/minute over 3 min and the catheter was flushed with normal saline (0.5 ml). The animal was given 10 to 15 minutes to recover from the effect of atenolol. The next high dose was given after the animal had reached the baseline blood pressure and heart rate level. In total, one rat was given seven different doses. Six rats were used for this set of experiments.

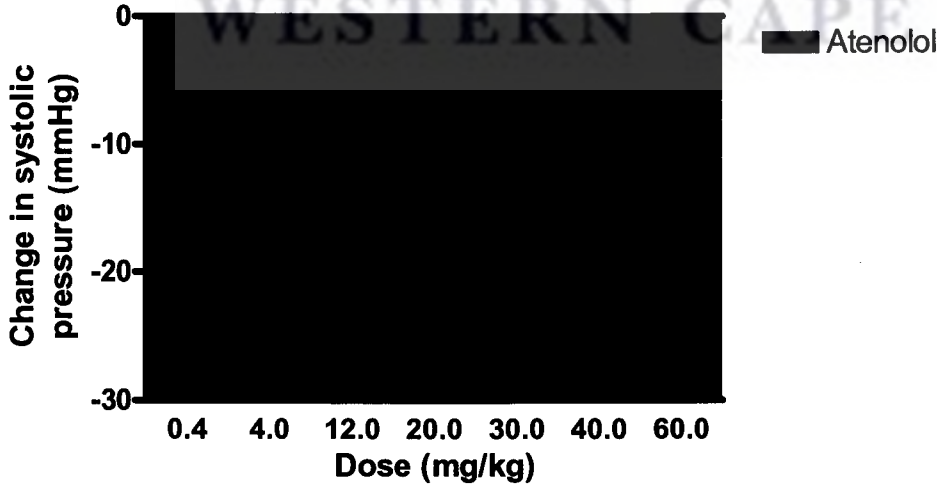


Fig 5.4 Effects of atenolol on systolic blood pressure

The systolic blood pressure decreased with increasing doses of atenolol. The minimum effect recorded was a decrease in blood pressure of 2.13 mmHg and the maximum effect was 21.23 mmHg. A lower significant (P = 0.05) difference was observed between the effects of the doses 0.4 mg and 12 mg/kg (4.983 ± 1.330) (P = 0.004) and highest significant (P = 0.0025) difference was observed between the effects of the doses 0.4 mg and 60 mg (19.1000 ± 3.414). However, at high doses i.e. between 12 mg and 60 mg there was no significant (P > 0.05) difference in decrease in diastolic blood pressure.

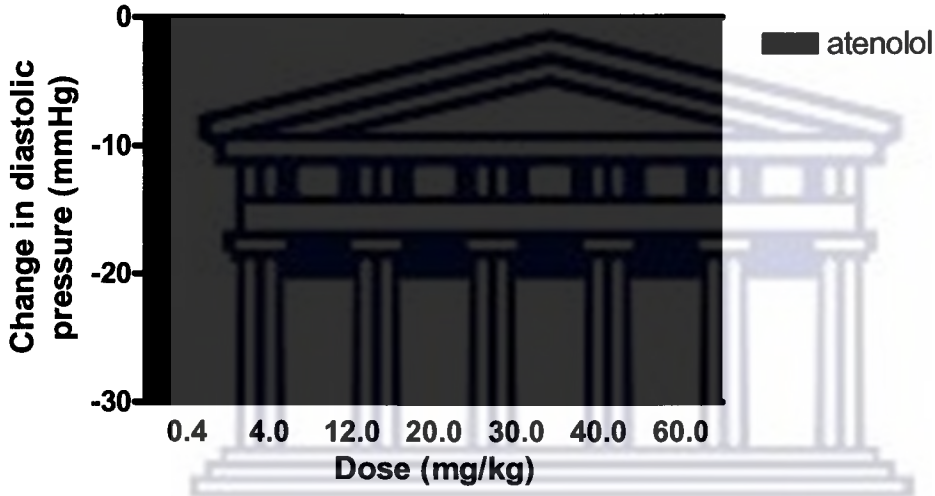


Fig 5.5 Effects of atenolol on diastolic blood pressure

Intravenous injection of increasing doses of atenolol increasingly decreased the diastolic blood pressure. The minimum effect recorded was a decrease in blood pressure of 0.833 mmHg and the maximum effect was 19.68 mmHg. The lowest significant (P = 0.005) difference was observed between the effect of the doses 0.4 mg and 12 mg/kg (7.816 ± 1.935 mmHg) and the highest significant (P = 0.0031) difference in diastolic blood pressure was observed between doses 0.4 mg and 60 mg (18.850 ± 3.715). However, at high doses i.e. between 20 mg and 60 mg there was no significant (P > 0.05) difference in decrease in diastolic blood pressure.

Dose (mg)	Δ systolic pressure (mmHg)	Δ diastolic pressure (mmHg)
0.4	-2.133 ± 1.009	-0.833 ± 0.739
4	-4.050 ± 0.799	-3.350 ± 1.239
12	-7.116 ± 0.866	-8.650 ± 1.788
20	-12.400 ± 2.587	-12.033 ± 2.388
30	-16.966 ± 2.572	-15.140 ± 2.375
40	-19.883 ± 3.375	-18.400 ± 3.318
60	-21.233 ± 3.261	-19.683 ± 3.640

Table 5.3 Means and SEM of change in systolic and diastolic pressure for atenolol.

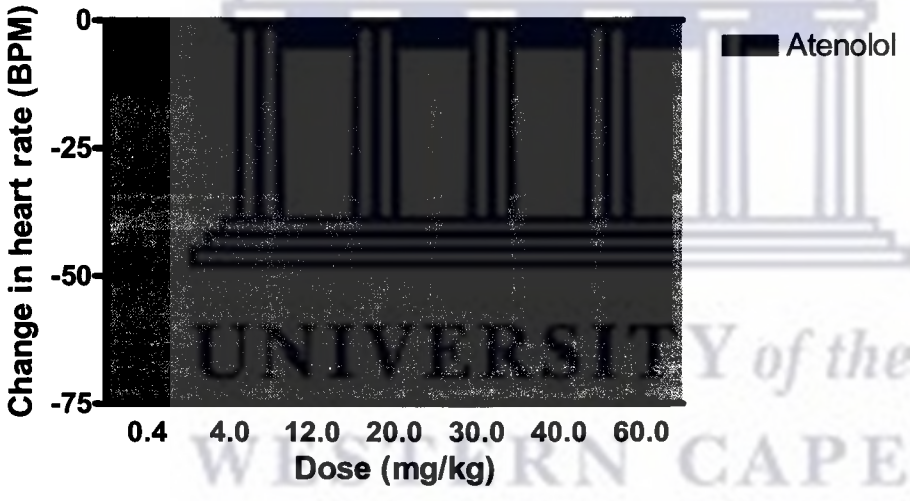


Fig 5.6 Effects of atenolol on heart rate

Atenolol significantly ($P < 0.05$) decreased the heart rate with increasing doses. The minimum effect recorded was a decrease in heart rate of 10.5 beats/minute and the maximum effect was 62.17 beats/minute. The lowest significant ($P = 0.0062$) difference was observed between the effects of the doses 0.4 mg and 30 mg/kg (49.666 ± 12.255 mmHg) and the highest significant ($P = 0.0071$) difference in diastolic pressure was observed between the effects of the doses 0.4 and 60 mg/kg (51.666 ± 12.317). However,

at high doses, i.e. between 12 and 60 mg/kg there was no significant ($P > 0.05$) difference in decrease in heart rate.

Dose (mg)	Δ heart rate (beats/minute)
0.4	-10.500 ± 2.552
4	-19.500 ± 3.052
12	-35.500 ± 8.114
20	-47.833 ± 10.014
30	-55.833 ± 11.668
40	-60.166 ± 11.987
60	-62.166 ± 12.051

Table 5.4 Means and SEM of change in heart rate for atenolol.

5.2.3 *Leonotis leonurus*

L. leonurus dose range used was determined by conducting trial of different doses. The lowest dose was determined by observation of the minimum effect on systolic and diastolic blood pressure and the maximum dose was determined by the highest dose that killed animals. *L. leonurus* was dissolved in normal saline using mortar and pastel and filtered through 0.45 μm filter. *L. leonurus* was used as indicated in the figure below at the doses of 200, 400, 600, 800 and 1g/kg. *L. leonurus* was infused at a rate of 0.16 ml / min over 3minutes and the catheter was flushed with normal saline (0.5 ml). The animal was given 10 to 15 minutes to recover from the effect of *L. leonurus*. The next high dose was given after the animal had reached the baseline blood pressure and heart rate level. In total, one rat was given five doses mentioned above.

