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Original Research Article

Acute toxicity study and prevention of Nω-nitro-L-arginine methyl ester-induced hypertension by *Osteopermum imbricatum*

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Abstract

Purpose: *To determine the phytochemical content, toxicity and hypertension prevention effects of Osteospermum imbricatum leaf and root extracts.*

Methods: *Phytochemical screening of leaf and root extracts of O. imbricatum was performed by colorimetric procedure. Acute toxicity of leaf and root extracts was conducted in two phases; phase I consisted of three groups of mice (n = 3) for each dose level of 10, 100 and 1000 mg/kg while phase II had three mice that received 1600, 2900 or 5000 mg/kg. Wistar rats were grouped into 7 groups that were co-treated with L-NAME and normal saline or L-NAME and hydroethanolic leaf extract (150 and 300 mg/kg) or L-NAME and hydroethanolic root extract (150 and 300 mg/kg) or L-NAME and amlodipine (5 mg/kg) or normal saline only for 4 weeks. Treatment was carried out via the oral route while blood pressure was measured weekly for 4 weeks by non-invasive tail cuff method.*

Results: *The phytochemical profile of the leaf and root extracts revealed the presence of phenols, terpenoids, flavonoids, glycosides, tannins, steroids and saponins. Both the leaf and root extracts were toxic at 5000 mg/kg with an LD_{50} of 3807.89 mg/kg. In the fourth week of the study, only the leaf extract significantly ($p < 0.01$) prevented the progression of L-NAME induced hypertension; systolic and diastolic blood pressure of the group treated with L-NAME and leaf extract (300 mg/kg) were 183 ± 1 and 140 ± 1 mmHg, respectively, compared to the group that was treated with L-NAME and normal saline which produced systolic and diastolic BP values of 213 ± 3 and 172 ± 4 mmHg, respectively. The extracts, especially OIR300, exhibited diuretic effects in the second and third week of study by promoting excretion of 16 and 19 ml urine, respectively, compared to 11 and 14 ml for LN group.*

Conclusion: *The results suggest that O. imbricatum is moderately toxic at a high dose and contains a wide range of phytochemicals which offer partial protection against the development of nitric oxide deficiency hypertension.*

Keywords: *Hypertension, Phytochemicals, Acute toxicity, Nω-Nitro-L-arginine methyl ester (L-NAME), Osteospermum imbricatum*

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INTRODUCTION

Hypertension (HTN) is a condition that afflicts almost 1 billion people world-wide, and is a leading cause of morbidity and mortality [1]. In South Africa for example, more than 6.2 million people have HTN [2,3].

The prevalence of herbal medicines use for the management of HTN has been reported in some African countries: Nigeria (80 %) [4], Morocco (21 %) [5], India (64 %) [6] and South Africa (21 %) [2]. The herbal medicines industry in South Africa is mostly informal with prescription made by personnel who have little or no knowledge of HTN. Knowledge on use of these herbal medicines is passed down from one generation to another. Because there is continued use of herbal medicines for the management of HTN and other ailments, it is imperative that safety limits as well as efficacy are investigated.

One of the plants commonly used in Eastern Cape of South Africa for the management of HTN is *Osteospermum imbricatum* (*Umashiqolo* in the Xhosa) of the family Asteraceae. Plants contain phytochemicals which are bioactive non-nutrient compounds [7]. Thus, crude plant extracts as used in the traditional settings, may have antihypertensive effects due to the contribution of a combination of phytochemical actions through different mechanisms.

It is well established from experimental and epidemiological studies that plant-based foods play an important role in the prevention of HTN and associated cardiovascular diseases [8]. Indeed, health-care systems emphasize providing medication for treatment and very rarely for prevention thus, missing important opportunities for risk and cost reduction [9]. Prevention of HTN by use of medicinal plants can be investigated in rat models that mimic essential HTN like the N^{ω}-nitro-l-arginine methyl ester (L-NAME) model Since it is likely that drugs are most efficient in preventing and reversing early rather than late vascular alterations in HTN, this model is suitable in testing the vascular protective effects of antihypertensive drugs in the context of NO deficiency [10].This study was therefore aimed at evaluating the qualitative phytochemical composition, acute toxicity and protective antihypertensive properties of *O. imbricatum* leaf and root extracts.

