

Antihypertensive action of *Launaea taraxacifolia* and its molecular mechanism of action

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Abstract: *Launaea taraxacifolia* has been traditionally used for the management of conditions such as cardiovascular, respiratory, and metabolic diseases. High blood pressure was established by oral administration of L-Nitro Arginine Methyl Ester (L-NAME) a non-selective inhibitor of endothelial nitric oxide synthase (eNOS). The antihypertensive action of the methanol leaf extract of *L. taraxacifolia* was examined. Fifty male Wistar rats were divided into 5 groups of 10 animals per group: Group A (Distilled water), Group B (Hypertensive rats; 40mg/kg L-NAME), Group C (Hypertensive rats plus 100 mg/kg extract), Group D (Hypertensive rats plus 200 mg/kg extract) and Group E (Hypertensive rats plus 10mg/kg of Lisinopril). The treatments were orally administered for five weeks. Haemodynamic parameters, urinalysis, indices of oxidative stress and immunohistochemistry were determined. Findings from this study showed that blood pressure parameters, urinary sodium and indices of oxidative stress increased significantly while *In-vivo* antioxidant defence systems decreased significantly in hypertensive rats. Immunohistochemistry revealed significant increases in expressions of mineralocorticoid receptor, angiotensin converting enzyme activity and kidney injury molecule-1 in kidney of hypertensive rats. Treatment with *Launaea taraxacifolia* normalized blood pressure parameters, urinary sodium, oxidative stress indices, antioxidant defence system, and serum nitric oxide bioavailability.

Keywords: *Launaea taraxacifolia*, hypertension, oxidative stress, antioxidant, anti-hypertensive.

INTRODUCTION

Elevated blood pressure has been reported to affect close to one billion people worldwide with attendant cardiovascular complications implicated in many deaths globally (Benjamin *et al.*, 2017). Previous research finding suggested that the use of appropriate drugs and lifestyle modifications could effectively help in reducing high blood pressure (Volpe *et al.*, 2019). Elevated blood pressure continues to be a major global problem due to various factors and it has been projected that approximately one quarter of the world's population may be affected in the next four years (Mills *et al.*, 2016).

Moreover, non-communicable disease-related deaths, majority of which are associated with elevated blood pressure and other cardiovascular derangements, reportedly account for 71% of deaths of hospitalized patients in the last two decades (Kearney *et al.*, 2005).

Hypertension therapy with orthodox medicine has continued to create a global economic burden with attendant side effects (Gheorghe *et al.*, 2018). Most of the currently available antihypertensive agents act either by modulating the vasodilatory apparatus of the vascular bed by modulating renin-angiotensin-aldosterone system or contractility of the heart (Ames *et al.*, 2019). In recent

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decades, the acceptance of medicinal plants or phytotherapy is becoming increasingly popular. *Launaea taraxacifolia* (LT), is widely distributed in West Africa (Dansi, *et al.*, 2008). It belongs to the Asteraceae or Compositae family. Ethnobotanical studies reported that LT has been used against cardiovascular and respiratory diseases (Asase and Yohonu, 2016). The leafy vegetable has been found to contain an array of phytoconstituents including flavonoids (Olugbenga *et al.*, 2015). Furthermore, the antioxidant (Adinortey *et al.*, 2018), anti-inflammatory (Datta *et al.*, 2014), cardio-protective (Larbie and Mensahm 2014), antimalarial, nephron-protective (Abouzeinab, 2015), anticancer (Thomford *et al.*, 2016), hypolipidaemic and neuroprotective (Koukou *et al.*, 2017) properties have been documented.

Based on the above literature search, antihypertensive effect of *Launaea taraxacifolia* has not being scientifically validated. This study explored molecular mechanism of antihypertensive efficacy of *Launaea taraxacifolia*.

Experimental framework

Preparation of plant extract

The fresh leaves of LT were authenticated at the Department of Botany, University of Ibadan, and specimen voucher number UIH-22760 was deposited at the herbarium. The LT leaves were subsequently dried at room temperature, pulverized, and subjected to cold extraction using analytical grade methanol.

Experimental design

Experimental animals used for this study were adult male rats (100±15g). Five experimental study groups were used following random selection. 50 male Wistar rats were distributed into 5 groups of 10 animals per group: Group A (Distilled water), Group B (40mg/kg L-NAME), Group C (40mg/kg L-NAME plus 100mg/kg extract), Group D (40mg/kg L-NAME plus 200mg/kg extract) and Group E (40mg/kg L-NAME plus 10mg/kg of Lisinopril). The experiment lasted for five weeks.

Ethical clearance

Experiments were carried out according to University of Ibadan Animal Care and Use Research Ethical Committee (ACUREC). The study was approved with approval number UI-ACUREC/18/0135.

In vitro antioxidant assays

The plant extract was evaluated for antioxidant power using ABTS and DPPH as described by RE *et al.* (1999) and (Liyana-Pathirana *et al.*, 2005), respectively.

Cytotoxicity

Cytotoxicity was determined using Tetrazolium Bromide (MTT) protocol (Mosmann, 1983). Tetrazolium bromide (MTT) was dissolved in Phosphate Buffered Saline at 5mg/ml and filtered. Ten microlitres of Stock MTT solution was added to microtitre plates, and the plates

were then incubated 4hr at 37°C. After the incubation period, a solution containing 100 µ of 0.04 N hydrochloric acid in isopropanol was added to the assay wells. The plates were read within 1hr following the addition of acid-isopropanol with an ELISA plate reader (Biotec, Synergy HT) at 570 nm wavelength (Mosmann, 1983).

In vitro measurement of nitrite

Nitrite was measured in culture media using Griess reagent, as earlier described (Yoon *et al.*, 2009) with modifications. The percentage of NO inhibition was calculated relative to the untreated LPS-induced cells and a NO inhibition greater than 70% was selected (Yang *et al.*, 2009). Quercetin, a potent NO inhibitor was used as a positive control.

Essential oil analysis

The essential oil of the plant extract was analyzed with Gas Chromatography-Mass Spectrometry (GC-MS) as earlier described (Ola-Davies *et al.*, 2019).

Urinalysis

Metabolism cages were used to house the rats for a twenty-four-hour period prior to termination of the experiment to collect urine sample. Metabolites in the urine were measured with Medi-Test Combi 9 urine strip (Germany).

Serum markers of renal damage

The qualitative estimation of the urinary content of total protein, albumin, creatinine, as well as blood urea nitrogen was done in accordance with the instruction manual of the Randox kits used for urinalysis.

Blood pressure measurement

The haemodynamic data were generated as reported by Oyagbemi *et al.* (2019).

Electrocardiography

Electrocardiograms were obtained in conscious rats placed on right lateral recumbence using a standard electrocardiograph (Oyagbemi *et al.*, 2019).

Serum preparation

Whole blood was collected into plain sample bottles and allowed to clot. The obtained serum was kept at a 4°C temperature.

Determination of serum testosterone

This assay was done with an enzyme-linked immunosorbent assay kit (DRG) Diagnostics, Germany) adhering to variation in the intra-assay coefficient following the manufacturer's instructions.

Renal and testicular cytosolic preparation

The kidney and testes were excised, rinsed, weighed, and homogenized with a 0.1M phosphate buffer, pH 7.4 using

a Teflon homogenizer. The resultant homogenates were centrifuged at 10,000g for 10 minutes at -4°C .

