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Acute and sub-chronic antihypertensive properties of *Taraxacum officinale* leaf (TOL) and root (TOR)

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Taraxacum officinale* (*T. officinale*) is a leafy vegetable that is commonly used traditionally as a diuretic agent. In the current study, we aimed at exploring the potential antihypertensive effect of *T. officinale* using two animal models of hypertension. Acute and sub-chronic antihypertensive effects of TOL and TOR at 500 mg/kg/bwt were evaluated in spontaneously hypertensive rats and L-NAME-induced hypertensive rats, respectively. Wistar rats were treated daily with L-NAME (40 mg/kg/bwt) until they became hypertensive and were then allocated to various treatment groups: control, furosemide (10 mg/kg/bwt) TOL (500 mg/kg/bwt) and TOR (500 mg/kg/bwt) for 4 weeks. Blood samples were collected for determination of the lipid profile. Results showed that one-off oral administration of TOL and TOR significantly ($p < 0.05$) reduced systolic, diastolic and mean arterial blood pressure in the 2nd and 4th hours. In the sub-chronic study, TOL and TOR significantly ($p < 0.05$) prevented an increase in blood pressure throughout the period of treatment. However, TOL and TOR increased HDL-cholesterol and triglyceride levels in this study. *T. officinale* possesses antihypertensive effects with the aerial part most active at the used dosage which however, supports further development of the extract as a potential therapeutically useful natural antihypertensive agent.

Keywords: *T. officinale*; **L-NAME; antihypertensive; Asteracea; lipids; oxidative stress**

INTRODUCTION

Hypertension (HTN) is one of the most imperative factors associated with the development of several cardiovascular diseases such as heart failure, renal failure, coronary heart disease, atherosclerosis, myocardial infarction and stroke (Mendis *et al.*, 2011). Globally, 970 million people have HTN, out of which 330 million and 640 million are attributed to the developed and developing countries, respectively; however an estimate has been reported to be 1.56 billion by 2025 (Campbell *et al.*, 2014). In South Africa, the incidence of HTN is estimated at 46% while the prevalence is expected to be 61% in 2030 (Dalal *et al.*, 2011). Prevention and treatment of HTN are therefore of great concern to public health (Kearney *et al.*, 2005).

Most patients in rural communities depend on various medicinal plants provided by traditional healers for the management of HTN. As this disease is asymptomatic in nature, inadequate awareness could result in a fatal health condition which could increase the global burden of heart diseases, stroke, premature death and disability (Steyn, 2006). Essential (or primary) HTN accounts for approximately 90% to 95% of patients diagnosed with HTN, and it is mainly triggered by endothelial dysfunction which results from NO deficiency (Nyadjeu *et al.*, 2013). Physiologically, reactive oxygen species (ROS) play an important role in the cardiovascular system through highly regulated redox-sensitive pathways (Montezano & Touyz, 2012). They do this to control endothelial function, vascular tone, cardiac function and pathophysiology of inflammation. ROS activate the redox-sensitive signalling pathways especially in the vasculature where nitric oxide (NO) is reduced and ROS production is increased, hence resulting in oxidative stress. Oxidative stress (an imbalance between the production and breakdown of ROS), promotes inflammation-induced vascular damage and endothelial dysfunction, which consequently result in NO deficiency, thereby facilitating vasoconstriction, leading to increased blood pressure (Iyer *et al.*, 2010). This occurs primarily through the inhibition of NO, via the direct chemical reaction of superoxide anion ($O_2^{-\cdot}$) with NO, resulting in the formation of peroxynitrite. Formation of peroxynitrite may result in further impairment of NO levels and enhance oxidative stress by inhibiting eNOS activity through oxidation of 4-tetrahydrobiopterin (BH_4), a co-factor of eNOS. This leads to eNOS uncoupling, where eNOS produces superoxide instead of NO (Ceriello, 2008). HTN and dyslipidemia are two important risk factors of cardiovascular diseases which cannot be underestimated. Therefore, in order to reduce cardiovascular risk, it is important to regulate HTN and dyslipidemia (Nyadjeu *et al.*, 2013).

