..

EVALUATION OF EFFECTS OF HEXANE AND METHANOL EXTRACTS OF *Trigonella Faenum-Graecum L.* ON SOME CARDIOVASCULAR PARAMETERS IN CATS AND RABBITS

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ZARIA, NIGERIA

DECEMBER, 2016

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BY

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MSC/MED/46973/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN HUMAN PHYSIOLOGY

DEPARTMENT OF HUMAN PHYSIOLOGY,

FACULTY OF MEDICINE

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

DECEMBER, 2016

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DECLARATION

I declare that the work in this dissertation entitled **EVALUATION OF EFFECTS OF HEXANE AND METHANOL EXTRACTS OF *Trigonella Faenum-Graecum L.* IN CATS AND RABBITS** on some cardiovascular parameters has been carried out by me in the Department of Human Physiology, Faculty of medicine, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any Institution.

Aisha IDRIS	Signature	Date

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CERTIFICATION

This dissertation entitled **EVALUATION OF EFFECTS OF HEXANE AND METHANOL EXTRACTS OF *Trigonella Faenum-Graecum L.* IN CATS AND RABBITS** on some cardiovascular parameters by Aisha IDRIS meets the regulations governing the award of M.Sc. Human Physiology at Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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AKNOWLEDGEMENT

My utmost gratitude to Allah (S.W.T) for His mercy, excellent health and courage to carried out this project work amicably and successfully.

My special thanks to my husband and parents for their unending love, support, prayers, guidance, encouragements morally, financially and emotionally throughout my knowledge pursuit and my whole life.

I wish to place on records my sincere thanks and appreciation to my supervisors Dr Abubakar Ahmad OF Department of Pharmacognosy and Drug Development and Dr. Rabiu AbduSSALAM Magaji of Department of Human Physiology; Prof. Aliyu Mohammed Head of Department of Human Physiology for their untiring efforts in guiding me to the successful completion of this thesis despite their tight schedules.

I value the aid and support of my lecturers: Professor Mabrouk Muhammad, Dr A.B Adelaiye, Dr M.I. Saleh, Dr Yusuf Tanko, Dr A. Abdulwahab, Dr Ibrahim Bako, Mrs A.N. Danborno, and Dr Maryam Dewu for carrying me along with my mates throughout the period of our program. I am grateful to all the technical staff in the Department of Pharmacology Ahmadu Bello University, Zaria for the role they played in the laboratory work during this research particularly Mr. John and Mallam Nasiru Shehu. I am also grateful to Malam Kabiru Laboratory Technologist of Department of Pharmacognosy and Drug development who helped with the extraction of the plant, Malam Musa of the

..

Department of Biological Science for identification of the plant seed. My profound gratitude goes to my friends and well-wishers at home as well as in the Faculty.

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List of Abbreviations

Adr	Adrenaline
Ach	Acetylcholine
ATP	Adenosine Triphosphate
BP	Blood pressure
CAT	Catalase
CP	Cyclophosphate
CVD	Cardiovascular Diseases
D.B.P	Diastolic Blood Pressure
DP	1,1, diphenyl-2picryl-hydrazyl
EDRF	Endothelium Derived Relaxing Factor
HDL	High Density Lipoprotein
HTN	Hypertension
GP	Glutathione Peroxide
GR	Glutathione Reductase
GSH	Glutathione
GST	Glutathione S-transferase
LDL	Low Density Lipoprotein
LPO	Lipid Peroxidation
M.A.B.P	Mean Arterial Blood Pressure
NCAM	Natural Center for Complemetary and Alternative Medicine
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
PABA	Para Amino Benzoic Acid

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P.P	Pulse Pressure
ROS	Reactive Oxygen Specie
S.B.P	Systolic Blood Pressure
SEM	Standard Error of Mean
THL	Total Haemoglobin Level
VLDL	Very Low Density Lipoprotein
WHO	World Health Organisation

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Abstract

Trigonella Foenum-gaecum L. contains a broad spectrum of therapeutic properties. It is used as galactagogue and antidiabetic among others in traditional medicine practice. Effects of methanol and hexane extracts of the seeds of *Trigonella Foenum-gaecum L.* on blood pressure was evaluated in normotensive male adult cats. Intravenously administered doses: (0.1 mg/ml, 1mg/ml, 10 mg/ml and 100 mg/ml) of the extracts to anaesthetized normotensive cats produced decrease in mean diastolic, and systolic and mean arterial pressures in a graded dose response manner but the decrease was significant as on the dose of 100mg/ml on diastolic blood pressure 14.50 ± 0.50 mmHg (hexane extract) and 13.50 ± 2.50 mmHg (methanol extract) while dose of Ach 10µg/ml produced a fall in mean diastolic, systolic, and means arterial pressure of 13.5 ± 2.50 mmHg, 22 ± 4.0 mmHg, and 16.3 ± 2.5 mmHg respectively. Atropine (25mg/ml) at all tolerable doses did not block the hypotensive effect of both extracts but rather potentiated the effect of the extracts. The methanol and hexane extracts of the seeds of *Trigonella Foenum-gaecum L*. decreases both the force and rate of myocardial contraction in a concentration dependent manner. The results obtained on Isolated Rabbit Prefused Heart Experiment shows that Methanol and Hexane extracts' blood pressure and myocardial relaxation effects may be by the combined effects of myocardial depression, restorative function of the plant high vitamin c content or/ and muscarinic receptor mediated vascular smooth muscle relaxation. Results of the experiment indicated that the plant *Trigonella Foenum-gaecum L.* has blood lowering properties properties, and therefore, lend pharmaco-physiological credence to folkloric, ethnomedical use of the plant in management and/or control of high blood pressure in some rural communities.

Keywords: Fenugreek, *Trigonella Foenum-gaecum,* blood pressure, myocardic contractility.

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CHAPTER ONE

1.0 Introduction

Cardiovascular diseases account for 17 million deaths annually worldwide and are known to be number one group of 'killer disease' (Ananya, 2014). Persistent high blood pressure is one of the leading causes of disability, mortality, and morbidity along the population through strokes and heart attacks It is the most common chronic illness among the world faces (Carretero and Oparil, 2001; Chobanian *et al.*, 2003).

Cardiovascular disease is a class of diseases that involve the heart or blood vessels (arteries, capillaries, and veins) (Malton *et al.*, 1993; WHO, 2014). Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease (Kelly and Valentin, 2010). The causes of cardiovascular disease are diverse but atherosclerosis and/or hypertension are the most common. Additionally, with aging come a number of physiological and morphological changes that alter cardiovascular function and lead to subsequently increased risk of cardiovascular disease, even in healthy asymptomatic individuals (Dantas *et al.*, 2012).

Cardiovascular deaths and disease have increased at a fast rate in low- and middle-income countries (Mendis *et al.*, 2011). Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis, begin in early life, making primary prevention efforts necessary from childhood.

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Free radicals produced at the end of metabolic processes: poly unsaturated fatty acids occur as a major part of low density lipoproteins in blood and oxidation of these LDL play a vital role in pathologies including atherosclerosis, coronary artery disease, myocardium infraction, heart failure, renal insufficiency, and stroke and dissecting aneurysm of aorta (Beevers *et al.* 2001), with high level of oxidized lipids, blood vessels are damaged due to the reactions and can lead to generation of foam cells and plaques damaging endothelial cells and hardening of the affected vessels with High blood pressure as the most common end result (Esterbuer *et al*., 1991).

Antioxidants like B-carotene, vitamin C and Vitamin E play important role in restorative and protection of the vessels (Neuzil *et al.,* 1997). An elevated arterial pressure is an important public health issue in developed countries. Although it is common, asymptomatic and readily detectable but it can often lead to lethal complication, if left untreated.

Evidence suggests a number of risk factors for heart disease: age, gender, high blood pressure, high serum cholesterol levels, tobacco smoking, excessive alcohol consumption, sugar consumption (Howard and Wylie-Rosett, 2002; Finks *et al.,* 2012), family history, obesity, lack of physical activity, psychosocial factors, diabetes mellitus, air pollution (Kelly and Valentin, 2010) While the individual contribution of each risk factor varies between different communities or ethnic groups the consistency of the overall contribution of these risk factors to epidemiological studies is remarkably strong (Yusuf *et al.,* 2004). Some of these risk factors, such as age, gender or family history, are immutable; however,

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many important cardiovascular risk factors are modifiable by lifestyle change, drug treatment or social change.

Because of high incidence and morbidity, various drugs and regimes have been advocated for the control of persistent high blood pressure. Many new drugs have been introduced which may demonstrate better efficacy but possess side effects. Recently attention has been focused towards herbal and mineral preparations which are traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases (Pierdomenico *et al.,* 2009).

Arterial Blood Pressure is determined by two parameters, systolic and diastolic, which depend on whether the heart muscle is contracting (systole) or relaxing between beats (diastole) and equate to a maximum and minimum pressure each in the heart during cardiac cycle, respectively. Normal blood pressure at rest is within the range of 100-140 mmHg systolic (top reading) and 60-90 mmHg diastolic (bottom reading). High blood pressure is said to be present if it is persistently at or above 140/90 mmHg (Chobanian *et al.,* 2003).

Persistent high blood pressure is one of the risk factors for strokes, heart attacks, heart failure, and arterial aneurysm, and is a leading cause of chronic kidney failure (Pierdomenico *et al.,* 2009). Moderate elevation of arterial BP leads to shortened life expectancy. Both dietary and lifestyle changes as well as medicines can improve BP control and decrease the risk of associated health complications.

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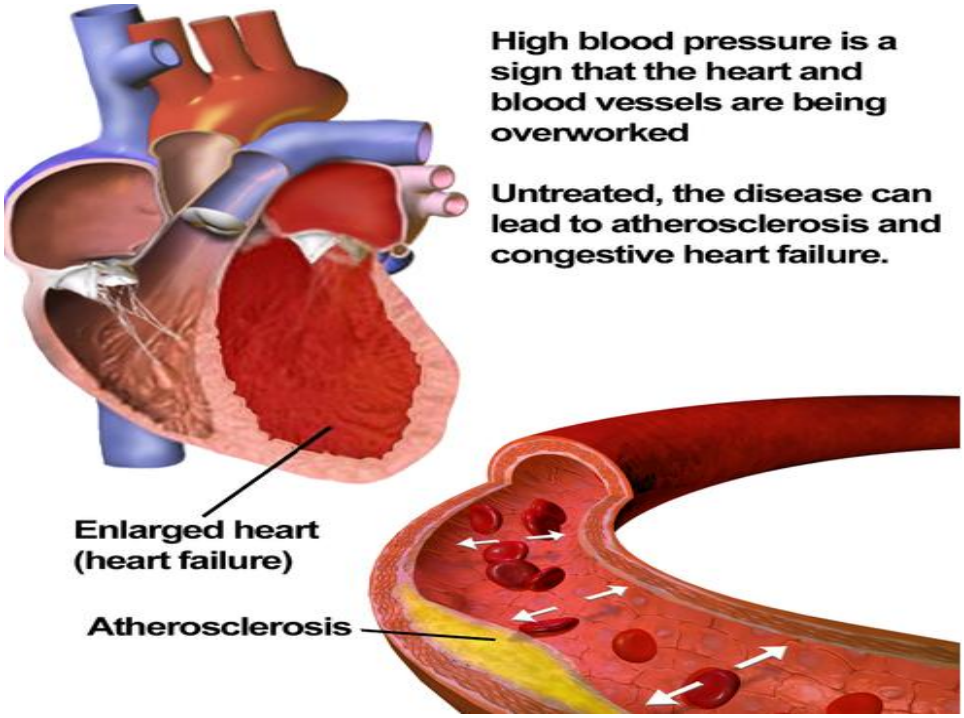


Figure 1.1: Some Factors Leading to High Blood Pressure (Blausen, 2014)

Evidence for genetic influence on blood pressure is reported from various studies (Corvol *et al.*, 1999). There is greater similarity in blood pressure within families than between families, which indicates a form of inheritance (Feinleib *et al.*, 1977). And it was proved that this finding wasn't due to shared environmental factors (Biron *et al.*, 1976). Single gene mutations are proved to cause Mendelian forms of high and low blood pressure. Almost 10 genes have been identified to cause these forms of hypertension (Lifton *et al.*, 2001; Wilson *et al.*, 2001).These mutations affect blood pressure by altering renal salt handling. Recently and with the aid of newly developed genetic analysis techniques researchers found statistically significant linkage of blood pressure to several chromosomal regions, including regions linked to familial combined hyperlipidaemia (Hsueh *et al.*, 2000; Hunt *et al.*, 2002). Overall, however, identifiable single-gene causes of hypertension are uncommon,

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consistent with a multifactorial cause of essential hypertension (Niu *et al.,* 1999; Luft, 2000).

The best studied monogenic cause of hypertension is the Liddle syndrome, a rare but clinically important disorder in which constitutive activation of the epithelial sodium channel predisposes to severe, treatment-resistant hypertension (Shimkets *et al.,* 1994). Epithelial sodium channel activation resulting in inappropriate sodium retention at the renal collecting duct level. Patients with the Liddle syndrome typically present with volume-dependent, low renin, and low aldosterone, and hypertension. Screenings of general hypertensive populations indicate that the Liddle syndrome is rare and does not contribute substantially to the development of hypertension in the general population (Melander *et al.*, 1998).

Autonomic nervous system plays a central role in maintaining cardiovascular homeostasis via pressure, volume, and chemoreceptor signals. It does this by modifying peripheral vasculature and the function of the kidneys, which affect cardiac output, vascular resistance, and fluid retention. Problems with this system, such as excess activity of the sympathetic nervous system, increase blood pressure and contribute to hypertension (Esler, 2000; Takahashi, 2008). In addition, increased activity of the sympathetic accompanied by reduced activity of the parasympathetic has been associated with many metabolic and hemodynamic abnormalities that result in increased cardiovascular morbidity and mortality (Esler, 2000; Brook and Julius 2000). Arterial baroreceptors are reset to a higher pressure in hypertensive patients, and this peripheral resetting reverts to normal when arterial pressure

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is normalized (Xue *et al.,* 2007; Lifton *et al.,* 2001). Furthermore, there is central resetting of the aortic baroreflex in hypertensive patients, resulting in suppression of sympathetic inhibition after activation of aortic baroreceptor nerves. Additional small-molecule mediators that suppress baroreceptor activity and contribute to exaggerated sympathetic drive in hypertension include reactive oxygen species and endothelin (Chapleau *et al.*, 1992), Some studies shown that hypertensive patients manifest greater vasoconstrictor responses to infused norepinephrine than normotensive controls (Ziegler *et al.,* 1991).And that hypertensive patients do not show the normal response to increased circulating norepinephrine levels which generally induces down regulation of noradrenergic receptor, and its believed that this abnormal response is genetically inherited (Calhoun *et al.,* 1993).

Exposure to stress increases sympathetic outflow, and repeated stress-induced vasoconstriction may result in vascular hypertrophy, leading to progressive increases in peripheral resistance and blood pressure (Carretero and Oparil, 2001).This could partly explain the greater incidence of hypertension in lower socioeconomic groups, since they must endure greater levels of stress associated with daily living. Persons with a family history of hypertension manifest augmented vasoconstrictor and sympathetic responses to laboratory stressors, such as cold pressor testing and mental stress that may predispose them to hypertension. This is particularly true of young African Americans. Exaggerated stress responses may contribute to the increased incidence of hypertension in this group (Fujino *et al.,* 2004).

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Another system maintaining the extracellular fluid volume, peripheral resistance and that if disturbed may lead to hypertension, is the renin-angiotensin-aldosterone system (Brenner and Rector, 2004; McConnaughey *et al.,* 1999; Segura and Ruilope, 2007). The activity of local renin–angiotensin systems and alternative pathways of angiotensin II formation may make an important contribution to remodeling of resistance vessels and the development of target organ damage (i.e. left ventricular hypertrophy, congestive heart failure, atherosclerosis, stroke, end-stage renal disease, myocardial infarction, and arterial aneurysm) in hypertensive persons (Hasegawa and Komuro, 2009; Saitoh, 2009).

The endothelium of blood vessels produces an extensive range of substances that influence blood flow and, in turn, is affected by changes in the blood and the pressure of blood flow. For example, local nitric oxide and endothelin, which are secreted by the endothelium, are the major regulators of vascular tone and blood pressure (O'Brien *et al.,* 2007).

Evidence suggests that oxidant stress alters many functions of the endothelium, including modulation of vasomotor tone. Inactivation of nitric oxide (NO) by superoxide and other reactive oxygen species (ROS) seems to occur in conditions such as hypertension (Nakazono *et al.,* 1991; Laursen *et al.,* 1997). Normally nitric oxide is an important regulator and mediator of numerous processes in the nervous, immune and cardiovascular systems, including smooth muscle relaxation thus resulting in vasodilation of the artery and increasing blood flow, suppressor of migration and proliferation of vascular smooth-muscle cells. It has been suggested that angiotensin II enhances formation of the oxidant superoxide at concentrations that affect blood pressure minimally (Cai and Harrison 2000).

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Endothelin is a potent vasoactive peptide produced by endothelial cells that has both vasoconstrictor and vasodilator properties. Circulating endothelin levels are increased in some hypertensive patients, particularly African Americans and persons with hypertension (Ergul *et al*., 1996; Campia *et al.*, 2004).

Persistent high blood pressure puts a strain on the heart, leading to hypertensive heart disease and coronary artery disease if untreated. It is also a major risk factor for stroke, aneurysms of the arteries (e.g. aortic aneurysm), and peripheral arterial disease and is a cause of chronic kidney disease. Even moderate elevation of arterial blood pressure is associated with a shortened life expectancy. Dietary and lifestyle changes can improve blood pressure control and decrease the risk of associated health complications, although drug treatment is often necessary in people for whom lifestyle changes are not enough or not effective. A proportion of people with high blood pressure report headaches (particularly at the back of the head and in the morning), as well as light headedness, vertigo, tinnitus (buzzing or hissing in the ears), altered vision or fainting episodes (Diao *et al.*, 2012). These symptoms, however, might be related to associated anxiety rather than the high blood pressure itself (Arguedas *et al.*, 2009).

On physical examination, hypertension may be suspected on the basis of the presence of hypertensive retinopathy detected by examination of the optic fundus found in the back of the eye using ophthalmoscopy. Classically, the severity of the hypertensive retinopathy changes is graded from grade I–IV, although the milder types may be difficult to

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distinguish from each other (Fisher and Williams, 2005). Ophthalmoscopy findings may also give some indication as to how long a person has been hypertensive (Diao *et al.,* 2012).

