Int. J. Med. Arom. Plants, ISSN 2249 – 4340
Vol. 2, No. 2, pp. 281-292, June 2012

RESEARCH ARTICLE

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

Pierre MUGABO[1*], Fatima KHAN[1], Andries BURGER[2]

[1]*Dept of Pharmacy, University of the Western Cape, Modderdam Road, Bellville South Africa*

[2]*Dept of Medical Biosciences, University of the Western Cape, Modderdam Road, Bellville, South Africa*

Corresponding author, Fax: +27-21-9591276, Tel: +27-21-959-2190

Article History: Received 20th March 2012, Revised 14th May 2012, Accepted 15th May 2012.

Abstract: The use the aqueous decoction of *Leonotis leonurus* (*L. leonurus*) *(Ll*) R. Br. (Lamiaceae) in the treatment of hypertension (HPT) in traditional medicine is well documented. The effect of the aqueous extract of *LI* on the blood pressure (BP) and heart rate (HR) has been investigated in normotensive rats. The aim of this study was to investigate the effect of *Ll* aqueous extract on the in isolated perfused rat heart (IPRH). Hearts were excised from male Wistar albino rats weighing 250-350g, aged less than 6 months. They were perfused at constant flow using the modified Langendorff perfused model of the heart. Effects of adrenaline on the left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), heart rate (HR), cardiac work (CW) and coronary perfusion pressure (CPP) were compared to that of *Ll*. Adrenaline (1µM) significantly ($p<0.05$) increased the LVSP by 40.6%, the LVDP by 43.9%, the HR by 22.5% and the CW by 89.4%. *Ll* (1.0 mg/ml and 2.0 mg/ml respectively and significantly ($p<0.01$) increased the LVSP by 25.36 and 14.91, the LVDP by 29.40 and 14.88. *Ll* (1.0 mg/ml and 2.0 mg/ml) significantly produced a negative chronotropic effect. Both adrenaline and *Ll* aqueous extract did not have any significant effect on the LVEDP. Adrenaline resulted in positive inotropic and chronotropic effects. At low concentrations *Ll* produced a positive inotropic and a negative chronotropic effect. At the concentration of 2.0mg/ml *Ll* decreased all parameters to zero. At higher concentrations higher than 2.0mg/ml, *Ll* seemed to have toxic effects on the heart.

Keywords: *Leonotis leonurus*; isolated perfused heart; left ventricular systolic pressure; left ventricular end-diastolic pressure; developed pressure; heart rate; cardiac work; coronary perfusion pressure.

Introduction

It is estimated that 80% of the South African black population consult traditional healers for advice and/or treatment of health concerns (Sofowora 1982; Du Toit 1998; Muller et al. 1999; George et al. 2001; Kelmanson et al. 2000). Traditional medicines like any other medicine require evaluation by scientific methods in order to be used to their full effect and safety.

The use of *Leonotis leonurus* (*L. leonurus*) (*Ll*) R. Br. (Lamiaceae) in the treatment of hypertension is well documented (Watt and Breyer - Brandwijk 1962; Hutchings et al. 1996; Van Wyk et al. 2000; Ojewole 2003; SATMERG 2003) and Tshambuluka et al. (2011)]. The modified isolated perfused rat heart has been used in previous studies in order to investigate the cardiovascular effects of plants used in traditional medicine. Pennacchio et al. (1995) investigated the cardioactive effects of an aqueous extract obtained from the leaves of the traditional Aboriginal medicinal plant of *Eremophilia alternofilia* on isolated hearts of normotensive male and female Wistar rats using the Langendorff heart preparation. The hearts were perfused retrogradely with modified Krebs-Henseleit (K-H) solution. A solution of the extract was administered through a polyethylene cannula over one minute. The results showed that the crude aqueous extract mediated an initial, but transient, positive inotropic effect followed by an immediate decrease. Khatib et al. (1998) investigated the cardiovascular effects of *Rosmarinus officinalis* aqueous extract on the isolated rabbit heart. The extract was dissolved in K-H solution and the hearts were perfused for a period of 10 minutes with the extract using the Langendorff method. The results showed that all

*Corresponding author: (E-mail) pmugabo<A.T.> uwc.ac.za

the tested concentrations produced a significant increase in LVP over 10 minutes. These findings motivate the need to investigate whether *Ll*, known with a hypotensive effect has any effect the heart.

Therefore, the aim of this study was to evaluate the effect of *Ll* aqueous extract on the LVSP, LVEDP, LVDP, HR, CW and CPP in the isolated perfused rat heart.