Biochemical parameters

Renal and testicular markers of oxidative stress

The protein carbonyl (PCO) contents of kidneys and testes were measured as described by Reznick and Packer (1994). Testicular and renal lipid peroxidation index (MDA), advanced oxidation protein product (AOPP), hydrogen peroxide (H_2O_2) generation and vitamin C contents were assayed as markers of oxidative stress (Wolff 1994; Jacques-Silva *et al.*, 2001; Kayali *et al.*, 2006).

Estimation of renal and testicular antioxidants defence status

The *in vivo* activity of superoxide dismutase, glutathione peroxidase, glutathione S-transferase, the content of reduced glutathione, as well as the thiol contents were determined using well-established methods (Misra and Fridovich, 1972).

Nitric oxide content and total protein concentration

The serum nitric oxide concentrations were measured with the standard method (Gornal *et al.*, 1949). Protein concentrations were estimated as earlier described (Drury and Wallington, 1979).

Histopathological examination

Kidney and testes were prepared for histopathologic evaluation by fixing in a 10% solution of formalin and subsequently embedding the tissues in paraffin wax. Thereafter, the paraffin wax embedded tissues were sectioned, stained with Haematoxylin and Eosin, and examined microscopically (Shin *et al.*, 2019).

Immunohistochemistry

The immunolocalization of the antigens with specific antibody probes was determined as earlier reported (Oyagbemi *et al.*, 2019). Quantitative analysis of immunostaining was performed with Image J software.

RESULTS

Gas chromatography-mass spectrometry (GC-MS) analysis

GCMS result revealed essential oil from LT with the confirmation of various phytochemicals/ phytonutrients with an abundance of butyl 2-ethylhexyl ester, diisooctyl hexanedioic acid, bis (2-ethylhexyl) ester and p-xylene (table 1).

In vitro antioxidant activity

The antioxidant capacity of LT against ABTS and DPPH radicals was comparable to that of Trolox and ascorbic acid, respectively (table 2). Figure 1 shows the free radical scavenging power of LT against DPPH and ABTS

radicals at different concentrations of LT. The IC_{50} values of LT were comparable to that of ascorbic acid and Trolox, respectively.

Cytotoxicity and inhibitory effects on LPS-induced NO production

As indicated in table 2, the cytotoxicity of LT against Vero cells demonstrated a dose-dependent manner, which was much lower than that of the cytotoxic agent doxorubicin with the lowest LC_{50} value. Furthermore, the potential of this extract to inhibit LPS-induced NO production is shown in table 3. The methanol leaf extract of LT demonstrated the capacity to reduce inflammation at 50 $\mu\text{g/ml}$ concentration (table 3).

Body weight gain / loss

From our results, a significant reduction in the percentage weight loss in hypertensive untreated rats and hypertensive-treated rats with 200mg/kg of LT was observed as indicated in table 4. However, there was no visible weight loss or gain in hypertensive rats administered 100mg/kg LT and 10mg/kg of lisinopril (table 4).

Electrocardiogram

The results of ECG indicated a significant increase in heart rate accompanied by significant reductions in P-wave and QRS durations, shortened QT and QTc intervals of hypertensive-untreated rats compared to the hypertensive-treated rats with LT (200 and 100mg/kg) and 10mg/kg of lisinopril (table 5). The ECG changes associated with L-NAME administration in this study were reversed by LT administration (table 5).

Urinalysis

Interestingly, urine content revealed high presence of bilirubin, urobilinogen, proteins, nitrates, leukocytes, and very low urine specific gravity of hypertensive rats (table 6). However, the presence of the parameters was mild in urine samples of hypertensive rats treated with LT (200 and 100 mg/kg body weight) and 10 mg/kg of lisinopril (table 6). Results from table 7 show a significant increase in urinary total protein and blood urea nitrogen, while urinary creatinine reduced significantly indicating impairment of creatinine clearance by the kidney of hypertensive rats. The difference in urinary total protein, sodium and urea were not significant, while urinary creatinine of hypertensive rats treated with LT and lisinopril was lowered than hypertensive untreated rats (table 7).

Sperm characteristics

The spermiogram revealed that sperm motility and counts decrease significantly in hypertensive-untreated rats (table 8). However, there was no significant difference in sperm livability in normotensive, hypertensive and hypertensive treated rats (table 8).

Blood pressure parameters

In fig. 3, 4 and 5, we observed statistically higher values of systolic, diastolic and mean arterial blood pressure of hypertensive rats at 1 week, 3 weeks and 5 weeks, respectively, relative to normotensive rats.

Kidney and testes oxidative stress indices and antioxidant status

Renal and testicular H_2O_2 generated and malondialdehyde (MDA) of hypertensive rats were elevated (fig. 6). The *in vivo* anti-oxidative power of LT was evident with significantly lower values of renal and testicular H_2O_2 generated and MDA. Similarly, the contents of protein carbonyl (PCO) and vitamin C were elevated in hypertensive animals relative to normotensive rats (fig. 7). Hypertensive rats treated with LT had a significant reduction in renal and testicular PCO content together with improvement in non-enzymic antioxidant vitamin C compared to hypertensive rats (fig. 7). Again, the total protein thiol (PSH) and non-protein thiol (NPSH) of testicular tissues showed statistically higher values while significant reduction in the PSH of renal tissues was obtained in rats that were hypertensive relative to the control rats and hypertensive rats treated with 100 and 200 mg/kg of LT (fig. 8). The testicular reduced glutathione (GSH) improved significantly in hypertensive rats. Further, there was appreciable improvement in both renal and testicular GSH of hypertensive rats administered LT and lisinopril (fig. 9). Also, the activity of superoxide dismutase (SOD) in both renal and testicular tissues of hypertensive rats decreased, with significant increases in the activity of SOD of hypertensive rats administered with 100 and 200mg/kg of LT and lisinopril in a dose-dependent fashion indicating the antioxidant activity of LT (fig. 9). The activity of glutathione S-transferase (GST) and glutathione peroxidase (GPx) in hypertensive rats were inhibited in comparison to normotensive rats (fig. 10). The activity of renal and testicular GST and GPx improved significantly in rats that were hypertensive but treated with either LT or lisinopril (fig. 10). Also, a dose-dependent increase in nitric oxide level of hypertensive rats treated with LT (100 & 200mg/kg) and 10 mg/kg lisinopril, while a concomitant significant reduction in advanced oxidative protein product in hypertensive rats treated with 10 mg/kg lisinopril was observed relative to hypertensive untreated and normotensive rats (fig. 11).

Serum testosterone

Fig. 11 shows a statistically significant reduction in the serum testosterone level of hypertensive rats while a dose-dependent increase was obtained in hypertensive rats administered LT (100&200mg/kg) and 10mg/kg lisinopril.

Histopathology

The histology of the kidney showed visible glomerular capillary congestion, patchy tubular epithelial

degeneration, attenuation of the tubular epithelial lining, lumina ectasia with a few peri-tubular inflammatory cells in hypertensive rats compared to normotensive rats and other treatment groups (fig. 12). The testicular architecture revealed spermatogenic arrest evident by irregularly shaped tubule outline, attenuation of germ cells and tubular ectasia (red arrows) in the hypertensive rats compared to other treated groups (fig. 13).

