EFFECTS OF *ALLIUM SATIVUM* EXTRACT TREATMENT ON CARDIAC AND RENAL REGULATION OF BLOOD PRESSURE IN HYPERTENSION-INDUCED LABORATORY RATS (*Rattus norvigecus*)

MUNYIKOMBO, WILLIAM MAKHACHA

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DECLARATION

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MUNYIKOMBO, WILLIAM MAKHACHA
SC/PHD/Z/010/12

Signature: _____ Date: _____

DECLARATION BY SUPERVISORS

The thesis has been submitted with our approval as University supervisors:

DR. DICKSON M. MWANIKI,
Department of Biological Sciences,
School of Science, University of Eldoret,
P.O Box 1125 – 30100
Eldoret, Kenya

Signature: _____ Date: _____

PROF. GIDEON A. M. NG'WENA
Department of Medical Physiology
School of Medicine, Maseno University,
P.O Box 333
Maseno, Kenya

Signature: _____ Date: _____

DEDICATION

I dedicate this work to

My wife Lindah K. Makhacha

and

Children, Neoshah E. Makhacha, Colby M. Makhacha and

Zinah A. Makhacha

ABSTRACT

Hypertension is a major cause of morbidity and mortality due to its association with coronary heart disease, cerebrovascular disease and renal disease. Fresh *Allium sativum* bulbs have been alleged to have blood pressure lowering effects. However, the exact mechanism and long-term effects are not fully been understood. The aim of this study was to investigate the effects *A. sativum* extract treatment on cardiac and renal regulation of blood pressure in hypertension-induced male DOCA-salt model wistar laboratory rats. Sixty four male DOCA-salt wistar rats weighing 250-300g, 36 weeks old were induced to hypertension and studied for 20 weeks. Doses of 50, 100 and 200 mg/kg body weight of *A. sativum* were prepared (FAGE-T1, CGE-T2 and CIGE-T3) and administered intraperitonially (IP) twice a day. Plasma levels of electrolytes (Na^+, K^+, Ca^{2+}, Cl^-), enzymes, heart histology and electrocardiogram recording were analysed. From the study, *A. sativum* extracts did not have effect on the mean plasma antidiuretic hormone (ADH) in comparison to the normotensive control group at $p<0.05$. However, FAGE 200mg/Kg body weight significantly decreased plasma *B*-type atrial natriuretic peptide (ANP) by 29.5% ($n = 4$; $p<0.05$) compared to the control group treated with normal saline (NS). The antihypertensive drugs Captoten (CPT) and Hydrochlorothiazide (HCT) lowered the mean plasma angiotensin II (Ang-II) levels by 30% ($n = 4$; $p<0.05$) and by 36.75% ($n = 4$; $p<.05$) of the control. No effect was noted in control groups C1, C2 and group T1 on mean plasma levels of aldosterone hormone (Ald). However, *A. sativum* extracts (CGE and CIGE) significantly lowered the mean plasma levels of Ald after longer treatment (20 weeks), compared to the control group ($n = 4$; $p<0.05$) by 7.67% ($n =4$; $p<0.05$) and 17% ($n = 4$; $p<0.05$) respectively. *A. sativum* extracts decreased mean plasma renin levels just as the antihypertensive drugs used in treatment groups C2 and C3 respectively, compared to the control group ($n = 4$; $p<0.05$). All *A. sativum* extracts showed significant decrease in mean plasma Na^+ ion ($p<0.05$) at all concentration levels in all hypertensive groups by 15.625% ($n = 4$; $p<0.05$) compared to the control group ($n = 4$; $p<0.05$). Seven out of 11 treatments significantly lowered mean plasma K^+ ion levels ($n =4$; $p<0.05 = 79.72\%$). All *A. sativum* extracts triggered significant decrease in mean plasma levels of Chloride (Cl^-) ions ($n = 4$; $p<0.05 = 23.25\%$). *A. sativum* extract concentrations did not show statistically significant change in mean plasma ionic Calcium (iCa^{2+}) levels compared to the control group ($p<0.05$). *A. sativum* extracts did not have significant effect on metabolites across all the treatment groups. *A. sativum* extract concentrations significantly reduced systolic blood pressure (SBP) from 150mmHg to 110mmHg (36.36% reduction) as compared to SBP in the normotensive control group at $p<0.05$ when used longer (20 weeks). All *A. sativum* extracts recorded significant decrease in mean diastolic blood pressure (DBP) with the least mean diastolic blood pressure of 63mmHg in the hypertensive treatment groups C2b, C3b and T1b (10.7% decrement) compared to the normotensive control group with 56.91mmHg at $p<0.05$ treated with NS. Electrocardiogram (ECG) recordings showed that *A. sativum* extracts reduced the RR intervals, QT intervals and Heart rate. Histological findings showed that FAGE had reversal effects on the myocardium post-hypertension as compared to the control, groups T2 and T3. These findings signify that garlic (*A. sativum*) use modulatory mechanisms including reduction of sodium ion concentration, reduction of systolic and diastolic blood pressure, plasma cholesterol levels, and renin.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ALT	-	Alanine aminotransferase test
ALD	-	Aldosterone
Ang-II	-	Angiotensin II
ACE	-	Angiotensin converting enzyme
ARCCG	-	Animal Rights and Care Committee Guidelines
ADH	-	Anti-diuretic hormone
AST	-	Aspartate aminotransferase test
B-ANP	-	*B*-type Atrial nautretic peptide
BP	-	Blood pressure
iCa^{2+}	-	Calcium ion
CI	-	Cardiac Index
CR	-	Cardiac rate
cTi	-	Cardiac troponin I
cTt	-	Cardiac troponin T
CNS	-	Central nervous system
Cl^-	-	Chloride ion
CKD	-	Chronic kidney disease
C1, C2, C3	-	Control groups
CD	-	Coronary diameter
CV	-	Coronary vasculature
CK-MB	-	Creatine kinase isoenzyme MB
CIGE	-	Crude industrial garlic extract
CGE	-	Crude garlic extract
DOCA	-	Deoxycorticosterone Acetate
PhD	-	Doctor of philosophy degree
ESRD	-	End-stage renal disease
ECF	-	Extracellular fluid
ESH-ESC	-	European Society of Hypertension and the European Society of Cardiology
EDTA	-	Ethylene-diamine tetra-acetic acid
ECG	-	Electrocardiography
FAGE	-	Fresh aqueous garlic extract
GC-MS	-	Gas-chromatography
GFR	-	Glomerular filtration rate
HF	-	Heart failure
HDL	-	High-density lipoproteins
HPLC	-	High-performance liquid chromatography
HCT	-	Hydrochlorothiazide
HTN	-	Hypertension /High blood pressure

IP	-	Intraperitonially
IND	-	Investigative new drugs
JGA	-	Juxtaglomerular apparatus
KeMU	-	Kenya Methodist University
LVT	-	Left ventricular thickness
LFMC	-	Length and force of myocardial contractility
LDL	-	Low-density lipoproteins
mg	-	Milligrams
mg/dL	-	Milligrams per deciliter
mmHg	-	Millimetres of Mercury
NIH	-	National Institutes of Health
nC3	-	Negative controls
NO	-	Nitric oxide
NMR	-	Nuclear magnetic resonance spectrometer
ANOVA	-	One-Way Analysis of Variance
pC3	-	Positive controls
K^+	-	Potassium ion
PV	-	Pulmonary vasculature
RAAS	-	Renin-Angiotensin-Aldosterone System
NaCl	-	Sodium Chloride
Na^+	-	Sodium ion
SEM	-	Standard error of Mean
SNS	-	Sympathetic nervous system
TLC	-	Thin-layers chromatography
TID	-	Transient ischemia disease
T1 – T3	-	Treatment groups
TPR	-	Total peripheral vascular resistance
UoE	-	University of Eldoret
UoN	-	University of Nairobi
WHO	-	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background information

Hypertension is a major cause of morbidity and mortality because of its association with coronary heart disease, cerebrovascular disease and renal disease. The extent of target organ involvement (i.e. heart, brain and kidneys) results to end-organ damage. North American studies have shown that hypertension is a major contributor to 500,000 strokes (250,000 deaths) and 1,000,000 myocardial infarctions (500,000 deaths) per annum (Cain and Khalil, 2002).

In general practice, the level of blood pressure above which treatment of hypertension is indicated is now set at 140/90 mmHg. Increased systemic vascular resistance, increased vascular stiffness, and increased vascular responsiveness to stimuli are central to the pathophysiology of hypertension. Newer antihypertensive agents such as ACE inhibitors and angiotensin II receptor antagonists are effective, but not more than diuretics and β-blockers (Foex and Sear, 2004).

National surveys continue to reveal that hypertension is often not detected and, where diagnosed, is often inadequately treated. Among hypertensive patients, only 25% appear to be well controlled. This is particularly true of isolated systolic hypertension. Yet the prevalence of isolated systolic hypertension increases with age. Indeed, the proportion of subjects suffering from isolated systolic hypertension, as opposed to systolic and diastolic hypertension, increases from 20% in the under 40 yr to 80% in the 60–69 yr old, and to 95% in those >80 yr. There is increasing emphasis on the risk associated with systolic hypertension as the level of systolic pressure is a good predictor of coronary and cerebrovascular risk, especially in the elderly. Treatment of systolic hypertension with its

wide pulse pressure is effective in terms of control of blood pressure and reduced morbidity, especially in older patients with high risk profile (James *et al.,* 2014).

Over the past decade the management of hypertension has changed with the recognition that there is no threshold below which elevated blood pressure causes no threat to health. Recent guidelines, including those of the British Hypertension Society, make it clear that treatment of isolated systolic hypertension is as important as that of systolic and diastolic hypertension (Williams *et al.*, 2004). The threshold above which hypertension should be treated to prevent long-term complications is now 140/90 mm Hg. Indeed, in Stage 1 hypertension, treatment of isolated systolic hypertension (systolic 140– 159 mm Hg, diastolic <90 mm Hg), reduces the prevalence of left ventricular hypertrophy, a predictor of future morbidity and mortality. There is also a 42% reduction of the risk of stroke and a reduction in the risk of dementia (James *et al.*, 2014).

The hypertension optimal treatment (HOT) study indicates that the treatment goal is to reduce blood pressure to 140/85 mm Hg (Hansson, 1998). It is also established that high normal blood pressure (130–139/85–89 mm Hg) progresses to Stage 1 hypertension (>140/>90 mm Hg) in >37% of individuals <64 yr and >49% of those >65 yr. The British National Formulary recommends the following approach (Williams *et al.,* 2004).

- blood pressure >220/>120 mm Hg: immediate therapy;
- blood pressure 200–219/110–119 mm Hg: confirm over 1–2 weeks, then treat; or
- blood pressure 160–199/100–109 mm Hg confirm over 3–4 weeks, then treat.

In patients with high blood pressure, the cumulative incidence of first cardiovascular events over 10 years is 10% in males and 4.4% in females. Even high normal blood

pressure is correlated with an increased risk of death attributable to coronary or cerebrovascular events.

In youth, the pulse pressure generated by the left ventricle is relatively low and the waves reflected by the peripheral vasculature occur mainly after the end of systole, thus increasing pressure during the early part of diastole and improving coronary perfusion. With ageing, stiffening of the aorta and elastic arteries increases the pulse pressure. Reflected waves move from early diastole to late systole. This results in an increase in left ventricular afterload, and contributes to left ventricular hypertrophy. The widening of the pulse pressure with ageing is a strong predictor of coronary heart disease (Franklin *et al.,* 1999).

The cardiac consequences of hypertension are left ventricular hypertrophy and coronary artery disease. Left ventricular hypertrophy is caused by pressure overload and is concentric. There is an increase in muscle mass and wall thickness but not ventricular volume. Left ventricular hypertrophy impairs diastolic function, slowing ventricular relaxation and delaying filling. Left ventricular hypertrophy is an independent risk factor for cardiovascular disease, especially sudden death. The consequences of hypertension are a function of its severity (Yusuf *et al.,* 2000).

High blood pressure can damage blood vessels in the kidneys, reducing their ability to work properly. When the force of blood flow is high, blood vessels stretch so blood flows more easily. Eventually, this stretching scars and weakens blood vessels throughout the body, including those in the kidneys. High blood pressure is the second leading cause of kidney failure in the United States after diabetes (National Institute of Diabetes and Digestive and Kidney Diseases, 2012 Report). Health care providers diagnose high blood

pressure when multiple blood pressure tests show that a systolic blood pressure is consistently above 140 or a diastolic blood pressure is consistently above 90. The best way to slow or prevent kidney damage from high blood pressure is to take steps to lower blood pressure. These steps include a combination of medication and lifestyle changes (Health, United States, 2011).