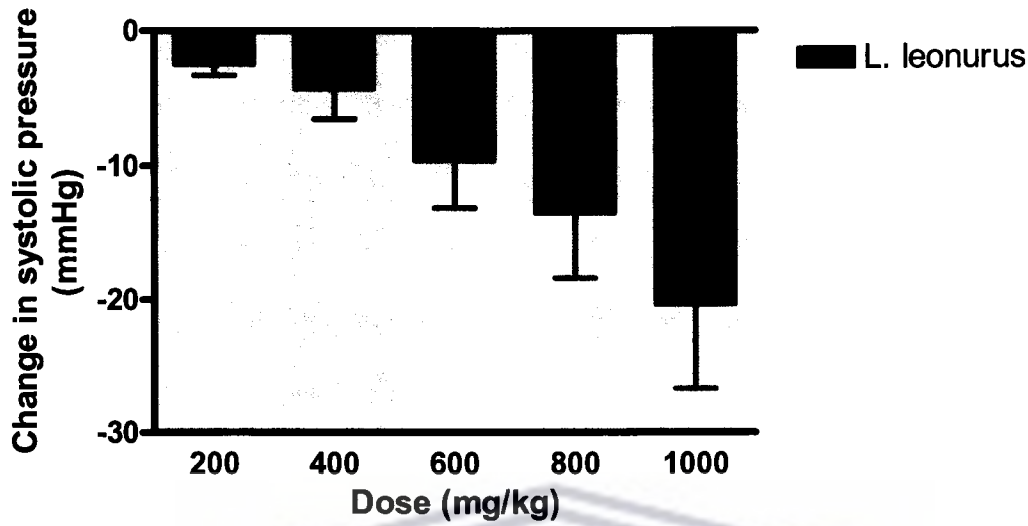


Fig 5.7 Effects of *L. leonurus* on systolic blood pressure

The systolic blood pressure increasingly decreased with increasing doses of *L. leonurus*. The minimum effect recorded was a decrease in blood pressure of 2.533 mmHg and the maximum effect was 20.4 mmHg. At the dose higher than 1g/kg the animal died. Therefore we considered 1g/kg as the maximum effective dose. The intravenous injection of the aqueous extract of *L. leonurus* produced a non-significant ($p < 0.0363$) difference in reduction of systolic blood pressure between doses 200 mg and 1g/kg (17.866 ± 5.293). The hypotensive effects were dose-dependent as shown in Fig 5.7.

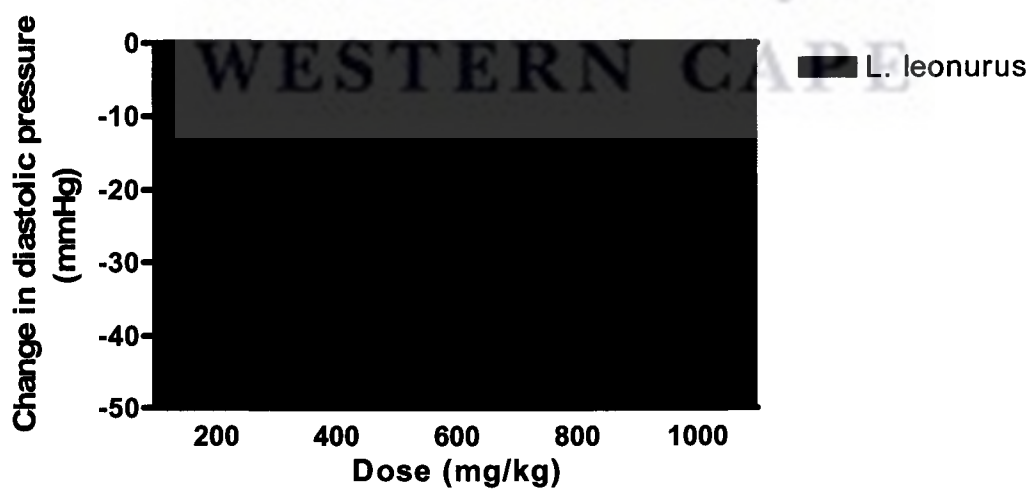


Fig 5.8 Effects of *L. leonurus* on diastolic blood pressure

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced an increasing decrease in diastolic blood pressure. The minimum effect recorded was a decrease in blood pressure of 6.633 mmHg and the maximum effect was 43.6 mmHg. The lowest significant (P = 0.0067) difference was observed between doses 200 mg and 600 mg (9.583 ± 2.794mmHg) and the highest significant (P = 0.0003) difference in diastolic pressure was observed between doses 200 mg and 1g/kg (36.966 ± 5.364).

Dose (mg)	Δ systolic pressure (mmHg)	Δ diastolic pressure (mmHg)
200	-2.533 ± 0.760	-6.633 ± 2.120
400	-4.333 ± 2.176	-10.966 ± 5.283
600	-9.666 ± 3.446	-16.216 ± 1.822
800	-13.533 ± 4.824	-24.600 ± 6.834
1000	-20.400 ± 6.307	-43.600 ± 4.924

Table 5.5 Means and SEM of change in systolic and diastolic pressure for *L. leonurus*.

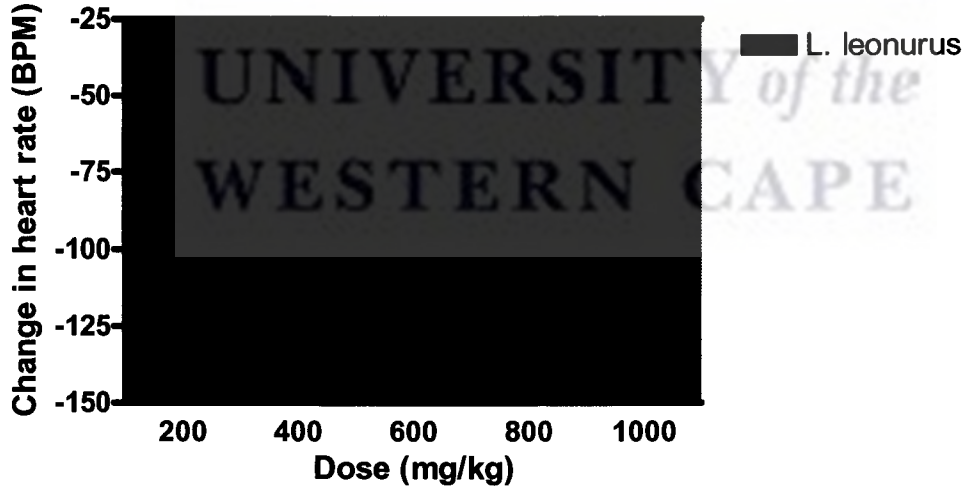


Fig 5.9 Effects of *L. leonurus* on heart rate

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate. The minimum effect recorded was a

decrease in heart rate of 55.83 beats/minute and the maximum effect was 122 beats/minute. The lowest significant ($P = 0.0007$) decrease was observed between doses 400 mg and 600 mg (35.8333 ± 7.470) and the highest significant ($P < 0.0001$) decrease in heart rate was observed between doses 200 mg and 1g/kg (66.166 ± 9.623). However, at high doses i.e. between 600 mg and 800 mg/kg and between 400 mg and 1g/kg, there was no significant ($P > 0.05$) difference in decrease in heart rate.

Dose (mg)	Δ heart rate (beats/minute)
200	-55.833 ± 7.959
400	-74.833 ± 5.528
600	-110.666 ± 5.024
800	-111.166 ± 5.996
1000	-122.000 ± 5.409

Table 5.6 Means and SEM of change in heart rate for *L. leonurus*

5.3 Effects of adrenaline on blood pressure and heart rate in pretreated rats

5.3.1 Rats pretreated with *L. leonurus*

L. leonurus was infused at a rate of 0.16 ml/minute over 3 minutes prior to administration of each dose of adrenaline. Adrenaline was also infused at the rate of 0.16 ml/minute over 3 minutes and thereafter the cannula was flushed with normal saline 0.5 ml. The animal was given 10 to 15 minutes to recover from the effect of *L. leonurus* and adrenaline. One rat was given five increasing doses of adrenaline. This experiment was done in six different rats.

Fig 5.10 Effect of adrenaline on systolic blood pressure in rats pre-treated with *L. leonurus.*

The systolic blood pressure increased with increasing doses of adrenaline. The minimum effect recorded was an increase in blood pressure of 41.2 mmHg and the maximum effect was 73.57 mmHg. A significant ($P < 0.05$) difference in increased systolic blood pressure was observed between doses 0.01 mg and 0.03 mg (13.500 ± 3.441 mmHg) ($P = 0.003$) and between doses 0.4 mg and 0.2 mg/kg (32.366 ± 5.675) ($P = 0.0005$).

