Evaluation of Antihypertensive, Hypotensive and Antihyperlipidemic Activities of Aqueous Crude Extract of *Thymus serrulatus* Leaves in Rats

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This is to testify that the thesis prepared by Hiwot Getachew which is entitled with "*Evaluation of Antihypertensive, Hypotensive and Antihyperlipidemic Activities of Aqueous Crude Extract of Thymus serrulatus Leaves in Rats*" And submitted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Abbreviations & Acronyms

ACEI	Angiotensin Converting Enzyme Inhibitors
AGES	Advanced Glycation End Products
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
Ang II	Angiotensin II
ANOVA	Analysis of Variance
AQ	Aqueous
AST	Aspartate Aminotransferase
BGL	Blood Glucose Level
BP	Blood Pressure
BUN	Blood Urea Nitrogen
COX2	Cyclooxygenase-2
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DALY	Disability Adjusted Life Years
DBP	Diastolic Blood Pressure
ENOS	Endothelial Nitric Oxide Synthase
ET-1	Endothelin-1
EtAc	Ethyl Acetate
GGT	Gamma-Glutamyl Transferase
ICAM-1	Inter- Cellular Adhesion Molecule 1
L-NAME	NG-Nitro-L-Arginine Methyl Ester
MAP	Mean Arterial Pressure
MG	Methylglyoxal
MMP2	Matrix Metalloproteinase- 2
NADPH-OX	Nicotinamide Adenine Dinucleotide Phosphate Oxidase;
NCDs	Non-Communicable Diseases
NHE3	Sodium-Hydrogen Exchanger 3
NO	Nitric Oxide
OECD	Organization for Economic Cooperation and Development

PAT1	Putative Anion Transporter 1
ROS	Reactive Oxygen Species
SBP	Systolic Blood Pressure
SSA	Sub Saharan Africa
TC	Total Cholesterol
TCAM	Traditional Medicine/Complementary and Alternative Medicine
TG	Triglyceride
TXA2	Thromboxane A2
UA	Uric Acid
WHO	World Health Organization

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ABSTRACT

Evaluation of Antihypertensive, Hypotensive and Antihyperlipidemic Activities of Aqueous Crude Extract of *Thymus serrulatus* Leaves in Rats

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In Ethiopia, the genus Thymus is represented by two indigenous species namely *Thymus serrulatus* and *Thymus schimperi* both of which are locally named as Tosign. Among numerous medicinal uses of *T. serrulatus* leaves treatments of renal diseases, liver diseases, hypertension and *Tinea capiti* are the most common. The main aim of the present study was to evaluate antihypertensive, hypotensive, hypolipidemic and antihyperlipidemic effects of the aqueous crude extract of *Thymus serrulatus* leaves and AQ residue against normotensive and fructose induced hypertensive rats. The blood pressure was measured from the tail of rats using non-invasive BP monitoring apparatus (Model 179, IITC Inc, U.S.A). Aqueous crude extract of *Thymus serrulatus* leaf and the AQ residue caused significant fall in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MABP) at doses of 250, 500 and 1000 mg/kg; in fructose induced hypertensive rats, where the highest reduction were seen by aqueous crude extract. The aqueous crude extract showed reduction of blood glucose and lipid profiles (Total cholesterol and Triglyceride) in a dose dependent manner. Aqueous residue at a dose of 1000 mg/kg showed comparable effect on alanine aminotransferase (ALT) compared with the Captopril treated groups and it also showed significant reduction compared to fructose induced hypertensive groups ($p<0.05$). The other liver parameter results were seen to have neither dose dependent pattern nor statistically significant difference with captopril treated groups and negative control ($p> 0.05$). The level of both urea and creatinine decreased dose dependently for all extracts; the higher doses of aqueous residue showed significant reduction compared to the negative control ($p<0.01$). The study findings indicate that *Thymus serrulatus* possesses a significant anti-hypertensive, anti-hyperlipidemic effects and kidney functions improvement; which may help in treatment of hypertension and its complication.

Key words: Hypertension, *Thymus serrulatus*, Antihypertensive effect, Antihyperlipidemic effect

1. INTRODUCTION

1.1 Overview of Hypertension

Non-communicable diseases (NCDs), such as cardiovascular diseases, cancers, diabetes, and chronic respiratory diseases, are now the leading cause of death in most regions of the world A cardiovascular disease (CVD) remains the leading cause of morbidity and mortality in the world (Naik & Kaneda, 2015).

CVD are a group of disorders with an abnormal functioning of the heart or blood vessels. CVDs include diseases of the heart, vascular diseases of the brain and diseases of blood vessels (WHO, 2011; Mendis S, *et al*., 2011; WHF, 2012). Risk factors associated with CVD include diabetes, inflammation, hypercholesterolemia, dementia, atherosclerosis and hypertension. Hypertension, in particular, is a major contributor to the pathogenesis of CVD and its associated mortality (Shouk, *et al*., 2014).

Hypertension is a hemodynamic disorder, associated with a rise in peripheral vascular resistance. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure(JNC7) classification in adults (age ≥18 years) is based on the average of two or more properly measured blood pressure (BP) readings from two or more clinical encounters. It includes four categories: normal, prehypertension, stage 1 hypertension and stage 2 hypertension.

Essential hypertension is characterized by a sustained systolic blood pressure (SBP) of greater than 140 mm Hg and a diastolic blood pressure (DBP) of greater than 90 mm Hg (Vikrant, *et al*., 2001; Joseph T., *et al*., 2008; Schellack, *et al*., 2016). The risk associated with increasing blood pressure is continuous, with 2mmHg rise in SBP associated with a 7% increased risk of mortality from ischaemic heart disease and a 10% increased risk of mortality from stroke (NICE clinical guideline 127, 2011).

The World Health Organization (WHO) estimates that by 2025, approximately 1.6 billion adults will suffer from hypertension and associated CVD complications (Shouk *et al*., 2014). Twice as many deaths from CVD now occur in developing countries as in developed countries (WHO, 2005). Africa, home to 54 low- and middle-income countries, is expected to have the world‟s largest increase in NCD deaths over the next decade. This will impose a significant burden to the

continent, which is also projected to see its population double within the next generation (Naik & Kaneda, 2015).

Cardiovascular diseases affect younger populations and lead to premature mortality in developing countries due to lack of prevention or effective management of CVD risk factors. Although the effect of risk factors on CVD in Africa is similar to that in other populations, the risk of CVD morbidity and mortality associated with hypertension may even be higher in Africans (Yusuf *et al*., 2004).

A report that presents updated estimates for the prevalence and control of hypertension in the United States for 2011–2014 shows the overall prevalence of hypertension among adults in the United States was 29.0% and was similar among men (30.0%) and women (28.1%) (Sug, *et al*., 2015). Hypertension is reported to be the fourth contributor to premature mortality in developed countries and the seventh in developing countries. Almost 12.8% (7.5 million) of the total deaths and around 3.7% of the total disability adjusted life years are due to raised BP (Jayashankar & Sankhla, 2015).

A review that was done in 2013 on the epidemiology, pathophysiology, treatment, complications, control and outcome of hypertension in the Sub-Saharan Africa (SSA) region estimated overall prevalence of hypertension in SSA for 2008 at 16.2% (95% CI 14.2% to 20.3%). The estimated number of hypertensive individuals in the region was put at 74.7 million. The calculated number of hypertensive individuals living in SSA by 2025 was estimated at 125.5 million, which corresponds to an increase of about 68% from 2008 to 2025. The study suggested the prevalence of hypertension in SSA will rise from 16.2% to 17.4% (Ogah & Rayner, 2013).

The review assessed 38 studies that were done in different parts of SSA and the lowest national estimated prevalence was 10.3% in Ethiopia and the highest was 23.0% in Ghana. The low prevalence of hypertension in Ethiopia may be due to the diet, which is low in sodium but high in potassium (Ogah & Rayner, 2013). Despite widespread skepticism about the public health importance of chronic disease in the country, the available data, although scanty, suggest that about one in four lives or DALYs in rural Ethiopia may be lost due to chronic diseases. This proportion gets even bigger when looking at the causes of DALYs lost among adults or in urban populations separately (Tesfaye, 2008).

1.2 Pathophysiology of Hypertension

Essentially, BP is the outcome of cardiac output and peripheral vascular resistance (BP=cardiac output × peripheral vascular resistance). Therefore, maintenance of a normal BP is dependent on the balance between the cardiac output and peripheral vascular resistance (Lakshmi, *et al*., 2011).

Many pathophysiologic factors have been implicated in the genesis of essential hypertension like: increased sympathetic nervous system activity; overproduction of sodium- retaining hormones and vasoconstrictors; long-term high sodium intake; inadequate dietary intake of potassium and calcium; increased or inappropriate renin secretion with resultant increased production of angiotensin II and aldosterone; deficiencies of vasodilators, such as prostacyclin, nitric oxide (NO), and the natriuretic peptides; diabetes mellitus; insulin resistance; obesity; increased activity of vascular growth factors; alterations in adrenergic receptors that influence heart rate, inotropic properties of the heart, and vascular tone; and altered cellular ion transport (S.Vikrant, *et al*., 2001; Oparil, *et al*., 2003).