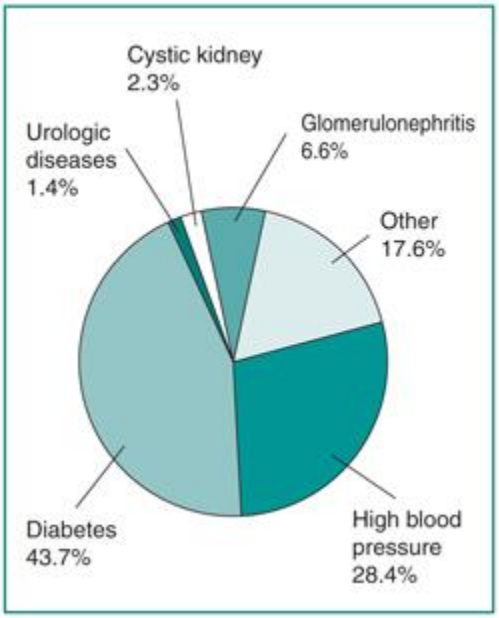


Figure 1.1 End-stage renal disease patients, by selected characteristics: United States, selected years 1980–2010. Centre for Disease Control and Prevention

Medications that lower blood pressure can also significantly slow the progression of kidney disease. Two types of blood pressure-lowering medications, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), have been shown effective in slowing the progression of kidney disease (Yusuf, 2000). Many people require two or more medications to control their blood pressure. In addition to an ACE inhibitor or an ARB, a health care provider may prescribe a diuretic—a medication that helps the kidneys remove fluid from the blood. A person may also need beta

blockers, calcium channel blockers, and other blood pressure medications (National Heart, Lung, and Blood Institute website, 2013).

Plant fibers and its extracts play an important dietary role in alleviation of diseases. Clinical studies indicate a fairly consistent blood pressure-lowering effect in hypertensive subjects. However, the diets observed in the majority of studies also differ in other respects that could influence blood pressure (e.g., lower in total calories, fats and animal protein, and atypical in sodium, potassium, chloride, and calcium content).

Physiologically, natural products with higher molecular weights and rotatable bonds with stereogenic centers are more readily absorbed compared to synthetic drugs. Thus, bioactive compounds isolated from the active extracts of the plants used in traditional medicines can be used to develop novel drugs by way of semi-synthesizing analogs of lead compounds for higher efficacy and/or lower toxicity. Human physiology is complex and the pathogenesis of any disease involves multiple pathways. Conventional medicines attempt to use a single compound to hit single target of a particular pathway for combating the related disease. This approach may be effective for an instantaneous relief but it may not be a long-term solution. Herbal formulations can be used as readily available way for investigative new drugs (IND) for the development of potent efficacious medicaments.

Garlic (*Allium sativum*) is one of the plants that have acquired reputation in different traditions as a prophylactic as well as therapeutic plant (Bakhshi *et al*., 2012) throughout history. Ancient Chinese and Indian medicine recommended garlic to aid respiration, digestion and to treat leprosy and parasitic infestation (Rivlrn, 1998). Avicenna (1988), recommended garlic as a useful compound in treatment of arthritis, toothache, chronic

cough, constipation, parasitic infestation, snake and insect bites, gynecologic diseases, as well as in infectious diseases (as antibiotic).

Garlic has attracted particular attention of modern medicine because of widespread belief about its effects in maintaining good health. In some Western countries, the sale of garlic preparations ranks with those of leading prescription drugs. There is appreciable epidemiologic evidence that demonstrates therapeutic and preventive roles for *Allium sativum*. Several experimental and clinical investigations suggest many favorable effects of *Allium sativum* and its preparations including; reduction of risk factors for cardiovascular diseases, reduction of cancer risk, antioxidant effect, antimicrobial effect and hepatoprotection (Colín-González, 2012; Aviello, 2009). These studies raised possibilities of revival of garlic's therapeutic values in different diseases.

1.1.1 Antihypertensive Properties of garlic

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular diseases including significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity (Chan *et al*., 2013). Both experimental and clinical studies on different garlic preparations demonstrate these favourable cardiovascular effects. In *in vivo* animal experiments, intravenous administration of garlic extracts produced slight reductions in both systolic and diastolic pressures (Sial and Ahmed, 1982) and oral ingestion of garlic extract in hypertensive animals brought the blood pressure back to the normal level (Chandekar and Jain, 1973). Several clinical studies showed that garlic reduced blood pressure in more than 80% of

patients suffering from high blood pressure (Auer et al., 1990; Petkov, 1979; Omar, 2013; Stabler *et al*., 2012).The present study investigated the mechanisms underlying *Allium sativum* regulation of blood pressure, with a focus on the cardiovascular and renal systems.

1.2 Statement of the problem

High blood pressure or Hypertension (HTN) is a main-stem condition that predisposes one to a trail of cardiovascular diseases. HTN is among the top five killer diseases worldwide. High blood pressure is a condition in which the blood pressure in the arteries is chronically elevated. This could lead to brain and cardiovascular damage e.g. heart attack, stroke, heart failure, aneurysm, or renal failure. The normal blood pressure (BP) is 100-140mmHg (Systollic) and 60-90mmHg (Diastolic). BP of 140/90 mmHg or above is considered hypertension (HTN). HTN has several sub-classifications including hypertension stage I, hypertension stage II, isolated systolic hypertension, exercise hypertension, pregnancy hypertension, primary hypertension and secondary hypertension. With these many sub-classifications, possibility of suffering from any form of HTN is so high, hence quite challenging to control. Result from studies on garlic and its effect on the cardiovascular system have been documented with authors supporting garlic's hypotensive activity (Chan *et al.,* 2013).

Firstly, it has not been clearly established how *Allium sativum* extract exerts its effects to be considered an antihypertensive herb. Secondly, data is also not available to show whether the various preparations of garlic, including commercial ones, have or can sustain the same potency value following long shelf life (6 months to a year - from the

time of manufacture, the time in warehouses to the time it reaches the consumer in that locality or even exported abroad) and still have similar effects to lower blood pressure (Efficacy). These reasons form basis for querry as compared to using fresh garlic. Further, none of these have been singly investigated to determine their mechanism of action and efficacy. Finally, it has not been established whether HTN causes any other alterations to the heart, apart from left ventricular hypertrophy (LVH), that subsequently predisposes one to other cardiovascular diseases like myocardial infarction (MI), ischemic heart disease (IHD), heart failure or heart attack and stroke, which have been and continue to be slow but causes of high cardiovascular morbidity and mortality in patients diagnosed with HTN.

1.3 Justification

Data from National Health and Nutrition Surveys have shown that those achieving target blood pressure values less than 140/90 mmHg are only 34% of the hypertensive population. Aging of individuals in a population, urbanization and socioeconomic changes in developing countries have led to increase in the prevalence of hypertension, with low control rates due to scarce health resources and insufficient health infrastructure. This situation is no better in the rest of the world and much worse in the developing countries. Thus prevention, detection, treatment and control of hypertension play a crucial role in protection of cardiovascular diseases.

According to the World Health Organization (WHO) statistics on the global status on HTN, about 70 million American adults (29%) have high blood pressure - that’s 1 of every 3 adults. Only about half (52%) of people with high blood pressure have their condition under control. Nearly 1 of 3 American adults has blood pressure values that are

higher than normal, but not yet in the high blood pressure range (Nwankwo *et al*., 2013). High blood pressure costs the nation $46 billion each year. This total includes the cost of health care services, medications to treat high blood pressure, and missed days of work (Mozzafarian *et al*., 2015). Women are about as likely as men to develop high blood pressure during their lifetimes. However, for people younger than 45 years old, the condition affects more men than women. For people 65 years old or older, high blood pressure affects more women than men. It has also been documented that Blacks develop high blood pressure more often, and at an earlier age, than whites and Hispanics do. More black women than men have high blood pressure (Mozzafarian *et al*., 2015).

Apart from deaths caused directly due to HTN, a huge mortality rate is recorded due to indirect effects of high blood pressure. It is noted that more than 360,000 American deaths in 2013 included high blood pressure as a primary or contributing cause. That is almost 1,000 deaths each day. High blood pressure increases the risk of other dangerous health conditions: About 7 of every 10 people having their first heart attack due to high blood pressure. About 8 of every 10 people have their first stroke due to high blood pressure. About 7 of every 10 people with chronic heart failure have high blood pressure. Kidney disease is also a major risk factor for high blood pressure (Mozzafarian *et al*., 2015). According to the World Health Organization (WHO, 2014) - 23% of Kenya's population have high blood pressure - that's 1 of every 3 adults. Apart from deaths caused directly due to HTN, a huge mortality rate is recorded due to indirect effects of high blood pressure, chronic heart failure and related cardiovascular conditions.

Though we cannot control all of our risk factors for high blood pressure, we can take measures to control high blood pressure and its complications, create awareness of the

condition and seek early treatment. Complementary/alternative medicine is increasingly becoming human's major pharmaceutical and therapeutic option of alleviating a lot of heart ailments. Some of the conventional antihypertensives are costly and involve tedious procedure to access. This raises anxiety and quest for alternative medicine, and for which this study aims at establishing the use of garlic in the management of high blood pressure.

1. 4 Broad Objective

To investigate the effects of *Allium Sativum* extract treatment on cardiac and renal regulation of blood pressure in hypertension-induced laboratory rats

1.4.1 Specific Objectives

1. To determine the effects of *Allium sativum* extract treatment on the plasma levels of antidiuretic hormone (ADH), B-type atrial natriuretic peptide (ANP), angiotensin II (Ang-II), aldosterone (ALD), and renin in hypertension-induced laboratory rats
2. To determine the effects of *Allium sativum* extract treatment on the plasma levels of electrolytes (Na^+, K^+, Cl^- and ionic Ca^{2+}) and metabolites (i.e. aspartate aminotransferase (AST), alanine aminotransferase (ALT), cardiac troponins (cTn), creatine kinase (CK-MB) and cholesterol) in hypertension-induced laboratory rats
3. To determine the effects of *Allium sativum* extract treatment on the electrical and mechanical activities of the heart in hypertension-induced laboratory rats
4. To determine the effects of *Allium sativum* extract treatment on the histology of the heart and the kidneys in hypertension-induced laboratory rats

1.5 Hypothesis:

H_0: *Allium sativum* extracts have no effect on cardiac and renal regulation of blood pressure, ADH, renin, Ald, ANP, electrolytes and metabolite in hypertension-induced laboratory rats.

H_1: *Allium sativum* extracts have effect on cardiac and renal regulation of blood pressure, ADH, renin, Ald, ANP, electrolytes and metabolite in hypertension-induced laboratory rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Hypertension (HTN) or High Blood Pressure

Hypertension is usually a slowly-developing disorder of middle to old age which predisposes to the cardiovascular disorders that cause most of the morbidity and mortality in the elderly (Weindruch, 1995). The incidence and sequelae of hypertension vary markedly by patient subgroup, particularly by gender and race.

High blood pressure is a condition in which the blood pressure in the arteries is chronically elevated. The heart usually works harder to pump blood to the rest of the body. This could lead to brain and cardiovascular damage e.g. heart attack, stroke, heart failure, aneurysm, or renal failure. The normal human blood pressure (BP) is 100-140mmHg (Systollic) and 60-90mmHg (Diastolic). BP of 140/90 mmHg or above is considered hypertension (HTN). HTN has several sub-classifications including hypertension stage I, hypertension stage II, isolated systolic hypertension, exercise hypertension, pregnancy hypertension, primary hypertension and secondary hypertension. Blood pressure is maintained by the force generated by a pump (the heart musculature), the resistance in the distribution system (the arteries = Vasculature), and the amount of intravascular fluid (Body fluids). Resistance is related to the size of the arterial bed. At the arteriole level, opening and dilating arterioles reduces pressure.

2.1.1 Blood Pressure and its Regulation

The main coordinating center is in the medulla oblongata of the brain; medullary cardiovascular control center. Reflex control of blood pressure involves the baroreceptor reflex is shown by Figure 2.1.1a.

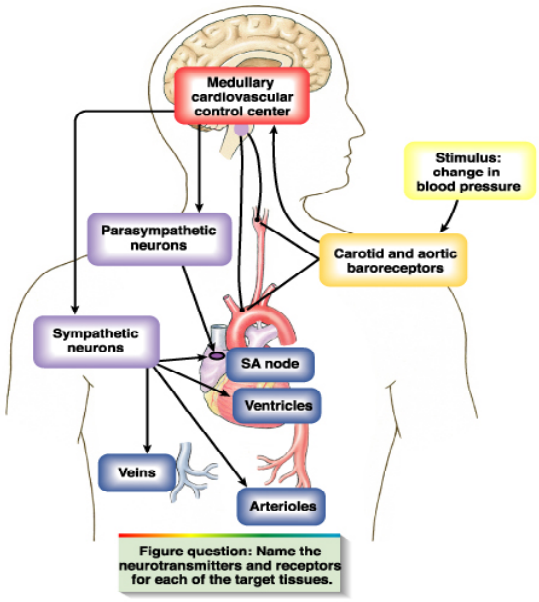


Figure 2.1.1a Coordination of Blood Pressure Regulation (Guyton, 1990)

2.1.1.1 The Heart and Blood Pressure

When a person is at rest, the heart pumps only 4 to 6 litres of blood each minute. During severe exercise, the heart may be required to pump four to seven times this amount. The basic means by which this volume is pumped out of the heart is regulated by (1) intrinsic cardiac regulation of pumping in response to changes in volume of blood flowing in to the heart and (2) control of the heart rate and strength of the heart pumping by the Autonomic nervous system (ANS) (Esler, 2010). Both divisions of the ANS, the Parasympathetic nervous systems (PNS) and Sympathetic nervous systems (SNS) regulate blood pressure via the Vagus nerve (Figure 2.1.1b). They act by increasing and decreasing the blood pressure when it begins to move outside of the bounds of normalcy (Guyton, 1990). For given levels of arterial blood pressure, the amount of blood pumped each minute (cardiac output) often can be increased more than 100 percent by sympathetic stimulation. By contrast, the output can be decreased to as low as zero or almost zero by vagal (parasympathetic) stimulation (Guyton, 1973).