Methods

Selection, collection and identification of Leonotis leonurus

The plant identified as **(***L. leonurus***) (*Ll*)** R. Br. (Lamiaceae) (Figure 1) is known in South Africa as "wilde dagga" in Afrikaans, "wild dagga" in English, "umunyane" in Zulu, "lebake" in Sotho and "umfincafincane" in Xhosa (Watt et al., 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. The hairy flowers resemble lion's ears, hence the name *"leonurus"* (Van Wyk et al., 2000).

The plant was collected from Kirstenbosch Botanical Garden, South Africa. The identification of the plant was confirmed by Dr. Gillian Scott, taxonomist of the 'South African Traditional Medicines Research Group (SATMERG), University of the Western Cape (UWC), South Africa. A voucher specimen (TRAD-10) was deposited at the Kirstenbosch Botanical Garden herbarium.

Preparation of the aqueous extract of Leonotis leonurus

According to SATMERG (2003), *Ll* is used by traditional healers in the form of an aqueous decoction. The decoction is 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. The choice of an aqueous extraction procedure in this study was based on the above information from SATMERG (2003). This procedure was previously described by Veale *et al*., 1989. The leaves were dried in a ventilated oven at a temperature not exceeding 35ºC and then allowed to regain the air-dry state (±8% moisture). 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 (Freeze Mobile 12SL, Virtis Company, Gardiner, New York. 12525) for 24 hours using 200ml boiling distilled water to 5mg powdered material. The yield of extract was ±1g/5g powder.

Figure 1: *Leonotis leonurus* (L.) R. Br. (With the permission of Frits van Oudtshoorn, Briza Publication)

Modified Krebs-Henseleit buffer solution

The modified K-H bicarbonate buffer solution was described in several previous studies 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 control perfusion solution had the following composition in mM: NaCl (118); $NaHCO_3$ (26.2); KCl(4.7); KH_2PO_4(0.9); $MgSO_4.7H_2O$(1.18); $CaCl_2$(1.8); D-glucose (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 monitored 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.

Experimental animals

Male Wistar albino rats weighing 250-350g, aged less than 6 months were obtained from the animal unit at the University of Stellenbosch, South Africa. Male Wistar albino rats were used in previous isolated heart studies by Venkataraman et al. (1993), Frolkis and Beruk (1998) and Altup et al. (2001). They were housed and fed on standard rat pellets (Epol, Westville, South Africa) and kept under a 12-hour light, 12-hour dark cycle at 25ºC and supplied with tap water. They were housed individually in a perspex animal cage for 30 minutes before starting experiment in order to habituate them to the new environment.

Animal preparation

The detailed methodology for the modified isolated heart perfusion system has previously been described by Man and Lederman (1985) and Hu et al. (1991). Rats were anaesthetised with 30mg/kg sodium pentobarbitone (Kyron Laboratories (Pty) Ltd, Benrose, South Africa) via the intraperitoneal route. Immediately after the abolition of deep pain reflexes the thorax was opened and the pericardium removed. The heart was then excised in less than 30 seconds as described by Sutherland et al., 2000 and placed in cool K-H solution (4°C) aerated with carbogen. It 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 PVC deflated balloon made from domestic cling wrap (Fresta Holdings Ltd., Brackenfell, South Africa) was inserted into the left ventricle through the bicuspid valve. This balloon was connected to a Deltran II pressure transducer (Utah Medical Products, West Midvale, Utah, USA) by means of a water filled stiff walled tube and was used to monitor the left ventricular pressure. The balloon was inflated. The volume of the left ventricle (preload) was adjusted by appropriate filling of this balloon with distilled water. A thermometer probe was inserted into the right ventricle to monitor the temperature.

Constant Flow

The perfusion system shown schematically in Figure 2 is a modified version of the isolated perfused rat heart at constant flow. The heart was mounted by securing the aorta on the cannula.

Drugs and chemicals

The following drugs were used in the study: Sodium pentobarbitone 6% solution [Kyron La-

boratories (Pty) Ltd] was used for anaesthesia in all the animals. Adrenotone (adrenaline) ampoules containing ADR base 1 mg/ml [SCP Pharmaceuticals (Pty) Ltd] was used as reference. Adrenaline was selected because of its known positive inotropic effect. Infusions of *Ll* 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.

