doi.org/10.36721/PJPS.2022.35.6.REG.1581-1594.1

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

Ademola Adetokunbo Oyagbemi[1], Fasilat Oluwakemi Hassan[1], Temitayo Olabisi Ajibade[1], Olufunke Eunice Ola-Davies[1], Temidayo Olutayo Omobowale[2], Olumide Samuel Ajani[3], Theophilus Aghogho Jarikre[4], Kehinde Olugboyega Soetan[1], Adebowale Benard Saba[5], Adeolu Alex Adedapo[5], Abimbola Obemisola Aro[6], Matthew Olugbenga Oyeyemi[3], Benjamin Obukowho Emikpe[4], Sanah Malomile Nkadimeng[7], Lyndy Joy McGaw[7], Prudence Ngalula Kayoka-Kabongo[6], Momoh Audu Yakubu[8] and Oluwafemi Omoniyi Oguntibeju[9]

[1]Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Theriogenology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[5]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[6]Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Florida, South Africa
[7]Phytomedicine Programme, Department of Paraclinical Science, University of Pretoria, Faculty of Veterinary Science, Old Soutpan Road, Onderstepoort, South Africa
[8]Department of Environmental & Interdisciplinary Sciences, College of Science, Engineering & Technology, Vascular Biology Unit, Center for Cardiovascular Diseases, Texas Southern University, Houston, TX, USA
[9]Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, South Africa

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 *Launeae 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

**Corresponding author:* e-mail: ademola.oyagbemi778@gmail.com

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 (Koukoui *et al.,* 2017) properties have been documented.

Based on the above literature search, antihypertensive effect of *Launeae taraxacifolia* has not being scientifically validated. This study explored molecular mechanism of antihypertensive efficacy of *Launeae 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 $4^{o}C$ 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 10,000*g* 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 histopathatologic 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 ug/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 5weeks, 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_8H_{10}	106
2	p-Xylene	6.156823	C_8H_{10}	106
3	P- Xylene	2.562371	C_8H_{10}	106
4	1,3,5-Pentanetriol, 3-methyl-	1.111766	$C_6H_{14}O_3$	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)
Launeae Taraxacifolia	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-picrylhydrazy

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
	100	39.54 ± 4.31	96.26 ± 2.31
Doxorubicin	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 *Launeae 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 *Launeae 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 (*Launeae taraxacifolia*)

Table 8: Effects of *Launeae 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 (*Launeae taraxacifolia*)

