Effects of *Leonotis leonurus* aqueous extract on the isolated perfused rat heart

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A mini - thesis submitted in partial fulfilment of the requirements for the degree of Magister Pharmaceuticae in the Faculty of Natural Sciences, School of Pharmacy, Department of Pharmacology, at the University of the Western Cape.

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KEYWORDS

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Langendorff perfusion model

Left ventricular systolic pressure

Left ventricular end-diastolic pressure

Left ventricular developed pressure

Heart rate

Coronary perfusion pressure

Cardiac work

ABSTRACT

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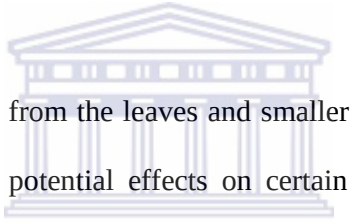

An aqueous extract prepared from the leaves and smaller stems of *Leonotis leonurus* was used to investigate the potential effects on certain cardiovascular parameters, such as left ventricular systolic pressure, end-diastolic pressure, developed pressure, heart rate, cardiac work and coronary perfusion pressure in isolated rat hearts. Hearts were perfused at constant flow for 3min using the modified Langendorff perfused model of the heart. Effects of adrenaline and digoxin solutions on the isolated heart were compared to that of the plant extract. Adrenaline produced both positive inotropic and chronotropic effects. Adrenaline increased ($p<0.01$) the left ventricular systolic pressure and hence the left ventricular developed pressure by 40.6% and 43.9% at peak, and 24.3% and 31.9%, after 3min, respectively. Simultaneously, the heart rate and the cardiac work were increased ($p<0.01$) by 22.5% and 89.4% at peak, and 24.6% and 63%, after 3min, respectively. There were no significant effects on the left ventricular diastolic pressure and the coronary perfusion pressure. Digoxin solution (2.5ng/ml) significantly ($p<0.01$) increased the left ventricular systolic

pressure by 5.1% after 3min and the left ventricular diastolic pressure by 9.7% at peak and 5.3% after 3min. The heart rate was significantly ($p<0.01$) decreased by 3.7% at peak. The cardiac work was increased by 4.5% after 3min. Digoxin did not significantly affect the left end diastolic pressure and the coronary perfusion pressure.

The extract of *Leonotis leonurus* at 0.1mg/ml increased ($p<0.01$) the left ventricular systolic pressure and hence the left ventricular diastolic pressure by 9.7% and 10.7% at peak, and 5.4% and 5.5% after 3min, respectively. The cardiac work was increased ($p<0.01$) by 10.1% at peak. *Leonotis leonurus* (0.1mg/ml) did not significantly affect the left ventricular end diastolic pressure, the heart rate and the coronary perfusion pressure. At 0.5mg/ml, the left ventricular systolic pressure and hence the left ventricular diastolic pressure were increased ($p<0.01$) by 14.8% and 15.4% at peak and 7.4% and 7.8% after 3min, respectively with a corresponding decrease ($p<0.01$) in the coronary perfusion pressure of 8.5% at peak and 4.4% after 3min. The cardiac work was increased ($p<0.01$) by 13.6% at peak and 5.2% after 3min. The extract at 1.0mg/ml increased ($p<0.01$) the left ventricular systolic pressure and hence the left ventricular diastolic pressure by 25.4% and 29.4% at Peak, and 23.1% and 26.3% after 3min, respectively. The heart rate was reduced ($p<0.01$) by 34.7% at peak and 28.3% after 3min. The cardiac work and the coronary perfusion pressure were decreased ($p<0.01$) by 15.9% and 12.1% at Peak and 3.3% and 11.4% after 3min. However, at 2.0mg/ml, the left ventricular systolic pressure and the left ventricular diastolic pressure were increased ($p<0.01$) by 14.9% at peak. The left ventricular diastolic pressure was decreased ($p<0.01$) by 9.8% over the 3min. The heart rate was drastically decreased ($p<0.01$) by 42.7% after 3min. The cardiac work was reduced

($p<0.01$) by 48.8% over the 3min period. Also, the coronary perfusion pressure was decreased ($p<0.01$) by 16.9% at peak.

Thus, *Leonotis leonurus* produced both positive inotropic and negative chronotropic effects after 3min perfusion, accompanied by a decreased coronary perfusion pressure. Thus, it appears that the extract seemed to contain certain constituents associated with positive inotropic and negative chronotropic agents as well as constituents associated with coronary vasodilation. However, at the higher concentration, it seemed to contain some constituents associated with toxic effects on the isolated heart.

Therefore, further studies are needed to isolate the various constituents and examine their possible pharmacological effects on the heart individually before it could be considered safe to recommend this plant for its use in the treatment of cardiovascular disease.

August 2007

DECLARATION

I declare that “Effects of a *Leonotis leonurus* aqueous extract on the isolated perfused rat heart” is my own work, that has not been submitted for any degree or examination at any other University, and that all sources I have used or quoted have been indicated and acknowledged by means of complete references.

DEDICATION

To my husband, Abdul Hamid Halday.

To my parents, Mr Ismail Khan and Mrs Gaironesa Khan.

To my sister, Ayesha Khan

To my daughter, Nisreen Halday.

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To my parents, whose encouragement, motivation, support and confidence in me have enabled me to acquire this achievement.

LIST OF ABBREVIATIONS

ADR:	Adrenaline
AV:	Aortic valve
CCF:	Congestive cardiac failure
CF:	Coronary flow
CO:	Cardiac output
CPP:	Coronary perfusion pressure
CW:	Cardiac work
EDV	End-diastolic volume
DIG:	Digoxin
HR:	Heart rate
IPH:	Isolated perfused heart
IVC:	Inferior vena cava
K-H:	Krebs – Henseleit
LA:	Left atrium
L. leonurus :	Leonotis leonurus
LV:	Left ventricle
LVEDP:	Left ventricular end-diastolic pressure
LVDP:	Left ventricular developed pressure
LVP:	Left ventricular pressure
LVSP:	Left ventricular systolic pressure
MOC:	Myocardial oxygen consumption
MV:	Mitral valve
NAPRALERT:	Natural Product Alert
PA:	Pulmonary artery

PV:	Pulmonary veins
RA:	Right atrium
SA :	Sino-atrial
SV:	Stroke volume
SVC:	Superior vena cava
TV:	Tricuspid valve

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Table 6.2: **Effects of adrenaline, digoxin and leonotis leonurus on the left ventricular systolic pressure**

Effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) [Regimens I-L] on LVSP of the isolated perfused rat heart. Data expressed as %change from baseline (mean ± SD) *****p<0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

Table 6.3: **Effects of adrenaline, digoxin and Leonotis leonurus on left ventricular end diastolic pressure**

Effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) [Regimens I-L] on LVEDP of the isolated perfused rat heart. Data expressed as %change from baseline (mean ± SD) *****p<0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

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Effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) [Regimens I-L] on CPP of the isolated perfused rat heart. Data expressed as %change from baseline (mean ± SD) *****$p<0.01$ versus baseline; Mann-Whitney U test (SPSS for Windows®).

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CHAPTER 1

INTRODUCTION

Traditional medicine and hence the use of medicinal plants have been in use for many years. It is estimated that 80% of the black population consult traditional healers for advice and/or treatment of health concerns (George et al. (2001); Kelmanson et al. (2000); Muller and Steyn (1999); Du Toit (1998); Felhaber and Mayeng (1997) and Sofowora (1982)). This could be due to the fact that traditional medicine is cheaper than modern medicine and that it is more accessible to most of the population of the third world. The main reasons, however, why such a large percentage of the population in every country of the developing world has to rely on traditional or indigenous forms of medicine are because of the shortage of hospitals and health facilities, as well as of the medical and paramedical staff needed to manage modern health care delivery systems (Sofowora (1982)). Traditional medicine has a wider acceptability among the people of developing countries than modern medicine mainly because it blends into the sociocultural life of the people in whose culture it is deeply rooted.

During the latter part of the 20th century, traditional medicine had become mainstream in developing countries. This is due in part to the recognition of the value of traditional medical systems, the identification of medicinal plants from indigenous pharmacopoeias that have been shown to have significant healing power, either in their natural state or as a source of new pharmaceuticals and the need to make health care affordable for all and the perception that natural remedies are somehow safer and more efficacious than remedies that are pharmaceutically derived.

Medicinal plants form a sizeable component of traditional medicine and are an important aspect of the daily lives of many people and an important part of South African cultural heritage. Plants were once a primary source of all the medicines in the world and they still continue to provide mankind with new remedies. Natural products and their derivatives represent more than 50% of all drugs in clinical use in the world (Van Wyk et al. (2000)). Well-known examples of plant-derived medicines include quinine, morphine, codeine, aspirin, atropine, reserpine and cocaine.

Recently, important new anti-cancer drugs such as taxol and vincristine have been developed. In South Africa, a large part of the day-to-day medicine is still derived from plants and large volumes of plants or their extracts are sold in the informal and commercial sectors of the economy. South Africa's contribution to world medicine include Cape aloes (*Aloe ferox*), buchu (*Agathosma betulina*) and devil's claw (*Harpagophytum procumbens*), but local equivalents exist for many of the famous remedies used elsewhere.

Traditional healers are an integral part of their people and society and they have flourished in the face of competition from modern medicine. Traditional healers in South Africa greatly out-number those who practise modern medicine. According to Van Wyk et al. (2000) and Kale (1995), in 1992 about 200 000 traditional healers practised in South Africa compared with 25 000 doctors of modern medicine.

Bye and Dutton (1991) recorded in 1982 the shortage of western doctors in the rural areas to be in the ratio of one medical practitioner for every 17 500 people. This forced rural inhabitants to consult with traditional healers, if not by choice, then certainly by necessity. According to the results obtained from a community survey conducted by Peltzer in 1998 in the Northern Province, South Africa, the ratio of medical doctors to the total population in the Northern Province was 1:20 000, compared with the ratio of traditional practitioners to the total population ratio of 1:200. The Northern Province is the most impoverished of the nine provinces in South Africa. The area is largely rural with 91% of the inhabitants living in the non-urban areas where access to health care facilities is inadequate. For example, in 1992 there were 2.4 hospital beds per 1000 people and a physician: population ratio of less than 1: 20 000. The inhabitants of this area are adversely affected by conditions such as overcrowding, lack of electricity and clean water, poor sanitation, poor roads and transport facilities, a high unemployment rate, and poorly equipped schools- all of which are impediments to the development of a healthy community. This survey once again confirmed the dependence of people from developing countries on the use of traditional medicine.

