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Acute Toxicity and Antihypertensive Effects of *Artemisia afra* and *Leonotis leonurus* in Spontaneously hypertensive rats

Tata Charlotte Mungho[1], Gwebu Ephraim Tobela[2], Aremu Olukayode Olasunkanmi[1], Sewani-Rusike Constance Rufaro[1], Oyedeji Adebola Omowumi[3], Oyedeji Opeopluwa Oyehan[4] and Nkeh-Chungag Benedicta Ngwenchi[5*]

1. Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, Mthatha 5117, SOUTH AFRICA.
2. Department of Chemistry, Faculty of Science and Technology, Rusangu University, Monze, ZAMBIA
3. Department of Chemistry, Faculty of Sciences, Walter Sisulu University, Mthatha 5117, SOUTH AFRICA
4. Department of Chemistry, Faculty of Science and Agriculture, University of Fort Hare, PBX1314 Alice, 5700 Eastern Cape Province, SOUTH AFRICA
5. Department of Biological and Environmental Sciences, Faculty of Natural Sciences, Walter Sisulu University, Mthatha 5117, SOUTH AFRICA
*bnkehchungag@wsu.ac.za

Abstract

Acute toxicity and antihypertensive effects of hydroethanolic extracts of Artemisia afra and Leonotis leonurus were studied in Swiss albino mice and spontaneously hypertensive rats (SHR) respectively. Phytochemical screening was determined by colorimetric techniques. Lorke's method for acute toxicity testing was carried out in two phases: in phase I three groups of mice (n = 3) were treated with 10, 100 or 1000 mg/kg of the extracts while in phase II mice were treated with 1600, 2900 or 5000 mg/kg of the extracts. Blood pressure, heart rate, blood flow and blood volume were measured using a non-invasive tail cuff method before treatment and 2, 4, 6, 8 and 24 hrs after treatment. Phytochemical screening revealed the presence of phenols, terpenoids, flavonoids, glycosides, tannins, steroids, triterpenoids and saponins. Both plant extracts were non-toxic with LD_{50} values greater than 5000 mg/kg.

Artemesia afra extracts had its greatest ($p<0.01$) antihypertensive effects at 2 and 4 hrs post treatment while the effects of Leonotis leonurus were weak at best. The antihypertensive effects of A. affra and L. leonurus were significantly higher ($p<0.01$) than the effects of furosemide 24 hrs post treatment. Results from this study suggest that even though A. afra and L. leonurus are used for hypertension treatment in South African traditional medicine, the former displayed better antihypertensive effects compared to the latter in SHR.

Keywords: Spontaneously hypertensive rats, *Leonottis leonurus*, *Artemisia afra*, Hydroethanolic, Antihypertensive, Phytochemicals.

Introduction

Hypertension is a multifactorial trait with both genetic and environmental influences and is an important risk factor for cardiovascular diseases.[6] More than one-fourth of the world's adult population suffers from hypertension.[21] The prevalence of hypertension in developing countries is increasing though awareness of the disease is low.[10] Several reports indicate that treatment and control of hypertension in sub-Sharan African countries is low.[12,14] Factors linked to the poor control rates are associated with availability of adequate medication and poor compliance with treatment regimen.[7,17] Even when medications are readily available, patients from rural communities have often practiced concomitant administration of pharmaceuticals and plant concoctions.[15]

Complementary and alternative therapies are important potential option for the treatment of hypertension which may contribute to reducing blood pressure levels and minimizing its complications.[21] Extracts from such plants should be investigated scientifically as they may provide alternatives to pharmaceuticals or may be candidate for adverse drug-drug interactions.[9] This study was therefore aimed at investigating the antihypertensive effects of *Artemisia afra* (umhlonyane) and *Leonotis leonurus* which are common plants in South African traditional pharmacopoeia.