EXPERIMENTAL

The whole plant (stems, leaves and roots) was collected in May 2015 from Eastern Cape Province of South Africa and supplied by Mr Fikile Mahlakata. It was authenticated by Dr Immelman of the Kei Herbarium, Walter Sisulu University where a voucher specimen (Tata 2/14009) was deposited. The roots and leaves of the plant were separated and dried in the laboratory, crushed and exhaustively extracted separately in 70 % ethanol. Ethanol was recovered under vacuum using a rotary evaporator (Laboroto 4000, Germany) at 35 ^{0}C and then oven dried at the same temperature. The plant extracts were stored in a refrigerator and reconstituted in distilled water before use.

Phytochemical screening of extract

Phytochemical screening of the root and leaf extracts was carried out following standard procedures [11]. The presence of flavonoids, tannins, saponins, terpenoids, alkaloids, glycosides and steroids was tested for the leaf and root extracts.

Animals

Swiss albino mice weighing 20-25 g were used for the acute toxicity test and female Wistar rats weighing 200-280 g were used for hypertension prevention study. Animals were housed in cages in animal rooms at Walter Sisulu University which is maintained at 24 ^{o}C. The room was lighted by day light and dark at night. The animals had free access to rat chow and water. All animal procedures were approved by the Faculty of Health Sciences Research and Ethics Committee of Walter Sisulu University (protocol # 051/15). The animals were treated according to National Research Council Guide for the use of Laboratory animals [12].

Acute toxicity study

Acute toxicity study was conducted in accordance with Lorke's method [13]. The study was conducted in two phases using a total of fifteen mice per extract. In the first phase, nine mice were divided into 3 groups of 3 mice each. Groups 1, 2 and 3 animals were treated orally with 10, 100 and 1000 mg/kg of the extract, respectively. In addition, a fourth group of three mice was used as control group and animals in this group were given distilled water. The second phase was done after 24hrs: 3 groups of 1 mouse each were given doses based on the findings of phase 1. No death was recorded in Phase 1, thus in phase II, higher doses (1600, 2900 and 5000 mg/kg) of the extract were administered to three mice (one mouse per dose) to determine the LD_{50} value The geometric mean of the least dose that killed and the highest dose

that did not kill was taken as the median lethal dose (LD_{50}) as in Eq 1.

$LD_{50} = \sqrt{(D_0 \times D_{100})}$ …… (1)

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

Anti-hypertension study

Animals and grouping

Forty-two female Wistar rats weighing 200-250 g were assigned to seven treatment groups of six rats each (n = 6). The rats were first treated with normal saline or amlodipine or plant extracts and 15 min later, L-NAME was administered [14].

Treatment groups were NT - (normotensive control)-normal saline only; LN - Normal saline and L-NAME (40 mg/kg); AMLO –Amlodipine (5 mg/kg) and L-NAME (40 mg/kg); OIL150 - Leaf extract(150 mg/kg) and L-NAME (40 mg/kg); OIL300 - Leaf extract (300 mg/kg) and L-NAME (40 mg/kg); OIR 150 - Root extract (150 mg/kg) and L-NAME (40 mg/kg); OIR300 - Root extract (300mg/kg) and L-NAME (40 mg/kg).

All treatments of animals were done orally daily for 4 weeks after which necropsy was performed.

Measurement of blood pressure

Blood pressure was measured in conscious rats, using non-invasive tail-cuff plethysmography (CODA^{TM} Blood Pressure System, Kent Scientific Co., USA) as per manufacturer's manual. Blood pressure (systolic and diastolic pressure) and heart rate was measured at time 0 before starting treatment and weekly for 4 weeks between 9 and 16 hours after administration of the extract. Results were expressed as absolute values.

Urine output

To investigate possible diuretic effects of OIL and OIR, urine output was measured weekly for 4 weeks by placing the rats individually in metabolic cages over a period of 21 hrs. The collected urine volume was measured in graduated measuring cylinder.

Termination of treatment

Treatment was stopped two days to termination. The rats were fasted for 16 h, weighed and terminated by CO_2 inhalation [15]. The kidneys, heart and liver were harvested and each was weighed. The percentage organ to body weight ratio was calculated:

% organ to body weight ratio = weight of organ/body weight *100.

Data presentation and statistical analysis

All data are presented as mean ± standard error of mean (SEM). Statistical analysis was carried out using GraphPad Prism version 5.03. One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons was used to determine differences between treatment groups.$P \leq 0.05$ was considered statistically significant.

RESULTS

Phytochemical profile of *O. imbricatum*

The phytochemical constituents of the leaves and roots of *O. imbricatum* are shown in Table 1. Steroids were present in the leaves but not in the roots.