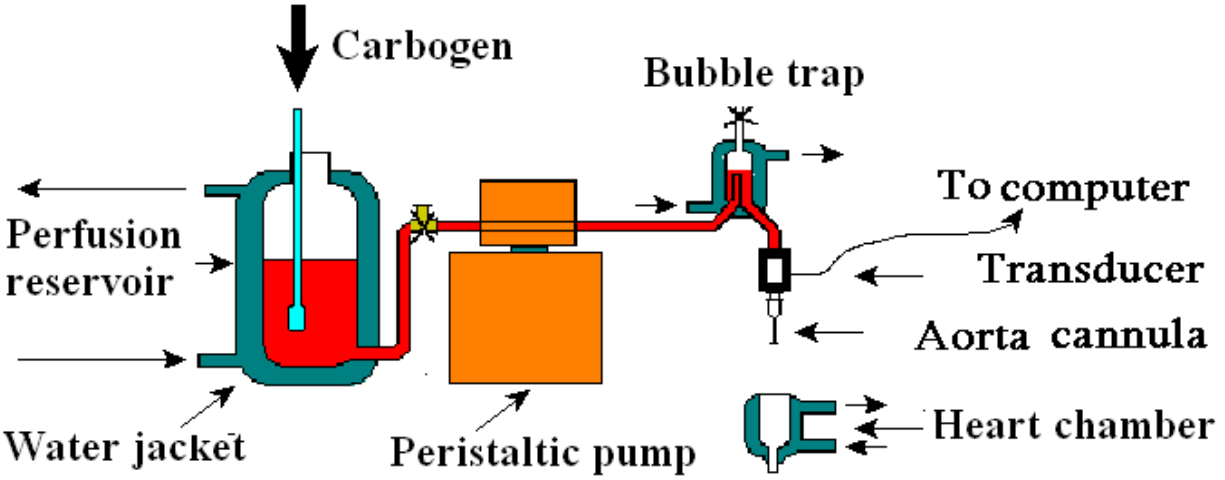

Figure 2: Modified constant flow perfusion apparatus.

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 *Ll* were dissolved in and diluted with K-H solution to concentrations of 0.1, 0.5, 1.0 and 2.0 mg/ml. ADR solutions were diluted with K-H solution to concentrations of 1.0 µM. 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 *Ll* 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 et al. (1998) perfused the hearts for 10 min.

Protocol

Rats hearts were randomly assigned to receive one of the infusion regimens listed in Table 1.

Table 1: Experimental schema.

Infusion regimen	Infusion I (3min)	Infusion II (3min)	Infusion III (3min)
A	K-H solution	K-H solution	K-H solution
B	ADR 1.0 µM	ADR 1.0 µM	ADR 1.0 µM
C	*Ll* 0.1 mg/ml	*Ll* 0.1 mg/ml	*Ll* 0.1 mg/ml
D	*Ll* 0.5 mg/ml	*Ll* 0.5 mg/ml	*Ll* 0.5 mg/ml
E	*Ll* 1.0 mg/ml	*Ll* 1.0 mg/ml	*Ll* 1.0 mg/ml
F	*Ll* 2.0 mg/ml	*Ll* 2.0 mg/ml	*Ll* 2.0 mg/ml

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.

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 ob-

tained. The infusions were then discontinued and a final set of measurements was obtained.

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 pressure transducer (MLT0670 disposable pressure transducer - ADInstruments Transducer Series – supplied by LASEC – Cape Town – South Africa) connected to the water-filled domestic wrap balloon inserted into the LV. The CPP was monitored via a similar 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. The 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 were determined. The 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.

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.

Ethics approval

Approval was obtained from the UWC Ethics Committee and the animals were treated according to the recommendations of the UWC's ethical regulations concerning animal experiments.

Results

Effects on the left ventricular systolic pressure

Table 2: Effects of adrenaline and *L. leonurus* extract on the left ventricular systolic pressure

Treatment	n	Baseline (mmHg)	Peak (mmHg)	Δ Peak % (mean ± SD)	Δ 3 min % (mean ± SD)
ADR 1.0 µM	7	90.59 ±7.35	127.41±9.15	40.64 ± 22.67 *	24.31 ± 21.29*
***Ll* 0.1 mg/ml**	5	103.21±9.78	113.26±10.04	9.73 ± 6.74 *	5.37 ± 2.98 *
***Ll* 0.5 mg/ml**	8	97.43±11.73	111.86±7.34	14.81 ± 5.93 *	7.35 ± 4.90*
***Ll* 1.0 mg/ml**	7	105.88±9.12	132.74±5.94	25.36 ± 8.10 *	23.13 ± 6.63*
***Ll* 2.0 mg/ml**	5	91.40±8.99	125.03±7.31	37.00 ± 12.18 *	- 4.17 ± 9.15

Table 2 summarises the effects of ADR [Regimen B], and *Ll* extract [Regimen C-F] on LVSP of the isolated perfused rat heart. Data (mean ± SD) are expressed as % change from

baseline with * = p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

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). 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.