The role of the traditional healer has also repeatedly been emphasised by the World Health Organisation (WHO). In the national health plan for South Africa [African National Congress (ANC)], 1994, it was stated that traditional health practitioners (THP) should be an integral and recognised part of health care. Consumers will be allowed to choose whom they want to consult for their health care, and legislation will be changed to facilitate controlled use of traditional healers.

Although traditional medicine is the primary form of medicine in many parts of the world, it suffers from many shortcomings. According to Kale (1995), there have been no studies on the efficacy of traditional remedies. Most of the claims made about the therapeutic effectiveness of medicinal plants are by traditional healers themselves (Sofowora, 1982). Another shortcoming is the imprecise diagnosis of a disease and this is due to the fact that the pathology of a certain disease is not always known to the traditional healer. The risk of patients being harmed by traditional healers has been highlighted in medical literature and was referred to by several South African doctors (Kale (1995)).

There is no regulatory system in place that ensures that any of the plant remedies do what is claimed or most importantly are safe. Inappropriate formulation or lack of understanding the plant and drug interactions have led to adverse reactions that are sometimes life-threatening or lethal. Many individuals are unaware of the problems associated with traditional use or the fact that their limited diagnostic skills or of those prescribing treatment for them, may prevent the detection of serious underlying conditions like malignancies. One must remember that the indications for the administration of traditional medicines are based on the patient's symptoms and not on the underlying disease, which is usually unknown.

Traditional medicines, as effective and potent medicines, require evaluation by scientific methods in order to be used to their full effect. Undoubtedly, many traditional remedies are beneficial and do have a definite role to play in rural health, but what is of concern is that many of the potentially toxic herbs are in fact used for their 'magical properties' and are doing very little to prevent disease.

CHAPTER 2

LITERATURE REVIEW

2.1 Lamiaceae family

L. leonurus belongs to the family of Lamiaceae. Lamiaceae (formerly Labiatae)- the Mint family, is a large family of mostly shrubs and herbs comprising about 200 genera and 3,200 species, commonly with aromatic, herbage, quadrangular stems, and verticillate inflorescences distributed all over the world. It includes many well-known herbs (Mint, Sage, Thyme, Basil), ornamental plants (*Coleus, Leonotis*) and weeds (Henbit, Ground Ivy, Self-Heal). The family is characterised by short-stalked epidermal glands bearing ethereal oils, often of the monoterpenoid, sesquiterpenoid or diterpenoid type (Hutchings et al. (1996)). Members of the family frequently produce triterpenoid substances but generally not saponins. Many of the species of the Lamiaceae family have been used in folk medicine (Ascensao et al. (1997)).

2.2 Distribution of *Leonotis leonurus*

L. leonurus has a wide natural distribution over large parts of South Africa and has become a popular garden plant (Watt and Breyer-Brandwijk (1962)). It is common at forest margins, on rocky hillsides and riverbanks and in tall grasslands of the Eastern and Western Cape Provinces, Kwazulu-Natal and Mpumalanga (Van Wyk et al. (2000) and SATMERG, (2003)).

2.3 Description of *Leonotis leonurus*

L. leonurus is commonly known as “wilde dagga” in Afrikaans, “wild dagga” in English, “umunyane” in Zulu, “lebake” in Sotho and “umfincafincane” in Xhosa (Watt and Breyer-Brandwijk (1962) and Van Wyk et al. (2000)). It is an attractive plant of 2-5 meters in height with a thick wood base and pale brown branches. All parts of the plant have a strong smell. The leaves are opposite each other on the stems, long and narrow, toothed in the upper half and distinctly hairy. Bright orange, tubular flowers are borne in characteristic rounded groups, which are neatly arranged around

the branch ends (Fig.2.1). The hairy flowers resemble lion's ears, hence the name *"leonurus"* (Van Wyk et al. (2000)).

Figure 2.1: *Leonotis leonurus* (L.) R. Br. (Van Wyk et al. (2000))

2.4 Active ingredients of *Leonotis leonurus*

The active component in Wild dagga is leonurine, the same alkaloid that is mildly psychoactive in the similar *Leonurus sibricus* (Marihuanilla). The chemical constituents isolated from the leaves included a reddish oil with a high boiling point, two phenolic compounds-$C_9H_{10}O_5$ and $C_8H_{10}O_5$ and 19.8% resin (Watt and Breyer-Brandwijk (1962) and Hutchings et al. (1996)). The *Leonotis* species also contain several unusual diterpenoids (labdane type lactones). The diterpene spiro ether, C-13 epimeric premarrubiin (a known precursor of marubiin), has also been isolated (Hutchings et al. (1996)). According to Van Wyk et al. (2000), marubiin is the main diterpenoid lactone in white horehound (*Marrubium vulgare*). This traditional European phytomedicine is used for the symptomatic treatment of coughs and acute bronchial disease. The actual pharmacological effect of *L. leonurus* is not known (Van Wyk et al. (2000)).

2.5 Medicinal uses of *Leonotis leonurus*

L. leonurus is used mainly in the form of an aqueous decoction, orally, per rectum and as a topical application (SATMERG (2003)). The plant is reputed to possess a great variety of medicinal properties. Natives have used the plant since the early times. Hottentots were particularly fond of smoking it instead of tobacco and used a decoction of the leaf as a strong purgative and as an emmenagogue (Watt and Breyer-Brandwijk (1962)).

Early colonists employed a decoction in the treatment of chronic cutaneous eruptions and possibly even in leprosy (Watt and Breyer-Brandwijk (1962)). The Zulus use an infusion of the leaf and stem, by the mouth and as an enema for coughs and colds in both human beings and stock (Watt and Breyer-Brandwijk (1962)). They also use a cold infusion of the leaf as a nasal douche to relieve headache in febrile attacks. Europeans and Natives frequently drink a decoction of the powdered stem or seed, with or without the flower, for the relief of haemorrhoids and apply it as a lotion for sores on the legs and head. Instead of the decoction, the fresh juice is sometimes applied to the sores and an infusion drunk for 'blood impurity' (Watt and Breyer-Brandwijk (1962)). The leaves are smoked by Europeans suffering from partial paralysis (Hutchings et al. (1996)). It has also been smoked for the relief of epilepsy (Van Wyk et al. (2000)). The leaves or roots are widely used as a remedy for snakebites and also to treat other bites and stings (Hutchings et al., 1996 and Van Wyk et al. (2000)). The Xhosas use a decoction of the plant or a tincture of the inner root-bark for snakebites (Watt and Breyer-Brandwijk (1962)). A preparation of either the root-bark or the fresh leaf is a Suto snakebite remedy (Watt and Breyer-Brandwijk (1962)). Externally, decoctions have been applied to treat boils, eczema, skin diseases, itching, and muscular cramps. Internally, decoctions are used for coughs, colds and influenza, and also for bronchitis, high blood pressure and headaches (SATMERG (2003); Van Wyk et al. (2000); Hutchings et al. (1996) and Watt and Breyer-Brandwijk (1962)). Leaf infusions have been used for asthma and viral hepatitis (Van Wyk et al. (2000); Hutchings et al. (1996) and Watt and Breyer-Brandwijk (1962)).

Infusions from flowers, leaves or stems are widely used in various other parts of Africa and Mauritius as purgatives and tonics and for influenza, tuberculosis, jaundice, muscular cramps, skin diseases, sores, bee and scorpion stings (Hutchings et al. (1996)). Decoctions have been used for the relief of cardiac asthma (Hutchings et al. (1996)). Ointments containing powdered leaves are applied for pain above the eye (Hutchings et al. (1996)). Teas have been used as diuretics and for obesity in Southern Africa (Hutchings et al. (1996)).

2.6 Dosage

One tablespoon of chipped dried herb (10g) added to three cups of boiling water is boiled for ten minutes and allowed to cool overnight, strained and used as a clear liquid for both internal and external use. If fresh material is used, 3-4 young twigs (leaf and stem) are boiled with one litre of water. For internal use, adults are recommended to drink half a cup (90ml), elderly patients a quarter cup (45ml) and children 2-6 years, two tablespoons of the liquid. It should be taken 2-3 times daily. For external use, the decoction may be applied to the affected area using a clean cloth (SATMERG (2003)).

2.7 Precautions

According to SATMERG (2003), *L.leonurus* is not recommended for use in pregnant women and the use of this plant to treat hypertension, epilepsy or snakebite cannot at this stage be recommended owing to lack of clinical data. First time users may experience dizziness, nausea and sweating.

2.8 Phytochemistry and documented effects of *Leonotis leonurus*

A considerable number of studies deal with the chemical composition of the essential oils produced by Lamiaceae. However, the morphology and particularly the ultrastructure of secreting glandular trichomes have been examined in only a few species. According to Ascensao et al. (1997), phytochemical studies conducted by Kaplan and Rivett (1968) and Purushothaman and Vasanth (1988), revealed that

L. leonurus produces labdane diterpenoids and an essential oil rich in terpene hydrocarbons, mainly β-caryophyllene and α-pinene. The glandular trichomes that produce essential oils are a general feature of the Mint family.

The vegetative and reproductive organs of *L. leonurus* bear numerous glandular trichomes of two morphologically distinct types (peltate and capitate) and also seem to have different secretion processes (Ascensao et al. (1997)). Ascensao and co-workers (1997) found that its secretion is an oleoresin containing terpenoids (essential oils and resiniferous acids) and flavonoid aglycones as its main constituents. Proteins, polysaccharides, alkaloids and tannins were not detected in the secretion.

Bienvenu et al. (2002) tested an aqueous extract of *L. leonurus* for anticonvulsant properties against seizures produced in mice by pentylenetetrazole, picrotoxin, bicuculline and N-methyl-DL-aspartic acid. The aim of the study was to verify the anticonvulsant properties of *L. leonurus* in order to prove and support its use as an antiepileptic remedy by the traditional medicine practitioners. Results showed that the aqueous extract protected some of the animals against seizures induced by the abovementioned agents and also delayed the latency of the seizures. Bienvenu and co-workers concluded that the results obtained are convergent to the confirmation that *L. leonurus* had anticonvulsant properties. Thus, this study contributes to the scientific proof of the efficacy of *L. leonurus* in the treatment of epilepsy.