1.3 Hypertension Management

A healthy lifestyle remains the cornerstone of managing hypertension, regardless of the BP level. In addition to decreasing BP, lifestyle changes may enhance antihypertensive drug effectiveness and decrease total CVD risk. It is estimated that dietary and exercise interventions may reduce BP by at least 10 mmHg in approximately one in four people with high BP (Smith, 2014).

Effective pharmacologic lowering of blood pressure has been shown to prevent damage of blood vessels and to substantially reduce morbidity and mortality rates. Unfortunately, several surveys indicate that only one third to one half of Americans with hypertension have adequate blood pressure control. Many effective drugs are available. Knowledge of their antihypertensive mechanisms and sites of action allows accurate prediction of efficacy and toxicity *(Katzung, et al*., 2012).

Antihypertensive drugs are the common approach employed to treat essential hypertension and are designed to control blood pressure with minimal long-term toxicity. These drugs include diuretics, angiotensin converting enzyme (ACE) inhibitors, vasodilators, as well as blockers of β-adrenoreceptors, calcium channels and α-adrenoreceptors. However, these drugs are not

without undesirable side effects, and they cannot be used to prevent the onset of hypertension (Joseph T. *et al*., 2008; Shouk *et al*., 2014). Due to the global impact of hypertension, investigation into new therapeutic alternatives, including the use of natural substances for drug development, merit discovery, evaluation and global distribution, especially to patient populations in developing countries is necessary (Simões *et al*., 2016).

Plant medicine has made many contributions to commercial drug preparations manufactured today. It has been losing ground to new synthetic medicines touted by scientists and physicians to be more effective and reliable. Traditional medicine encompasses knowledge systems that have been established over generations based on the concepts, beliefs and practices native to diverse cultures prior to the modern medicine era (Rawat, *et al*., 2016). Fortunately, the importance of phytotherapy has been established in pharmacological and clinical studies for the primarily healthcare and as a recognized tool for modern medicine (Simões *et al*., 2016).

In some Asian and African countries, about 80% of the populations depend on the indigenous traditional remedies for their primary health care needs which mainly involve the crude plant-preparations. The low living standard and the relatively high cost of hypertension treatment make access to medical care difficult. Thus, a significant proportion of hypertensive patients used medicinal plants for their treatment (Lawson, *et. al*., 2016).

In the recent past, there has been a growing interest in Traditional medicine/Complementary and Alternative Medicine (TCAM) and their relevance to public health both in developed and developing countries. Diversity, flexibility, easy accessibility, broad continuing acceptance in developing countries and increasing popularity in developed countries, relative low cost, low levels of technological input, relative low side effects and growing economic importance are some of the positive features of traditional medicine (Yadav, 2013).

Medicinal plants are more easily accessible, more affordable and useful for both prevention and treatment of hypertension. These facts, along with accumulated conventional wisdom from folk medicine, have led to an increasing interest in botanicals for their potential antihypertensive benefits. Many drugs with antihypertensive effects were originally derived from plants. Examples include aspirin (from *Salix alba*), reserpine (from *Rauwolfia serpentia*), digitoxin

(from *Digitalis purpurea*), and tetrandine (from *Stephenia tetradra*) (Shouk *et al*., 2014).

There are different antihypertensive medicinal plants that different researchers studied about in the recent past few years. *Reta maraetam* Forssk (Maghrani, 2007), *spicigera* (Esquivel-Gutiérrez *et al*., 2013), *Eclipta alba* (Mishra & Pal, 2013), *Thymus serrulatus* (Geleta *et al*., 2015), *Hancornia speciosa* (Silva, *et al*., 2016), *Thymus schimperi* (Haji *et al*., 2016), *Gmelina arborea*(Lawson *et al*., 2016) are some of them.

Traditionally used medicinal plants like *Allium sativum, Centella asiatica, Ginkgo biloba, Crataegus oxycantha, Crataegus monogyna, Passiflora edulis, Hibiscus sabdariffa, Elaeocarpus ganitrus, Hypericum perforatum,* and *Achillea millefolium* are used in treatment of hypertension (Lakshmi *et al*., 2011).

Though traditional medicines are considered safe, optimization of their appropriate doses is worth considering, as inappropriate use of plant medicines may have adverse effects. Studies to establish the safety and effectiveness of traditionally used medicinal plants should be carried out.

1.4 Overview of the Experimental Plant

The Lamiaceae/Labiatae is, a large plant family represented by about 236 genera and above 7200 species. The genus Thymus, under family lamiaceae, is noteworthy for its numerous species and varieties. The genus Thymus includes about 249 species worldwide and is distributed mainly in temperate Eurasia. It is found in Ethiopia and Eritrea but seems to be absent in tropical Africa (Damtie & Mekonnen, 2016). The Thymus species in Ethiopia and Eritrea are restricted to afromontane and afroalpine region. In Ethiopia, this genus is represented by two indigenous species namely *T. serrulatus* and *T. schimperi* both of which are locally named as Tosign (Amharic) and Tesni/Thasne (Tigrigna) (Asfaw, *et al*., 1999; Damtie & Mekonnen, 2016).

They grow on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes. Both species are perennial herbs, woody at the base and are 5-40 cm high (Dagne, *et al*., 1998; Geleta, *et al*., 2015). Among numerous plants used traditionally for medicinal purpose *T. serrulatus* is the most common one in Ethiopia (figure 1). Its leaf is used for treatment of renal diseases, liver diseases, hypertension, and *Tinea capitis* (Debelo *et al*., 2015).

Figure 1 Photograph of *Thymus serrulatus* plant

1.5 Induction of Hypertension in Rats

There are different models of inducing hypertension, like; Renovascular hypertension, Dietary hypertension, Endocrine hypertension, neurogenic hypertension, psychogenic hypertension, Genetic hypertension and other models.

Increased dietary carbohydrate intake can raise blood pressure in experimental animals. The increased intake of either sucrose or glucose was shown to enhance the development of either spontaneous hypertension or salt hypertension, in rats and it is reported that hypertension could be induced in normal rats by feeding a high-fructose diet. Fructose feeding was also found to cause insulin resistance, hyperinsulinemia, and hypertriglyceridemia in normal rats (Badyal, *et al*., 2003; Rana, *et al*., 2011; Jadhav, *et al*., 2014).

Studies to date show that the mechanisms by which excess fructose increases BP categories into three broad mechanisms. The first mechanism is through increment of salt absorption, the second mechanism is because of endothelial dysfunction which intern result alter the synthesis of

nitric oxide, angiotensin II, uric acid, thromboxane A2 and endothelin I and the third mechanism is by overstimulation of the sympathetic nervous system (Klein & Kiat, 2015).

1.6 Ethnobotanical Studies on Different Herbs with Antihypertensive Effect

Seven days of continuous administration of Ethanolic Extract of *Eclipta Alba* (EEEA) to fructose induced hypertensive rats exhibited antihypertensive effect in the form of a significant lowering in systolic, diastolic and mean arterial pressure (MAP). And 21 days of continuous administration of the plant to the hypertensive rats revealed a decrease in systolic, diastolic, mean arterial pressure and heart rate in a dose dependent manner which was highly significant with 200 & 400 mg/kg doses (Mishra & Pal, 2013).

Another study revealed that daily administration of aqueous extract of *Reta maraetam* Forssk (RR) (20 mg/kg) to both normotensive and spontaneously hypertensive rats for 3 weeks exhibited significant reduction in blood pressure on the 7^{th} day; significant reduction in systolic blood pressure compared to baseline starting day 7 (197.5 ± 4.03 mmHg, $P < 0.01$) and continue to fall through day 21 (171 ± 4.03 mmHg, $P< 0.001$) (Maghrani, 2007).

Four weeks NG-Nitro-L-Arginine Methyl Ester (L-NAME) induced hypertensive rats showed a significant blood pressure reduction (204 mmHg to 160 mmHg) when olive leaf extracts (*Oleaeuropaea*), was administered at a dose of 100mg/kg. Oral administration of the extract at different dose levels at the same time as L-NAME for a period of 8 weeks showed a dose dependent prophylactic effect against the rise in blood pressure induced by L- NAME (Okpanyi & Kreuter, 2002).

Another study by Esquivel-Gutiérrez et al. (2013) reported the antihypertensive activity of the chloroform extract of *Justicia spicigera* in L-NAME treated rats by lowering the blood pressure from 180/164± 1.7/3.2 mm Hg to 149/133 ± 4.0/3.7mmHg attributed to the flavonoids present in the extract (Esquivel-Gutiérrez *et al*., 2013).

A randomized double blind controlled trial showed that *A. graveolens* (as a formulation Tensigard® comprising of *A. graveolens* and *Orthosiphonstamineus* Benth) reduced the blood pressure significantly (Supari, 2002). The vasorelaxant activity of *A. graveolens* was also observed in an *ex vivo* study conducted on pre-contracted rat aortic rings with and without endothelium (Jorge, *et al*., 2013).

After induction of hypertension in mice by surgical removal of a kidney and by subcutaneous administration of a pellet with deoxycorticosterone, traditionally used herb, *Hancorniaspeciosa* Gomes (0.03, 0.1 or 1 mg/kg; po), produced a dose-dependent, long-lasting reduction in systolic blood pressure; and also induced a concentration-dependent vasodilatation of mesenteric resistance arteries contracted with phenylephrine (Silva, *et al*., 2016).