Rats pre-treated with *L. leonurus* 800 mg/kg produced a non-significant ($P > 0.05$) difference on systolic blood pressure when compared to non pre-treated rats as seen in Fig 5.10. The lower doses of 0.04 to 0.016 mg/kg produced a non-significant ($P > 0.05$)

increase and the highest dose of 0.2 mg/kg produced a significant (P = 0.0043) difference of 29.983 ± 7.280 mmHg.

Dose (mg)	Δ systolic pressure (mmHg) pre *L. Leonurus* administration	Δ systolic pressure (mmHg) post *L. leonurus* administration	P value
0.04	41.200 ± 2.693	44.300 ± 6.603	0.3095
0.08	47.250 ± 1.1505	61.033 ± 8.311	0.0584
0.12	54.700 ± 2.140	67.583 ± 12.335	1.0000
0.16	64.866 ± 4.050	75.066 ± 6.833	0.2403
0.2	73.566 ± 4.995	43.583 ± 5.293	0.0043

Table 5.7 Means, SEM and P values of systolic pressure for adrenaline pre and post administration of *L. leonurus*.

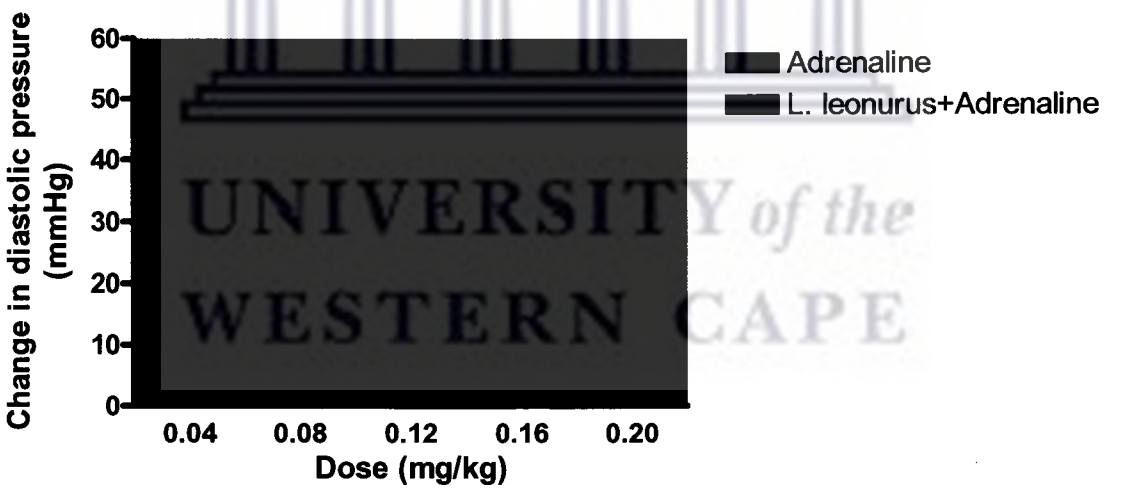


Fig 5.11 Effect of adrenaline on diastolic blood pressure in rats pre-treated with *L. leonurus*.

The diastolic blood pressure increased with increasing doses of adrenaline. The minimum effect recorded was an increase in blood pressure of 16.45 mmHg and the maximum effect was 45.07 mmHg. A significant (P < 0.05) difference in increased diastolic blood

pressure was observed between doses 0.04 and 0.16 mg/kg (14.866 ± 5.323 mmHg) (P = 0.05) and between doses 0.08 and 0.2 mg/kg (25.933 ± 11.813) (P = 0.05).

Rats pre-treated with *L. leonurus* 800 mg/kg produced a non-significant (P > 0.05) difference on diastolic blood pressure when compared to non pre-treated rats as seen in Fig 5.11. Doses 0.08 and 0.12 mg/kg produced a significant (P < 0.05) difference in diastolic pressure and doses 0.04 and 0.12 mg/kg produced a non-significant (P > 0.05) difference in diastolic pressure.

Dose (mg)	Δ diastolic pressure (mmHg) pre *L. leonurus* administration	Δ diastolic pressure (mmHg) post *L. leonurus* administration	P value
0.04	16.450 ± 2.135	14.683 ± 3.070	0.9372
0.08	19.133 ± 1.675	28.216 ± 3.220	0.0043
0.12	22.666 ± 2.0569	46.133 ± 2.528	0.0022
0.16	31.316 ± 4.871	28.966 ± 3.215	0.7857
0.2	45.066 ± 11.709	26.500 ± 3.264	0.3095

Table 5.8 Means, SEM and P values of diastolic pressure for adrenaline pre and post administration of *L. leonurus*

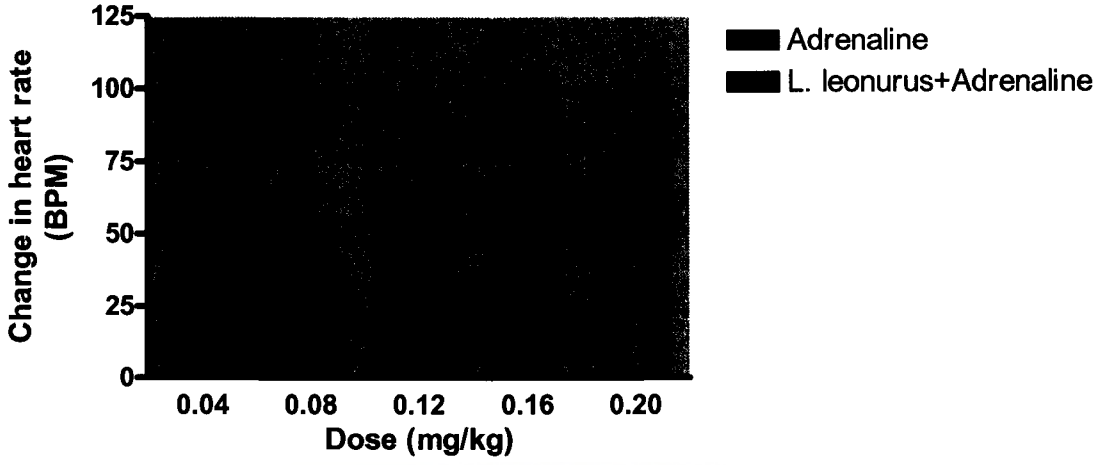


Fig 5.12 Effect of adrenaline on heart rate in rats pre-treated with *L. leonurus*

The heart rate increased with increasing doses of adrenaline. The minimum effect recorded was an increase in heart rate of 40.83 beats/minute and the maximum effect was 69.67 beats/minute. A significant ($P = 0.0063$) difference was observed between the effects obtained with the doses of 0.04 and 0.2 mg/kg (26.833 ± 8.760).

Rats pre-treated with *L. leonurus* 800 mg/kg produced a significant ($P < 0.05$) difference on heart rate when compared to non pre-treated rats as seen in Fig 5.12. The lowest dose of 0.04 mg/kg produced an increase of 32.666 ± 8.836 beats/minute and the highest dose of 0.2 mg/kg produced an increase of 45.666 ± 5.914 beats/minute.

Dose (mg)	Δ heart rate (beats/minutes) pre *L. leonurus* administration	Δ heart rate (beats/minutes) post *L. leonurus* administration	P value
0.04	40.833 ± 6.620	61.500 ± 17.876	0.5628
0.08	49.166 ± 5.746	38.166 ± 10.870	0.6688
0.12	53.333 ± 7.172	87.333 ± 10.065	0.026
0.16	61.000 ± 8.1731	86.666 ± 17.880	0.3939
0.2	69.666 ± 5.736	100.166 ± 11.665	0.0455

Table 5.9 Means, SEM and P values of heart rate for adrenaline pre and post administration of *L. leonurus*.

5.3.2 Rats pretreated with reserpine

Animals were pretreated with reserpine at the dose of 5 mg/kg. Reserpine was injected intraperitonially (i.p) 16 to 24 hours prior to adrenaline dose response experiments (Klemola *et al* 1999). Reserpine was dissolved in 1ml DMSO and made up to 10ml with normal saline. Adrenaline was infused at a rate of 0.16 ml/minute over 3 minutes. The animal was given 10 to 15 minutes to recover from the effect of adrenaline. The same procedure was followed for all the different doses used. One rat was given five more different doses. This experiment was done in six different rats.