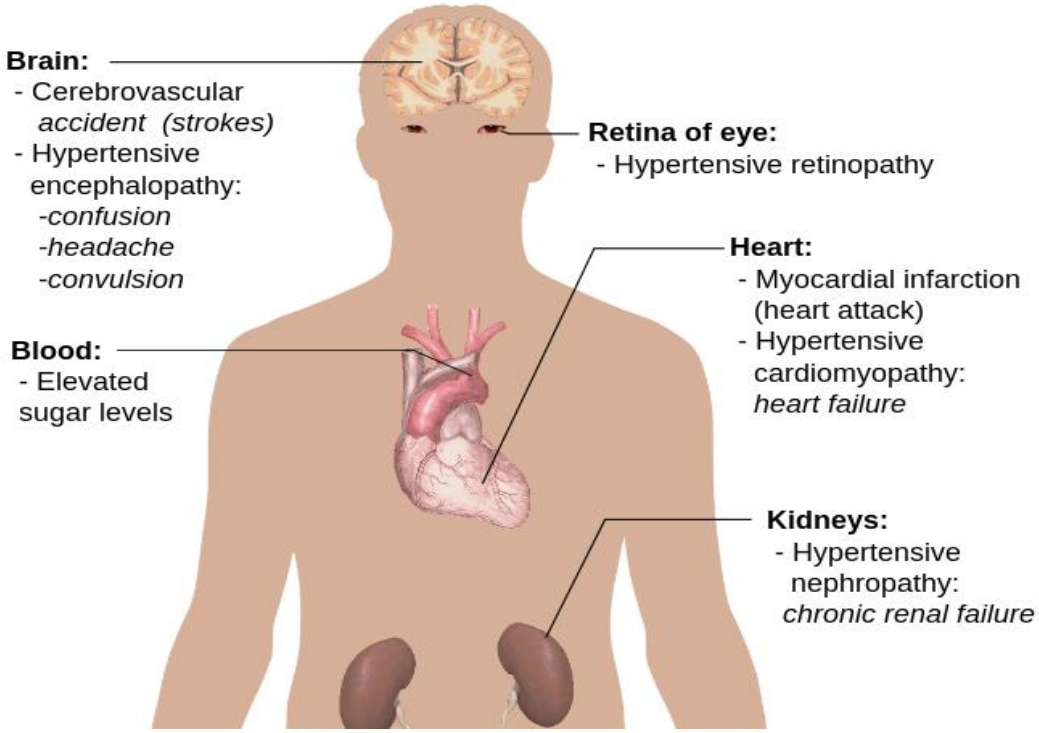


Figure 1.2: Complications of persistent high blood pressure (Haggstrom, 2014)

1.1 Trigonella Foenum-graecum L.

Trigonella foenum-graecum L. (also known as Fenugreek in English, methi in India, Erun in Yoruba and Kimba in Hausa) is an annual plant in the family Fabaceae. The plant has small round leaves, is cultivated worldwide as a semi-arid crop, and is a common ingredient in dishes from the Indian Subcontinent. Major fenugreek-producing countries are India, Iran, Nepal, Bangladesh, Pakistan, Argentina, Egypt, France, Spain, Turkey, Morocco and China (Parthasarathy *et al.,* 2008). The largest producer of fenugreek in the world is India, fenugreek grows an average height of two feet. The leaves consist of three small obovate to

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oblong leaflets; leaves and seeds mature in long pods, are used to prepare extracts or powders for medicinal use (Deepika *et al.,* 2014). Fenugreek has three culinary uses: as a herb (dried or fresh leaves), as a spice (seeds), and as a vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell. Fenugreek seeds are used both whole and in powdered form and are often roasted to reduce their bitterness and enhance their flavor (Sharma *et al.*, 1990).



Figure 1.3: Fenugreek- leaves and seeds (Anitha, 2013)

Fenugreek has been used for its medicinal properties for thousands of years. The earliest recorded use of fenugreek dates back to Egypt in 1500 B.C., according to the National Center for Complementary and Alternative Medicine (NCAM). The Food and Drug Administration has not approved the use of fenugreek for medicinal purposes, but some doctors and pharmacists recommend it for certain medical conditions. Fenugreek is often

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used as a food flavoring (Jill, 2014); They have a strong aroma and a bitter taste. But when used in small quantities they impart flavour to food (Anitha, 2013).

The chemical constituents of fenugreek include: Alkaloides such as trimethylamine, neurin, trigonelline, choline, gentianine, carpaine and betain. Amino acids including isoleucine, 4-Hydroxyisoleucine, histidine, leucine, lysine, L-tryptophan, argenine. Saponins such as graecunins, fenugrin B, fenugreekine (Colin, 2013; Deepika *et al.,* 2014), trigofoenosides A-G. Steroidal sapinogens **:** yamogenin, diosgenin, smilagenin, sarsasapogenin, tigogenin, neotigogenin,gitogenin, neogitogenin, yuccagenin, saponaretin. Flavonoids: quercetin,rutin,vetixin isovetixin. Fibers: gum, neutral detergent. Fibres. Other constituent include coumarin, lipids, vitamins, minerals. 28% mucilage; 22 % proteins; 5 % of a stronger-swelling, bitter fixed oil (Oncina *et al.,* 2000).

The phytochemistry of Fenugreek reveals**:** a. stem: Fenugreek contains a number of steroidal sapogenins. The diosgenin were found in the oily embryo. Two furastanol glycosides, F-ring opened precursors of diosgenin have been reported, as also hederagin glycosides. The alkaloid trigonelline, trigocoumarin, trimethyl coumarin and nicotinic acid are present in stem (Shailendra and Garima 2014). Mucilage is a prominent constituent of the seeds. About 28 % mucilage; 5 % of a stronger-smelling, bitter fixed oil, 22 % proteins; a volatile oil; two alkaloids, Trigonelline and Choline, and a yellow colouring substanceare present in stem (Helambe and Dand, 2003). b. leaf**:** The leaves contain 7 saponins, known as graecunins. These compounds are glycosides of diosgenin. Leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals 1.5%, fiber 1.1%, and carbohydrates 6%. The

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mineral and vitamins contents are calcium, iron, phosphorous, carotene, thiamine, riboflavin, niacin and vitamin C (Mehrafarin *et al.*, 2011). c. seed: Fenugreek Seeds are aromatic, bitter, carminative, galactogouge, antibacterial and may be eaten raw or cooked. Bulk of the seed is dietary fiber (50%) and protein (30%) both of which have no taste or flavor. The chemical components of fenugreek seeds include a large carbohydrate fraction (mucilaginous fiber, galactomannan); 20-30% proteins high in tryptophan and lysine; pyridine-type alkaloids; flavonoids; free amino acids (4-hydroxyisoleucine, arginine, lysine, histidine); saponins; glycosides; vitamins, minerals, (28%) mucilage, (22 %) proteins, 5 % of a stronger-smelling, bitter fixed oil. volatile oils. Bitterness is mainly due to the oil, steroidal saponins and alkaloids. Historically used as a culinary and medicinal herb, recent research studies have shown its effectiveness in reducing blood glucose levels, promoting lean body mass, lowering cholesterol, and treating gastrointestinal disorders. Studies has shown the role of Fenugreek seeds in adjustment of some enzymes, including those related to glucose and lipid metabolism. Preliminary research with type-1 diabetics suggest that fenugreek may aid insulin secretion and may reduce total cholesterol and LDL cholesterol levels (Vats *et al.*, 2002 and Zia *et al.*, 2001).

Seeds of Fenugreek contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis. Plant tissue cultures from seeds grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller amounts of gitongenin and trigogenin. The seeds also contain the saponin fenugrin B. Several coumarin compounds have been identified in fenugreek seeds as well as a number of alkaloids (eg, trigonelline, gentianine, carpaine) (Ragni *et al*, 2016). A large proportion of the trigonelline is degraded to nicotinic

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acid and related pyridines during roasting. These degradation products are, in part, responsible for the flavor of the seed. The seeds also yield as much as 8% of a fixed, foul-smelling oil. Three minor steroidal sapogenins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin (Oncina *et al.,* 2000).

Fenugreek seeds contain moisture 13.7%, protein 26.2%, fat5.8%, minerals (calcium, carotene, thiamine, niacin and riboflavin) 3.0%, fiber 7.2% and carbonhydrates 44.1% per 100g. Fenugreek leaves contain moisture 86.1%, protein 4.4%, fat 0.9%, minerals(calcium, carotene, thiamine, niacin and riboflavin phosphorous vitaminC) 1.5%, fiber 1.1%, and carbonhydrates 6.0 per 100g (Mullaicharam *et al.,* 2013). It also contains lecithin, choline, minerals, B. Complex, iron, Phosphates, PABA (Para-Amino Benzoic Acid) and vitamins A, D and a rich source of vitamin K (Anwesha, 2011).

1.2 Statement of the Research Problem

By 2030, more than 23million people may die annually of CVD worldwide. The major causes of CVD are tobacco use, physiocal inactivity, an unhealthy diet and harmful use of alcohol (WHO, 2014). Of WHO's six regions, Africa has the highest prevelence of CVD particularly High blood pressure of extimated 46% of adults aged ≥25 The problem caused by high blood pressure worsen on those who are unaware of the risk or cannot afford checkups (WHO, 2011).

Although oxidation reaction is crucial to life, it produces free radicals that damage cells and body function in general. The systems of complex and multiple types of antioxidants, such

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as glutathione, vitamin C, vitamin A, vitamin E as well as enzymes like catalase, peroxidases and superoxide dismutase, are widely used in dietary supplements and have been investigated for prevention of diseases like cancer, coronary heart diseases and neurodegenerative diseases (Jha *et al.*, 1995).

In the last three decades, a lot of concerted efforts have been channeled into researching into local plants with hypotensive or antihypertensive therapeutic values. The hypotensive or antihypertensive effects of some of these medicinal plants have been validated and others disproved. Attempts by the low-income group, particularly the rural dwellers in the developing countries, to control high blood pressure and its attendant complications in the face of the scarce socioeconomic resources, have led more people opting for herbal remedy. However, more scientific research needs to be done to verify the effectiveness and elucidate the safety profile of such herbal remedies (Farnworth *et al.*, 2001).

1.3 Justification of the Study

People in low and middle-income countries who suffer from CVDs and other no communicable diseases have less access to effective and equitable health care service which responds to their needs. As a result, many people in low and middle-income countries are detected late in the course of the disease and die younger from CVDs and other non communicable diseases, often in their most productive years (WHO, 2014).

Attempts by the low-income group, particularly the rural dwellers in the developing countries, to control high blood pressure and its attendant complications in the face of the

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scarce socioeconomic resources, have led more people opting for herbal remedy (WHO, 2011).

Fenugreek is known to contain many medicinal properties. The seed extract displayed a positive effect as positive cardiotonic, hypoglycaemic, diuretic, antiphlogistic and hypotensive agents (Duke and Ayensu, 1985). Moreover, a more recent research suggests that *Trigonella foenum-graecum L.* has antioxidant, antihypertensive, anticancer, antithromolic effect (Doshi *et al.,* 2012). There different ways of preventing and treating CVDs, beside drug therapy and life style changing, current study's interest focus on plant based natural drug treatment exploiting the said properties of Fenugreek.

Also, the chemical constituents of fenugreek include: B-carotene, vitamin C, Vitamin E and Vitamin A; all of which are potent antioxidants and are expected to preserve endothelial function via free radical scavenging activity among others (Muhammad *et al.,* 2013).

Therefore with the dearth of information suggests that Trigonella *foenum-graecum L.* would have not only Blood Pressure lowering effect but also possesses preventive and restorative effect on cardiovascular system.

1.4 Aim and Objectives

1.4.1 Aim

This research work was undertaken to determine effects of Hexane and Methanol extracts of *Trigonella foenum graecum L.* on blood pressure and cardiac contractility.

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1.4.2 Objectives

i. To determine acute toxicity level of Hexane and Methanol extracts of *Trigonella foenum graecum L.*

ii. To determine effects of Hexane and Methanol extracts of *Trigonella foenum graecum L.* on cat blood pressure.

iii. To determine effects of Hexane and Methanol extracts of *Trigonella foenum graecum L.* on cardiac contractility of rabbit.

1.5 Research Hypothesis

Hexane and Methanol extracts of *Trigonella foenum graecum L.* have no effect on cardiovascular system.

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CHAPTER TWO

2 Literature Review

The renewed interest in the search for new drugs from natural sources, especially from plant sources, has gained global attention during the last two decades. 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. Thus, natural plants and herbs can be our source of drugs, with fewer side effects and better bioavailability for treatment of HTN in future (Nahida and Feroz, 2011)

Many herbs have been used locally to decrease high blood pressure, these include: *Radish Rauwolfia serpentine, Sesamum Indicum, Solanum sisymbriifolium, Theobroma cocoa, Titicum aestivum, Uncaria tomentosa, Viscum album, Prunus domestica, Zingiber officinale e.t.c* (Sade, 2014).

Glycine max (Family: Fabaceae Common name: Soybean). Soybean has been found to be effective as a hypotensive agent. One study has shown a very modest reduction in BP (Sacks *et al.,* 2006)

Hibiscus sabdariffa (Family: Malvaceae; Common name: Roselle). This happens to be one of the most extensively studied plants for positive cardiotonic properties. The leaves, calyx, and corolla of this plant are used traditionally in many West African countries for various

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medicinal purposes and as edibles. Effects of this plant extracts on blood pressure have been variously studied (Sarr *et al.,* 2009).

Lycopersicon esculentum (Family: Solanaceae; Common name: Tomato). Tomato extract contains carotenoids, such as lycopene, beta carotene, and vitamin E, which are known as effective antioxidants, to inactivate free radicals and to slow the progress of atherosclerosis (Engelhard *et al.,* 2006).

Musanga cecropiodes (Family: Cecropiaceae; Common name: Umbrella tree, Cork Wood). It is a rapidly growing plant ubiquitous to the tropical rain forests, particularly of West Africa. The water extract of the stem bark has been reported to produce a dose-dependent reduction in mean arterial BP (Adeneye *et al.,* 2006).

Phyllanthus amarus (Family: Euphorbiaceae; Common name: Nela nelli). This plant is used as a diuretic and also been used to lower BP in traditional medicine practice (Fabian and Eric, 2007).

Raphanus sativus (Family: Cruciferae; Common name: Radish): The plant has been found to have positive cardiotonic activity. Isolated tissue preparations were suspended in tissue baths containing Krebs solution, while acute toxicity study was performed in mice for 24 hours. The extract caused a dose-dependent (0.1-3 mg/kg) caused fall in BP and HR of rats that was mediated through an atropine-sensitive pathway (Ghayur and Gilani, 2006).

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Zingiber officinale (Family: Zingiberaceae; Common name: Ginger). Ginger root is commonly used in Asian cooking. It acts to improve blood circulation and relaxes muscles surrounding blood vessels (Nicoll and Henein, 2009) e.t.c

Trigonella foenum-graecum is greatly used as medicinal plant, particularly in North Africa, the Middle East and India. It contains a broad spectrum of therapeutic properties (Atefeh, 2013). In 1995, Pierre *et al.* reported that the steroidal saponins in the plant enhance food consumption, reduce plasma cholesterol levels and motivation to each. It is a highly potent female herb, since it helps relaxing the uterus and relieving menstrual pains, (Health from Nature, 2011) and is an excellent stimulator of milk production (galactagogue) in nursing mothers and increase breast size (Chevallier,1996; Chantry *et al.,* 2004; Turkyilmaz *et al.*, 2011 ; Jill, 2014). Also, Fenugreek seed has been found to be rich in protein with essential amino acids, Iron, Ascorbate and Folate content and may have restorative and nutritive properties; this might help to prevent and cure anemia and help maintain good health for a long time (Doshi *et al.,* 2012).

Report showed that diosgenin is a compound that has properties comparable to the primary female sex hormone estrogen. Because it has properties similar to estrogen, fenugreek is hailed for enhancing libido (Basch *et al.,* 2003; John, 2011; Steels *et al.,* 2011 and Colin, 2013) and protective effect on thioacetamide induced hepatotoxicity (Zargar, 2009). Both oral and I.P administration of the plant glycoside extract exert toxic effect on central nervous system (Jamal *et al.,* 2000).

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Several human intervention trials demonstrated that the antidiabetic effects of fenugreek seeds ameliorate most metabolic symptoms associated with type-1 and type-2 diabetes in both humans and animals by reducing serum glucose and improving glucose tolerance (Sharma and Raghuram, 1990; Hamodi, 2006, Xue *et al.*, 2007; Gall and Zerihum, 2009; Gupta *et al.*, 2001), anti-inflammatory (Duke and Ayensu, 1985) and preventive and curative effect of the plants has been shown on C57BL/6J models of Type 2 diabetic induced high fat diet (Nawal *et al.*, 2012).

Furthermore, the herb is believed to be a natural hormone replacement therapy for women experiencing menopause. It is potent for treatment of body odour produced by thick mucus and toxins in nasal and oral passages, leucorrhea. It is helpful in lowering blood sugar and cholesterol levels (Himalaya, 2012)

Extracts of *Trigonella foenum-graecum L.* seeds and its saponin constituents have been revealed to have anticarcinogenic ability. The seed extract has been assessed in the Ehrlich ascites carcinoma model in BALB/c mice and it demonstrated 70% inhibition of tumor cell growth compared to control group (Sur *et al.,* 2001 and Hibasami *et al.,* 2003). Also. In 1999 Viswaria et al reported that the debitterised fenugreek powder lacks propensity to induce acute toxic effect on both mice and rats.

Pandian *et al.* 2002 speculated that the polysaccharide compositions of the gel and/or the flavonoids are responsible for the gastroprotective and anti-secretory activities of the seeds.

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Trigonella foenum-graecum L. seeds have been proved to influence immune system (Bin-Hafeez *et al.,* 2003). The seed is extremely nutritious and are suggested to improve body weight gain, particularly in anorexia nervosa. It also improved feed intake and protein utilization in rats (Chevallier, 1996), it has insecticidal activity (Pemonge *et al.,* 1997), Alkaloid derived from fenugreek's seed showed high capacity in cancer treatment and prevent hyperlipidaemia and atherosclerosis in diabetic cases (Raghuram *et al.,* 1994). A study at the Australian Centre for Integrative Clinical and Molecular Medicine found that men aged 25 to 52 who took a fenugreek extract twice daily for six weeks scored 25% higher on tests gauging libido levels than those who took a placebo (John, 2011; Steels *et al.,* 2011).

On external application, Fenugreek helps heal superficial wounds more quickly and because of its rich protein content, the herb is an excellent ingredient in hair care and skincare like skin abscesses, boils, burns, eczema and gout. Fenugreek has soluble fiber acclaimed for promoting weight loss because it causes your digestive tract to enlarge and successfully control appetite (Colin, 2013).

Anti-microbial effect of all samples from essential oils of the plant *Trigonella focnum greacum L.* have shown interesting biological activity of the five bacterial strains and one fungal strain (Mehani and Segni 2012).

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Fenugreek has been found to have hypoglycemic effect on normal mice (Tayyaba *et al* 2001). Also, the plant has shown anticataract activity of *Pterocarpus marsupian* bark and fenugreek seeds in alloxan induced rats.

Pemonge *et al*. (1997) reported the antioxidant and antifatigue properties of Fenugreek in rats subject to weight loaded forced swim test where they showed that the plant enhances physical activity.

In 2004, Flammang *et al.* studied the potential genotoxicity of a fenugreek seed extract (THL), containing a minimum of 40% 4-OH-ILE, using the standard battery of tests (reverse mutation assay; mouse lymphoma forward mutation assay; mouse micronucleus assay). THL was determined not to be genotoxic under the conditions of the tested genetic toxicity battery. The negative assay results provide support that addition of THL to foodstuffs formulated for people with diabetes is expected to be safe.

In 2006 Bathia *et al.* reported protective effect of fenugreek, on lipid peroxidation (LPO) and on enzymatic anti-oxidants. Cyclophosphamide treated animals exhibited a significant decrease in the activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GP) and catalase (CAT) when compared to the controls. Level of reduced glutathione (GSH) was also reduced with an increase in LPO in CP-treated animals. L-buthionine-SR-sulfoximine (BSO) treatment depicted an additive toxic effect in CP-treated animals. Pre-treatment of herbal extract restored activities of all the enzymes and thus showed an overall protective effect on additive effect of CP and BSO. Restoration

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of GSH by extract treatment may play an important role in reversing CP-induced apoptosis and free radical mediated LPO in urinary bladder.

Sayeda *et al*. (2008) reported that Fenugreek seeds extract such as methanol, ethanol, dichloromethane, acetone, hexane and ethyl acetate have a chelating activity, reducing power and antioxidant/radical scavenging activity (1,1-diphenyl-2-picryl-hydrazyl (DPPH°) free radical scavenging activity).