One of the previous studies reported that administration of L-NAME (20 mg/kg/day) for seven days caused an increase in mean arterial blood pressure from 99 ± 4mmHg to 155 ± 2mmHg in rats. However, simultaneous administration of *Gmelina arborea* (500 mg/kg/day) with hypertensive agent, suppressed the rise in a blood pressure (126±13 mmHg). In addition, the *G. arborea* extract revealed a significant reduction in a blood pressure from 179±10 mmHg to 147±7 mmHg up on administration in L-NAME treated rats for 14 days (Lawson *et al*., 2016).

After three hours administration of Buckwheat (*Fagopyrumesculentum*), at a dose of 0.1mg/kg, there were a significant decrease in blood pressure compared to negative control group ($p<0.010$). The maximum effects were observed 9 h after the single oral administration, and the means of SBP and DBP had decreased by 31.3 and 23.9mmHg compared to the negative control group, respectively (Nakamura, *et al*., 2012).

Evaluation of antihypertensive effect of aqueous methanol extract of *Carallumatuberculata* on hypertensive (egg-fed diet, glucose-induced and cadmium-induced hypertensive rats) and normotensive rats showed a significant dose-dependent, ($p < 0.05$) decrease in SBP, DBP, MBP, and HR ($p < 0.01$) of normotensive rats, at all test doses(100, 300 and 500 mg/kg body weight, p.o.) (Ahmad *et al*., 2015). The 500 mg/kg dose produced a highly significant effect (mm Hg, $p < 0.001$) in SBP (85.9 ± 7.2), DBP (71.86 ± 12.1), MBP (75.1 ± 11.7) and HR (238.08 ± 8.3 beats/min), in comparison to 100 and 300 mg/kg doses (Ahmad *et al*., 2015).

An in vitro study done in Ethiopia revealed that 0.5-5 mg/mL *Thymus serrulatus produced* a significant ($p<0.001$) reduction of KCl-induced contractions of guinea pig thoracic aorta (Geleta, *et al*., 2015). Antihypertensive activity evaluation of aqueous extract of leaves of *Thymus Schimperi*, that was done in Ethiopia, leaves against salt-sucrose induced hypertensive rats produced significant ($p<0.01$) reduction of systolic blood pressure with the higher dose (500mg/kg) but there was no significant ($p>0.05$) reduction of SBP for the lower dose (250mg/kg) (Haji, *et al*., 2016).

1.7 Significance of the Study

Ethnobotanical studies are often significant in revealing locally important plant species especially for the discovery of crude drugs. The tropical rain forests have become an important point of this activity, primarily due to the rich biodiversity they harbor, which promises a high diversity of chemicals with the potential novel structures. However, of this rich biodiversity, only a small portion has been studied for its medicinal potential.

Since plants can be our source of drugs, with fewer side effects and better bioavailability for treatment of HTN in future, this study will provide useful information for the development of new pharmacotherapies from medicinal plants for use in hypertension and which also allow us to isolate and identify the active compound that can be used as a potential drug or a lead compound. Moreover, this study will generate evidence-based information to our people using this plant for management of different diseases such as hypertension and diabetes. The study might also initiate further research to be conducted on the genus thymus and other plants found in Ethiopia.

2. OBJECTIVES

2.1 General Objective

To evaluate antihypertensive, hypotensive, hypolipidemic and antihyperlipidemic effects of the aqueous crude extract and aqueous residue of *Thymus serrulatus* leaves against fructose induced hypertensive rats.

2.2 Specific Objectives

- ⇨ To evaluate antihypertensive activity of aqueous crude extract and aqueous residue of *T. serrulatus* leaves in fructose induced hypertensive rats.
- ⇨ To evaluate hypotensive activity of aqueous crude extract of *T. serrulatus* leaves in normotensive rats.
- ⇨ To evaluate the effect of aqueous crude extract and aqueous residue of *T. serrulatus* leaves on serum lipid profile (TC and TG) and Blood Glucose (BG).
- ⇨ To evaluate the effect of aqueous crude extract and aqueous residue of *T. serrulatus* leaves on kidney parameters (BUN and Creatinine).
- ⇨ To evaluate the effect of aqueous crude extract and aqueous residue of *T. serrulatus* leaves on liver parameters (AST, ALT, ALP and GGT).
- ⇨ To perform preliminary phytochemical screening on the aqueous crude extract and aqueous residue of *T. serrulatus* leaves
- ⇨ To conduct acute toxicity study of *T. serrulatus* leaves in rats.

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Drug, Chemicals and reagents

For this experiment, drugs and chemicals such as Ethyl Acetate, Petroleum Ether, Dinitro-2-4-Phenylhydrazine, Chloroform, Sulfuric acid, Ferric acid, Ammonia Solution, Mayer`s Reagent, Dragendorff`s Reagent, D-Fructose, Tween-80 and Captopril are used. All the drugs, chemicals, and reagents that are used for the experiment meet the required standards and were of analytical grade.

3.1.2 Instruments and Apparatus

Materials like Balance, Whatman filter paper, Orbital shaker, Heparinized capillary tube with plastic sealing, cages, feeding bottle, Water bath, Lyophilizer/ Freeze dry system, Centrifuge, desiccator, Clinical chemistry analyze, BP analyzer (Model 179, IITC Inc, USA) were used to carry out the experiment.

3.1.3 Plant Materials

The fresh leaves of *T. serrulatus* was collected from its natural habitat around Debre Sina, a town about 180 Km North-East of Addis Ababa, capital of Ethiopia. Identification and authentication of the plant specimens was done by a botanist in the Directorate of Traditional and Modern Drug Research, Ethiopian Public Health Institute, where a voucher specimen was deposited for future reference (TS-2180).

3.1.4 Experimental Animals

Healthy adult Wistar rats of both sex weighing (210-250 g), and aged 8-10 weeks were obtained from Ethiopian Public Health Institute. The rats were grouped (n=5) in stainless steel cage and maintained at room temperature (22 ± 3°C), relative humidity (40-70%) and with 12 h light-dark cycle. They were acclimatized for a week before the actual experiment and they were provided with a standard pellet food and water *ad libitum*. The animals were familiarized with the BP measuring environment by keeping them in the holder for about 10 min every day for a week before experiment. All procedures and techniques used in this study were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (*Guide for the Care and Use of Laboratory Animals*, 1996).

3.2 METHODS

3.2.1 Plant preparation and extraction

The dust materials were removed from the leaves of the plant by washing with tap water and gently rinsing it with distilled water. It was cut into pieces and dried under shade and then, pulverized using a mortar and pestle to get a coarse powder used for the extraction. The air-dried and powdered plant materials defatted with petroleum ether and extracted by cold maceration technique with distilled water for 4 hrs to give us the aqueous (AQ) crude extract. This procedure was repeated two times by adding another fresh solvent into marcs. Then the resulting liquid extract was combined and filtered with gauze followed by filter paper, allowed to freeze overnight and dried using lyophilizer. Finally, the dried extract combined and transferred into vials and kept in desiccator until used for the actual experiment.

To remove some nonpolar compounds that diffuse during extraction process the AQ crude extract was suspending in 300ml of distilled water and shaken to mix completely with solvent and then equal amount of ethyl acetate (EtAc) was added to it. Then shaken gently to mix and allowed to settle for some times until it forms two layers and the ethyl acetate layer was removed. The remaining pure aqueous extract (AQ residue) was collected and freeze dried with lyophilizer. Finally, the dried extract was kept in a desiccator until the actual experiment.

3.2.2 Grouping and Dosing

i)**For antihypertensive and antihyperlipidemic study**

The rats were randomly assigned into eight groups of five animals (n=5) each to perform antihypertensive and antihyperlipidmic activities**.** All Animals were loaded with 66% w/v D-Fructose *ad libitum*. Group 1, was treated as negative control and received only 66%w/v D-Fructose *ad libitum,* group 2, was considered as positive control, and received captopril (20 mg/kg/day).

There were 6 experimental groups; group 3, 4 and 5 received three different doses (250, 500 and 1000 mg/kg, respectively) of the AQ crude extract and group 6, 7 and 8 received three different doses (250, 500 and 1000 mg/kg, respectively) of the AQ residue.

ii)**For hypotensive and hypolipidemic study**

Rats with normal BP (SBP around 125-130 mmHg) were selected and randomly assigned into 4 groups of 5 animals (n=5) as follows: the 1stgroup, serving as negative control, received distilled water *ad libitum* only and the 2nd, 3rd, and 4th experimental animal groups received the AQ crude extract at three different test doses (250, 500 and 1000 mg/kg), respectively.

Doses were chosen based on previous efficacy and safety study done on the experimental plant (Debelo et al., 2015).

Assay of serum lipid level, hepatic enzymes and renal function tests

After 15 days plant administration the rats were fasted overnight; blood samples were collected in a test tube by cardiac puncture under ether anesthesia and left to stand at room temperature for 2 h, then centrifugation at 3000 rpm for 10 minutes. The serum was immediately separated from the test tube after centrifugation in order to determine serum lipid profile (triglyceride (TG), total cholesterol (TC), blood glucose (BG)), liver parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma gluthathionetransferase (GGT)), and kidney profile (urea and creatinine) using automated chemistry analyzer.