Another mechanism in blood pressure control by the heart is natriuretic factor released from the atria of the heart, which senses filling of blood and increased volume. Subsequent increased filling, results in release of this factor, which inhibits sodium reabsorption at the distal renal tubule. If a sudden change in blood pressure occurs it is controlled in the short term by the sympathetic nervous system which alters three things: Total peripheral resistance, Capacitance and Cardiac output. It is only in the long term in response to chronic changes in blood pressure that the kidney works to alter the balance between fluid intake and output in order to regulate blood pressure (DiBona, 2005).

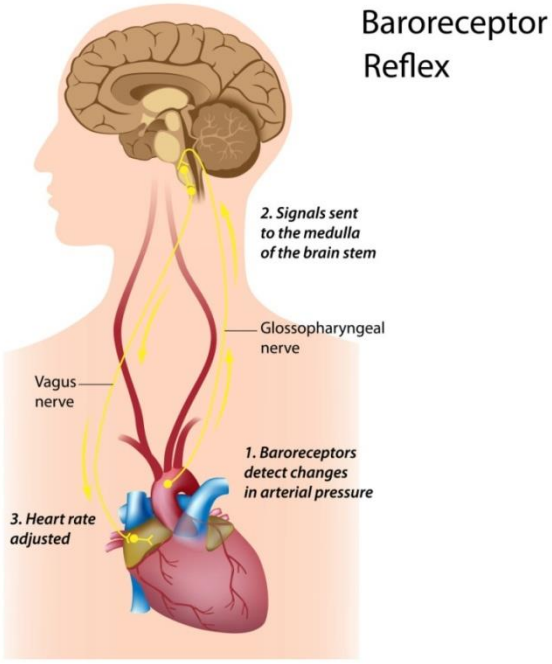


Figure 2.1.1b Regulation of the heart by the Autonomic nervous system (Esler, 2010)

When we think about blood pressure it is the mean arterial pressure that is monitored and regulated by the body. It is the force of blood pushing against the walls of the arteries as

the heart pumps blood to the systemic tissues of the body. It is not the arterial systolic or diastolic or pulse-pressure, nor the pressure found in any other part of the vascular tree. However, when blood pressure is checked, those measurements record the arterial systolic and diastolic pressures, which are used as markers to assess mean arterial pressure (Figure 2.1.1c) (Guyton, 1961). The limit for normal blood pressure as designated by the National Institutes of Health (NIH) is less than 120/80 mmHg. So, blood pressure is simply the force exerted on the blood vessels by blood being pumped by the heart, during contraction and relaxation.

2.1.1.2 Physiological Determinants of Blood Pressure

There are four major determinants of blood pressure. They include:

1. **Blood Volume** – the more volume of blood present means that the vessels and heart have to work hard to pump that blood through the Circulatory system.
2. **Overall Compliance** – the elastic characteristics of the vessels contribute to the overall pressure in the vessels. When the vessels have expanded the blood pressure is lowered and if it recoils blood pressure will increase.
3. **Cardiac Output** – CO is related to two other factors: heart rate and stroke volume. When the heart rate is fast, CO is increases and when stroke volume is high, CO also increases. Therefore when CO increases, then the arterial pressure will also increase.
4. **Peripheral Resistance** – the resistance of the arteries is related to the Overall Compliance characteristic. When peripheral resistance increases, the overall compliance decreases and thus the arterial blood pressure increases.

2.1.1.3 Importance of Blood Pressure Regulation

As blood is pumped from the heart to the various blood vessels, enough pressure is generated in order to send blood to all parts of the body. As the blood travels further from the heart, they branch off and gradually decrease in size, much like the branches of the tree. One branch may travel to the stomach, while another may transport blood to the muscle and yet another to the brain, etc. Blood pressure keeps the blood flowing through all these branches so that the cells of the body can receive the oxygen and nutrients needed, and get rid of the waste products from respiring and metabolizing tissues and organs to sustain life.

When the heart contracts, pressure builds up in the blood vessels as blood passes through, while the opposite is true when the heart relaxes, in between heart beats. For the blood to be able to reach all of the vital organs, healthy, elastic blood vessels that will stretch and recoil as the pressure goes up and down respectively, are needed. Persons who suffer from **hypertension**, their small blood vessels in vital organs are most often affected over time. These vessels become scarred, harden and inelastic, which means they are more likely to get blocked or worse rupture which could lead to organ damage and even the failure of these organs in some cases. So it is important to regulate hypertension to reduce the risks of: organ damage, heart attacks and strokes.

In the case of **hypotension**, the blood pressure is abnormally low. When the pressure is this low, blood is not pumped effectively through the systemic circuit of the body. This leaves the body with a lack of blood supply getting to major/vital organs. With organs not

receiving optimal blood supply, it's cells do not receive the proper amounts of oxygen and cannot carry out fundamental metabolic processes efficiently, which reduces the amount of energy the cells produce to power the body. This will lead to a host of problems such as, fainting, dizziness, seizures etc. So, regulation of blood pressure is of utmost importance for our survival!

2.1.2 The Kidney and Blood Pressure

In the renal system, when the glomerular filtration rate (GFR) drops, the stretch receptors in the macula densa signal cells of the juxtaglomerular apparatus (JGA) secrete renin (Figure 2.1.2). Renin stimulates the liver to secrete Angiotensinogen which is converted to angiotensin I. Angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE) secreted by the lungs capillaries endothelial tissues. Angiotensin II is a powerful vasoconstrictor, mainly in peripheral arterioles, which increases peripheral vascular resistance, thereby elevating blood pressure. It also ensures tubular sodium and chloride reabsorption, potassium excretion and water retention. In addition, renin stimulates release of aldosterone by zona glomerulosa of adrenal cortical cells. Aldosterone exerts an effect on the distal renal tubules, causing them to increase sodium reabsorption while secreting potassium. Retention of sodium leads to an increase in fluid (Blood volume) in the vascular system to maintain pressure. (Manrique *et al*., 2009).

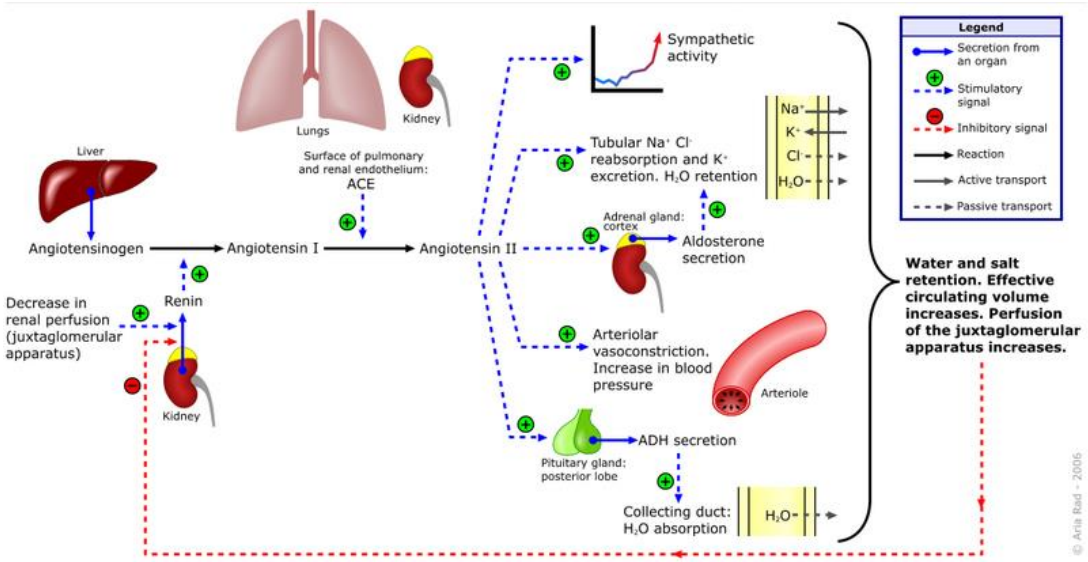


Figure 2.1.2 The Renin-Angiotensin-Aldosterone System that contributes to regulation of Blood Pressure (Hani and Stephen, 2012)

Another factor in blood pressure control is natriuretic factor released from the atria of the heart, which senses filling of blood and increased volume. Subsequent increased filling, results in release of this factor, which inhibits sodium reabsorption at the distal renal tubule.

2.1.3 Role of the kidney in regulating blood pressure

The kidneys perform many functions that are important to your body. They include controlling the composition of your blood and eliminate wastes -- filtration/reabsorption/secretion method, influencing blood pressure -- renin secretion and helping to regulate body's calcium and vitamin D activation.

The kidney particularly plays a central role in the regulation of arterial blood pressure. A large body of experimental and physiological evidence indicates that renal control of extracellular volume and renal perfusion pressure are closely involved in maintaining the arterial circulation and blood pressure. Renal artery perfusion pressure directly regulates sodium excretion—a process known as pressure natriuresis—and influences the activity

of various vasoactive systems such as the renin–angiotensin–aldosterone system (Hani and Stephen, 2012). As a result, many researchers argue that identifying any marked rise in blood pressure requires resetting of the relationship between arterial blood pressure and urinary sodium excretion, which can occur by an array of systemic or local mechanisms (Nature Reviews Nephrology, 2012).

If the kidneys fail to function, then renal dialysis methods (artificial filtration methods) can be used to help you survive by cleansing the blood. This is especially necessary when both kidneys fail (Patschan, 1997). Although you have two kidneys, it is possible to live with only one. One healthy kidney can be donated and transplanted into a compatible person with total kidney failure. Kidney transplants are a common way to help those people survive and live a normal life (Simoes, 2011)

People with chronic high blood pressure (**hypertension**) often take a class of drugs called **diuretics** to control their blood pressure. Diuretics reduce sodium ion (Na^+) reabsorption from the lumen of the nephron. Water reabsorption is also reduced. Therefore, Na^+ and water are lost in the urine, which increases urine flow. The decreased reabsorption of Na^+ and water from the nephron reduces blood volume, thereby reducing blood pressure (Pope, 1993).

2.1.4 Renal Mechanism of Blood Pressure Regulation

Increased pressure has a direct effect on the kidney and is shown by the following equation.

$$Q = (PA - PE) \div R$$

Q = Flow, PA = Pressure in afferent arteriole, PE = Pressure in efferent arteriole,

R = Resistance

Three mechanisms of Renal Regulation

1. Pressure diuresis: As arteriolar blood pressure increases, so flow through the kidneys also increases - see above formula. This increases filtration rate and increases urinary output (Weinberger, 1993; Howard and Mitchell, 2012).
2. Pressure natriuresis: If renal perfusion pressure is increased then sodium excretion increases i.e. sodium excretion increases when blood pressure increases. If more sodium is excreted less water is reabsorbed therefore the extracellular fluid (ECF) volume decreases and blood pressure decreases. The actual mechanism is not clear but it is thought to involve a direct effect of the pressure on the renal interstitium (Wang, 2000).
3. Renin-Angiotensin-Aldosterone System (RAAS): The RAAS was first identified as having a role in regulating arterial blood pressure and maintaining fluid and electrolyte homeostasis (Simões and Flynn, 2011; Bhave and Neilson, 2011). Specialized cells in the distal tubule called the macula densa sense the concentration of sodium and chloride. If blood pressure falls there is a reduction in concentration of sodium and chloride in the distal tubule which is sensed by the macula densa. The macula densa releases prostaglandins which act on the juxtaglomerular apparatus which releases renin into the bloodstream. The drop in blood pressure is also detected by baroreceptors in the aortic arch, carotid sinus

and the afferent renal arteriole which stimulates renin release by the juxtaglomerular apparatus (Guyton, 1990). Circulating renin is secreted by the juxtaglomerular cells in the afferent arteriole in response to multiple stimuli, including renal ischaemia, increased sympathetic nervous system (SNS) activity and reduced salt intake. Once secreted, renin stimulates the liver to produce Angiotensinogen which is activated to angiotensin I, which is later converted to angiotensin II by angiotensin-converting enzyme. Angiotensin II is a powerful vasoconstrictor that stimulates aldosterone secretion from the zona glomerulosa, which in turn leads to salt and water retention. Activation of the RAAS causes hypertension not only by increasing total vascular resistance and stimulating aldosterone-induced salt retention, but also by increasing tubular sodium reabsorption via direct action on the proximal tubules, by potentiating the activity of the SNS and by regulating glomerular filtration rate (GFR).