Kumar *et al.* (2012) reported reversed hyperglycemia by decrease lipid peroxidation in diabetic rats brain, restoring membrane fluidity, activities of membrane bound and antioxidant enzymes, thereby ameliorating diabetic complications. While in 2012 also, Ali *et al.* reported the antinociceptive and anti-inflammatory effect of Alkaloid and Flavonoid fractions of fenugreek powder.

Taku *et al.* (2014) found that fenugreek ameliorates hepatic steatosis and hyperlipidemia by suppressing the mRNA expression of lipogenic genes in the liver of obese diabetic mice while Omezzumi *et al* (2013) reported the antifungal activities against phytopathogenic fungai in vitro and have the potential to be used as antifungal agent for control of FORL & FOL on tomato plant.

Deepika *et al.* (2014) reported the cardioprotective property of fenugreek against Isoproterenol Induced Myocardiac Infarction Rats from biochemical and histopathological evidence in animals pre-treated with 200mg/kg and 400mg/kg of ethanol extract of

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Trigonella foenum-graecum L. Also, Nithya *et al.* (2014) reported the effect of fenugreek seed on glycemic control in patient with diabetis mellitus.

Enas *et al.* (2015) reported antispasmodic, antioxidant and antimicrobial effects of Ethyl acetate, Acetone and Aquatic extracts of *Trigonella foenum-graecum L.* and *Artemisia annua* using rabbit jejunum, DPPH method and paper disc method respectively. The same year Thoutreddy *et al.* reported anti-obesity and anticoagulant activity of ethanol extracts of *Trigonella foenum-graecum L.* in diet induced weight wistar rats measuring TC, LDL, VLDl and HDL levels on the 8^{th} day as well as clotting time on the 48^{th} day.

Fenugreek is considered to be nutraceutical plant (Shivangi *et al.,* 2016) for its role in increase health benefit and reduces risk of carsiovascular diseases by its cholesterol lowering effect, free radical scavenging role and/or antioxidant role (Ashwani and Peiman 2014)

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CHAPTER THREE

3 Materials and Methods

3.1 Materials

3.1.1 Plant materials

Trigonella foenum-graecum seeds were purchased from a farm in Tudun Wada, Kaduna North Local Government of Kaduna State, Nigeria. Authentication and identification was done by Malam Musa of Department of Biological Sciences, Ahmadu Bello University, Zaria. A specimen was deposited and a voucher number 494 assigned for future reference.

3.1.2 Experimental animals

A total of 4 adult rabbits (1 -1.2 kg) 4 young adult male cats (0.9-1.5 kg) and 13 adult albino Wistar rats (150-200g) were weighed using Gallme Mattler Balance and were used for the experiments. The animals were obtained and maintained in the animal house of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, where they were fed with standard rabbit pellets and mouse cubes (Livestock Feed) and allowed free access to water.

3.1.3 Drugs and chemicals

Thiopental sulphate**,** Atropine sulphate 25mg/ml, Promethaxine maleate, heparin, Methanol 95% v/v (2.5L), tween 80, dried seeds of *Trigonella foenum-graecum L.* (140g).

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3.1.4 Laboratory Tools and Equipments

21-G butterfly canula, Pressure transducer, Ugo basile twin channel recorder, thermometer, mercury sphygmomanometer , Petri-dish, Ringer-Locke solution, glass canula, force displacement transducer, thermostated water bath, Gallme Mattler Balance , mortar, pestle, water bath, beaker, dessert spoon, Soxhlet apparatus, timble, grease, distilled water, anti-bubble chips, separating funnel, cotton wool, evaporating dish, Viking Joncod electric milling machine (model: 9809005), cages and feeders.

3.2 Methodology

3.2.1 Plant preparation

3.2.1.1 Methanol extraction

The dried seeds of *Trigonella foenum-graecum L.* were powdered using mortar and pestle and then sieved. The weighed powdered seed sample (approx. 140g) was poured into a separating funnel. Then the solvent (95% v/v Methanol) was poured sufficiently to cover the plant and it was allowed to stand for 24hours after which it was drained and rewashed with more ethanol. The filtrate was poured into an evaporating dish and placed on a water bath at about 60^0C until when all water molecules had evaporated leaving behind the extract residue dark brown in colour. The extract residue was craved and poured into a suitable container (about 78g). The extraction was adopted from the method of cold maceration (woo *et al.*, 1980).

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3.2.1.2 Hexane extraction

The dried seeds of *Trigonella foenum-graecum L.* were reduced to fine powder using a Viking Joncod electric milling machine (model: 9809005). The powdered seed (140 g) was be extracted using a Soxhlet apparatus. Oils from the seed was extracted by repeated washing or percolation with hexane under reflux in a special glass ware.

3.2.2 Acute toxicity test

The lethal dose (LD_{50}) of the plant extract was determined using the method of Lorke (1983) using 13 rats. A total nine rats were grouped into 3 groups of 3 each for the first phase of the LD_{50}. 5g of the extract was diluted with 45ml of distilled water to make100 mg/ml stock solution, 1000mg/ml and 10mg/ml were diluted from the stock concentration. Group 1 was administered intraperitoneally with dose of 1000 mg/kg of body weight, Group 2 was given 100 mg/kg and Group 3 was given 10mg/kg.

Four rats were grouped into four (one for each) for the second phase of the LD_{50}, each was administered intraperitoneally with 140 mg/kg, 225 mg/kg, 370 mg/kg and 600 mg/kg per group respectively. The median lethal dose (LD_{50}) was calculated using the second phase.

3.2.3 Cat blood pressure experiment

The experiment was in vivo and 4 cats were used. The cat was anesthetized by injecting Thiopentane sodium at the dose of 50mg/kg via intraperitoneal route. The animal lost consciousness after 20 minutes of administration, after which it was properly secured to the dissecting table by using twine to tie its limbs, so as to prevent unwanted movement of the

animal during the experiment. The left femoral vein was exposed and tied with peripheral ligature. Also, the right internal carotid artery was exposed and cannulated with plastic 21-G butterfy cannula for blood pressure measurement using Ugo Basile twin channel recorder (model 7090). The basal blood pressure was recorded on a filter paper of the micro-dynamometer after the administration of the heparinized normal saline to prevent blood clothing. The drugs and extract were administered through the cannula inserted through the left femoral vein just above the cannula. The speed and sensitivity of the machine were 95mm/minute and 1 respectively. The first set of drugs recorded were the standard drugs (Ach and Adrenaline), followed by graded dose response for each extract (0.1ml, 0.4 ml and 0,8 ml respectively), and the dose which caused maximum effect was chosen as experimental dose. Flushing was properly done after every administration of extract and drugs till blood pressure was brought back to normal.

One centimetre (1cm) of the standard ruler used in measuring the systolic and diastolic blood pressure change corresponds to 10mmHg pressure change in glass sphygmomanometer. On the tracing the values from the baseline to the lowest border of the tracing represent the diastolic pressure while from the baseline to upper border represent the systolic pressure (Ahmed *et al.,* 1972; Fabian and Eric, 2007; Bako *et al.,* 2010)

3.2.4 Isolated rabbit perfused heart experiment

The experiment was carried out in accordance with the Langendorff, procedure (Langendorff, 1983). Each rabbit was injected with 1000 IU of heparin intravenously through the marginal ear vein. Five minutes later, the rabbit was sacrificed, dissected and

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the heart with about 1 cm of the aorta attached, was removed as quickly as possible and transferred into a Petri-dish containing Ringer-Locke solution (NaCl; 45.0 g, NaHCO3; 1.0 g, D glucose; 5.0 g, KCl; 2.1 g, CaC_{12}·2H2O; 1.6 g, in 5 L of distilled water). The heart preparation was gently squeezed several times to remove as much as possible of residual blood. The aorta was re moved just below the carotid bifurcation. The heart was be transferred to the perfusion apparatus and tied on to the glass canula. A small spring clip was attached to the apex of the ventricle and was connected to a force displacement transducer (model 82125) via two pulleys. The perfusate fluid was Ringer-Locke solution, which was continuously bubbled with oxygen. The fluid was allowed constant flow rate from a reservoir maintained at 37^0C by water circulated through thermostat water bath. The first set of drugs recorded were the standard drugs (Ach and Adrenaline), followed by graded dose response for each extract (0.1ml, 0.4 ml and 0.8 ml respectively), and the dose which caused maximum effect was chosen as experimental dose. Flushing was properly done after every administration of extract and drugs till heart beat was brought back to normal.

3.2.5 Interactive reactions

The observed effect of the Methanol and Hexane extracts of *Trigonella foenum-graecum L.* was similar to that of Acetylcholine, therefore, Machanism of action of Acetylcholine was blocked with Atropine Sulphate and interaction was attempted using high volume of Methanol extract i.e 0.8 ml of 100mg/ml that produce highest observed action (Appendix xxiii and xxiv).

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3.3 Statistical Analysis

Data collected were analysed using SPSS version 20 and expressed as Mean ± SEM. All analysis of significance was done using one way ANOVA of variance and dependant Paired *t* test; values with $p \leq 0.05$ were considered significant

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CHAPTER FOUR

4 Results

4.1 Acute Toxicity Study (LD_{50})

The acute study on both Hexane and Methanol Extracts of *Trigonella foenum graecum L.* in albino rats was found to be above 5000 mg/kg according to the method of Lorke (1983).

4.2 Isolated Rabbit Perfused Heart

Both the rate and force of myocardial contraction were depressed in a dose dependent manner, with a greater effect on the rate than on the strength of contraction. Hexane extract of *Trigonella Foenum-gaecum L.* at dose 0.1 ml decreases the strength of contraction from 4.8 ± 0.3 to 2.7 ± 0.3 cm^{-1}, Methanol extract of *Trigonella Foenum-gaecum L.* at dose 0.8 ml decreases it from 4.5 ± 0.0 to 3.6 ± 0.0 cm^{-1} which was similar to Acetylcholine 10 µg/ml whose decrease was from 6.0 ± 0.15 to 2.55 ± 0.15 cm^{-1} (Table 4.1). This explains that the plant seed of *Trigonella Foenum-gaecum L.* causes decrease in strength of contraction of rabbit myocardium.

Ach 10 µg/ml at dose of 0.1 ml decreases the speed of contraction from 2.02 ± 0.03 to 1.94±0.05 number of beats/sec, hexane extract 10 mg/ml at dose of 0.8 ml decreases the speed of contraction from 0.83 ± 0.04 to0.49 ± 0.04 beats/min, while methanol extract of 10 mg/ml at dose of 0.4 ml decreases the speed of contraction from 1.70 ± 0.0 to 1.11 ± 0.08 beats/sec respectively (Table 4.1). This means that the plant of *Trigonella Foenum-gaecum L.* has vasorelaxant effect that slows rate of contraction s^{-1} on the rabbit myocardium.

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Table 4.1: Effects of Hexane and Methanol Extracts of *Trigonella foenum graecum L.* on Strength of Contraction cm^{-1} of Rabbit Isolated Heart tracing.

Concentrations (mg/ml)	**Before administration**	**After administration**
Ach (10 µg/ml)	6.00 ± 0.00	2.55 ± 0.15**
Adr (10 µg/ml)	4.35 ± 0.45	1.56 ± 0.24**
Hexane Extract (0.1 mg/ml)	4.80 ± 0.30	2.70 ± 0.30**
Hexane Extract (1 mg/ml)	5.10 ± 0.00	6.15 ± 0.15**
Hexane Extract (10 mg/ml)	4.80 ± 0.30	10.35 ± 7.65*
M. Extract (0.1 mg/ml)	5.40 ± 0.60	4.80 ± 0.90*
M. Extract (1 mg/ml)	4.50 ± 0.00	3.60 ± 0.00**
M. Extract (10 mg/ml)	4.95 ± 0.15	1.80 ± 0.00**

** Statisitically significant $P \leq 0.05$ * Not statistically significant $P \geq 0.05$ (paired *t- test*)

n=3

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Table 4.2: Effects of Hexane and Methanol Extracts of *Trigonella foenum graecum L.* on Speed of Contraction Number of Beat/sec of Rabbits Isolated Heart.

Concentrations (mg/ml)	**Before administration**	**After administration**
Ach (10 µg/ml)	2.02 ± 0.03	1.94 ± 0.05*
Adr (10 µg/ml)	2.16 ± 0.30	2.32 ± 0.30*
Hexane Extract (0.1 mg/ml)	1.42 ± 0.08	1.15 ± 0.04*
Hexane Extract (1 mg/ml)	5.10 ± 0.00	1.54 ± 0.04*
Hexane Extract (10 mg/ml)	0.83 ± 0.04	0.49 ± 0.07**
M. Extract (0.1 mg/ml)	1.66 ± 0.00	1.49 ± 0.07*
M. Extract (1 mg/ml)	1.70 ± 0.00	1.11 ± 0.08**
M. Extract (10 mg/ml)	0.46 ± 0.02	0.87 ± 0.08**

** Statisitically significant $P \leq 0.05$ * Not statistically significant $P \geq 0.05$ (paired *t- test*)

n=3

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4.3 Cat Blood Pressure

The results of the present study has shown that, the Hexane and Methanol extracts of *Trigonella foenum-graecum L.* demonstrate blood pressure lowering effect in normotensive cats where statistical difference of ($P \leq 0.05$) was considered significant as obtained from the micro dynamometer (Table 4.3).

The effect of methanol and hexane extracts of the seeds of *Trigonella Foenum-gaecum L.* on blood pressure was evaluated in normotensive cats. Intravenously administered doses (0.1ml, 0.4 ml and 0.8 ml) of the extracts to anaesthetized normotensive cats produced a fall in mean diastolic and systolic and mean arterial pressures in a graded dose response manner with significant fall at dose of 0.8 ml in hexane extract diastolic (22.50 ± 4.50 mmHg) and methanol extract diastolic (13.50 ± 2.50 mmHg) respectively when compared to Acetylcholine which is a standard drug. This proves that the plant of *Trigonella Foenum-gaecum L.* has blood pressure lowering effect either by direct effect reducing the myocardiac contractility or decrease sympathetic activity. The dose of Acetylcholine 10μg/ml produced significant fall in mean systolic, diastolic, and mean arterial pressures of 22.0 ± 4.00 mmHg, 13.50 ± 2.50 mmHg, and 16.3 ± 2.5 mmHg which is expected as the acetylcholine was used as standard or positive control (Table 4.4).

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Table 4.3: Comparison of blood pressure among Normal Basal Rhythm, Acetylcholine and Hexane Extract of *Trigonella foenum graecum L.*

B. P	**Basal**	**Ach**		**Hexane Extracts**	
(mmHg)	**Rhythm**	**10µ/ml**	**1 mg/ml**	**10 mg/ml**	**100 mg/ml**
Systolic B.P	27.00 ± 1.00	22.00 ± 4.00^{S}	29.50 ± 2.50^{NS}	29.50 ± 2.50^{NS}	29.50 ± 2.50^{NS}
Diastolic B.P	25.50 ± 1.50	13.50 ± 2.50^{S}	22.50 ± 4.50^{NS}	24.50 ± 5.50^{NS}	14.50 ± 0.50^{S}
Pulse Pressure	1.50 ± 0.50	13.50 ± 1.50^{NS}	10.00 ± 3.00^{NS}	5.00±1.50^{NS}	6.50±1.50^{NS}
M.A.B.P	26.00 ± 1.00	16.30 ± 2.30^{S}	24.60 ± 2.80^{NS}	25.85 ± 0.85^{NS}	18.00 ± 0.00^{NS}

S = Statistically Significant $P \leq 0.05$ NS= Not Statistically Significant $P \geq 0.05$

(ANOVA) n=3

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Table 4.4: Comparison of Blood Pressure Among Normal Basal Rhythm, Acetylcholine and Methanol Extract of *Trigonella foenum graecum L.*

B. P	**Basal**	**Ach**	**Methanol Extracts**		
(mmHg)	**Rhythm**	**10µ/ml**	**1 mg/ml**	**10 mg/ml**	**100 mg/ml**
Systolic B.P	27.00 ± 1.00	22.00 ± 4.00^{S}	16.50 ± 1.00^{NS}	19.50 ± 2.50^{NS}	22.50 ± 2.50^{NS}
Diastolic B.P	25.50 ± 1.50	13.50 ± 2.50^{S}	16.00 ± 4.50^{NS}	19.50 ± 5.50NS	13.50 ± 0.50^{S}
Pulse Pressure	1.50 ± 0.50	13.50 ± 1.50^{NS}	9.00 ± 1.00^{NS}	5.00±1.50^{NS}	4.50 ± 1.50^{NS}
M.A.B.P	26.00 ± 1.00	16.30 ± 2.30^{S}	16.50 ± 2.80^{NS}	19.80 ± 1.15^{NS}	16.20 ± 1.10^{NS}

S = Significant $P \leq 0.05$ NS= Not Significant $P \geq 0.05$ (ANOVA) n=3

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CHAPTER FIVE

5 Discussion

Pharmacologically, orthodox drugs exert hypotensive effects by various mechanisms. Hypotensive drugs could lower blood pressure by a direct effect on the heart, in which case the force and rate of myocardial contraction are decreased, e.g. propranolol, a beta-receptor antagonist, some may relax the vascular smooth muscle, in which case the total peripheral resistance is decreased; e.g. alpha adrenoceptor antagonists like prazosin, and nifedipine a calcium ion channel blocker.

The Acute Toxicity Study on both hexane and methanol extracts of *Trigonella foenum-graecum L.* seeds showed that the plant has LD_{50} greater than 5000 mg/kg, this is safe to say that the plant is safe for consumption, thus consumption of *Trigonella foenum-graecum L.* seeds in large quantity has not been reported to have any fatal or adverse effect. This is supported by Fedelic *et al.* (2009) as Acute Toxicity study and result of their experiment has shown that the plant is not toxic and that taking it in large dose has no fatal effect after administration for 90 days. While in the study of Latha *et al.* (2016) on Acute and Repeated Toxicity reported slight signs of toxicity at the dose of 3000mg and 5000 mg/kg.

The decrease in blood pressure observed in the present study in intravenously administered graded doses of the hexane and methanol extracts *Trigonella foenum-graecum L.* in our result is contrary to the report on Fabian and Eric (2007) and Bako *et al* (2010) where they all reported significant fall in blood pressure at lower doses of their extracts with

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similar chemical constituents as *Trigonella foenum graecum L.* The Achetycholine used produced significant fall in mean diastolic, systolic, and mean arterial pressure.

Since *Trigonella foenum graecum L.* contains numerous chemical compounds, including tetracyclic compounds known as, saponins, diosgenin, choline and trigonelline, glucosides, and other organic acids, and so on (Ross, 1999), anti-cholesterol: terpenes, antioxidants : polyphenols and ascorbic acid (Ashwani and Peiman 2014) chemical compounds might probably be responsible for the observed blood pressure lowering effects of the plant extracts. However, the pharmacological actions of the extracts unveiled in this study appear to provide some rational explanations and justifications for the therapeutic applications of the herb in anecdotal, folkloric, African traditional medicine.The result of the research work is similar to a work published by Fabian and Eric (2007) on the hypotensive of aqueous extract of phyllathus amarus and euphorbiaseae at a dose dependant manner. Also, the potent vasodilation effect might have resulted from relaxation of smooth muscle of the blood vessels in a similar manner to its effects on other smooth muscles as reported by Yahia *et al* (2010) that Flavonoids in general have almost consistently and caused hypotension under different conditions using different models of study.

