Effects of a Methanol Fraction of the Leaves of *Leonotis leonurus* on the Blood Pressure and Heart Rate of Normotensive Male Wistar Rats

K. Obikeze, P. Mugabo, I. Green, D. Dietrich, and A. Burger

Abstract*—Leonotisleonurus* a shrub indigenous to Southern Africa is widely used in traditional medicine to treat a variety of conditions ranging from skin diseases and cough to epileptic fits and 'heart problems'. Studies on the aqueous extract of the leaves have indicated cycloxegenase enzyme inhibitory activity and an antihypertensive effect.

Five methanol leaf extract fractions (MLEa - MLEe) of *L. leonurus* were tested on anaesthetized normotensive male Wistar rats (AWR) and isolated perfused working rat hearts (IWH). Fraction MLEc (0.01mg/kg – 0.05mg/kg) induced significant increases in BP and HR in AWR and positive chronotropic and inotropic effects in IWH (1.0mg/ml – 5.0mg/ml). Pre-administration of atenolol (2.0mg/kg) and prazosin (60μg/kg) significantly inhibited MLEc effect on HR and MAP respectively *in vivo*, while atenolol (7.0mg/ml) pre-perfusion significantly inhibited MLEc effect *in vitro*.

The hypertensive effect of MLEc is probably via $\beta_1$agonism. Results also indicate the presence of multiple cardioactive compounds in *L. leonurus*.

***Keywords*—**Cardiovascular effect, *in vitro, in vivo,* isolated perfused working heart, *Leonotis leonurus,* rat.

I. Introduction

ESTIMATES by the world health organization (WHO) indicate that approximately 60% of the world's population depends on traditional medicine for their healthcare needs[1]. This figure rises up to about 80% in Sub-Saharan Africa with 70% of South Africans consulting traditional medicine healers for the treatment of chronic conditions including hypertension and 'heart problems [2].South Africa is a country with a large biodiversity of over 30 000 higher plant species, with approximately 3000 species belonging to a few plant families such as the Laminaceae finding extensive use indigenous traditional medicines formularies. Used extensively presently used as medicines [3]. *Leonotisleonurus* (Laminaceae) is a shrub with characteristic bright orange lion ear shaped flowers whose use in traditional medicine ranges from the treatment of epilepsy and skin conditions to bronchitis and 'heart conditions [2], [3].

Previous studies on the plant have reported an antiepileptic effect in mice treated with the aqueous extract of the leaves, while the ethanol extract of the leaves were reported to inhibit cycloxygenase enzyme *in vitro* [4], [5]. Studies focused on the cardiovascular effects of the plant's extracts have variously reported a hypotensive effect in hypertensive and normotensive male Wistar rats, a vasorelaxant effect *in vitro* and a positive inotropic, negative chronotropic effect in isolated Langendorff perfused rat hearts [6], [8]. Although many compounds have been isolated from the plant, only one diterpenoid compound has been reported to affect the cardiovascular system with a negative chronotropic effect in normotensive Wistar rats [2], [9]-[11]. Though the above studies indicate that *L. leonurus* has some effect on the cardiovascular system, there is no indication of the effect of the organic extracts of the plant, simulating its traditional medicinal use as an alcohol leaf decoction. This study focused on the evaluation of the cardiovascular effects of fractions of a methanol leaf extract (MLE) of *L. leonurus* in normotensive male Wistar rats using the anaesthetized rat model and the isolated perfused working heart model.

II. Materials and Methods

A. Plant Material

Plants were collected from Montague botanic gardens, Cape Town, South Africa, identified and a voucher specimen (No. 6859) deposited at the herbarium of the University of the Western Cape (UWC). Fresh leaves of the plant were washed, dried in a ventilated oven at 30°C for 72 hours, and ground to a fine powder. The powdered leaves (53 g) were continuously extracted for 5 hours with 1.5L of methanol, and excess solvent removed using a rotovapor (RE 300; Biddy Stirline). The dry extract was chromatographed on a silica gel (70 - 230 mesh grade) column, with ethyl acetate: hexane (1:4) combination as the mobile phase. Five fractions were collected, evaporated and labeled MLEa - MLEe.

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B. Animals

Normotensive male Wistar rats weighing 250 - 350 g were used for *in vitro* and *in vivo* experiments. The animals were obtained from the Medical Research Council (MRC), Tygerberg, South Africa and kept at the animal room at the school of pharmacy, UWC, where they were allowed free access to food and water. Ethical approval for the study was obtained and all animals were treated according to ethical regulations as required by the University of Western Cape ethics committee.