Lack of reliable clinical data about the effects of *L. leonurus* in the treatment of hypertension exists. However, Mugabo et al. (2002) found that at low dose an aqueous extract obtained from the leaves of *L. leonurus* had a positive inotropic and positive chronotropic effect in the isolated perfused rat heart and had no effect on the BP and HR in normotensive rats. Using the same aqueous extract, Ojewole, J. A. O. (2003) found that at high dose *L. leonurus* aqueous extract decreased the BP and HR in spontaneously hypertensive rats.

As discussed previously, most of the therapeutic successes of this plant are based on claims made by traditional healers. The scientific proof of the pharmacological effects of this plant still needs to be proven. Thus the need arose to scientifically scrutinise and validate these claims made by traditional healers and to introduce scientific

expertise and modern conventional techniques designed to increase the quantity and quality of the product to traditional medicine.

2.9 Traditional medicine and cardiovascular disease

For cardiovascular diseases, herbal treatments have been used in patients with CHF, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency and arrhythmia (Mashour et al. (1998)). According to Elwin-Lewis (2000), care should be taken when using herbal medications to treat cardiovascular problems. While some herbs may be worthwhile, many contain natural cardiac glycosides, blood thinners, or affect the blood pressure and are not only bioreactive on their own but can work with prescribed medications to potentiate or diminish their action. For example, ginger contains a potent inhibitor of thromboxane synthetase and thus prolongs bleeding time. Today many herbal remedies are being used prophylactically to maintain and enhance good health or prevent certain conditions from occuring. Since many of these herbal medications are popular and promoted as both safe and efficacious, it is not always possible for a long-term user to understand why this practice could be harmful. Symptoms can vary from trivial to severe and are particularly disconcerting when they affect the heart or blood pressure.

2.10 Traditional medicine for the treatment of hypertension

The objective of this section is to show the large amount of research being conducted by other researchers on plant extracts for the treatment of hypertension. Hypertension and the heart disease that inevitably proceeds from it is given credit for killing more people each year than virtually all other natural causes of death combined (Nyerges, 1997). It is one of the most prevalent and important health problems in developed as well as developing countries. Overall 18-54% of the world's population is hypertensive, 12% of deaths are caused by hypertension and its direct effects and 20% of the general population should expect to have high blood pressure during their life (Faraji and Tarkhani, (1999)). In 90% of cases the etiology of hypertension is unknown (essential hypertension).

Reserpine was one of the first drugs used on a large scale to treat systemic hypertension (Mashour et al. (1998)). It lowers blood pressure by decreasing cardiac output, peripheral vascular resistance, heart rate and renin secretion. Several other plants are also used to treat hypertension today. For example, *Stephania tetandra, Uncaria rhynchophylla* and the root of *Lingusticum walliichii* are sometimes used in traditional Chinese medicine to treat hypertension (Mashour et al. (1998)). Tetrandrine, an alkaloid extract of *S. tetrandra*, has been shown to be a calcium ion channel antagonist, paralleling the effects of verapamil (Mashour et al. (1998)).

Screening of plants for antihypertensive effects in traditional medicines has been performed over many years and several models have been utilised (Hansen et al. (1995)). In the treatment of hypertension, inhibition of the angiotensin-converting enzyme (ACE) is established as one modern therapeutic principle (Hansen et al. (1995)). Elbl and Wagner (1991) introduced an in vitro assay for the detection of ACE inhibitors in plant extracts (Hansen et al. (1995). By utilising this technique, a number of plant species, namely, *Allium ursinum* (Liliaceae), *Amelanchier ovalis* (Rosaceae), *Cistus clusii* (Cistaceae), *Lespedezae capitata* (Fabaceae) have been found to be active and 3 classes of compounds with potential ACE-inhibitory activity have been isolated: flavonoids, procyanidins and peptides.

Hansen and co-workers (1995) investigated 31 plant species from different types of traditional medicine as potential ACE inhibitors. A list of some of the plants selected is given below: *Desmodium styracifolium* was used as a diuretic (Hansen et al., 1995), *Crataegus pinnatifida* had been used as decoction for the treatment of hypertension in China for thousands of years (Hansen et al., 1995), and *Fructus Crataegi* (*C. pinnatifida*) had a cardiovascular effect.

Nyman and co-workers (1998) investigated plants utilised as traditional medicines in India for ACE inhibition. Some of the plant species collected are mentioned below: *Adhatoda vasica* (Acanthaceae), *Aerva tomentosa* (Lamiaceae),

Alternanthera sessilis (L.) DC, *Amaranthus spinosus* L., *Cyperus rotundus* L. and *Scleria lithosperma* Sw were used as diuretics for the treatment of hypertension.

Duncan and co-workers (1999) investigated 20 plants used by traditional healers in South Africa for the treatment of hypertension for their anti-hypertensive properties, utilising the ACE assay. Some of the plants used are mentioned below: *Adenopodia spicata*, *Agapanthus africanus*, and *Hypoxis colchicifolia*, have been used in the treatment of heart disease and blood pressure disorders.

Noumi and co-workers (1999) conducted a study on plants used for the treatment of hypertension. Some of the plants collected were *Annona muricata* L. (Annonaceae) used in the treatment of hypertension, *Cymbopogan citrates*, *Milletia sanagana* (Fabaceae) and *Jatropha curcas* L. (Euphorbiaceae) used as diuretics. It was found that 26 plants were traditionally used to *cure* hypertension.

Faraji and Tarkhani (1999) evaluated the effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension. Sour tea was used traditionally to treat hypertension (Faraji and Tarkhani, 1999).

Salah and co-workers (2001) analysed *Ruellia praetermissa* for its constituents and inhibitory effects on ACE.

Guerrero and co-workers (2002) assessed the antihypertensive and vasodilator effects of ethanolic extracts of some Colombian medicinal plants. *Calea glomerata* Klatt, Croton schiedeanus Schlecht, *Curatella americana* L., *Lippia alba* (Mill)n N.E.Br. and *Lupinus amandus* were among the medicinal plants used in Colombian folk medicine for the treatment of hypertension (Guerrero et. al., 2002). *C. schiedeanus*, *C. Americana* and *L. amandus* showed significant antihypertensive activity.

2.11 Traditional medicine and the modified isolated perfused rat heart

The objective of this section was to establish that the method of using the isolated perfused heart as a model, was acceptable.

Pennacchio and co-workers (1995) investigated the cardioactive effects of an aqueous extract obtained from the leaves of the traditional Aboriginal medicinal plant of *Eremophilia alternofilia (E. alternofilia)* on isolated hearts of normotensive rats using

the Langendorff heart preparation. Male and female Wistar rats of body mass between 400 and 600g were used. The hearts were perfused retrogradely with modified Krebs-Henseleit (K-H) solution. A solution of the extract was administered retrogradely through a polyethylene cannula over a period of one minute. The results showed that the crude aqueous extract mediated an initial, but transient, positive inotropic effect followed by an immediate decrease. In 1996, in an extension of the above-mentioned study, Pennacchio and co-workers described the cardioactive effects induced by the aqueous and methanolic extracts of the leaves of *E. alternofilia*. Isolated hearts were prepared in a similar manner as described in the abovementioned study. Verbascoside and geniposidic acid were identified from two different species of *Eremophila* and caused significant, but opposite, changes in the Langendorff rat heart preparation.

Khatib and co-workers (1997) investigated the cardiovascular effects of *Rosmarinus officinalis (R. officinalis)* extract on the isolated intact rabbit heart. An aqueous extract prepared from Rosemary, *R. officinalis* L. (Lamiaceaea), was prepared and its potential effects on certain cardiovascular parameters such as LVP, CF and HR were studied. The hearts were perfused according to the Langendorff method. The LVP was recorded through a glass cannula inserted into the LV via the LA and linked to a recorder via a pressure transducer. The extract was dissolved in K-H solution and the hearts were perfused for a period of 10 minutes with the extract after 30 minutes stabilisation period. Extract concentrations of 1.4, 14 and 140mg/l were tested. Results showed that all the tested concentrations produced a significant increase in LVP over the entire 10 minutes period. None of the concentrations induced any significant effect on HR.

Thus, the method of using the isolated perfused rat heart as a model for this study was indeed acceptable.

2.10 Aim of the study

The aim of this study was to evaluate the effect of *L. leonurus* aqueous extract on the isolated perfused rat heart in order to make recommendations regarding the use of this plant in traditional medicine for the treatment of hypertension and heart failure.

2.11 Hypothesis

L. leonurus increases the heart rate and force of myocardial contraction of the isolated perfused rat heart.

CHAPTER 3

CARDIAC FUNCTION

The objective of this section is to describe how a normal mammalian heart functions within the body and how its function or performance can be influenced or affected by certain factors such as preload, afterload, heart rate and myocardial contractility.

3.1 Coronary blood flow

The flow of blood through the many vessels that pierce the myocardium is called the coronary (cardiac) circulation (Tortora and Grabowski (1993)). Blood enters the heart via the SVC and IVC into the RA. From the RA the blood flows through the TV into the RV that serves to pump blood to the lungs for the exchange of gases. The blood is ejected through valves in the PA. Upon return from the pulmonary vascular system the blood enters the LA via the PV. Blood flows through the MV to the thicker walled LV that serves to forcefully pump blood through the AV into the aorta and to the systemic vascular system (Fig 3.1, Mc Cance and Huether (1990)).

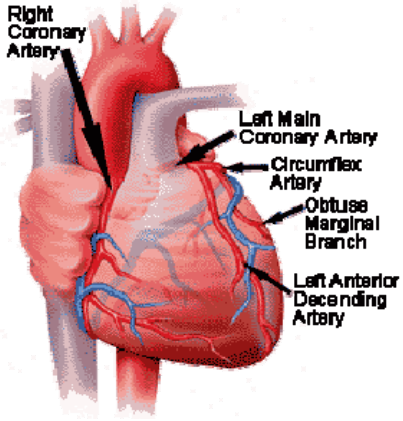

Figure 3.1: Blood flow in the heart (anterior view)
(Mc Cance and Huether (1990)).

3.2. Cardiac output

The CO is the volume of blood put out by one half (left or right) of the heart in unit time (Ross (1972)). *In vivo*, this must obviously be the same for the left and right ventricles, since they are directly related via the pulmonary vasculature (Ross (1972)).