4. Additional roles for RAAS activation within tissues and local sites have been identified. All components of the RAAS are synthesized locally in the kidney. Angiotensinogen is synthesized in the proximal tubules and is converted to angiotensin I by renin travelling along the vascular compartment or present in the renal interstitium (Wang *et al.,* 1994). Angiotensin-converting enzyme has been localized within the proximal tubules, distal tubules and collecting ducts, and facilitates local production of angiotensin II (Thethi *et al.,* 2012). In addition to its known vasoconstrictive properties, angiotensin II also stimulates release of prorenin and renin from the collecting duct principal cells and alters the pressure–natriuresis relationship (Kang *et al.,* 2008). Finally, aldosterone synthesis is

believed to occur locally within the kidney, independent of the adrenal glands, and the renal aldosterone synthesis is increased in diabetic animals (Xue and Siragy, 2005).

5. Current knowledge supports a role for intrarenal RAAS in arterial blood pressure regulation. For example, urinary angiotensinogen level is elevated in patients with hypertension and in those with chronic kidney disease (Kobori *et al.,* 2003; Jang *et al.,* 2012). Intrarenal renin expression is increased following pharmacological infusion of angiotensin II and after subtotal nephrectomy (Prieto *et al.,* 2004). Selective renal renin inhibition using intrarenal aliskiren infusion induces diuresis and natriuresis and prevented the development of hypertension in an angiotensin II-dependent animal model of hypertension (Howard and Mitchell, 2012). Overall, evidence in favour of a role of intrarenal RAAS activation in the generation and maintenance of chronic hypertension is increasing (Kobori *et al.,* 2007; Navar *et al.,* 2011).

2.1.5 Regulation of Renal Blood Flow and Salt

It is essential that renal blood flow is maintained to ensure that adequate filtration of toxins from the blood takes place. Changes in pressure affect renal blood flow. Increase in the salt intake of an animal increases blood pressure in the short term. It increases the osmolarity of the blood which therefore increases water movement from tissues to the blood causing an increased circulating volume. As a result of this increased osmolarity more ADH is released as the osmoreceptors in the hypothalamus are triggered. This

results in increased water retention in the kidneys further increasing the circulating volume. Secondary to the increase in salt, the thirst centre is stimulated to increase fluid intake to try and counter act the increased osmolarity. This would increase blood volume and therefore pressure temporarily decreases until this will be corrected by the compensatory mechanisms (Burnier, 1994).

2.2 Epidemiology of Hypertension

The prevalence of hypertension is higher in men than age-matched premenopausal women, but similar for 70-year-old men and postmenopausal women (Hanes *et al*., 1996). Physiological levels of oestrogen exert a cardio-protective effect, with postmenopausal women being two to three times less likely to develop heart disease if receiving oestrogen replacement therapy (Stampfer *et al*., 1991). Importantly, the prevalence of hypertension is closely associated with the level of kidney function, and the proportion of patients with systemic hypertension increases with diminished levels of kidney function irrespective of the cause of kidney disease (Udani, 2011).

Based on the National Health and Nutrition Examination Survey III data set of 15,600 individuals, more than 40% of those with a glomerular filtration rate (GFR) of 60–90 ml/min/1.73 m^2 had a blood pressure level of ≥140/90 mmHg; the prevalence of patients with hypertension increased to more than 75% in those with a GFR of <30 ml/min/1.73 m^2 (National Kidney Foundation, 2002). It is important to emphasize that the prevalence of stage 2 hypertension with a systolic blood pressure of ≥160 mmHg reaches 20% in patients with more advanced renal failure (GFR <15 ml/min/1.73 m^2) (National Kidney Foundation, 2002). Despite increased awareness and improved antihypertensive drug therapy in recent years, improvements in rates of blood pressure control have been

limited for patients with chronic kidney disease (CKD), indicating that hypertension associated with kidney disease is often difficult to control (Sarafidis, *et al*., 2008; Plantinga, *et al*., 2009).

Despite overall health problems, control of HTN is far from being achieved. Data from National Health and Nutrition Surveys have shown that those achieving target blood pressure values less than 140/90 mmHg are only 34% of the hypertensive population. This situation is no better in the rest of the world and much worse in the developing countries. Aging of populations, urbanization and socioeconomic changes in developing countries have led to increase in the prevalence of hypertension, with low control rates due to scarce health resources and insufficient health infrastructure. Thus prevention, detection, treatment and control of hypertension plays a crucial role in protection of cardiovascular and neuro-diseases. Not only in developing countries but also in developing world, implementation of hypertension guidelines should be reinforced around the world.

2.3 Global Status on Hypertension

Hypertension remains a global health problem affecting more than 25% of the world's population (Kearney *et al*., 2005), and is a leading cause of morbidity and mortality in both developing and developed countries (Udani *et al.*, 2011). Hypertension remains the most common treatable risk factor for many cardiovascular conditions, including stroke, atherosclerotic vascular disease, and congestive heart failure. In the USA, hypertension is listed as the second most prevalent cause of end-stage renal disease (ESRD) and is the listed primary diagnosis in more than 125,000 patients on dialysis (US Renal Data

System, 2011). Hypertension is also the second leading cause of incident ESRD and every year more than 25,000 patients in the USA start dialysis for hypertension-related renal damage. Controlling blood pressure is a complex process as many different systems either directly or indirectly contribute to the regulation of blood pressure.

According to the World Health Organization (WHO) statistics on the global status on HTN, about 70 million American adults (29%) have high blood pressure - that's 1 of every 3 adults. Only about half (52%) of people with high blood pressure have their condition under control. Nearly 1 of 3 American adults has pre-hypertension - blood pressure numbers that are higher than normal, but not yet in the high blood pressure range (Nwankwo *et al*., 2013).

High blood pressure costs the nation $46 billion each year. This total includes the cost of health care services, medications to treat high blood pressure, and missed days of work (Mozzafarian *et al*., 2015).

The European Society of Hypertension and the European Society of Cardiology (ESH-ESC) guidelines for the management of arterial hypertension define a pre-hypertensive state in two categories: (1) normal blood pressure with systolic blood pressure of 120 to 129 mmHg or diastolic blood pressure of 80 to 84 mmHg; and (2) high-normal blood pressure with systolic blood pressure of 130 to 139 mmHg or diastolic blood pressure of 85 to 89 mm Hg. The Joint National Committee 7 from the USA has combined the normal and high-normal blood pressure categories into a single entity termed 'pre-hypertension'. Persons with prehypertension are at increased risk for cardiovascular diseases. (Kokubo and Kamide, 2009).

2.4. Blood Pressure Levels Vary by Age, Race and Ethnicity

Women are about as likely as men to develop high blood pressure during their lifetimes. However, for people younger than 45 years old, the condition affects more men than women. For people 65 years old or older, high blood pressure affects more women than men (Mozzafarian *et al*., 2015).

Table 2.4.1 Blood Pressure Levels Vary by Age

Age	Men (%)	Women (%)
20-34	11.1	6.8
35-44	25.1	19.0
45-54	37.1	35.2
55-64	54.0	53.3
65-74	64.0	69.3
75 and older	66.7	78.5
All	34.1	32.7

Blacks develop high blood pressure more often, and at an earlier age, than whites and Hispanics do. More black women than men have high blood pressure (Mozzafarian *et al*., 2015).

Table 2.4.2 Blood Pressure Levels Vary by Race and Ethnicity

Race of Ethnic Group	Men (%)	Women (%)
African Americans	43.0	45.7
Mexican Americans	27.8	28.9
Whites	33.9	31.3
All	34.1	32.7

More than **360,000** American deaths in 2013 included high blood pressure as a primary or contributing cause. That is almost **1,000 deaths each day**. High blood pressure increases your risk for dangerous health conditions. About 7 of every 10 people having their first heart attack have high blood pressure. About 8 of every 10 people having their first stroke have high blood pressure. About 7 of every 10 people with chronic heart failure have high blood pressure. **Kidney disease** is also a major risk factor for high blood pressure (Mozzafarian *et al*., 2015).

Although all risk factors for high blood pressure cannot be controlled, prevention, control and its complications can be done. Unfortunately, according to Heidenreich (2011), about 1 of 5 U.S. adults with high blood pressure still do not know that they have it. Using team-based care that includes the patient, primary care provider, and other health care providers is a recommended strategy to reduce and control blood pressure (Guide to Community Preventive Services, 2015). Reducing the average amount of salt or sodium that people eat from 3,400 milligrams (mg) to 2,300 mg per day—the level recommended in the Dietary Guidelines for Americans, 2010—may reduce cases of high blood pressure by 11 million and save 18 billion health care dollars every year **(**Palar and Sturm, 2009).

The only reliable way to detect hypertension is to regularly check blood pressure. This should be done as part of a physical exam on every adult. If hypertension is not treated, there will be organ damage to kidneys, heart, and brain which is generally not reversible. Death in persons with hypertension most often occurs from heart failure, chronic renal failure, and stroke (Law *et al.*, 2009).

2.5 Causes of Hypertension

Over 90% of the time, an identifiable cause for hypertension cannot be found. This is known as "primary" or "essential" hypertension. The term "essential" arose from a belief long ago that an increased pressure was essential to maintain blood perfusion through an abnormal arterial system. Autoregulation of blood pressure is based upon vascular changes, and dietary sodium may play a major role in this process. Increased sodium intake leads to increased intravascular fluid volume with resultant increased cardiac output that leads to increased peripheral resistance and an increase in blood pressure. This increased blood pressure then increases renal perfusion pressure that should trigger increased excretion of sodium with water. In essential hypertension the process of sodium excretion is impaired, probably due to multigenic influences. (Sacks and Campos, 2010)

The onset of hypertension is typically in middle age (Padwal *et al*., 2008). Some factors that may contribute to primary hypertension include: environmental influences such as increased salt intake, obesity and lack of exercise acting on a genetic predisposition (Bohr *et al*., 1991). The specific genes responsible for hypertension have not been identified but epidemiological, family and twin studies suggest that a substantial portion of the phenotypic variation in blood pressure is genetically determined (Dzau *et al*., 1995). Long-term hypertensives often have other cardiovascular risk factors including elevated cholesterol levels, reduced high-density lipoproteins, diabetes, left ventricular hypertrophy and obesity (Weindruch, 1995). Untreated hypertensives present acutely with stroke, coronary artery disease leading to myocardial infarction or acute renal failure. Most patients have essential hypertension, where no cause can be determined,

which leads to many abnormalities in the physiological regulatory systems for blood pressure including neurotransmitters and humoral factors with abnormalities of the cardiac and vascular smooth muscle and endothelium. It is often unclear which of these changes are causative and which are secondary to the hypertension (Bohr *et al*., 1991). These are the characteristics of human hypertension which rat models should mimic.

Less than 10% of the time, hypertension has unidentifiable underlying cause, though this does not necessarily mean that recognition will provide a cure for hypertension. Unidentifiable underlying cause for hypertension may include:

1. Renal Diseases: just about any renal disease leading potentially to renal failure can result in hypertension. Such diseases can include:
    - Diabetic nephropathy
    - Glomerulonephritis
    - Renal vascular diseases (renal artery stenosis, fibromuscular dysplasia, vasculitis)
    - Dominant polycystic kidney disease
    - Renal cell carcinoma
2. Endocrine Diseases:
    - Cushing's syndrome with increased cortisol
    - Pheochromocytoma, with increased catecholamines (tends to be episodic)
    - Aldosterone secreting neoplasm (adrenal cortical adenoma)
3. Neurogenic Causes: such as increased intracranial pressure (tends to be of sudden onset)
4. Vascular Diseases:

- Aortic coarctation
- Vasculitis (such as polyarteritis nodosa)
- Fibromuscular dysplasia of renal arteries

Many studies have been undertaken using rat models of hypertension and heart failure (De Jong, 1996). This study uses a rat model of hypertension with focus on systemic hypertension, but some consideration of renal and pulmonary hypertension is inevitable. Excessive vasoconstriction, commonly involving the endogenous peptides, angiotensin II and endothelin, or deficient vasodilatation, often involving nitric oxide (NO), are common mechanisms in hypertension, whether defined as systemic, pulmonary or renal.

2.6 Pathophysiology of Hypertension

Renal Disease: the renal vasculature shows changes with hypertension.

- "Benign" nephrosclerosis: modest elevations in blood pressure over the years result in thickening of small renal arteries and arterioles, known as hyaline arteriolosclerosis. This vascular disease leads to formation of small cortical scars, with reduction in renal size.
- "Malignant" nephrosclerosis: in a small number of persons with previously mild hypertension, or as the initial event, there is a marked rise in blood pressure. Diastolic pressure may exceed 120 to 150 mmHg. The changes seen in arterioles may include:
    - Fibrinoid necrosis
    - Necrotizing arteriolitis
    - Hyperplastic arteriolosclerosis

Heart Disease: the pressure load placed on the left ventricle results in left ventricular hypertrophy. The heart enlarges and dilates, with hypertrophy more marked than dilation, until the left heart begins to fail, particularly when the heart reaches 500 gm in size. Congestive heart failure and cardiac arrhythmias may result from the failing heart.