Lipids are naturally occurring molecules such as fats, triglycerides and phospholipids, and are hydrophobic in nature. Due to their hydrophobicity, lipids are transported through

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packaging in LDL-cholesterol and very low density lipoproteins (Iwalokun *et al.*, 2011). This they do by narrowing blood vessels and increasing resistance because of plaque formation. Plaque formation involves atherogenic lipid modification by peroxidation and proliferation of underlying smooth muscle cells and foam cell formation coupled with NO consumption to form ROS, thereby resulting in endothelial dysfunction, and hence a vasoconstriction effect on the system.

L-NAME-induced HTN is a suitable model for studying the cardiovascular effect of antihypertensive agents and medicinal plants (El-nezhawy *et al.*, 2014; Kodavanti *et al.*, 2013; Badary *et al.*, 2013). L-NAME-induced HTN is a rat model of HTN which is widely used to mimic HTN in humans because it competes for the binding site on endothelial nitric oxide synthase (eNOS) and, as a result, inhibits the synthesis of NO (Zhao *et al.*, 2013). Several drugs are currently available for the clinical management of HTN. It is however reported that many hypertensive patients either prefer to use medicinal plant products or use them in addition to pharmaceuticals for treatment (Azam *et al.*, 2014; Talha *et al.*, 2011), as they believe that these plant extracts are more effective and have fewer side effects (Chow, 2013). Among these medicinal plants is *Taraxacum officinale*.

T. officinale is an herbaceous perennial flowering plant, native to Eurasia and North and South America; although it is found as a weed in all parts of the world. It is also widely distributed and consumed as a vegetable in the Eastern Cape region of South Africa. Traditionally, the plant is used for treating abscesses, reducing eye inflammation, and provoking diuresis in Chinese, Arabian and Native American traditional medicine (Clare *et al.*, 2009). However, in South Africa, the leaf and root decoction are used as a diuretic, an expectorant, a laxative and a liver tonic. A survey of the literature showed that the antihypertensive effect of *T. officinale* has not been explored by any study. Therefore, the present study was undertaken to explore the antihypertensive activity of *T. officinale* in L-NAME-induced HTN, wherein nitric oxide (NO) is completely blocked.

MATERIALS AND METHODS

Drugs and chemicals

Nω-nitro-L-arginine methyl ester (L-NAME) was purchased from Sigma-Aldrich (St Louis, MO, USA). Furosemide (Pharmacare Ltd, South Africa), and sodium chloride (NaCl). Ethyl alcohol and diethyl ether were supplied by Shalom laboratories, Durban, South Africa.

Experimental animals

Male Swiss albino mice (20–25 g), spontaneously hypertensive rats (230–300 g) and Wistar rats (250–350 g) were purchased from the South African Vaccine programme, Johannesburg. The animals were housed and allowed to acclimatise for two weeks in the Department of Biological and Environmental Sciences animal holding facility. Animals were kept under normal lighting conditions and temperature was maintained at 24 °C. Wood shavings mixed with shredded paper were used as bedding and pacifier, respectively. Animals had free access to rat pellets (Epol SA: protein 180 g/kg, moisture 120 g/kg, fat 25 g/kg, fibre 60 g/kg, calcium18 g/kg and phosphorus 7 g/kg) and water *ad libitum*. Animal cages were cleaned with water and disinfectant and bedding replaced two times per week.

Plant material

T. officinale was collected from Ginsburg, in the Eastern Cape with the assistance of Mr Reuben Matewu, a traditional healer. The collected plant was taxonomically identified and authenticated by Dr Immelman of the KEI herbarium, Department of Biological and Environmental Sciences, Walter Sisulu University. The leaves and the roots were thoroughly washed in water and air-dried. The leaves were separated from roots, chopped into pieces and weighed. The dried leaves and roots were extracted in 70% ethanol.