Both the rate and force of myocardial contraction were depressed in a concentration dependent manner, with a greater effect on the rate than on the force of contraction. Hexane extract at lowest dose that decreases the strength of contraction this was contrary to Methanolic extract decreases myocardial contraction significantly at the highest dose similar to Ach 10 µg/ml. the different effects observed using different method of extraction

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could be as a result of different active constituents present in both extracts that act on different pathways

The Ach in the study decreases the speed of contraction significantly while Hexane and Methanol extracts have similar effect; both decrease the speed and strength of contraction in a dose dependant manner. The observed effect may be due to flavonoids, lysine, L-tryptophan and steroidal saponins as reported by Colin (2013). This is in line with a report by Chukwuemeka *et al* (2012) where they confirmed the role of flavonoids in endothelium dependant vasorelaxant effect. However, this finding is contrary to the report of Yahia *et al* (2010) who reported increase in cardiac contractility in an isolated perfused heart experiment.

The Atropine used at tolerable doses of 0.1 ml and 0.4 ml block the effect of Ach but did not block the hypotensive effect of both extracts – both potentiated the effect of the extracts, This indicates that the extract may be relaxing vascular smooth muscle via muscarinic receptor stimulation instead of competitive antagonism, with the possibly release of endothelium derived relaxing factor (EDRF), and possibly by the inhibition of the influx and utilization of extra-cellular calcium ions through the calcium channels. With the results obtained from the various experiment carried out it follows that the extract has blood pressure lowering effect, which may be due to the combined effects of vascular smooth muscle relaxation, occasioned by both the muscarinic receptor mediated release of endothelium derived relaxing factor, inhibition of influx calcium ion and utilization of

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extracellular calcium ion through the vascular smooth muscle, and caused depression of both the force and rate of contraction of myocardium (Fedelic *et al.*, 2009).

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CHAPTER SIX

6 Summary, Conclusion and Recommendation

6.1 Summary

Intravenously administered aqueous doses of the extracts on anaesthetized normotensive cats produced an insignificant fall in mean diastolic, and systolic and mean arterial pressures in a graded dose response manner with more significant on diastolic blood pressure. The dose of 0.1ml of Acetylcholine 10 µg/ml produced a fall in mean diastolic, systolic, and mean arterial pressure, the dose of 1 mg/ml of Methanolic extract of *Trigonella Foenum-gaecum L.* produced a fall in mean diastolic, systolic, and mean arterial pressure.

The extract had a greater blood pressure depressant effect on the diastolic blood pressure than on the systolic blood pressure. Atropine (25 mg/ml) at all tolerable doses did not block the hypotensive effect of both extracts but potentiated the effect of the extracts. The extracts were found to decrease both the force and rate of myocardial contraction in a concentration dependent manner.

6.2 Conclusion

Results of the experiment indicate that the plant *Trigonella Foenum-gaecum* has hypotensive properties, and therefore, justified its use to folkloric, ethnomedical in management and/or control of high blood pressure in some rural communities,

The results obtained show that the extracts have hypotensive effect which may be due the combined effects on myocardial depression and muscarinic receptor mediated vascular smooth muscle relaxation.

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6.3 Recommendations

i. Fenugreek seed could be exploited as an important supplement in food manufacturing such as functional foods and possible antihypertensive effect.

ii. Further studies is needed to establish the possibility of other plausible mechanisms of the hypotensive action involve the fenugreek.

Iii. The plant's crude protein content suggests its potential as another source of human protein consumption.

6.4 Contributions to knowledge

i. The *Trigonella Foenum-gaecum* caused decrease in speed and strength of contraction of myocardium Hexane and Methanol Extracts of *Trigonella Foenum-gaecum L.* ($p = 0.04$ and 0.05 respectively) in Cat Blood Pressure Experiment compared to Acetycholine.

ii. There was a decrease in normal mean arterial blood pressure of Methanol Extract at dose 100 mg/ml in Cat Blood Pressure Experiment compared to Acetycholine ($p= 0.04$).

iii .*Trigonella Foenum-gaecum* may not be toxic. According to toxicity studies carried out on the plant with LD_{50} above 5000 mg/kg

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Appendix I

Appendix I: P values for Isolated Perfuse Heart Experiment among Hexane Extract of *Trigonella foenum-graecum*, Methanol Extract of *Trigonella foenum-graecum* and Acetylcholine.

Doses Administered	Strength of Contraction/cm	Speed of Contraction/sec
Ach (10μg/ml)	0.00	
Hexane Extract (0.1mg/ml)	0.02	0.09
Hexane Extract (1mg/ml)	0.04	0.28
Hexane Extract (10mg/ml)	0.54	0.05
M. Extract (0.1mg/ml)	0.63	0.14
M. Extract (1mg/ml)	0.00	0.02
M. Extract (10mg/ml)	0.02	0.04

$P \leq 0.05$ = Significant, $P \geq 0.05$ = Not Significant

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Appendix II

Appendix II: P values for Cat Blood Pressure Experiment among Hexane Extract of *Trigonella foenum-graecum*, Methanol Extract of *Trigonella foenum-graecum* and Acetylcholine.

Doses Administered	S.BP	D.B.P	P.B.P	M.A.B.P
Ach (10µg/ml)	0.00			
Hexane Extract (1mg/ml)	1.00	0.87	0.10	0.14
Hexane Extract (10mg/ml)	1.00	0.28	0.83	0.88
Hexane Extract (100mg/ml)	0.10	1.00	0.44	0.04
M. Extract (1mg/ml)	0.79	0.10	0.92	1.00
M. Extract (10mg/ml)	1.00	0.38	0.32	0.81
M. Extract (100mg/ml)	0.97	0.16	0.08	0.05

Key: S.B.P (Systolic Blood Pressure), D.B.P (Diastolic Blood Pressure), P.P (Pulse Pressure), M.A.B.P (Mean Arterial Blood Pressure).

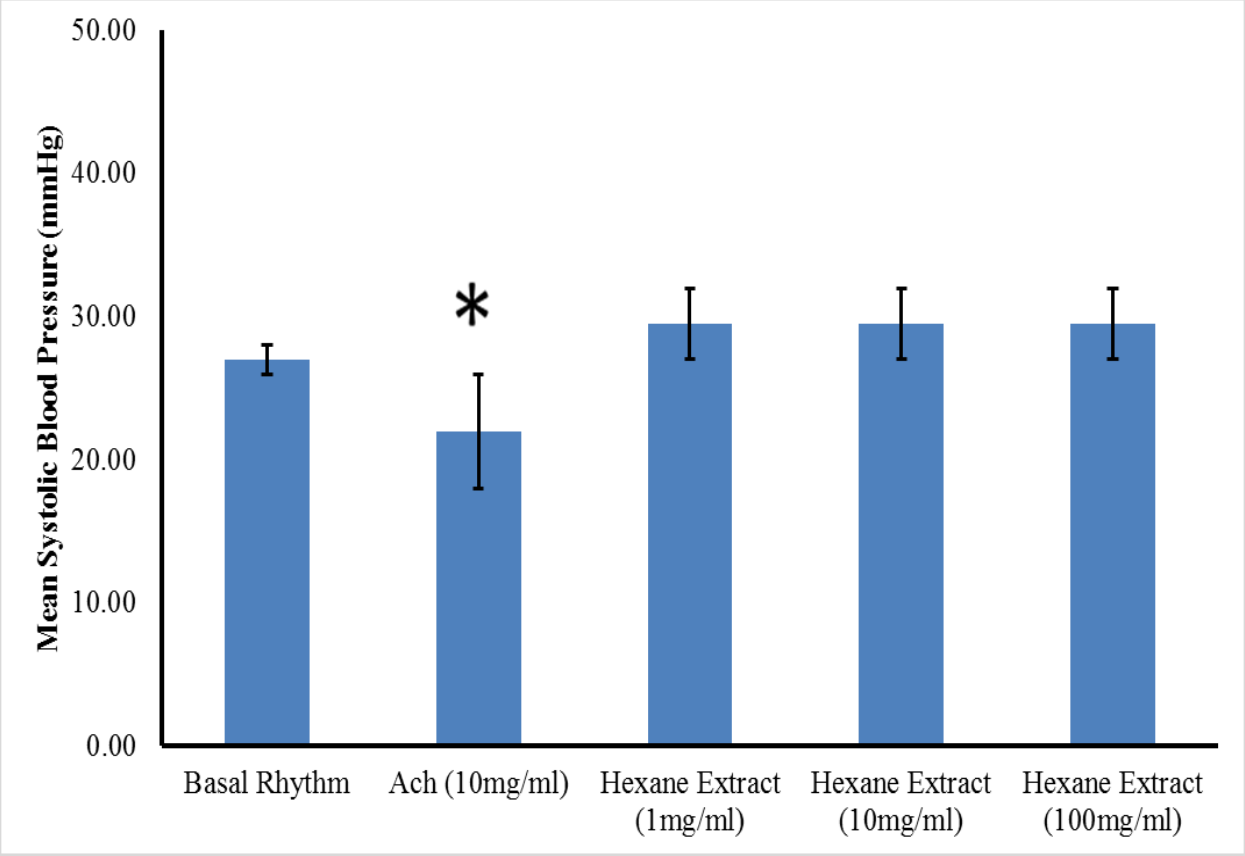
*$P \leq 0.05$ = Significant, $P \geq 0.05$ = Not Significant

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Appendix III

Appendix III: Mean Systolic Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Hexane Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

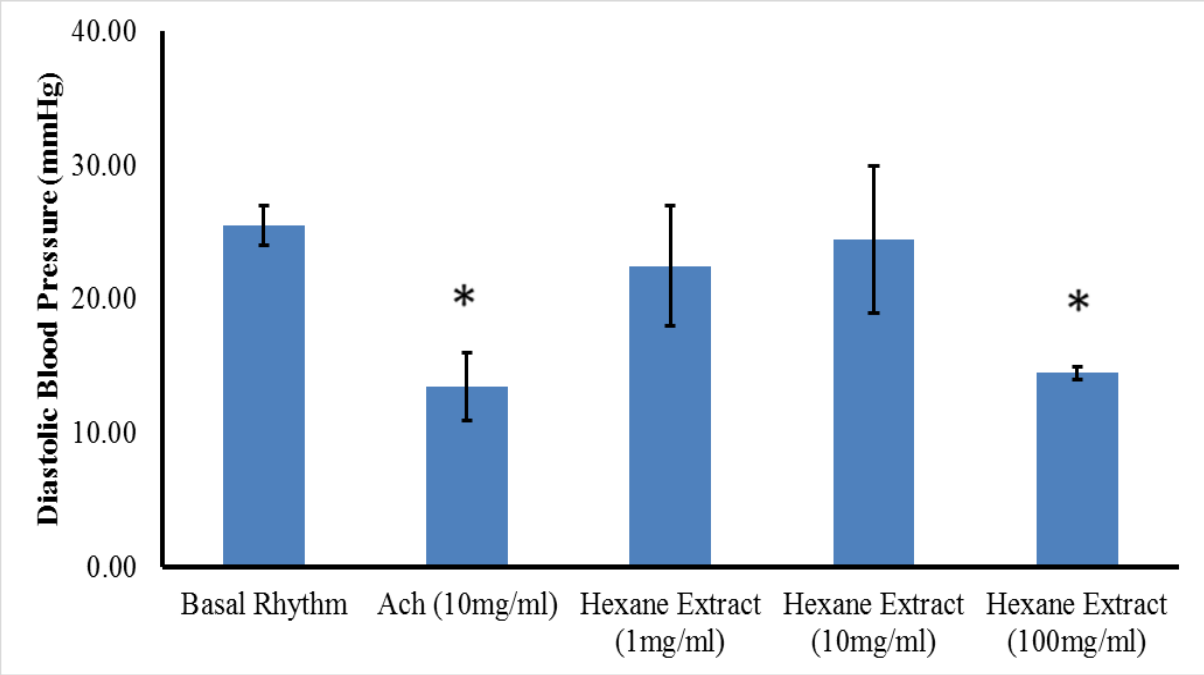


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Appendix IV

Appendix IV: Mean Diastolic Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Hexane Extract of *Trigonella foenum graecum L.*One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

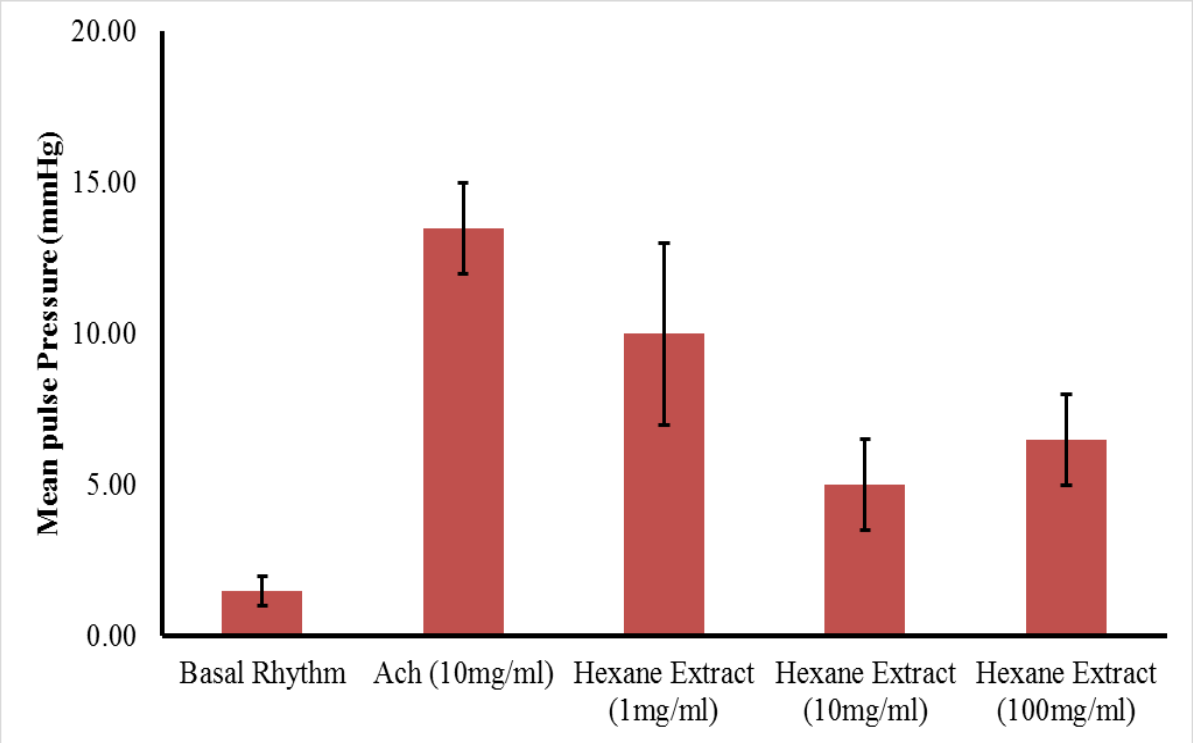


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Appendix V

Appendix V: Mean Pulse Pressure (mmHg) for Acetylcholine 10µg/ml, Hexane Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

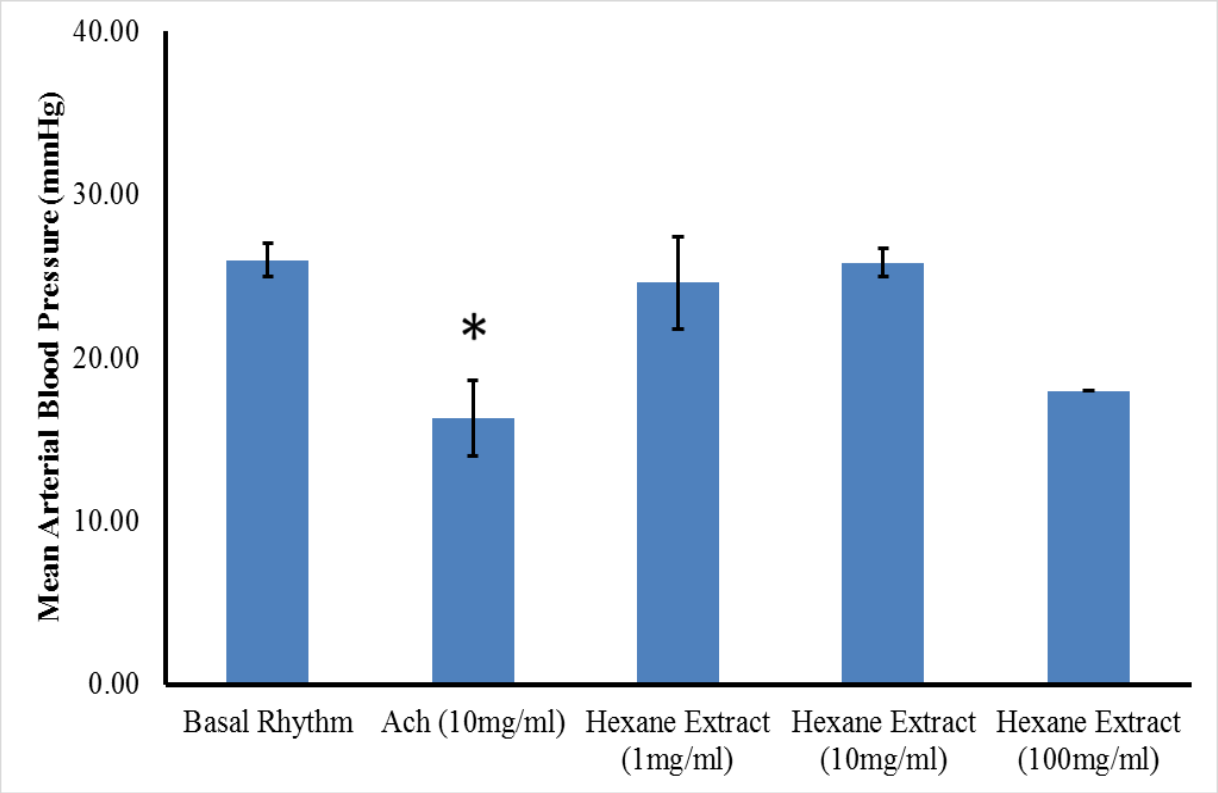


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Appendix VI

Appendix VI: Mean Arterial Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Hexane Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

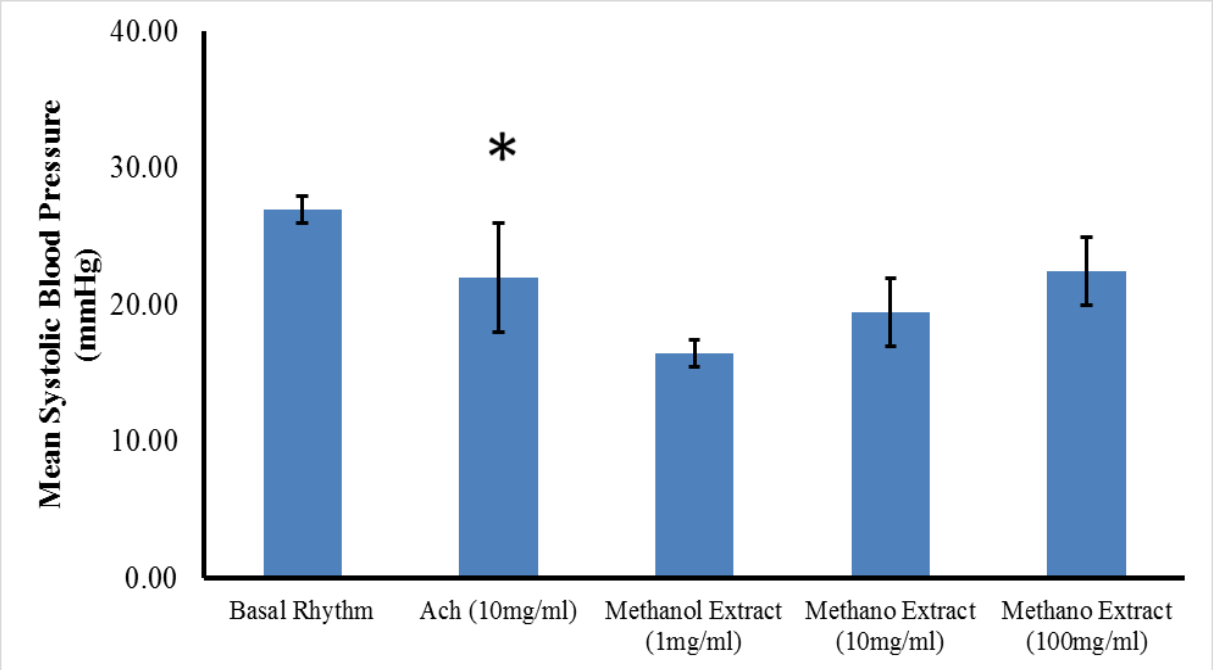


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Appendix VII

Appendix VII: Mean Systolic Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Methanol Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