Blood pressure measurement

The blood pressure was measured from the tail of rats using non-invasive BP monitoring apparatus (Model 179, IITC Inc, USA) (Fig.2). For testing, the animals were placed in the prewarmed holder. The appropriate cuff with sensor (photoelectric) was then mounted on their tails and warmed to about 32–35°C. When they were relaxed and became calm the tail cuff was inflated to a pressure well above the expected SBP and slowly released during which the pulse was recorded by the BP analyzer. The tail-cuff was inflated and deflated automatically at 1-min intervals. The highest and the lowest readings were not counted, and the average of the remaining three readings was accepted as the measurement. The SBP and mean BP (MBP) were

read from the pulse tracings (Fig 2). The diastolic BP (DBP) was calculated from SBP and MBP using the equation: DBP = (3MBP – SBP)/2. The measurements were made in triplicate and the average values were reported for all experiments.

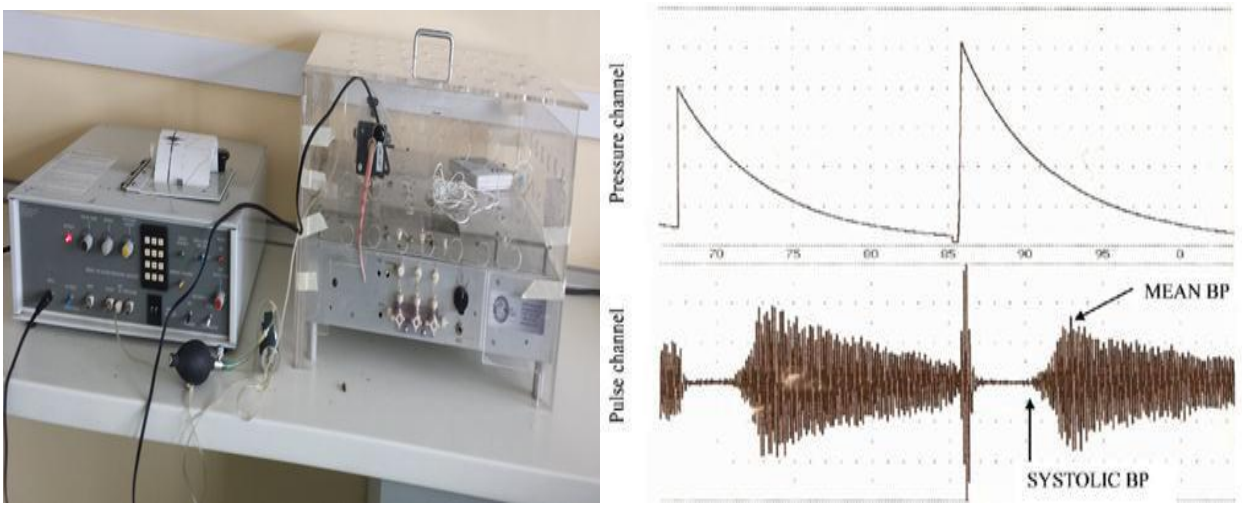

Figure 2 Model 179, IITC Inc, USA BP apparatus and a tracing of rat BP measurement showing the pressure of tail cuff and pulse

Phytochemical Screening

Aqueous extracts and aqueous residue of *T. serrulatus* leaves were screened for the presence of secondary metabolites to relate the activities of the plant with the presence or absence of these constituents. Thus, the test for presence of alkaloids, saponins, flavonoids, terpenoids, phenols and tannins was performed as indicated below (Kalita *et al.*, 2011; Sasidharan *et al*., 2011).

a) Alkaloids

One and half milliliter of 10% HCl was added to 0.5 mg of the extracts in a test tube. The mixture was heated for 20 minutes. It was then cooled and filtered. To 1ml of the filtrate 5 drops Mayers and Draggendorff's reagents each were added. Formation of cream and orange colored precipitates respectively indicates the presence of alkaloids in the extracts.

b) Saponins

Froth test: An aqueous solution of 0.5 mg of the extract in a test tube was vigorously shaken for 2 minutes. Foam which persisted for 30 minutes and doesn't disappear upon warming was taken as an indication of the presence of saponin in the extract.

c) Polyphenols (Phenolic compounds)

Three drops of a mixture of 1 ml 1% FeCl3 and 1% K3Fe(CN)6 each were added to 2 ml of extracts. Formation of green or blue color was taken as an indication of the presence of polyphenols.

d) Flavonoids

To 2 ml of aqueous solution of the extract 4 drops of 2% lead acetate solution was added. Development of yellow or orange color confirms the presence of flavonoids.

e) Terpenoids (Ketonic)

One milliliter of 2, 4-dinitrophenylhydrazine solutions (0.5g dissolved in 100ml of 2M HCl) was added to 2ml aqueous solution of the extract. Formation of yellow-orange coloration indicates the presence of a ketonic terpenoids.

f) Anthraquinones

Borntrager's test: Five milliletr of the extract was dried and shaken with 3ml petroleum ether. The filtrate was added to 2ml of a 25% ammonia solution. The mixture was shaken and formation of a red coloration was taken as an indication of the presence of free anthraquinones.

g) Tannins

Three drops of 5% ferric chloride solution was added to 1ml of the extract solution in water. A greenish or blue coloration or precipitation was taken as indication of the presence of tannins.

h) Phytosterols

Five drops of 3% vanillin in conc. H2SO4 was added to a concentrated chloroform solution of extracts. Formation of a rose or reddish brown color indicates the presence of phytosterols.

i) Test for Glycosides (Keller-Killiani Test)

To 0.5 g of each extract suspended in 5 ml water, 2 ml of glacial acetic acid containing one drop of ferric chloride hexahydrate (FeCl3.6H2O) solution was added. This was mixed with 1 ml of concentrated sulfuric acid and observed for a brown ring at the interface or a violet ring below the brown ring; alternatively acetic acid was added and observed for a greenish ring above the brown ring which gradually spread throughout this layer.

Acute Toxicity Test

Acute toxicity test was performed using the limit test dose of 5000 mg/kg on five female rats as described by Organization for Economic Corporation and Development (OECD) 425 guidelines for the testing of chemicals. Immediately after administration of the extract, the animals were carefully observed continuously for the first 4 hours for any overt signs of toxicity, i.e., morbidity and mortality then for the next 24 hours and daily thereafter, for a total of 14 days (OECD, 2008).

Data Analysis

All experimental data are expressed as mean ± standard error of the mean (S.E.M) and data were processed using SPSS version 20. Analysis was conducted with one-way analysis of variance (ANOVA) followed by Tukey *post-hoc* test for multiple comparisons of the mean differences and responses of different extracts with controls. The analysis is performed with 95% confidence interval and the significance are set at $p<0.05$. The findings are presented using tables, charts, and graphs.

4. RESULTS

4.1 Acute Toxicity Test

After administration of the oral limit dose of 5000 mg/Kg of aqueous crude extract of *T. serrulatus*, all animals didn`t show any overt sign of behavioral abnormality as well as morbidity during the observation period. Furthermore, no mortality was observed during the same period indicating that the LD50 of the extract is greater than the limit dose employed here.

4.2 Effect of *T. serrulatus* on Renal Function and Hepatic Enzymes Tests in Hypertensive Rats

The level of both urea and creatinine decreased dose dependently for AQ crude extract and AQ residue. Compared with the groups that were treated with Captopril; administration of 1000 mg/kg of AQ residue showed significant difference in the serum concentration of urea and creatinine ($p<0.01$). The reduction in concentrations of urea and creatinine by the end of administration was 35.8% and 40%, respectively. For all test doses of the crude extract and AQ residue, even if their value showed some reduction, the levels of creatinine and urea were not significantly different to that of hypertensive groups ($p > 0.05$, Table 1).

The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma glutha thionetransferase (GGT) were significantly elevated in fructose induced hypertensive rats. The effect of groups treated with Captopril and AQ residue at a dose of 1000 mg/kg on ALT is comparable and they also showed significant reduction compared to hypertensive negative control groups ($p<0.05$). ALT level of the AQ crude extract at a dose of 1000 mg/kg showed significant increase compared to the Captopril treated groups ($p<0.01$). Effect of all test doses of the crude extract and AQ residue (except 1000 mg/kg of AQ residue) on other liver parameters were seen to have neither dose dependent pattern nor statistically significant difference compared to the negative control ($P> 0.05$, Table1).

Table 1 Effect of crude extracts and AQ residue of *T. serrulatus* leaves on Liver and kidney parameters in D-Fructose (66% w/v *ad libitum*) induced hypertensive rats.

Substance administered	Dose mg/kg		Liver and kidney parameters				
		ALT (U/L)	ALP (U/L)	GGT (U/L)	AST (U/L)	Urea (mg/dl)	Cr (mg/dl)
D-Fructose	66% w/v *ad libitum*	74.4±5.1	149.2±0.9	4.7±1.8	191.9±4.4	32.5±0.9	0.34±0.02
Captopril	20	49.3±5.3a***	102.5±0.5	2.7±0.3	150±12.5	35.1±1.2	0.4±0.02
AQ Crude	250	69.2±3.2	90.5±11.7	3.3±0.7	176±2.1	33±0.6	0.35±0.02
AQ Crude	500	76.3±6.8	101±14.7	3.6±1.3	189±4.3	29.4±0.7	0.33±0.03
AQ Crude	1000	80.9±4.8b**	102±10.9	3.9±1.9	217±27.7	28.2±2.8	0.34±0.01
AQ residue	250	41.6±5.1a**	148.4±15.7	2.2±1.8	147±37.1	29.6±3.2	0.32±0.01
AQ residue	500	44.3±4.4a**	149.6±11.6	2.5±1.6	153±26.2	25.6±3.7b***	0.3±0.05
AQ residue	1000	49±2.6a***	155±3.8	2.7±2.1	164±12.3	22.5±0.6b**	0.24±0.01b*

Data are expressed as mean ± SEM, n=5; * = p<0.001, **=p<0.01, ***p<0.05 a= compared to negative control, b= compared to Captopril treated group. aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutha thionetransferase (GGT).