Preparation of 70% ethanol extract

70% ethanol extraction of TOL and TOR was prepared by diluting 700 ml of absolute ethanol with 300 ml of distilled water in a volumetric flask. 100 g of the samples were extracted in 500 ml of 70% ethanol for 48 h undergoing a shaking process (Gyro-Rocker, STR9, UK). The extracts were filtered with a Buchner funnel and Whatman No. 1 filter paper. Excess ethanol in the filtrate was concentrated using a rotary evaporator (Laborota 4000 efficient, Heldoph, Germany) under a reduced pressure at 40 °C. The extracts were freeze-dried, and dry extracts were used for the experiment.

Phytochemical screening of *T. officinale*

Qualitative analysis of secondary metabolites in extracts was performed. 70% ethanol extracts of TOL and TOR were screened for the presence of tannins, saponins, flavonoids, terpenoids, alkaloids, glycosides and phenolic compounds as described by Mir *et al.* (2013).

Tannins

0.5 g of powdered samples of TOL/TOR was boiled in 20 ml of distilled water in a test tube and filtered. 0.1% FeCl was added to the filtered samples. Brownish green or blue-black colouration indicated the presence of tannins.

Saponins

2 g of powdered samples of TOL/TOR was boiled in 20 ml of distilled water. 10 ml of the filtered samples was shaken vigorously to obtain an emulsion. Formation of stable foam indicated the presence of saponins.

Terpenoids

5 ml of ethanol extracts of TOL/TOR was added to 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added to the mixture to form a layer. Formation of an interface with a reddish-brown colouration confirmed the presence of terpenoids.

Steroids

2 ml of filtrate was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour in the lower chloroform layer indicated the presence of steroids.

Glycosides

1 ml of concentrated H_2SO_4 was prepared in a test tube with 5 ml of ethanol extracts of TOL/TOR. The samples were mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_2$. The above mixtures were carefully added to concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture.

A brown ring appearance indicated the presence of cardiac glycosides.

Alkaloids

1 ml of ethanol extracts of TOL/TOR was added to 2 ml of Dragendoff's reagent. 1 ml of the extracts was also mixed with 2 ml of Meyer's reagent. With Dragendoff's reagent, a turbid orange colour indicated the presence of alkaloids. While with Meyer's reagent, a yellow precipitate confirmed the presence of alkaloids.

Phenolic compounds

500 mg of TOL/TOR extracts was dissolved in 5 ml of distilled water. To this, a few drops of neutral 5% FeCl3 solution were added. A dark colouration indicated the presence of phenolic compounds.

Acute toxicity (LD_{50})

The acute toxicity study was performed using Lorke's method as modified by Maikai and Kobo (2009). The study was divided into two phases. In the first phase, nine mice were assigned to three treatment groups of three mice each. Groups 1, 2 and 3 received 10, 100 and 1000 mg/kg/bwt of the extract, respectively, to determine the range of doses producing observable toxic effect in the animals. Animals were observed for behavioural changes (such as excitement, restlessness, difficulty in breathing and loss of appetite), and mortality within 24 hours. In the second phase of the toxicity test, crude extracts were administered orally at three higher dose-levels starting with the highest dose in phase 1 (i.e. 1600, 2900 and 5 000 mg/kg/bwt) to three mice ($n = 1$ per group). The LD_{50} values were calculated for each extract using

$$LD_{50} = \sqrt{(A \times B)} \quad (1)$$

Where *A* is the maximum dose producing no mortality and *B* is the dose that produces 100% death (Lorke, 1983).

Acute hypertensive study

Twenty four spontaneously hypertensive rats were used for the study. The animals were divided into four groups of six rats each.

- Group I – The control group (normal saline).
- Group II – Furosemide-treated group (10 mg/kg/bwt),
- Group III – TOL-treated group (500 mg/kg/bwt).
- Group IV – TOR-treated group (500 mg/kg/bwt).

Baseline blood pressure was measured for each rat before extract administration. Blood pressure was measured 2 and 4 hours after drug/extract administration. However, because we were not able to procure sufficient numbers of SHRs for the whole study, the rest of the study was carried out using Wistar rats which were more readily available.