C. Drugs and Chemicals

Atenolol (Astra Zeneca; Cape Town, South Africa) was dissolved in normal saline, while prazosin (Sigma; Cape Town, South Africa) and fractions MLEa - MLEe were suspended with 1% dimethylsulfoxide (Sigma; Cape Town, South Africa) in normal saline and further diluted with either normal saline (for *in vivo* experiments) or Krebs-Henseleit buffer solution (for *in vitro* experiments). Fresh Krebs-Henseleit buffer solution containing (in mM); 118.5 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 10.0 g glucose (all Sigma; Cape Town, South Africa) was prepared daily.

D. Measurement of Blood Pressure and Heart Rate in vivo

The effects of the methanol fractions on blood pressure (BP) and heart rate (HR) was evaluated using the anaesthetized rat model as described by Liviu*set al* (2000) [12]. Normotensive male Wistar rats 3 - 4 months old and weighing between 250 g and 350 g, were anaesthetized using sodium pentobarbital (30 mg/kg IP), restrained and tracheotomised to facilitate breathing. The external jugular vein was cannulated for the infusion of drug substances, while the femoral artery was cannulated for the measurement of BP and HR. Systolic pressure (SP), diastolic pressure (DP), MAP and HR were monitored continuously on the Chart 4.0 software running on a computer with input from a PowerLab® (T20) connected to the arterial cannula via a BP transducer and BP amplifier (all AD instruments, Australia).

Randomized doses of the fractions MLEa – MLEe, as well as randomized MLEc doses following atenolol and prazosin pre-treatment were administered via the venous cannula to the animals.

E. Measurement of Cardiovascular Effects in vitro

The isolated perfused working heart (IWH) model was used to evaluate the in vitro effects of the methanol fractions [12]-[15]. The model was however modified to enable the perfusion of the working heart from two distinct working heart systems to reduce the risk of cross-contamination of the perfused drug/extracts [16]. Male Wistar rats weighing between 250 g and 350 g and not older than four months were anaesthetized with an overdose of sodium pentobarbitone. The beating hearts were rapidly excised and immediately immersed in cold (<4^{o}C) Krebs-Henseleit buffer. Excised hearts were then mounted and cannulated for working heart perfusion according to the method described by Sutherland and Hearse [13], [14]. After cannulation, the hearts were perfused retrogradely (Langendorff perfusion) for a ten minute resting period with Krebs-Henseleit buffer, following which ten minute working heart perfusion with either control(Krebs-Henseleit buffer) or test substances (fractions MLEa – MLEe and atenolol) occurred. Working heart perfusion with fraction MLEc after an initial five minute atenolol pre-perfusion was also carried out. Pressure developed in the aortic cannula (dP) and HR were recorded using the Chart Recorder Module (Gentronics, USA) software on a desktop computer connected to the aortic cannula via a BP transducer positioned at the same level as the heart. Coronary flow (Qe) and aortic output (Qa) were measured by collecting perfusate (over one minute) from the organ chamber and aortic output cannula respectively. Cardiac output (CO) was calculated as the sum of Qe and Qa [13].

III. Statistical Analysis

Results of measured parameters were expressed as mean± S.E.M. (n= 6). Blood pressure readings were presented as MAP calculated from SP and DP data. Statistical significance (p< 0.05) of the differences in the means for control and test substances were analyzed using the unpaired student's t–test.

IV. Results

A. Effects on Anaesthetized Normotensive Rats

In anaesthetised normotensive rats, methanol leaf fractions MLEa, MLEb, MLEd and MLEe induced no observable changes to the cardiovascular parameters monitored (results not included). Fraction MLEc (0.01 mg/kg – 0.05 mg/kg) induced dose dependent increases in SP, DP and MAP (SP and DP results not shown) that were significant with MLEc doses above 0.02 mg/kg (Fig. 1).