3.3. Stroke volume

The SV is the output of one ventricle in a single contraction cycle (Ross (1972)), therefore SV x HR = CO. The determinants of the SV are: preload, afterload, inotropic state of the myocardium (cardiac contractility), frequency of contraction (HR), and uniformity (or lack of it) of electrical and mechanical function of the ventricle. (Horacio and Donald (1995)).

3.4 Factors affecting cardiac function

Four factors affect cardiac function or performance directly: preload, afterload, HR and myocardial contractility (Mc Cance and Huether (19900). Preload and afterload depend on both the heart and the vascular system. HR and myocardial contractility are characteristics of the cardiac tissue and are influenced by neural and humoral mechanisms (Mc Cance and Huether (1990)). To understand the role of these factors in cardiac performance, it is first necessary to understand two physical laws that explain the mechanisms of heart action: the Frank Starling law of the heart and Laplace's law.

3.4.1 Frank-Starling's law of the heart

The energy of contraction is a function of the initial length of muscle fibres: in the case of the heart, the force of ventricular contraction is proportional to the volume of that chamber at the end of diastole (Ross (1972)). This is Starling's law of the heart and is a simple explanation of the extra work done by the perfused heart when a medium is allowed to flow into the LV from the left atrial cannula (working heart). In the Langendorff preparation, little or no ventricular filling occurs (Ross (1972)).

In this specific study, the cardiac function was indeed determined from pressure differences generated by the LV. The volume of the LV was determined by the volume or the degree of inflation of the balloon in the LV.

3.4.2 Laplace's law

In Laplace's law, wall tension is related directly to the product of intraventricular pressure and internal radius and inversely to the wall thickness (Mc Cance and Huether (1990)). In other words, the amount of tension generated in the wall of the ventricle (or any chamber or vessel) to produce a given intraventricular pressure depends on the size of the ventricle (Mc Cance and Huether (1990)).

3.5 Preload

Preload depends on the volume of blood that fills the ventricles at the end of the diastole, the EDV (Tortora and Grabowski (1993)). The greater the EDV, within limits, the more forceful the contraction. The length of ventricular diastole and venous pressure are the two key factors that determine EDV (Tortora and Grabowski (1993)). In this study, the preload depends on the volume of the balloon that fills the LV at the end of the diastole.

3.6 Afterload

Ejection of blood from the heart begins when pressure in the right ventricle exceeds the pressure in the pulmonary trunk (about 20 mmHg) and the pressure in the LV exceeds the pressure in the aorta (about 80 mmHg), (Tortora and Grabowski, 1993). Thus afterload is the pressure that must be overcome before the semilunar valves can open. Afterload involves a force-velocity relationship, that is, the lighter the afterload, the faster the contraction, and the heavier the afterload, the slower the contraction (Mc Cance and Huether (1990)).

3.7 Heart rate

Homeostatic mechanisms strive to maintain an adequate CO by increasing HR and strength of contraction (Tortora and Grabowski (1993)). The SA node initiates contraction, and left to itself would set a steady HR.

Several factors contribute to the regulation of HR, the most important being the neural factors, including neural reflexes, and hormonal and chemical factors. Neural control is exerted by both the central and autonomic nervous systems. Hormonal factors include the catecholamines (epinephrine and norepinephrine), thyroid hormones, growth hormones and pancreatic hormones (Tortora and Grabowski (1993)). The isolated heart is denervated allowing the separation of cardiac from sympathetic and vagal stimulation. However, denervation and the absence of other peripheral factors can often be compensated for, thus catecholamines or other neurotransmitters may be included in the perfusates and many other peripheral factors can be added exogenously and in a controlled manner.

3.8 Myocardial contractility

Stroke volume, or the volume of blood ejected during systole, depends on the force of contraction, which depends on myocardial contractility, or the degree of myocardial fiber shortening (Mc Cance and Huether (1990)).

Two major factors determine the force of contraction: (1) changes in the stretching of the ventricular myocardium caused by changes in ventricular volume (preload) and (2) alterations in the sympathetic activation of the ventricles (Mc Cance and Huether (1990)). Myocardial contractility is difficult to measure because measurement requires keeping preload, afterload and HR constant. Factors affecting contractility are called inotropic agents. Positive inotropic agents increase the velocity of myocardial contraction and SV. These include excess thyroid hormone, epinephrine, norepinephrine, dopamine or isoprotereol infusion and calcium salt infusion. Negative inotropic agents decrease the velocity of myocardial contraction and SV. These include alcohol, procainamide, quinidine and propranolol (Mc Cance and Huether (19900).

Myocardial contractility is also affected by the oxygen and carbon dioxide levels in the coronary blood (Mc Cance and Huether (1990)). With severe hypoxemia (arterial oxygen saturation less than 50%), contractility is decreased. With less severe hypoxemia (saturation more than 50%), contractility is stimulated (Mc Cance and Huether (1990)).

CHAPTER 4

THE MODIFIED LANGENDORFF HEART MODEL

This section gives a detailed description of isolated perfused heart models that have been employed in previous studies and how the donor heart, perfusion solution and the type of isolated heart preparation were selected.

4.1 The isolated perfused heart model

Isolated heart preparations are used to study physiological and metabolic parameters of the heart independently of its environment (Depre (1998)). Several preparations of IPH are currently used. These are the retrograde perfusion system originally developed by Langendorff (1895) and the working heart models, developed by Neely et al. (1967). In the 1960s and 1970s, such models led to the understanding of metabolic regulation in the heart. These models were also used to investigate the influence of heart work and hormones on cardiac metabolism and the effects of substrate supply on heart function and metabolism. Now these models are used to investigate the effects of various drugs on the heart. IPH preparations have been useful in studies designed to elucidate cardiac function and metabolism, in part, because they permit precise control of the determinants of MOC and CF, of concentrations of substrates, other metabolites and hormones and of pH, P_{O2} and P_{CO2} in the perfusate (Bergmann et al. (1979)). Conventionally, such preparations are perfused with blood-free media for convenience and to avoid difficulties related to thrombosis or metabolic effects of anticoagulants, aggregation of cellular elements, of haemolysis (Bergmann et al. (1979)).

At a practical level, the isolated heart, especially from small mammals, provides a highly reproducible preparation that can be studied quickly and in large numbers and at relatively low cost (Sutherland et al. (2000)). It allows the examination of cardiac chronotropic, inotropic and vascular effects without the complications of an intact model (Johnson et al., 1991 and Chinchoy et al. (2000)).

The isolated perfused non-ischaemic rat heart model employed in this study was based on the technique first described by Langendorff (1895) and subsequently modified and improved by many other workers. Definitive interpretation of data in studies of this type with IPH requires precise control of factors that influence cardiac performance, oxygen consumption, metabolism and CF (Bergmann, et al. (1979)).

4.2 Basic principles

During a normal cardiac contraction in a mammalian heart, the blood stored in the LV is ejected at a pressure of about 80-100 mmHg into the aorta. At the base of the aorta is an ostium (hole) that feeds blood under this pressure into the coronary arteries. Langendorff maintained the isolated heart through the use of a reservoir with a pressure head that was connected via a tube to the aortic cannula. When the reservoir was opened, the perfusate was forced through the ostia into the coronary bed. This is often termed retrograde perfusion, in the sense that the perfusate flows down into the aorta rather than out the LV through the aorta, as blood does *in situ.*

4.3 Selection of the donor heart

The heart from any mammalian species together with non-mammalian hearts such as those from frogs or birds may be perfused (Sutherland et al. (2000)). However, isolated perfusion of large animal hearts such as pigs, as described by Chinchoy et al. (2000)), monkeys, sheep, dogs and even man have been reported. These are less frequently used on account of high cost, greater variability, large volumes of perfusion fluids and cumbersome equipment that is required. Also, the isolated perfused rabbit heart has been shown to be a reliable model for the assessment of electrophysiologic activity of pharmacologic agents (Johnson et al. (1991)) but this species suffers problems with anaesthesia. The rat heart is most frequently studied and has a great advantage over smaller hearts such as the mouse where intraventricular pressure recordings are more difficult. However, the rat also suffers from a limitation, namely its very short action potential duration that can limit its value of some studies of arrthymogenesis and anti-arrhythmic drugs.

4.4 Selection of perfusion solution

The majority of studies in literature are based on a bicarbonate perfusion fluid as defined by Krebs and Henseleit (1932). This perfusion fluid mimics the key ionic content of blood or plasma and has a pH of 7.4 at 37ºC and has the following composition (in mM): NaCl l18.5, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.0 and $CaCl_2$ 2.5 (Krebs and Henseleit (1932)). The ionic components of the media are important and vary with the species; the potassium and calcium are the most variable and critical of the ions.

The calcium component is added last and the pH of the solution is lowered by gassing with 95% O_2 + 5% CO_2 (carbogen) before adding the calcium. All media are filtered before use to remove particulate impurities that can be present in even the purest commercial chemical. Substrates are added to support the large energy requirements of cardiac contractile function. High concentrations of glucose are usually added as the sole substrate. The choice of glucose as the sole substrate in most heart perfusion relies on the heart's ability to utilise almost any substrate as an efficient energy source. This is despite the fact that, *in vivo*, fatty acids are the predominant energy source. The general practice of avoiding fatty acids as an energy source results primarily from the difficulty of dissolving these agents in aqueous solutions and the complication of frothing when the fatty acid-containing solutions are gassed. Insulin can be added to aid in glucose utilisation (Chinchoy et al. (2000)). Sodium pyruvate can be added as an additional energy substrate and mannitol added to increase the perfusate osmolarity, thus reducing cardiac oedema (Chinchoy et al. (2000)). Drugs or other agents can be added to any perfusion solutions.

K-H solution has been adopted, almost without exception, as the physiological perfusion medium by most workers employing the isolated rat heart (Perkin (1987)). The osmotic pressure of this buffer is 79 kPa at 37ºC that equates to that of blood plasma (75 kPa), (Perkin (1987)). This pressure is sufficient to ensure that the cells are not damaged by osmotic imbalance when they are exposed to K-H solution (Perkin, (1987)).