Effects of Leonotis leonurus aqueous extract on left ventricular systolic pressure

Ll 0.1, 0.5, 1.0 and 2.0 mg/ml solution transiently increased LVSP from baseline and produced a maximum response (Peak) within the 1st minute. At Peak, all the tested concentrations of the aqueous extract significantly (p<0.01) increased LVSP by 9.7%, 14.8%, 25.3% and 37.0%, respectively. After 3min, the responses were reduced and LVSP was only significantly (p<0.01) increased by 5.3%, 7.3% and 23.1%, respectively [p < 0.01 baseline versus. (0.1, 0.5 and 1.0 mg/ml) *Ll*]. On withdrawal of the extract, the hearts recovered with the physiological measurements returning to approximately initial levels.

LVSP for *Ll* 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 F], the hearts remarkably recovered and the physiologic measurements returned to approximately pre-administration levels.

Effects on the left ventricular end-diastolic pressure

Table 3 shows the effects of ADR [Regimens B] and *Ll* extract [Regimens C-F] on LVEDP of the isolated perfused rat heart. Data (mean ± SD) are expressed as % change from baseline with *p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

Table 3: Effects of adrenaline and *Leonotis leonurus* on the left ventricular end diastolic pressure

Treatment	n	Baseline (mmHg)	Peak (mmHg)	Δ Peak % (mean ± SD)	Δ 3min % (mean ± SD)
ADR 1.0 µM	7	4.41 ±10.39	3.76±13.29	- 14.67 ± 9.96	- 9.77 ± 31.99
***Ll* 0.1 mg/ml**	5	4.75±8.29	3.97±15.37	- 16.44 ± 49.31	- 10.86 ± 24.40
***Ll* 0.5 mg/ml**	8	3.48±11.92	3.47±14.49	- 0.28 ± 40.61	- 9.29 ± 13.29
***Ll* 1.0 mg/ml**	7	4.64±5.97	9.22±23.48	98.62 ± 35.90	37.79 ± 82.66
***Ll* 2.0 mg/ml**	5	4.01±7.23	5.34±43.21	33.00 ± 58.76	101.50 ± 11.74

Effects of adrenaline on the left ventricular end diastolic pressure

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

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

As indicated in Table 3, *Ll* aqueous extract produced no significant effect on LVEDP.

Effects on the left ventricular developed pressure

The effects of ADR and *Ll* extract on LVDP of the isolated perfused rat heart are summarised in Table 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 4: Effects of adrenaline and *Leonotis leonurus* extract on the left ventricular developed pressure

Treatment	n	Baseline (mmHg)	Peak (mmHg)	Δ Peak% (mean ± SD)	Δ 3min % (mean ± SD)
ADR 1.0 µM	7	86.18 ±5.34	124.01 ±29.25	43.90 ± 13.41*	31.93 ± 11.33*
Ll 0.1 mg/ml	5	98.46±8.31	109.00±15,92	10.71 ± 7.69*	5.49 ± 3.65*
Ll 0.5 mg/ml	8	93.95±11.37	108.40±19,32	15.38 ± 7.30*	7.79 ± 4.64*
Ll 1.0 mg/ml	7	101.24±14.29	130.95±13.23	29.36 ± 12.25*	26.35 ± 9.10*
Ll 2.0 mg/ml	5	87.39±10.31	99.39±18.28	14.88 ± 12.53*	-9.80 ± 10.56

Table 4 shows the effects of ADR [Regimens B] and *Ll* extract [Regimens C-F] on LVDP of the isolated perfused rat heart. Data

(mean ± SD) are expressed as % change from baseline with *****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

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 2.

Effects of Leonotis leonurus aqueous extract on the left ventricular developed pressure

The tested concentrations of *Ll* extract (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 versus baseline (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.*

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]*. These changes in LVDP followed similar patterns as was described for changes in LVSP [Table 2] and decreased to zero within the 2nd minute of constant flow perfusion.