Artemisia afra, also known as African wormwood (*umhlonyane* in Xhosa, *mlonyane* in Zulu and *zengana* in Southern Sotho) is a medium sized multi-stemmed, clump-forming woody perennial shrub which grows up to 2 m in height with a leafy, hairy ridged stem. It belongs to the family Asteraceae and is traditionally used either alone or in combination with other plants for the treatment of respiratory tract related problems, gastrointestinal disorders, skin afflictions, gynaecological problems, fever, diabetes and cardiovascular disorders like hypertension.[19]

Leonotis leonurus (*umfincafincane* in Xhosa, *umuyane* in Zulu and *lebake* in Sotho) on the other hand is a shrub widely known as '*wild dagga*' found in most parts of the world and belongs to the Lamiaceae family.[16] It has many reputed traditional uses including treatment of cough, cold, influenza, chest infections, diabetes, hypertension, eczema, epilepsy, delayed menstruation, intestinal worms, constipation, spider bites and scorpion stings and as an antidote for snakebite.[16]

Although these plants are used regularly in traditional medicine, studies validating their safety and

antihypertensive effects are few. The present study therefore evaluated the acute toxicity and antihypertensive effects of the hydroethanolic extracts of *A. afra* and *L. leonurus* in Swiss albino mice and spontaneously hypertensive rats respectively in order to validate the use of these plants as pharmacological tools in ethnomedicine.

Material and Methods

Drugs, chemicals and reagents: Glacial acetic acid, ammonia, ferric chloride, sulphuric acid, trichloromethane, Dragendoff's reagent and Meyer's reagent were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA) while furosemide was obtained from Pharmacare Ltd. (South Africa). All chemicals including solvents were of analytical grade.

Plant Collection and Extraction: *Artemisia afra* (voucher specimen number: Aremu 4/14/90) and *Leonotis leonurus* (voucher specimen number: Aremu4/14/91) were collected from Mandela Park, Mthatha – South Africa. Both plants were identified by Dr. Immelman of the KEI Herbarium, Walter Sisulu University. Plant material was air-dried, crushed and extracted in 70% ethanol. The ethanol was recovered using a rotator evaporator (Laborator 4000, Germany) and the extract dried in a fan oven at 35°C. Plant materials used in the phytochemical study were separated by parts such as leaves, spines and roots while whole plant extracts were used for *in vivo* studies.

Qualitative Phytochemical Screening of Extracts: Qualitative phytochemical screening of both extracts was determined using standard procedures as described by Amin et al.[3]

Animal Handling: Swiss albino mice weighing 20-25 g were obtained from the South African Vaccine Initiative while spontaneously hypertensive rats weighing 180-200 g were obtained from the University of KwaZulu Natal Animal unit, South Africa. All animals were housed at 24°C. Lighting to animal facility was exclusively by day light. The animals were fed normal rat chow and water *ad libitum*. All animal procedures were approved by the Research and Ethics Committee of the Institution (Protocol # 051/15).

Acute toxicity: Acute toxicity study was conducted in accordance with Lorke's method as described by Bulus et al.[8] The study was conducted in two phases using a total of fifteen mice per extract. In the first phase, nine mice were randomly distributed into 3 groups (n = 3) per dose level of 10, 100 and 1000 mg/kg respectively to establish the range of doses producing a toxic effect. The second phase consisted of 3 categories (n = 1) per dose level of 1600, 2900 and 5000mg/kg respectively to determine the correct LD_{50} value.[2] A control group of 3 mice was treated with distilled water.

All animals were observed continuously during the first 30 mins after dosing for immediate effects and then periodically (with special attention given to the first 4 hrs) for the next 24 hrs and then for 2 weeks. Animals were observed for changes in breathing pattern, appetite, general activities, paralysis and mortality. Changes in wellness parameters were compared with those of control animals.[1] The mean of the least dose that killed the mice and the highest dose that did not kill any mouse was taken as the median lethal dose (LD_{50}):

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

where D_0 is the maximum dose that caused no mortality and D_{100} is the lowest dose that caused 100% mortality.

