Evaluation of Diuretic and Antihypertensive Activity of Leaf Extracts of Thymus Schimperi in Rats

Article · June 2016

CITATIONS
4

READS
428

4 authors, including:

Eyasu Makonnen
Addis Ababa University
243 PUBLICATIONS **3,855** CITATIONS
SEE PROFILE

Asfaw Debela
117 PUBLICATIONS **2,346** CITATIONS
SEE PROFILE

Bekesho Geleta
Children's Cancer Institute Australia
42 PUBLICATIONS **496** CITATIONS
SEE PROFILE

British Journal of Pharmacology and Toxicology 7(1): 1-8, 2016
DOI:10.19026/bjpt.7.2779
ISSN: 2044-2459; e-ISSN: 2044-2467

Submitted: October 31, 2015 Accepted: January 8, 2016 Published: February 25, 2016

Research Article

Evaluation of Diuretic and Antihypertensive Activity of Leaf Extracts of *Thymus Schimperi* in Rats

[1]Hussein Haji, [2]Eyasu Makonnen, [3]Asfaw Debella and [3]Bekesho Geleta
[1]Department of Pharmacology, College of Health Sciences, Arsi University, P.O. Box 396, Asella, Ethiopia,
[2]Department of Pharmacology, School of Medicine, College of Health Sciences, Addis Ababa University, P.O. Box 9080, Ethiopia,
[3]Directorate of Traditional and Modern Medicine Research, Ethiopian Public Health Institute, P.O. Box 1242, Addis Ababa, Ethiopia

Abstract: The present study was performed to evaluate diuretic activity of aqueous and essential oil extract and antihypertensive activity of the aqueous extract of *Thymus Schimperi* leaves against salt-sucrose induced hypertension in rats. Aqueous extract of *T. Schimperi* leaves at the doses of 250, 500, 750 and 1000 mg/kg and the essential oil at 1.0 and 1.5 mL/kg were administered orally with normal saline to male wistar rats (n = 6). Normal saline and hydrochlorothiazide were used as negative and positive control, respectively. Urine volume and its electrolyte contents (Na^+, K^+ and Cl^-) were measured to study diuretic activity of *T. Schimperi* leaves. On the other hand, for antihypertensive study, rats were randomly divided into control and treatment groups (n = 6). Treatment groups were given daily aqueous extracts (250 and 500 mg/kg) orally with 2% NaCl and 10% sucrose solution. Whereas, positive, negative and normal control received captopril (20 mg/kg/day with 2% NaCl and 10% sucrose solution), only 2% NaCl and 10% sucrose solution *ad libitum* and water *ad libitum* for 6 weeks, respectively. The blood pressure was measured every week using tail cuff blood pressure analyzer and after 6 weeks the blood was sampled to evaluate effect on serum lipid profile (TC, TG and BG) and liver function indicator enzymes (ALT & ALP) using clinical chemistry analyzer. The oral administration of aqueous extract of *T. Schimperi* leaves (500 mg/Kg/day) for six weeks produced significant ($p<0.05$) prevention in systolic blood pressure increment. The oral administration of aqueous extract of *T. Schimperi* (250, 500, 750 and 1000 mg/kg) showed positive diuretic activity at 5 h. The aqueous extract of *T. Schimperi* (750 and 10000 mg/kg, orally) significantly increased Na^+, K^+ and Cl^- excretion in 5 h and additionally essential oil of *T. Schimperi* (1 and1.5 mL/kg) showed significant kaliuretic effect. The results of the present study revealed the antihypertensive and diuretic activity of aqueous extract of *T. Schimperi* leaves. These effects might be attributed to the presence of the potassium or phenolic components of the thymus leaves.

Keywords: Antihypertensive, diuretic, salt-sucrose, *T. schimperi*

INTRODUCTION

Hypertension is a chronic disorder characterized by a persistently elevated Blood Pressure (BP) exceeding 140/90 mmHg or greater (Vasant *et al*., 2012). It has been named the "silent killer," as it is asymptomatic and the major contributor or risk factor to cardiovascular morbidity and mortality (Gavras, 2009). Risks of stroke, heart failure, kidney failure, coronary heart disease, or cerebrovascular disease or early death from a cardiovascular cause are directly correlated with BP (Foex and Sear, 2004).

Hypertension in patients with diabetes causes a significant increase in risk of cerebrovascular disease, coronary heart disease, stroke, heart failure, renal disease, retinopathy and sexual dysfunction. The association with co-morbidities such as dyslipidemia, prothrombotic state and autonomic dysfunction contributes to an increase in morbidity and mortality (López-Jaramillo *et al*., 2013).

Hypertension affects about 1 billion people globally and accounts for approximately 7.1 million deaths annually. It is prevalent not only among developed nations but also in developing countries (Kumar *et al*., 2012). It is 1.5 to 2.0 times more common in patients with diabetes than without diabetes and more so in females than males (Tashko and Gabbay, 2010).