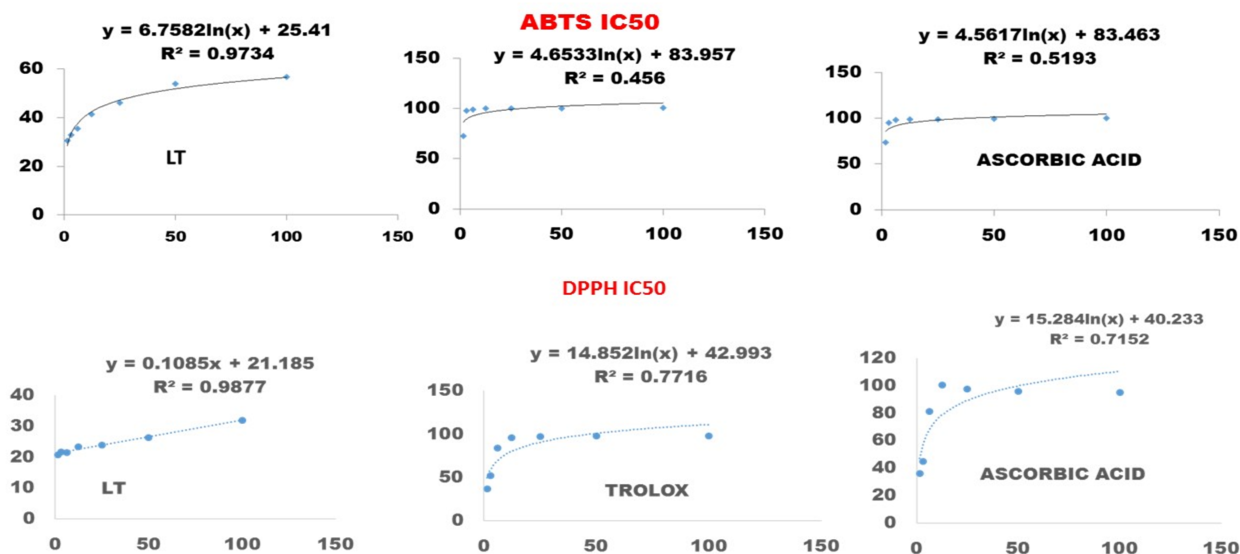

Fig. 1: Effect of *Launeae taraxacifolia* on ABTS and DPPC IC50

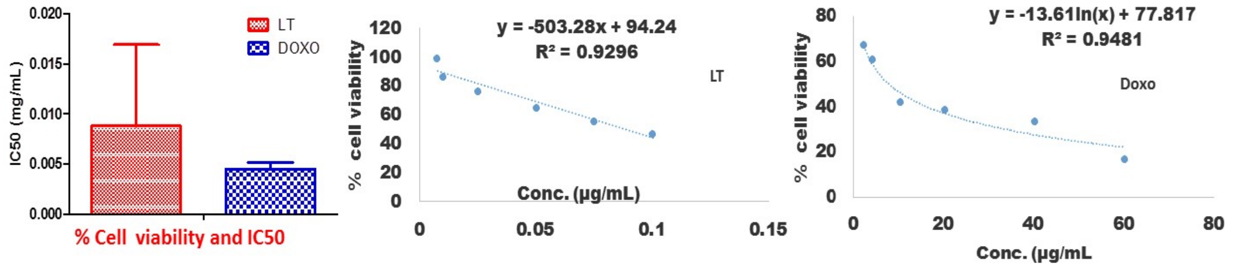

Fig. 2: Effect of *Launeae taraxacifolia* on call viability and cytotoxicity

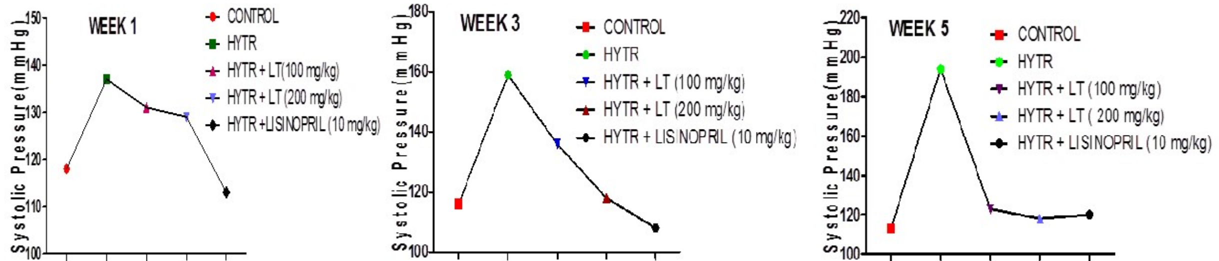

Fig. 3: Effect of *Launeae taraxacifolia* on systolic blood pressure (mmHg) across five weeks in L-NAME induced hypertension.

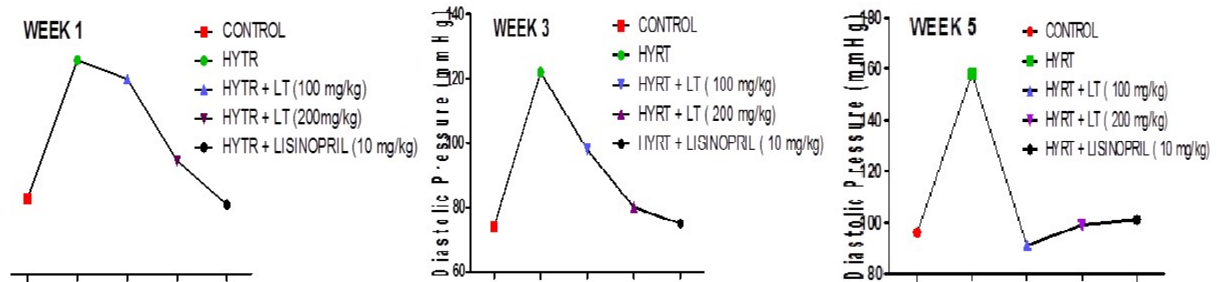

Fig. 4: Effect of *Launeae taraxacifolia* on diastolic blood pressure (mmHg) across five weeks in L-NAME induced hypertension.

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

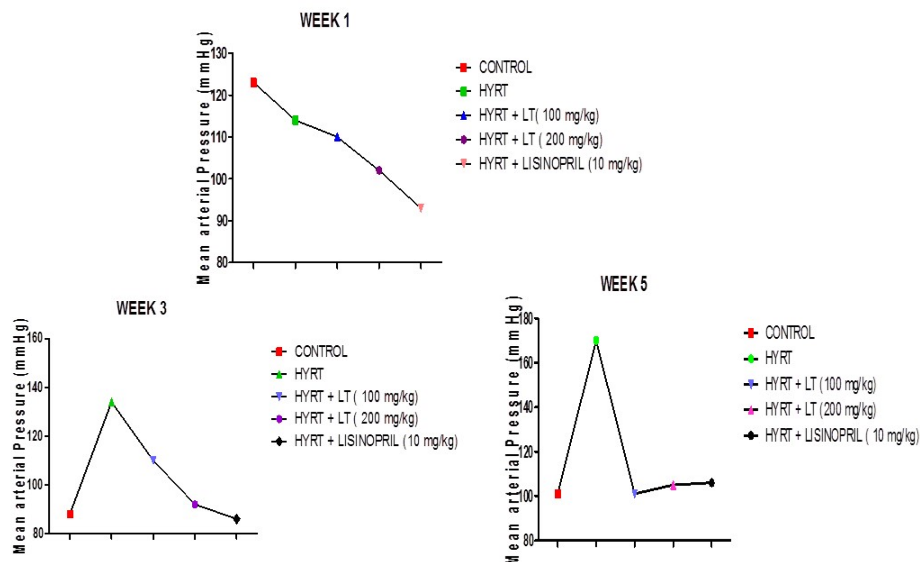

Fig. 5: Effect of *Launeae taraxacifolia* on mean arterial blood pressure (mmHg) across five weeks in L-NAME induced hypertension. Values presented as Mean ± SD HYTR (Hypertensive rats), LT (*Launeae taraxacifolia*)