CNS Disease: the effect of hypertension on small arteries and arterioles in the brain is to cause thickening and loss of resilience. This hypertensive hyalinization may produce occlusion with resultant small lacunar infarcts, or "lacunes" that appear most commonly in the region of the basal ganglia, internal capsule, thalamus, basis pontis, and hemispheric white matter. This arteriolar sclerosis also results in vessels that are more prone to rupture. The most common site for rupture is the region of the basal ganglia. The hypertensive hemorrhage that results from rupture is one of the causes for a "stroke".

2.7 Treatment of Hypertension

Treatment of hypertension depends upon severity and response to interventions. Lifestyle changes like eating healthy diet with less salt, exercising regularly, quit smoking and maintain a healthy weight are recommended. But sometimes lifestyle changes aren't enough. Mild hypertension may respond to lifestyle changes with diet and exercise. Dietary changes include reducing intake of sodium and increasing intake of fruits and vegetables. Weight reduction to a BMI of <25 is beneficial (Sacks and Campos, 2010).

High sodium intake, above 2 grams per day, is implicated in 1.65 million cardiovascular deaths worldwide in 2010. During that year, the mean level of sodium consumption per person on earth was estimated to be almost 4 grams per day. (Mozaffarian *et al.*, 2014). A reduction in dietary salt intake by 9.5%, which has been achieved in the United Kingdom

over the past 5 years, could prevent a million deaths from stroke and myocardial infarction, and reduce health care costs by over 32 billion dollars, among the current U.S. adult population aged 40 to 85 years. (Smith-Spangler *et al*., 2010)

For milder hypertension with blood pressures above 140 mm Hg systolic and/or 90 mm Hg diastolic, if lifestyle changes are not followed or not effective, then pharmacologic therapy can be instituted. One may begin with a single antihypertensive agent. If that doesn't work, the medication can be continued longer to determine if there is an effect, the dose may be increased, or another agent added (Chobanian, 2009).

For more severe hypertension with systolic pressures above 160 mm Hg or diastolic above 100 mm Hg, pharmacologic therapy is instituted with two antihypertensive agents. If that doesn't work, another agent is added (Frank, 2008).

2.7.1 Blood Pressure (BP) Treatment Goals

Table 2.7 Blood pressure treatment goal depends on the healthy status

Blood Pressure	**Treatment Goal**
Less than150/90 mm Hg	If you're a healthy adult age 60 or older
Less than140/90 mm Hg	If you're a healthy adult younger than age 60
Less than140/90 mm Hg	If you have chronic kidney disease, diabetes or coronary artery disease or are at high risk of coronary artery disease

Blood pressure treatment goal depends on the healthy status of an individual (Table 2.7). BP of 120/80 mm Hg or lower is the ideal blood pressure goal. An age of 60 or older, and use of medications produces lower systolic blood pressure (such as less than 140 mm

Hg), medications won't need to be changed unless they cause negative effects to your health or quality of life. People older than 60, commonly have isolated systolic hypertension — when diastolic pressure is normal but systolic pressure is high.

2.7.2 Pharmacological Agents used for treatment of high blood pressure

Thiazide diuretics: Diuretics, also known as water pills, are medications that act on the kidneys to excrete sodium and water, reducing blood volume. Thiazide diuretics are major drugs for HTN, but not the only option. Examples are hydrochlorothiazide (Microzide) and chlorthalidone. Diuretics or calcium channel blockers function best for Africans than do angiotensin-converting enzyme (ACE) inhibitors alone. A common side effect of diuretics is increased micturition.

Beta blockers: These drugs reduce the workload on the heart and causes vasodilation, with reduced heart rate and force of contractility. Examples of beta blockers include acebutolol and atenolol. Beta blockers don't work as well, especially in older adults, but may be effective when combined with other blood pressure medications.

Angiotensin-converting enzyme (ACE) inhibitors: These medications — such as lisinopril, benazepril and captopril relax blood vessels by blocking the formation of a natural chemical that narrows blood vessels.

Angiotensin II receptor blockers (ARBs): These medications relax blood vessels by blocking the action, not the formation, of a natural chemical that narrows blood vessels.

ARBs include candesartan (Atacand) and losartan (Cozaar). People with chronic kidney disease may benefit from having an ARB as one of their medications.

Calcium channel blockers: These drugs like amlodipine (Norvasc), diltiazem (Cardizem and Tiazac relax the muscles of your blood vessels. Some slow the heart rate. Calcium channel blockers function well in older people than do ACE inhibitors alone. Grapefruit juice interacts with some calcium channel blockers, increasing blood levels of the medication and puts one at higher risk of side effects.

Renin inhibitors: Aliskiren (Tekturna) slows down the production of renin, an enzyme produced by the kidneys that starts a chain of chemical steps that increases blood pressure. Tekturna works by reducing the ability of renin to begin this process. Due to a risk of serious complications, including stroke, aliskiren shouldn't be taken with ACE inhibitors or ARBs.

2.7.3 Additional medications used to treat high blood pressure

If blood pressure goals cannot be achieved with combinations of the above medications, the doctor may prescribe:

Alpha blockers: These drugs reduce nerve impulses to blood vessels, reducing the effects of natural chemicals that narrow blood vessels. Alpha blockers include doxazosin and prazosin.

Alpha-beta blockers: In addition to reducing nerve impulses to blood vessels, alpha-beta blockers slow the heartbeat to reduce the amount of blood that must be pumped through the vessels. Examples of alpha-beta blockers include carvedilol and Labetalol.

Central-acting agents: These drugs prevent the brain from signalling the nervous system to increase heart rate and narrow blood vessels. Examples include clonidine, guanfacine and methyldopa.

Vasodilators: These medications, including hydralazine and minoxidil, function directly on the muscles in the walls of your arteries, preventing the muscles from tightening and arteries from narrowing.

Aldosterone antagonists: Examples are spironolactone (Aldactone) and eplerenone (Inspra). These drugs block the effect of a natural chemical that can lead to salt and fluid retention, which can contribute to high blood pressure.

2.7.4 Resistant hypertension: When your blood pressure is difficult to control!

If blood pressure remains stubbornly high despite taking at least three different types of high blood pressure drugs, one of which usually should be a diuretic, this is resistant hypertension. People who have controlled high blood pressure but are taking four different types of medications at the same time to achieve that control also are considered to have resistant hypertension (Plantinga, 2009). The possibility of a secondary cause of the high blood pressure generally should be reconsidered (National Kidney Foundation, 2002).

Adjustment of medications is required to come up with the most effective combination and doses. Adding an aldosterone antagonist such as spironolactone (Aldactone) often leads to control of resistant hypertension. Some experimental therapies such as catheter-based radiofrequency ablation of renal sympathetic nerves (renal denervation) and

electrical stimulation of carotid sinus baroreceptors are being studied (Esler, 2010; Rafiq *et al.,* 2012; Mark, 1996).

In addition, medications being taken can be reviewed due to other conditions. Some medications, foods or supplements can worsen high blood pressure or prevent your high blood pressure medications from working effectively (Sarafidis *et al.,* 2008). Lack of taking blood pressure medications exactly as directed, skipping doses because you can't afford the medications, because you have side effects or because you simply forget to take your medications, can worsen the condition.

2.8 Biology of *Allium sativum* L. (Garlic)

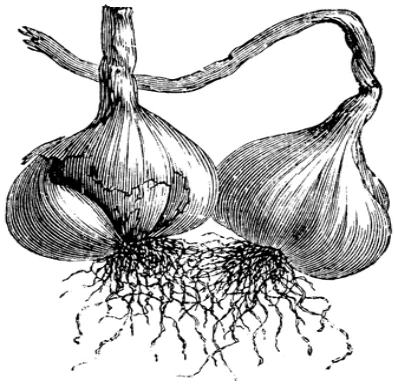


Figure 2.8.1 Garlic plant (Iciek *et al*., 2009)

Garlic is a bulbous plant; grows up to 1.2m in height. Garlic is easy to grow and can be grown in mild climates (Figure 2.8.1). There are different types or subspecies of garlic, most notably hard-neck garlic and soft-neck garlic. Botanically, *Allium sativum* is a member of the Lillaceae family, along with onions, chives, and shallots (Iciek *et al*., 2009; Lanzotti, 2006).

Figure 2.8.2 *Allium sativum* bulbs (Source: Author, 2016)

2.8.1 *Allium sativum's* Chemical Constituents

Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfonate) are the principal bioactive compounds present in the aqueous extract of *Allium sativum* or raw *Allium sativum* homogenate. When garlic is chopped or crushed, allinase enzyme is activated and converts allicin from alliin (present in intact garlic). Other important compounds present in garlic homogenate are 1-propenyl-allyl-thiosulfonate, allyl-methyl-hiosulfonate, (E, Z)-4, 5, 9-trithiadodecal, 6,11-triene 9- oxide (ajoene), and y-L-glutamyl-S-alkyl- L-cysteine. The adenosine concentration increases several folds as the homogenate is incubated at room temperature for several hours.

Another widely studied garlic preparation is aged garlic extract. Sliced raw garlic stored in 15-20% ethanol for more than $1_{1/2}$ years is refereed to aged garlic extract. This whole process causes considerable loss of allicin and increases activity of certain newer compounds, such as S-allylcysteine, sallylmercaptocysteine, allixin, N-0 -(Ideoxy- D-fructos- 1 -yl)-Larginine, and selenium which are stable and significantly antioxidant. Medicinally used, garlic oil is mostly prepared by steam distillation process. Steam-

distilled garlic oil consists of the diallyl, allyl methyl, and dimethyl mono to hexa sulfides (Lawson and Bauer, 1998).

2.8.2 Effects of *Allium sativum* on cardiovascular diseases

Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular diseases. The wealth of scientific literature supports the proposal that garlic consumption have significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity (Chan *et al.*, 2013). Garlic is known in many countries as a strong inhibitor of several diseases (Pedraza-Chaverii and Tapia, 1998)). Garlic has selenium, oligosaccharides and flavonoids, which can promote health. Garlic, onion, green onions and scallion are major sources of *Allium* in human diet. However, the mechanisms are not well documented.

2.8.3 Effect of *Allium sativum* on HTN

HTN is an important disease, which is commonly diagnosed in developing countries (Srinath and Katan, 2004). HTN can induce degenerative diseases especially cardiovascular diseases. Documentation has indicated the effects of some functional foods and nutrient on blood pressure, for example, garlic decreases blood pressure and aortic rigidity due to aging (Auer et al., 1990). Both experimental and clinical studies on different garlic preparations demonstrate these favorable cardiovascular effects. In a research done on a group of patients with high diastolic blood pressure, garlic powder consumption for 12 weeks could decreased diastolic blood pressure compared to control

(Auer et al., 1990). Another research on men with hypercholesterolemia indicated that garlic extract decreased systolic blood pressure compared to the control group (Steiner et al., 2001). In a double blind study in healthy adults, consumption of garlic pills three times per day for 12 weeks could not change blood pressure. Changes in blood pressure were not observed in another study on patients with hypercholesterolemia who consumed garlic (Jain et al., 1977).

In *in vivo* animal experiments, intravenous administration of garlic extracts produced slight reductions in both systolic and diastolic pressures (Sial and Ahmed, 1982) and oral ingestion of garlic extract in hypertensive animals brought the blood pressure back to the normal level (Chandekar and Jain, 1973). Several clinical studies showed that garlic reduced blood pressure in more than 80% of patients suffering from high blood pressure (Auer *et al.*, 1990; Petkov, 1979; Omar, 2013; Stabler *et al*., 2012). In one trial, investigation on 47 hypertensive patients showed that garlic significantly decreased the mean systolic blood pressure by 12 mmHg and the mean supine diastolic blood pressure by 9 mmHg versus placebo. The authors stated that garlic was free from side effects and no serious complication was reported (Auer *et al*., 1990).

In another study, 200 mg of garlic powder was given three times daily, in addition to hydrochlorothiazide-triamterene baseline therapy, produced a mean reduction of systolic blood pressure by 10-11 mmHg and of diastolic blood pressure by 68 mmHg versus placebo (Kandziora, 1988). However, these data are insufficient to determine if garlic provides a therapeutic advantage versus placebo in terms of reducing the risk of cardiovascular morbidity in patients diagnosed with hypertension (Stabler *et al*., 2012).

It has been suggested that the mechanism of antihypertensive activity of garlic is due to its prostaglandin-like effects, which decrease peripheral vascular resistance (Rashid and Khan, 1985). Aged garlic extract was superior to placebo in lowering systolic blood pressure in patients suffering from uncontrolled hypertension. A dosage of 240-960 mg of aged garlic extract containing 0.6-2.4 of S-allylcysteine significantly lowered blood pressure by about 12 mmHg over 12 weeks (Ried *et al*., 2013a).