Immunohistochemistry

The renal immunostaining of angiotensin-converting enzyme (ACE) revealed significantly higher expression in hypertensive-untreated rats relative to hypertensive treated LT (100 & 200mg/kg) and 10 mg/kg lisinopril (fig. 14) groups. Interestingly, hypertensive treated LT (100 & 200 mg/kg) and 10mg/kg lisinopril groups showed similar expressions of ACE indicating the possible mechanism of action of LT. Fig. 15 shows the renal immunolocalization of mineralocorticoid receptor (MCR) of hypertensive rats which is more significantly expressed than in normotensive rats. The expression of MCR reduced significantly in hypertensive rats administered LT (100&200mg/kg) and 10mg/kg lisinopril (fig. 15). In another experiment, an appreciable rise in expression of kidney injury molecule (Kim-1) of hypertensive rats relative to normotensive animals was obtained (fig. 16). However, the expression in the immunolocalization of Kim-1 became significantly reduced in hypertensive rats administered with LT (100&200mg/kg) and 10 mg/kg lisinopril (fig. 16). In hypertensive rats, the testicular caspase-3 expression was found to increase significantly when compared to normotensive and hypertensive treated rats (fig. 17). This is indicative of testicular apoptosis. However, the observable increase in the expression of caspase-3 in hypertensive rats was reduced significantly in hypertensive rats administered with LT (100&200mg/kg) and 10 mg/kg lisinopril, like normotensive rats (fig. 17).

DISCUSSION

A consistent reduction in arterial blood pressure was associated with the administration of methanol leaf extract of LT for five weeks, comparable to the effects observed following treatment with the antihypertensive drug, lisinopril. The reduction in blood pressure was consistent with the improvement in nitric oxide bioavailability. Recent findings reported that a low level of nitric oxide bioavailability is inversely proportional to the hypertensive state (Konukoglu *et al.*, 2017; Stanhewicz *et al.*, 2017). Nitric oxide is needed for the maintenance of vascular tone and relaxation (Dharmashankar and Widlansky 2010), therefore, a decrease in NO availability might precipitate endothelial dysfunction, hypertension, and arterial stiffness as earlier reported (Kaliora *et al.*, 2007). This aberration in the NO signaling observed in hypertensive rats was ameliorated

Table 1: Showing GC-MS analysis of plant constituent and percentage

PEAK	Compound names	%Area	Chemical Formula	weight
1	Ethylbenzene	1.621058	C ₈ H ₁₀	106
2	p-Xylene	6.156823	C ₈ H ₁₀	106
3	P- Xylene	2.562371	C ₈ H ₁₀	106
4	1,3,5-Pentanetriol, 3-methyl-	1.111766	C ₆ H ₁₄ O ₃	134
5	Decane	3.328609	C ₁₀ H ₂₂	142
6	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.899492	C ₁₆ H ₂₂ O ₄	278
7	Phytol	1.921502	C ₂₀ H ₄₀ O	296
8	Benzyl butyl phthalate	0.937665	C ₁₉ H ₂₀ O ₄	312
9	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	58.91385	C ₂₀ H ₃₀ O ₄	334
10	Hexanedioic acid, bis(2-ethylhexyl) ester	7.728716	C ₂₂ H ₄₂ O ₄	370
11	Diisooctyl phthalate	13.81815	C ₂₄ H ₃₈ O ₄	390

Table 2: *In vitro* antioxidant properties of methanol leaf extract of *launeae taraxacifolia*

Sample	ABTS IC50 (µg/ml)	DPPH IC 50 (µg/ml)
<i>Launeae Taraxacifolia</i>	38.03637 ± 15.92304	265.5760 ± 28.9632
Trolox	0.00245 ± 0.000355	1.6029 ± 0.7780
Ascorbic Acid	0.00065 ± 0.00033	1.8947 ± 1.1898

Mean values are presented as Mean±S.D.

Abbreviations: ABTS: 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 3: Macrophage RAW 264.7 cells 40 000 cells/well over 24hrs

Samples	Conc (µg/mL)	%Cell Viability	%NO Inhibition
LT	1.6	100.25 ± 8.97	36.04 ± 8.45
	12.5	91.90 ± 8.56	52.58 ± 4.31
	50	82.94 ± 15.89	78.65 ± 5.73
	100	62.55 ± 9.92	92.48 ± 2.08
Quercetin	Conc (µg/mL)	%Viability	%NO Inhibition
	1.6	99.82 ± 5.42	53.84 ± 6.37
	12.5	83.35 ± 7.66	89.18 ± 7.32
	50	63.97 ± 3.77	93.46 ± 1.71
Doxorubicin	100	39.54 ± 4.31	96.26 ± 2.31
	Conc (µM/mL)	%Viability	
	2	79.36 ± 9.34	
	4	62.51 ± 13.87	
	10	9.42 ± 4.95	
	20	1.46 ± 0.80	

Note: Assays were read in triplicates

Table 4: Effect of *Launeae Taraxacifolia* on percentage weight gain in L-NAME induced hypertension.

GROUPS	INITIAL WEIGHT	FINAL WEIGHT	% WEIGHT GAIN
Control	103.8±9.85	153.6±15.45	32.4
HYTR	125.1±11.33	162.7±18.46	23.1
HYTR + LT (100 mg/kg)	122.8±7.24	172.3±9.79	28.7
HYTR + LT (200 mg/kg)	122.3±13.43	158.6±8.36	22.5
HYTR + Lisinopril (10 mg/kg)	112.1±11.61	151.2±22.34	25.9

Values are presented as Mean±SD (n=5. HYTR (Hypertensive rats), LT (*Launeae taraxacifolia*)

Table 5: Effect of *Launeae Taraxacifolia* on the electrocardiogram (ECG) changes

GROUPS	Heart rate	P Wave duration	QRS duration	QT interval	QT corrected	R-wave amplitude
Control	257.3 ± 21.1	20.3 ± 2.63	15 ± 0.82	70.8 ± 14.86	150.8 ± 28.74	0.89 ± 0.08
HYTR	266 ± 26.72 ^a	14.3 ± 6.34 ^a	14.3 ± 2.22 ^a	51 ± 21.26 ^a	105.5 ± 39.62 ^a	0.41 ± 0.17 ^a
HYTR + LT (100 mg/kg)	217.3 ± 88.6	20 ± 6.08	17 ± 3.00	70.7 ± 11.06	129 ± 15.00	0.61 ± 0.14
HYTR + LT (200 mg/kg)	229.5 ± 79.5	17.5 ± 5.2	15.8 ± 3.77	61 ± 18.01	122.8 ± 20.06	0.58 ± 0.07 ^a
HYTR + Lisinopril (10 mg/kg)	250.3 ± 46.5	23 ± 7.35	16.3 ± 1.26	66.25 ± 1.25	133.8 ± 6.89	0.66 ± 0.17

Values are presented as Mean±SD (n=5. Alphabets indicates significant difference across groups at p<0.05. P-wave (m/s), QRS duration (m/s), QT interval (m/s), QTC (m/s), R wave amplitude (m/s). HYTR (Hypertensive rats), LT (*Launeae taraxacifolia*)

Table 6: Effect of *Launea Taraxacifolia* on qualitative urinalysis in L-NAME-induced hypertension.