Sub-chronic study

Induction of HTN in rats

HTN was induced in Wistar rats using L-NAME (40 mg/kg/bwt) administered orally daily for 5 weeks. Blood pressure was measured at baseline and monitored weekly during the L-NAME treatment period using the non-invasive blood pressure machine CODA 8 (Kent Scientific Corporation, Connecticut, USA). Blood pressure was measured on the same day of the week between 09h00 and 12h00. Rats were first pre-warmed at 37 °C for 30 min and allowed to rest in a plastic restrainer equipped with a conical piece that allows rats to breathe freely and stay relaxed. Animals were handled humanely (Leary *et al.*, 2016) and in accordance with the South African National Standard (2008) for the care and use of animals for scientific purposes. Ethical clearance for this study was obtained from the Walter Sisulu Research Ethics Committee (clearance number: 14-0003-11).

Biochemical assays

At the end of 21 days of treatment, animals were lightly anaesthetised using diethyl ether. Blood was collected by cardiac puncture with a 10 ml syringe and needle into 5 ml pre-labelled vacutainer gel-separating tubes (BD Plymouth, UK) for serum collection. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10 minutes (Table Top centrifuge, Taiwan). Using a 3 ml Pasteur pipette, serum was collected and aliquoted into three labelled Eppendoff tubes and, stored at -70 °C until needed for lipid profile assays.

Lipid profile determination

Serum levels of high density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride were determined using an automatic chemistry analyzer (Mayamed, China) with a purchased reagent kit (Biosinol, China). Total cholesterol (TC) level was calculated using the formula of Friedewald *et al.* (1972). Atherogenic indices were calculated from the TC, and HDL-C.

$$AIS = \text{Total Cholesterol/High Density Lipoprotein} \quad (2)$$

Statistical analysis

GraphPad prism (version 5) was used for statistical analysis of all data. Analysis of Variance (ANOVA) and t-test were used to compare means and determine statistical differences in treatment groups. The t-test was used to compare treatment groups versus control; while ANOVA followed by Tukey's multiple comparison tests was used to compare means of one group with every other group. Data were presented as mean ± Standard Error of Means (SEM). P value < 0.05 was considered significant.

RESULTS

Phytochemical analysis of *T. officinale* leaf and root extracts

Qualitative phytochemical evaluation of *T. officinale* 70% ethanol extract showed that phenols, tannins, glycoside, steroids, terpenoids and saponins were present in both TOL and TOR while alkaloids were present in TOR but absent in the TOL (Table 1).

Acute toxicity study

In the first phase, doses up to 1000 mg/kg/bwt did not cause mortality when administered via the oral route. In the second phase, there was also no mortality at 1600, 2900 and 5000 mg/kg/bwt. The LD_{50} was estimated to be ≥5000 mg/kg/bwt, p.o. The results are presented in Table 2.

Table 1. Phytochemical composition of *T. officinale* leaf and root extracts.

Phytochemical	TOL	TOR
Alkaloids	-	+
(A) Meyer's reagent	-	+
(B) Draggendoff's reagent	-	+
Phenols	+	+
Tannins	+	+
Glycoside	+	+
Steroids	+	+
Terpenoids	+	+
Saponins	+	+

+, phytochemicals present; - phytochemicals absent.

Antihypertensive studies

Effect of treatment on systolic blood pressure

The acute effect of treatment on systolic blood pressure (SBP) examined *in vivo* in spontaneously hypertensive rats shows that while 500 mg/kg/bwt TOL significantly ($p < 0.001$) reduced systolic blood pressure at the 2nd and 4th hours post treatment, TOR (500 mg/kg/bwt) showed significant ($p < 0.05$) SBP at the 4th hour (Table 3). In animals that received TOL (500 mg/kg/bwt), SBP dropped from 180.7 ± 4.23 to 139.2 ± 3.38 mmHg, corresponding to a reduction in SBP by 41.5 ± 0.5 mmHg at the 2nd hour; while at the 4th hour, SBP decreased by 43.8 ± 0.8 mmHg. On the other hand, TOR (500 mg/kg/bwt) reduced SBP by 14.4 ± 8.0 mmHg at the 2nd hour and 23.9 ± 3.4 mmHg at the 4th hour, respectively.