Table 1: Qualitative phytochemical profile of the leaves and roots of *Osteospermum imbricatum*

Phytochemical	Leaf	Root
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Alkaloids	+	+
Tannins	+	+
Cardiac glycosides	+	+
Steroids	+	-
Phenols	+	+

+ = phytochemical present; - = phytochemicals absent

Acute toxicity

During the two weeks study period, the animals in phase I (10, 100 and 1000 mg/kg) and the lowest phase II dose (1600 mg/kg) of both leaf and root treatment groups showed no observable adverse reactions. Parameters used for evaluation of toxicity like sleep, behavioral pattern, skin, fur and appetite were found to be normal. General weakness, lacrimation, tremor or paralysis did not occur in any of the animals in phase I. Both phase I and II animals had normal skin and fur with no fur loss or development of skin sores after 14 days. However both extracts showed toxicity at in phase II as shown in Table 2.

Table 2 shows the changes that were observed in phase II; in the first 30 mins after treatment, lethargy was observed in mice that received root extract in the ratio 1/3. 4 hrs later, ratios 2/3 and

1/3 lethargy were observed in mice that received root and leaf extract respectively. 72 hrs after treatment, mortality in the ratio 1/3 was found in both cases. Changes in body weights after two weeks were similar to controls (Tables 3 and 4). The calculated LD_{50} value of the hydroethanolic extract of both the leaves and roots of *O. imbricatum* was found to be 3807.89 mg/kg.

Table 5 represents organ (harvested after HTN prevention) to body weight ratio. Rats treated with *O. imbricatum* leaf extract (OIL), *O. imbricatum* root extract (OIR) and amlodipine (AMLO) had significantly larger hearts ($p<0.01$) compared to those from the normotensive (NT) control group (table 5). The hearts were however not different from those of the L-NAME (LN) control group. There was no significant difference in kidney sizes from OIL, OIR and AMLO treatment groups compared to those from the NT and LN control groups. Livers from the OIL300 (2.48 mg) was significantly smaller ($p < 0.05$) than those from the LN treatment groups (2.62 mg).

Protective effect *O. imbricatum* leaf and root extracts in L-NAME induced hypertension

At the beginning of the experiment, baseline SBP (149±4 to 151±2 mmHg) and DBP (111±1 to 120±3 mmHg) were similar among groups and L-NAME administration induced a progressive increase in SBP (171±2 to 191±2 mmHg) and DBP (131±3 to 154±1 mmHg) which was significantly ($p<0.01$) higher than pressures in NT control group (Table 6 and 7).

Table 3: Effect of oral administration *Osteospermum imbricatum* leaf extract on the body weights of mice

Experiment	Dose (mg/kg bw)	Weight gain/g (mean ± SEM)
Phase I	10	$4.23±0.25_a$
	100	$4.35±0.38_a$
	1000	$1.43±1.90_a$
Control	0	1.91±0.64
Phase II	1600	4.83
	2900	3.06
	5000	/

Same subscripts indicate no significant difference ($p > 0.05$)

Table 4: Effect of oral administration *Osteospermum imbricatum* root extract on the body weights of mice

Experiment	Dose (mg/kg)	Weight gain/g (mean ± SEM)
Phase I	10	$4.09±0.54_a$
	100	$3.62±0.59_a$
	1000	$2.87±0.38_a$
Control	0	1.91±0.64
Phase II	1600	4.38
	2900	4.59
	5000	/

Same subscripts indicate no significant difference ($p > 0.05$)

Table 2: Changes observed after administration of *Osteospermum imbricatum* leaf and root extracts

Variable	Phase II (leaf extract)						Phase II (root extract)					
	30min	*4h*	*24h*	*48h*	*72h*	*2W*	*30min*	*4h*	*24h*	*48h*	*72h*	*2W*
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	A,N	A,N	A,N	N	N	A,N	A,N	A,N	A,N	N	N
General weakness	0	Z	Z	Z	0	0	Z	YZ	Z	Z	0	0
Loss of appetite	0	Z	Z	Z	0	0	Z	YZ	Z	Z	0	0
Breathing difficulties	0	Z	Z	Z	0	0	0	Z	Z	Z	0	0
Lacrimation	0	0	0	0	0	0	0	Z	0	0	0	0
Tremor	0	0	0	Z	0	0	0	Z	Z	Z	0	0
Paralysis	0	0	0	Z	0	0	0	0	0	Z	0	0
Mortality	0	0	0	0	Z	0	0	0	0	0	Z	0