GROUPS	Control	HYTR	HYTR + LT (100 mg/kg)	HYTR + LT (200 mg/kg)	HYTR + Lisinopril (10 mg/kg)
Ascorbic acid	-	-	-	-	-
Blood	-	-	-	-	-
Bilirubin	-	+1	-	-	-
Urobilinogen	0.2	0.2	0.2	0.2	0.2
Ketone	-	-	+1	-	-
Glucose	-	-	-	-	-
Protein	+1	+3	+2	+1	+1
Nitrate	+	++	+	+	+
Leucocyte	-	+1	-	-	-
pH	7	8	8	9	8
Specific gravity	1.015	1.005	1.010	1.010	1.015

Note: -Ve (Absence) & +Ve (Present)

Table 7: Effect of *Launea Taraxacifolia* on urinary markers of renal damage in L-NAME Induced Hypertension

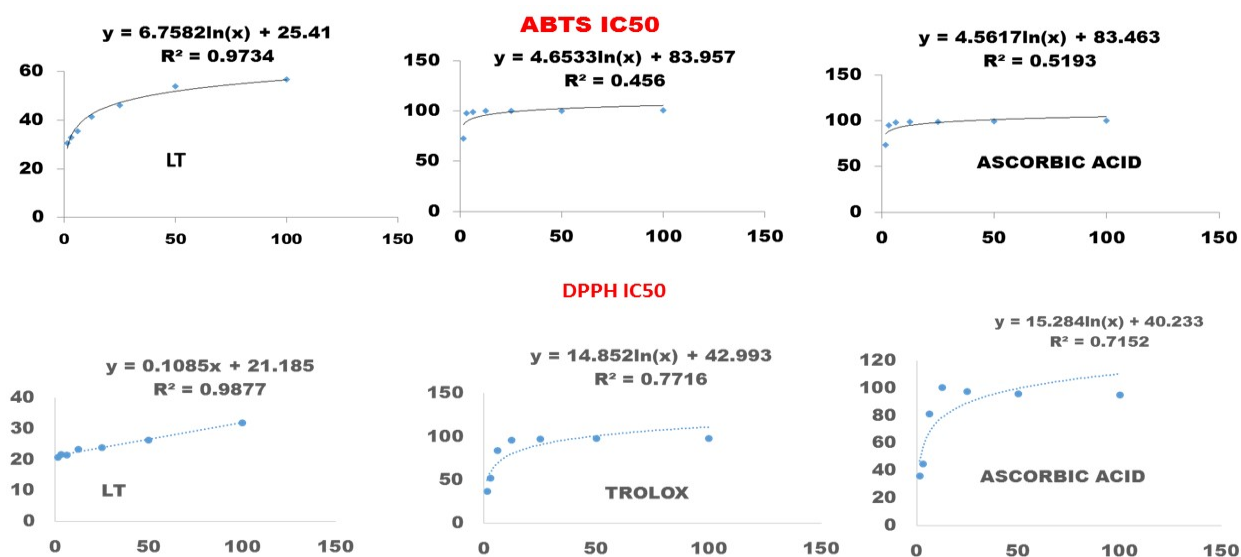
GROUPS	Total protein (mg/mL)	Urea (U/L)	Creatinine (U/L)	Sodium (U/L)
Control	1.9 ± 0.33	172.7 ± 42.41	7.9 ± 1.83	132.3 ± 13.51
HYTR	2.7 ± 0.56 ^a	207.3 ± 3.58 ^a	12.6 ± 3.49 ^a	146.8 ± 14.05 ^a
HYTR + LT (100 mg/kg)	2.1 ± 0.64	174.8 ± 38.50	26.8 ± 7.30	133.6 ± 15.23
HYTR + LT (200 mg/kg)	2.0 ± 0.76	200.9 ± 3.96	21.8 ± 6.31	141.0 ± 19.13
HYTR + Lisinopril (10 mg/kg)	2.4 ± 0.79	184.3 ± 19.37	18.5 ± 4.73	144.28 ± 8.41

Values are presented as Mean±SD (n=5). Alphabets indicates significant difference across groups at p<0.05. Protein (mg/dL), Urea (mg/dL), Creatinine (mg/dL), Sodium (mg/dL). HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

Table 8: Effects of *Launea Taraxacifolia* on Sperm parameters in L-NAME Induced Hypertension

GROUPS	Sperm motility (%)	Sperm livability (%)	Sperm count (X10 ⁶ Sperm/mL)
Control	93.0 ± 2.74	96.8 ± 1.64	137.4 ± 8.35
HYTR	82.0 ± 4.47 ^a	96.2 ± 1.64	119.4 ± 7.33 ^a
HYTR + LT (100 mg/kg)	74.0 ± 5.48 ^a	96.8 ± 1.64	96.2 ± 7.46 ^{a, b}
HYTR + LT (200 mg/kg)	72.0 ± 4.47 ^{a, b}	96.8 ± 1.64	91.2 ± 7.79 ^{a, b}
HYTR + Lisinopril (10 mg/kg)	76.0 ± 5.48 ^a	96.8 ± 1.64	96.2 ± 7.46 ^{a, b}

Values are presented as Mean±SD (n=5). Alphabets indicates significant difference across groups at p<0.05. HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

**Fig. 1:** Effect of *Launea taraxacifolia* on ABTS and DPPC IC50

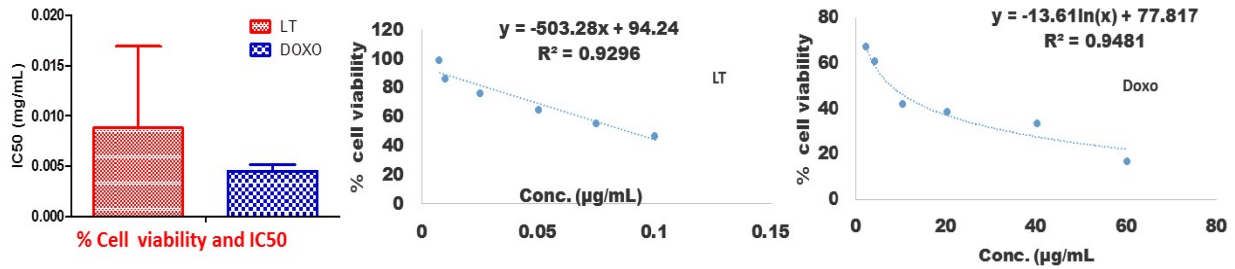


Fig. 2: Effect of *Launea taraxacifolia* on call viability and cytotoxicity

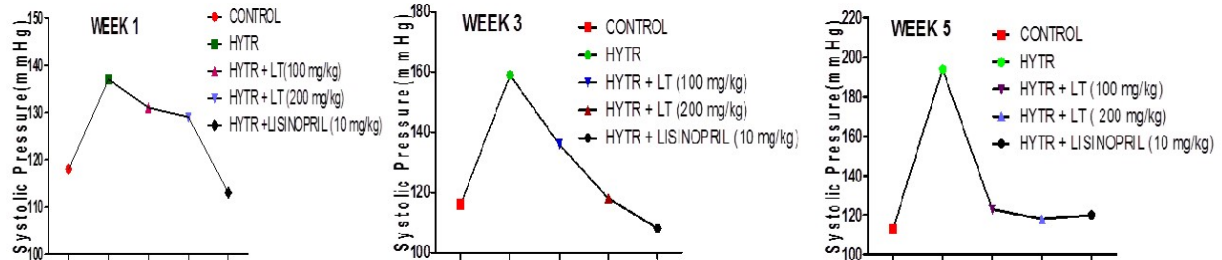


Fig. 3: Effect of *Launea taraxacifolia* on systolic blood pressure (mmHg) across five weeks in L-NAME induced hypertension.