Effect of treatment on diastolic blood pressure

Following oral administration of extracts of TOL and TOR on spontaneously hypertensive rats, a significant reduction in diastolic blood pressure (DBP) was observed in the TOL-treated group; while diastolic blood pressure in the TOR-treated group was similar to the control (Table 4). TOL reduced DBP from 125.2 ± 3.7 to 82.6 ± 3.65 and 93.3 ± 0.09 mmHg at the 2nd and 4th hours, respectively with resultant decreases in DBP of 42.6 ± 0.6 and 31.8 ± 0.09 mmHg, respectively. TOR however reduced DBP by 14.45 ± 4.71 mmHg (from 120.07 ± to 105.22 ± mmHg).

Effect of treatment on mean arterial blood pressure

TOL significantly ($p < 0.001$) reduced the mean arterial blood pressure from 143.3 ± 3.6 to 101.1 ± 0.4 and 107.6 ± 3.2 mmHg (by 42.3 ± 1.3 and 35.3 ± 0.4 mmHg) at the 2nd and 4th hours, respectively while TOR failed to significantly reduce mean BP (Table 5).

Table 2. Acute toxicity test of *T. officinale* in mice.

Dose mg/kg	Death patterns after 24 hours
	Phase 1
10	0/3
100	0/3
1000	0/3
1000	0/3
	Phase 2
1600	0/1
2900	0/1
5000	0/1
LD_{50}	≥5000 mg/kg/bwt, p.o.

Table 3. Acute effect of TOL and TOR on SBP.

Groups	Baseline	2H	4H
NS	202.2 ± 1.9	198.0 ± 1.6	182.8 ± 0.8
TOL	180.7 ± 4.2	139.2 ± 4.8$^{***\#\#}$	136.8 ± 3.4$^{***\#\#}$
TOR	181.1 ± 8.6	166.7 ± 0.6	157.2 ± 5.3*
FURO	170.3 ± 3.5	166.1 ± 5.6*	163.1 ± 4.1**

NS, untreated controls; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals.
Data are presented as mean ± SEM for six rats. *$p < 0.05$, **$p < 0.01$ statistically compared to control. $^{\#\#}$ $p < 0.01$ statistically compared to furosemide-treated animals.

Table 4. Acute effect of TOL and TOR on DBP.

Groups	Baseline	2H	4H
NS	156.0 ± 6.7	156.1 ± 6.6	137.8 ± 3.3
TOL	125.2 ± 3.6	82.6 ± 3.1$^{***\#\#}$	93.3 ± 3.7$^{*\#\#}$
TOR	120.7 ± 7.8	110.4 ± 0.1	106.2 ± 3.2
FURO	122.1 ± 5.1	116.6 ± 4.5*	113.2 ± 3.4

NS, untreated controls; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals.
Data are presented as mean ± SEM for six rats. *$p < 0.05$, **$p < 0.01$ statistically compared to control. $^{\#\#}p < 0.01$ statistically compared to furosemide-treated animals.

Results of sub-chronic studies

Effect of sub-chronic treatment with TOL and TOL on SBP

The sub-chronic effect of TOL and TOR on the SBP of L-NAME-induced hypertensive rats is hereby presented. Blood pressures of rats receiving distilled water/extracts/drugs were measured in the first week of experimentation (Table 6). SBP(s) were normal and ranged between 115.4 ± 3.1 and 124.1 ± 4.1 mmHg. After four weeks of daily treatment with 40 mg/kg/bwt L-NAME, rats became hypertensive with blood pressures ranging from 180.3 ± 0.8 to 191.0 ± 3.6 mmHg. Weekly measurement of BP showed that TOL (500 mg/kg/bwt) significantly ($p < 0.05$ to 0.001) reduced SBP in the 1st, 2nd and 3rd weeks of treatment with SBP in this group reduced by 35 ± 14.1, 52 ± 5.7 and 63 ± 3.9 mmHg, respectively (Table 6). On the other hand, treatment with TOR (500 mg/kg/bwt) induced a later onset blood of BP lowering effects which became significant ($p < 0.01$ to 0.05) in the 2nd and 3rd weeks of treatment. In this group SBP was reduced by 43 ± 11.5 and 45 ± 5.7 mmHg, respectively. The antihypertensive drug (furosemide (10 mg/kg/bwt)) also