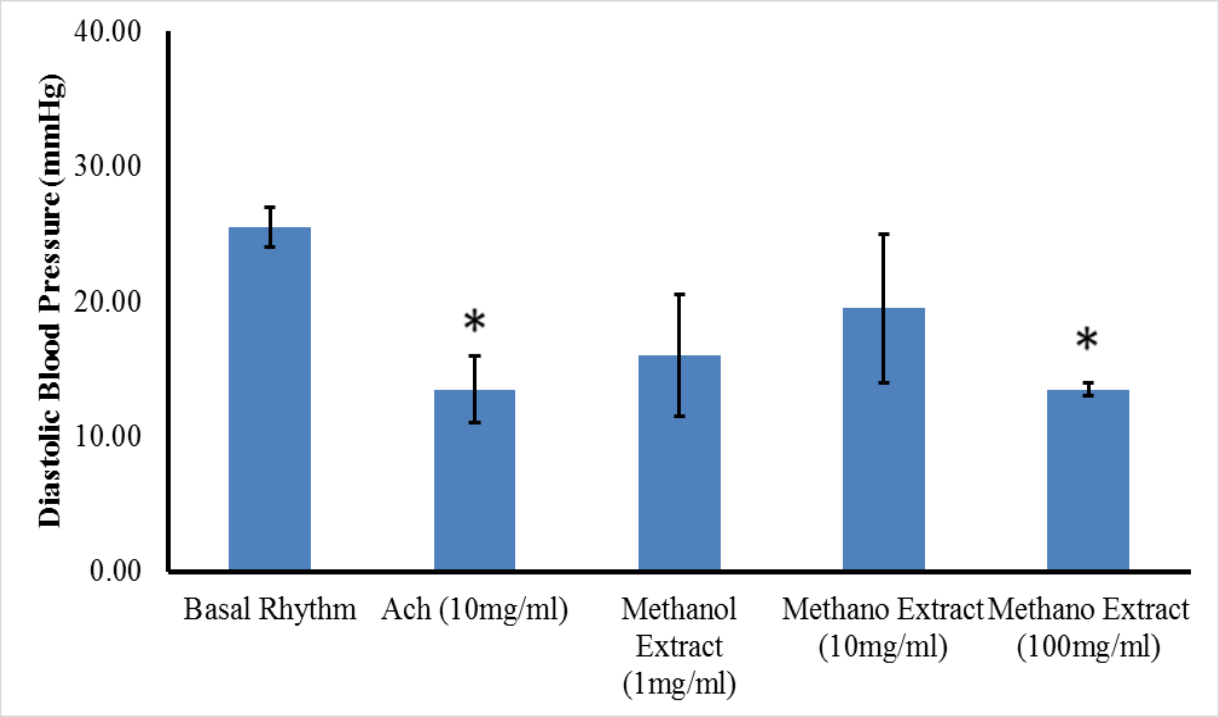


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Appendix VIII

Appendix VIII: Mean Diastolic Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Methanol Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

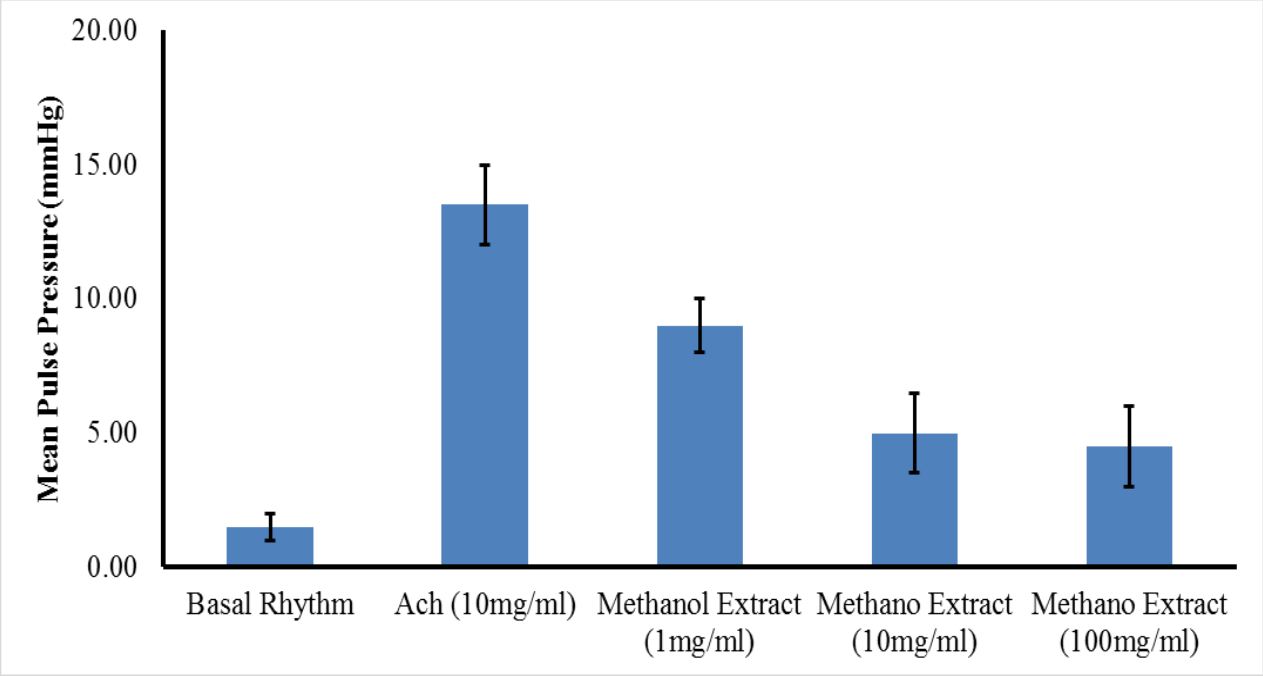


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Appendix IX

Appendix IX: Mean Pulse Pressure (mmHg) for Acetylcholine 10µg/ml, Methanol Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

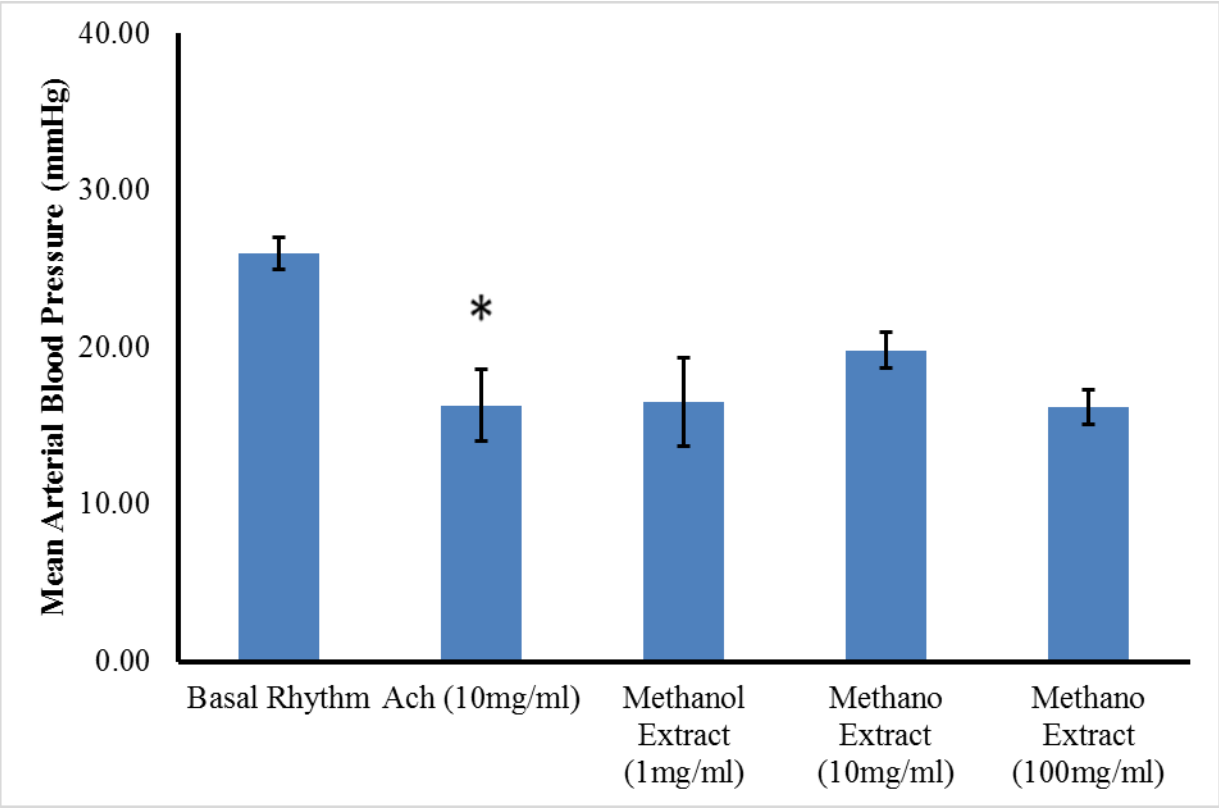


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Appendix X

Appendix X: Mean Arterial Blood Pressure (mmHg) for Acetylcholine 10µg/ml, Methanol Extract of *Trigonella foenum graecum L.* One Way ANOVA in Cat Blood Pressure Experiment.

* denotes a statistically significant difference.

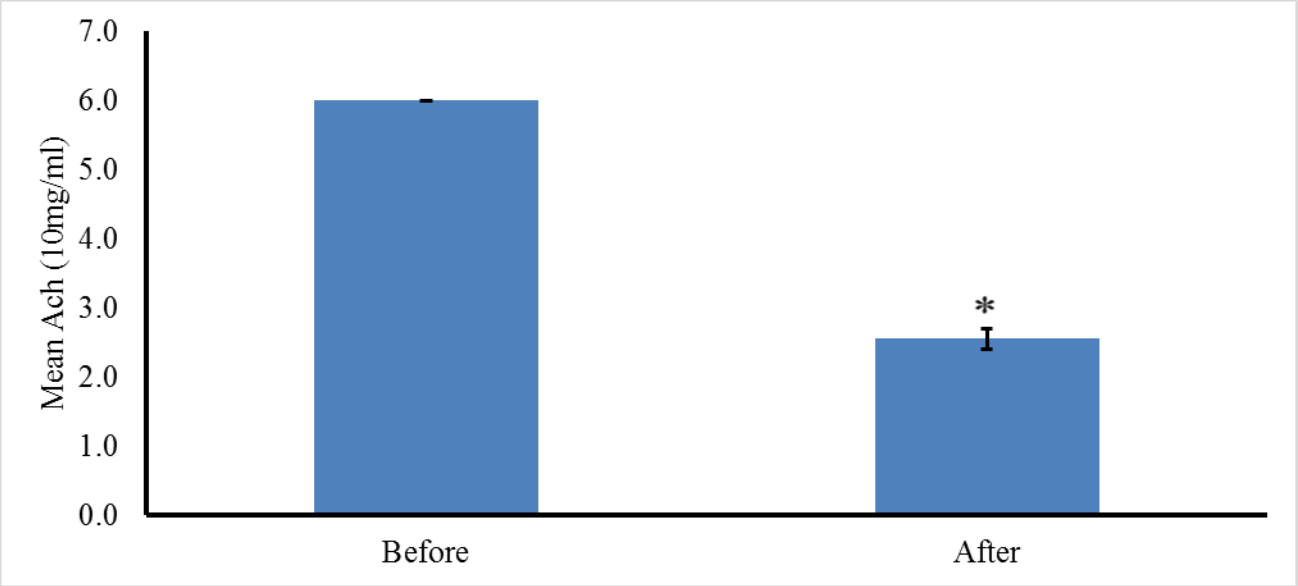


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Appendix XI

Appendix XI: Acetylcholine 10µg/ml paired *t* test in Isolated Perfuse Heart Experiment.

*p<0.05 (paired t test)

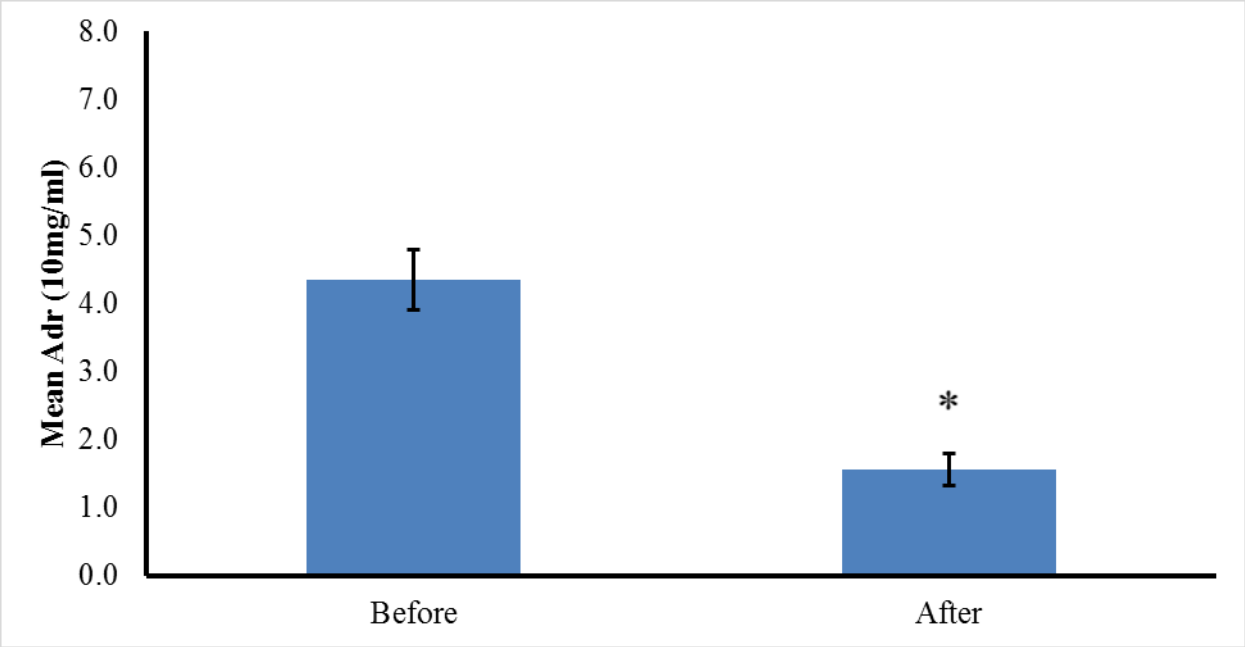


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Appendix XII

Appendix XII: Adrenaline paired *t* test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)

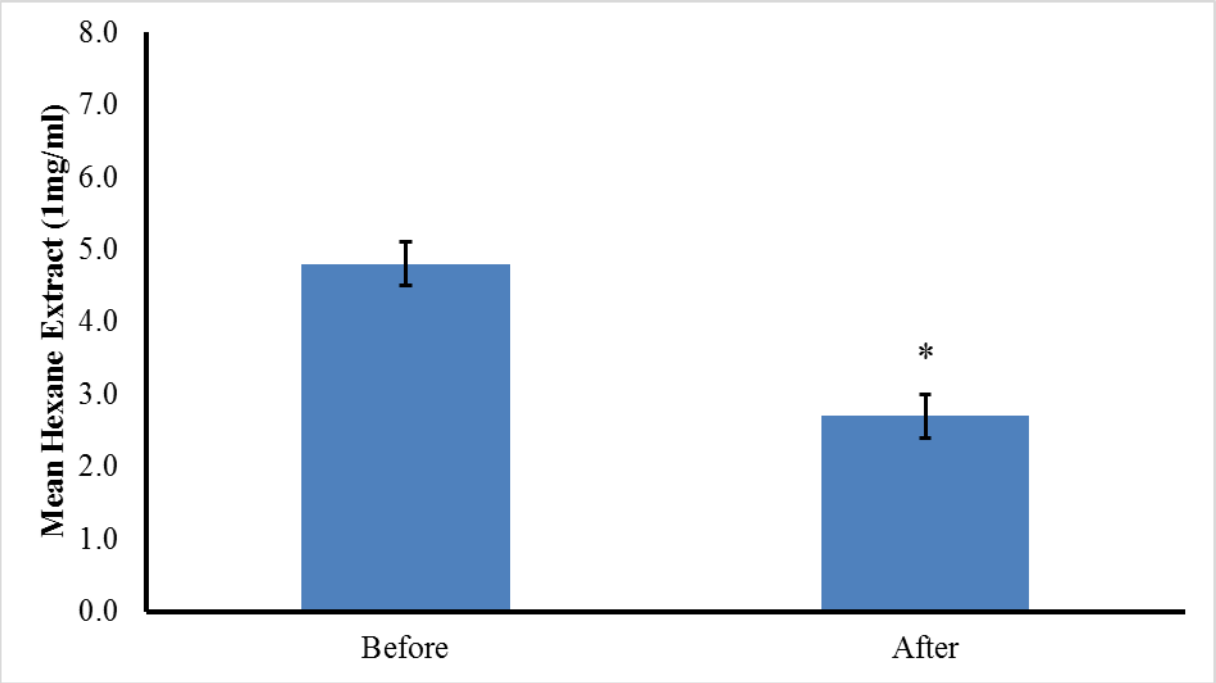


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Appendix XIII

Appendix XIII: H. Extract *Trigonella foenum-graecum L.* 0.1mg/ml paired t test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)

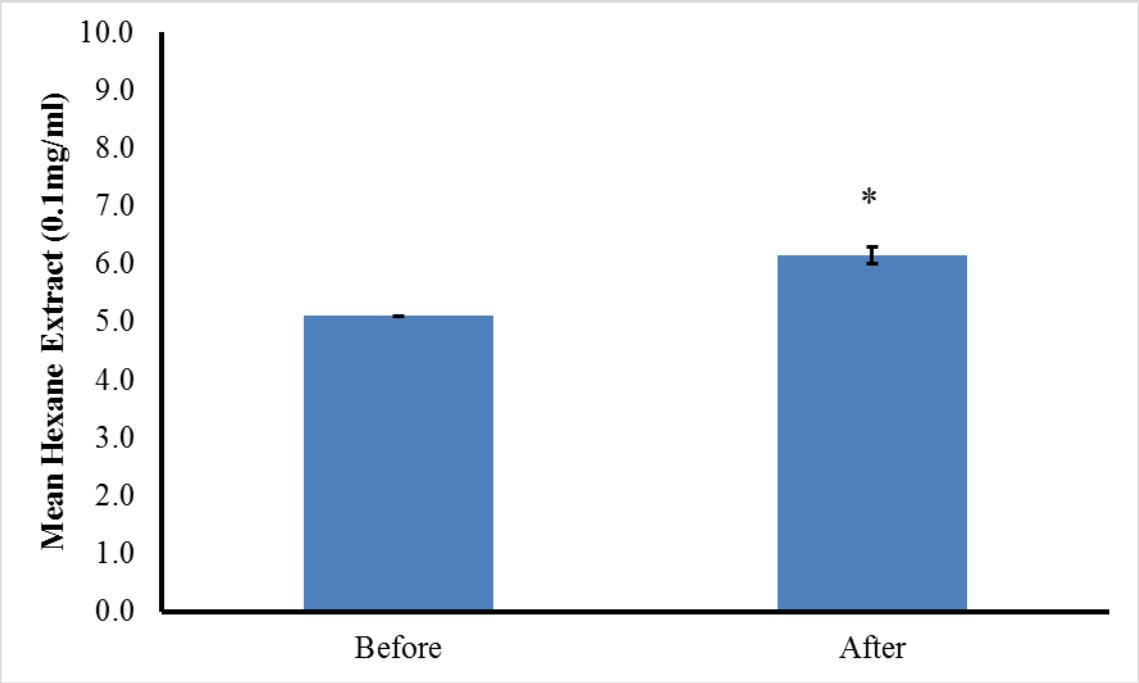


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Appendix XIV

Appendix XIV: H. Extract *Trigonella foenum-graecum L.* 1mg/ml paired t test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)