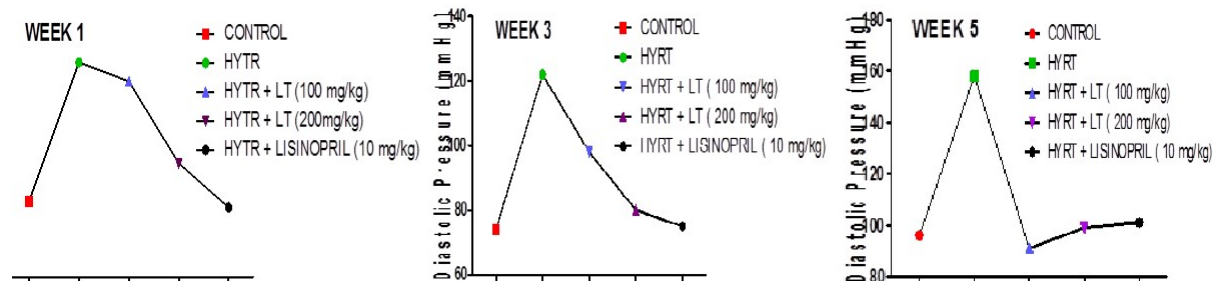


Fig. 4: Effect of *Launea taraxacifolia* on diastolic blood pressure (mmHg) across five weeks in L-NAME induced hypertension.

Values presented as mean+SD. HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

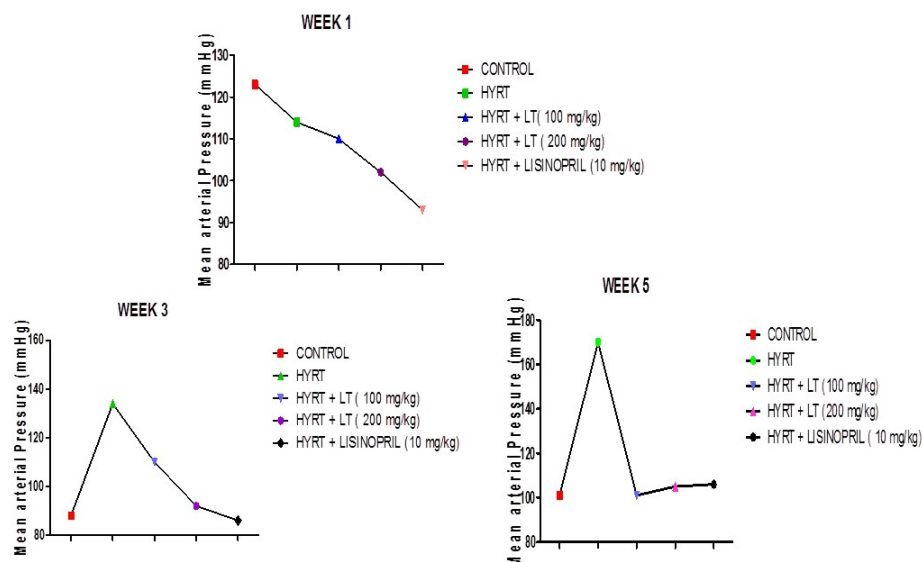


Fig. 5: Effect of *Launea taraxacifolia* on mean arterial blood pressure (mmHg) across five weeks in L-NAME induced hypertension. Values presented as Mean $\pm$ SD HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

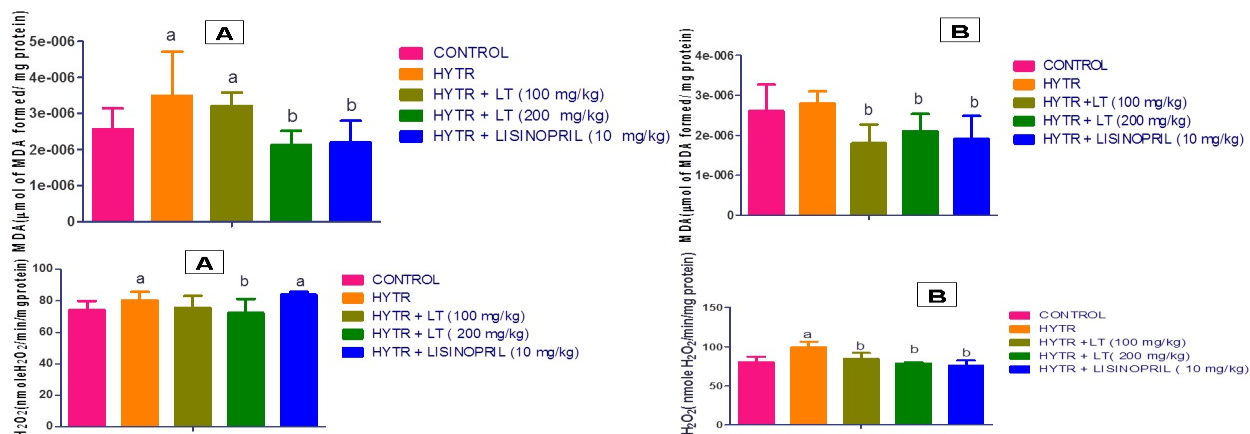


Fig. 6: Effect of *Launaea taraxacifolia* on renal and testicular malondialdehyde (MDA) levels in L-NAME induced hypertension.

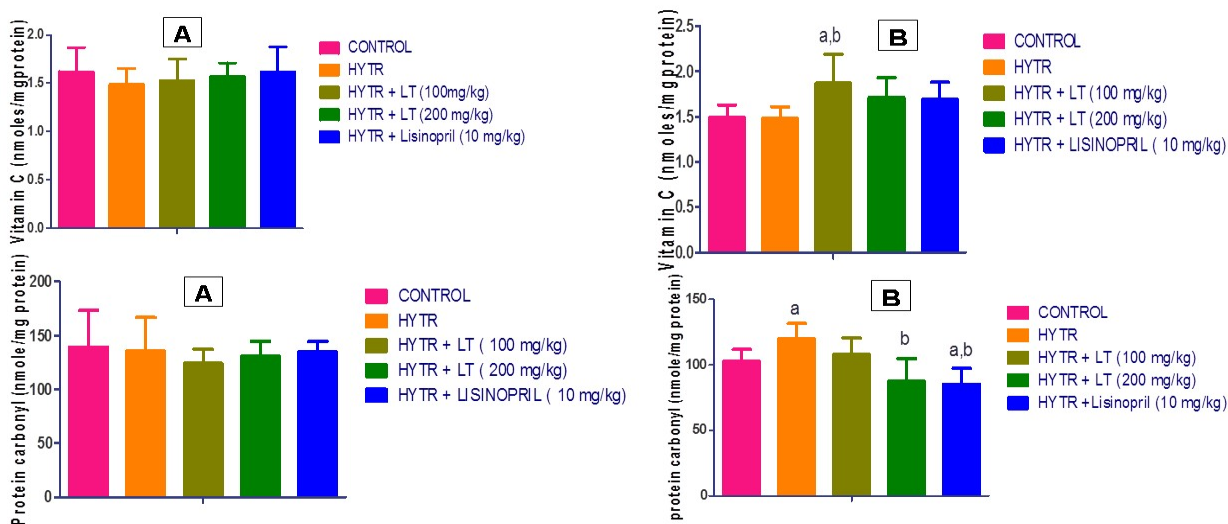


Fig. 7: Effect of *Launaea taraxacifolia* on renal and testicular Vitamin C and protein carbonyl content in L-NAME induced hypertension.