4.5 Selection of the type of isolated heart preparation

The IPH preparation facilitates the perfusion of the physiological salt solution either under constant hydrostatic pressure or at constant volume. Isolated hearts are typically perfused either at constant pressure using a large perfusate reservoir positioned above the preparation or constant flow using a peristaltic pump (Shattock et al. (1997)). Both approaches have their advantages and disadvantages – for example, while constant pressure may be more physiological, constant flow is easier to use with blood-based perfusates or expensive drugs where perfusate volumes are restricted. Switching between constant flow and constant pressure modes of perfusion is not straightforward and, with simple apparatus, may not be feasible within a single experimental protocol. Shattock et al. (1997) developed an electrical feedback system designed to control an IPH perfused with a peristaltic pump. The constant flow heart perfusion method was successfully employed in previous studies by Man and Lederman (1985) and Altup et al. (2001) and the constant pressure heart perfusion method was successfully employed by Johnson et al. (1991) in the isolated rabbit heart.

4.6 Anaesthesia and excision of the donor heart

The anaesthetic(s) to be used depends on the donor, potential problems with side effects in the experimental protocol, the extent of surgical procedures and the regulations of your Animal Care and Use Committee (Sutherland et al. (2000)). Anaesthesia can be induced by inhalation of agents such as ether, halothane and methoxyflurane. Ether is hazardous as it is highly flammable and irritant to the animal and must only be used in a well-ventilated area. The most common and widely used anaesthetic is pentobarbitone via an intravenous or intraperitoneal route (Sutherland et al. (2000)). Whatever the choice of procedure, stress to the animal should be minimised by keeping the animal in a quiet environment prior to anaesthesia and minimum handling of the animal. Induction of anaesthesia should be as swift as possible, with the perception of pain completely suppressed (Sutherland et al. (2000)). Once the animal is anaesthetised the heart can be excised. The thorax is opened by an incision along the lower margin of the last to the first ribs, exposing the heart.

The pericardium is removed and the organ supported by cradling the heart between the fingers and cutting across the arch of the aorta and vena cava. This entire process can be accomplished in less than 30 seconds although some investigators report times as long as 10 minutes (Sutherland et al. (2000)). The heart is immediately immersed in cold perfusion solution (4ºC) gassed with carbogen to limit any ischaemic injury during the period between excision and the restoration of the vascular perfusion (Sutherland et al. (2000)).

4.7 Cannulation and re-establishment of vascular perfusion

The aortic perfusion cannula can be constructed from a variety of materials including glass, plastic or thin walled stainless steel. The external diameter is typically similar to, or slightly larger than that of the aorta (about 3mm for a heart from a 250g rat). According to Sutherland et al. (2000), it is advisable to have the perfusion fluid gently dripping from the aortic cannula prior to cannulation since this helps to minimise the chance of air emboli at the time the heart is attached to the cannula. A water-jacketed reservoir, situated above the aortic cannula contains the perfusion solution that is oxygenated via a sintered glass gas distributor with carbogen. The cannula is inserted and the aorta is clamped with an artery clip or with a blood vessel clamp such as Diffenbach serafine (Sutherland et al. (2000)). Care must be taken not to puncture the aorta. The aorta is then securely tied and the clamp removed. Full flow of the perfusate is initiated once the heart is mounted on the cannula. The most critical part of the preparation is the delay in time from the removal of perfusion in the donor to the reperfusion of the heart, since this organ has only the oxygen and substrate contained in the vessels at the time of removal to sustain itself.

4.8 Insertion of balloon

Once cannulation is completed and coronary perfusion initiated, contractile function and regular heart rhythm will return within a few seconds but it may be 10 minutes or more before maximum function is established. Contractile activity can be assessed via a side strain gauge attached to the apex of the heart or an open tip pressure transducer inserted into the LV.

The preferred procedure involves the insertion of a compliant intraventricular balloon. This method was previously employed by Bergmann et al. (1979), Inamdar et al. (1994), Frolkis and Beruk (1998) and Depre (1998). The balloons are often made from thin silicone rubber or domestic food wrap and provide an ideal means of measuring isovolumic left ventricular function and HR, as is required in the present study. The left atrial appendage is removed and a deflated balloon is inserted into the LV via the MV. This method is described in detail by Sutherland et al. (2000). Care should be taken while inserting and securing the balloon since it is very easy to damage the heart. The balloon is inflated with water until a LVEDP of 4-8 mmHg is obtained. The LVEDP is achieved by appropriate adjustment of the volume of the balloon. Left ventricular systolic, diastolic and developed pressures can be measured once the balloon is in position.

4.9 Measurement of the left ventricular pressure and the heart rate

The fluid-filled latex balloon inserted into the LV is connected to a pressure transducer via a short length of polyethylene tubing. The balloon is inflated to induce a LVEDP and is adjusted by changing the volume of the balloon. As the heart contracts, it develops a LVSP which can be recorded along with the HR, dP/dt and CPP (Bergmann et al. (1979)). LVP and dP/dt are obtained by electronic differentiation and are recorded continuously. CPP is monitored continuously with the use of a side arm cannula immediately distal to the AV (Bergmann et al. (1979)). The volume rate of coronary flow can be measured by the volume of fluid flowing out of the isolated heart per minute (Depre (1998)).

CHAPTER 5

MATERIALS AND EXPERIMENTAL METHODS

This section gives a detailed description of the model that was employed in the present study and information regarding the selection criteria of the plant material, perfusion solution and the donor animal, the preparation of the plant extract and the physiological parameters monitored throughout the study.

5.1 Plant Material

5.1.1 Selection, Collection and Identification of *Leonotis leonurus*

According to Williamson et al. (1996), one major criterion for the selection of a plant for such study is traditional healers' claims for its therapeutic usefulness. If a traditional healer claims success in the treatment of a particular disease, the scientist working from this selection criterion would expect to find, in the plant extract, a chemical constituent with an appropriate pharmacological activity. *L. leonurus* has become a popular medicinal plant in South Africa. Many traditional uses for *L. leonurus* have been recorded and its usefulness in the treatment of hypertension is well documented (SATMERG (2003), Hutchings et al. (1996), Watt and Breyer - Brandwijk (1932), Van Wyk et al. (2000)). One major problem, however, in using literature for selection of plants is that in many cases the exact use of the medicinal plants is not described in as much detail as required, neither dosages, dosage forms, nor the kind of diseases treated is reported in any detail (Verproote (1989)).

L. leonurus was selected on the basis of traditional usage. This is a popular basis for investigation, especially in societies where traditional medicine of some sort is a major form of health care. The plant material was collected from Kirstenbosch Botanical Garden, Kirstenbosch, South Africa. The identification of the plant material was confirmed by Dr. Gillian Scott, taxonomist of the 'South African Traditional Medicines Research Group (SATMERG)', School of Pharmacy, University of the Western Cape (UWC), South Africa.

5.1.2 Preparation of an aqueous extract of *Leonotis leonurus*

According to SATMERG (2003), *L. leonurus* is mainly used in the form of an aqueous decoction, orally, per rectum and as a topical application. The decoction is usually prepared by adding one tablespoon of chipped dried herb (10.0g) to 3 cups of boiling water and boiled for 10 minutes. The decoction is then cooled overnight, strained and used as a clean liquid for both internal and external use.

There are various extraction methods for screening plants for activity. However, the choice of an aqueous extraction procedure in this study was based on the above information from SATMERG (2003) regarding its preparation for internal use. This procedure was previously described by Veale et al., 1989. The leaves and smaller stems were dried in a ventilated oven. For the sake of reproducibility in the laboratory, only material that was dried in a ventilated oven at a temperature not exceeding 35ºC and then allowed to regain the air-dry state (±8% moisture) was used. The material was milled and the powder was passed through a sieve of mesh size of 850µm. Boiling distilled water was added and the mixture was stirred and then strained through glass wool. The filtrate was freeze-dried for 24 hours using 200ml boiling distilled water to 5mg powdered material. The yield of extract was ±1g/5g powder.

5.2 Modified Krebs-Henseleit buffer solution

The modified K-H bicarbonate buffer solution was previously discussed and described by Bergmann et al. (1979), Belo and Talesnik (1982), Man and Lederman (1985), Perkin (1987), Johnson et al. (1991), Hu et al. (1991), Venkataraman et al. (1993), Inamdar et al. (1994), Fujita et al. (1998), Khatib et al. (1998), Chinchoy et al. (2000), Altup et al. (2001) and Zhang et al. (2002). It has been recorded that it is a suitable and reliable medium for retrograde perfusion.

The solution had the following composition in mM:

Sodium Chloride	(NaCl)	:	118
Sodium Bicarbonate	($NaHCO_3$)	:	26.2
Potassium Chloride	(KCl)	:	4.7
Potassium Dihydrogen Phosphate	(KH_2PO_4)	:	0.9
Magnesium Sulphate	($MgSO_4.7H_2O$)	:	1.18
Calcium Chloride	($CaCl_2$)	:	1.8
Anhydrous D-glucose	($C_6H_{12}O_6$)	:	11.2

The perfusion solution was pumped through a Millipore filter of pore size 0.45µm to minimise particulate embolisation. It was oxygenated with carbogen, supplied by Afrox Limited®, to maintain an arterial P_{O2} of 400-500 mmHg and a pH of 7.35-7.40 (Bergmann et al. (1979), Belo et al. (1982) and Johnson et al. (1991)). The hearts were perfused with K-H solution at 37.0-37.5˚C controlled by a thermostatically-controlled water-jacketed system in which all glass reservoirs, the heart perfusion chamber and as many of the delivery lines as possible are surrounded by rapidly flowing water at 37.0-37.5ºC. All solutions were freshly prepared and stored at 4ºC between experiments.

5.3 Experimental animals

Male Wistar albino rats weighing approximately 250-350g in body weight, aged less than 6 months were obtained from the animal unit at the Department of Physiology, University of Stellenbosch, South Africa. Male Wistar albino rats were used in previous isolated heart studies described by investigators such as Altup et al. (2001), Frolkis and Beruk (1998) and Venkataraman et al. (1993). The animals were housed with a 12-hour light, 12-hour dark cycle at 25ºC and supplied with standard laboratory diet and tap water. They were housed individually in a perspex animal cage for 30 minutes before starting an experiment in order to habituate them to the new environment.