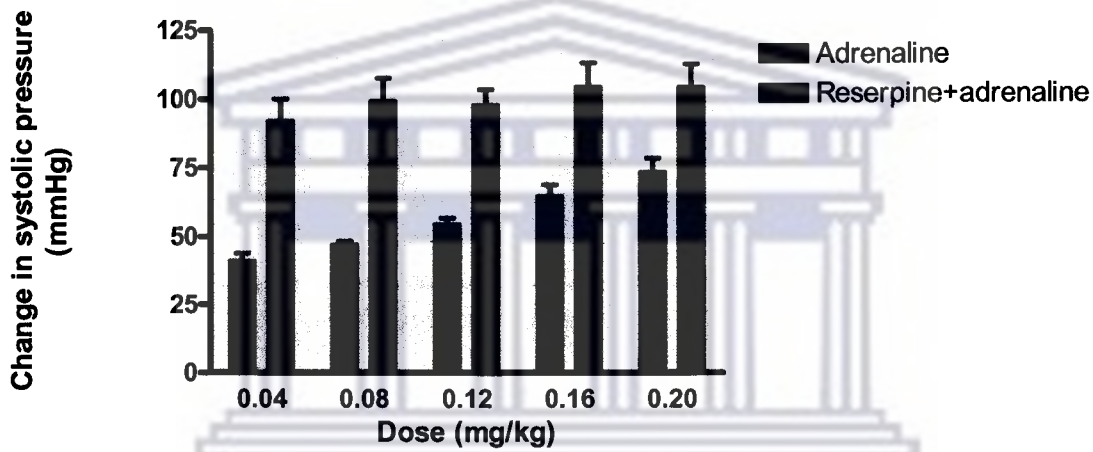


Fig 5.13 Effect of adrenaline on systolic blood pressure in rats pre-treated with reserpine

The systolic blood pressure increased with increasing doses of adrenaline. The minimum effect recorded was an increase in blood pressure of 41.2 mmHg and the maximum effect was 73.57 mmHg. A significant ($P < 0.05$) difference in increased systolic blood pressure was observed between doses 0.04 and 0.12 mg/kg (13.500 ± 3.441 mmHg) ($P = 0.003$) and between doses 0.4 and 0.2 mg/kg (32.366 ± 5.675) ($P = 0.0005$).

Rats that were pre-treated with reserpine 5 mg/kg produced significant ($P < 0.05$) increases in systolic blood pressure when compared with adrenaline alone as shown in shown in Fig 5.13. The lowest dose of 0.04 mg/kg produced a significant ($P = 0.002$) change of 51.033 ± 8.364 mmHg and the highest dose of 0.2 mg/kg produced a significant ($P = 0.0043$) increase of 30.966 ± 9.690.

Dose (mg)	Δ systolic pressure (mmHg) pre reserpine administration	Δ systolic pressure (mmHg) post reserpine administration	P value
0.04	41.200 ± 2.693	92.233 ± 7.918	0.0022
0.08	47.250 ± 1.1505	99.533 ± 8.191	0.0022
0.12	54.700 ± 2.140	98.033 ± 5.526	0.0022
0.16	64.866 ± 4.050	104.783 ± 8.577	0.0022
0.2	73.566 ± 4.995	104.533 ± 8.302	0.0043

Table 5.10 Means, SEM and P values of systolic pressure for adrenaline pre and post administration of reserpine.

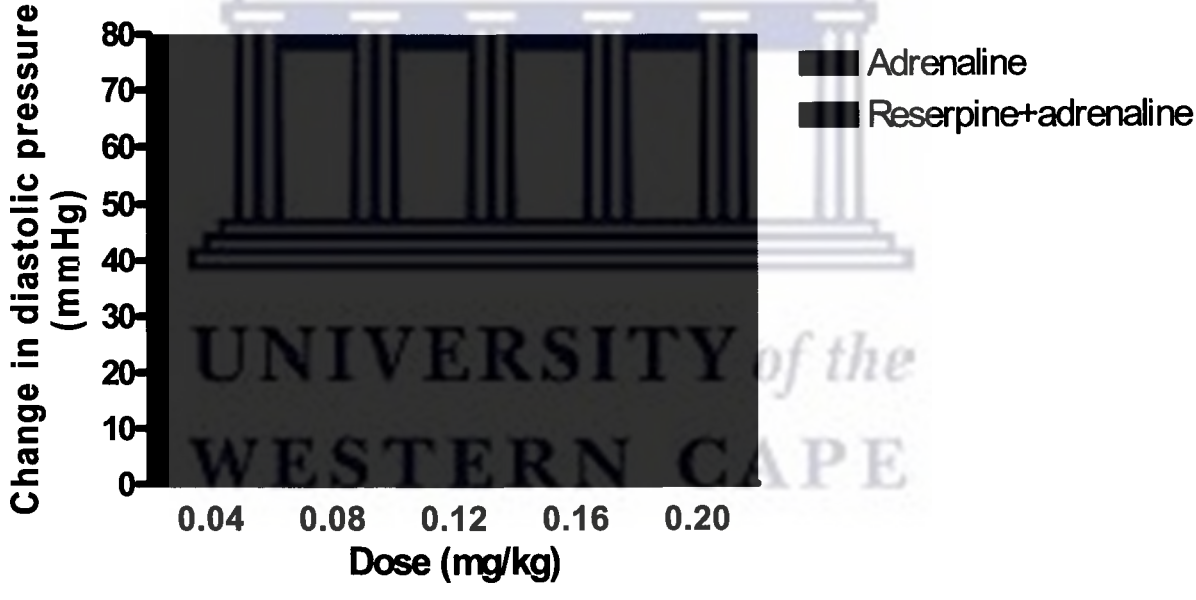


Fig 5.14 Effect of adrenaline on diastolic blood pressure in rats pre-treated with reserpine

The diastolic blood pressure increased with increasing doses of adrenaline. The minimum effect recorded was an increase in blood pressure of 16.45 mmHg and the maximum effect was 45.07 mmHg. A significant ($P < 0.05$) difference in increased diastolic blood

pressure was observed between doses 0.04 and 0.16 mg/kg (14.866 ± 5.323 mmHg) (P = 0.05) and between doses 0.08 and 0.2 mg/kg (25.933 ± 11.813) (P = 0.05).

Rats that were pre-treated with reserpine 5 mg/kg produced significant (P < 0.05) increases in diastolic blood pressure when compared with adrenaline alone as shown in shown in Fig 5.14. The lowest dose of 0.04 mg/kg produced a change of 35.233 ± 4.563 mmHg and the highest dose of 0.2 mg/kg showed an increase of 24.700 ± 13.389 mmHg.

Dose (mg)	Δ diastolic pressure (mmHg) pre reserpine administration	Δ diastolic pressure (mmHg) post reserpine administration	P value
0.04	16.450 ± 2.135	51.683 ± 4.029	0.0022
0.08	19.133 ± 1.675	65.050 ± 3.213	0.0022
0.12	22.666 ± 2.056	62.866 ± 3.103	0.0022
0.16	31.316 ± 4.871	71.850 ± 5.639	0.0022
0.2	45.066 ± 11.709	69.766 ± 6.511	0.1320

Table 5.11 Means, SEM and P values of diastolic pressure for adrenaline pre and post administration of reserpine.

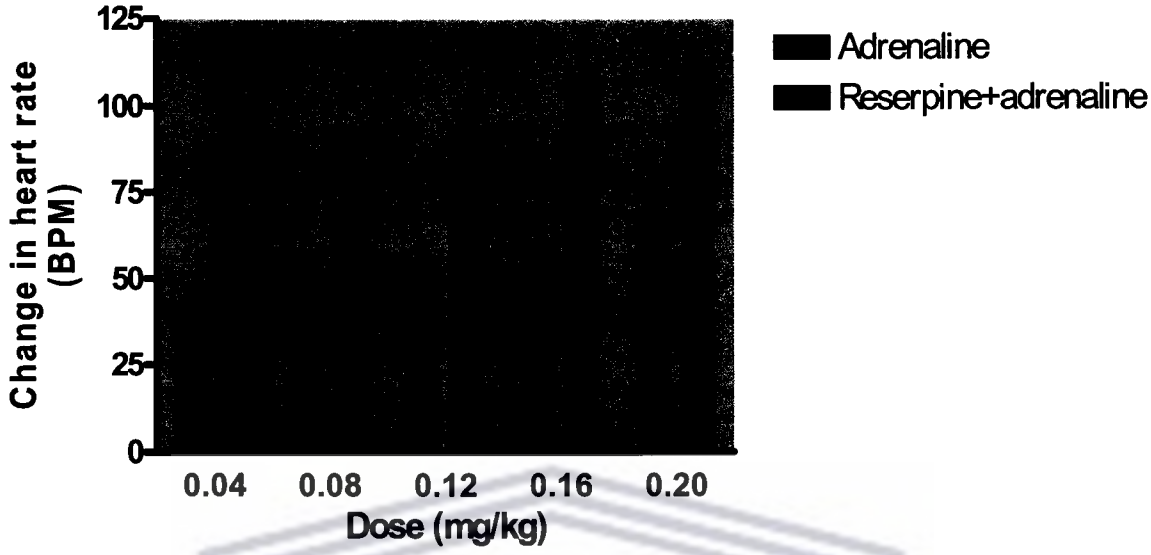


Fig 5.15 Effect of adrenaline on heart rate in rats pre-treated with reserpine

The heart rate increased with increasing doses of adrenaline. The minimum effect recorded was an increase in heart rate of 40.83 beats/minute and the maximum effect was 69.67 beats/minute. A significant (P = 0.0063) difference was observed between the effects obtained with the doses of 0.04mg and 0.2 mg/kg (26.833 ± 8.760).