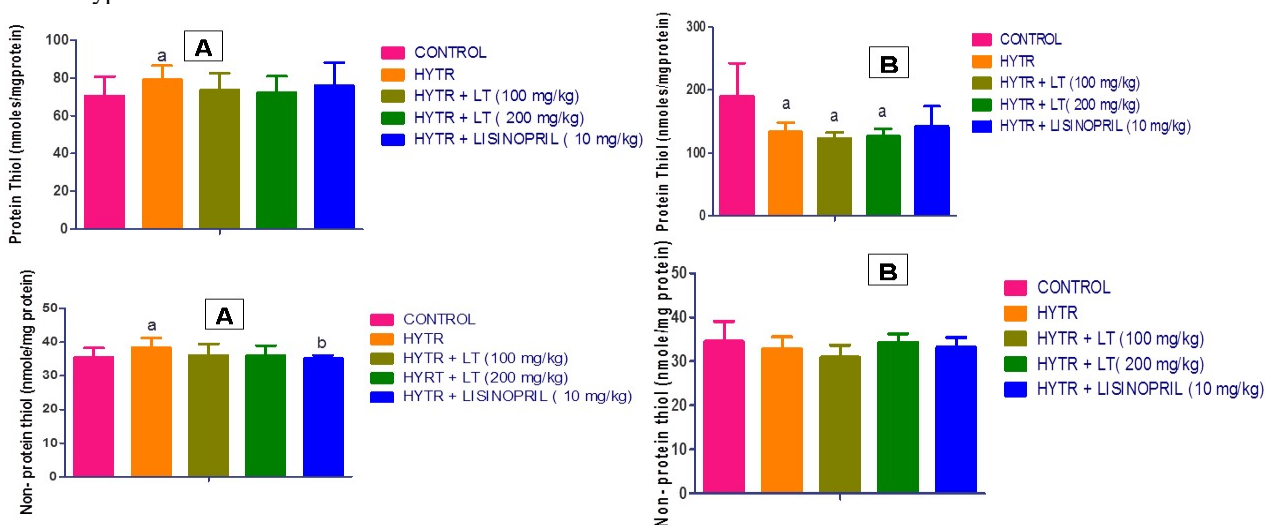


Fig. 8: Effect of *Launaea taraxacifolia* on renal and testicular protein thiol (PSH) in L-NAME induced hypertension.

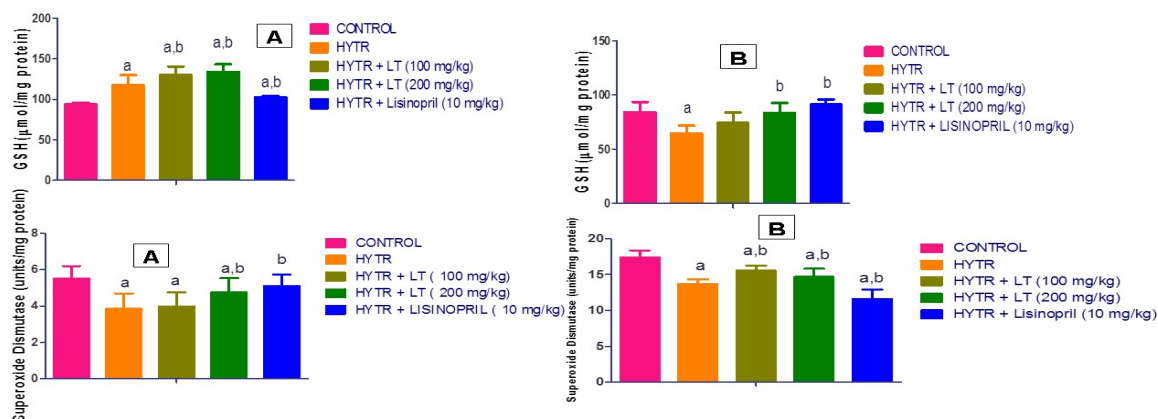


Fig. 9: Effect of *Launea taraxacifolia* on renal and testicular reduced glutathione and superoxide dismutase (SOD) activity in L-NAME induced hypertension.

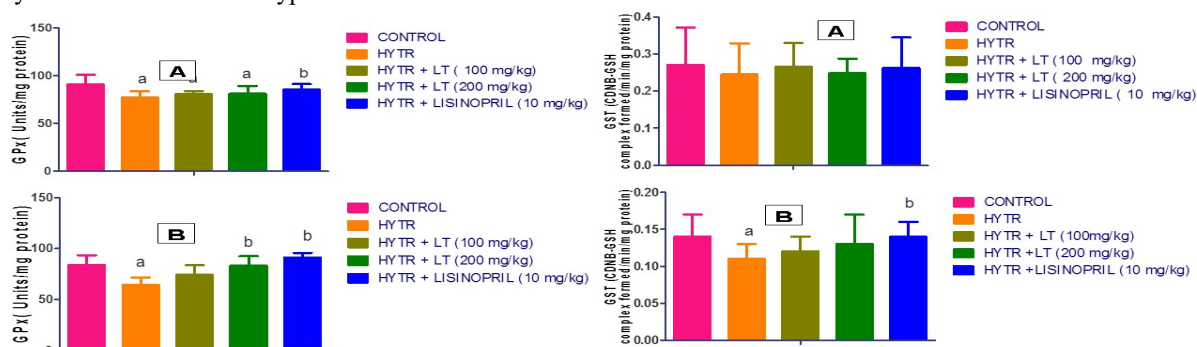


Fig. 10: Effect of *Launea taraxacifolia* on testicular and renal glutathione peroxidase (GPx) activity in L-NAME induced hypertension.

Values are presented as mean±SD. Alphabets indicates significant difference across group at $p < 0.05$. HYTR (Hypertensive rats; 40mg/kg L-NAME), LT (*Launea taraxacifolia*). MDA (malondialdehyde; $\mu\text{mol/mg}$ protein). A: Testis, B: Kidney. HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

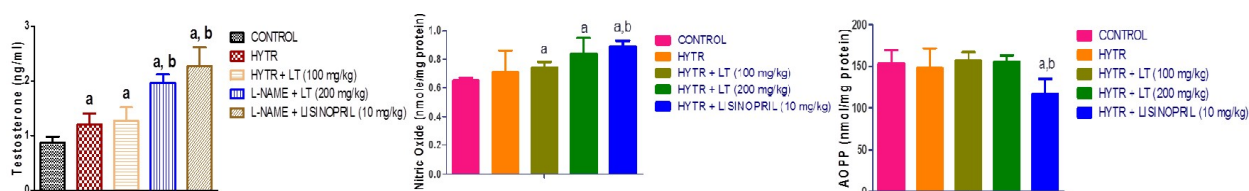


Fig. 11: Effect of *Launea taraxacifolia* on serum testosterone, nitric oxide and advanced oxidation protein product (AOPP) contents in L-NAME induced hypertension.