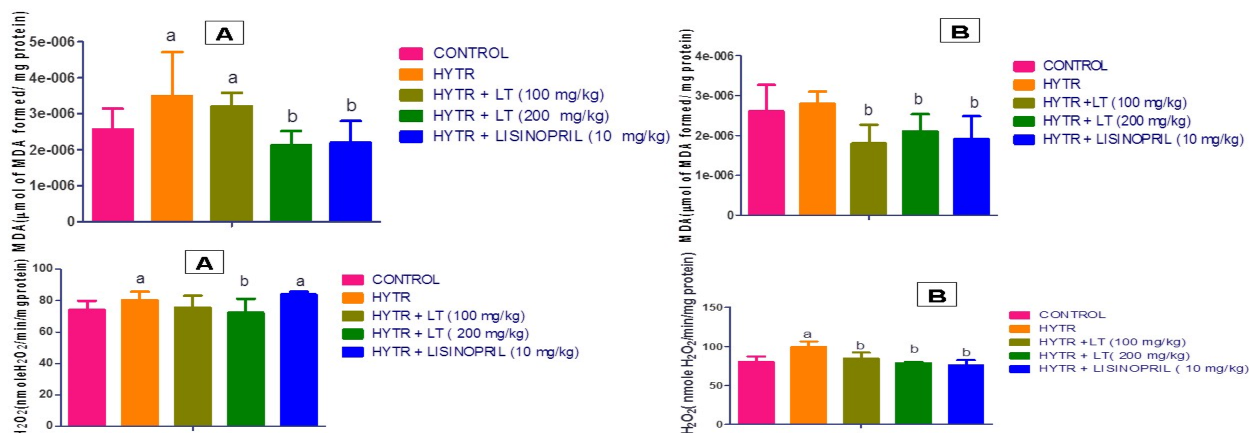

Fig. 6: Effect of *Launeae taraxacifolia* on renal and testicular malondialdehyde (MDA) levels in L-NAME induced hypertension.

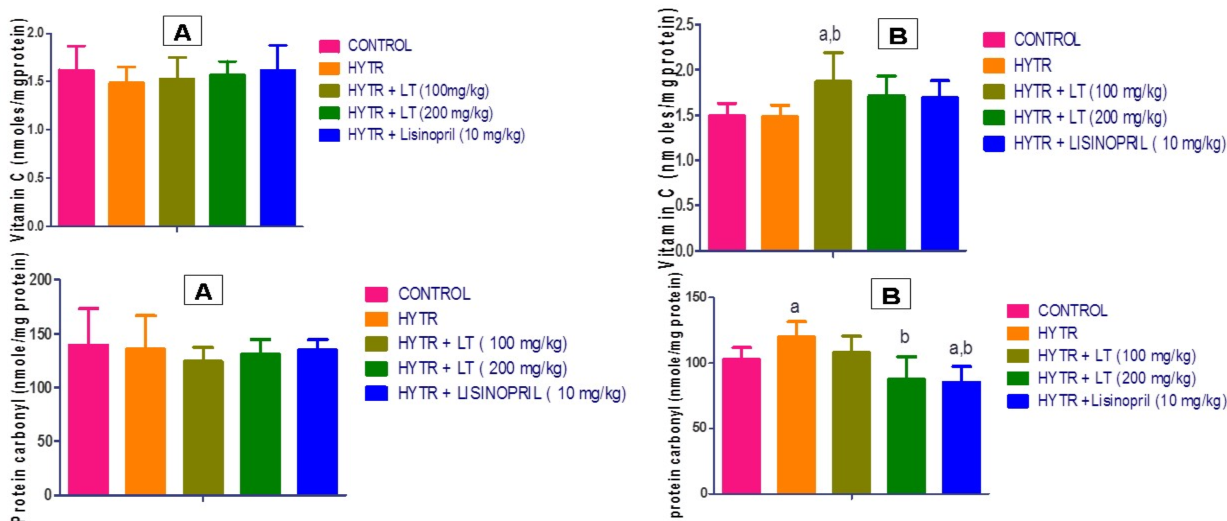

Fig. 7: Effect of *Launeae taraxacifolia* on renal and testicular Vitamin C and protein carbonyl content in L-NAME induced hypertension.