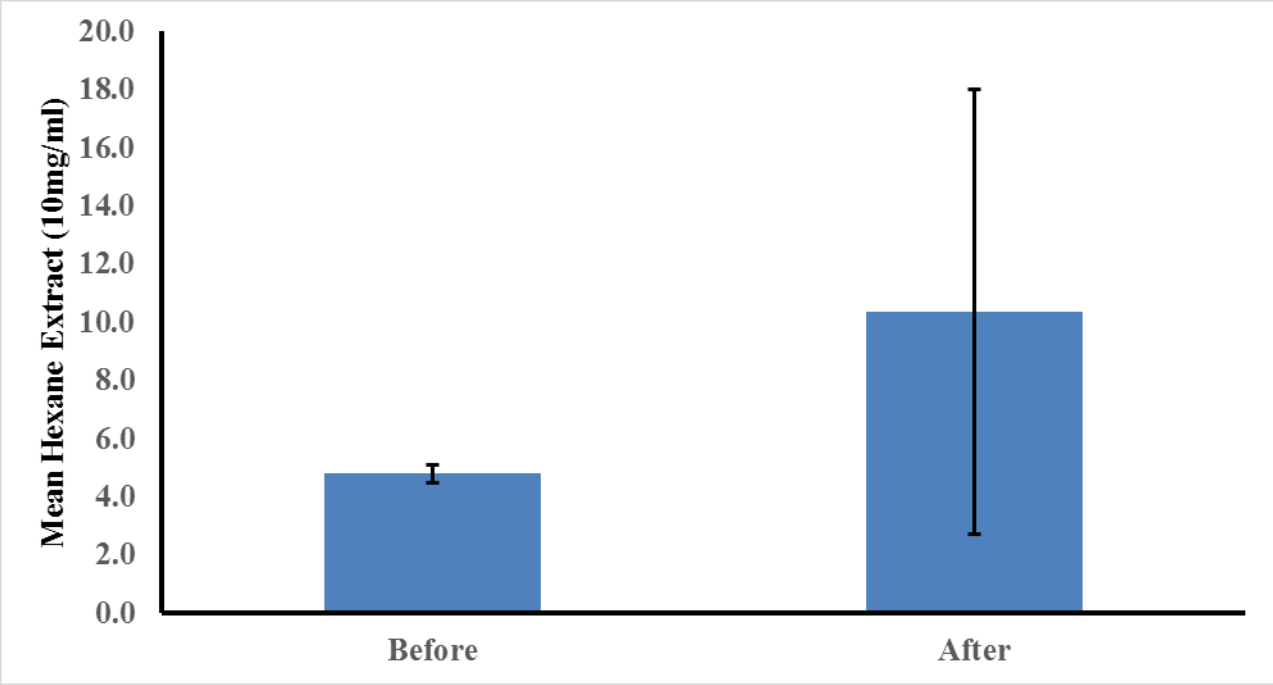


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Appendix XV

Appendix XV: H. Extract *Trigonella foenum-graecum L.* 10mg/ml paired t test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)



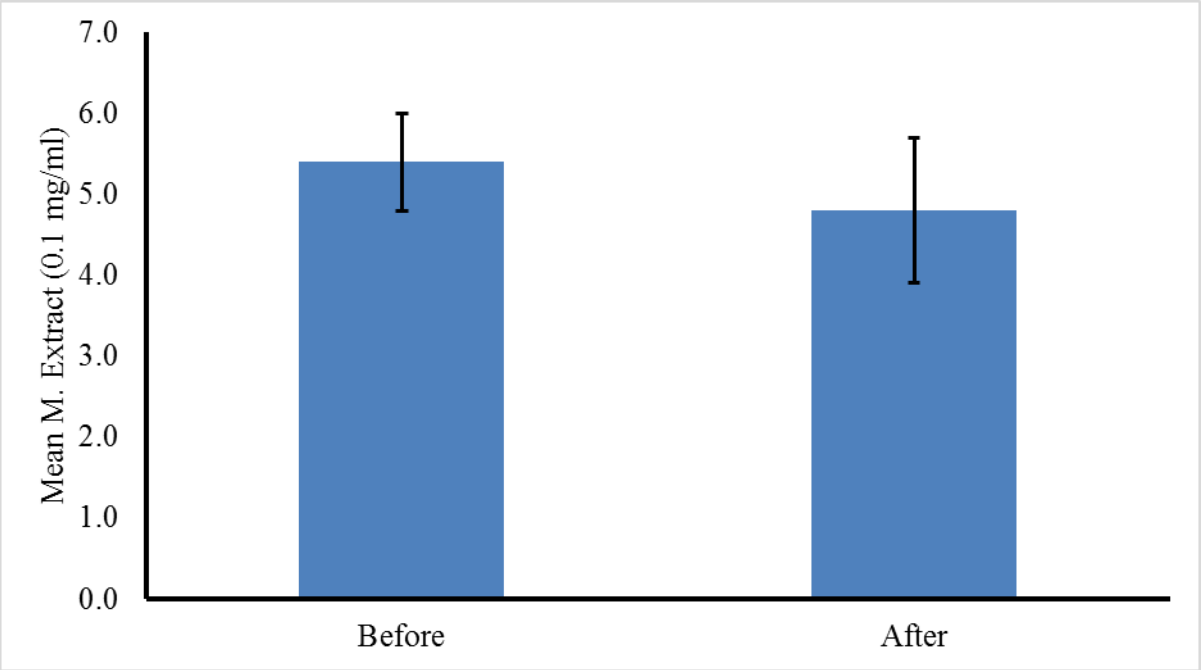
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Appendix XVI

Appendix XVI: M. Extract of *Trigonella foenum-graecum L.* 0.1mg/ml paired t test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)

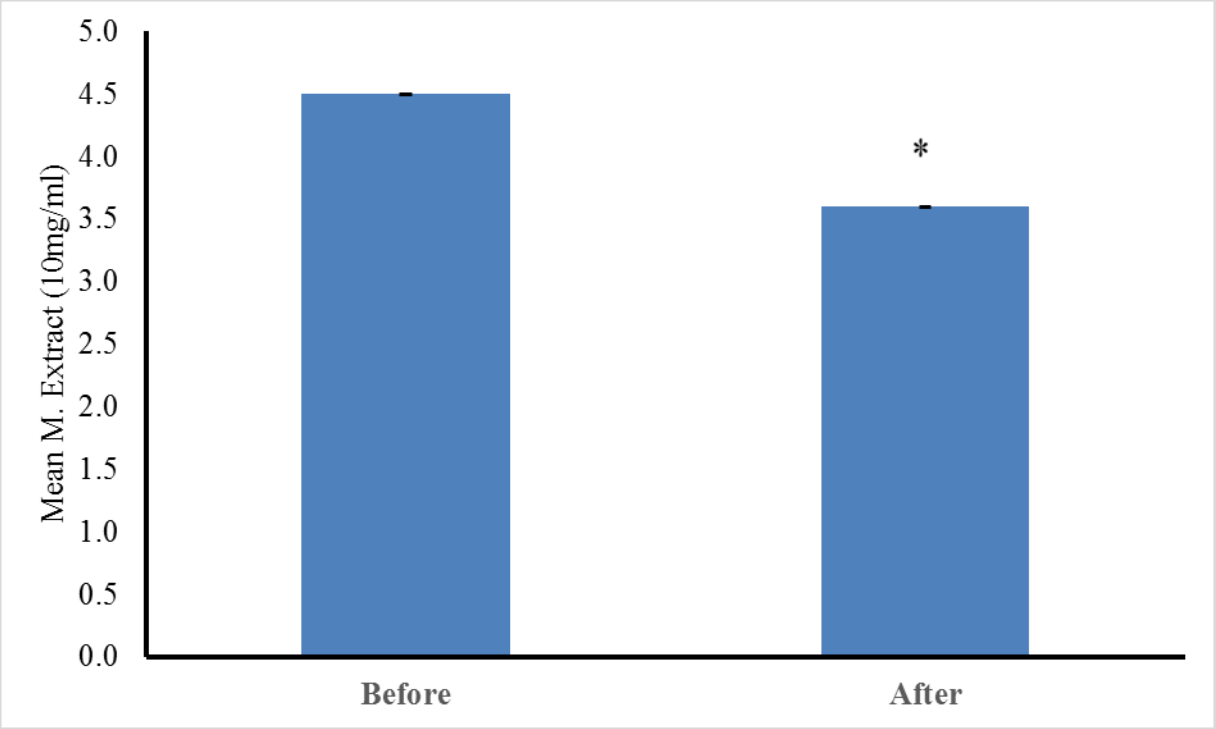


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Appendix XVII

Appendix XVII: M. Extract *Trigonella foenum-graecum* 1mg/ml paired t test in Isolated Perfuse Heart Experiment.

* P < 0.05 (paired t test)

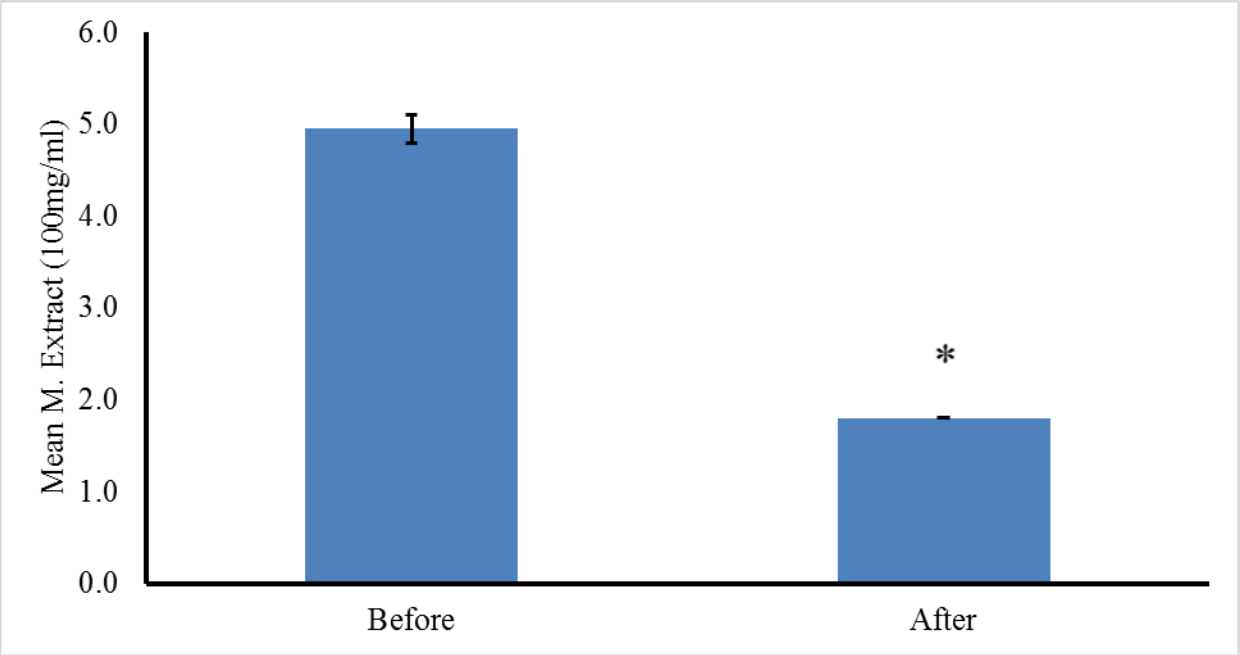


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Appendix XVIII

Appendix XVIII: M. Extract *Trigonella foenum-graecum* 10mg/ml paired t test in Isolated Perfuse Heart Experiment.

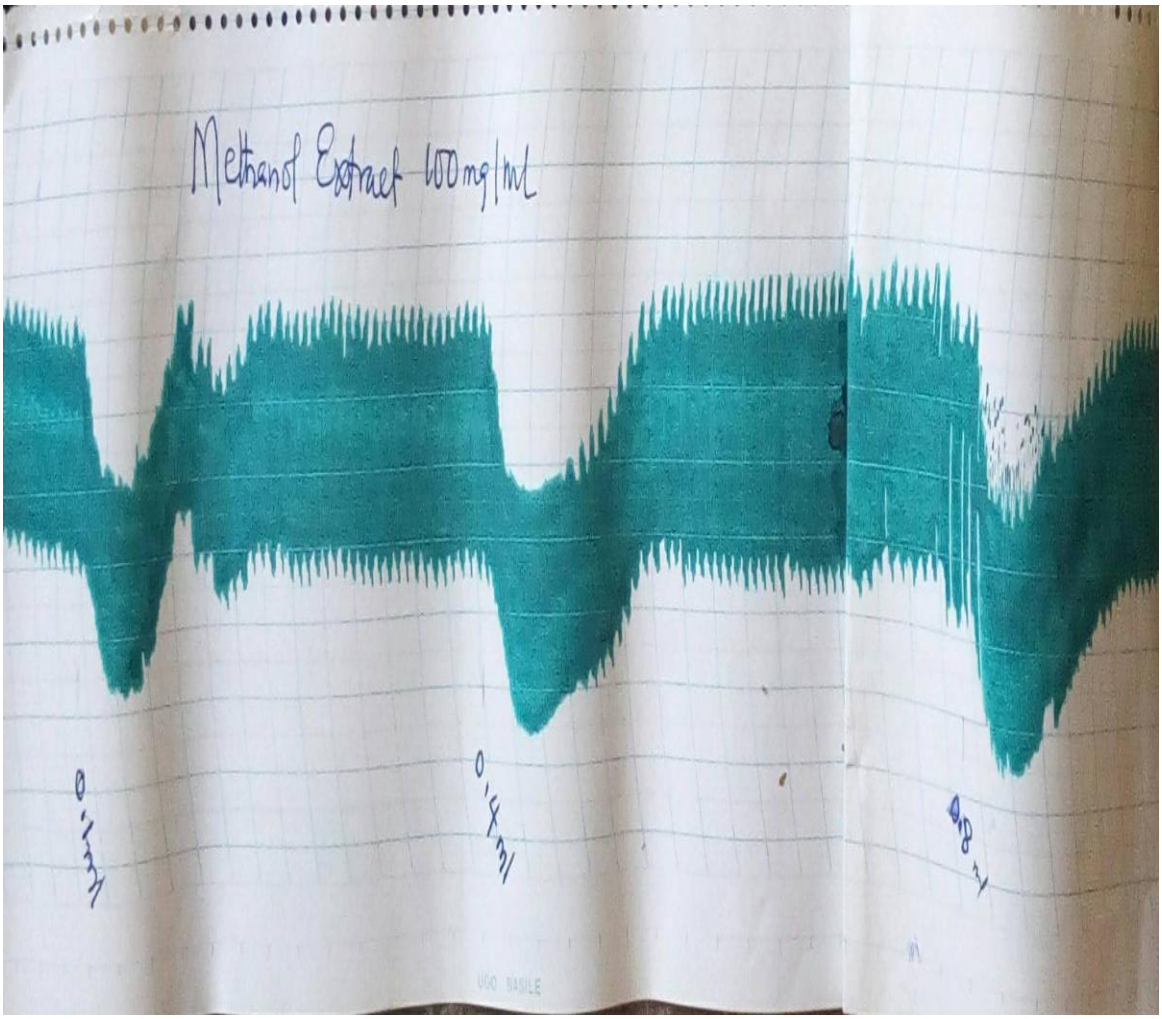
* P < 0.05 (paired t test)



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Appendix XIX

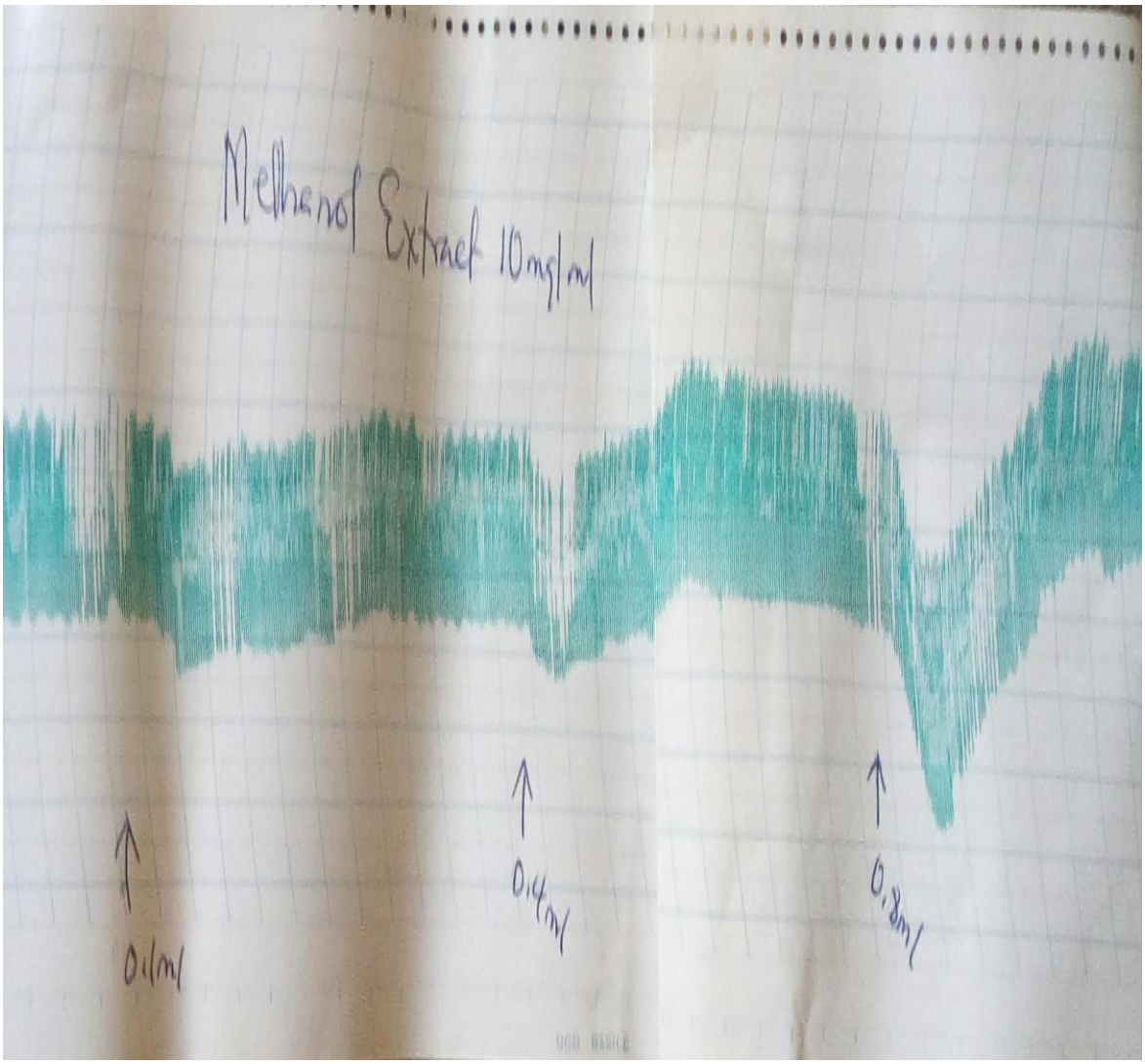
Appendix XIX: Tracing of 100mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XX