Corresponding Author: Bekesho Geleta, Directorate of Traditional and Modern Medicine Research, Ethiopian Public Health Institute, P.O. Box 1242, Addis Ababa, Ethiopia, Tel.: +251911091969

Clinically, various antihypertensive drugs have been used to manage hypertension and to alleviate symptoms. However, the efficacy of these drugs is only 40-60% and usually two or more antihypertensive drugs from different categories are needed to be combined to achieve the optimal results and thus, this ultimately increases the cost of treatment and side effects. The frequent side effects of synthetic antihypertensive drugs includes dry mouth, dizziness, visual disorders, headache, cough, emotional distress, gastrointestinal disturbance, peripheral circulatory symptoms like cold hands, feet and swollen ankles. These distressing side effects can lead to non-compliance and adversely affects health-related quality of life (Vasant *et al*., 2012). Hence, newer antihypertensive agents are needed to expand therapeutic options, increase treatment efficacy, decrease side effects and enhance patient adherence (Patel *et al*., 2012). Thus there is a need to explore alternative therapies particularly from herbal sources as these are cost effective and possess minimal side effects.

Even though, there are several allopathic antihypertensive medications are available, most people living in developing countries rely on traditional medicine. Various herbal preparations have been used and claimed to have benefit for hypertension. The diuretic, hypotensive, antihypertensive and *in vitro* vasodilatory effects of some of these plants such as *Thymus serrulatus* (Geleta *et al*., 2015), *Moringa stenopetala* (Mengistu *et al*., 2012), *Syzygium guineense* (Ayele *et al*., 2010), *Tetrapleura tetraptera* (Thierry *et al*., 2012) and *Colocasia esculenta* (Vasant *et al*., 2012) have been validated and others disproved.

One of the plants used for treatment of hypertension in Ethiopia is *Thymus schimperi* Ronniger. *T. schimperi* Ronniger locally known as 'Tossign' (Amharic) is endemic to the Ethiopian highlands, growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes. It is perennial herb, woody at the base and 5-40 cm high (Asfaw *et al*., 2000; Dagne *et al*., 1998). *T. schimperi* was found to have antifungal activity. The studies shows that the *in vitro* vasodilatory effect of *T. serrulatus* (Geleta *et al*., 2015), antihypertensive activity of *T. vulgaris* (Kensara *et al*., 2013) and *T. serpyllum* (Mihailovic-Stanojevic *et al*., 2013) which has similar genius with *T. schimperi*.

The volatile oils from *T. schimperi* have been examined by means of gas chromatography and gas chromatography-mass spectrophotometery and the main constituents of the essential oils of *T. schimperi* from four regions such as Bale, Gonder, Shewa and Wollo were identified as *p*-cymene (9-23%), γ-terpinene (8-17%), thymol (6-38%) and carvacrol (5-63%) (Asfaw *et al*., 2000).

As to my knowledge since the diuretic and antihypertensive activity of this plant has not been scientifically evaluated, the present study was undertaken to investigate the possible diuretic and antihypertensive activity of aqueous extract of *T. schimperi* leaves in different experimental models taking the community claim in consideration. This study may help to investigate diuretic and antihypertensive agents with greater efficacy and lower adverse effects than the existing drugs.

MATERIALS AND METHODS

Experimental animals: Normal Wistar rats, bred in the animal house of the Ethiopian Public Health Institute, age of 6 to 8 weeks and weighing 250 to 300 g were used for the experiment. They were kept for a week in a controlled environment (25±1°C and 12 h/12 h light/dark cycle) with 6 rats per cage. The animals were allowed free access to tap water and standard laboratory animal food. The care and handling of the animals were in accordance with the internationally accepted standard guidelines for use of animals (National Research Council, 2011). The study was submitted to and approved by, Ethiopian Public Health Institute ethics committee.

Plant material collection: The fresh *T. schimperi* leaves were collected from South Eastern Ethiopia around Chilalo Mountain about 175 km far from Addis Ababa in September 2013. The plant was identified by a taxonomist at the Directorate of Modern and Traditional Medicine Research of Ethiopian Public Health Institute and a voucher number (HH001) was deposited in the herbarium for future reference.

Extraction of the plant material:

Isolation of essential oil: About 440 g of fresh leaves and 571 g of semi-dried leaves of *T. schimperi* were put for hydro-distillation in a clevenger-type apparatus for 3 h, in a reduced pressure at a temperature of 70°C. The essential oils obtained were separated from water and stored in refrigerator until needed for use. The percent yield was 1.48% (v/w) for fresh leaves and 1.02% (v/w) for semidried leaves.

Aqueous extraction: About 1600 g of dried leaves of the *T. schimperi* were extracted by heating at 70°C for 15 min (infusion method). The extract was filtered through Whatman filter paper No. 1 and freeze dried using lyophilizer (12L Console Freeze Dry 230v-60 (7754040) Freeze Dry System, Labconco, USA). A brownish dried extract was collected, weighed and kept in a desiccator until used for experiment. The percent yield was 8.4% (w/w).

Phytochemical screening: Phytochemical analysis of the major secondary metabolites such as alkaloids, saponnins, flavonoids, tannins and polyphenols of the plant extracts was undertaken using standard qualitative color tests (Trease and Evans, 1989).