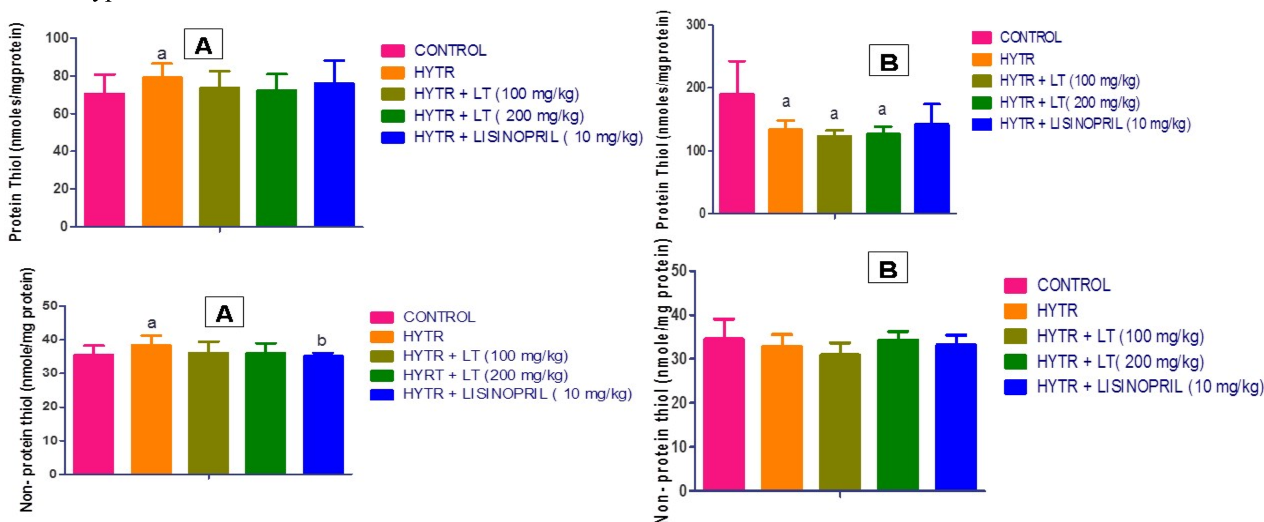

Fig. 8: Effect of *Launeae taraxacifolia* on renal and testicular protein thiol (PSH) in L-NAME induced hypertension.

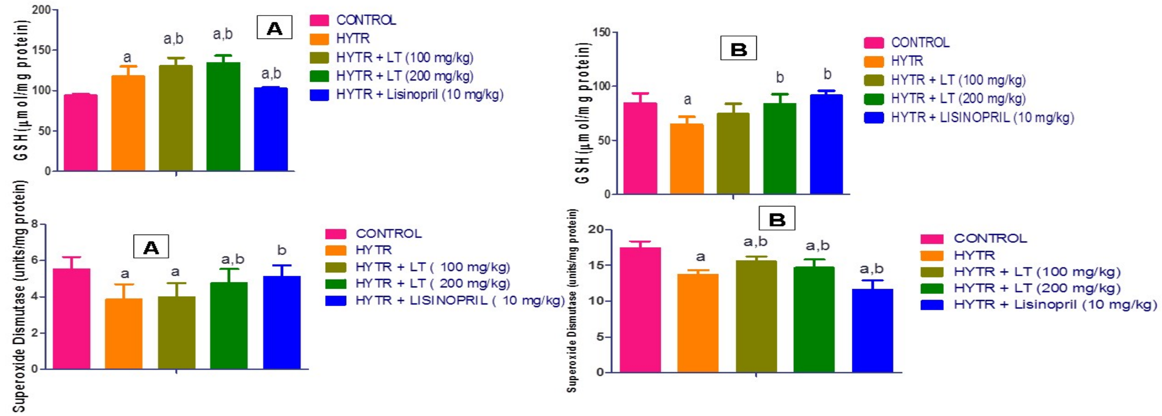

Fig. 9: Effect of *Launeae taraxacifolia* on renal and testicular reduced glutathione and superoxide dismutase (SOD) activity in L-NAME induced hypertension.

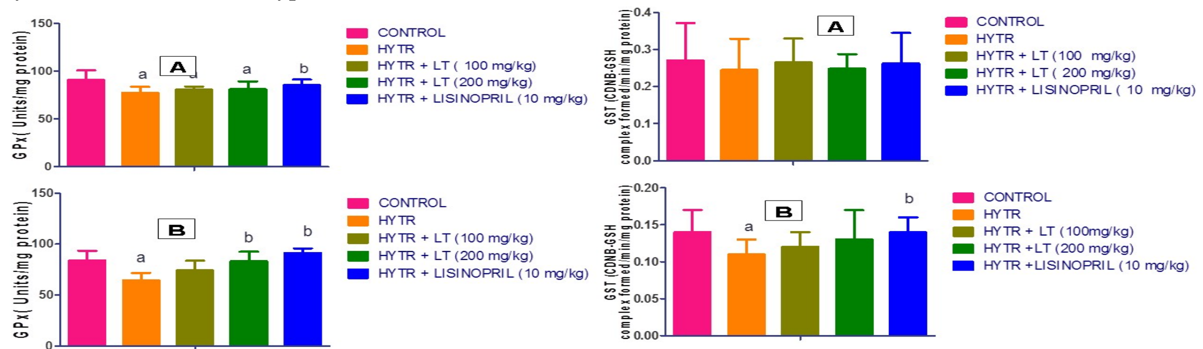

Fig. 10: Effect of *Launeae taraxacifolia* on testicular and renal glutathione peroxidase (GPx) activity in L-NAME induced hypertension.

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

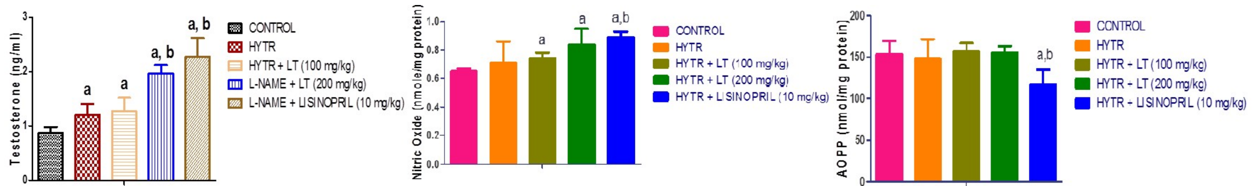

Fig. 11: Effect of *Launeae taraxacifolia* on serum testosterone, nitric oxide and advanced oxidation protein product (AOPP) contents in L-NAME induced hypertension.