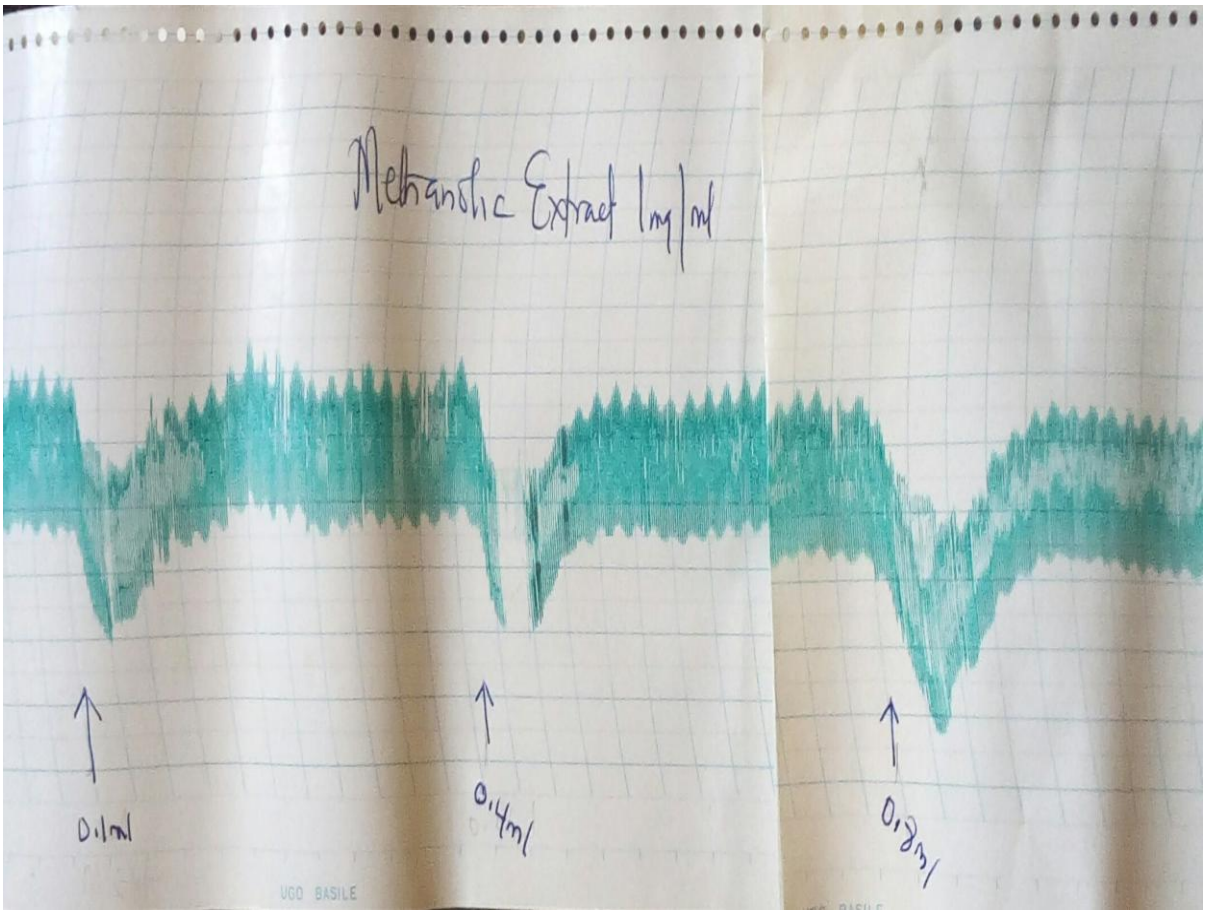
Appendix XX: Tracing of 10mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XXI

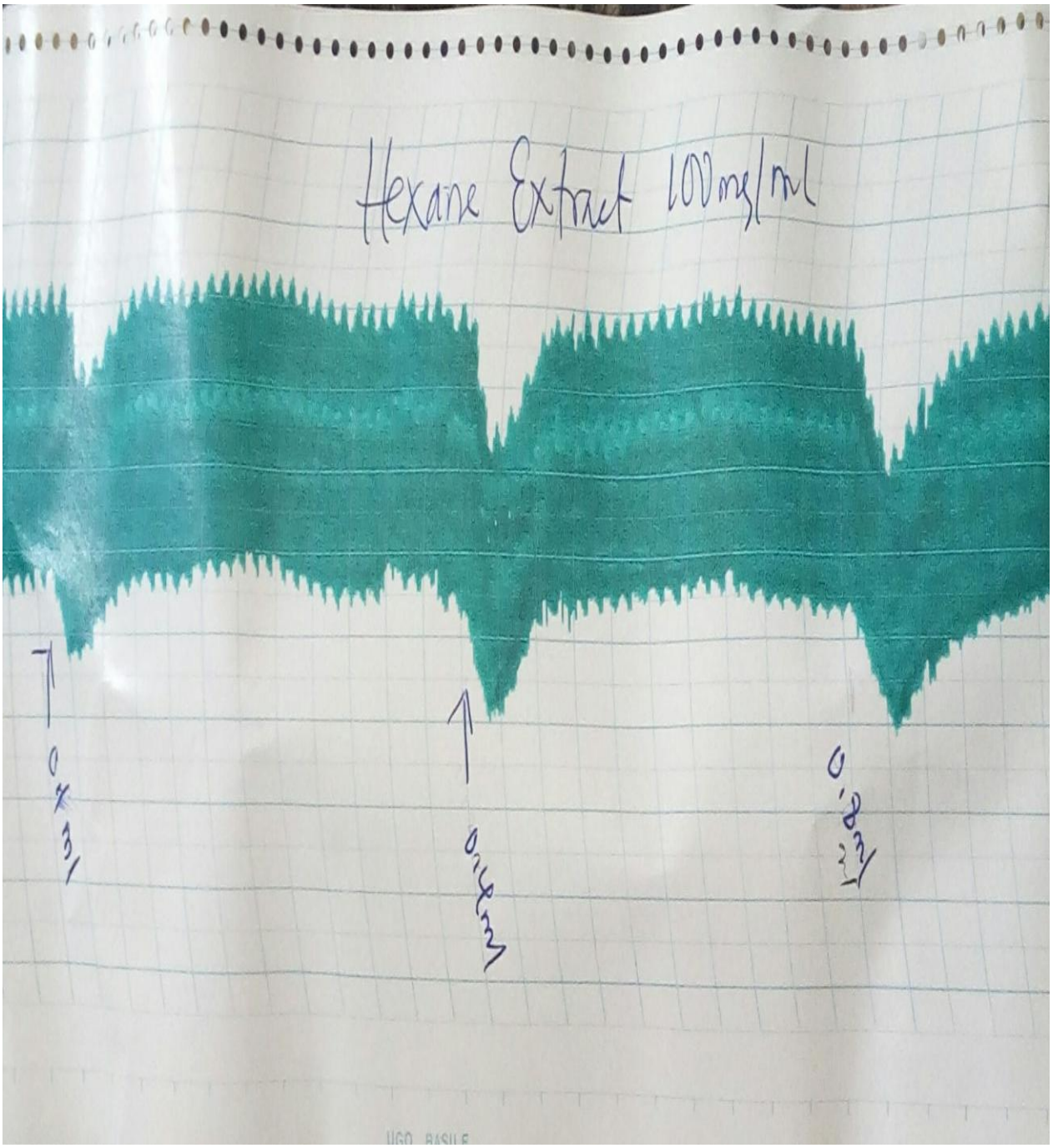
Appendix XXI: Tracing of 1mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XXII

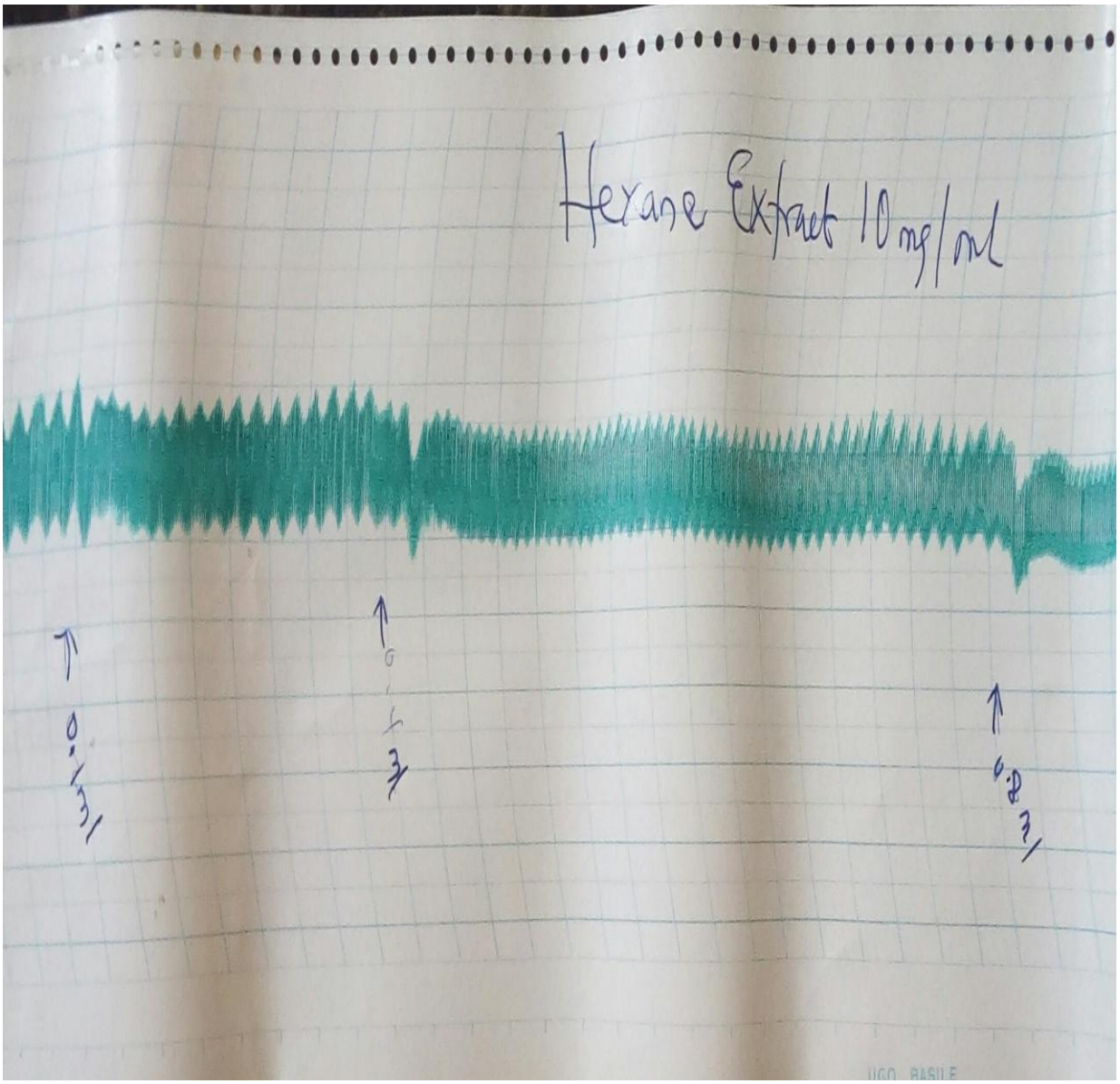
Appendix XXII: Tracing of 100mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XXIII

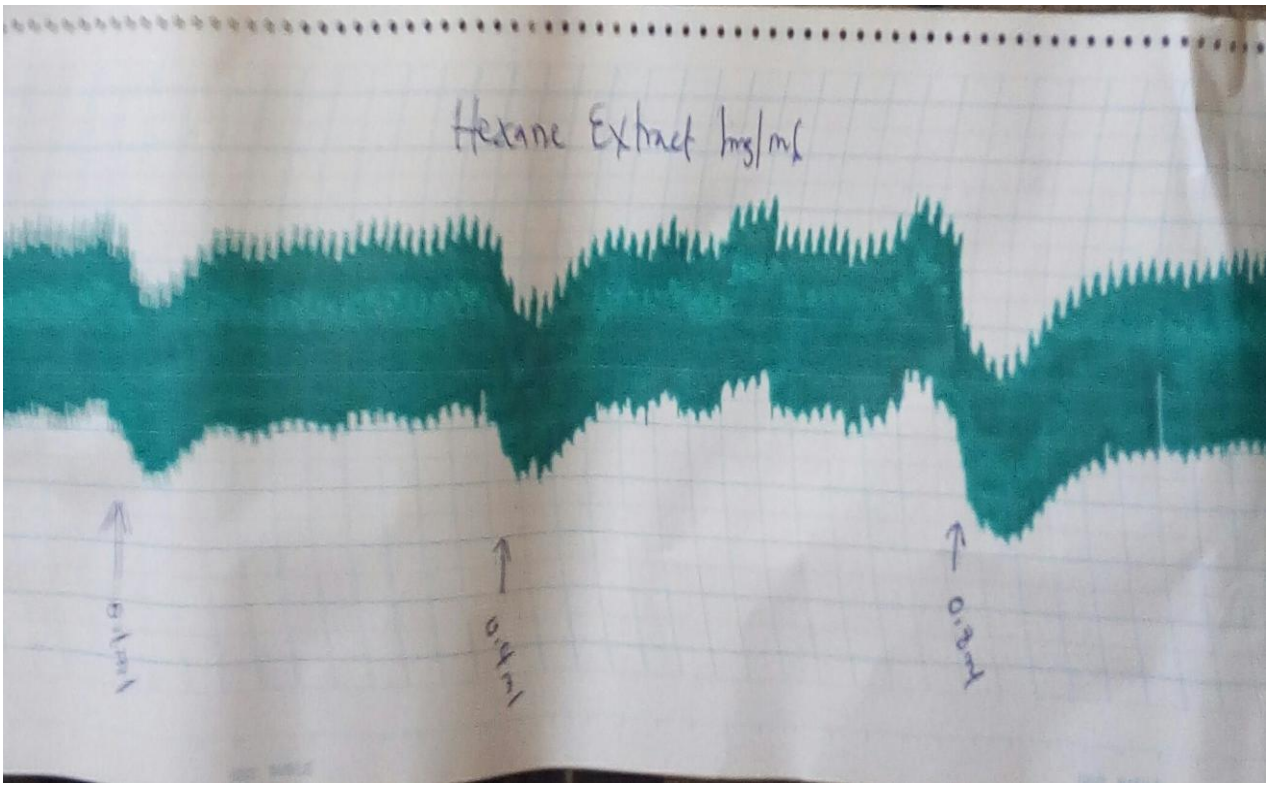
Appendix XXIII: Tracing of 10mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XXIV

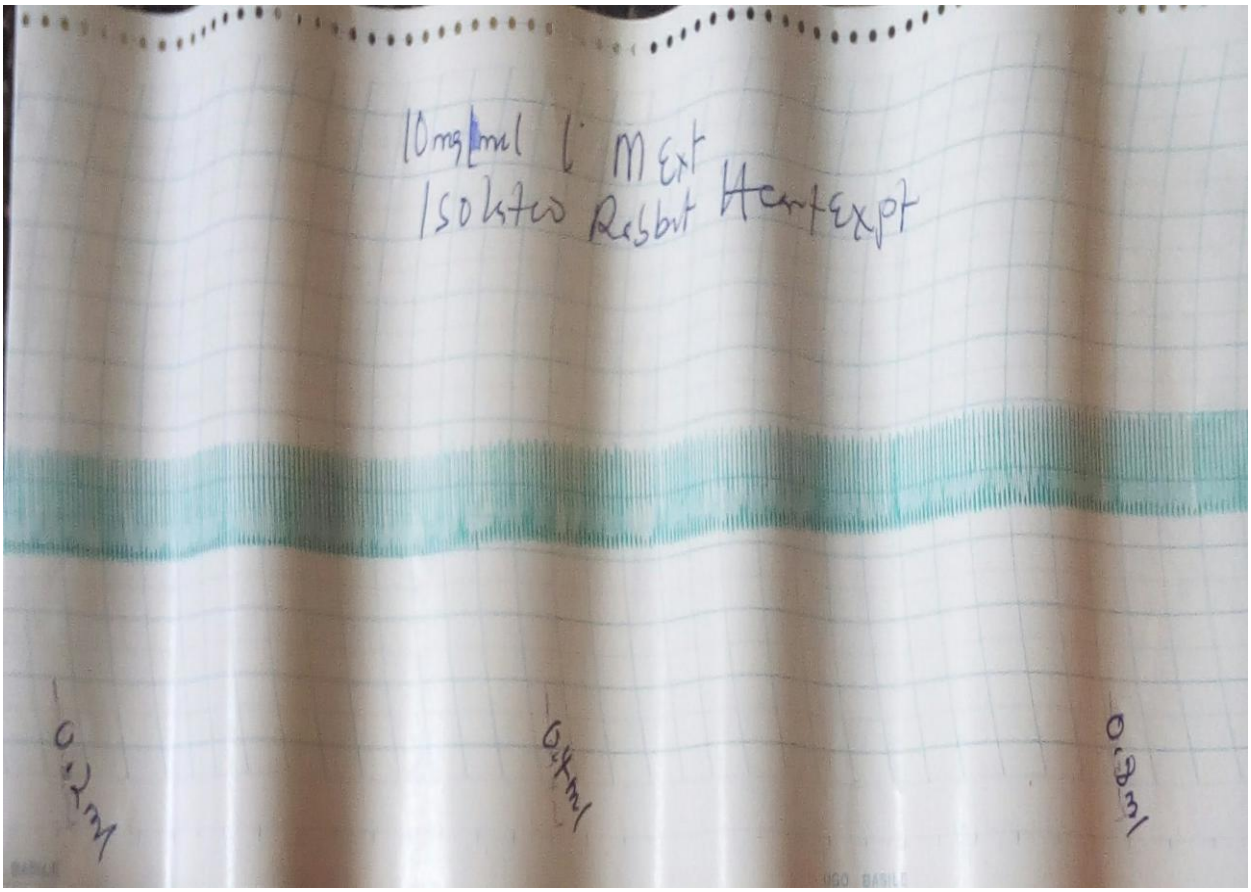
Appendix XXIV: Tracing of 1mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.1ml, 0.4ml and 0.8ml in Cat Blood Pressure Experiment.