Antihypertensive effects: Twenty-four SHRs were assigned to four treatment groups of six rats each (n=6) as follows:
Group I- Normal saline
Group II-Furosemide
Group III- *A. afra* hydroethanolextract
Group IV – *L. leonorus* hydroethanol extract
Each group received assigned treatment once off after baseline blood pressure was measured.

Blood Pressure Measurement: Blood pressure was measured in conscious rats using non-invasive tail-cuff plethysmography (CODA™ Blood Pressure System, Kent Scientific Co., USA). Blood pressure was determined at baseline and then 2, 4, 6, 8 and 24 hrs after administration of assigned treatments.[23] At the beginning of the experiments, rats were given 2 ml of water and allowed to rest for 30 mins. They were restrained in glass restrainers each having a black conical plastic piece with a nose opening, placed over the head region of the rat to cover the eyes of the rats and reduce stress.

The restrained rats were placed on a warming platform at 35 - 38°C and allowed to acclimatize to the holder for at least 5 minutes before fitting the occlusion cuff (O-cuff) and volume pressure recording (VPR) sensor. They were allowed to acclimatize to the warming platform for 30 minutes, then the occlusion cuffs were inflated to impede blood flow to the tails. As these cuffs deflated slowly, the VPR sensors measured the physiological characteristics of the returning blood. Systolic BP was automatically measured at the first appearance of tail swelling and diastolic BP was measured when the increasing rate of swelling ceased in the tail. In addition to SBP and DBP, mean arterial blood pressure and heart rate were also automatically measured. 5 to 8 consistent readings were selected for analyses.[20]

Statistical analysis: GraphPad Prism, version 5 was used for data analysis. ANOVA followed by ad hoc tests was performed to determine differences between treatment groups at selected time intervals. $P<0.05$ was considered significant. Results are presented as the mean ± SEM of the percentage change in BP.

Results and Discussion

Phytochemical content: The phytochemical constituents of the extracts contained variable numbers of phytochemicals. Whereas the flower extract of *L. leonurus* had many more secondary metabolites than the leaf and spine extracts, the leaf extract of *A. afra* had many more phytochemicals. All extracts contained saponins, terpenoids and phenols while all extracts were void of steroids.

On the other hand, glycosides were present in both extracts of *L.leonurus* though it was absent in the spines of *L. leonurus*. Phenols and flavonoids have antioxidant properties.[22] Polyphenols have vasorelaxant effects[24] while flavonoids are associated with antihyperlipidermic effects.[5] All the aforementioned effects of phytochemicals have a role in lowering BP thus making extracts of *L. leonurus* and *A. afra* important candidates for anti-hypertensive studies.

Acute Toxicity: The hydroethanolic extract of *A. afra* and *L. leonurus* did not cause any observable changes in the mice from 30 mins to 2 weeks after treatment. The skin and fur of treated animals remained normal, sleeping patterns were unchanged and animals maintained normal activity patterns. Amount of food consumed was similar between treated and control animals. Neither respiratory nor nervous system effects were observed and no mortality was noted. The LD_{50} of the hydroethanolic extract of these plant extracts was considered to be greater than 5000 mg/kg b.w. which is considered to be non-toxic.

According to Konate and collegues[13] pharmacological substances with LD_{50} less than 5 mg/kg are classified in the range of highly toxic substances, those with LD_{50} between 5 mg/kg and 5000 mg/kg are classified in the range of moderately toxic substances and those with LD_{50}more than 5000 mg/kg are not toxic. Thus, they suggested that these extracts were non-toxic and could be considered safe for use in traditional medicine.