N-normal sleep, A,N - some are normal and others abnormal, 0 - no observable change, Y - the mouse treated with 2900 mg/kg; Z - the mouse treated with 5000 mg/kg

Table 5: Organ/body weight ratio after treatment with *Osteospermum imbricatum* leaf and root extracts

Organ	NT	LN	AMLO	OIL150	OIL300	OIR150	OIR300
Heart	0.37	0.42##	0.42##	0.41##	0.41##	0.44##	0.41##
Kidney	0.59	0.58	0.6	0.58	0.58	0.59	0.61
Liver	2.53	2.62	2.66	2.59	$2.48^{\#}$	2.54	2.51

Values are expressed as mean ±SEM. n = 6; NT= normotensive control; LN= L-NAME control; AMLO = amlodipine and L-NAME; OIL=*O.imbricatum* leaf and L-NAME; OIR =*O. imbricatum* root and L-NAME #$p<0.05$, ##$p< 0.01$ compared to normotensive control group

Rats receiving L-NAME with concurrent administration of leaf and root extracts showed increased SBP (OIL150 - 187±2, OIL300 - 183±1 mmHg; OIR150 - 203±4, OIR300 - 192±3 mmHg) compared to normotensive controls (153±3 mmHg) but lower SBP compared to L-NAME (213±3 mmHg) controls but in the fourth week, only OIL150 and OIL300 significantly ($p<0.01$) prevented increase in SBP compared to LN group (Table 6). The DBP only appeared lower than the LN control in the fourth week of treatment (Table 7). Both OIL extracts had significant ($p < 0.01$) effect on SBP but OIL300 had significant effect ($p < 0.01$) on both SBP and DBP while OIR extracts did not.

Treatment with L-NAME significantly lowered heart rate. There was a significant decrease ($p<0.05$) in heart rates in the LN and AMLO treatment groups in the 2nd (LN - 380±6 bpm) and 3rd weeks (LN - 377±6, AMLO - 374±2 bpm) of treatment compared to the baseline heart rate (LN - 406±3, AMLO 393±3 bpm) at week 1. The OIL150 treatment group had a significant decrease ($p<0.01$) in heart rate (397±4 to 356±4 bpm) in the 2nd week of treatment. In the OIR150 treatment group, a significant decrease ($p<0.01$) in heart rate (142±1 to 404±6 bpm) was recorded throughout the treatment weeks while significant decrease ($p<0.05$) was observed in the OIR300 treatment group (372±1 to 348±6 bpm) only in the second week of treatment (Table 8).

Table 8: Effect of *Osteospermum imbricatum* leaf and root extracts on heart rate

Treatment	Heart rate			
	Week 1	***Week 2***	***Week 3***	***Week 4***
NT	413±3	413±3	408±4	408±4
LN	406±3	380±6*	377±6*	385±4
AMLO (5mg/kg)	393±3	379±1	374±2*	380±5
OIL150 mg/kg	397±4	356±4**	389±5	414±8
OIL300 mg/kg	396±5	379±5	393±4	378±4
OIR150 mg/kg	452±1	388±3**	380±6**	404±6**
OIR300 mg/kg	372±1	348±6*	386±5	381±4

Values are expressed as mean ±SEM. n = 6; NT= normotensive control; LN= L-NAME control; AMLO= amlodipine and L-NAME; OIL= *O.imbricatum* leaf and L-NAME; OIR =*O. imbricatum* root and L-NAME * $p< 0.05$, ** $p < 0.01$ compared to baseline heart rate

Urine output increased progressively in all the treatment groups; OIL150 (13±0.4 to 20±1 ml), OIL300 (11±0.7 to 16±0.9 ml), OIR150 (8±0.2 to 17±0.9 ml), OIR300 (10±0.5 to 21±1 ml).