Effects on the heart rate

Table 5: Effects of adrenaline and *Leonotis leonurus* extract on the heart rate

Treatment	n	Baseline (bpm)	Peak (bpm)	Δ Peak % (mean ± SD)	Δ 3min % (mean ± SD)
ADR 1.0 µM	7	270.16 ±5.69	330.92 ±18.21	22.49 ± 12.48 *	24.57 ± 10.78*
Ll 0.1 mg/ml	5	229.03±9.56	227.86±24.02	- 0.51 ± 0.99	- 2.47 ± 2.07
Ll 0.5 mg/ml	8	257.85±11.34	255.45±17.32	- 0.93 ± 2.42	- 2.37 ± 2.50
Ll 1.0 mg/ml	7	296.67±7.34	193.64±5.67	- 34.73 ± 3.70*	- 28.28 ± 4.94 *
Ll 2.0 mg/ml	5	315.60±16,31	262.39±9.85	- 16.86 ± 22.38	- 42.71 ± 8.02 *

Table 5 shows the effects of ADR [Regimens B] and *Ll* extract [Regimens C-F] on HR of the isolated perfused rat heart. Data (mean ± SD) are expressed as % change from baseline with *****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®)

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]. 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]. When perfusion with ADR solution was stopped, the heart rate recovered but remained at a level above pre-administration levels.

Effects of Leonotis leonurus extract on the heart rate

The aqueous extract of *Ll* 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*]. At Peak, the 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*) 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. At lower concentrations the aqueous extracts of *Ll* (0.1 and 0.5 mg/ml) did not significantly affect HR.

Effects on the cardiac work

The cardiac work (CW) was expressed as the product of heart rate and left ventricular developed pressure (HR x LVDP).

Table 6: Effects of adrenaline and *Leonotis leonurus* extract on the cardiac work

Treatment	n	Baseline	Peak	Δ Peak % (mean ± SD)	Δ 3min % (mean ± SD)
ADR 1.0 µM	7	23282.39±11.42	44089.86±11.32	89.37 ± 20.97*	63.01 ± 1.54*
Ll 0.1 mg/ml	5	22550.00±7.34	24836.74±7.39	10.17 ± 8.23*	2.91 ± 4.81
Ll 0.5 mg/ml	8	24225.00±9.28	27690.78±13.21	13.59 ± 8.60*	5.18 ± 3.23*
Ll 1.0 mg/ml	7	30034.87±11.24	25357.16±8.40	- 15.89 ± 8.40*	- 13.27 ± 6.89*
Ll 2.0 mg/ml	5	26157.66±13.47	24705.91±10.37	- 5.54 ± 24.17	- 48.84 ± 4.89*

This table shows the effects of ADR [Regimens B] and *Ll* extract [Regimens C-F] on CW of the isolated perfused rat heart. Data (mean ± SD) are expressed as % change from baseline with *****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

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]. The lower dose of ADR had no significant effect on CW.

Effects of Leonotis leonurus extract on the cardiac work

The aqueous extract of *Ll* (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). 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*). At the 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*). 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*.

Effects on the coronary perfusion pressure

The effects of ADR and *Ll* aqueous extract solutions on CPP of the isolated heart are summarised in Table 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 the 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 7: Effects of adrenaline (ADR) and *Leonotis leonurus* on the coronary perfusion pressure

Treatment	n	Baseline (mmHg)	Peak (mmHg)	Peak (mean ± SD)	3min (mean ± SD)
ADR 1.0 µM	7	69.25±7,34	63.69±4.59	- 8.02 ± 8.35	- 6.58 ± 7.10
***Ll* 0.1 mg/ml**	5	66.43±5.27	64.45±6.39	- 2.98 ± 2.57	- 1.61 ± 2.41
***Ll* 0.5 mg/ml**	8	62.10±10.34	56.79±9.31	- 8.54 ± 6.27	- 4.44 ± 5.72*****
***Ll* 1.0 mg/ml**	7	64.50±15.03	56.71±12.21	- 12.09 ± 8.58*	- 11.45 ± 7.74*****
***Ll* 2.0 mg/ml**	5	80.46±10.23	66.88±14.32	- 16.87 ± 9.21*	- 15.86 ± 12.51

Table 7 shows the effects of ADR [Regimens B] and *Ll* extract [Regimens C-F] on CPP of the isolated perfused rat heart. Data (mean ± SD) are expressed as % change from baseline with*****p < 0.01 versus baseline; Mann-Whitney U test (SPSS for Windows®).

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.

Effects of Leonotis leonurus aqueous extract on the coronary perfusion pressure

The aqueous extract of *Ll* 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*. 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*. These effects on CPP corresponded with the positive inotropic effects of the extract as it was previously described. 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.