Evaluation for diuretic activity: The assessment of diuretic activity was employed with slight modification

of methods used by Vogel *et al*. (2008). In this method, male Wistar rats weighing between 230-280 g deprived of food and water for 15 h prior to the experiment, were divided into eight groups (n = 6). The 1st group of animals, serving as control, received normal saline (25 ml/kg, orally); the 2nd group received hydrochlorthiazide (10 mg/kg, orally) in saline; the 3rd, 4th, 5th and 6th groups were given crude aqueous extracts orally at a dose of 250 mg/kg (TS250), 500 mg/kg (TS500), 750 mg/kg (TS750) and 1000 mg/kg (TS1000) was administered orally in a volume of 1 ml/100 g, respectively. Group VII and VIII received essential oil orally at a dose of 1 mL/kg (TS1) and 1.5 mL/kg (TS1.5), respectively. Immediately after oral administration, the animals were placed individually in metabolic cages kept at 20°C±0.5°C. The volume of urine output after 5 h was measured and finally stored at -20°C for further analysis. During this study, no food and water were made available to animals. The concentrations of Na^+, K^+ and Cl^- in the urine and K^+ in water (distilled and deionized) dissolved aqueous extract of *T. schimperi* were determined using methods described by the manufacturer (Roche diagnostics, Germany) using ROCHE 9180 electrolyte analyzer. Diuretic index, diuretic activity, saluretic index and Na^+/K^+ were calculated in order to compare the effects of the test substances and hydrochlorthiazide on urine excretion.

Induction of hypertension in rats: Male Wistar rats were randomly divided into five groups (n = 6). Normal control rats (Group 1) received tap water and other groups (2-5) were given 2% NaCl and 10% sucrose solution *ad libitum* daily for six weeks. The salt-sucrose treated animals (Groups 3, 4 and 5) were also given orally 250 mg/kg/day aqueous extract of T. *schimperi*, 500 mg/kg/day aqueous extract of *T. schimperi* and 20 mg/kg/day of captopril, respectively for six weeks.

Blood pressure measurement: The BP was measured from the tail of rats using non-invasive BP monitoring apparatus (Model 179, IITC Inc, USA) before induction (basal BP) and weekly for six weeks. For testing, the animals were placed in the pre-warmed holder and appropriate cuff with sensor (photoelectric) was then mounted on their tails and warmed to about 32-35.4°C of tail temperature. When they were relaxed and became calm the tail cuff was inflated to a pressure well above the expected systolic blood pressure (SBP) (200 mmHg) and slowly released during which the pulse was recorded by the BP analyser. The SBP was read from the pulse tracings. Every time the measurements were made in triplicate and the average values were reported (Ayele *et al*., 2010).

At end 6th week, the blood was collected in vacutainer tube by cardiac puncture from night fasted cervical dislocated rats. The serum was separated after centrifugation at 3000 rpm for 10 min. The serum lipid profile (Total Cholesterol (TC), Glucose (BG), Triglycerides (TG)), liver function indicators (Alanine Transaminase (ALT), Alkaline Phosphatase (ALP)) were assayed using methods described by the manufacturer (Roche diagnostics, Germany) using COBAS-e-411 clinical chemistry analyzer instrument.

Acute oral toxicity: The aqueous extract of *T. schimperi* leaves was evaluated for possible toxic effect in female Wistar rats orally at a dose of 5 g/kg body weight according to OECD guidelines No. 425 (OECD, 2008). Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. The changes in skin, fur, eyes, respiratory and behavioral patterns were noted.

Statistical analysis: All data were expressed as mean+SEM. The statistical significance was evaluated by using SPSS version 16. The difference between the groups was compared using repeated measures of ANOVA. A value of $p<0.05$ was considered statistically significant.

RESULTS

Phytochemical screening: The leaf crude aqueous extract of *T. schimperi* and powdered leaves phytochemical screening by chemical methods (Table 1) showed the presence of phenols, tannins and saponins. Both the crude extract and powdered leaves, however, did not reveal the presence of alkaloids, phytosterols and glycosides.

Diuretic activity: Administration of *T. schimperi* aqueous extract (250, 500, 750 and 1000 mg/kg) showed significant ($p<0.05$) increase in urine volume compared to normal control. However, the diuretic effect was not linearly related to dose. The diuretic effect observed by the extract was less than that of the standard drug and highest diuretic index of the extract was observed at 500 mg/kg (Table 2). Essential oil, however, did not show significant diuresis ($p>0.05$) at either of the doses (1.0 mL/kg or 1.5 mL/kg).