Table 6: Protective effect of *Osteospermum imbricatum* leaf and root extracts on systolic blood pressure

Treatment	Systolic blood pressure (mmHg)			
	Week 1	***Week 2***	***Week 3***	***Week 4***
NT	149±4	149±4	153±3	153±3
LN	151±1	185±1$^{\#\#}$	198±2$^{\#\#}$	213±3$^{\#\#}$
AMLO (5 mg/kg)	149±1	171±2*$^{\#\#}$	189±0.4$^{\#\#}$	180±4**$^{\#\#}$
OIL150 mg/kg	150±1	191±2$^{\#\#}$	195±3$^{\#\#}$	187±2**$^{\#\#}$
OIL300 mg/kg	150±3	189±3$^{\#\#}$	184±3*$^{\#\#}$	183±1**$^{\#\#}$
OIR150 mg/kg	151±0.4	175±2$^{\#\#}$	205±2$^{\#\#}$	203±4$^{\#\#}$
OIR300 mg/kg	151±2	174±2$^{\#\#}$	184±1$^{\#\#}$	192±3$^{\#\#}$

Values are expressed as mean ±SEM. n = 6; NT= normotensive control; LN= L-NAME control; AMLO= amlodipine and L-NAME; OIL= *O.imbricatum* leaf and L-NAME; OIR =*O. imbricatum* and L-NAME root* $p< 0.05$, ** $p < 0.01$ compared to L-NAME (LN) control group; #$p<0.05$, ##$p< 0.01$ compared to normotensive control group

Table 7: Protective effect of *Osteospermum imbricatum* leaf and root extracts on diastolic blood pressure

Treatment	Diastolic blood pressure (mmHg)			
	Week 1	***Week 2***	***Week 3***	***Week 4***
NT	111±4	111±4	108±3	108±3
LN	115±1	141±1$^{\#\#}$	151±3$^{\#\#}$	172±4$^{\#\#}$
AMLO (5 mg/kg)	111±1	131±3$^{\#\#}$	149±0.4$^{\#\#}$	149±5*$^{\#\#}$
OIL150 mg/kg	115±2	154±1$^{\#\#}$	165±3$^{\#\#}$	153±3$^{\#\#}$
OIL300 mg/kg	120±3	152±3$^{\#\#}$	148±4$^{\#\#}$	140±1**$^{\#\#}$
OIR150 mg/kg	117±0.1	142±2$^{\#\#}$	169±2$^{\#\#}$	158±4$^{\#\#}$
OIR300 mg/kg	115±2$^{\#\#}$	134±2$^{\#\#}$	158±4$^{\#\#}$	147±3$^{\#\#}$

Values are expressed as mean ±SEM. n = 6; NT= normotensive control; LN= L-NAME control; AMLO=amlodipine and L-NAME; OIL=*O.imbricatum* leaf and L-NAME; OIR =*O. imbricatum* root and L-NAME * $p< 0.05$, ** $p < 0.01$ compared to L-NAME (LN) control group; #$p<0.05$, ## $p< 0.01$ compared to normotensive control group

Table 9: Effect of *Osteospermum imbricatum* leaf and root extracts on urine output

Wk	NT (ml)	LN (ml)	AMLO (ml)	OIL150 (ml)	OIL300 (ml)	OIR150 (ml)	OIR300 (ml)
1	11±0.3	9±0.5	16±1**$^{\#\#}$	9±0.3	11±0.7	8±0.2	10±0.6
2	13±0.2	11±0.3	16±0.9**	13±0.4	12±0.9	14±0.7	16±1**
3	14±0.7	14±0.5	17±0.6*	12±0.3	14±0.8	16±0.5	19±1**$^{\#\#}$
4	13±0.5	18±0.5	17±0.6	20±1.5$^{\#}$	16±0.9	17±0.9	21±1.4$^{\#\#}$

Values are expressed as mean ±SEM. n = 6; NT= normotensive control; LN= L-NAME control; AMLO=amlodipine and L-NAME; OIL=*O.imbricatum* leaf and L-NAME; OIR =*O. imbricatum* root and L-NAME; *p< 0.05, **p < 0.01 compared to L-NAME (LN) control group; #p<0.05, ## p< 0.01 compared to normotensive control group

High volume of urine was produced by rats treated with the standard drug amlodopine (AMLO) from the first to the last week of treatment. Increased urine volume was also observed in the LN treatment group in the 4th week of treatment (Table 9).

DISCUSSION

This study examined the phytochemicals, acute toxicity and preventive effect on hypertension of the hydroethanol extracts of *Osteospermum imbricatum* in L-NAME induced hypertensive rats. The findings reveal that *O. imbricatum* has saponins, tannins, flavonoids and phenolic compounds. It was discovered that although this plant appeared to be safe at low doses, it showed marked adverse effects at higher doses. The study confirmed that L-NAME induced hypertension as seen by the marked elevation of SBP and DBP which was significantly reduced by concurrent treatment with *O. imbricatum* leaf extract. Heart rates were lower in all L-NAME treated groups compared to control.