Table 5. Acute effect of TOL and TOR on MABP.

Groups	Baseline	2H	4H
NS	171.1 ± 5.1	169.7 ± 4.9	152.2 ± 0.6
TOL	143.3 ± 3.7	101.1 ± 2.3$^{***\#\#}$	107.5 ± 3.2$^{**\#\#}$
TOR	140.4 ± 8.1	128.8 ± 0.2	122.9 ± 3.7
FURO	137.9 ± 4.5	132.9 ± 4.8	129.6 ± 3.3

NS, untreated controls; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals.
Data are presented as mean ± SEM for six rats. *$p < 0.05$, **$p < 0.01$ statistically compared to control. $^{\#\#}$ $p < 0.01$ statistically compared to furosemide-treated animals.

Table 6. Sub-chronic effect of TOL and TOR on SBP.

			Week of treatment		
Group/SBP (mm/Hg)	Pre-induction	HTN	1	2	3
Normotensive control	122.5 ± 2.1	131.1 ± 0.5	131.1 ± 0.5**	129.9 ± 1.6***	159.1 ± 1.5
Control HTN	124.1 ± 4.1	186.1 ± 2.8	188.7 ± 10.4	197.8 ± 12.7	150.1 ± 2.7
TOL	115.4 ± 3.1	180.3 ± 0.8	145.8 ± 14.1*	128.5 ± 5.7***	117.2 ± 3.9***#
TOR	119.5 ± 5.0	180.5 ± 1.1	164.8 ± 5.1	137.2 ± 11.5***	135.9 ± 5.6*##
FURO	123.9 ± 4.1	187.8 ± 2.6	133.6 ± 11.9**	150.0 ± 7.3**	139.6 ± 0.3##

Control, normotensive controls; NS + L-NAME, hypertensive control; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically compared to hypertensive control; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ statistically compared to normotensive control.

significantly ($p < 0.05$ to 0.01) lowered SBP. At the doses used, TOL (500 mg/kg/bwt) had better blood pressure lowering effect on SBP than furosemide (Table 6).

Effect of sub-chronic treatment with TOL and TOR on DBP

When TOL and TOR were investigated sub-chronically in L-NAME-induced hypertensive rats, results show that TOL (500 mg/kg/bwt) significantly ($p < 0.001$) reduced DBP in the 1st, 2nd and 3rd weeks of treatment by 36 ± 12.2, 39 ± 6.1, and 49 ± 2.3 mmHg, respectively; while the TOR-treatment induced a later onset of BP lowering effects. In this group, DBP was significantly ($p < 0.01$) lowered in the 2nd and 3rd weeks of treatment by 38 ± 12.5 and 39 ± 5.3 mmHg, respectively compared with the untreated control (Table 7). Although furosemide (10 mg/kg/bwt) also lowered DBP significantly, its effects were weaker (at used doses) than the effects of TOL (500 mg/kg/bwt).

Effect of sub-chronic treatment with TOL and TOR on mean arterial blood pressure

TOL (500 mg/kg/bwt) significantly ($p < 0.01$) dropped mean arterial blood pressure in the 1st, 2nd and 3rd weeks of treatment. The observed blood pressure lowering effect increased with time from the 1st to the 3rd weeks (35 ± 11.7, 43 ± 5.9 and 53 ± 2.6 mmHg, respectively). Treatment with TOR (500 mg/kg/bwt) reduced mean arterial blood pressure by 40 ± 12.2 and 42 ± 5.4 mmHg, respectively during the 2nd and 3rd weeks of treatment (Table 8). Furthermore, unlike TOL (500 mg/kg/bwt), the effects of TOR (500 mg/kg/bwt) on mean arterial blood pressure were weaker than the effects of furosemide (10 mg/kg/bwt) in the 3rd week of treatment.