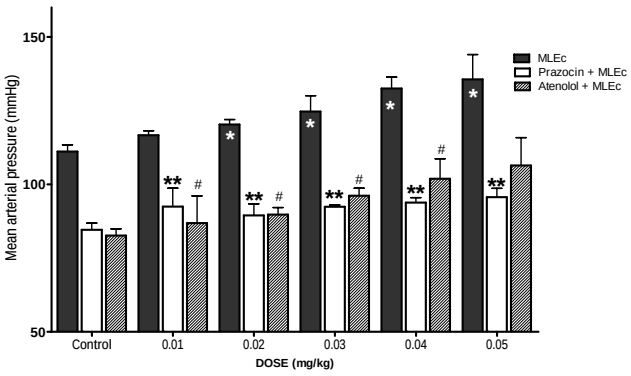

Fig. 1 Effect of MLEc (0.01mg/kg - 0.05mg/kg), atenolol (2.0mg/kg) + MLEc, and prazosin (60µg/kg) + MLEc on mean arterial pressure in anaesthetized normotensive rats.* P <0.05 with respect to control (n=6); # P <0.05 with respect to MLEc-only (n=6); **P <0.05 with respect to MLEc-only (n=6)

MLEc also induced dose-dependent increases in HR, with significant increases occurring with doses above 0.03 mg/kg (Fig. 2). Prazosin (60 µg/kg) pre-administration led to statistically significant reductions in the increases in MAP induced by all doses of MLEc (Fig. 1). Prazosin pre-

administration also led to non-dose-dependent changes in HR, with slight but non-significant increases occurring with the lower (0.01-0.02mg/kg) MLEc doses and slight non-significant decreases occurring with the higher (0.03-0.05mg/kg) MLEc doses (Fig. 2). Pre-administration of atenolol (2 mg/kg) led to reductions in the increases in MAP induced by MLEc administration, with statistically significant reductions occurring with all but the highest (0.05 mg/kg) MLEc dose (Fig. 1). MLEc-induced increases in HR were also significantly reduced by atenolol pre-administration (Fig. 2).

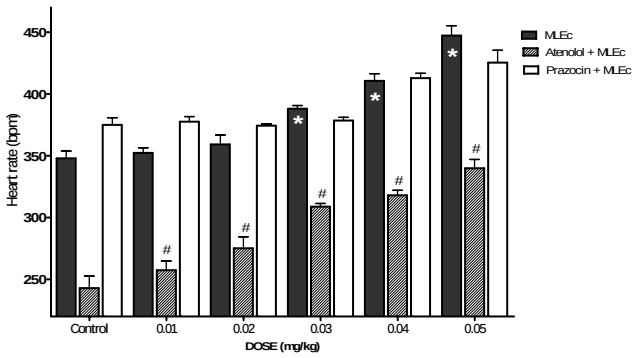

Fig. 2 Effect of MLEc (0.01 mg to 0.05 mg), atenolol (2.0mg/kg) + MLEc, and prazosin (60µg/kg) + MLEc on heart rate in anaesthetised normotensive rats. * P <0.05 with respect to control (normal saline) (n=6); $^{\#}P$ <0.05 with respect to MLEc only (n=6)

B. Effects on the Isolated Perfused Working Heart

Isolated working hearts (IWH) were perfused for 10 minutes with different doses (1.0mg/ml – 5.0mg/ml) of MLEc. Perfusion with MLEc led to significant dose-dependent increases in dP, Qe, CO, Qa (results not shown) and HR (table I). A five minute pre-perfusion of atenolol (7.0µg/ml) led to significant reductions in the increases in dP, CO, and HR induced by all doses of MLEc (Table I). Atenolol pre-perfusion also led to reductions in Qe with the 1.0mg/ml, 2.0mg/ml, and 4.0mg/ml doses of MLEc, while increases in Qe occurred with the 3.0mg/ml and 5.0mg/ml doses, with significant changes occurring only with the highest dose (Table I).

TABLE I
EFFECT OF ATENOLOL (7.0µG/ML) PRE-PERFUSION ON MLEC (1.0MG/ML - 5.0MG/ML) INDUCED CHANGES IN CARDIAC PARAMETERS MEASURED IN ISOLATED WORKING HEARTS
(N=6).