Effect on urinary electrolyte: The effects of different doses of aqueous extract and essential oil of the leaves of *T. schimperi* on urinary electrolyte (Na^+, K^+ and Cl^-) excretion in rats (Table 3). The aqueous extract increased urinary Na^+, K^+ and Cl^- excretion in a dose dependent manner. The two higher doses of the

Table 1: Chemical tests on the crude extract and powdered plant of *T. schimperi* for the major classes of secondary metabolites

S. No	Metabolites tested for	Tested sample	
		Powdered plant	Crude aqueous extract
1	Alkaloids	-	-
2	Phenols	+	+
3	Tannins	+	+
4	Saponins	-	-
5	Glycosides	-	-
6	Phytosterols	-	-

Key: (+) = present (-) = absent

Table 2: Effect of Aqueous Extract (AE) and Essential Oil (EO) of the leaves of *Thymus Schimperi* (TS) on urine volume

Group	Cumulative urine volume (mL)	Diuretic index	Diuretic activity
Normal saline	0.42±0.26	1.0	-
TS250	1.50±0.44*	3.57	0.29
TS500	2.46±0.38*	5.86	0.48
TS750	1.83±0.49*	4.36	0.35
TS1000	1.63±0.19*	3.88	0.32
TS1	0.88±0.31	2.1	0.17
TS1.5	1.29±0.2	3.07	0.25

Results are expressed as mean±SEM, (n = 6); *p<0.05, **p<0.001 compared with control; Diuretic index = volume treated group/volume control group; Diuretic activity = volume treated group/volume standard group

Table 3: Effect of aqueous extract and essential oil of the leaves of *Thymus schimperi* (TS) on electrolyte excretion in normal rats (n = 6)

Groups	Urinary electrolyte concentration			Saluretic index			
	Na^+(mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)	Na^+	K^+	Cl^-	Na^+/K^+
Normal saline	73.5+10.5	83.12+10.05	92.5+8.5	-	-	-	0.88
HCTZ10	144.17+1.25*	62.63+4.89	106.33+3.34	1.55	0.75	1.15	1.82
TS250	120+3.96	83.13+10.05	138.8+9.45	1.63	1	1.50	0.86
TS500	125.5+12.45	131.82+8.99	158.17+8.55	1.7	1.58	1.71	0.95
TS750	155.67+9.96*	198.55+7.71**	162.67+10.11*	2.12	2.38	1.76	0.78
TS1000	199.5+13.71**	305.62+4.89**	278+10.75**	2.71	3.66	3	0.65
TS1	108.33+8.48	157.22+7.97*	113.17+7.76	1.47	1.88	1.22	0.69
TS1.5	107.75+5.41	177.01+8.74**	105.25+1.03	1.46	2.12	1.14	0.61

Results are expressed as mean±SEM, (n = 6); * = p<0.05, ** = p<0.001 compared with control group

Table 4: Concentration of K^+in different doses of T. schimperi aqueous extract

Dose of TS (mg/kg)	Conc. of K^+(mmol/L)
250	36.9
500	75.9
750	102.3
1000	146.0

aqueous extract significantly (p<0.05) elevated urinary excretion of Na^+, K^+ and Cl^- compared to the control. At high dose (1000 mg/kg) of extract the urinary excretion of Na^+ was significantly (p<0.01) greater than the standard. The urinary excretion of K^+ produced by the extract (500, 750 and 1000 mg/kg) and that of Cl^- (750 and 1000 mg/kg) was significantly (p<0.01) higher than that of the standard. The essential oil did not cause significant urinary Na^+ and Cl^- excretion at any of the doses though it increased urinary excretion of K^+ significantly (p<0.05) at both doses compared to the control.

The concentration of K^+ found in aqueous extract of *T. schimperi* leaves is high and increase in a dose dependent manner (Table 4).

Effect on serum enzymes and lipid profiles: ALP and ALT levels were not significantly (p>0.05) different in salt-sucrose group, *T. schimperi* (250 and 500 mg/kg) and standard compared to the control group. There was no significant (p>0.05) differences in TC, TG and HDL levels of salt-sucrose, *T. schimperi* (250 and 500 mg/kg) and standard group compared to control. However, the level of HDL was significantly (p<0.05) decreased in *T. schimperi* (250 and 500 mg/kg) compared to salt-sucrose group. The level of LDL is significantly (p<0.05) increased in *T. schimperi* (250 and 500 mg/kg) groups compared to control (Table 5).

Effects on blood pressure: The effect of aqueous extract of *T. schimperi* on SBP is shown in Fig. 1. After 6 weeks of treatment SBP was significantly (p<0.01) increased in salt-sucrose group compared to control. The increase in SBP was significantly (p<0.01) inhibited by higher dose of *T. schimperi* (500 mg/kg) and standard drug compared to salt-sucrose group. However, the increase in SBP was not significantly (p>0.05) inhibited by lower dose (250 mg/kg) of the extract compared to salt-sucrose group.

Acute toxicity study: The animals showed no clinical signs of toxicity and any behavioral change at oral limit

Table 5: Effects of *T. schimperi* aqueous extract on serum enzymes, lipid profile and body weight

		Treatment			
Parameters	Control	Salt-sucrose	TS250	TS500	Captopril
BW (%)	12.07±1.99*	-15.965±1.68	-19.98±1.68*	-21.02±1.92*	-26.55±3.06*
ALP (U/L)	277.3+15.21	264.2+13.05	261.5+19.29	267.67+19.07	220.2+16.4
ALT (U/L)	88.27+9.17	70.68+10.03	65.30+13.85	81.75+12.56	63.11+9.94
TC (mg/dL)	112.2+9.59	105.75+8.72	132+8.6	128.42+16.06	143.67+12.37
TG (mg/dL)	121.32+14.95	84.93+6.54	122.53+15.3	134.07+19.85	139.8+10.33
HDL (mg/dL)	28.32+1.46	34.5+1.73	26.38+1.26***	26.98+2.19***	33.77+0.99
LDL (mg/dL)	9.89+0.25	19.53+1.84	22.53+1.78*	24.2+4.82**	20.4+1.51
BG (mg/dL)	107.93+5.55	129.3+4.93*	112.87+3.07	101.77+6.28	116.12+4.96