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

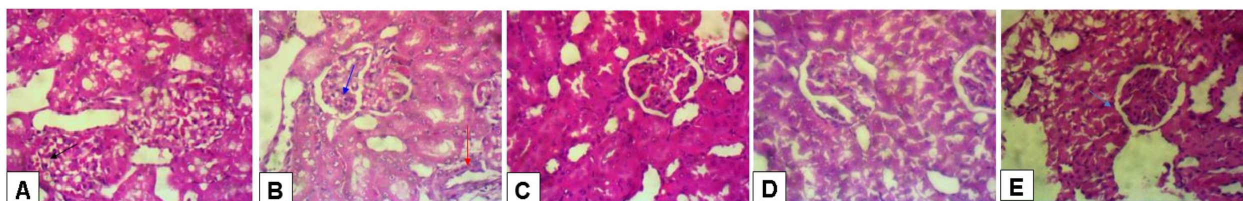

Fig. 12: Histology of the kidney showing the effect of Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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.

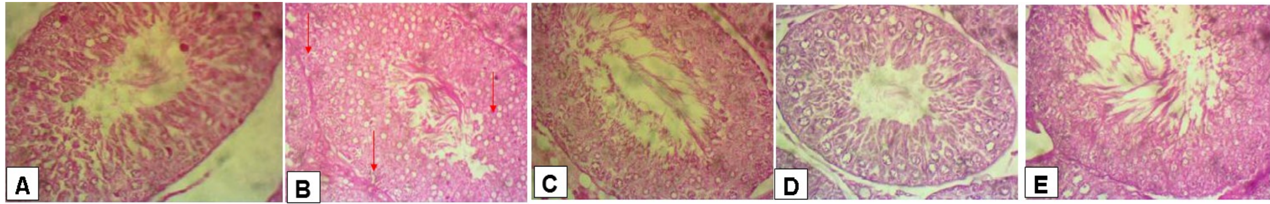

Fig. 13: Histology of the testes showing the effect of Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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.

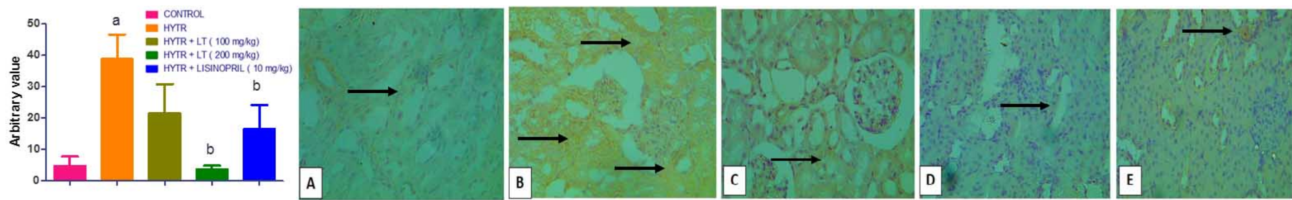

Fig. 14: The immunohistochemistry of angiotensin converting enzyme (ACE) on Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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.

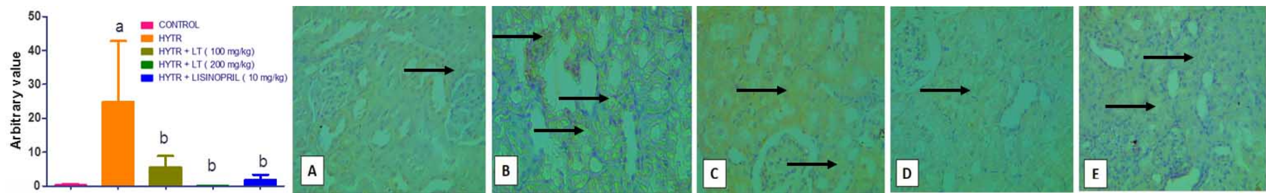

Fig. 15: The immunohistochemistry of mineralocorticoid receptor on Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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.

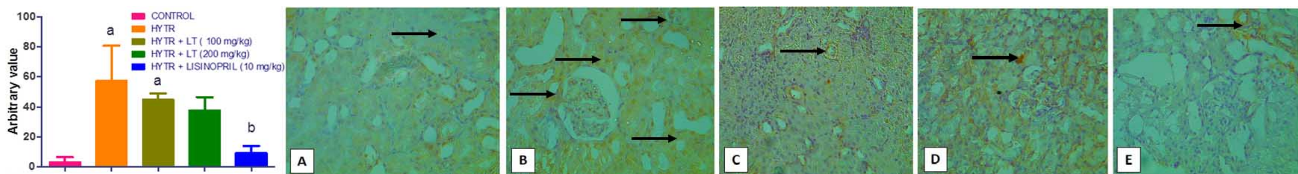

Fig. 16: The immunohistochemistry of kidney injury molecule 1 (Kin 1) on Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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.