Values are presented as mean±SD. Alphabets indicates significant difference across group at $p < 0.05$. HYTR (Hypertensive rats; 40mg/kg L-NAME), LT (*Launea taraxacifolia*). A: Testis, B: Kidney. HYTR (Hypertensive rats), LT (*Launea taraxacifolia*)

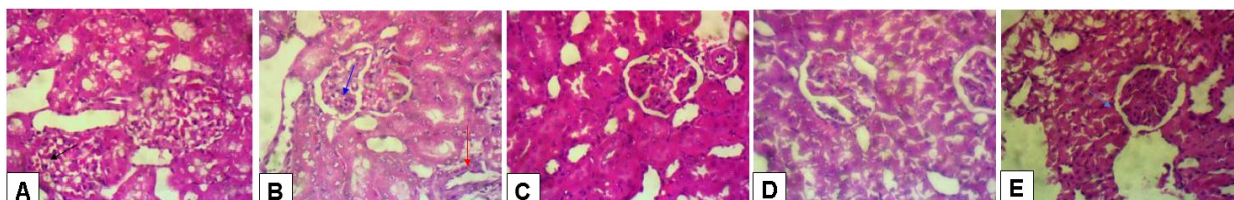


Fig. 12: Histology of the kidney showing the effect of $N\omega$ -nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

A (Control), B (HYTR; 40mg/kg L-NAME), C (HYTR+LT 100mg/kg), D (HYTR+LT 200mg/kg), E (HYTR+Lisinopril; 10mg/kg). There was visible glomerular capillary congestion, patchy tubular epithelial degeneration (blue arrow), attenuation of tubular epithelial lining, lumina ectasia (white arrow) with a few peri-tubular inflammatory cells (red arrow) in hypertensive rates compared to other treatment groups.

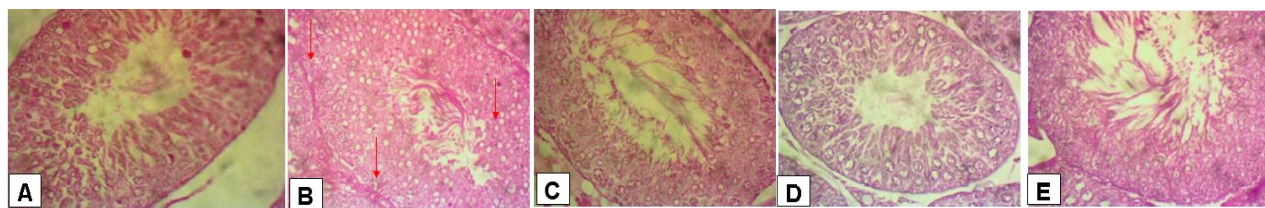


Fig. 13: Histology of the testes showing the effect of Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

A (Control), B (HYTR), C (HYTR+LT 100mg/kg), D (HYTR+LT 200mg/kg), E (HYTR+Lisinopril; 10mg/kg). There is spermatogenic arrest evident by irregularly shaped tubule outline, attenuation of germ cells, tubular ectasia (red arrow) in the hypertensive rat compared to other treatment groups.

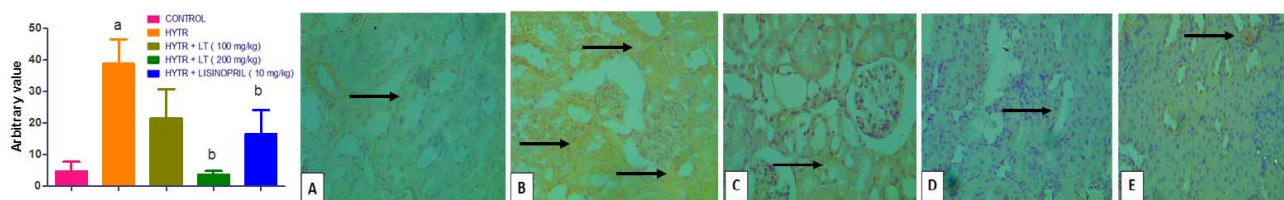


Fig. 14: The immunohistochemistry of angiotensin converting enzyme (ACE) on Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

A (Control), B (HYTR), C (HYTR+LT 100mg/kg), D (HYTR+LT 200mg/kg), E (HYTR + Lisinopril; 10mg/kg). There are expressions of angiotensin converting enzymes in hypertensive rat compared to control however groups treated with LT and Lisinopril shows reduced expression.

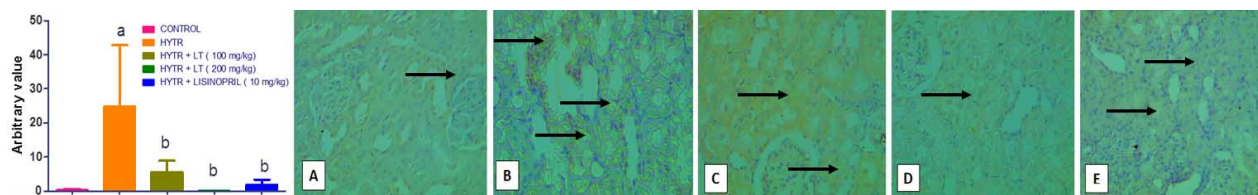


Fig. 15: The immunohistochemistry of mineralocorticoid receptor on Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

A (Control), B (HYTR), C (HYTR+LT 100mg/kg), D (HYTR+LT 200mg/kg), E (HYTR+Lisinopril; 10mg/kg). There are significant increase in the expression of mineralocorticoid receptors in hypertensive rats compared to other treatment groups.

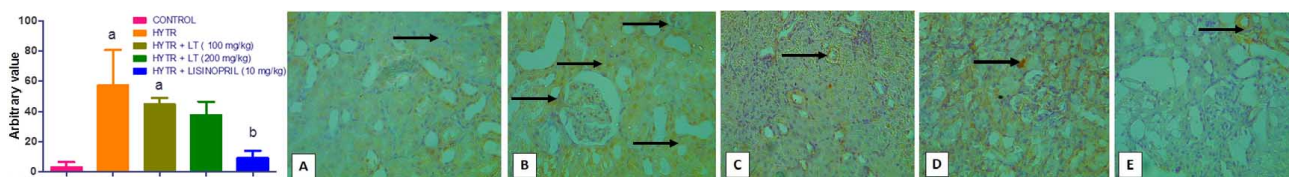


Fig. 16: The immunohistochemistry of kidney injury molecule 1 (Kin 1) on Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

A (Control), B (HYTR), C (HYTR+LT 100mg/kg), D (HYTR+LT 200mg/kg), E (HYTR+Lisinopril; 10mg/kg). There are significant increase in renal damage and expression of kidney injury molecule in the hypertensive rats compared to other treatment groups.

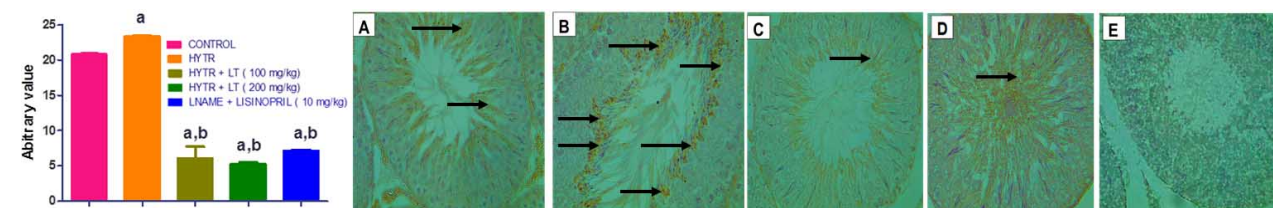


Fig. 17: The immunohistochemistry of caspase-3 receptor on Nω-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

in hypertensive rats treated with the leaf extract of LT. Also, our study revealed that hypertension precipitated a significant increase in heart rate accompanied by a reduction in duration of QRS, QT and QTc intervals, respectively. The relationship between hypertension, cardiovascular mortality, and morbidity has been established (Ku *et al.*, 2019). The cardio-protective effect of LT was evident by the restoration of electrocardiographic changes to near normal values. Therefore, the leaf extract of LT is believed to play a dual role, i.e., both as an antihypertensive and a cardio-protective agent.