2.8.4 Effect of *Allium sativum* on HTN with focus on Lipids and Cardiovascular Health

Garlic administration in rats suffering from hypercholesterolemia, induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride, and LDL, but there was no effect on serum HDL (Kamanna and Chandrasekhara, 1982). In *in vitro* experiments, garlic administration suppressed LDL oxidation and increased HDL, which may be one of the protective mechanisms of the beneficial effects of garlic in cardiovascular health (Rahman and Lowe, 2006). Long term application of garlic and its preparations on experimental atherosclerosis induced by a high cholesterol diet, showed 50% reduction in atheromatous lesions, particularly in the aorta (Jain, 1977).

Most of human studies on lipid lowering effects of garlic and garlic preparations described significant decrease in serum cholesterol and triglyceride (Gardner *et al*., 2001; Ziaei *et al*., 2001). A meta-analysis including 39 primary trials of the effect of 2 months administration of garlic preparations on total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides was performed (Ried *et al*., 2013b). The results suggest garlic is effective in reduction of total serum cholesterol by 17±6 mg/dL and low-density lipoprotein cholesterol by 9 ± 6 mg/dL in

subjects with elevated total cholesterol levels (>200mg/dL). An 8% reduction in total serum cholesterol is of clinical relevance and is associated with a 38% reduction in risk of coronary events at 50 years of age.

High-density lipoprotein cholesterol levels improved only slightly, and triglycerides were not influenced significantly. Garlic was highly tolerable in all trials and was associated with minimal side effects. This meta-analysis study concluded that garlic should be considered as an alternative option with a higher safety profile than conventional cholesterol-lowering medications in patients with slightly elevated cholesterol (Ried *et al*., 2013b). However, a few studies using garlic powder, having low allicin yields, failed to show any lipid lowering effects (Luley *et al*., 1986). It has been suggested that different people might have different responses to garlic, thus garlic may be more beneficial for some specific groups (Zeng *et al*., 2013). Preventive effect of garlic on atherosclerosis has been attributed to its capacity to reduce lipid content in arterial membrane. Allicin, S-allyl cysteine, presented in aged garlic extract and diallyl disulfide, presented in garlic oil are the active compounds responsible for anti-atherosclerotic effect (Gebhardt and Beck, 1996; Yu-Yah and Liu, 2001).

2.9 Scientific and Medical Contributions

This aims of the study is to find out the mechanisms used by *Allium sativum* herb to mediate its hypotensive effects. A combined knowledge of Histopathology of kidney and myocardial tissue, electrophysiological (understanding the Heart structure and function), and analysis of hormones, metabolites, electrolytes and enzyme levels in plasma will unravel the concept that has for a long time not been understood. The description and analysis from this study will provide authentic evidence and support to the interaction of

antihypertensives and cardiovascular antiarrhythmic drugs. Controlling an overloaded heart, fluid volume and high blood pressure (HTN) reduces cardiomegaly. Predisposition to ischemic heart disease (IHD) is usually due to blocked coronary blood vessels leading to myocardial infarction, hence heart failure or heart attack. The mechanism of action, safety and efficacy of herbal drugs must be established through research experiments like this one. This study provides the mechanism of action utilized by garlic's active compounds to reduce and/or control HTN.

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.1 Study Sites

The research project was carried out at the University of Eldoret (UOE) in the Department of Biological Sciences animal house at the farm. The processing of various *Allium sativum* extracts, isolation and determination of concentrations were done at the Department of Chemistry and Biochemistry, University of Eldoret. The biochemical and Pathophysiological analysis took place at Nairobi Annex Laboratory (Eldoret branch). Electrophysiological procedures were conducted at the Department of Medical Physiology, University of Nairobi (UoN). All laboratory requirements and chemicals were supplied by ScieLab Chemical Suppliers (Nairobi).

3.2 Study Design

This research aimed to determine possible mechanisms of action of garlic on blood pressure via analysis of the cardiovascular and renal systems. The study used was a Laboratory-based Randomized Controlled Experimental design involving a control and treatment groups. It constituted a group of Normotensive (C1) and five groups of Hypertension-Induced Wistar laboratory rats (C2, C3, T1, T2 and T3). Sixty four (64)Wistar laboratory rats were randomly divided into 16 smaller groups dependent on the concentrations of the extracts, with four Wistar rats (n = 4) per group as follows;

Group C1: Normotensive rats - treated with normal physiological saline

Group C2: Treated with Captoten (CPT 50, 100, and 200mg/Kg body weight)

Group C3: Treated with Broad-spectrum 1st liner Antihypertensive drug (Hydrochlorothiazide = HCT 50, 100 and 200mg/Kg body weight). ***Group C2 and C3 were used as Positive Controls but hypertensive.***

Group T1: treated with Fresh Aqueous Garlic Extract – FAGE 50, 100 and 200mg/Kg body weight

Group T2: treated with Crude Garlic Extract - CGE 50, 100 and 200mg/Kg body weight

Group T3: treated with Crude Industrial Garlic Extract – CIGE 50, 100 and 200mg/Kg body weight. ***Groups T1, T2 and T3 were also Hypertensive.***

3.3 Sample Size Calculation

The sample size was calculated using the formula cited by Muganda (2003) as shown below;

$$n = \frac{Z^2Pq}{d^2}$$

where n = the desired sample size

Z = the Standard Normal deviation at the required confidence level

P = the Population in the target group estimated to have characteristics being measured

q = 1-P

d = the level of statistical significance test

3.4 Experimental Animal Model

In cardiovascular research, animal models have allowed the study of cardiovascular disease in the early stages, as well as the investigation of the mechanisms of the pathogenesis of cardiovascular disease and the effects of drug intervention (Crofton and

Share, 1997). The aim of these studies is to provide clear concepts for selected investigations in humans. An ideal animal model for any cardiovascular disease in humans should have five characteristics: (i) mimic the human disease, (ii) allow studies in chronic, stable disease, (iii) produce symptoms which are predictable and controllable, (iv) satisfy economical, technical and animal welfare considerations, and (v) allow measurement of relevant cardiac, biochemical and hemodynamic parameters. One of the recommended models for experiments of Hypertension, cardiac hypertrophy and failure is the DOCA–salt model Wistar rats (Schenk and McNeill, 1992).

3.4.1 Animal Acquisition, Feeding and Handling

Sixty four (64) male DOCA-salt Model wistar laboratory rats of 36 weeks old and weighing between 250-300g, were used for the study for a period of 20 weeks. They were purchased from the Animal house at Kenya Medical Research Institute (KEMRI). They were housed in cages of 4 animals each at the University of Eldoret (UoE) in the Department of Biological Sciences Animal House at the Teaching and Research farm. The animals were maintained in an animal room well ventilated with 12 hours light/dark cycle with temperatures ranging between 22 and 25°C monitored by a room Thermostat connected to heating bulbs with the mentioned set-points. During this period rats were fed with standard rat chow and access to tap water *ad libitum.* These animals were genetically similar, so issues of genetic indifferences would not interfere with the research. After one month of acclimatization, the 64 male wistar rats were randomly divided into 6 major groups. Five treatment groups were further divided into 3 smaller groups each, forming a further 15 small groups corresponding to the concentrations.

The aim of further division was to find out whether there were Dose-Response effects among the groups. The first 3 groups were controls (C1, C2 and C3), while the last 3 groups were treated with various preparations of *Allium sativum* (T1, T2 and T3) with concentrations of *Allium sativum* extract of 20% (50mg/Kg), 40% (100mg/Kg) and 80% (200mg/Kg) body weight of 4 animals each in the treatment groups. All animal experiments complied with the "Principles of Laboratory Animal Care" United States National Institute of Health Publication (National Institute, 2012).

3.5 Garlic Extract and Administration

A **B**

Figure 3.5.1a The initial steps of *Allium sativum* preparation (A and B) in Chemistry and Biochemistry laboratory, University of Eldoret (Source: Author, 2016)

3.5.1 *Allium sativum* Extract preparations:

Fresh *Allium sativum* bulbs and powdered industrial garlic were purchased from one supplier in Eldoret market and supermarket respectively (Figure 3.5.1a). These two types

of garlic products were to be used to compare and establish whether there were any differences in their activities and composition between the fresh bulbs and the industrially processed type. The *Allium sativum* species required contain 8-12 cloves. The cloves were chopped into smaller pieces and openly air-dried in a room at room temperature (Figure 3.5.1a). They were ground into powder and socked in a capped glass container and mixed with 96% Ethyl acetate. The mixer was allowed to mix well for 72 hours. After that, it was first filtered then centrifuged. The supernatant was placed in a hot bath (80^{o}C) to allow its alcohol evaporate completely. Including the industrial powdered garlic, the two were filtered and concentrated at 40^{o}C using a rotary evaporator. After filtration, the residues were resocked and filtered four more times to ensure complete extraction and isolation of compounds (O'Gara, 2000).

a **b** **c** **d**

Figure 3.5.1b Garlic bulb (a), Cloves (b), Chopped and dried cloves (c) and Ground cloves (d) (Source: Author, 2016)

The extracts included fresh aqueous garlic extract (FAGE), crude garlic extract (CGE), and crude industrial garlic extract (CIGE). Fractions of isolated active compounds from the crude garlic extracts as F1, $F2_{a}$, $F2_{b1}$, $F2_{b2}$. From the crude extracts, separation and purification were done using column chromatography with a determined ratio of hexane:

ethyl acetate for a good elusion. The column was packed with Silca gel 60, 0.06 – 0.2mm (70-230 mesh ASTM), with eluents of hexane and ethyl acetate. The thin-layers chromatography (TLC) was used to determine the profiles of the isolated fractions, using a ratio of 50% Hexane: 50% Ethyl acetate.

3.5.2 *Allium sativum* Extract Administration

Percentages of the *A. sativum* extract preparations were prepared as 20%, 40%, and 80% concentration from 100% stock solution. Each treatment group (T1, T2 and T3) was intraperitonially treated with preparations of *Allium sativum* with concentrations of 20% (50mg/Kg), 40% (100mg/Kg) and 80% (200mg/Kg) body weight of 4 animals each in the treatment groups. These *Allium sativum* preparations were administered intraperitonially (IP) once a day at 9:00am using insulin syringe.

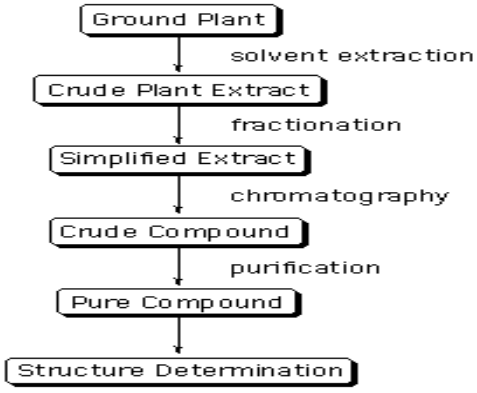


Figure 3.5.2 Schematic Flow of Processing *Allium sativum* Extract (O'Gara, 2000).

3.6 Inclusion and Exclusion criteria

3.6.1 Inclusion Criteria

The animals that were used for this study were only adult male wistar laboratory rats, weighing between 250 – 300g. They were inbred, healthy, with normal temperature and active.

3.6.2 Exclusion Criteria

Those excluded were females Wistar rats, sick, less or excess weight, dormant and non-healthy.

3.7 Laboratory procedures

3.7.1 ***Phase 1:*** Induction of Hypertension and Measurements of Blood Pressure

According to Crofton and Share (1997), Salt retention and fat accumulation is characteristic of human hypertension and can be achieved rapidly in uninephrectomised rats by mineralocorticoid administration (Kandlikar and Fink, 2011). Hypertension was induced to 5 treatment groups out of 6 groups using special diet (Fortified pellets with high lipids (20%) and weekly subcutaneous injections Deoxycorticosterone Acetate (DOCA) (Bell, 1979) salt (10%) and salt loading of 1% Sodium Chloride (NaCl) in drinking water (Mozaffarian *et al.*, 2014). The study was conducted for a period of 20 weeks (6 weeks of HTN induction and 14 weeks of treatment, observation and analysis). Blood pressures were measured using the tail-cuff method via a digital Powerlab recorder against the tail artery. A systolic blood pressure of 150mmHg and a diastolic blood pressure of 100mmHg were achieved to ascertain the induction of hypertension, with the rats' normal BP of 100/70 mmHg, to tract of the experiment. The treatment groups were treated with various preparations and concentrations of *Allium sativum* intraperitonially using insulin syringe. Normal physiological saline and known broad-spectrum antihypertensive drugs were used for the control groups and positive controls respectively as described earlier.

3.7.2 *Phase 2:* Blood Sample Collection and Biochemical Analysis

Total blood volume of a rat is approximately 6.0 ml per 100 g body weight. Not more than 10% of the total volume per rat was collected every two weeks. The tails were sterilized using 70% alcohol while the animals were conveniently restrained. A small piece of the tail-end was cut using surgical blades, and approximately 1ml of blood drawn into Ethylene-diamine tetra-acetic acid (EDTA) treated vials for each of the tests conducted with averagely three repeats.

The blood samples were used to analyze and record biochemical changes in the heart and kidney using biochemical markers and assays of plasma levels of hormones, metabolites and electrolytes [Sodium ions (Na^+), Potassium ions (K^+), Chloride ions(Cl^-) and ionic Calcium (iCa^{2+})], to depict the status of the myocardium and the kidney pre- and post-HTN (Harvey, 1990).