Antihypertensive effect of *A. afra* and *L. leonurus* in SHR

Effect of extracts on blood pressure: The structural and functional vascular alterations that occur during hypertension are important pathological mechanisms that lead to the increase in BP and are the targets of antihypertensive therapy.[21] In this study, we observed the antihypertensive properties of *A. afra* and *L. leonurus* extracts in spontaneously hypertensive rat models. Spontaneously hypertensive rats are good experimental models to investigate hypertension phenotype very similar to essential hypertension. Hypertension in these animals is characterized by an increase in vascular reactivity accompanied by hyper-responsiveness to vasoconstrictor agonists.[11] Extracts of *A. afra* and *L. leonurus* significantly ($p<0.01$) lowered SBP 2 hrs after treatment. While the SBP lowering effects of *A. afra* were sustained through the 24 h period decreasing progressively over time, the effects of *L. leonurus* were very weak (Table 2).

Furosemide on the other hand lowered SBP significantly only during the 6 and 8 hrs after treatment. Throughout the study period, *A. afra* showed superior blood pressure lowering effects compared to *L. leonurus*. The extract of *A. afra* induced significant decrease in DBP. The greatest effects of the extract were observed during 2 and 4 hrs post treatment. Results obtained with extract of *L. leonurus* were weak and not time dependent (Table 3).

Mean arterial blood pressure on the other hand was significantly decreased by the extract of *A. afra* (23.6% and 20.1%) compared to either furosemide (3.3±0.8% and 7.1±0.2%) or *L. leonurus* (0.4±4% and 7.1±1%) treatment 2 and 4 hrs after treatment respectively (Table 4). Since the extracts of *A. afra* and *L. leonurus* exerted a substantial BP lowering effect in SHR, it could be suggested that these extracts may have a role on vascular alterations. Increased renal reactive oxygen species is implicated in the pathogenesis of hypertension in SHRs.[4]

Table 1
Phytochemical constituents of *A. afra* and *L. leonurus*

	L. leonurus			*A. afra*	
Phytochemical	**Leaf**	**Flower**	**Spine**	**Leaf**	**Stem**
Saponins	+	+	+	+	+
Flavonoids	_	_	_	_	_
Terpenoids	+	+	+	+	+
Glycosides	+	+	_	+	+
Phenols	+	+	+	+	+
Steroids	-	-	-	-	-
Alkaloids	_	+	_	_	_
Phytosteroids	_	+	_	+	_
Phlabotannins	-	-	-	+	-
Tannins	+	-	+	+	+

+ phytochemical present; - phytochemical absent

Table 2
Percentage change in mean SBP with treatment

Time(hrs)	Percentage change in SBP from baseline			
	Normal saline	Furosemide	*A. afra*	*L. leonurus*
2	-1.1±0.6	1.6±2	20.5±0.8**##	6.0±4**
4	5.5±1	5.0±1	14.8±0.7**##	7.9±3
6	-8.3±0.2	5.8±0.4**	4.3±3**##	1.0±3**
8	0.1±0.4	11.6±2**	9.2±4*	1.9±1
24	-5.8±1	-7.5±2	7.2±0.4**	7.6±2**

Negative values indicate an increase in SBP from baseline values. Percentages were obtained by computing (((SBP at baseline - SBP at given times after treatment)/SBP at baseline) *100). Values are expressed as mean ± sem, n = 6; * indicates comparisons between treatment groups and normal saline group. * p< 0.05; ** p < 0.01; # indicates comparisons between *A. afra* and *L. leonurus* treatment groups. #p<0.05; ##p<0.01.