Rats that were pre-treated with reserpine 5 mg/kg produced non-significant (P > 0.05) increases in heart rate when compared with adrenaline alone as shown in shown in Fig 5.15. The lowest adrenaline dose of 0.04 mg/kg produced a change of 27.333 ± 17.128 beats/minute and the highest adrenaline dose of 0.2 mg/kg showed an increase of 42.000 ± 8.956 beats/minute.

Dose (mg)	Δ heart rate (beats/minutes) pre reserpine administration	Δ heart rate (beats/minutes) post reserpine administration	P value
0.040	40.833 ± 6.620	68.166 ± 15.799	0.1212
0.08	49.166 ± 5.746	71.166 ± 5.375	0.0173
0.12	53.333 ± 7.1724	93.000 ± 7.852	0.0108
0.16	61.000 ± 8.1731	108.833 ± 13.300	0.0260
0.2	69.666 ± 5.736	111.666 ± 6.401	0.0043

Table 5.12 Means, SEM and P values of heart rate for adrenaline pre and post administration of reserpine.

5.4 Effects of *L. leonurus* on blood pressure and heart rate in pretreated rats

5.4.1 Rats pretreated with dobutamine

Dobutamine 20 µg/kg was initially infused at a rate of 0.16 ml / minute over 3 minutes followed by a dose of *L. leonurus* which was infused simultaneously at the same rate over 3 minutes. The cannula was then flushed with normal saline (0.5 ml). The animal was given 10 to 15 minutes to recover from the effect of dobutamine and *L. leonurus*. The same procedure was followed for increasing doses of *L. leonurus* used. One rat was given five more different doses. This experiment was done in six different rats.

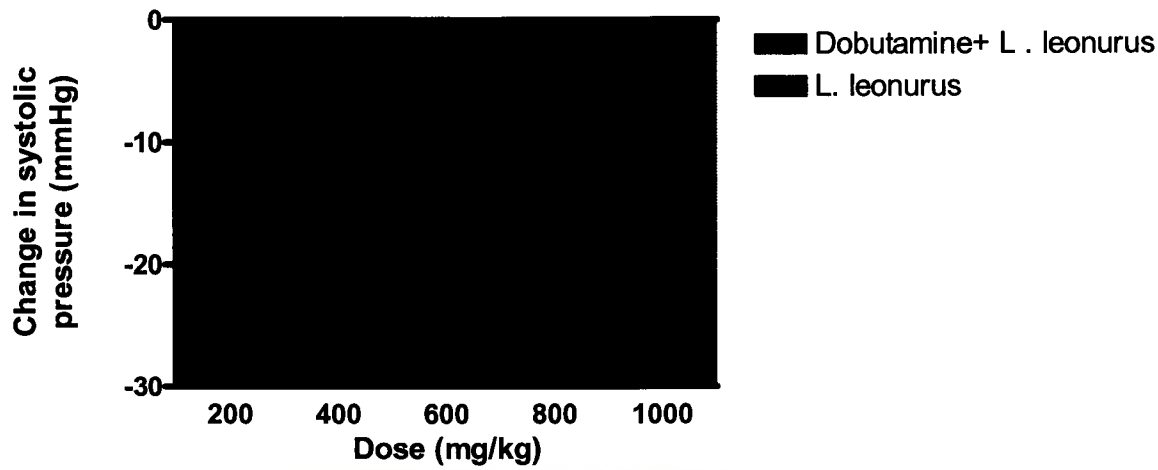


Fig 5.16 Effect of *L. leonurus* on systolic blood pressure in rats pre-treated with dobutamine.

The systolic blood pressure increasingly decreased with increasing doses of *L. leonurus*. The minimum effect recorded was a decrease in blood pressure of 2.533 mmHg and the maximum effect was 20.4 mmHg. The intravenous injection of the aqueous extract of *L. leonurus* produced a non-significant ($p < 0.0363$) difference in reduction of systolic blood pressure between doses 200 mg/kg and 1g/kg (17.866 ± 5.293).

In rats treated with dobutamine 20 μg/kg, *L. leonurus* at increasing dosages induced a non-significant ($P > 0.05$) fall in systolic pressure. The minimum dose of 200 mg/kg produced 11 mmHg and the maximum dose of 1 g/kg produced a change of 18.9 mmHg in systolic pressure 7.315 ± 1.648.

Dose (mg)	Δ systolic pressure (mmHg) pre dobutamine administration	Δ pressure (mmHg) post dobutamine administration	P value
200	-2.533 ± 0.760	-5.416 ± 2.423	0.0208
400	-4.333 ± 2.176	-6.025 ± 2.694	0.1032
600	-9.666 ± 3.446	-7.350 ± 3.291	0.0377
800	-13.533 ± 4.824	-8.333 ± 4.114	0.0378
1000	-20.400 ± 6.307	-9.450 ± 4.659	0.0231

Table 5.13 Means, SEM and P values of systolic pressure for *L. leonurus* pre and post administration of dobutamine.

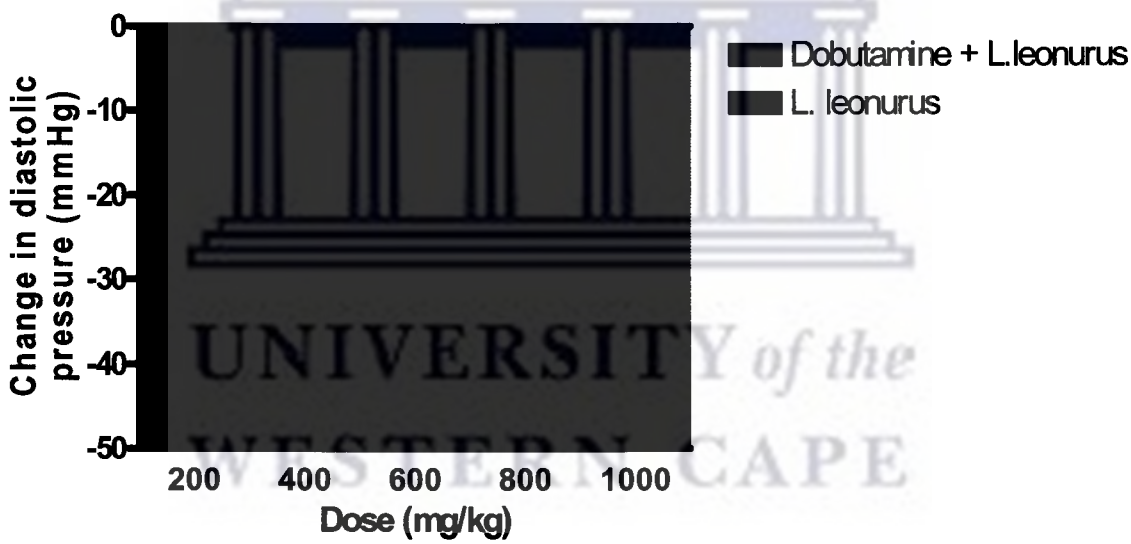


Fig 5.17 Effect of *L. leonurus* on diastolic blood pressure in rats pre-treated with dobutamine.

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced an increasing decrease in diastolic blood pressure. The minimum effect recorded was a decrease in blood pressure of 6.633 mmHg and the maximum effect was 43.6 mmHg.

In rats treated with dobutamine 20 µg/kg, *L. leonurus* produced a non-significant (P > 0.05) decrease in diastolic blood pressure with an increase in dose 7.998 ± 2.9. The minimum dose of 200 mg/kg produced 8.05 mmHg and the maximum dose of 1g/kg produced a change in diastolic pressure of 25.2 mmHg.