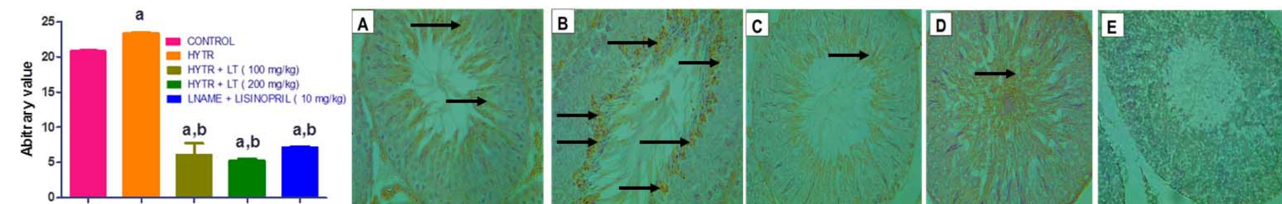

Fig. 17: The immunohistochemistry of caspase-3 receptor on Nɯ-nitro-L-arginine methyl ester (L-NAME) induced hypertension in Wistar rats.

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 IC_{50} of 38µg/mL was recorded. According to Yang *et al.* (2019), an extract with IC_{50} >100µg/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 LC_{50} of 87.9µg/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 50µg/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 (Yılmaz, 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 *Launeae taraxacifolia* exhibited relatively low cytotoxicity, but potent antihypertensive effects. The antihypertensive effect occurred through inhibition of kidney angiotensin converting enzyme and mineralocorticoid receptor. *Launeae 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, *Launeae taraxacifolia* will be subjected to further studies towards drug development and commercialization as a novel antihypertensive agent from a medicinal plant.

ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance of Dr. Falayi and Dr. Ogunpolu of the cardio-renal Laboratory, Department of Veterinary Physiology and Biochemistry, faculty of Veterinary Medicine, University of Ibadan, Nigeria. The authors also appreciate the final support of Cape Peninsula University of Technology (CPUT), Phytomedicine and Phytochemistry Group, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Bellville 7535, South Africa.

REFERENCES

Abouzeinab NS (2015). Antioxidant effect of silymarin on cisplatin-induced renal oxidative stress in rats. *J. Pharmacol. Toxicol*., **10**(1): 1-19.

Adinortey MB, Ansah C, Weremfo A, Adinortey CA, Adukpo GE, Ameyaw EO and Nyarko AK (2018). DNA damage protecting activity and antioxidant potential of *Launaea taraxacifolia* leaves extract. *J. Nat. Sci. Biol. Med*., **9**(1): 6-13.

Ames MK, Atkins CE and Pitt B (2019). The renin-angiotensin-aldosterone system and its suppression. *J. Vet. Intern. Med*. **33**(2): 363-382.

Arai K, Tsuruoka H and Homma T (2015). CS-3150, a novel non-steroidal mineralocorticoid receptor antagonist, prevents hypertension and cardiorenal injury in Dahl salt-sensitive hypertensive rats. *Eur. J. Pharmacol.* **769**: 266-273.

Asase A and Yohonu DT (2016). Ethnobotanical study of herbal medicines for management of diabetes mellitus in Dangme West District of southern Ghana. *J. Herbal Med*. **6**(4): 204-209.

Benjamin EJ, Blaha MJ, Chiuve SE, Cushman M, Das SR, Deo R, de Ferranti SD, Floyd J, Fornage M and Gillespie C, et al. (2017). Heart disease and stroke statistics-2017 update: a report from the American heart association. *Circulation*. **135**(10): e146-e603.

Bratsos S (2019). Efficacy of angiotensin converting enzyme inhibitors and angiotensin receptor-neprilysin inhibitors in the treatment of chronic heart failure: A review of landmark trials. *Cureus.* **11**(1): e3913.

Dansi A, Adjatin A, Adoukonou-Sagbadja H, Faladé V, Yedomonhan H, Odou D and Dossou B (2008). Traditional leafy vegetables and their use in the Benin Republic. *Genetic Res Crop Evol*. **55** (8): 1239-1256.

Datta S, Kundu S, Ghosh P, De S, Ghosh A and Chatterjee M (2014). Correlation of oxidant status with oxidative tissue damage in patients with rheumatoid arthritis. *Clin. Rheumatol.* **33**(11): 1557-1564.

de Alencar MVOB, Islam MT, de Lima RMT, Paz MFCJ, dos Reis AC, da Mata AMOF, Filho J WGO, Cerqueira GS, Ferreira PMP, Sousa JMC, Mubarak MS and Melo-Cavalcante AAC (2018). Phytol as an anticarcinogenic and antitumoral agent: An *in vivo*

study in swiss mice with DMBA-Induced breast cancer. *IUBMB Life,* **71**(2): 200-212.

Dharmashankar K and Widlansky ME (2010). Vascular endothelial function and hypertension: insights and directions. *Curr. Hypertens. Rep.* **12**(6): 448-455.

Drury RA, Wallington EA and Cancerson R (1976). Editors Carlton's Histopathological Techniques 4th edition, Oxford University Press, London, UK, pp.139-142.