Discussion and conclusions

The present study was designed to investigate the potential effects of the aqueous extracts of the leaves of *L. leonurus* of the family Lamiaceae (Hutchings et. al. 1996; Van Wyk *et al*. 2000), on certain cardiovascular parameters such as LVSP, LVEDP, LVDP, HR, CW and CPP in the isolated perfused rat heart.

L. leonurus has numerous traditional uses (Hutchings et. al. 1996; Van Wyk *et al*. 2000), as it was previously described. Its use for the treatment of hypertension was not recommended (SATMERG, 2003) as a result of a lack of clinical data regarding the mechanism of action and potential adverse effects of this plant.

As in previous studies of the isolated perfused heart (Man and Lederman, 1985; Hu et al.1991; Venkataraman et al. 1993; Inamdar et al.1994 and Khatib et al. 1998), a constant flow rate was used in the present study. A non-recirculating system was employed so that the steady state concentrations of the substrates in perfusion medium could be maintained, and potential effects of unrecognised metabolites released from the heart could be avoided (Bunger et al. 1979). This system was capable of indicating the effects of agents having either positive or negative inotropic actions (Gamble et al. 1970). Furthermore, the results of this study are comparable to those obtained in the above mentioned studies. This is indicative of the validity of the experimental model used. The present study did not provide us with information regarding the mechanism of positive/negative inotropism and chronotropism of this plant extract. More detailed studies need to be undertaken to identify its mechanism of action.

Rationale for using adrenaline as test drug

The rationale for using ADR solutions as reference drugs was to compare its effects with that of the plant extract. Also, if in this study, we could reproduce the effects of ADR obtained by other researchers (Meyers *et al*. 1976), the experimental system would, furthermore, prove to be a reliable one.

Large standard deviation values obtained for the left ventricular end diastolic pressure

It is seen that large SD values were obtained for % change in LVEDP relative to the other parameters [Table 3]. This large change is due to the expression of the data as % change from baseline. Actual small changes in LVEDP divided by the very low control value will give large and fluctuating values for % change in LVEDP. For example, a 2mmHg increase in LVEDP starting from a baseline value of 0.1mmHg, will be equivalent to a $2x10^3$% change in LVEDP, while a 2mmHg increase starting with a baseline value of 8mmHg, will only be equivalent to a 25% change in LVEDP.

Effects of adrenaline solution on the isolated heart

Adrenaline is a powerful cardiac stimulant (Westfall and Westfall 2006). It acts directly on the predominant beta -1 (β_1) receptor of the myocardium and on the cells of the pacemaker and the conducting tissues (Westfall and Westfall 2006). Intravenous infusions results in a rise in blood pressure, predominantly systolic pressure, increased HR and vasoconstriction of the cutaneous, renal and splanchnic blood vessels (Westfall and Westfall 2006).

Adrenergic stimulation of the isolated heart by an ADR concentration of 1.0µM drastically increased LVSP and similarly LVDP with a corresponding acceleration of HR, thus reflecting the positive inotropic and chronotropic effect of the hormone (Tables 2, 4 and 5). ADR acts directly on receptors of the effectors tissues, for example, the heart. Sympathetic innervations are not necessary for its action, on the contrary, denervation increases the sensitivity of effectors of directly-acting amines such as ADR (Meyers et al. 1976). ADR acts to elevate LVSP by increasing SV and its effect on HR is the resultant of its direct action and of reflex slowing due to the rise in blood pressure (Meyers et al.1976). According to Meyers et al. (1976), a comparative study of the effects of L-arterenol, epinephrine and isopropylarterenol on the isolated perfused rabbit heart demonstrated that ADR increased the rate and force of ventricular contraction in hearts which were independent of other

hydrodynamic influences. Also, this positive inotropism and chronotropism was associated with a decreased CPP. Thus, the system proved to be a reliable one since it was able to reproduce findings of previous researchers (Meyers et al. 1976).

Effects of Leonotis leonurus extract on the isolated perfused rat heart

In the present study, we found that the tested concentrations of the aqueous extract of *Ll* produced a positive inotropic effect on the isolated heart with a corresponding negative chronotropic effect over the 3min period (Tables 2 and 5). The abovementioned effects were accompanied by a decreased CPP from baseline over the 3min period (Table 7). Thus, the aqueous extract of *Ll* contains constituents associated with positive inotropic and negative chronotropic agents as well as constituents associated with coronary vasodilation.