4.3 Effect of *T. serrulatus* on Renal Function and Hepatic Enzymes in Normotensive Rats

Blood creatinine insignificantly decreased in rats treated with 500 mg/kg and increased in rats treated with 1000 mg/kg of the extract. And serum urea level also insignificantly decreased at the doses of 500 and 1000 mg/kg of the plant extract as compared to the normal control (Table 2).

As shown in the same Table, serum ALT level of rats treated at a dose of 500 mg/kg of the extract increased insignificantly but ALT level of rats treated at a dose of 250 mg/kg of the extract showed non-significant decrease. In the same way, serum AST level increased in a dose dependent manner when treated with 500 and 1000 mg/kg of the extract. There was insignificant (p>0.05) decrease of alkaline phosphatase activities in the liver of the animals following the administration of the extract (Table 2).

Table 2 Effects of crude extracts of *T. serrulatus* leave on Liver and kidney parameters of normotensive rats.

Substance administered	Dose mg/kg	Liver and Kidney parameters					
		ALT (U/L)	ALP (U/L)	GGT (U/L)	AST (U/L)	Urea (mg/dl)	Cr (mg/dl
Distilled water	*Ad Libitum*	61.3±4.2	109±9.4	2.9±0.9	159.6±22.1	26.8±2.8	0.3±0.02
AQ Crude	250	59.4±3.7	83.7±6.1	2.8±1.2	117.6±19.2	29±1.56	0.35±0.02
AQ Crude	500	73±5.1	97.2±13.5	3.1±2.4	147.3±16	24.4±3.7	0.29±0.03
AQ Crude	1000	69.6±8.3	98.1±1.8	3.0±1.7	184.4±21.2	26.1±1.1	0.32±0.1

Data are expressed as mean ± SEM (n=5), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutha thionetransferase (GGT).

4.4 Effect of *Thymus serrulatus* on Fructose Induced Hypertensive Rats

4.4.1 Antihypertensive Effect

AQ crude extract showed dose dependent reduction of Systolic Blood Pressure (SBP) compared to negative control by 24.2% ($p<0.001$), 15.9% ($p<0.001$) and 9.3% ($p<0.001$) with doses of 1000 mg/kg, 500 mg/kg and 250 mg/kg, respectively (Table 3). A dose of 1000 mg/kg of the AQ crude extract also showed comparatively similar reduction of SBP with the positive control.

The AQ crude extract (especially with 1000 mg/kg) kept reducing the SBP from the day of administration until the last day of the experiment but for AQ residue, even if it showed significant reduction of SBP compared to the negative control ($p<0.001$); there was an increment of SBP from the basal value (from day 1 through day 15). AQ residue also showed significant reduction of SBP in a dose dependent manner; compared to the negative control. A dose of 1000 mg/kg of AQ residue produced 9.3%, reduction ($p<0.001$) and 500 mg/kg of AQ residue produce 7.2% reduction ($p<0.001$).

Table 3 Effect of AQ crude extracts of *T. serrulatus* leaves and AQ residue on SBP in D- Fructose (66% w/v ad libitum) induced hypertensive rats.

Substance administered	Dose (mg/kg)	SBP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
D-Fructose	66% w/v *ad libitum*	129.4±0.53	157±0.99	172±0.35	182±0.56
Captopril	20	129±0.44	138±0.47a*	137±0.96a*	134±0.89a*
AQ Crude	250	128±0.32	156±1.03b*	164±0.6a*	165±0.8a*b*
AQ Crude	500	128.2±0.37	152.2±1.2a*b*	157±0.8a*	153±0.3a*b*
AQ Crude	1000	127.4±0.24	145.4±0.4a*b*	144±0.6a*b*	138±1.3a*b*
AQ residue	250	130.8±2.3	153±0.32	165±0.72a*	172±0.49a*b*
AQ residue	500	128.6±0.51	153±0.54a*b*	164±1.24a*b*	169±0.65a*b*
AQ residue	1000	128.2±0.58	150±0.32a*b*	161±0.8a*b*	165±0.54a*b*

Data are expressed as mean ± SEM, n=5; SBP= systolic blood pressure, * = $p<0.001$, **=$p<0.01$, a= compared to negative control, b= compared to positive control.

Daily oral administration of 250 and 500 mg/kg of AQ crude extract and all the three test doses of AQ residue showed significant increase in Mean Arterial Blood Pressure (MABP) compared to positive control ($p<0.001$), but they produced significant reduction compared to negative control in the 10^{th} day of the experiment, whereas, 1000 mg/kg oral administration of AQ crude extract prevented a rise in MABP in a similar manner with positive control (Table 4).

After consecutive oral daily administration for 15 days, AQ crude extract of 1000 mg/kg produced 22.1% ($p<0.001$) reduction of MABP compared to negative control and it showed significant reduction of MABP in a similar manner as that of positive control ($p<0.001$). A dose of 1000 mg/kg of AQ residue produced higher reduction percentage (8.9% ($p<0.001$)) compared to negative control ($p<0.001$).

Table 4 Effect of AQ crude extracts and AQ residue of *T. serrulatus* leaves on MABP in D- Fructose (66% w/v ad libitum) induced hypertensive rats.

Substance administered	Dose (mg/kg)	MABP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
D-Fructose	66% w/v *ad libitum*	97.4±0.25	123±0.56	137±0.45	145±0.76
Captopril	20	97±0.44	107.34±0.38a*	107.8±0.58a*	105.08±0.42a*
AQ Crude	250	96.4±0.51	120±0.72b*a*	129±0.72a*	133±0.89a*b*
AQ Crude	500	96.2±0.37	118±0.22b*	124±0.82a*	125±0.12a*b*
AQ Crude	1000	95.4±0.244	114±0.32a*b*	114±0.62a*b*	113±0.72a*b*
AQ residue	250	96.8±0.58	118±0.32b*	129.5±0.82a**b*	138±0.56a*b*
AQ residue	500	96.6±0.51	119±1.2a*b*	130±0.25a*b*	136±1.8a*b*
AQ residue	1000	96.8±0.58	117±0.9a*b*	128.3±0.7a*b*	132±1.9a*b*

Data are expressed as mean ± SEM, n=5; MABP= mean arterial blood pressure, * = p<0.001, **=p<0.01, ***p<0.05, a= compared to negative control, b= compared to positive control.

Both AQ crude extract and the AQ residue showed significant reduction in Diastolic Blood Pressure (DBP) as compared to negative control in a dose dependent manner. Effect of AQ crude extract (with all tested doses) has much higher percentage of reduction than the residue (Table 5). Higher dose (1000 mg/kg) of AQ crude extract produced a 20.2% ($p< 0.001$) fall in hypertensive rats, while 500 mg/kg and 250 mg/kg produced an 11.8% ($p < 0.01$) and 9.1% ($p< 0.01$) reduction in DBP, respectively (Table 5).

Table 5 Effect of AQ crude extracts and AQ residue of *T. serrulatus* leaves on DBP in D- Fructose (66% w/v ad libitum) induced hypertensive rats.

Substance administered	Dose (mg/kg)	DBP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
D-Fructose	66% w/v *ad libitum*	81.4±0.24	106±0.9	119±0.8	126.5±0.78
Captopril	20	81±0.44	91.46±0.31a*	92±0.89a*	89.76±0.25a*
AQ Crude	250	80.4±0.51	102±0.42	111.5±0.92a*	115±0.84a*b*
AQ Crude	500	80.2±0.37	101±0.58a*b*	107±0.32b*a*	111±0.92a*b*
AQ Crude	1000	79.4±0.24	97.8±0.2a*b*	99±0.72a*b*	100.5±0.32a*b*
AQ residue	250	81.2±0.37	100±0.32	112.16±0.23a**b*	121±0.32a*b*
AQ residue	500	80.6±0.51	103±0.84a*b*	114±0.75a*b*	117±0.76a*b*
AQ residue	1000	80.2±0.58	101±0.54a*b*	112±0.98a*b*	115±0.64a*b*

Data are expressed as mean ± SEM, n=5; DBP= diastolic blood pressure, * = p<0.001, **=p<0.01, ***p<0.05, a= compared to negative control, b= compared to positive control.

4.4.2 Effect of *T. serrulatus* on Serum lipid profile and Blood Glucose

Compared to the negative control; TG level was significantly decreased with 1000 mg/kg AQ crude extract and 1000 mg/kg AQ residue administration ($p<0.001$). The other test doses of the crude extract and the AQ residue showed increment of TG level but it is not significant ($p>0.05$, Figure 3).