Biochemical studies

Effect of treatment with TOL and TOR on lipid profile

Sub-chronic oral administration of L-NAME (40 mg/kg/bwt) caused an increase in LDL-C and TG in all the experimental groups. Increased HDL-C but no significant changes in triglyceride or cholesterol levels were observed in animals treated with 500 mg/kg/bwt TOL and TOR when compared with hypertensive control animals. However, TOL significantly increased HDL-cholesterol when compared with normotensive animals, while there was a significant ($p < 0.05$) increase in triglyceride level in the furosemide-treated group (Table 9). Atherogenic indices in animals that received only L-NAME were higher than other treatment groups. Although the atherogenic indices of the animals treated with TOL and TOR are close to those observed in the normotensive control animals, they were still similar to those observed in animals that received only L-NAME.

DISCUSSION

The phytochemical evaluation of *T. officinale* 70% ethanol extract showed that phenols, tannins, glycosides, steroids, terpenoids and saponins were present in both TOL and TOR while alkaloids were present in TOR but absent in TOL. The Dragendoff and Meyer's tests for the presence of alkaloids showed similar results. Acute and sub-chronic antihypertensive effects of TOL and TOR were studied using SHRs and L-NAME-induced hypertensive rat models, respectively. Acute antihypertensive results revealed that TOL significantly reduced blood pressure parameters when statistically compared with control and used reference drugs. The presence of secondary metabolites contributes to the medicinal properties of the plant (Ahmed & Gilani, 2014; Mir *et al*., 2013). Saponins, alkaloids, phenols and flavonoids have been shown to possess antihypertensive properties

Table 7. Sub-chronic effect of TOL and TOR on DBP.

			Week of treatment		
Group/DBP (mm/Hg)	Pre-induction	HTN	1	2	3
Normotensive control	87.7 ± 4.3	93.2 ± 0.8	93.2 ± 0.9**	94.4 ± 0.9***	126.9 ± 11.7
Control HTN	79.9 ± 4.3	148.8 ± 1.8	147.6 ± 10.5	151.4 ± 10.4	114.1 ± 3.8
TOL	77.5 ± 4.5	134.4 ± 0.6	98.8 ± 12.2***	95.8 ± 6.1***	85.9 ± 2.3***###
TOR	78.5 ± 5.0	135.8 ± 1.4	118.6 ± 4.1	97.7 ± 12.5**	95.9 ± 5.3**###
FURO	80.5 ± 3.1	147.8 ± 2.5	97.3 ± 0.2***	110.3 ± 6.9*	108.3 ± 0.1##

Control, normotensive controls; NS + L-NAME, hypertensive control; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals. Data are presented as mean ± SEM.*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically compared to hypertensive control; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ statistically compared to normotensive control.

Table 8. Sub-chronic effect of TOL and TOR on MABP.

			Week of Treatment		
Group/SBP (mm/Hg)	Pre-induction	HTN	1	2	3
Normotensive control	98.9 ± 3.4	105.6 ± 0.7	105.6 ± 0.7***	105.9 ± 0.8***	137.5 ± 1.5
Control HTN	94.3 ± 4.1	160.8 ± 2.1	161.06 ± 15.3	166.5 ± 11.2	125.9 ± 3.4
TOL	89.8 ± 3.9	149.3 ± 0.6	114.2 ± 11.8**	106.3 ± 5.9***	96.1 ± 2.6***###
TOR	91.9 ± 5.1	150.4 ± 1.2	131.8 ± 2.9	110.5 ± 12.2**	108.9 ± 5.3**###
FURO	94.6 ± 3.5	160.8 ± 2.5	109.0 ± 0.2**	123.3 ± 6.9*	118.3 ± 0.1###

Control, normotensive controls; NS + L-NAME, hypertensive control; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals. Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ statistically compared to hypertensive control; ###$p < 0.001$ statistically compared to normotensive control.