Results are expressed as mean±SEM, (n = 6); * = p<0.05; ** = p<0.01compared to control (tap water); *** = p<0.05 compared to salt-sucrose group

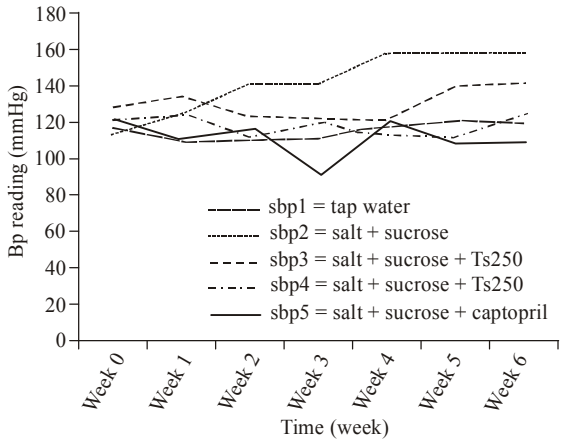

Fig. 1: Effect of *Thymus schimperi* aqueous extract on systolic blood pressure against salt-sucrose induced hypertension in rats (n = 6)

dose of 5 g/kg and all survived during the observation period.

DISCUSSION

Diuretics are drugs that act on the kidney and are able to increase the volume of urine excreted and that is why they are used in cardiac failure, acute oedema of the lung, nephritic oedema syndrome, arterial hypertension and diseases related with the retention of fluids (Devi, 2011). These effects may be produced by stimulation of regional blood flow or initial vasodilation, or by producing inhibition of tubular reabsorption of water and ions (Patel *et al*., 2012).

Hydrochlorthiazide was used as a standard in this diuretic study because it can increase the urinary flow rate along with a strongly increased Na^+ and K^+ excretion. It is also one of the most clinically used diuretics for treatment of patients with mild or moderate hypertension and normal renal and cardiac function and other ailments. Captopril, angiotensin converting enzyme inhibitor, was used as a standard drug in this antihypertensive study because it is a first line antihypertensive drug (Katzung *et al*., 2012).

There are several phytochemicals such as phenols, saponins and other polar compounds which are responsible for diuretic effects of plants. The significant diuretic effect of the aqueous extract of *T. schimperi* observed at all dose levels in the present investigation might be attributed to the presence of one or more water soluble active components in the extract. Phytochemical screening of *T. schimperi* leaves has confirmed the presence of tannins, saponins and phenols.

The diuretic effect of the extract was not dose dependent which might be explained in terms of interaction of co-extracted substance (s) of the extract which might get high enough concentration to interfere with the diuretic activity of responsible component (s) at higher doses of the extract. The essential oil of the plant did not produce diuresis perhaps due to absence of active component (s) responsible for diuresis.

The diuretic effect of aqueous extracts of *T. schimperi* was accompanied with increased urinary Na^+, K^+ and Cl^- excretion. The concentration of these electrolytes increased as the doses of the extract increased and significant excretion was observed at higher doses which might be due to an increase in the concentration of active components of the extract at these higher doses.

The increase in the ratio of concentration of excreted $Na^+$and K^+ indicates that the extract increases Na^+ excretion to a greater extent than K^+, which is a very essential quality of a good diuretic with lesser hypokalaemic side effect (Sweazea *et al*., 2010). However, the ratio of concentration of excreted $Na^+$and K^+ in this study for both aqueous and essential oil extracts were not increased as the dose of the extract increases. This implies the extract causes kaliuretic effect and hypokalimic complications.

Renal K^+ excretion is affected by distal Na^+ delivery, the renin angiotensin aldosterone system, vasopressin, dietary K^+ intake, acid-base status, urine flow and serum K^+ concentration. In this study the excessive excretion of K^+ (kaliuretic effect) of the extract might be due to high concentration of the K^+ in the extract or renal effects of the components of the extract.

Many commonly ingested nutrients or dietary elements (fatty acids and sugars) known to augment insulin resistance are also associated with elevated BP.

Ingestion of sugars (sucrose, fructose, glucose) by various rat strains is associated with perturbations in the glucose/insulin system and higher systolic blood pressure.

Feeding rats a high sucrose diet (34.5%) has been found to elicit hypertriglyceridemia, impaired glucose tolerance, hyperleptinemia, enhanced oxidative stress and impaired acetylcholine-mediated vasodilation, which are characteristics of metabolic syndrome and pre-diabetes. Insulin resistance, dyslipidemia and hypertension lead to type 2 diabetes and its associated cardiovascular complications, collectively known as metabolic syndrome (Claudea *et al*., 2013).

About 5% sucrose administration for six weeks in rats was found to induce significant increase in systolic arterial pressure and cause significant increases in lipid peroxidation and reduce superoxide dismutase and glutathione levels in the heart, liver and kidney (Johnson *et al*., 2007).