Total cholesterol (TC) level in animal treated with 1000 mg/kg of AQ crude extract showed significant reduction compared to hypertensive control groups; whereas TC level of the residue (with all test doses) didn`t show significant difference ($p>0.05$) compared to both Captopril treated groups and negative controls but there is a dose dependent reduction after its administration.

Blood glucose level decreased dose dependently for AQ crude extract and its residue and it was significant compared to Captopril treated groups ($p<0.001$). AQ crude extract at 1000 mg/kg showed the highest reduction percentage of Blood glucose (42.1%, $p<0.001$) compared to the negative control followed by 1000 mg/kg of AQ residue (22.1%, $p<0.01$); as shown in figure 3.

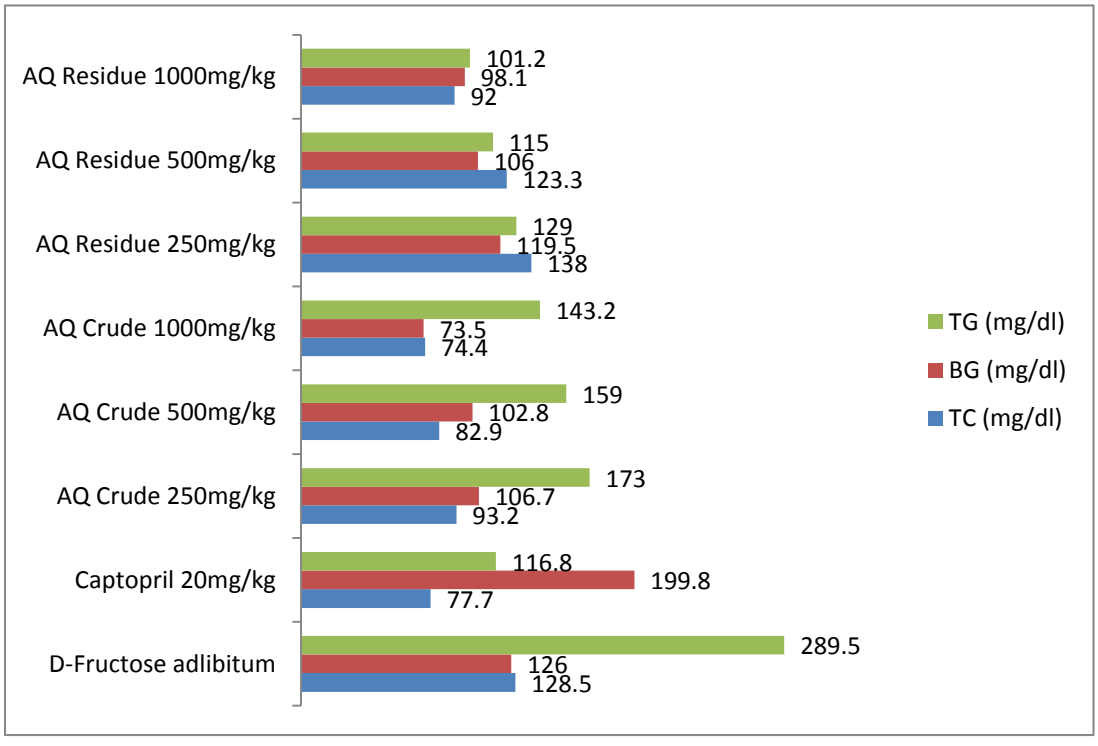

Figure 3 Effect of AQ crude extracts and AQ residue of *T. serrulatus* leaves on blood glucose and lipid profile in Hypertensive rats

4.5 Effect of *Thymus serrulatus* on Normotensive Rat

4.5.1 Hypotensive Effect

Lower doses (250 mg/kg and 500 mg/kg) of *T. serrulatus* AQ crude extract did not produce significant hypotensive effect until the 10^{th} day of extracts administration. On the 15^{th} day of the experiment, 1000 mg/kg AQ crude extract showed significant hypotensive effect with a reduction of Systolic Blood Pressure (SBP) by 7.8% compared to negative control ($p<0.001$), and the overall reduction of SBP for 250 mg/kg and 500 mg/kg was 2.9% and 4.2%, respectively ($p<0.001$) (Table 6).

Table 6 Effect of crude extracts of *T. serrulatus* leaves on SBP in adult Normotensive rats.

Substance administered	Dose (mg/kg)	SBP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
Distilled water	*ad libitum*	128.4±0.4	127.8±0.36	128.6±0.6	129±0.85
AQ Crude	250	128.2±0.5	128.7±0.6	127.8±0.8	125.2±0.48c*
AQ Crude	500	128±0.52	126.8±0.4c**	124.9±0.37c*	123.6±0.8c*
AQ Crude	1000	127.8±0.37	125.3±0.63c*	123.2±0.37c*	119.4±0.7c*

Data are expressed as mean ± SEM, n=5; SBP= systolic blood pressure, * = $p<0.001$, **=$p<0.01$, c= compared to negative control

As shown in table 7, all test doses of the AQ crude extract showed significant reduction of Mean Arterial Blood Pressure (MABP) compared to the negative control ($p<0.001$). The reduction of MABP was 10.9 mmHg and 4.8 mmHg, respectively, for 1000 mg/kg and 250 mg/kg doses of the extract.

Table 7 Effect of crude extracts of *T. serrulatus* leaves on MABP in adult Normotensive rats.

Substance administered	Dose (mg/kg)	MABP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
Distilled water	*ad libitum*	93.4±0.4	92.2±0.37	92.6±0.5	94.1±0.47
AQ Crude	250	92.2±0.7	93.5±0.45c***	92.8±0.5	89.3±0.3c*
AQ Crude	500	93.4±0.5	88.5±0.6c*	86.3±0.5c*	85.5±0.37c*
AQ Crude	1000	92.8±0.2	88.1±0.8c*	85.2±66c*	83.2±0.5c*

Data are expressed as mean ± SEM, n=5; MABP= mean arterial blood pressure, * = $p<0.001$, ***=$p<0.05$, c= compared to negative control.

Throughout the experiment period (from day 1 to day 15), reduction of Diastolic Blood Pressure (DBP) in normotensive rats became more significant compared to both the negative control and the baseline (Table 8). The cumulative effect of the higher dose of the AQ crude extract (1000 mg/kg) showed significant reduction of diastolic blood pressure (15.2% ($p<0.001$)) compared to the negative control.

Table 8 Effect of crude extracts of *T. serrulatus* leaves on DBP in adult Normotensive rats

Substance administered	Dose (mg/kg)	DBP			
		Day 1 (mmHg)	Day 5 (mmHg)	Day 10 (mmHg)	Day 15 (mmHg)
Distilled water	*ad libitum*	75.9±0.4	74.4±0.37	74.5±0.5	76.8±0.47
AQ Crude	250	74.2± 0.8	75.9±0.54c***	75.3±0.5	71.4±0.4c*
AQ Crude	500	76.1±0.6	69.4±0.6c*	67±0.76c*	66.5±0.36c*
AQ Crude	1000	75.3±0.2	69.5±0.9c*	66.2±0.66c*	65.1±0.5c*

Data are expressed as mean ± SEM, n=5; DBP= diastolic blood pressure, * = $p<0.001$, ***= $p<0.05$, c= compared to negative control.

4.5.2 Assay of Blood Glucose and Serum lipid profile

Lipid profile (TC and TG) results were seen to have neither dose dependent pattern nor statistically significant difference with the negative control and between doses ($P> 0.05$) as shown in figure 4. There is non-significant decrease in BG level which follows a dose dependent pattern. The decrease in BG level was relatively high for those treated at a dose of 1000 mg/kg of the extract as shown in figure 4.

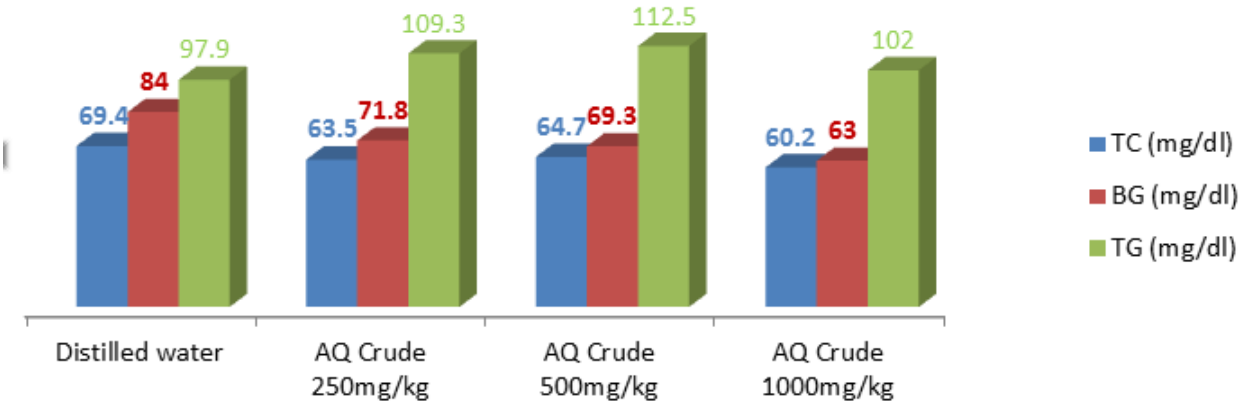

Figure 4 Effect of crude extracts of *T. serrulatus* leaves on blood glucose and lipid profile in normotensive rats

4.6 Phytochemical Screening

Screening the plant extracts for presence of secondary metabolites using chemical method of detection revealed the presence of alkaloids, tannins, polyphenols, phytosterols, and flavonoids as shown in table 9. However cardiac glycosides, terpenoids and anthraquinones were absent in both crude extract and AQ residue.