The medicinal value of a plant lies in its phytochemicals. Alkaloids, phenols, saponins, and flavonoids have been shown to have antihypertensive properties, free radical extinguishing properties and ability to inhibit lipid peroxidation [16]. *Osteospermum imbricatum* possesses these phytochemicals which may be responsible for the antihypertensive property shown by the leaf extract. The root extract of *O. imbricatum* exhibited some toxic effects when given orally at concentration of 2900mg/kg and above, while the leaf extract showed toxicity at 5000 mg/kg. However the insignificant changes in parameters like normal breathing, movement, feeding and body weights in phase I of the acute toxicity test, reveals the safety of the extract at doses below 2900 mg/kg. Recovery and weight gain after treatment indicated that the mice survived acute toxicity [13].The toxicity of *O. imbricatum* may be as a result of cyanide poisoning [17]. Studies have shown that *Osteospermum* is a toxic plant genus and when the plant cell is damaged, linamarase causes linamarin to split into glucose, hydrocyanic acid and acetone [17]. The hydrocyanic acid is likely responsible for the toxicity observed in this study. According to Konate *et al* [18], 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. This classification suggests that *O. imbricatum* is moderately toxic and would be regarded as safe or having low toxicity.

The results of the present study showed that oral administration of L-NAME resulted in marked and sustained HTN and decreased heart rate. Nitric oxide is a potent vasodilator, it maintains vascular tone, inhibit platelet aggregation, monocyte adhesion, smooth muscle proliferation/migration and regulates BP [10,19]. Therefore loss or decrease of NO production due to inhibition of nitric oxide synthase by L-NAME plays an important role in pathogenesis of HTN. Lack of NO in the circulatory system induces vasoconstriction leading to an increase in peripheral resistance eventually leading to HTN [20].The decreased in heart rate is likely due to baroreflex activity [21] suggesting that L-NAME-induced increase in total peripheral resistance results in decrease heart rates and probably decrease cardiac output. Previous studies have provided compelling evidence that chronic L-NAME-induced HTN is caused by widespread vasoconstriction associated with a decrease in cardiac output [22]. The ability of the leaf extract to prevent decrease in heart rate induced by L-NAME suggests that its mode of action maybe via adrenergic receptors. *Osteospermum imbricatum* extract may also exhibit its BP lowering properties by up-regulating nitric oxide synthase or serving as a NO donor thus reducing total peripheral resistance and causing diuresis. Studies have shown that NO exerts direct inhibitory effects on renal tubular function through the inhibition of both the Na^+/K^+-ATPase and the Na^+/H^+ exchanger therefore, reducing sodium reabsorption and increasing

natriuresis/diuresis [23]. Excessive production of reactive oxygen species is proposed to be a major factor in mediating the pathogenesis of HTN [19]. In the L-NAME model, a large quantity of superoxide production suppresses nitric oxide bioavailability [19].

Osteospermum imbricatum extract exhibited little or no protection of the development of cardiomegaly secondary to sustained peripheral resistance as shown by failure of the extract to completely prevent the development of HTN. The sustained increase in BP due to absence of NO may have resulted in hypertrophy of the heart confirmed by increase heart sizes [24].

Diuretics act by diminishing sodium reabsorption at different sites in the nephron, thereby increasing urinary sodium and water losses hence decreasing blood volume and the high blood pressure in HTN [25]. The leaf and root extracts of *O. imbricatum* caused progressive increase in urine output throughout the four weeks treatment period implying possible diuretic effects by the plant. Possible site of action may be the loop of henle, distal tubule or collecting duct. It may be prudent in future studies to assess electrolytes since some diuretics are associated with fluid and electrolyte complications, including volume depletion, azotemia, hypokalemia, metabolic alkalosis, hyponatremia, hyperuricemia, and hypomagnesemia [25]. The L-NAME control group only showed diuresis in the fourth week of treatment. This may be associated with HTN induced kidney disease. The decrease in liver size from the group that was treated with higher dose of the leaf extract maybe as a result of reduction in fat content in liver cells.

CONCLUSION

The results of this study demonstrate that *O. imbricatum* has a wide range of secondary metabolites, and that the leaf extract offers partial protection against the development of NO deficiency hypertension. This study therefore validates the use of the leaf extract of this plant in the treatment of hypertension in combination with other plants. Warnings of toxicity at high concentration should be communicated to practitioners.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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