It has been demonstrated that hypertension develops when normal rats are fed a fructose-enriched diet as early as two weeks after initiation of the diet (Bharti *et al*., 2012). Chronic fructose feeding leads to insulin resistance, glucose intolerance, hyperinsulinemia, hyperglycemia and hypertri glycerdemia in a relatively short time in normal rats. This metabolic change leads to essential hypertension. It also favors hepatic lipogenesis and is responsible for the development of nonalcoholic fatty liver disease along with cardiovascular complications (Fariba *et al*., 2013).

Artificial sweeteners (sucralose and aspartame) provide the sweetness of sugar without the calories. They reduce BW; do not increase appetite, sensation of hunger, or food intake (Whitehouse *et al*., 2008). In this study rats BW and food intake were reduced in all salt-sugar treated groups which might be due to limited calories in the sugar used and the sugar used might be sweetened low calorie sugar. High salt-sucrose feeding appears to reduce HDL levels and increase the fraction of LDL, both of which may impact adversely on vascular diseases (Thierry *et al*., 2012).

In this study, salt-sucrose feeding does not cause significant changes in TC, TG, HDL and LDL compared to the control. However, there was a significant decrease in LDL and increase in HDL in groups given *T. schimperi* compared to salt-sucrose group and compared to control group, respectively. This might be because of limited fructose content of the used sugar and the extract might facilitate the breakdown of the stored lipid.

Damage to tissue can release different types of enzymes based on their location. For example, mild inflammation of the liver reversibly increases the permeability of the cell membrane and releases cytoplasmic enzymes such as Lactate Dehydrogenase (LD), ALP and Aspartate Transaminase (AST), while necrosis will release mitochondrial sources of ALT as well as Aspartate Transaminase (AST) (Arneson and Brickell, 2007). However, in this study serum ALP and ALT levels were not significantly different in all groups.

Serum glucose level of salt-sucrose feeding rats was significantly increased compared to control group. However, there was no significant differences observed in serum glucose level of *T. schimperi* and captopril treated groups compared to salt-sucrose groups. This might be due to lack of glucose lowering activity of the extract.

Na^+ and K^+ have been implicated in the etiology of hypertension (Geleijnse *et al*., 2003). The consequences of an excess of Na^+ and K^+ deficit in the body could be largely responsible for the hypertension and associated tissue injury.

Physiologically, normal kidney has the ability to excrete easily the daily salt load without allowing a marked rise in extracellular volume. Excess salt intake produces hypertension in rats, which mimics human hypertension. High salt intake hypertension has been produced in rats, rabbits and chicks by replacing drinking water with 1-2% NaCl for 9-12 months (Kaur *et al*., 2011).

Addition of high salt to high sucrose causes marked elevation of BP in wistar rats. High sucrose intake appears also to elevate BP only with normal or high dietary salt intake (Thierry *et al*., 2012). In this study the rise in SBP in salt-sugar treated groups compared to water treated groups might be due to salt.

A high-potassium diet and increases in serum K^+, even within the physiologic range, cause endothelium-dependent vasodilatation by hyperpolarizing the endothelial cell through stimulation of the Na^+ pump and opening K^+ channels. Endothelial hyperpolarization is transmitted to the vascular smooth-muscle cells, resulting in decreased cytosolic Ca^{2+}, which in turn promotes vasodilation (Adrogue and Madias, 2000).

High K^+ may reduce the risk of stroke; lowers BP; inhibits free radical formation, vascular smooth muscle proliferation and arterial thrombosis. It has also been shown experimentally that K^+ may reduce macrophage adherence to the vascular wall (an important factor in the development of arterial lesions, oxidative stress of the endothelium, or vascular eicosanoid production) (Cohn *et al*., 2000). In this study leaves extract of *T.schimperi* contains higher concentration of K^+ which might be responsible for antihypertensive and diuretic activity of the extract.

CONCLUSION

The aqueous extract of the dried leaves of *T. schimperi* has diuretic activity while the essential oil did not produce diuresis at the dose employed. The

aqueous extract of dried leaves *T. schimperi* was found to possess inhibitory activity in salt-sucrose raise in systolic blood pressure.

Further studies are necessary to be performed for the antihypertensive activity of the extract against other hypertension models and effect of the extract on liver. Purification, isolation and characterization of the phytoconstituents responsible for the antihypertensive and diuretic effect and exploring the exact mechanism of action are needed.

Conflict of interest: The authors declare that they have no conflict of interest.

AKNOWLEDGEMENT

The authors are grateful for financial support of this study which was provided by Ministry of Finance and Economic Development (project number OBN6.34/2006) through Ethiopian Public Health Institute and the School of Graduate Studies, Addis Ababa University. We also like to appreciate technical assistance of Mr. Abiy Abebe during laboratory work. Staffs of the Traditional and Modern Medicine Research Directorate and Finance, plan and monitoring sincerely appreciated for their direct and indirect contribution during the study.

REFERENCES

Adrogue H.J. and N.E. Madias, 2000. Mechanisms of disease: Sodium and potassium in the pathogenesis of hypertension. N. Engl. J. Med., 356: 1966-1978. http://www.nejm.org/doi/full/10.1056/NEJMra064486.