El-Sayed AEB, Aboulthana WM, El-Feky AM, Ibrahim NE and Seif MM (2018). Bio and phyto-chemical effect of Amphora coffeaeformis extract against hepatic injury induced by paracetamol in rats. *Mol. Biol. Rep*., **45**(6): 2007-2023.

Gheorghe A, Griffiths U, Murphy A, Legido-Quigley H, Lamptey P and Perel P (2018). The economic burden of cardiovascular disease and hypertension in low- and middle-income countries: A systematic review. *BMC Public Health*. **18**(1): 975.

Gomez-Sanchez E and Gomez-Sanchez CE (2014). The multifaceted mineralocorticoid receptor. *Compr. Physiol.* **4**(3): 965-994.

Gornal AG, Bardawill JC and David MM (1949). Determination of serum proteins by means of biuret reaction. *J. Biol. Chem.* **177**(2): 751-766.

Guo D, Li S, Behr B and Eisenberg ML (2017). Hypertension and Male Fertility. *World J. Mens Health*. **35**(2): 59-64.

Hassan EM, Matloub AA, Aboutabl ME, Ibrahim NA and Mohamed SM (2016). Assessment of anti-inflammatory, antinociceptive, immunomodulatory, and antioxidant activities of Cajanus cajan L. seeds cultivated in Egypt and its phytochemical composition. *Pharm. Biol*. **54**(8): 1380-1391.

He HL, Liu D and Ma CB (2013). Review on the angiotensin-I-converting enzyme (ACE) inhibitor peptides from marine proteins. *Appl. Biochem. Biotechnol.* **169**(3): 738-749.

Islam MT, Ali ES, Uddin SJ, Shaw S, Islam MA, Ahmed MI, Chandra Shill M, Karmakar UK and Yarla NS, et al. (2018). Phytol: A review of biomedical activities. *Food Chem. Toxicol.* **121**: 82-94.

Jacques-Silva MC, Nogueira CW, Broch LC, Flores EMM and Rocha JBT (2001). Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. *Pharmacol. Toxicol.* **88**(3): 119-125.

Jaisser F and Farman N (2016). Emerging Roles of the Mineralocorticoid Receptor in Pathology: Toward New Paradigms in Clinical Pharmacology. *Pharmacol. Rev.* **68**(1): 49-75.

Kaliora AC and Dedoussis GV (2007). Natural antioxidant compounds in risk factors for CVD. *Pharmacol. Res*., **56**(2): 99-109.

Kayali R, Cakatay U, Akcay T and Altug T (2006). Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissues of ageing rat. *Cell Biochem. Funct*., **24**(1): 79-85.

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK and He J (2005). Global burden of hypertension: Analysis of worldwide data. *Lancet*. **365**(9455): 217-223.

Konukoglu D and Uzun H (2017). Endothelial dysfunction and hypertension. *Adv. Exp. Med. Biol*. **956**: 511-540.

Koukoui O, Senou M, Agbangnan P, Seton S, Koumayo F, Azonbakin S, Adjagba M, Laleye A and Sezan A (2017). Effective *in vivo* cholesterol and triglycerides lowering activities of hydroethanolic extract of *Launaea taraxacifolia* leaves. *Int. J. Pharmaceut. Sci. Res.,* **8**(5): 2040-2047.

Ku E, Lee BJ, Wei J and Weir MR (2019). Hypertension in CKD: Core Curriculum 2019. *Am. J. Kidney Dis*., **74(1)**: 120-131.

Lainscak M, Pelliccia F, Rosano G, Vitale C, Schiariti M, Greco C, Speziale G and Gaudio C (2015). Safety profile of mineralocorticoid receptor antagonists: Spironolactone and eplerenone. *Int. J. Cardiol*. **200**: 25-29.

Larbie C and Mensah DA (2014). Botanicals for managing cardiovascular disorders: A review of medicinal weeds on KNUST campus. *Global J. Res. Med. Plants Indigenous Med.,* **3**(9): 349-358

Liu D and Lv LL (2019). New Understanding on the Role of Proteinuria in Progression of Chronic Kidney Disease. *Adv. Exp. Med. Biol***. 116**: 487-500.

Liyana-Pathirana CM and Shahidi F (2005). Antioxidant activity of commercial soft and hard wheat (Triticum aestivum L.) as affected by gastric pH conditions. *J. Agric Food Chem*. **53**(7): 2433-2440.

Lu Q, Davel AP, McGraw AP, Rao SP, Newfell BG and Jaffe IZ (2019). PKC-delta mediates mineralocorticoid receptor activation by angiotensin II to modulate smooth muscle cell function. *Endocrinol*., **160**(9): 2101-2114.

Lv JC and Zhang LX (2019). Prevalence and Disease Burden of Chronic Kidney Disease. *Adv Exp. Med. Biol*. **1165**: 3-15.

Macchiavello S, Fardella C and Baudrand R (2019). Update in the clinical management of low renin hypertension. *Rev. Med. Chil.,* **147**(4): 490-498.

Marushchak M, Maksiv K and Krynytska I (2019). The specific features of free radical oxidation in patients with chronic obstructive pulmonary disease and arterial hypertension. *Pol. Merkur. Lekarski.,* **47**(279): 95-98.

Meister TA, Rimoldi SF, Soria R, von Arx R, Messerli FH, Sartori C, Scherrer U and Rexhaj E (2018). Association of Assisted Reproductive Technologies with Arterial Hypertension during Adolescence. *J. Am. Coll. Cardiol.* **72**(11): 1267-1274.