Other tests included Atrial Natriuretic peptide (ANP), Aldosterone (ALD) (Drury, 1985), Anti-diuretic hormone (ADH), Angiotensin II (ANGII), Alanine Aminotransferase test (ALT), Aspartate Aminotransferase test (AST), serum Creatine Kinase Isoenzyme-MB (CKMB), myocardial protein tests (i.e. serum Cardiac Troponin- I (cTi) and Troponin-T (cTt), and cholesterol-glycerol levels (Ried, 2013a). ALT and AST, though used to check liver functions, they are also produced by damaged heart, brain and kidney tissues.

3.7.2.1 Protocol for Quantitative determination of ALT and AST content in Plasma

ALT and AST were determined calorimetrically according to the method of Reitman and Frankel (Reitman and Frankel, 1957). Plasma ALT and AST levels were quantitatively measured using a spectrophotometer (Biowave S2000 made in England) and commercial

Reflotron diagnostic Kits (made by Biochemistry Company, German) packs of 30 Test strips, REF 10745120. Blood samples were collected as described in protocol section 3.7.2. Using the Reflotron pipette, the sample was drawn (avoiding bubbles) and applied as a drop to the centre of the red application zone. The Plasma sample was allowed to flow into the reaction zone. The required sample volume was 30 microlitres.

In the presence of AST or ALT, alpha-ketoglutarate and Alanine sulfinate are converted to pyruvate and glutamate respectively. In the second reaction step, catalyzed by pyruvate oxidase, the resulting pyruvate is cleaved into acetyl phosphate, carbon dioxide and hydrogen peroxide. In the presence of pyruvate oxidase, the hydrogen peroxide converts an indicator into its oxidised blue form. At temperatures of 37^0C the formation of the dye was measured kinetically at 567 nm as a measure of the enzyme activity of AST or ALT and the result displayed after 124 seconds. The enzyme activity was shown for 37^0C in U/L. All biochemical results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.2 Protocol for Quantitative determination of Cholesterol in Plasma

Plasma cholesterol levels were quantitatively determined by enzymatic calorimetric methods, using commercial Reflotron diagnostic Cholesterol kits. Briefly, blood samples were transferred directly into centrifuge tubes, allowed to clot at room temperature for 20 minutes, and centrifuged for 20 minutes at 2000 rpm. The supernatants were transferred into test tubes for lipid analysis. All Reflotron tests require a sample volume of 30

microlitres. The cholesterol concentrations (proportional to the dye formed) were measured at wavelengths of 642 nm and 37^0C, and readings were displayed after about 150 seconds in mg/dL. All biochemical results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.3 Protocol for Quantitative determination of Creatine Phosphokinase Isoenzyme-MB (CK-MB) in Plasma

***i*-CHROMA™ CK-MB** along with ***i*-CHROMA™ Reader** is a fluorescence immunoassay that measures concentration of Creatine Kinase Isoenzyme-MB (CK-MB) in whole blood/serum/Plasma. It is based on an immunoassay system using antigen-antibody reaction and fluorescence technology. The blood sample was collected and prepared as described in section 3.7.2. The test sample and the detection buffer were mixed thoroughly and then loaded into the sample wells on the test cartridge, forming a complex of antibody (anti-CK-MB) – antigen (CK-MB) – antibody (anti-CK-MB) producing fluorescence on the membrane of the test cartridge. Thus, the more the CK-MB in the test sample, the more the complexes get accumulated on the test cartridge membrane.

The ***i*-CHROMA™** Reader was used to scan the intensity of the fluorescence on the test cartridge membrane, and then displays CK-MB concentration on the LCD screen of the reader in ng/ml. All biochemical results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA),

followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.4 Protocol for Quantitative determination of Cardiac Troponin-I (cTi) in Plasma

***i*-CHROMA™ Tn-I** along with ***i*-CHROMA™ Reader** is a fluorescence immunoassay that measures concentration of Cardiac Troponin-I (cTi) in whole blood/serum/Plasma. It is based on an immunoassay system using antigen-antibody reaction and fluorescence technology. The blood sample was collected and prepared as described in section 3.7.2. The test sample and the detection buffer were mixed thoroughly and then loaded into the sample wells on the test cartridge, forming a complex of antibody (anti-Tn-I) – antigen (Tn-I) – antibody (anti-Tn-I) producing fluorescence on the membrane of the test cartridge. Thus, the more the Tn-I in the test sample, the more the complexes get accumulated on the test cartridge membrane.

The ***i*-CHROMA™** Reader was used to scan the intensity of the fluorescence on the test cartridge membrane, and then displays Tn-I concentration on the LCD screen of the reader in ng/ml. All biochemical results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.5 Protocol for Quantitative determination of Cardiac Troponin-T (cTt) in Plasma

***i*-CHROMA™ Tn-T** along with ***i*-CHROMA™ Reader** is a fluorescence immunoassay that measures concentration of Cardiac Troponin-T (cTt) in whole blood/serum/Plasma. It is based on an immunoassay system using antigen-antibody reaction and fluorescence technology. The blood sample was collected and prepared as described in section 3.7.2. The test sample and the detection buffer were mixed thoroughly and then loaded into the sample wells on the test cartridge, forming a complex of antibody (anti-Tn-T) – antigen (Tn-T) – antibody (anti-Tn-T) producing fluorescence on the membrane of the test cartridge. Thus, the more the Tn-T in the test sample, the more the complexes get accumulated on the test cartridge membrane.

The ***i*-CHROMA™** Reader was used to scan the intensity of the fluorescence on the test cartridge membrane, and then displays Tn-T concentration on the LCD screen of the reader in ng/ml. All biochemical results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.6 Protocol for Quantitative determination of Electrolytes in Serum

3.7.2.6.1 Serum preparation

A day after the last day of *Allium sativum* extract administration, blood samples were drawn from the tails as described in section 3.7.2. Portions of blood were directly collected in non-heparinised tubes and were allowed to clot. The serum was separated by centrifugation using Denley BS400 centrifuge (England) at 3000 rpm for 10 minutes and the serum collected and the subjected to electrolyte analysis.

3.7.2.6.2 Determination of Serum Electrolytes

Serum Sodium (Na^+) and Potassium ions (K^+) were measured by the flame photometric method of Vogel (1960). The Chloride ions (Cl^-) were analyzed using the method of Schales and Schales (1941). Ionic Calcium (iCa^{2+}) was determined according to laboaratory procedures of Randox Laboratories Limited Kits, United Kingdom. The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.7 Protocol for Quantitative determination of Antidiuretic Hormone (ADH) (Arginine Vasopressin = AVP) in Plasma

The quantitative determination of ADH is measured using an Arg-Vasopressin ELISA kit. It is a competitive immunoassay for the quantitative determination of Vasopressin in plasma, serum, and conditioned cell media. All components of this kit, except the standard, are stable at 4°C until the kit's expiration date. Upon receipt, the standard must be stored frozen at -20°C.

Reconstitution and Preparation of Sample

Vasopressin samples should be reconstituted in kit Assay Buffer 28 for extracted serum and plasma samples. Due to the low endogenous levels of native Arg8-Vasopressin, sample extraction is recommended, thereby concentrating them and allowing for accurate determinations of Arg8-Vasopressin. Blood samples were drawn from the DOCA-salt

Wistar rats as described in section 3.7.2 into chilled EDTA (1mg/ml blood) collecting tubes. The samples were centrifuged at 1,600 rpm for 15 minutes at 4°C. The plasma was transferred into plastic tubes and stored at -70°C. The stability of peptides was improved by the addition of protease inhibitor cocktail in the samples before freezing.

Assay Procedure

Assay Buffer 28 (100µl) was added into the NSB and the Bo (0pg/ml Standard) wells. 100µl of standards were added to tube No.1 through No. 7 into the appropriate NSB Wells, followed by 100µl of the Samples. An additional 50µl of Assay Buffer 28 was added into the NSB wells. 50µl of the blue Conjugate was added into each well, except the Blank wells, followed by 50µl of the yellow Antibody into each well, except the Blank and NSB wells. Every well used should be **Green** in color except the NSB wells which should be **Blue**. The Blank wells should be empty at this point and have no color.

The plate tapped gently to mix. It was then sealed and incubated at 4°C for 18-24 hours. The contents of the plates were emptied and washed by adding full well volume (~400 µl) of wash solution to every well. The wash repeated 2 more times for a total of 3 washes. After the final wash, the wells were emptied by aspiration and the plate firmly tapped on a lint free paper towel to remove any remaining wash buffer. 200µl of streptavidin conjugated to horseradish peroxidase (SA-HRP) was added to every well, except the Blank wells. The plates were sealed and incubated at room temperature on a plate shaker for 30 minutes at 500 rpm*.Wash as above (step 8) to remove unbound HRP conjugate and add 200µl of the TMB Substrate to every well. The plates were sealed and incubated at room temperature on a plate shaker for 30 minutes at 500 rpm. A 100µl of Stop

Solution 2 was added to every well. This stops the reaction and the plate should be read immediately. The plate reader was blanked against the Blank wells, and the optical density was recorded at **450nm** in pmol/L

The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the statistical package software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.8 Protocol for Quantitative determination of B-Type Atrial Natriuretic Peptide (B-type ANP) Hormone in Plasma

Reagents and Sample preparation

All reagents of the kit are stable at 4°C (2-8°C) until the expiry date stated on the label of each reagent. B-type ANP fragments are stable in whole blood, serum or plasma for several hours at room temperature or 4°C (2-8°C). Plasma was separated immediately by centrifugation for 20 minutes at 2000 rpm at 4^0C (2-8^0C). Required Plasma samples were aliquotted and stored at -25^0C. Before assaying, samples were mixed well.

Reconstitution of Sample

Diluted Wash buffer (WASHBUF) concentrate was used as 1:20 (1+ 19) in tap water. Crystals in the buffer concentrate dissolve at room temperature. Buffer is stable at 4°C (2-8°C) until expiry date. For the Standard (STD): 200µl of tap water was pipette into each vial. It was left at room temperature (18-26°C) for 20 min followed by gentle swirling. Reconstituted standard is stable at -25°C or lower until expiry date. For the Control (CTL): 200µl of tap water was pipette into the vials and left at room temperature

(18-26°C) for 20 min followed by gentle swirls. The Reconstituted control is stable at -25°C or lower until expiry date.

Assay protocol

All reagents and samples were raised to room temperatures (18-24°C) before use in the assay. The positions for Blank/ Standard/Sample/ Control were marked on the protocol sheet. Microtiter strips were used with a minimum of one well as Blank. 150µl Assay buffer was pipette into all wells, except the blank well. 30µl of Standards/Sample/Control were added in duplicates into respective well, except blank, followed by 50µl of Conjugate into each well, except blank, with a gentle swirl. It was covered tightly and incubated overnight (16-25 hours) at 4°C (2-8°C) in the dark. The wells were washed well 5 times with 300µl diluted washbuffer aspirate. Any remaining washbuffer was removed by hitting the plate against paper towel after the last wash. 200µl Substrate was added into each well and incubated for 20 min at room temperature (18-26°C) in the dark. 50µl of Stop solution was added into each well and shaken well. The optical density was measured immediately at 450nm with reference optical density at 630nm in pmol/L.

The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.9 Protocol for Quantitative determination of Angiotensin-II (Ang-II) Hormone in Plasma

The Euro-Diagnostica angiotensin II kit was used for the quantitative measurement of angiotensin II in plasma. After extraction the angiotensin II concentrations were measured by radioimmunoassay (RIA). This radioimmunoassay uses a rabbit anti-angiotensin II antiserum and a radio-iodinated angiotensin II tracer. Bound and free phases were separated by a second antibody bound to solid phase particles, followed by a centrifugation step. Radio-activity in the bound fractions were measured. Angiotensin II radioimmunoassay has its established application in the treatment and monitoring of hypertension.

Sample collection

Careful standardization of the animals and sampling conditions were observed. Due to the extreme liability of angiotensin II in biological fluid much care was taken to ensure that the blood sample was collected properly. Blood was collected directly from the DOCA-salt Wistar rats into cold tubes containing EDTA. The samples were then centrifuged immediately at 4° C to separate the plasma. They were frozen immediately in plastic tubes at -20° C until assay time.

Assay Procedure

During all pipetting steps, the assay tubes and reagents were kept in an icebath. 400 µL of each standard, 400 µL of controls and 400 µL of each plasma extract were pipetted in duplicate into the corresponding labelled polystyrene tubes. 400 µL of assay buffer (Reagent D) was added to the max. binding tubes (0 pmol/L), followed by addition of 600 µL of assay buffer to the NSB (blank) tubes. 200 µL of angiotensin II antiserum (Reagent A) was added to each tube, except blank and TC-tubes, followed by Vortex and

incubation for 6 hours at 4° C. 200 µL of 125I-Angiotensin II tracer (Reagent B) was then added to all tubes. Vortex was done to all tubes then incubation at 4° C for 18-22 hours. While stirring continuously, 100 µL of the double antibody solid phase (Reagent C) was added to all tubes, except TC- tubes, followed by Vortex and incubation for 30-60 minutes at 4° C. All the tubes were centrifuged for 15 minutes at 1700 rpm at 4° C. The supernatants was carefully decanted the residue counted for 1-2 minutes in a gamma counter.