Arneson, W. and J. Brickell, 2007. Clinical Chemistry: A Laboratory Perspective. F.A. Davis Company, Philadelphia. http://www.worldcat.org/title/clinical-chemistry -a -laboratory-perspective/oclc/162150117.

Asfaw, N., H.J Storesund., L. Skattebol, F. Tonnesen and A.J. Aasen, 2000. Volatile oil constituents of two thymus species from Ethiopia. Flavour. Frag. J., 15(2): 123-125. http://onlinelibrary.wiley.com/doi/10.1002/(SICI)1099-1026(200003/04) 15: 2%3C123::AID-FFJ879%3E3.0.CO;2-8/abstract.

Ayele, Y., K. Urga and E. Engidawork, 2010. Evaluation of *in vivo* antihypertensive and i*n vitro* vasodepressor activities of the leaf extract of *Syzygium guineense* (Willd) D.C. Phytother. Res., 24(10): 1457-1462. http://onlinelibrary.wiley.com/doi/10.1002/ptr.3141/abstract.

Bharti, D., P. Jagtap, V. Undale and A. Bhosale, 2012. Aerial parts of aqueous extract of *Cynodon dactylon* shows hypotensive effect in high fructose treated Wistar rats. Int. J. Res. Pharm. Biomed. Sci., 3(2): 585-591. http://www.ijrpbsonline.com/files/21-3225.pdf.

Claudea, B.D., A.M.Y. Désiréa, D.D.P. Désiréa, A.A.G. Blaiseb, K. Pierrea and D. Theophile, 2013. Methanol extract of *Allanblackia gabonensis* (Guttiferacae) prevents hypertension and oxidative stress induced by chronic sucrose consumption in rat. Pharmacologyonline, 1: 194-200. http://pharmacologyonline.silae.it/files/archives/2013/vol1/PhOL_2013_1_A027_006_Dimo.pdf.

Cohn, J.N., P.R. Kowey, P.K. Whelton and L.M. Prisant, 2000. New guidelines for potassium replacement in clinical practice: A contemporary review by the National Council on Potassium in Clinical Practice. Arch. Intern. Med., 160(16): 2429-2436. http://www.ncbi.nlm.nih.gov/pubmed/10979053.

Dagne, E., S. Hailu, D. Bisrat and T. Worku, 1998. Constituents of the essential oil of *Thymus schimperi*. Bull. Chem. Soc. Ethiop., 12(1): 79-82. http://www.ajol.info/index.php/bcse/article/view/21037/18809.

Devi, M.S.S., 2011. Acute toxicity and diuretic activity of *Mangifera indica* L. bark extracts. Int. J. Pharm. Biol. Sci., 2(3): 141-146. http://ijpbs.net/vol-2_issue-3/pharma_science/19.pdf.

Fariba, B., S. Hassan, G. Shahin and R. Yavar, 2013. Effects of dietary fructose, sucrose and lactose in induction of nonalcoholic fatty liver in rat. World Appl. Sci. J., 22(3): 368-373. http://connection.ebscohost.com/c/articles/90449984/effects-dietary-fructose-sucrose-lactose-induction-nonalcoholic-fatty-liver-rat.

Foex, P. and J.W. Sear, 2004. Hypertension: Pathophysiology and treatment. CEACCP, 4(3): 71-75. http://ceaccp.oxfordjournals.org/content/4/3/71.abstract.

Gavras, H., 2009. Pathogenesis of hypertension: A review. J. Med. Sci., 2(1): 25-28. http://hamdanjournal.org/journal/index.php?journal=HAMDAN&page=article&op=view&path%5B%5D=51.

Geleijnse, J.M., F.J. Kok and D.E. Grobbee, 2003. Blood pressure response to changes in sodium and potassium intake: A metaregression analysis of randomised trials. J. Hum. Hypertens., 17(7): 471-480. http://www.ncbi.nlm.nih.gov/pubmed/12821954.

Geleta, B., M. Eyasu, S. Kebamo, A. Debella, E. Makonnen and A. Abebe, 2015. *In vitro* vasodilatory effect of aqueous leaf extract of *Thymus serrulatus* on thoracic aorta of Guinea pigs. Asian Pac. J. Trop. Biomed., 5(1): 15-18. http://www.sciencedirect.com/science/article/pii/S2221169115301647.

Johnson, R.J., M.S. Segal, Y. Sautin, T. Nakagawa, D.I. Feig, D.H. Kang, M.S. Gersch, S. Benner and L.G. Sánchez-Lozada, 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am. J. Clin. Nutr., 86(4): 899-906. http://www.ncbi.nlm.nih.gov/pubmed/17921363.

Katzung, B.G., H.E. Ives, S.B. Masters and A.J. Trevor, 2012. Diuretic Agents. 12th Edn., In: Basic and Clinical Pharmacology. Lange Medical Books/ McGraw-Hill, NY.

Kaur, M., A.C. Rana and S. Kumar, 2011. Induction of hypertension by various animal models. Int. J. Pharm. Biol. Sci., 1(3): 335-340. http://www.ijpbs.com/ijpbsadmin/upload/ijpbs_50c84594acfa5.pdf.