5.4 Animal preparation

The detailed methodology for the modified isolated heart perfusion system has previously been described by Hu et al. (1991) and Man and Lederman (1985). Non-fasted, male Wistar albino rats were anaesthetised with 30mg/kg sodium pentobarbitone via the intraperitoneal route. The thorax was opened and the pericardium removed. The heart was then rapidly excised in less than 30 seconds (Sutherland et al., 2000) and placed in cool K-H solution (4°C) aerated with carbogen. The heart was cannulated at the aorta as described by Langendorff (1895) and immediately perfused retrogradely with oxygenated non-recirculating K-H solution for coronary perfusion. The perfusate was gassed with carbogen and a thermostatically-controlled water-jacketed system maintained the temperature of the circulated water that enveloped the perfusate chambers, the oxygenator and the chamber containing the heart. The aorta was tied securely and any excess tissue was trimmed. A water filled domestic wrap balloon connected to a pressure transducer was inserted into the LV. The balloon was inflated. A thermometer probe was inserted into the RV to monitor the temperature.

5.5 Constant Flow

The preparation developed schematically in Fig 5.1 was a modified version of the isolated perfused rat heart at constant flow.

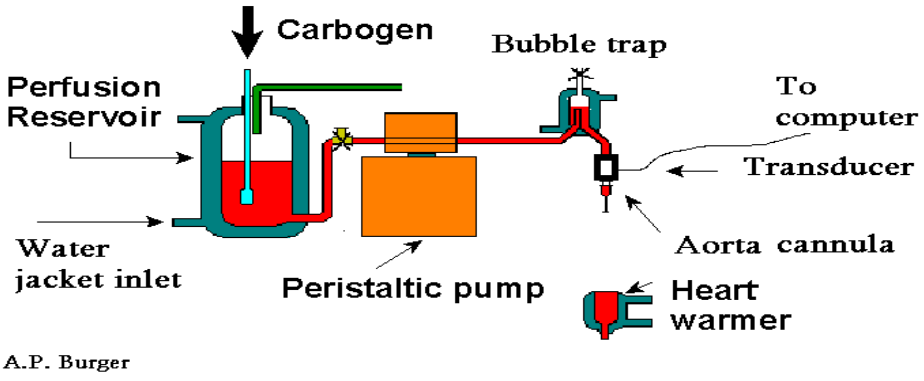

Figure 5.1: Modified constant flow perfusion apparatus (Figure supplied by A.P. Burger-personal communication)

The constant flow model above was employed in this study. There were many reasons for choosing this model, one reason being the availability of equipment for accurately detecting changes in coronary resistance. Also, due to the limited availability of the extract and the high cost of the reference drugs. A constant pressure model could have provided more useful information. The constant model is, however, in itself a well-accepted model for doing this type of study. The advantages and disadvantages of both models were previously discussed.

5.6 Drugs and chemicals

The following drugs were used in the study: Sodium pentobarbitone 6% solution [Kyron Laboratories (Pty) Ltd] was used for anaesthesia in all the animals. Adrenotone (adrenaline) ampoules containing ADR base 1 mg/ml [SCP Pharmaceuticals (Pty) Ltd] and DIG intravenous injections each 2ml containing 0.5mg DIG [GlaxoWellcome)] were used as reference. They were selected because of their known positive inotropic effects.

Infusions of *L. leonurus* extract for heart perfusion were freshly prepared daily by dissolving a given quantity of the dried extract in a pre-filtered solution of K-H solution.

5.7 Perfusion of the heart

The target perfusion pressure range was 70-80 mmHg and was obtained by appropriate adjustment of the pump flow rate. Predetermined quantities of the *L. leonurus* extract were dissolved in and diluted with K-H solution to concentrations of 0.1, 0.5, 1.0 and 2.0 mg/ml. ADR and DIG solutions were diluted with K-H solution to concentrations of 0.1 and 1.0 µM. and 0.25, 2.5, 25, 250 and 2500 ng/ml, respectively. All solutions were filtered through a Millipore filter with a pore size of 0.45 µm and were heated in a water bath at a constant temperature and equilibrated for 15 minutes. The temperature of all solutions was maintained at 37 ± 0.5°C and the hearts were perfused at constant flow with samples for a period not exceeding 3 minutes.

This time period was chosen because at low dose *L .leonurus* aqueous extract increases the systolic BP and has no effect on the HR in normotensive rats after 3 minutes intravenous infusion (Mugabo et al. (2002)). However, Khatib and co-workers (1998) perfused the hearts for 10 min.

5.8 Protocol

Hearts were randomly assigned to receive one of the twelve infusion regimens listed in Table 5.1

Table 5.1: Experimental schema

Infusions I, II and III were performed by switching from the main perfusion line (K-H solution) to the side-arm line (test material). The system was allowed 20min recovery period between each infusion.

Infusion regimen	**Infusion I (3min)**	**Infusion II (3min)**	**Infusion III (3min)**
A	K-H solution	K-H solution	K-H solution
B	ADR 0.1 µM	ADR 0.1 µM	ADR 0.1 µM
C	ADR 1.0 µM	ADR 1.0 µM	ADR 1.0 µM
D	DIG 0.25 ng/ml	DIG 0.25 ng/ml	DIG 0.25 ng/ml
E	DIG 2.5 ng/ml	DIG 2.5 ng/ml	DIG 2.5 ng/ml
F	DIG 25 ng/ml	DIG 25 ng/ml	DIG 25 ng/ml
G	DIG 250 ng/ml	DIG 250 ng/ml	DIG 250 ng/ml
H	DIG 2500 ng/ml	DIG 2500 ng/ml	DIG 2500 ng/ml
I	LL 0.1 mg/ml	LL 0.1 mg/ml	LL 0.1 mg/ml
J	LL 0.5 mg/ml	LL 0.5 mg/ml	LL 0.5 mg/ml
K	LL 1.0 mg/ml	LL 1.0 mg/ml	LL 1.0 mg/ml
L	LL 2.0 mg/ml	LL 2.0 mg/ml	LL 2.0 mg/ml

In Regimen A, one group of rats received an infusion of drug-free K-H solution in a manner comparable to the drug-infused hearts, to evaluate the preparation stability (Johnson et. al. (1991)).

In all regimens, physiologic measurements ('Baseline values') were obtained after 20 minutes recovery period before the initial infusion was started. After 3 minutes of infusion I, physiologic measurements were repeated. Infusion II was then started and after 3min, physiologic measurements were again repeated. Infusion III was then started for 3min and once again the physiologic measurements were obtained. The infusions were then discontinued and a final set of measurements was obtained.

5.9 Physiological parameters monitored

The following physiological parameters were monitored and recorded in each experiment, throughout the study: left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), heart rate (HR), cardiac work (CW), coronary perfusion pressure (CPP) and the perfusate temperature.

The LVSP, LVEDP and LVDP were continuously monitored via a Statham^{R} (SP1400) pressure transducer connected to the water-filled domestic wrap balloon inserted into the LV. CPP was monitored via a Statham^{R} pressure transducer connected to the side-arm of the aortic cannula. A computer-based system was used for continuous data acquisition. The software determined the HR from the different pressure readings. Temperature was continuously monitored via a thermometer probe inserted into the right ventricle of the heart. The raw data was analysed using a custom component of the software. This software averaged the values for a particular selection of data points. The raw data was exported to Microsoft Excel® and the means of each variable was determined. CW was determined by calculating the product of the LVDP and the HR in Microsoft Excel®.

The following values were determined for data analysis using Microsoft Excel®: 'Control, Baseline, Peak and 3 min'. The 'Control value' was the value in the parameter during heart perfusion with pure K-H solution. The 'Baseline value' was determined, for all physiological parameters, during the recovery period just before the perfusion of the aqueous extract or the control drug.

The ‘Peak value’ was measured over 10 seconds at the point of maximum increase or decrease in the parameter assessed. The ‘3min value’ was the time period for heart perfusion with either the reference drugs or the plant extract. All the drugs exerted their Peak effects before the 3min perfusion period.

Data was expressed as the percentage (%) change from the ‘Baseline value’. The % changes for LVSP, LVEDP, LVDP, HR, CW and CPP from ‘Baseline to Peak’ and ‘Baseline to 3min’ were calculated.

The equation for calculating % change that was used in this study is shown below:

$$\% \text{ change} = \frac{(\text{experimental value} - \text{baseline value}]}{\text{baseline value}} \times 100\%$$

The ‘Experimental value’ was calculated as the percentage change value from baseline to peak and from baseline to 3min for all physiological parameters being assessed.

5.10 Statistical analysis

The Mann-Whitney U test (2-tailed) was used to compare the data for 2 independent samples (SPSS for Windows®). The results were expressed as mean ± standard deviation (mean ± SD). The data with p values equal or less than 1% ($p < 0.01$) were considered statistically significant and marked with an asterisk (*) in the tables of results.

5.11. Ethics approval

Approval was obtained from the Ethics Committee of the University of the Western Cape (UWC) and the animals were treated according to the recommendations of the UWC’s ethical regulations concerning animal experiments.

CHAPTER 6

RESULTS

This chapter only includes the presentation and description of the results obtained in the study with the associated p-values. The results are fully discussed in the following chapter.

6.1 Krebs-Henseleit (K-H solution): The possible influence of switching to a new solution

The data recorded in Table 6.1 shows the % change from baseline (mean ± SD) in the parameters under investigation resulting from switching between the main perfusion line containing K-H solution and the side-arm line also containing K-H solution. Six hearts were randomly assigned and perfused according to Regimen A (Table 5.1).

Table 6.1: ***K-H solution***

This table shows the percentage change resulting from switching between K-H solution contained in both perfusion lines of the experimental set-up of the isolated perfused rat heart [Regimen A]. Data expressed as % change from baseline (mean ± SD) *****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

n	LVSP	LVEDP	LVDP	HR	CW	CPP
1	-0.89	2.80	-0.45	1.58	1.12	-0.38
2	-0.59	2.45	-0.55	-0.50	-1.05	1.75
3	0.14	5.87	0.678	0.33	1.01	2.07
4	0.58	2.75	-2.55	-0.85	-3.37	-1.04
5	0.59	1.43	-0.01	-1.76	-1.77	0.88
6	0.36	0.58	-0.22	0.93	0.70	0.47
Mean	**0.03**	**2.65**	**-0.52**	**-0.05**	**-0.56**	**0.63**
SD	**0.63**	**1.80**	**1.09**	**1.23**	**1.82**	**1.20**

6.1.1 Effects of switching between two perfusion lines

Table 6.1 shows the possible influence of switching between the main perfusion line and the side-arm line of the experimental set-up, on the abovementioned parameters. Isolated hearts were perfused according to Regimen A [Table 5.1] and the results [Table 6.1] showed that there were no significant changes from baseline in any of the parameters under investigation after 3min perfusion with K-H solution. Also, no peak values were obtained since the responses were flat.