Dose (mg)	Δ diastolic pressure (mmHg) pre dobutamine administration	Δ diastolic pressure (mmHg) post dobutamine administration	P value
200	-6.633 ± 2.120	-4.829 ± 2.317	0.026
400	-10.966 ± 5.283	-6.683 ± 3.210	0.315
600	-16.216 ± 1.822	-7.300 ± 4.178	0.655
800	-24.600 ± 6.834	-8.575 ± 4.223	0.0259
1000	-43.600 ± 4.924	-12.600 ± 5.635	0.0003

Table 5.14 Means, SEM and P values of diastolic pressure for *L. leonurus* pre and post administration of dobutamine.

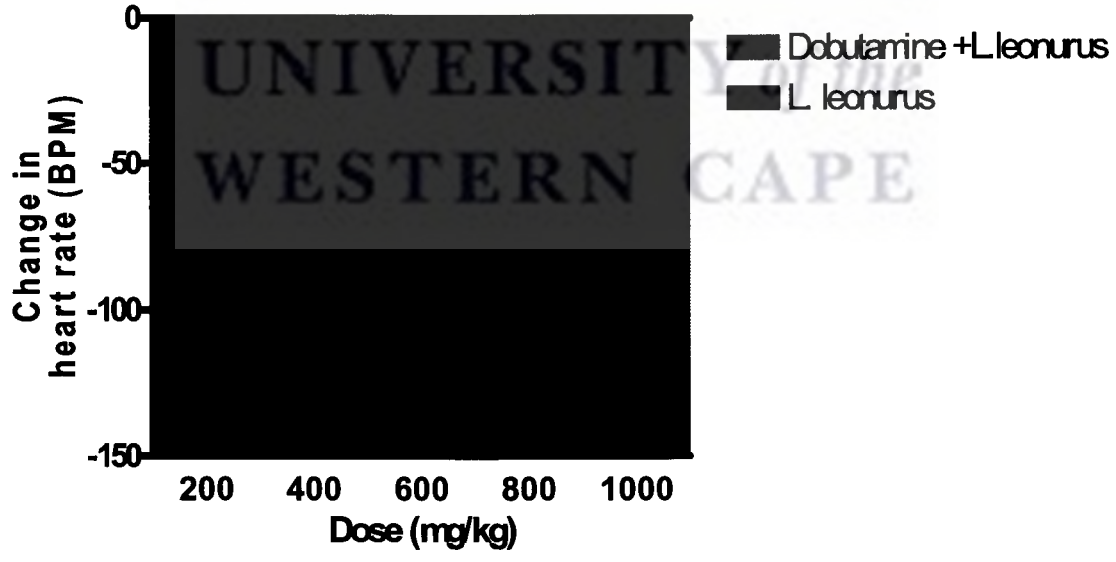


Fig 5.18 Effect of *L. leonurus* on heart rate in rats pre-treated with dobutamine

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate. The minimum effect recorded was a decrease in heart rate of 55.83 beats/minute and the maximum effect was 122 beats/minute.

In rats treated with dobutamine 20 µg/kg, *L. leonurus* produced a significant ($P < 0.05$) fall in heart rate with an increase in dose of *L. leonurus* (50 mg to 250 mg / 250 g rat equivalent to 200 mg to 1 g/kg rat) 12.67 ± 9.19. The minimum dose of 50mg produced 6.0 mmHg and the maximum dose of 250mg produced a change of 47.5 beats/minute in heart rate.

Dose (mg)	Δ heart rate (beats/minutes) pre dobutamine administration	Δ heart rate (beats/minutes) post dobutamine administration	P value
200	-55.833 ± 7.959	-3.000 ± 1.437	0.0009
400	-74.833 ± 5.528	-4.500 ± 2.028	0.0007
600	-110.666 ± 5.024	-12.000 ± 5.662	<0.0001
800	-111.166 ± 5.996	-20.166 ± 9.020	<0.0001
1000	-122.000 ± 5.409	-23.666 ± 10.716	<0.0001

Table 5.15 Means, SEM and P values of heart rate for *L. leonurus* pre and post administration of dobutamine.

5.4.2 Rats pretreated with atenolol

Atenolol 12 mg/kg was infused at a rate of 0.16 ml/minute over 3 minutes, a standard dose of 800 mg/kg was immediately infused at the same rate and the catheter was flushed with normal saline (0.5 ml). The animal was given 10 to 15 minutes to recover from the effect of atenolol and *L. leonurus*. This experiment was done in six different rats. Another set of six animals was infused with 800 mg/kg *L. leonurus* at the rate of 0.16 ml/minute.

Fig 5.19 Effect of *L. leonurus* on systolic blood pressure in rats pre-treated with atenolol

L. leonurus 800 mg/kg induced a significant ($P < 0.05$) fall in the systolic blood pressure -13.533 ± 4.824. When pre-treated with atenolol 12 mg/kg, rats displayed significant ($P < 0.05$) differences in systolic blood pressures -7.567 ± 0.815 when compared to *L. leonurus* on its own. The hypotensive effect of *L. leonurus* on systolic blood pressure was blocked with pretreatment with atenolol.

Dose (mg)	Δ systolic pressure (mmHg) pre atenolol administration	Δ systolic pressure (mmHg) post atenolol administration	P value
800	-13.533 ± 4.824	-7.566 ± 0.815	0.0002

Table 5.16 Means, SEM and P values of systolic pressure for *L. leonurus* pre and post administration of atenolol.

Fig 5.20 Effect of *L. leonurus* on diastolic blood pressure in rats pre-treated with atenolol.

L. leonurus 800 mg/kg induced a significant ($P < 0.05$) fall in the diastolic blood pressure -24.53 ± 6.819. When pre-treated with atenolol 12 mg/kg, rats displayed significant ($P < 0.05$) differences in diastolic blood pressures -9.183 ± 2.532 when compared to *L. leonurus* on its own. The hypotensive effect of *L. leonurus* on diastolic blood pressure was blocked with pretreatment with atenolol.

Dose (mg)	Δ diastolic pressure (mmHg) pre atenolol administration	Δ diastolic pressure (mmHg) post atenolol administration	P value
800	-24.533 ± 6.819	-9.1833 ± 2.532	0.0002

Table 5.17 Means, SEM and P values of diastolic pressure for *L. leonurus* pre and post administration of atenolol.

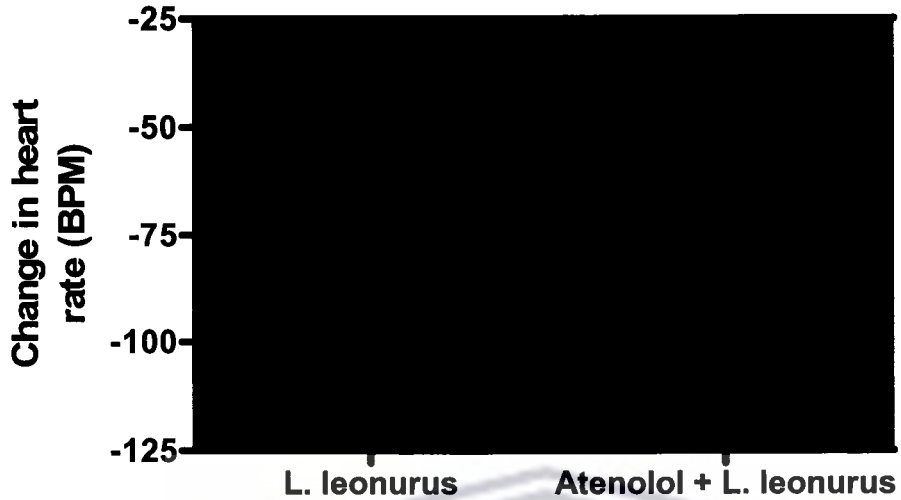


Fig 5.21 Effect of *L. leonurus* on heart rate in rats pre-treated with atenolol

L. leonurus 800 mg/kg induced a significant ($P < 0.05$) fall in the heart rate -111.2 ± 5.996. When pre-treated with atenolol 12 mg/kg, rats displayed significant ($P < 0.05$) differences in heart rate -49.83 ± 8.753 when compared to *L. leonurus* on its own. The hypotensive effect of *L. leonurus* was blocked by the pre-treatment with atenolol.

Dose (mg)	Δ heart rate (beats / minute) pre atenolol administration	Δ heart rate (beats / minute) post atenolol administration	P value
800	-111.166 ± 5.996	-49.833 ± 8.753	0.0023

Table 5.18 Means, SEM and P values of heart rate for *L. leonurus* pre and post administration of atenolol.