Table 9. Effect of treatment on serum lipid profile of L-NAME-induced hypertensive Wistar rats.

Groups/parameter (mg/dl)	HDL-C	LDL-C	TG	T-CHO	AIS
Control normotensive	24.7 ± 0.04	24.5 ± 0.07	21.4 ± 0.03	53.2 ± 0.04	2.2 ± 1.0
Control HTN	29.2 ± 5.96	57.4 ± 6.9	53.9 ± 4.1	97.4 ± 11.2	3.3 ± 1.9
TOL	38.8 ± 2.9[b]	54.1 ± 6.1[c]	51.3 ± 2.8[c]	103.2 ± 6.5	2.7 ± 2.2
TOR	32.4 ± 2.5	51.4 ± 1.8[b]	43.9 ± 1.9[c]	92.6 ± 4.3	2.9 ± 1.7
FURO	45.5 ± 4.6[a]	41.2 ± 3.7	68.8 ± 5[#]	100.5 ± 4.1	2.2 ± 0.9

Control, normotensive controls; Control HTN, hypertensive control; TOL, *T. officinale* leaf extract-treated animals; TOR, *T. officinale* root extract-treated animals; FURO, furosemide-treated animals. *$p < 0.05$ statistically compared to hypertensive control. #$p < 0.05$ statistically compared to hypertensive control animals.
Data are presented as mean ± SEM. [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ statistically compared to normotensive control.

(Kurmukov, 2013). Tannins reduced blood pressure in NG-nitro-L-arginine (L-NNA)-induced hypertensive rats (Turgut Coşan *et al*., 2013). Moreover, glycosides have been known to lower blood pressure, although its individual activity is still questionable (Ornish, 2015). *T. officinale* possesses these phytochemicals which may be responsible for the antihypertensive properties shown by the leaf and the root extracts (Table 1).

The L-NAME-induced HTN depends on a chronic inhibition of NO production by L-arginine analogue, therefore resulting in arterial HTN (Bernatova, 2014). The present sub-chronic study provides evidence that chronic inhibition of NO synthesis in L-NAME-induced hypertensive rats leads to marked elevation of systemic blood pressure. This could be attributed to a blockade of NO synthesis by NO inhibitors like L-NAME, resulting in endothelial dysfunction, elevation of blood pressure and further pathological damage to the cardiovascular system and kidneys, which could lead to aggravation of HTN (Nyadjeu *et al*., 2013). This study shows that TOL and TOR significantly reduced systolic, diastolic and mean blood pressures in experimental rats. These findings suggest that TOL and TOR could possibly enhance NO synthesis by upregulating eNOS and increased vasodilation in different parts of the vascular tree. Our findings are consistent with reported work on rats treated with L-NAME (Bernatova, 2014; Veerappan & Senthilkumar, 2015).

Cholesterol is not only a significant biomolecule, but also a very important marker of cardiovascular disease. Cholesterol increase has been implicated in HTN and other cardiovascular diseases and diabetes (Akpanabiatu *et al*., 2013). One major pathway suggesting the cardioprotective function of HDL is the reverse cholesterol transport (RCT) pathway (Kontush, 2006). In this study, *T. officinale* did not lower TC and LDL-C levels, although there was an increase in HDL-C level. Atherogenic indices in TOL and TOR-treated animals were also in close range to normotensive animals, but similar to those in hypertensive control animals. This might suggest a weak hypolipidemic effect of *T. officinale*.

CONCLUSION

We conclude that *T. officinale* possesses effective antihypertensive, and increased HDL-C, a good form of cholesterol. The aerial (leaf) part was more active than the root at the used dosage which, however, supports further development of the extract as a potential therapeutically useful natural antihypertensive agent.

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DECLARATION OF INTEREST

None.

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