The pathophysiology of chronic kidney disease and hypertension is multifactorial (Liu *et al.*, 2019). Some of these factors include increased sodium retention, modulation of the renin-angiotensin-aldosterone system and endothelial dysfunction (Liu *et al.*, 2019). In fact, hypertension is a known cause of end-stage kidney failure (Sun, 2019). For this reason, the Kim-1 as a molecular marker of renal damage was evaluated. Kim-1 is a membrane-bound protein that is highly expressed in acute kidney injury (Zappitelli *et al.*, 2015). The observed significant increase in the expressions of Kim-1 in hypertension might be due to persistent high blood pressure, oxidation, and inflammation. The Kim-1 is classified as one of the new biomarkers for acute kidney injury and glomerular filtration rate, better than creatinine (Wang *et al.*, 2019). The elevated Kim-1 level observed was indicative of renal damage and reduced glomerular filtration rate. This was also accompanied with proteinuria and reduced urine specific gravity, which are indications of renal damage. The protection of the kidney by LT was indicated by reduction in the urinary sodium, protein and an improvement of glomerular filtration rate in the hypertensive treated rats with LT.

Again, we observed a significant increase in urinary sodium, oxidative stress indices and reduction of glutathione peroxidase, glutathione S-transferase, superoxide dismutase activity, reduced glutathione, vitamin C contents, nitric oxide bioavailability levels and urinary creatinine clearance in rats that are hypertensive but not treated. However, treatment of hypertensive rats with the methanol leaf extract increased both the enzymic and non-enzymic antioxidants. In fact, the pathogenesis and pathophysiology of hypertension have been associated with the involvement of oxidative stress (Schultz *et al.*, 2019; Marushchak *et al.*, 2019). The antioxidant power of LT was demonstrated by increasing the activities of antioxidant enzymes and increasing the contents of GSH and vitamin C with concomitant depletion of markers of oxidative stress of renal and testicular tissues. This attests to the antioxidant capacity of LT observed *in vitro* with the ABTS radical scavenging activity as recorded in this study. Although, the extract did not show significant scavenging activity comparable to

the positive controls, a better antioxidant action with ABTS IC₅₀ of 38 µg/mL was recorded. According to Yang *et al.* (2019), an extract with IC₅₀ >100 µg/mL was considered to have weak antioxidant activity. Interestingly, the extract however, showed a weak radical scavenging activity.

Toxicity of the LT extract was tested on normal Vero cells, and the extracts proved relatively non-toxic compared to the positive control, doxorubicin. From our result, this extract with LC₅₀ of 87.9 µg/mL is considered safe to be investigated further for use as an antihypertensive potential therapy. On the other hand, the extract showed a potent inducible NO inhibition activity at 50 µg/mL concentration which was not due to toxicity based on the cell viability >80%, demonstrating the beneficial anti-inflammatory properties of LT. Meanwhile, renal fibrosis has been implicated as one of the culprits that can facilitate end-stage renal disease in hypertensive patients (Macchiavello *et al.*, 2019).

The findings from the present study revealed that hypertension activated MCR. Previous reports have documented that activation of MCR is directly proportional to the hypertensive state (Jaisser *et al.*, 2016). Meanwhile, a statistically significant reduction in the expression of MCR in hypertensive rats treated with LT was recorded and this reduction was like that of Lisinopril, the standard antihypertensive drug. In fact, 200mg of LT gave a better deactivation of MCR. We speculated that L-NAME activated renal RAS as previously reported (Arai *et al.*, 2015). Therefore, the plant extract of LT and lisinopril might be classified as novel MCR antagonists. Elsewhere, it was reported that RAS was activated in salt-sensitive hypertensive rats demonstrating an increase in ACE activity (Arai *et al.*, 2015). Lainscak *et al.* (2015) reported a non-steroidal mineralocorticoid receptor antagonist with antihypertensive property. Oxidative stress, inflammation and fibrosis have been reported to contribute to inappropriate activation of MCR (He *et al.*, 2013; Gomez-Sanchez and Gomez-Sanchez, 2014). Interestingly, increase ACE activity has been associated with the activation of MCR (Jaisser *et al.*, 2016). MCR receptor antagonists are known to have cardioprotective effects. The usefulness of the methanol leaf extract of LT as seen in the present study indicates the potential therapeutic applications of plant-based-pharmacological MCR antagonists.

From the present study, higher immunolocalization of renal ACE was recorded in hypertensive untreated rats. However, the 200mg/kg methanol leaf extract of LT reduced the expressions of the renal ACE comparable to the normotensive rats. This confirms the novel antihypertensive action of LT on hypertensive rats. The biological action of ACE is demonstrated by converting angiotensin I to angiotensin II (Yilmaz, 2019). Hence, the

inhibition of ACE is linked to the maintenance of blood pressure. Therefore, the plant extract of LT was shown to inhibit renal ACE which indicates the mechanism of action of its antihypertensive effect. We demonstrated for the very first time the antihypertensive efficacy of LT through the inhibition of MCR and ACE, respectively. The extract of LT demonstrated an antihypertensive effect which was comparable to that of lisinopril as shown by our findings. The inhibitors of ACE have been reported to have therapeutic implications in myocardial infarction, hypertension, and congestive heart failure (Ohtsubo *et al.*, 2019; Bratsos *et al.*, 2019).

Another complication that may arise from hypertension is reproductive impairment as evidenced by a significant reduction in sperm counts, motility, serum testosterone and higher immunolocalization of testicular caspase-3 of hypertensive rats. Interestingly, there were significant improvements in the serum testosterone level and testicular cell death as indicated in the activity of caspase-3 in hypertensive rats treated with LT. Previous and current findings have reported a direct relationship between hypertension and male infertility (Guo *et al.*, 2017; Meister *et al.*, 2018). The observed testicular apoptosis in our study confirms an earlier report on the reduction of semen quality and alterations in the testicular microcirculation in hypertensive rats (de Alencar *et al.*, 2018). The high indices of oxidative stress and apoptosis together with the depletion of antioxidants recorded in this study must have contributed significantly to the reduction in sperm counts, viability, motility, and serum testosterone.

Gas chromatography-mass spectrometry analysis confirmed phytol, a well-established phytochemical with an array of biological activities including anti-inflammatory, anxiolytic, cytotoxic, anticancer, antimutagenic, antimicrobial, anticonvulsant, antinociceptive, antioxidant, antidepressant and immunostimulatory effects (Hassan *et al.*, 2018; Islam *et al.*, 2018; El-Sayed *et al.*, 2018). It is therefore hypothesized that phytol might be responsible for most of the biological activities observed in this study.

CONCLUSION

Methanol leaf extract of *Launaea taraxacifolia* exhibited relatively low cytotoxicity, but potent antihypertensive effects. The antihypertensive effect occurred through inhibition of kidney angiotensin converting enzyme and mineralocorticoid receptor. *Launaea taraxacifolia* as leafy vegetable is worthy of further development as a safe and effective antihypertensive especially in poor resource settings like Africa. In our future research, *Launaea taraxacifolia* will be subjected to further studies towards drug development and commercialization as a novel antihypertensive agent from a medicinal plant.

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