Mills KT, Stefanescu A and He J (2020). The global epidemiology of hypertension. *Nat. Rev. Nephrol.,* **16**(4): 223-237.

Misra HP and Fridovich I (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.,* **247**(10): 3170-3175.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol Methods*, **65**(1-2): 55-63.

Ohtsubo T, Shibata R, Kai H, Okamoto R, Kumagai E, Kawano H, Fujiwara A, Kitazono T, Murohara T and Arima H (2019). Angiotensin-converting enzyme inhibitors versus angiotensin receptor blockers in hypertensive patients with myocardial infarction or heart failure: A systematic review and meta-analysis. *Hypertens Res*. **42**(5): 641-649.

Olugbenga DJ, Ukpanukpong RU and Ngozi UR (2015). Phytochemical screening, proximate analysis and acute toxicity study of *Launaea taraxacifolia* ethanolic extract on albino rats. *Intern. J. Sci. Technol.* **3**(6): 199.

Oyagbemi AA, Omobowale TO, Awoyomi OV, Ajibade TO, Falayi OO, Ogunpolu BS, Okotie UJ, Asenuga ER, Adejumobi OA, Hassan FO, Ola-Davies OE, Saba AB, Adedapo AA and Yakubu MA (2019). Cobalt chloride toxicity elicited hypertension and cardiac complication via induction of oxidative stress and upregulation of COX-2/Bax signaling pathway. *Hum. Exp. Toxicol*., **38**(5): 519-532.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*. **26**(9-10): 1231-1237.

Reznick AZ and Packer L (1994). Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol*. **233**: 357-363.

Schultz A, Olorundami OA, Teng RJ, Jarzembowski J, Shi ZZ, Kumar SN, Pritchard K Jr, Konduri GG and Afolayan AJ (2019). Decreased OLA1 (Obg-Like ATPase-1) expression drives ubiquitin-proteasome pathways to downregulate mitochondrial SOD2 (Superoxide Dismutase) in persistent pulmonary hypertension of the newborn. *Hypertension***, 74**(4): 957-966.

Shin YK, Han AY, Hsieh YS, Kwon S, Kim J, Lee KW and Seol GH (2019). Lancemaside A from Codonopsis lanceolata prevents hypertension by inhibiting NADPH oxidase 2-mediated MAPK signalling and improving NO bioavailability in rats. *J. Pharm. Pharmacol.,* **71**(9): 1458-1468.

Stanhewicz AE and Kenney WL (2017). Role of folic acid in nitric oxide bioavailability and vascular endothelial function. *Nutr. Rev.* **75**(1): 61-70.

Sun HJ (2019). Current Opinion for Hypertension in Renal Fibrosis. *Adv. Exp. Med. Biol.,* **1165**: 37-47.

Thomford NE, Mkhize B, Dzobo K, Mpye K, Rowe A, Parker MI, Wonkam A, Skelton M, September AV and Dandara C (2016). African Lettuce (*Launaea taraxacifolia*) displays possible anticancer effects and herb-drug interaction potential by CYP1A2, CYP2C9, and CYP2C19 inhibition, *OMICS: J. Integ. Biol.,* **20**(9): 528-537.

Volpe M, Gallo G, Battistoni A and Tocci G (2019). Implications of guidelines for hypertension management in Europe. *Circ. Res*., **124**(7): 972-974.

Wang D, Li H, Weir EK, Xu Y, Xu D and Chen Y (2019). Dimethylarginine dimethylaminohydrolase 1 deficiency aggravates monocrotaline-induced pulmonary oxidative stress, pulmonary arterial hypertension and right heart failure in rats. *Int. J. Cardiol*., **295**: 14-20.

Wolff SF (1994). Ferrous ion oxidation in the presence of ferric ion indicator xylenol orange for measurement of hydrogen peroxides. *Methods Enzymol.,* **233**: 182-189.

Yang EJ, Yim EY, Song G, Kim GO and Hyun CG (2009). Inhibition of nitric oxide production in lipopolysaccharide-activated RAW 264.7 macrophages by Jeju plant extracts. *Interdisc Toxicol*., **2**(4): 245-249.

Yang L (2019). How Acute Kidney Injury Contributes to Renal Fibrosis. *Adv. Exp. Med. Biol.,* **1165**: 117-142.

Yılmaz İ (2019). Angiotensin-Converting Enzyme Inhibitors Induce Cough. *Turk. Thorac. J.* **20**(1): 36-42.

Yoon WJ, Kim SS, Oh TH, Lee NH and Hyun CG (2009). *Abies koreana* essential oil inhibits drug-resistant skin pathogen growth and LPS-induced inflammatory effects of murine macrophage. *Lipids*., **44(5)**: 471-476.

Zappitelli M, Greenberg JH, Coca SG, Krawczeski CD, Li S, Thiessen-Philbrook HR, Bennett MR, Devarajan P and Parikh CR (2015). Translational research investigating biomarker endpoints in acute kidney injury (TRIBE-AKI) consortium. association of definition of acute kidney injury by cystatin C rise with biomarkers and clinical outcomes in children undergoing cardiac surgery. *JAMA Pediatr*., **169**(6): 583-591.