However, at higher concentration (2.0mg/ml) the extract dropped the values of all parameters to zero within the 2nd minute and remained at this level over the 3min period (Tables 2 and 5). It appears that the extract at this concentration possibly contains some constituents with toxic effects on the isolated heart. Therefore, further studies would be recommended to isolate the various constituents and examine their possible pharmacological effects on the heart individually before it would be undoubtedly safe to recommend this plant for its use in the treatment of cardiovascular disease.

The abovementioned effects of *Ll* extract were described for the isolated heart, but we do not know what its effect would be on an intact heart. If *Ll* increased LVSP it would therefore be contraindicated for its use in the treatment of hypertension. We also recommend that more doses of the extract be tested both in the isolated and intact hearts and perfused for longer periods.

Acknowledgements: The project was funded by the National Research Foundation (Grant # 2069540)

References

Altup S, Demiryurek AT, Tungel M, Kanz I. 2001. Contribution of peroxynitrite to the beneficial effects preconditioning on ischaemia-induced arrhythmias in rat isolated hearts. *European Journal of Pharmacology*, 415: (2-3): 239-246.

Belo, S.E. and Talesnik, J. 1982. Coronary vasoconstrictor and vasodilator actions of arachidonic acid in the isolated perfused heart of the rat. *British Journal of Pharmacology*, 75: 269-286.

Bergman, S.R.; Clark, R.E. and Sobel, B.E. 1979. An improved isolated heart preparation for external assessment of myocardial metabolism. *American Journal of Physiology*, 236: H644-H651.

Bunger, R.; Sommer, O.; Walter, G.; Stiegler, H and Gerlach, E. 1979. Functional and metabolic features of an isolated perfused guinea pig heart performing pressure-volume work. *Pflugers Archives*, 380: 259-266.

Chinchoy,E.; Soule, C.L.; Houlton, A.J.; Gallagher, W.J.;Hjelle, M.A.; Laske, T.G.; Morissette, J. and Iaizzo, P.A. 2000. Isolated four-chamber working swine heart model. *The Annals of Thoracic Surgery*, 70: 1607-1614.

Cocks, M. and Dold, A. 2000. The role of 'African Chemists' in the health care system of Eastern Cape Province of South Africa. *Social Science and Medicine*, 51 (10): 1505-1515.

Du Toit, B.M. 1998. Modern Folk Medicine in South Africa. *South African Journal of Ethnology*, 21 (4): 145-158.

Frolkis, V.V and Beruk, O.V. 1998. Age peculiarities of changes in contractile function of isolated rat hearts during prolonged perfusion. *Mechanisms of Ageing and Development*, 106 (1-2): 173-182.

Fujita, S.; Roerig, D.L.; Bosnjak, Z.J. and Stowe, D.F. 1998. Effects of vasodilators and perfusion pressure on coronary flow and simultaneous release of nitric oxide

from guinea pig isolated hearts. *Cardiovascular Research*, 38 (3): 655-667.

Gamble, W.J.; Conn, P.A.; Kumar, A.E.; Plenge, R. and Monroe, R.G. 1970. Myocardial oxygen consumption of blood-perfused, isolated, supported, rat heart. *American Journal of physiology*, 219 (3): 604-612.

Hu, W., Kinnaired, A.A.A. and Man, R.Y.K. 1991. Mechanisms of coronary vascular effects of platelet-activating factor in the rat perfused heart. *British Journal of Pharmacology*, 103:1097-1102.

Hutchings, A.; Scott, A.H.; Lewis, G and Cunningham, A.B. 1996. Zulu Medicinal Plants, An Inventory. Natal University Press, Pietermaritzburg, 266-267.

George,J.; Laing,M.D. and Drewes,S.E. 2001. Phytochemical research in South Africa. *South African Journal of Science*, 97(3/4): 93-106.

Inamdar, N.; Venkataraman, B.V. and Aleem, A. 1994. A Simple and Improved perfusion Apparatus for Isolated Hearts. *Indian Journal of Pharmacology*, 26:262-265

Johnson, K.E.; Balderston, S.M.; Pieper, J.A.; Mann, D.E. and Reiter, M.J. 1991. Electrophysiologic effects of Verapamil Metabolites in the isolated heart. *Journal of Cardiovascular Pharmacology*, 17: 830-837.

Khatib, S.; Alkofahi, A.; Hasan, M. and Najib, N. 1998. Cardiovascular effects of *Rosmarinus officinalis* extract on isolated intact rabbit heart. *Fitoterapia*, LXIX (6), 502-505.