Kensara, O.A., N.A. ElSawy, A.G. El-Shemi and E.A. Header, 2013. *Thymus vulgaris* supplementation attenuates blood pressure and aorta damage in hypertensive rats. J. Med. Plants Res., 7(11): 669-676. https://www.researchgate.net/publication/235966975_Thymus_vulgaris_supplementation_attenuates_blood_pressure_and_aorta_damage_in_hypertensive_rats.

Kumar, B.P.S., K. Jyothi, N. Sravya, Rajveersingh, M.K. Swamy and S. Mathew, 2012. Evaluation of risk factors in hypertension patients visiting to rural tertiary care teaching hospital. J. Pharm. Sci., 2(2): 57-61. http://journaldatabase.info/articles/evaluation risk factors_hypertension.html.

López-Jaramillo, P., R.A. Sánchez, M. Diaz, L. Cobos, A. Bryce, J.Z. Parra Carrillo, F. Lizcano, F. Lanas, I. Sinay, I.D. Sierra, E. Peñaherrera, M. Bendersky, H. Schmid, R. Botero, M. Urina, J. Lara, M.C. Foss, G. Márquez, S. Harrap, A.J. Ramírez and A. Zanchetti, 2013. Latin American consensus on hypertension in patients with diabetes type 2 and metabolic syndrome. J. Hypertens., 31(2): 223-238. http://www.ncbi.nlm.nih.gov/pubmed/23282894.

Mengistu, M., Y. Abebe, Y. Mekonnen and T. Tolessa, 2012. *In vivo* and *in vitro* hypotensive effect of aqueous extract of *Moringa stenopetala*. Afr. J. Health Sci., 12: 545-551. http://www.ncbi.nlm.nih.gov/pubmed/23515422.

Mihailovic-Stanojevic, N., A. Belščak-Cvitanović, J. Grujić-Milanović, M. Ivanov, D. Jovović, D. Bugarski and Z. Miloradović, 2013. Antioxidant and antihypertensive activity of extract from *Thymus serpyllum* L. in experimental hypertension. Plant Foods Hum. Nutr., 68(3): 235-240. http://www.ncbi.nlm.nih.gov/pubmed/23828496.

National Research Council, 2011. Guide for the Care and Use of Laboratory Animals. 8th Edn., National Academic Press, Washington, D.C. https://grants.nih. gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf.

OECD, 2008. OECD Guideline for the Testing of Chemicals. Acute Oral Toxicity: Up-and-Down Procedure. Test No. 425 (1st Adoption), Paris, France. http://www. keepeek.com/Digital-Asset-Management/oecd/environment/test-no-425-acute-oral-toxicity-up-and-down-procedure_97892 6407 1049-en#page1.

Patel, P., J. Vaghasiya, A. Thakor and J. Jariwala, 2012. Antihypertensive effect of rhizome part of *Acorus calamus* on renal artery occlusion induced hypertension in rats. Asian Pac. J. Trop. Dis., 2(1): S6-S10. http://www.sciencedirect.com/science/article/pii/S2222180812601145.

Sweazea, K.L., M. Lekic and B.R. Walker, 2010. Comparison of mechanisms involved in impaired vascular reactivity between high sucrose and high fat diets in rats. Nutr. Metab., 7: 48. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2887873/.

Tashko, G. and R.A. Gabbay, 2010. Evidence-based approach for managing hypertension in type 2 diabetes. Integr. Blood Press. Control, 3: 31-43. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3172068/.

Thierry, B.N.M., N.L.T. Esther, A.O.B. Farouck, T.D. Emery and D. Theophile, 2012. Aqueous extract of *Tetrapleura tetraptera* (Mimosaceae) prevents hypertension, dyslipidemia and oxidative stress in high salt-sucrose induced hypertensive rats. Pharmacologia, 3(9): 397-405. https://www.researchgate.net/publication/236308211_Aqueous_Extract_of_Tetrapleura_tetraptera_Mimosaceae_Prevents_Hypertension_Dyslipidemia_and_Oxidative_Stress_in_High_Salt-sucrose_Induced_ Hypertensive_Rats.

Trease, G.E. and W.C. Evans, 1989. Pharmacognosy. 13th Edn., Bailliere Tindall, London.

Vasant, O.K., B.G. Vijay, S.R. Virbhadrappa, N.T. Dilip, M.V. Ramahari and B.S. Laxamanrao, 2012. Antihypertensive and diuretic effects of the aqueous extract of *Colocasia esculenta* Linn. leaves in experimental paradigms. Iran J. Pharm. Res., 11(2): 621-634. http://www.ncbi.nlm.nih.gov/pubmed/24250487.

Vogel, H.G., W.H. Vogel, B.A. Scholkens, J. Sandow, G. Muller and W.F. Vogel, 2008. Drug Discovery and Evaluation: Pharmacological Assays. 2nd Edn., Johann Wolfgang Goethe Universität, Frankfurt am Main, Germany.

Whitehouse, C.R., J. Boullata and L.A. McCauley, 2008. The potential toxicity of artificial sweeteners. AAOHN J., 56(6): 251-259.