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Appendix XXV

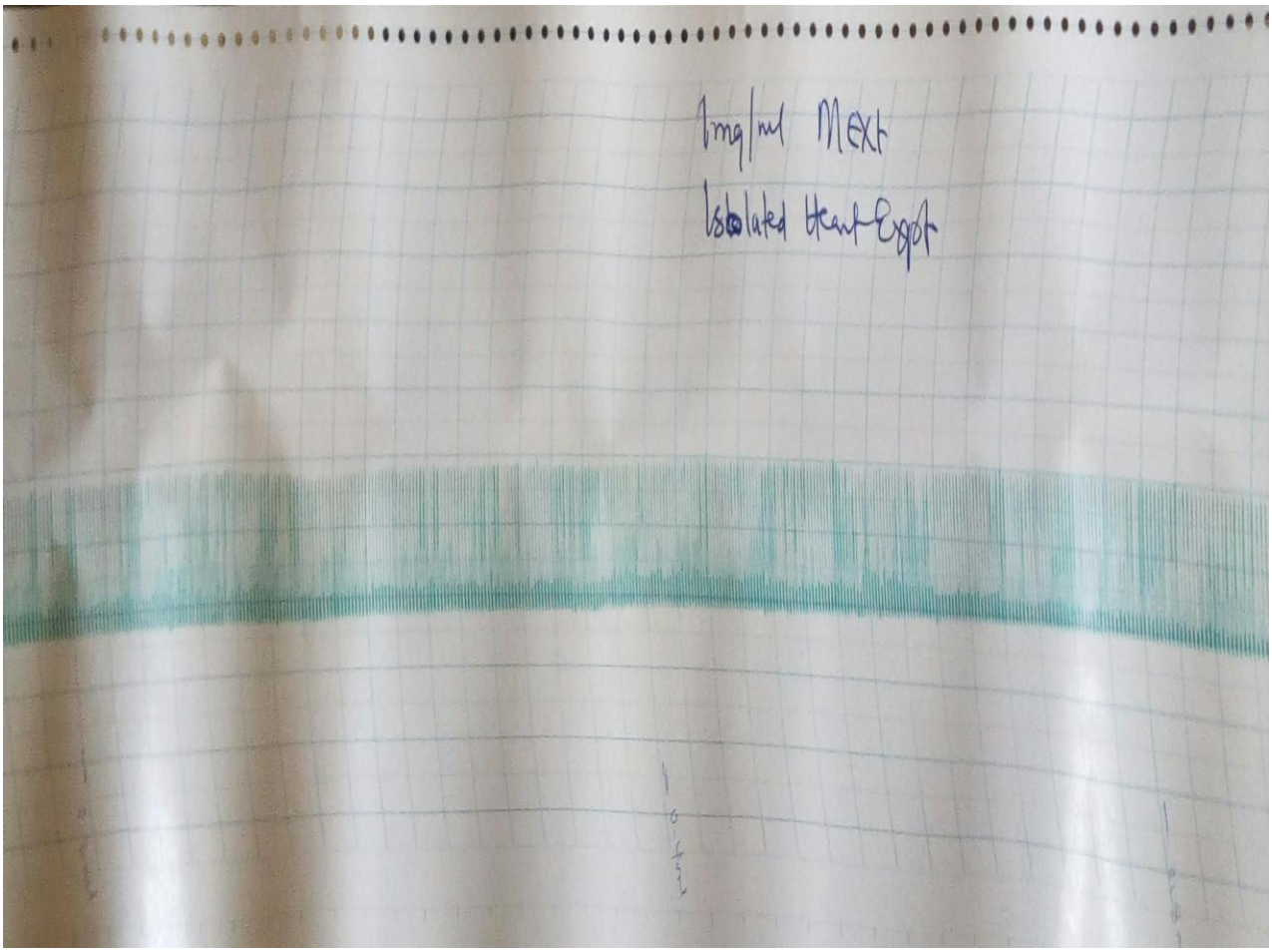
Appendix XXV: Tracing of 10mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml in Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXVI

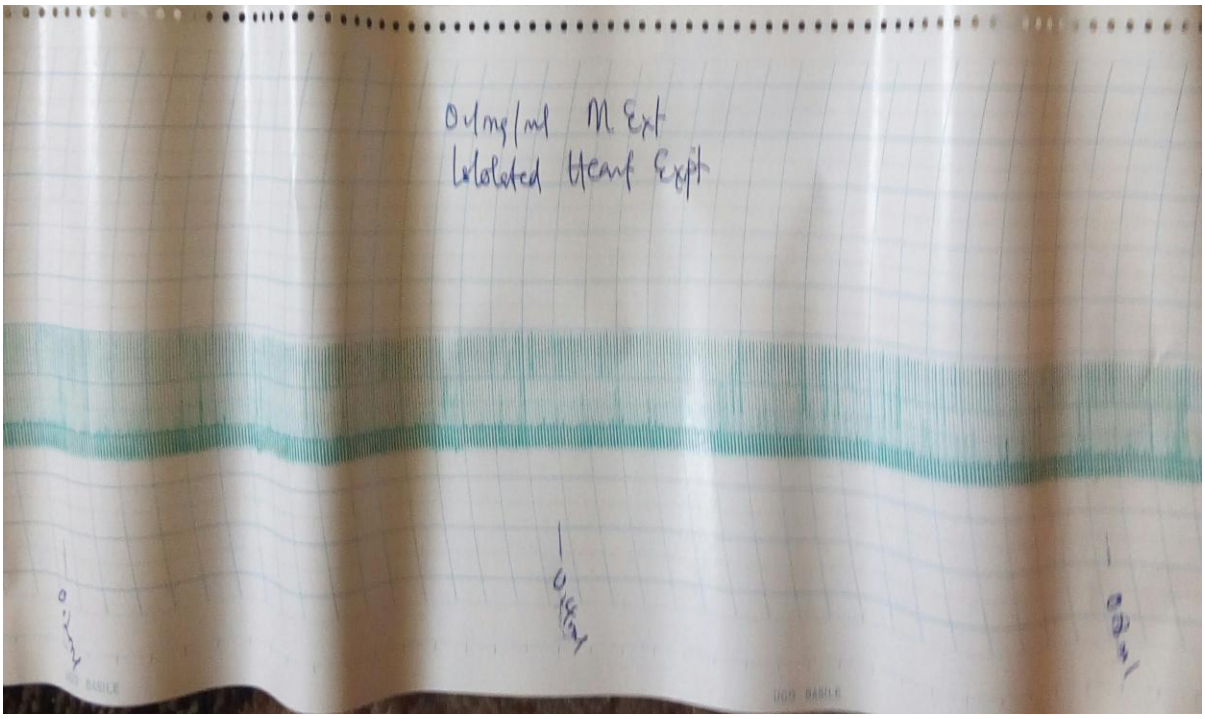
Appendix XXVI: Tracing of 1mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml in Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXVII

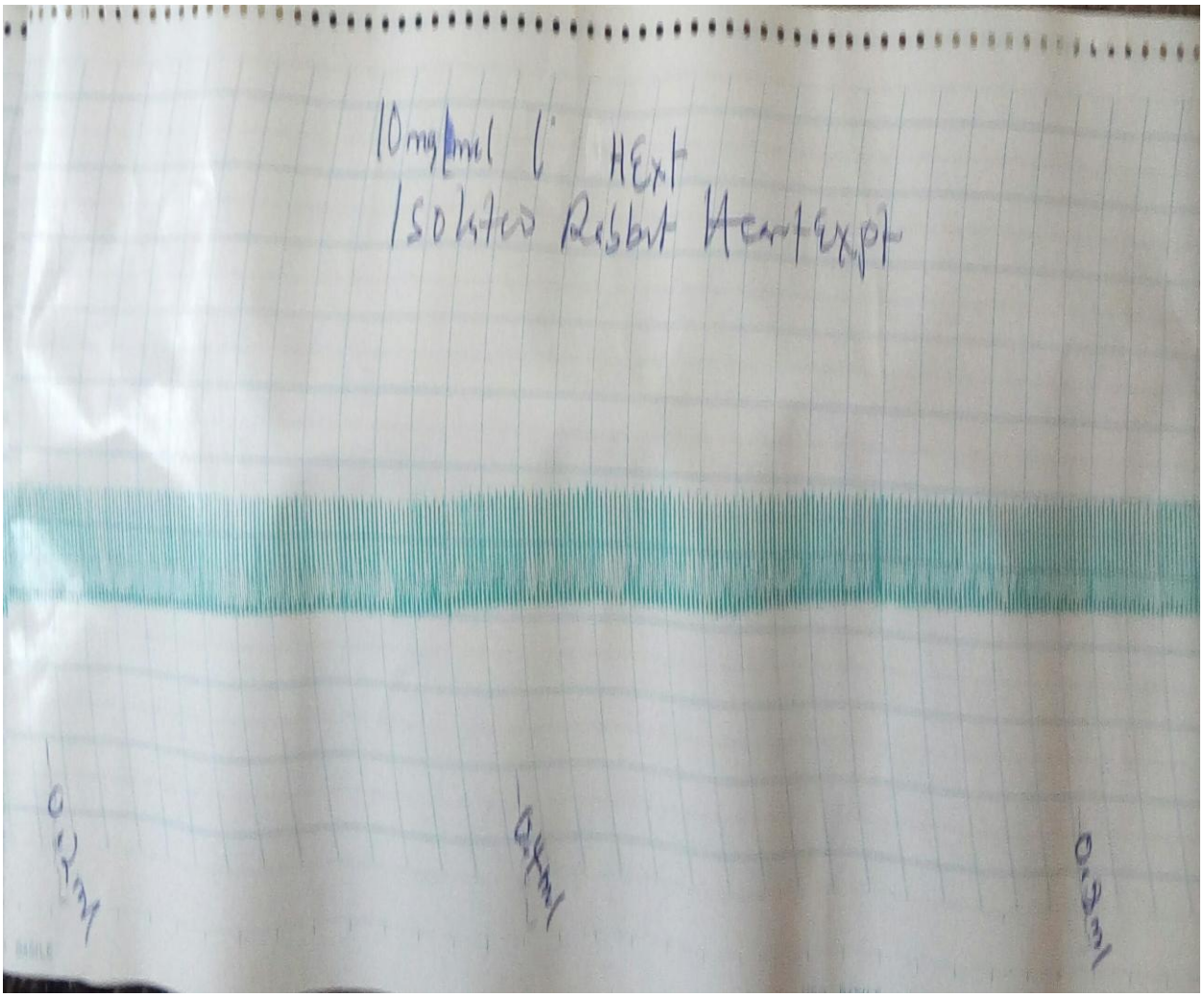
Appendix XXVII: Tracing of 0.1mg/ml of Methanol Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml for Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXVIII

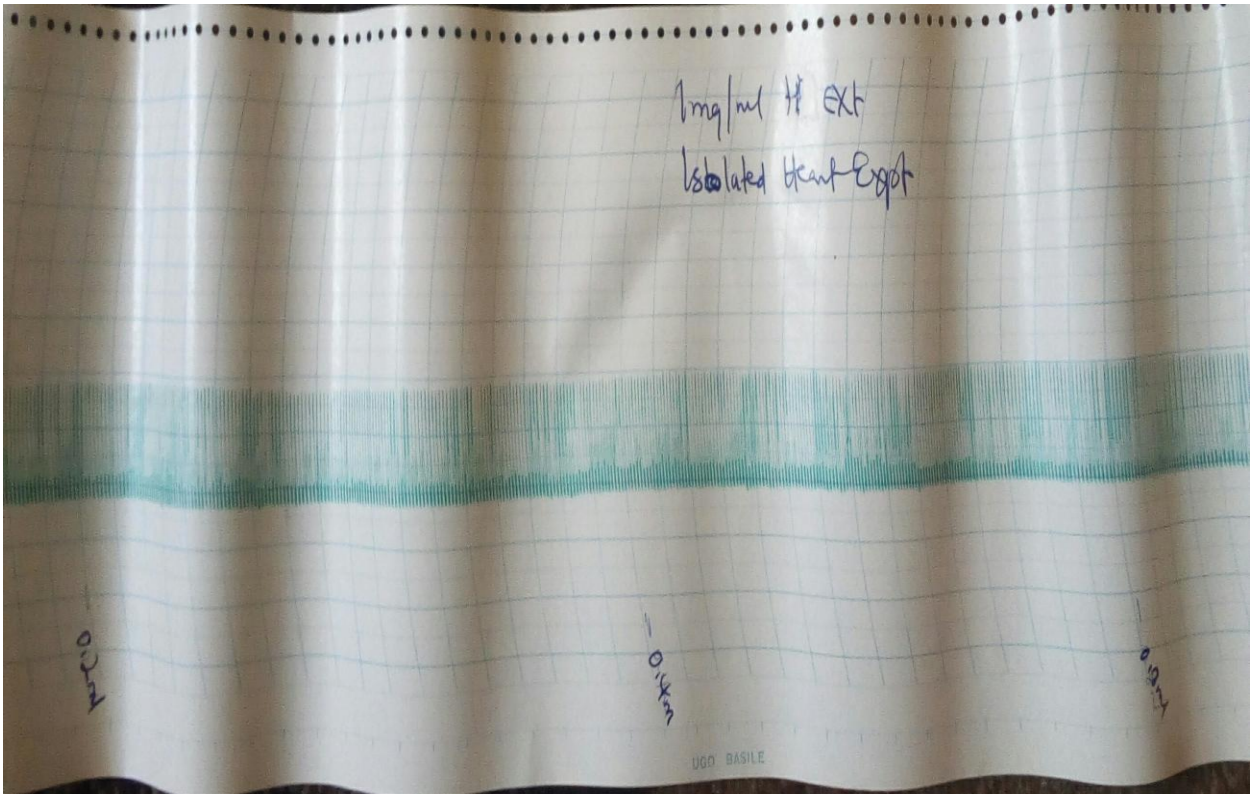
Appendix XXVIII: Tracing of 10mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml in Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXIX

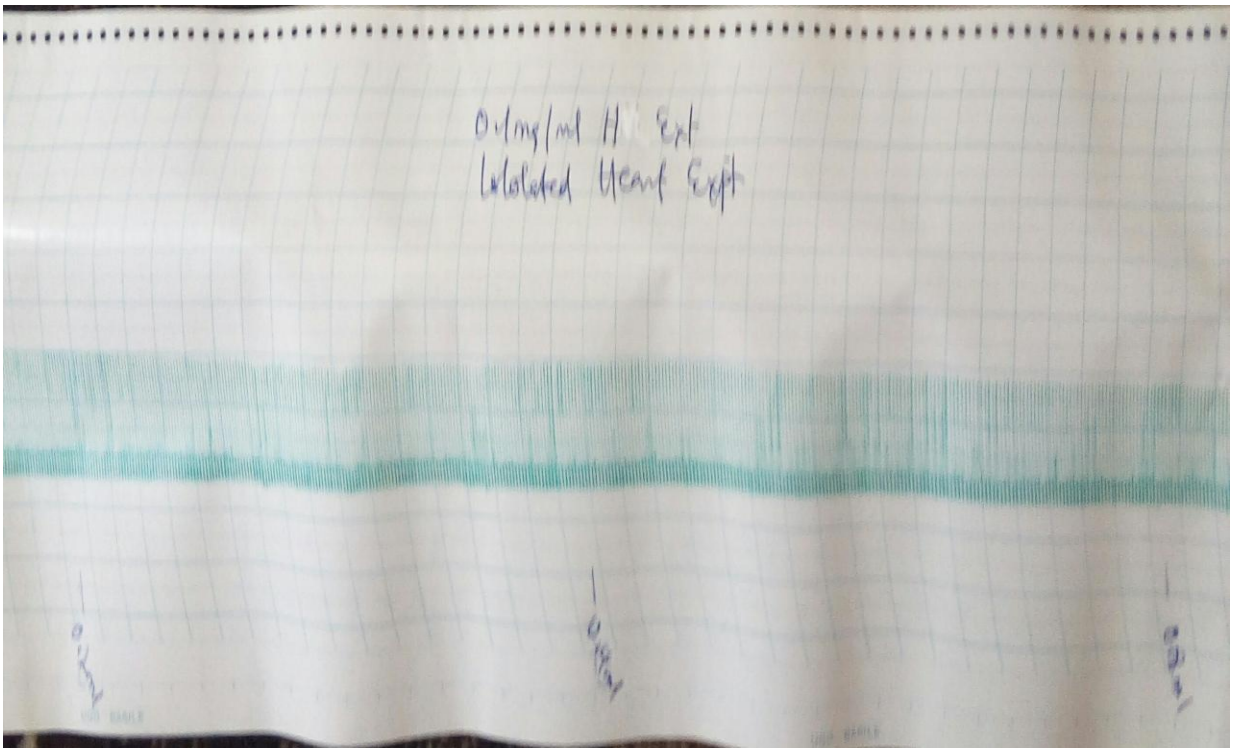
Appendix XXIX: Tracing of 1mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml in Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXX

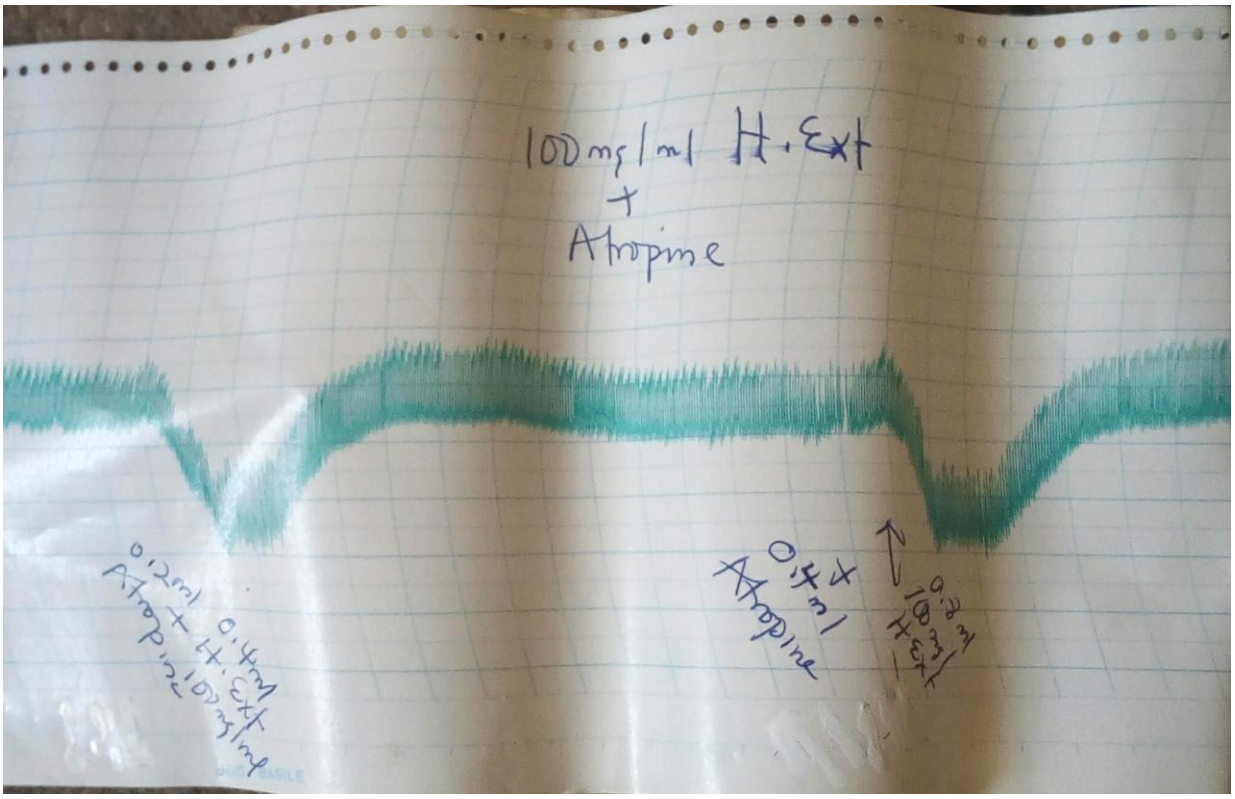
Appendix XXX: Tracing of 0.1mg/ml of Hexane Extract of *Trigonella foenum-graecum L.* with volume 0.2ml, 0.4ml and 0.8ml for Isolated Rabbit Perfuse Heart Experiment.



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Appendix XXXI

Appendix XXXI: Drug interaction between 0.2ml Atropine and 0.4ml of 100mg/ml of Hexane Extract *Trigonella foenum-graecum L.*, then, 0.4ml of Atropine and 0.8ml of 100mg/ml Hexane Extract in Cat Blood Pressure Experiment.



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Appendix XXXII

Appendix XXXII: Action of 10µg/ml of Acetylcholine in Isolated Rabbit Perfuse Heart Experiment.