Table 9 Secondary metabolites of the extracts of *T. serrulatus* detected by the chemical method.

Secondary metabolites	Type of Plant extracts	
	AQ crude extracts	AQ residue
Alkaloid	+	+
Tannins	+	+
Polyphenols	+	+
Saponins	+	–
Cardiac glycosides	–	–
Phytosterols	+	+
Flavonoids	+	+
Terpenoids	–	–
Anthraquinones	–	–

Note: +: present; -: absent

5. DISCUSSION

From thousands of years medicine and natural products have been closely interconnected through the use of traditional medicine (Tiwari, 2015). Despite competition from other drug discovery methods, natural products are still providing their fair share of new clinical candidates *(Chin. et al., 2006).*

In both fructose induced hypertensive rats and normotensive rats, the effects of the aqueous crude extract and fractions of *T. serrulatus* leaves on blood pressure were determined at various doses using non-invasive BP monitoring apparatus (Model 179, IITC Inc, USA). The model of using fructose to induce experimental hypertension has become a widely accepted method for testing antihypertensive agents. Previously, it has been reported that 10% and 20% of fructose administration to normotensive rats results an increment of SBP by 20mmHg and 26mmHg, respectively (Dai & Mcneil, 1995). Hwang et al. reported that the SBP of rats fed a 66% fructose diet for 2 weeks rose from 124 to 145mmHg (I S Hwang, H Ho, 2014)(Klein & Kiat, 2015). Oral administration of 20 mg/kg of Captopril, an FDA approved angiotensin converting enzyme inhibitor was used as comparator to investigate the relative efficacy of the observed reductions on Systolic Blood Pressure, Mean Arterial Blood Pressure and Diastolic Blood Pressure.

5.1 Phytochemical screening

Phytochemical screening of the AQ crude extract of *T. serrulatus* showed presence of different secondary metabolites, such as Alkaloid, Tannins, Polyphenols, Saponins, Phytosterols and Flavonoids. This finding is in line with the study done on the phytochemical constituent of *T. serrulatus by* (Melka, *et al*., 2016). The AQ residue contains all secondary metabolites that are present in the crude extract except saponins. Phytochemical constituent of *Thymus vulgaris* also showed the presence of alkaloid, tannin, and polyphenols, saponins and flavonoids and absence of cardiac glycosides as secondary metabolites (Asha & Mathew, 2017; Ghalem & Ali, 2017).

The difference in the results obtained might possibly be due to the different method of extraction and solvents polarities. Some volatile active compounds may be also destroyed or evaporated from the samples during the sample processing and drying (Mohammad A. *et al*, 2013).

5.2 Antihypertensive Effect

Fructose induced hypertensive negative control group showed increment of on Systolic Blood Pressure (SBP), Mean Arterial Blood Pressure (MABP) and Diastolic Blood Pressure (DBP), by 52.6 mmHg, 47.6 mmHg and 45.1 mmHg respectively; from the basal value. The present findings indicate that extract of *T. serrulatus* leaves had a definite dose-dependent effect in preventing the rise in SBP, MABP and DBP in hypertensive rats; when both the extract and fructose were given concomitantly. The best dose which almost completely prevented the rise in blood pressure was 1000 mg/kg of the AQ crude extract. Accordingly, the three test doses of the crude extract was used in an effort to see whether giving the extract to animals with established antihypertensive effect would still be capable of producing hypotension in normotensive rats.

Unlike the AQ crude extract, AQ residue decreased blood pressure only at the highest dose employed and the lower doses failed to show appreciable antihypertensive effect. The observed difference in pharmacological response might be due to differential distribution of active phytoconsituents.

All test doses of the aqueous crude extract and AQ residue showed significant and dose dependent reduction of Systolic blood pressure, Mean arterial blood pressure and Diastolic blood pressure after the 10^{th} days of administration, which is in line with studies done on antihypertensive effect of *Thymus Schimperi* leaves against salt-sucrose induced hypertensive rats (Haji, *et al*., 2016), ethanolic extract of *Eclipta Alba* leave (Mishra & Pal, 2013) and on *Retama raetam* Forssk (RR) leave (Maghrani, 2007). The AQ crude extract at the dose of 1000mg/ kg showed higher percentage reduction of SBP (24.2%), MABP (22.1%) and DBP (20.2%); compared to the negative control followed by AQ residue of 1000mg/kg.

A study on *in vitro* vasodilatory effect of aqueous leaf extract of *Thymus serrulatus* on thoracic aorta of Guinea pigs indicated that *T. serrulatus* aqueous leaf extract possessed endothelium-dependent vasodilatory effect *(*Geleta *et al*., 2015), therefore these result may support the present findings as it may also possess endothelium-dependent antihypertensive effect. On the other hand having comparatively similar reduction pattern on blood pressure of the larger doses of the AQ crude extract and captopril; the present study may suggest the plant extract may have same

antihypertensive mechanism as captopril, i.e., improving endothelium-dependent coronary vasodilatation (Ibrahim, Sulaiman, & Al-saffar, 2010). Since one mechanism of hypertension induction by fructose is by endothelial dysfunction; the present study finding may also indicate that *T. serrulatus* leave extract possess endothelium-dependent antihypertensive effect (Klein & Kiat, 2015).

Different studies indicated plant species of the *Thymus* genus are characterized by chemical polymorphism, meaning that several chemotypes exist; where carvacrol and thymol are the most common constituent (Snezana Jaric, *et al.,* 2015). Miloradovic *et al*. proved the pronounced antihypertensive activity of wild thyme aqueous extracts containing phenols and flavonoids in terms of their potential antihypertensive effect on spontaneously hypertensive and normotensive rats (Miloradović, *et al.*, 2013) and hence the present study finding showed the presence of phenols and flavonoids in both the crude extract and AQ residue.

However, the antihypertensive activity of the aqueous crude extract of *T. serrulatus* may not be only due to the mere presence of certain dominant components but is the result of the synergism of a larger number of components, including some which are present only in small amounts. And this is in agreement with previous studies carried out on antihypertensive effect of *Thymus Schimperi* leaves against salt-sucrose induced hypertensive rats (Haji, *et al*., 2016), and vasodilatory effect of *Thymus serrulatus* on thoracic aorta of Guinea pigs (Geletat, *et al*., 2015).

In the cerebral artery of rats, carvacrol showed endothelium-dependent vasodilatory effect (Earley *et al*., 2010; Santos et al., 2011; Luna-vázquez *et al*., 2013), so this might be one mechanism for reduction of blood pressure by *T. serrulatus* extracts; since one way of induction of hypertension by fructose is endothelium dysfunction. Even if there is significant reduction of Systolic blood pressure, Mean arterial blood pressure and Diastolic blood pressure for all test dose of AQ residue compared to negative control, there is an increment from there basal value.

The reduction of blood pressure, especially with the higher dose of AQ residue, might be because of the presence of phenolic compounds thymol and carvacrol (Asfaw N. *et al,* 1999b); (Nagle et al., 2013). This result is in line with the study done on 2007 by Aydin *et al,* which explained carvacrol inhibited the hypertension induced by L-NAME (Aydin, *et al*., 2007).

The minimum doses of the extracts produce less significant effect and this could be accounted by the lack of enough concentration of active components which were responsible for antihypertensive activity at these lower doses. Increasing the dose did affect the antihypertensive effect produced especially by the aqueous extract.

5.3 Hypotensive Effect

The extract lowered the systolic, diastolic and mean arterial pressure of normotensive rats in a dose dependent manner. This result is in agreement with previous *in vivo* hypotensive studies on leaves of *Moringa stenopetala* normotensive anaesthetized guinea pigs (Mengistu, *et al*., 2005). The extract exerted a greater blood pressure lowering effect on the diastolic blood pressure than on the systolic blood pressure. The highest dose of crude extract (1000 mg/kg) caused 15.2% fall in diastolic pressure, compared to 7.8% fall in systolic pressure by the same dose. This result is in agreement with previous study on hypotensive effect of aqueous extract of the leaves of *Phyllanthusamarus* schum and thonn (*euphorbiaceae*) (Amaechina & Omogbai, 2007).

The hypotensive effect of *T. serrulatus* may instead be attributed to the alkaloid compounds present in the leaves of the plant. The alkaloids have been documented to have a transient depressive effect on the blood pressure (Nwokocha *et al*., 2012).

The presence of phenolic compounds thymol and carvacrol (Asfaw N. *et al*,1999b); (Nagle et al., 2013) may explain the hypotensive effect of the higher dose of the crude extract. And this result is in agreement with studies done in normotensive rats which explained carvacrol at a dose of 100 mg/kg (*i.p*.) reduced blood pressure (Aydin, *et al*., 2007).

p-cymene and α-pinene monoterpenes are present in the essential oil of *T. serrulatus* constituent (Asfaw *et al*., 1999b), which may explain significant hypotensive effect of the higher dose of the crude extract. This effect was also seen in studies about the pharmacological actions of the volatile oil of the Black seed *Nigella sativa* in rats which revealed its ability to decrease the arterial blood pressure (in doses of 4-32 μL/kg (*i.v*.)); in a dose dependent manner (El Tahir, 2003). A review in cardiovascular effects of monoterpenes (Santos *et al*., 2011), was also reported that intravenous administration of *p*-cymene and α-pinene in urethane anaesthetized rats results hypotension.