Kelmanson,J.E.; Jager,A.K. and Van Staden,J. 2000. Zulu medicinal Plants with Antibacterial Activity. *Journal of Ethnopharmacology*, 69 (3): 241-246

Langendorff, O. 1895. Untersuchungen am uberlebenden Saugethierherzen. *Pflugers Archives fur die Gesante Physiologie des Menschen and der Tiere*, 61: 291-332.

Lindsay, S.J.; Kearney, M.T.; Prescott, R.J.; Fox, A.A. and Nolan, J. 1999. Digoxin and mortality in chronic heart failure. *The Lancet*, 354 (9183): 1003

Man, R.Y.K. and Lederman, C.L. 1985. Effect of Reduced Calcium on Lysophosphatidylcholine-Induced Cardiac Arrhythmias. *Pharmacology*, 31:11-16.

Massie, B.M. 1998. 15 years of heart failure trials: What have we learned? *Lancet* 352 (9129), pp. 129-134.

Meyers, F.H.; Jawertz, E. and Goldfien, A. 1976. Review of medical pharmacology. Lange Medical Publications, pp7-79; 133-135.

Mugabo, P.; Njagi, A.; Dietrich, D.L.L. and Syce, J. 2002. Cardiovascular effects of *Leonotis leonurus* in the normotensive rat. Revista de Fitoterapia, 2 (SI), p 163

Muller.and Steyn,M. 1999. Culture and the feasibility of a partnership between westernized medical practitioners and traditional healers. *Society in Transition*, 30 (2): 142

Ojewole, J. A. O. 2003. Hypotensive effect of *Leonotis leonurus* aqueous leaf extract in rats. *American Journal of Hypertension.* 16 (5) Supplement 1, A40.

Pennacchio, M.; Syah, Y.M.; Ghisalberti,E.L. and Alexander, E. 1996. Cardioactive compounds from *Eremophila* species. *Journal of Ethnopharmacology*, 53: 21-27.

Richmond, G.S. 1995. Cardioactive effects of *Eremophila alternifolia* extracts. *Journal of Ethnopharmacology*, 47: 91-95.

Perkin, M.F. 1987. The cardiac effects and arrhythmogenic potential of selected beta-adrenoceptor agonists in the isolated perfused rat heart. Magister Pharmaceuticae thesis. University of the Western Cape, South Africa.

Rang, H.P.; Dale, M.M. ; Ritter, J.M.; Flower,R.J. 2007. RANG and DALE'S Pharmacology, sixth edition. Churchill Livingstone.

Sofowora,A. 1982. Medicinal Plants and traditional medicine in Africa. Chichester: John Willey, 2-243.

South African Traditional Medicines Research Group [SATMERG] .2003. UWC Pharmacopoeia Monograph Project. Bellville: School of Pharmacy, University of the Western Cape (UWC), South Africa.

Sutherland, F.J. and Hearse, D.J. 2000. The Isolated Blood and Perfusion fluid Perfused Heart. *Pharmacological Research*, 41 (6): 613-630.

Tshambuluka,N.D.; Mugabo,P.; Green,I 2011. Cardiovascular effects of *leonotis leonurus* in anaesthetized rats. LAP LAMBERT Academic Publishing,96p.

Van Wyk, B.E.; Van Oudtshoorn, B. and Gericke, N. 2000. Medicinal Plants of South Africa. Briza Publications (SA), 8-22/166-167.

Veale, D.J.H.; Oliver, D.W.; Arangies, N.S. and Furman, K.I. 1989. Preliminary isolated organ studies using an aqueous extract of *Clivia miniata* leaves. *Journal of Ethnopharmacology*, 27: 341-346.

Venkataraman, B.V.; Dulchavsky, S.A. and Dutta, S. 1993. Measurement of physiologic parameters from isolated perfused rat heart computerised system. *Indian Journal of Pharmacology* 25: 193-198.

Watt, J.M. and Breyer-Brandwijk, M.G. 1962. Medicinal and Poisonous Plants of Southern Africa. Edinburg: E & S Livimgstone, 156-157.

Westfall T.C and Westfall D.P. 2006. Adrenergic agonists and antagonists, In: Brunon, L.L, Lazo J.S, and Parker K.L. Goodman and Gilman's The pharmacological basis of therapeutics. McGraw-Hill, Eleventh edition, 237-245.

Zhang, L.; Parratt, J.R.; Beastall, G.H.; Pyne, N.J. and Furman, B.L. 2002. Streptozocin diabetes protects against arrhythmias in rat isolated hearts: role of hypothyroidism. *European Journal of Pharmacology*, 435 (1-2): 269-276.