6.2 Effects on the Left ventricular systolic pressure

Table 6.2: Effects of adrenaline, digoxin and *L. leonurus* extract on the left ventricular systolic pressure

This table summarises the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on LVSP of the isolated perfused rat heart. Data expressed as % change from baseline (mean ± SD)
*****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

	n	Baseline (mmHg)	Peak (mmHg)	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1 µM	4	94.36	99.81	5.77 ± 2.65	4.74 ± 7.11
ADR 1.0 µM	7	90.59	127.41	40.64 ± 22.67 *****	24.31 ± 21.29*
DIG 0.25 ng/ml	5	124.39	128.09	2.97 ± 2.22	1.26 ± 1.49
DIG 2.5 ng/ml	6	114.34	125.17	9.46 ± 5.04	5.14 ± 1.49*
DIG 25 ng/ml	6	125.15	123.67	- 1.18 ± 5.39	- 0.33 ± 2.46
DIG 250 ng/ml	5	118.17	118.01	- 0.13 ± 3.27	- 0.89 ± 3.73
DIG 2500 ng/ml	6	104.23	103.86	- 0.35 ± 2.46	- 2.76 ± 2.36
LL 0.1 mg/ml	5	103.21	113.26	9.73 ± 6.74 *	5.37 ± 2.98 *
LL 0.5 mg/ml	8	97.43	111.86	14.81 ± 5.93 *	7.35 ± 4.90*
LL 1.0 mg/ml	7	105.88	132.74	25.36 ± 8.10 *	23.13 ± 6.63*
LL 2.0 mg/ml	5	91.40	105.03	14.91 ± 12.18 *	- 4.17 ± 9.15

6.2.1 Effects of adrenaline on the left ventricular systolic pressure

ADR 1.0 µM solution significantly increased LVSP at Peak by 40.6% and 24.3% after 3min [$p < 0.01$ baseline versus 1.0 µM ADR, Table 6.2]. The onset of this effect was rapid and quickly reached a Peak value within 30 seconds. LVSP started decreasing almost immediately to a level above pre-administration levels. When perfusion with ADR solution was stopped, the hearts recovered and the physiologic parameters returned to almost initial levels except, however, for LVEDP that continued to gradually increase to above pre administration levels. No statistically significant effect on LVSP was produced by the lower concentration of ADR solution [Table 6.2].

6.2.2 Effects of digoxin on the left ventricular systolic pressure

The results showed that DIG solution at a dose of 2.5 ng/ml increased LVSP by 5.1% after 3min [$p < 0.01$ baseline versus 2.5 ng/ml DIG, Table 6.2] with no significant effect at Peak. LVSP transiently increased to a Peak value and started to decrease to a level above pre-administration levels. All the other tested concentrations of DIG solution produced varying and insignificant effects on LVSP [Table 6.2].

6.2.3 Effects of *Leonotis leonurus* aqueous extract on left ventricular systolic pressure

The results showed that the extract transiently increased LVSP from baseline and produced a maximum response (Peak) within the 1st minute. At Peak, all the tested concentrations of the extract had increased LVSP by 9.7%, 14.8%, 25.4% and 14.9%, respectively [$p < 0.01$ baseline versus (0.1, 0.5, 1.0 and 2.0 mg/ml) *LL*; Table 6.2]. However, the tested concentrations (0.1, 0.5 and 1.0 mg/ml LL) gradually decreased LVSP to a level above pre-administration levels. Thus, after 3min, the responses were much reduced and LVSP was increased by 5.4%, 7.4% and 23.1%, respectively
[$p < 0.01$ baseline versus. (0.1, 0.5 and 1.0 mg/ml) LL; Table 6.2]. On withdrawal of the extract, the hearts recovered with the physiological measurements returning to approximately initial levels.

However, LVSP for *L. leonurus* extract at a dose of 2.0 mg/ml started decreasing almost immediately from the Peak value to zero. LVSP remained at this level over the 3min perfusion period. When perfusion with this extract was stopped [Regimen L], the hearts remarkably recovered and the physiologic measurements returned to approximately pre-administration levels.

6.3 Effects on the Left ventricular end-diastolic pressure

Table 6.3: Effects of adrenaline, digoxin and *Leonotis leonurus* on the left ventricular end diastolic pressure

This table shows the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on LVEDP of the isolated perfused rat heart. Data are expressed as % change from baseline (mean ± SD)**. ***p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

	n	Baseline (mmHg)	Peak (mmHg)	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1 µM	4	5.30	4.71	- 11.06 ± 37.16	- 7.28 ± 6.78
ADR 1.0 µM	7	4.41	3.76	- 14.67 ± 9.96	- 9.77 ± 31.99
DIG 0.25 ng/ml	5	4.41	4.01	- 8.97 ± 5.99	- 9.40 ± 11.09
DIG 2.5 ng/ml	6	6.31	6.55	3.73 ± 4.62	2.51 ± 0.49
DIG 25 ng/ml	6	4.12	4.55	10.33 ± 43.75	28.48 ± 36.85
DIG 250 ng/ml	5	7.04	6.92	- 1.79 ± 9.93	0.14 ± 14.26
DIG 2500 ng/ml	6	4.38	4.55	3.73 ± 5.47	8.18 ± 11.08
LL 0.1 mg/ml	5	4.75	3.97	- 16.44 ± 49.31	- 10.86 ± 24.40
LL 0.5 mg/ml	8	3.48	3.47	- 0.28 ± 40.61	- 9.29 ± 13.29
LL 1.0 mg/ml	7	4.64	9.22	98.62 ± 211.90	37.79 ± 82.66
LL 2.0 mg/ml	5	4.01	5.34	33.00 ± 58.76	101.50 ± 11.74

6.3.1 Effects of adrenaline on the left ventricular end diastolic pressure

When isolated hearts were perfused with ADR, it decreased LVEDP. However, as indicated in Table 6.3, these decreases in LVEDP were not statistically significant.

6.3.2 Effects of digoxin on the left ventricular end diastolic pressure

All the tested concentrations of DIG produced varying and inconsistent effects on LVEDP all of which were not statistically significant [Table 6.3].

6.3.3 Effects of *Leonotis leonurus* extract on the left ventricular end diastolic pressure

When isolated hearts were perfused with *L. leonurus* aqueous extract, it seemed to reduce LVEDP [Table 6.3]. Also, when hearts were perfused according to Regimens K & L [Table 5.1], there appeared to be an elevation in LVEDP. However, as indicated in Table 6.3, the results showed that the aqueous extract produced no significant effect on LVEDP.

6.4 Effects on the left ventricular developed pressure

The effects of ADR, DIG and *L.leonurus* aqueous extract on LVDP of the isolated perfuse rat heart are summarised in Table 6.4. LVDP was one of the indices of contractility that was investigated. LVDP was expressed as the difference between LVSP and LVEDP and was calculated before doing the statistics.

Table 6.4: Effects of adrenaline, digoxin and *Leonotis leonurus* extract on the left ventricular developed pressure

This table shows the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on LVDP of the isolated perfused rat heart. Data are expressed as % change from baseline (mean ± SD)**.** ***p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

	n	Baseline (mmHg)	Peak (mmHg)	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1µM	4	89.06	95.10	9.45 ± 4.13	2.52 ± 1.52
ADR 1.0 µM	7	86.18	124.01	43.90 ± 13.41*****	31.93 ± 11.33*****
DIG 0.25 ng/ml	5	119.98	123.72	3.12 ± 2.66	1.44 ± 1.94
DIG 2.5 ng/ml	6	108.04	118.46	9.65 ± 5.11*	5.27 ± 1.57*
DIG 25 ng/ml	6	121.02	119.12	- 1.01 ± 5.73	- 0.11 ± 2.14
DIG 250 ng/ml	5	111.13	110.77	- 0.21 ± 3.30	- 1.39 ± 3.74
DIG 2500 ng/ml	6	99.85	98.44	- 1.54 ± 2.11	- 1.96 ± 3.64
LL 0.1 mg/ml	5	98.46	109.00	10.71 ± 7.69*	5.49 ± 3.65*****
LL 0.5 mg/ml	8	93.95	108.40	15.38 ± 7.30*	7.79 ± 4.64*****
LL 1.0 mg/ml	7	101.24	130.95	29.36 ± 12.25*	26.35 ± 9.10*****
LL 2.0 mg/ml	5	87.39	99.39	14.88 ± 12.53*	-9.80 ± 10.56

6.4.1 Effects of adrenaline on the left ventricular developed pressure

ADR solution evoked significant increases in LVDP from baseline of 43.9% at Peak and 31.9% over the entire 3min heart perfusion [$p < 0.01$ baseline versus 1.0 µM ADR]. These changes in LVDP corresponded with the changes in LVSP and followed similar patterns as was described for Table 6.2.

6.4.2 Effects of digoxin on the left ventricular developed pressure

Table 6.4 shows that DIG (0.25 and 2.5 ng/ml) increased LVDP both at Peak (9.7% increase) and over the entire 3min perfusion period (5.3%). The effect obtained with DIG 2.5 ng/ml was statistically significant [$p < 0.01$ baseline versus 2.5 ng/ml DIG]. The remaining tested concentrations of DIG did not significantly affect the LVDP [Table 6.4].

6.4.3 Effects of *Leonotis leonurus* extract on the left ventricular developed pressure

The tested concentrations of the aqueous extract of *L. leonurus* (0.1, 0.5 and 1.0 mg/ml) produced significant increases in LVDP at Peak, of 10.7%, 15.4% and 29.4%, respectively [$p < 0.01$ baseline versus (0.1, 0.5 and 1.0 mg/ml) *LL*, Table 6.4]. Similarly, these effects on LVDP were significantly lower after 3min and the extract increased LVDP by 5.5%, 7.8% and 26.3%, respectively [$p < 0.01$ baseline versus (0.1, 0.5 and 1.0 mg/ml) *LL*, Table 6.4].

However, the extract at a dose of 2.0 mg/ml significantly increased LVDP at Peak by only 14.9% and decreased LVDP by 9.8% after 3min [$p < 0.01$ baseline versus 2.0 mg/ml *LL*, Table 6.4]. These changes in LVDP followed similar patterns as was described for changes in LVSP [Table 6.2] and decreased to zero within the 2nd minute of constant flow perfusion.