Table 3
Percentage change in mean DBP with treatment over time

Time (hrs)	Percentage change in DBP from baseline			
	Normal saline	Furosemide	*A. afra*	*L. leonurus*
2	-7.1±1	4.6±2**	25.7±1**##	-3.8±4
4	4.5±3	8.7±0.1	20.0±1**##	6.4±0.6
6	-2.1±0.3	-2.7±0.2	2.4±2**#	-0.7±0.1
8	-2.9 ±2	1.5±1	6.6±3*##	-2.3±0.7
24	-3.7±0.6	-12.3±2	7.8±0.2**	6.2±0.6**

Negative values indicate an increase in DBP from baseline values. Percentages were obtained by computing (((DBP at baseline - DBP at given times after treatment)/DBP at baseline) *100). Values are expressed as mean ± sem, n = 6; * indicates comparisons between treatment groups and normal saline group. * p< 0.05; ** p < 0.01; # indicates comparisons between *A. afra* and *L. leonurus* treatment groups. #p<0.05; ##p<0.01.

Table 4
Percentage change in mean arterial blood pressure (MABP) with treatment over time

Time (hrs)	Percentage change in mean arterial blood pressure from baseline			
	Normal saline	Furosemide	*A. afra*	*L. leonurus*
2	-4.6±1	3.3±0.8	23.6±0.1**##	0.4±4
4	5.0±2	7.1±0.2	20.1±0.8**##	7.1±1
6	-4.6±0.2	0.8±0.1	3.1±2*	0.1±1*
8	7.7±2	13.6±1	7.7±3##	-0.4±0.1
24	-4.5±1	-10.3±2	7.6±.3**	7.0±1**

Negative values indicate an increase in MABP from baseline values. Percentages were obtained by computing (((MABP at baseline - MABP at given times after treatment)/MABP at baseline) *100). Values are expressed as mean ± sem, n = 6; * indicates comparisons between treatment groups and normal saline group. * p< 0.05; ** p < 0.01; # indicates comparisons between *A. afra* and *L. leonurus* treatment groups. #p<0.05; ##p<0.01.

Thus, the positive antihypertensive effects of these plant extracts suggest that their mechanism of action may be through free radical scavenging by antioxidants thus supporting earlier publications[18,22] on their antioxidant capacity.

Effect of extracts on heart rate: Table 5 illustrates the effect of the plant extracts on heart rate during the 24 hrs experimental period. Heart rate tended to increase in untreated control animals during the 24 hr test period. *L. Leonorus* extracts decrease heart rate throughout the 24 hr period. However significant decreases were noted during the 2, 4 and 8 hrs post treatment (Table 5). Treatment with *A. afra* on the other hand resulted in an initial increase in heart rate from 373±5 per minute to 418±6 per minute (p<0.01) 2 hrs after treatment and then decreased significantly during the 6thhr (324±5 per minute; p<0.01). Heart rate at 24 hrs was lower in all groups compared to baseline heart rates.

The overall decrease in heart rates in the extract treated animals compared to the controls suggested that one of the modes of action of these plant extracts may be by targeting β-adrenergic receptors. Binding of components of the plant extract to these receptors may have prevented neurotransmitters from the sympathetic nervous system from binding and this may have resulted in decreasing heart rate, cardiac output and total peripheral resistance and consequently decrease in BP.

Table 5
Effect of *A. afra* and *L. leonurus* on heart rate

	Time (hrs)					
Treatment groups	**0**	**2**	**4**	**6**	**8**	**24**
Normal saline	360±5	341±4	390±15	370±6	370±1	421±9**
Furosemide	380±6	360±5*	410±5	402±9	374±1	362±5
A. afra	373±5	418±6**	347±1	324±5**	348±10	367±9
L. leonurus	420±7	365±4**	385±7*	388±7	385±8*	402±2

Values are expressed as mean ± sem, n = 6; * indicates comparisons between baseline heart rate and heart rates at different times after treatment. * $p < 0.05$; ** $p < 0.01$.

Conclusion

A. afra and *L. leonurus* are non-toxic and exhibit antihypertensive properties, hence they may be potential candidates for antihypertensive therapy. The effects produced by these extracts in SHRs suggest that they could offer cheaper and safer potential therapeutic benefits in the prevention and treatment of HTN and other associated disorders in the traditional healing community supporting their use in traditional medicine.

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