5.4.3 Rats pretreated with atropine

Animals were pretreated with 2.4 mg/kg of atropine. The recommended pretreating dose of 1 to 4 mg/kg atropine was obtained from literature (Abdul-Ghani, 1997). Atropine was infused at a rate of 0.16 ml / min over 3 min. *L. leonurus* was immediately infused at the same rate. The cannula was then flushed with normal saline (0.5 ml). The animal was given 10 to 15 min to recover from the effect of atropine and *L. leonurus*. The same procedure was followed for increasing doses of *L. leonurus* used. One rat was given five more different doses. This experiment was done in six different rats. Atropine infusion line was completely covered with foil as atropine gets degraded on exposure to light.

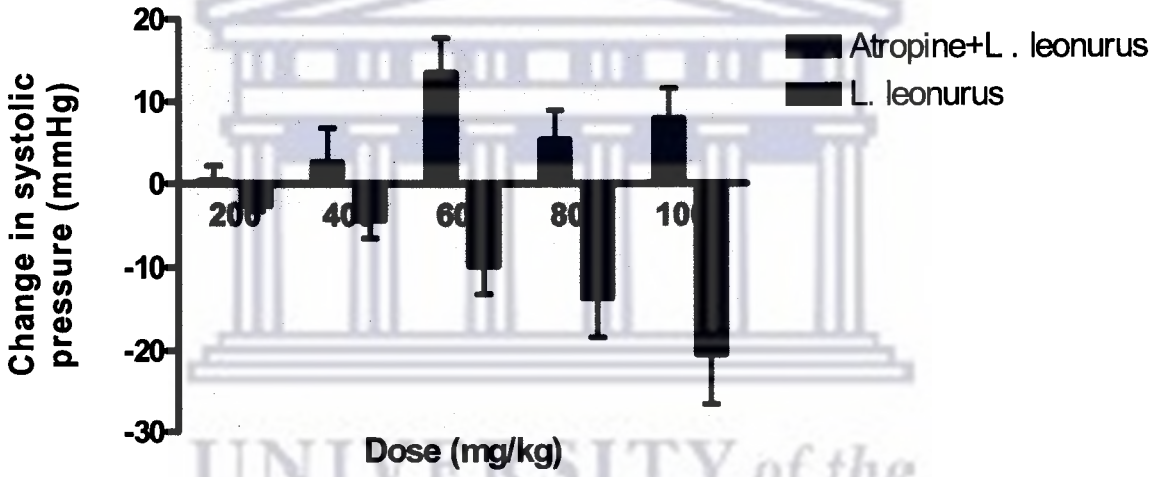


Fig 5.22 Effect of *L. leonurus* on systolic blood pressure in rats pre-treated with atropine

The systolic blood pressure increasingly decreased with increasing doses of *L. leonurus*. The minimum effect recorded was a decrease in blood pressure of 2.533 mmHg and the maximum effect was 20.4 mmHg. The intravenous injection of the aqueous extract of *L. leonurus* produced a non-significant ($p < 0.0363$) difference in reduction of systolic blood pressure between doses 200 mg and 1g/kg (17.866 ± 5.293). When pre-treated with Atropine (2.4 mg/kg), rats displayed significant ($P < 0.05$) differences in systolic pressures when compared to *L. leonurus* on its own. *L. leonurus* induced a dose-dependent (200 mg to 1g/kg) fall in the systolic blood pressure. When this hypotensive

effect of *L. leonurus* was challenged with atropine, a blockade was observed in every dose.

Dose (mg)	Δ systolic pressure (mmHg) pre atropine administration	Δ systolic pressure (mmHg) post atropine administration	P value
200	-.533±0.760	0.300±1.890	0.3095
400	-4.3333±5.332	2.550±4.243	0.2922
600	-9.666±2.176	13.483±4.302	0.0022
800	-13.533±4.824	5.316±3.664	0.0087
1000	-20.400±6.307	7.900±3.735	0.0022

Table 5.19 Means, SEM and P values of systolic pressure for L. leonurus pre and post administration of atropine.

Fig 5.23 Effect of *L. leonurus* on diastolic blood pressure in rats pre-treated with atropine

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced an increasing decrease in diastolic blood pressure. The minimum effect recorded was a decrease in blood pressure of 6.633 mmHg and the maximum effect was 43.6 mmHg. In rats pre-treated with atropine (2.4 mg/kg), non-significant ($P > 0.05$)

changes are seen in lower doses (200 - 400 mg/kg) and significant ($P < 0.05$) changes can be seen in higher doses (600 mg /kg to 1g/kg) when compared to *L. leonurus* on its own. At the lowest dose (200 mg/kg) a non-significant ($P = 0.09$) change of 4.300 ± 2.277 and the highest dose of (1g/kg) produced a significant ($P = 0.002$) change of 44.200 ± 5.842.

Dose (mg)	Δ diastolic pressure (mmHg) pre atropine administration	Δ diastolic pressure (mmHg) post atropine administration	P value
200	-6.633±2.120	-1.566±1.135	0.931
400	-10.966±5.283	-0.199±2.615	0.0844
600	-16.216±1.822	4.433±2.482	0.0022
800	-24.600±6.834	0.650±3.080	0.0087
1000	-43.600±4.924	0.600±3.143	0.0022

Table 5.20 Means, SEM and P values of diastolic pressure for *L. leonurus* pre and post administration of atropine.

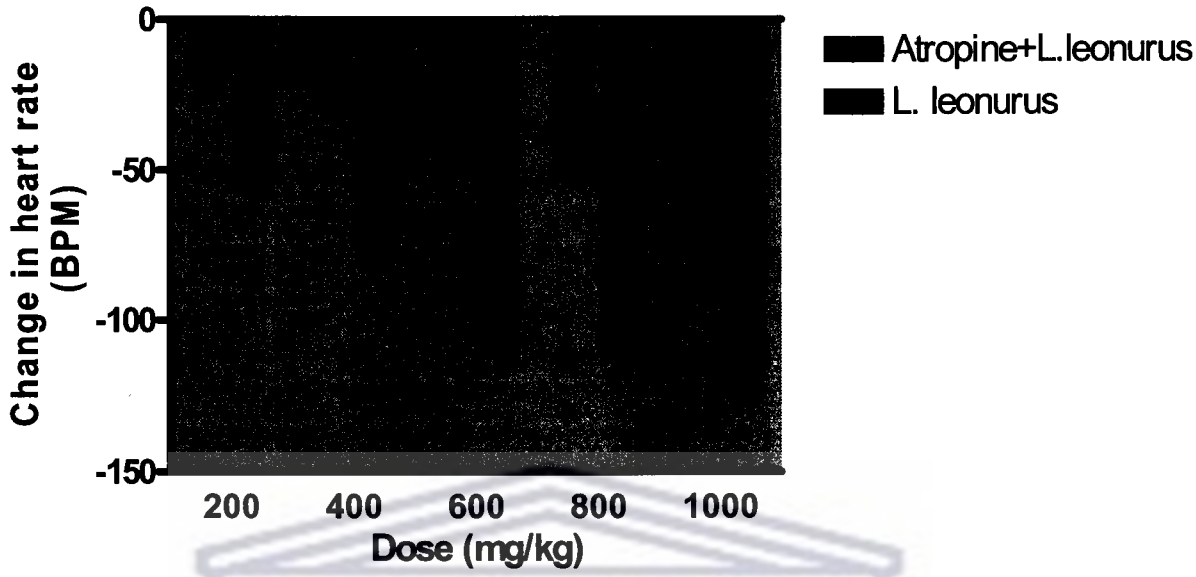


Fig 5.24 Effect of *L. leonurus* on heart rate in rats pre-treated with atropine

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate. The minimum effect recorded was a decrease in heart rate of 55.83 beats/minute and the maximum effect was 122 beats / minute. The lowest significant ($P = 0.0007$) decrease was observed between doses 400 mg and 600 mg/kg (35.8333 ± 7.470) and the highest significant ($P < 0.0001$) decrease in heart rate was observed between doses 200 mg and 1g/kg (66.166 ± 9.623

When pre-treated with atropine (2.4 mg/kg), rats displayed significant ($P < 0.05$) differences in fall in heart rate when compared to *L. leonurus* on its own. *L. leonurus* induced a dose-dependent (200 mg to 1g/kg) fall in heart rate. When this hypotensive effect of *L. leonurus* was blocked with atropine, a decrease in fall in heart rate was observed in every dose.