6.5 Effects on the heart rate

Table 6.5: Effects of adrenaline, digoxin and *Leonotis leonurus* extract on the heart rate

Table 6.5 shows the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on HR of the isolated perfused rat heart. Data are expressed as % change from baseline (mean ± SD)
*****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

	n	Baseline (bpm)	Peak (bpm)	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1 µM	4	311.82	315.97	1.33 ± 14.58	1.18 ± 4.24
ADR 1.0 µM	7	270.16	330.92	22.49 ± 12.48 *	24.57 ± 10.78*
DIG 0.25 ng/ml	5	294.17	289.46	- 1.60 ± 3.80	- 0.12 ± 2.86
DIG 2.5 ng/ml	6	271.60	261.50	- 3.72 ± 0.98 *	- 0.68 ± 0.59
DIG 25 ng/ml	6	275.97	273.49	- 0.90 ± 3.60	- 0.60 ± 2.63
DIG 250 ng/ml	5	266.80	263.80	- 1.11 ± 2.86	- 0.52 ± 2.12
DIG 2500 ng/ml	6	300.00	298.86	- 0.38 ± 2.89	- 1.52 ± 1.36
LL 0.1 mg/ml	5	229.03	227.86	- 0.51 ± 0.99	- 2.47 ± 2.07
LL 0.5 mg/ml	8	257.85	255.45	- 0.93 ± 2.42	- 2.37 ± 2.50
LL 1.0 mg/ml	7	296.67	193.64	- 34.73 ± 3.70*	- 28.28 ± 4.94 *
LL 2.0 mg/ml	5	315.60	262.39	- 16.86 ± 22.38	- 42.71 ± 8.02 *

6.5.1 Effects of adrenaline on the heart rate

ADR solution produced a significant increase in HR of 22.5% at Peak [p < 0.01 baseline versus 1.0 µM ADR, Table 6.5]. This increasing effect on HR remained constant throughout the perfusion and after 3min, ADR solution had significantly increased HR by 24.6% [p < 0.01 baseline versus 1.0 µM ADR, Table 6.5]. When perfusion with ADR solution was stopped, the heart rate recovered but remained at a level above pre-administration levels.

6.5.2 Effects of digoxin on the heart rate

DIG solution slightly but significantly decreased HR at Peak by 3.7% [$p < 0.01$, baseline versus 2.5 ng/ml DIG] with no significant effect over the 3min period. The other tested concentrations of DIG did not significantly affect HR [Table 6.5].

6.5.3 Effects of *Leonotis leonurus* extract on the heart rate

The aqueous extract of *L. leonurus* at a dose of 1.0 mg/ml produced a significant negative chronotropic effect both at Peak and over the entire 3min heart perfusion [$p < 0.01$, baseline versus 1.0 mg/ml *LL*, Table 6.5]. At Peak, HR was reduced by 34.7% and over the 3min period by 28.3%. This HR-lowering effect approached a minimum within the 1st minute and remained constant until perfusion with the extract was stopped. After switching to the main perfusion line, the hearts recovered and the levels for HR remained below pre-administration levels. However, at a dose of 2.0 mg/ml, the extract drastically decreased HR by 42.7% over the 3min period [$p < 0.01$ baseline versus 2.0 mg/ml *LL*, Table 6.5] with no significant effect at Peak. This decrease occurred rapidly and within the 2nd minute it had decreased to zero. This effect corresponded with the inotropic effect evoked by the extract at this concentration. The aqueous extracts of *L. leonurus* (0.1 and 0.5 mg/ml) did not significantly affect HR [Table 6.5].

6.6 Effects on the Cardiac work

Another index of contractility that was investigated was cardiac work (CW), expressed as the product of heart rate and left ventricular developed pressure (HR x LVDP). The double product (HR x LVSP) is an important parameter *in vivo* and i*n vitro*.

Table 6.6: Effects of adrenaline, digoxin and *Leonotis leonurus* extract on the cardiac work

This table shows the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on CW of the isolated perfused rat heart. Data are expressed as % change from baseline (mean ± SD)
*****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

	n	Baseline	Peak	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1 µM	4	27770.69	30048.75	10.90 ± 4.24	3.73 ± 2.3
ADR 1.0 µM	7	23282.39	44089.86	89.37 ± 20.97*****	63.01 ± 1.54*****
DIG 0.25 ng/ml	5	35294.52	35812.00	1.48 ± 5.35	1.42 ± 4.62
DIG 2.5 ng/ml	6	29343.66	30977.29	5.55 ± 4.33	4.54 ± 1.33*****
DIG 25 ng/ml	6	32869.04	32578.13	- 1.55 ± 7.94	- 0.49 ± 3.47
DIG 250 ng/ml	5	29569.35	29152.42	- 1.41 ± 2.80	- 0.90 ± 3.92
DIG 2500 ng/ml	6	29955.00	29419.78	- 1.68 ± 2.54	- 0.51 ± 2.71
LL 0.1 mg/ml	5	22550.00	24836.74	10.17 ± 8.23*	2.91 ± 4.81
LL 0.5 mg/ml	8	24225.00	27690.78	13.59 ± 8.60*	5.18 ± 3.23*****
LL 1.0 mg/ml	7	30034.87	25357.16	- 15.89 ± 8.40*	- 13.27 ± 6.89*****
LL 2.0 mg/ml	5	26157.66	24705.91	- 5.54 ± 24.17	- 48.84 ± 4.89*****

6.6.1 Effects of adrenaline on the cardiac work

ADR drastically increased CW at Peak by 89.4% and 63% over the 3min period [p<0.01 baseline versus 1.0 µM ADR, Table 6.6]. The lower dose of ADR had no significant effect on CW [Table 6.6].

6.6.2 Effects of digoxin on the cardiac work

DIG slightly increased CW by 4.5% over the 3min period [p < 0.01 baseline versus 2.5 ng/ml DIG, Table 6.6] with no significant effect at Peak. All the other tested concentrations of DIG did not significantly affect CW [Table 6.6].

6.6.3 Effects of *Leonotis leonurus* extract on the cardiac work

L. leonurus aqueous extract (0.1 and 0.5 mg/ml) significantly increased CW at Peak by 10.1% and 13.6%, respectively [p < 0.01 baseline versus 0.1 and 0.5 mg/ml *LL*, Table 6.6]. Only 0.5 mg/ml *LL* significantly increased CW by 5.2% over the 3min period [p < 0.01 baseline versus 0.5 mg/ml *LL*, Table 6.6]. However, at a dose of 1.0 mg/ml, the aqueous extract decreased CW at Peak by 15.9% and by 13.3% over the 3min period [p < 0.01 baseline versus 1.0 mg/ml *LL*, Table 6.6]. Similarly, the extract at a dose of 2.0 mg/ml reduced CW by 48.8% over the 3min period with no significant effect at Peak [p < 0.01 baseline versus 2.0 mg/ml *LL*, Table 6.6].

6.7 Effects on the coronary perfusion pressure

The effects of ADR, DIG and *L. leonurus* aqueous extract solutions on CPP of the isolated heart are summarised in Table 6.7. The changes in CPP that were produced by the aqueous extract/ drugs were followed by typical autoregulatory flow responses. For example, a reduction in CPP was associated with an initial decrease of flow followed by an increase towards a control value. Conversely, an elevation of the CPP resulted in an initial increase in flow that subsequently declined towards the flow value measured before the change in CPP. These responses were consistent with that of previous researchers (Bunger et. al. (1979)).

Table 6.7: Effects of adrenaline, digoxin and *Leonotis leonurus* on the coronary perfusion pressure

This table shows the effects of Adrenaline (ADR) [Regimens B&C], Digoxin (DIG) [Regimens D-H] and *L. leonurus* (*LL*) extract [Regimens I-L] on CPP of the isolated perfused rat heart. Data are expressed as % change from baseline (mean ± SD)
*****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

	n	Baseline (mmHg)	Peak (mmHg)	Peak (mean ± SD)	3min (mean ± SD)
ADR 0.1 µM	4	79.59	71.71	- 9.89 ± 6.39	- 6.95 ± 5.31
ADR 1.0 µM	7	69.25	63.69	- 8.02 ± 8.35	- 6.58 ± 7.10
DIG 0.25 ng/ml	5	75.79	75.85	0.08 ± 1.28	0.37 ± 0.47
DIG 2.5 ng/ml	6	71.09	75.22	5.80 ± 3.61	4.72 ± 2.21
DIG 25 ng/ml	6	82.03	83.08	1.15 ± 2.52	1.95 ± 1.91
DIG 250 ng/ml	5	76.33	77.02	0.90 ± 4.05	3.04 ± 6.22
DIG 2500 ng/ml	6	72.66	73.04	1.03 ± 2.35	2.10 ± 3.53
LL 0.1 mg/ml	5	66.43	64.45	- 2.98 ± 2.57	- 1.61 ± 2.41
LL 0.5 mg/ml	8	62.10	56.79	- 8.54 ± 6.27	- 4.44 ± 5.72*****
LL 1.0 mg/ml	7	64.50	56.71	- 12.09 ± 8.58*	- 11.45 ± 7.74*****
LL 2.0 mg/ml	5	80.46	66.88	- 16.87 ± 9.21*	- 15.86 ± 12.51

6.7.1 Effects of adrenaline on the coronary perfusion pressure

ADR (0.1 and 1.0 µM) reduced CPP both at Peak and over the 3min period. However, this reduction was not statistically significant [Table 6.7].

6.7.2 Effects of digoxin on the coronary perfusion pressure

None of the tested concentrations of DIG significantly affected CPP [Table 6.7].

6.7.3 Effects of *Leonotis leonurus* aqueous extract on the coronary perfusion pressure

The aqueous extract of *L. leonurus* produced significant decreases in CPP at Peak of 8.5%, 12.1% and 16.9%, respectively [$p < 0.01$ baseline versus (0.5, 1.0 and 2.0 mg/ml) *LL*, Table 6.7]. Only the tested concentrations (0.5 and 1.0 mg/ml) significantly reduced CPP by 4.4% and 11.4%, respectively, over the 3min period [$p < 0.01$ baseline versus (0.5 and 1.0 mg/ml) *LL*, Table 6.7]. These effects on CPP corresponded with the positive inotropic effects of the extract as it was previously described [Table 6.2]. The CPP started to decrease with an increase in LVSP. It reached a minimum level within the 1st minute and maintained this level throughout perfusion with the extract. On withdrawal of the aqueous extract, the hearts recovered with CPP levels rising to above pre-administration levels.