Drug perfused	Developed pressure (mmHg)	Coronary flow (ml/min)	Cardiac output (beats/min)	Heart rate
Control	36.2 ± 4.6	14.6 ± 2.8	51.0 ± 2.3	235.0 ± 14.4
Atenolol perfusion	29.5 ± 5.4	16.2 ± 0.2	29.2 ±1.4	203.0 ± 11.3
MLEc (1.0mg/ml)	46.6 ± 4.9*	20.9 ±1.3*	58.7 ±0.7*	253.0 ±12.3*
Atenolol + MLEc	31.9 ± 2.3**	16.8 ±3.6	27.2 ±2.3**	200.0 ±15.6**
MLEc (2.0mg/ml)	49.0 ± 1.1*	22.3 ±2.8*	60.4 ±1.9*	258.0 ±11.9*
Atenolol + MLEc	34.4 ± 2.1**	19.6 ±1.4	29.3 ±2.3**	218.0 ±19.2**
MLEc (3.0mg/ml)	51.5 ± 1.9*	23.7 ±1.0*	62.1 ±4.2*	261.0 ±9.5*
Atenolol + MLEc	38.1 ± 0.4**	24.9 ±3.5	36.5 ±2.8**	227.0 ±14.8**
MLEc (4.0mg/ml)	57.8 ± 3.1*	25.7 ±1.7*	66.2 ±2.3*	267.0 ±14.9*
Atenolol + MLEc	43.6 ± 5.2**	25.6 ±2.8	43.9 ±1.2**	239.0 ±7.2**
MLEc (5.0mg/ml)	65.3 ± 6.3*	29.5 ±1.0*	69.7 ±1.7*	270.0 ±12.3*
Atenolol + MLEc	48.6 ± 2.0**	25.9 ±0.6**	49.0 ±3.4**	248.0 ±11.4**

*p< 0.05 when compared to control
** p<0.05 when compared to MLEc-only perfusion

V. DISCUSSION

Of the five fractions of the methanol extract of *L. leonurus* leaves tested *in vivo* and *in vitro,* fraction MLEc was the only fraction to induce changes in the cardiovascular parameters measured. Increases in BP and HR induced by MLEc were the opposite of previously reported effects of extracts from the plant [6], [7]. It is to be noted however the previous studies reported on the effects aqueous extracts of the plant, while fraction MLEc was from a methanol extract. Solvents choice is an important determinant of the nature of compounds extracted from plant material due to the different solubility profiles of the constituents. The differences in solvents used

and results obtained from this study and previous studies suggests that MLEc contains different cardioactive compounds from those found in aqueous extracts of the plant [17]. Phytochemical analysis however indicated that MLEc contained alkaloids, cardiac glycosides, diterpenoids and tannins, a profile that is very similar to that reported of the aqueous extract [4]. The cardiovascular effects observed with the extracts could be attributed to any one or a combination of these groups of compounds since alkaloids, cardiac glycosides, diterpenoids and tannins extracted from different plants have all been shown to exhibit cardioactivity [4], [18], [19], [20]. Significant increases in HR observed with MLEc are indicative of a positive chronotropic and/or inotropic effect while the increases in MAP observed may be due to the resulting increased cardiac output. Increases in MAP may also have been due to vasoconstriction. Prazosin an a1 receptor antagonist significantly attenuated the MLEc-induced increase in MAP, even in the presence of increased HR suggesting that MLEc possess a vasoconstrictive effect. Atenolol pre-administration also attenuated the MLEc-induced increase in MAP and HR, suggesting that increases in MAP induced by MLEc were due to both vasoconstriction and a positive chronotropic/inotropic effect. In IWH, MLEc perfusion led to significant increases in dP, Qa, Qe, CO, and HR indicative of a positive chronotropic and inotropic effect. The increases in HR were consistent with those obtained with MLEc in our *in vivo* experiments, but once again differ from the effects reported with the aqueous extracts of the plant [8]. MLEc produced increases in all cardiac parameters similar to the effect of adrenaline an α and β adrenoceptor agonist suggesting that MLEc contains compounds possessing β agonist activity [21]. Pre-administration of atenolol, a β1 blocker led to significant reductions in the positive chronotropic and inotropic effect of MLEc. The significant inhibition of the positive chronotropic and inotropic effects *in vitro* by atenolol strongly suggests that MLEc acts via β1 receptors. MLEc induced increases in Qe suggesting vasodilatation of coronary vessels which would negate the suggestions of a vasoconstrictive effect leading to increased MAP. Other studies have demonstrated that in working hearts, increased vascular pressure could lead to the increase in coronary flow observed [22].

In conclusion, a fraction of the methanol extracts of the leaves of *L. leonurus* exhibited a positive chronotropic and inotropic effect both *in vivo* and *in vitro* suggestive of a β1 agonist effect and direct vasoconstrictive effect. This gives good indication for further studies to isolate possible lead compounds with cardiovascular activity from the plant.

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