Dose (mg)	Δ heart rate (beats/min) pre atropine administration	Δ heart rate (beats/min) post atropine administration	P value
200	-55.833 ±7.959	-16.833±8.088	0.0043
400	-74.833 ±5.528	-24.666 ± 4.716	0.0022
600	-110.666 ±5.024	-44.500 ± 5.596	0.0022
800	-111.166 ±5.996	-48.666 ± 4.216371	0.0022
1000	-122.000 ± 5.409	-76.333 ± 2.389	0.0022

Table 5.21 Means, SEM and P values of heart rate for *L. leonurus* pre and post administration of atropine.

The next chapter will be discussing the results obtained from this chapter, conclusions and recommendations for future studies.

CHAPTER 6

6.1 Discussions

The results of this investigation showed that the aqueous extract of the leaf of *L. leonurus* lowered systolic, diastolic blood pressure and heart rate in rats. The dose-dependent nature of the effects of the leaf extract of the plant on blood pressure and heart rate of the rat suggests a cumulative action of the active substance(s) present in the leaves of the plant. This observation agrees with the earlier reports by Ojewole *et al* 2003.

Literature has shown that antihypertensive agents lower blood pressure by interfering with any of the blood pressure regulatory mechanisms as discussed in chapter 2. The possibility that the extract could be inducing its hypotensive effect via autonomic, and muscarinic systems was examined in this study.

Reserpine depletes adrenergic nerves of noradrenaline by blocking or destroying the storage mechanism within the nerve ending, so that there is less transmitter amount available for release.

Klemola *et al 1999* investigated the presence and source of catecholamines in pericardial fluid of normotensive, and spontaneously hypertensive reserpine-treated rats. He discovered that noradrenaline is the only detectable catecholamine present in rat pericardial fluid. The effect of reserpine at 6, 12, and 24 hrs after pre-treatment with 5 mg/kg intraperitonialy (i.p) shown that, the concentration of noradrenaline in pericardial fluid reflects the amount of noradrenaline released within the heart rather than the amount of noradrenaline in plasma.

Previous investigations indicate that the spontaneously hypertensive rat (SHR) has elevated sympathetic tone at rest. The present study shows exaggerated sympatho-adrenal activation in response to sympathetic stimuli in normotensive rats. This was observed when the systolic, diastolic blood pressure, heart rate and preganglionic adrenal sympathetic nerve activity (SNA) in normotensive Wistar rats increased after pre-treating with reserpine, suggesting that there was an increased noradrenaline overflow in the rats.

Depletion of norepinephrine and sensitivity to adrenaline in rat atria were observed after pretreatment of rats with reserpine at 16 to 24hrs. Pre-treatment with reserpine 5 mg/kg produced supersensitivity to the chronotropic responses to adrenaline. This was in line with earlier observations by Rice *et al* (1987); Duckles, 1991; Klemola *et al* 1999; and Raasch 2004).

The same response was observed when the adrenergic system was challenged with *L. leonurus*. Pre-treatment with *L. leonurus* 800 mg/kg produced an exaggerated sympatho-adrenal response.

It is however been reported that a variety of sympathetic stimuli, including ganglionic blockade, mental stress and neuronglucopenia, cause exaggerated activation of preganglionic adrenal SNA in SHR indicating that adrenal SNA is hyper-responsive (Rice *et al* 1987).

The present study also investigated the effects of the β-agonist dobutamine, which also hastens the onset of Left Ventricular relaxation, on the Left Ventricular contractile response to receptor-mediated coronary endothelial stimulation. Heart rate, diastolic and systolic pressures were recorded during *L. leonurus* 800 mg/g infusion alone and during the concurrent intravenous administration of dobutamine 20 μg/kg. The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate, diastolic and systolic pressure. The hyportensive effect of the aqueous leaf extract of *L. leonurus* was blocked by the concurrent treatment of rats with dobutamine 20 μg/kg. This antagonism of the hypotensive effect of *L. leonurus* extract by dobutamine suggests the involvement of β-adrenergic mechanism in the action of the leaf extract.

Atenolol selectively block the β_1 receptor effect of noradrenaline and adrenaline in the heart. The β-adrenoceptor cardiovascular effects are dependent on the amount of sympathetic tone present. The main cardiac effects resulting from the reduction of sympathetic drive are reduced myocardial contractility (rate of rise of pressure in the ventricles) and reduced automaticity (heart rate).

The intravenous injection of increasing doses of the aqueous extract of *L. leonurus* produced a dose dependent decrease in heart rate, diastolic and systolic pressure. The hypotensive effect of the aqueous leaf extract of *L. leonurus* was blocked by pre-treatment of rats with atenolol 12 mg/kg. This antagonism of the hypotensive effect of *L. leonurus* extract by atenolol pretreatment suggests the involvement of β-adrenergic mechanism in the action of the leaf extract.

During dobutamine and atenolol pretreatment experiments, there were interesting observations, in that, both dobutamine (beta agonist) and atenolol (beta antagonist) seemed to inhibit the effects of *L. leonurus* in blood pressure and heart rate. It is suspected that, dobutamine inhibit the effects of *L. leonurus* by inhibiting effect of L. leonurus on beta receptors due to the fact that at some dosages it acts as beta antagonist. The (-) isomer of dobutamine is a potent agonist at *alpha*-1-receptors and is capable of causing marked pressor response while the (+) isomer is a potent *alpha*-1-receptor antagonist which can block the effect of dobutamine. The effects of these two isomers are mediated via *beta* receptors. The relatively constant peripheral resistance presumably reflects counterbalancing of alpha receptor-mediated vasoconstriction and beta receptor mediated vasodilation (Brunton *et al;* 2006).

Also, a synergistic effect of *L. leonurus* was rather expected when animals were pretreated with atenolol, but inhibition of *L. leonurus* effects on beta blockers was observed.

Atropine acts on muscarinic receptors and cause reversible blockade of the actions of cholinomimetics. The atria of the heart are richly innervated by parasympathetic (vagal) nerve fibres, and the sinoatrial node is therefore sensitive to muscarinic receptor blockade. The effect of moderate to high therapeutic doses of atropine in the innervated and spontaneously beating heart is a clear blockade of vagal slowing and a relative tachycardia. Low doses of atropine cause parasympathetic stimulation and often result in initial bradycardia before the effects of peripheral vagal block become manifest. This effect has been ascribed to central stimulation of the vagal nucleus, although other evidence suggests that it may be due to block of presynaptic muscarinic receptors on

vagal postganglionic fibres that normally limit acetylcholine release in the sinal node. The same mechanism operates in the control of atrioventricular node function, in the presence of high vagal tone. Administration of atropine can significantly reduce the PR interval of the (electrocardiogram) ECG by blocking muscarinic receptors in the AV node.

Atropine reduces the effect of vagal nerve stimulation, primarily on the SA node. Since stimulation of the vagus nerve slows heart rate, atropine increases heart rate by blocking this effect. Atropine also speeds-up conduction through the AV node, and thus lessening heart block in certain cases. Cholinergic compounds are known to cause a fall in BP by activation of muscarinic receptors located on the epithelium of blood vessels (Furchgott and Zawadski 1980).

When the hypotensive effect of *L. leonurus* was challenged with atropine (2.4 mg / kg), a partial blockade in heart rate ($P < 0.05$) with even a complete blockade in systolic and diastolic pressure ($P < 0.05$) was observed.
The antagonism of the hypotensive effect of the extract by atropine pretreatment seems to suggest the involvement of cholinergic mechanism in the action of the leaf extract. It is however well known that the stimulation of the cholinergic system in many animal species results in both hypotension and bradycardia (Ganong, 1993).

Gilani et al (1994) reported the ability of saponins in other plants to exhibit hypotensive and vasodilator activities and the presence of such compounds in *L. leonurus* might possibly contribute in the cardiovascular effects of *L. leonurus.*

6.2 Conclusions

In conclusion, the results of this study supported the traditional claim that the aqueous leaf extract of *L. leonurus* has a dose dependent blood pressure lowering effect. This hypotensive effect is probably mediated through 1) inhibition of sympathetic system and 2) cholinergic control of the arterial pressure and most significantly through muscarinic receptor blockade.

6.3 Recommendations

Further studies are necessary to;

1) elucidate the phytochemistry of the active principles in the leaf extract of *L. leonurus*.

2) determine *alpha*-receptor, calcium channel blocking and angiotensin converting enzyme effect of *L. leonurus*.

3) determining the actual mechanism by which a *beta*-blocker atenolol and a *beta*-agonist dobutamine bring about the same effect in rats treated with *L. leonurus*.

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