5.4 Effect of *T. Serrulatus* on Blood Parameters

Measurement of the activities of marker enzymes in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues (Malomo, 2000; Yakubu *et al*., 2003). Such measurement can also be used to indicate tissue cellular damage caused by a chemical compound long before it is revealed by histological techniques. Compared to histopathological and biochemical assessment of chronic oral administration of aqueous leaf extract of *Thymus Serrulatus* in Mice (Debelo *et al*., 2015), the present study result shows similar effect on creatinine and urea level, which indicate reduction in both markers in dose dependent manner.

Treatment of the hypertensive rats with *T. serrulatus* caused reduction in the serum creatinine and uric acid levels, especially in group that received AQ residue compared to the fructose induced hypertensive group. This may be due to the presence of polyphenols and flavonoids in the extract which might be responsible for the antioxidant activities and the reduction of serum creatinine and uric acid levels which is also seen in *Thymus Vulgaris* (El-newary, *et al.*, 2017; Print, *et al*., 2016). Reduction in the levels of serum urea and creatinine is an indication that *Thymus serrulatus* enhanced the clearance functioning of the kidney, and this result is in line with studies done on effect of aqueous extract of *c. planchonii* (Nafiu, *et al*., 2011).

When the liver cell is damaged, the AST and ALT level in liver cells will be released to serum. Therefore, levels of AST and ALT are the most commonly used biochemical indexes for evaluating the damage of liver. The findings of this research indicated that AQ crude extract of *T. serrulatus* leaves is effective in the control of hepatic dysfunction that may occur during hypertension. Protective effect of *T. serrulatus* extract is evident by amelioration in serum liver functions ALP, AST and ALT activities. Compared to negative control; the AQ crude extract, both solvent fractions and Captopril reduced AST, GGT, ALT and ALP activities in hypertensive rats, in comparable rate.

AQ residue showed comparable reduction of ALT at a dose of 1000 mg/kg with Captopril, and it also showed significant reduction compared to fructose induced hypertensive groups ($p<0.05$). This result is in line with a research report by El-newary and omer 2017 explained that *T. vulgaris* leaves extract also showed significant reduction in hepatic function tests and antioxidant activity close to the standard drug, which could be attribute to its polyphenols

and flavonoids contents (El-newary and omer 2017). Hepatoprotective effect of Saponins in *Thymus serrulatus* leaves may also have contribution for the reduction of liver parameters in both normotensive and fructose induced hypertensive rats (Ghalem & Ali, 2017).

Thymus serrulatus is rich in flavonoids and phenolic compounds, especially thymol and carvacrole (Asfaw *et al*., 1999b). This may be due the reduction in blood glucose by the crude extract and AQ residue to the action of carvacrole or thymol, which have insulin mimetic effect (Print *et al*., 2016). The present study also showed *Thymus serrulatus* was associated with lower serum levels of TC and TG. The decline in the level of cholesterol perhaps due to the thyme oil contain flavone, which is characterized by possessing antioxidant qualities and depressor of blood lipids (Print et al., 2016).

The medicinal use of saponins in hypercholesterolaemia and hyperglycaemia is reported in different studies. Terpenoids also have been found to be useful in the prevention and therapy of several diseases, including hypertension, and also to have antihyperglycemic effect (Asha & Mathew, 2017). Thus the presence of these secondary metabolites in *T. serrulatus* may impart for its effect on the reduction of TC and TG level. Reduction of TC and TG level by T. *serrulatus* leave is in agreement with hypolipidmic effect of *T. vulgaris* which is represented as decreasing on TC level (El-newary & Omer, 2017).

6. CONCLUSION

According to the in vivo experiment, both normotensive and fructose induced hypertensive rat groups administered with AQ crude extract of *Thymus serrulatus* leaves showed a reduction in Systolic Blood Pressure, Mean Arterial Blood Pressure and Diastolic Blood Pressure. In fructose induced hypertensive rats, all test doses of *T. serrulatus* leave crude extract and AQ residue showed a significant decrease in SBP, MABP and DBP starting from the 10^{th} day of administration. The reduction in SBP was greater in fructose induced hypertension than in normotensive rats. Thus, the antihypertensive effect of *T. serrulatus* aqueous extract seems to be more pronounced than the hypotensive effect. *Thymus serrulatus* has antihypertensive, antihyperglycemic effect and kidney functions improvement, which may help in treatment of hypertension and its complications.

7. RECOMENDATION

Based on the findings of the present study, the following are recommended:

1. Further studies should be directed to identify the main constituents of *T. serrulatus* that are responsible for the antihypertensive activity.

2. Further studies are required to ascertain the precise mechanism of action of the antihypertensive activity of the plant

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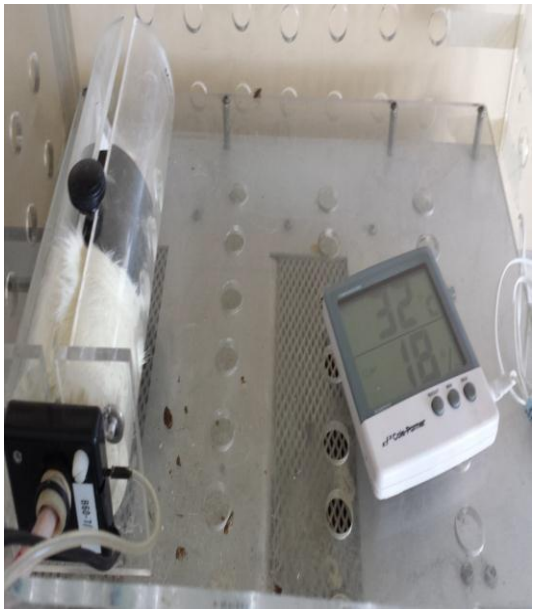
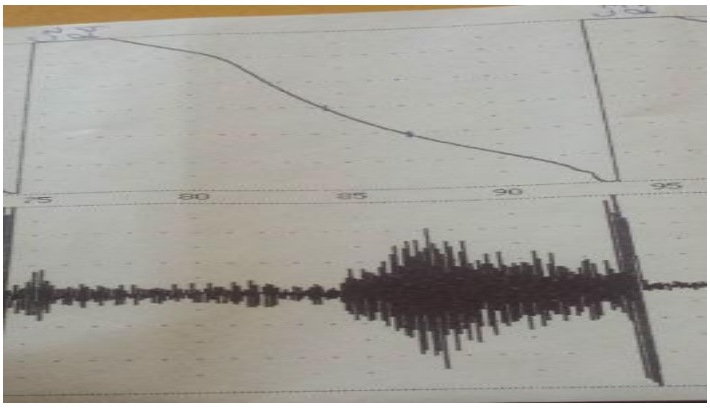
9. ANEX

1.Solvent Fractionation of *Thymus serrulatus*

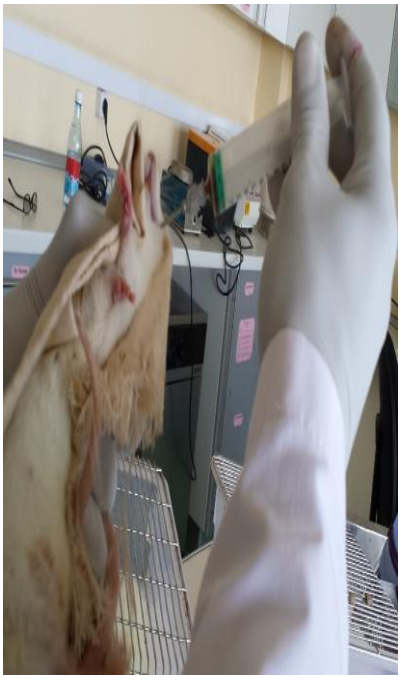


2. Blood Pressure Method by Using non-invasive BP Monitoring Apparatus (Model 179, IITC Inc, USA)

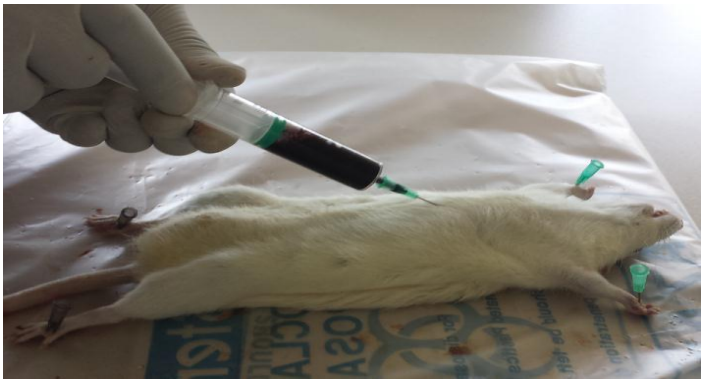


3. Tracing of BP from the BP Monitoring Apparatus





4. Administration of AQ Crude Extract and Solvent Fraction *T. serrulatus*




5. Collection of Serum Sample