The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.10 Protocol for Quantitative determination of Aldosterone (Ald) Hormone in Plasma

The Aldosterone Enzyme Immunoassay (ELISA) Kit is a complete kit for the quantitative determination of aldosterone in plasma, serum, and urine samples. Measurement of serum aldosterone in conjunction with plasma renin is used clinically to differentiate between primary and secondary aldosteronism

Sample Preparation

Plasma samples were diluted in Assay Buffer and ran directly in the assay with a minimum 1:4 dilution required for rat plasma. These are the minimum dilutions required to remove matrix interference of these samples.

Assay procedure

1000 mg C18 solid phase system columns was conditioned on a vacuum manifold by passing 5-10ml of 100% methanol through the columns, followed by 5-10ml of dH_2O. The Plasma samples were added and the columns washed with 5-10ml dH2O. Water was allowed to drain completely from columns until dry. 2ml of Diethyl ether was used to elute the samples. The samples were then dried down in a speedvac for 2-3 hrs. Using the assay buffer, samples were rehydrated at room temperature. A minimum of 250µl of the assay buffer was used to reconstitute and allow for duplicate sample measurement. Stop solution was added. The yellow color was read at 405nm in ng/dl. The amount of signal is indirectly proportional to the amount of aldosterone in the sample.

The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. $p<0.05$ was considered statistically significant.

3.7.2.11 Protocol for Quantitative determination of Renin Hormone in Plasma

The Renin ELISA is an Enzyme immunoassay for the quantitative in vitro diagnostic measurement of active Renin in plasma. Renin measurements are used in the diagnosis and treatment of certain types of hypertension.

Specimen Collection, Storage and Preparation

Plasma: Whole blood samples were collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection. Specimens were capped and stored at room temperature and NOT stored at 2-8°C prior to processing, since

cryoactivation of prorenin may occur in the temperature range of 2-8°C, giving false positive active renin values.

Assay Procedure

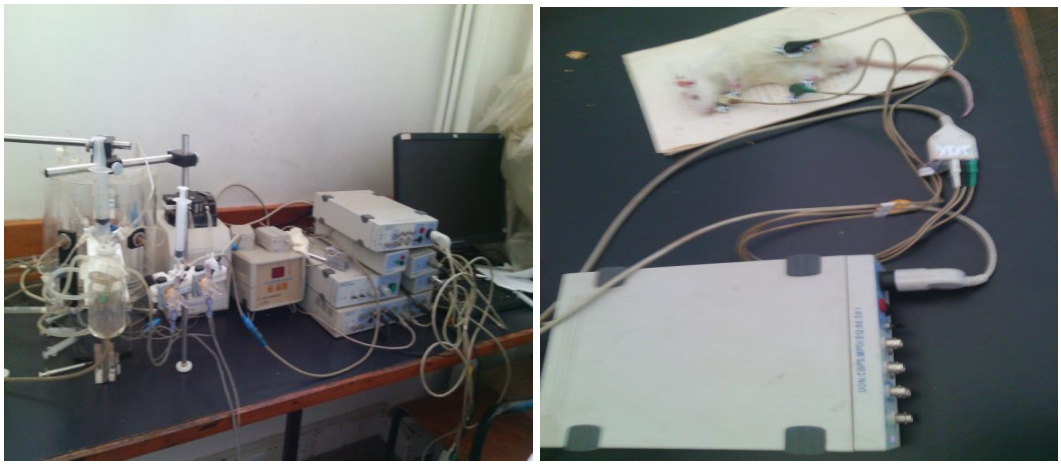
All reagents and specimens were allowed to come to room temperature before use. All reagents were mixed without foaming and all steps were completed to the end without interruption. The desired number of Microtiter wells in the frame holder was determined. 150 µL of Assay Buffer was dispensed into all wells, followed by addition of 50 µL of each Standard, Control and samples with new disposable tips into appropriate wells. Incubation was done for 90 minutes at room temperature on a plate shaker with ~700 rpm. The content of the wells were shaken out and rinsed 4 times with 300 µL diluted Wash Solution. The wells were stricken on the absorbent paper to remove residual droplets. 100 µL Enzyme Conjugate was added in all wells, followed by incubation for 90 minutes at room temperature on a plate shaker with ~ 700 rpm. The contents of the wells were Briskly shaken out and the wells rinsed 4 times with 300 µL diluted Wash Solution. The wells were sharply stricken on blotting paper to remove residual droplets. 100 µL of Substrate Solution was added to each well then incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µL of Stop Solution to each well. The optical density of each well was read at 450 ± 10 nm with a microtiter plate reader as ng/dl. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD

test suing the statistical package software (SPSS) under windows XP. Statistical significance was considered at $p<0.05$.

3.7.3 ***Phase 3:*** Electrophysiology and Conductivity Analysis using a Real-time Electrocardiogram (ECG)

Cardiovascular parameters were used to study in-vivo effects of *Allium sativum* on the electrical and mechanical activity of the myocardium from 11 sampled out animal from the 6 groups (1 from the control group and 2 from each of the five treated groups) using Bohr's procedure (1991). The assessments included measurements of the cardiac rate (CR), systolic (SBP) and diastolic blood pressure (DBP) and electrocardiography (ECG) recordings. In-vivo analysis of the ECGs included the P duration, PR intervals, RR intervals, QRS intervals, all using the PowerLab machine (Figure 3.7.3). The left ventricular thickness (LVT), coronary diameter and pulmonary vessel thickness were analyzed histologically as described by Chan, 2013.



A **B**

Figure 3.7.3 The PowerLab machine with NIBP system used for cardiovascular recording and analysis, courtesy of Medical Physiology Department, University of Nairobi (A). An anaesthetized DOCA-salt Wistar rat connected to the Cardiac transducer via the Three-surface limb-lead electrodes (B) (Source: Author, 2016)

3.7.3.1 Animal Preparation for Electrophysiology

11 selected and carefully marked male DOCA-salt Wistar rats were taken for electrophysiological studies to the University of Nairobi's department of Medical Physiology animal house where the only working PowerLab machine 2/26 (Non-Invasive Blood Pressure measurement with a pulse transducer MLT125/M) was available. Using their cages, the animals were transported during the night to avoid hyperactivity. The tests were conducted between 8:00am and 9:00am in the morning. Data was collected at the beginning of the study after the animals had acclimatized before induction of hypertension, after 3 weeks following induction of hypertension and an average of 8 tests during the 12-weeks experimental period as the groups were being treated with *Allium sativum* extracts.

3.7.3.2 Procedure

The electrophysiology study was based on clinical protocols used to evaluate cardiac conduction in human patients. Three Surface-limb-lead ECG data from 11 DOCA-salt Wistar rats were analyzed. Normal *in vivo* cardiac conduction properties for 11 of 64 rats (n =11) that underwent the procedure were summarized. For each study, an animal was irreversibly anaesthetized using Ketamine 75mg/Kg BW + Diazepam 5mg/Kg BW intraperitonially (Lasting 20min with a 2-3 hours recovery time). A surface Three-limb-lead ECG was then obtained by placement of surface electrodes on the paw of each limb, secured with tape as follows; the White electrode on the Right Forelimb, the Green electrode on the Right hind limb and the Black electrode on the Left hind limb. The ECG channels were amplified (0.1 mV/cm) and filtered between 10 and 100 Hz, and a stable

signal was reliably obtained before the electrogram recording. Body temperature, cardiac rhythm, and heart rate were monitored on the transmission screen during the recording. A warming light was used to maintain body temperature within a range of 34°C to 37°C on the warming plate for prevention of hypothermia since the weather was a bit chilly at the time of the experiment.

The PowerLab machine 2/26 (Non-Invasive Blood Pressure measurement with a pulse transducer MLT125/M) was set at a recommended manufacturer's pace necessary for rats. Cardiac rhythm was continuously monitored and recorded (at 100 mm/s), and all ECG frontal axes (P and QRS complex) and time intervals (PR, QRS, QT, RR and P duration) were calculated for each animal in standard fashion (Zipes, 1992; Hiss and Lamb, 1962), using the electrogram tracing and the digital data that were generated by the computer real-time.

3.7.3.3 Statistical Analysis

Data were presented as the Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test using the Statistical Package Software (SPSS) under windows XP. A value of $P<.05$ was considered statistically significant.

3.7.4 ***Phase 4:*** Anatomical and Histological studies of the Heart Tissues

3.7.4.1 Sacrifice of Animals

At the end of the experiment, 11 selected animals from the 6 groups (1 from the control group and 2 from each of the five treated groups), were irreversibly anaesthetized using Ketamine 75mg/Kg BW + Diazepam 5mg/Kg BW intraperitonially (Lasting 20min with

a 2-3 hours recovery time). The hearts were harvested for further anatomical and histological studies.

3.7.4.2 Protocol for determination of Histomorphological Examination

At sacrifice, the weight, length, diameter, ventricular thickness, aortic root dilation and perfusion verse size of cardiomyocytes were measured and recorded (Esler, 2010). The heart weights were determined using a top loader sensitive balance (Mettler-Toledo Garvens GmbH, Giesen, Germany). The relative weights of the heart (%) to the body weight at sacrifice were evaluated. The hearts were trimmed to remove excess fat, blood vessels or remnants of the lungs. The large vessels were clipped at their roots. The heart dimensions are measured and recorded using anatomical calipers. Two parallel lines were drawn on a polystyrene board 10cm from each other. The identification case numbers were written along the two lines at 4 – 5cm intervals. A needle was driven through each heart at the superior end of the ventricular septum to impale the heart to the board such that the upper border of the atria just touches the horizontal line drawn on the board and the apex of the heart points downwards at an angle perpendicular to the horizontal line. Each heart was fixed to its assigned position corresponding to its identification case number. Transverse sections were made across each ventricle at 3mm and 6mm from the apex of the heart. The left ventricular thickness was measured and recorded. The tissue sections from the apex and the mid-ventricular region were placed in cassettes and processed histologically, then examined by a pathologist.

3.7.4.3 Histological techniques

The tissues were stored in 10% neutral-buffered formalin and transported to Lancet Company in Nairobi. Different cross-sectional planes of the cardiac tissues were sectioned and tissue slides prepared for Histomorphological reading and analysis following prolonged hypertension. Sections of 5 micrometer were cut from the paraffin embedded tissue and stained with Haemotoxylin and Eosin (H&E Stain) to demonstrate the general histoarchitecture of the heart (Drury and Wallington, 1985). Masson's trichrome stain was used to demonstrate collagen fibers in the myocardium of the left ventricles of the heart, while Verhoeff-Van Gieson stain was used to demonstrate elastic fibers in myocardium of the left ventricles.

3.7.4.4 Photomicrography

H&E Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at X400 magnification. Photomicrographs of H&E stained sections were imported on to the Motic Images Plus, Version 2.0 software for histomorphometric analysis, to measure the ventricular thickness and diameter of the cardiomyocytes. The results were expressed as Mean ± SEM. Significance among the groups were determined by One-Way Analyses of Variance (ANOVA), followed by Turkey's HSD test suing the Statistical Package Software (SPSS) under windows XP. A value of $P<.05$ was considered statistically significant.

3.8 Ethical Issues

All procedures and care to the animals in accordance to and approved by the University of Eastern Africa, Baraton Ethics Committee, and approval by University of Eldoret Board of Postgraduate Studies.

3.9 Data collection, Statistical analysis and Presentation

Various physiological parameters were analyzed using the PowerLab machine 2/26 (Non-Invasive Blood Pressure measurement with a pulse transducer MLT125/M), to establish the blood pressure, the hearts structural and functional status. Data was recorded in tables and presented using bar graphs and pie-charts. The results were expressed as means $\pm$ SEM and were analyzed using One-Way Analysis of Variance (ANOVA), followed by Turkey's Multiple range test as a post-hock test for significant difference. Paired or unpaired Student's t-test was used for the Statistical analysis. A $\boldsymbol{p}$ value of less than 0.05 was considered significant. All statistical procedures were performed by statgraphics software version 5.0 (STSC, Inc., Rockville, MD, USA).

3.10 Quality Assurances

All the chemical supplies were of analytical grade and were purchased from one supplier (ScieLab Chemical Suppliers, Nairobi). The equipment used in the laboratories were also standardized and calibrated for physiological measurements. They are constantly used hence well maintained for accuracy and reliability. The measurements were recorded in two decimal points in triplicates, with averages calculated and recorded as Mean ± (SEM). The animals used were inbred strains.

CHAPTER FOUR

RESULTS

4.1. Plasma antidiuretic hormone, atrial natriuretic peptide, aldosterone, angiotensin II and renin levels in hypertension-induced laboratory rats following *Allium sativum* Extract Treatment

4.1.1 Effect of *Allium sativum* Extract Treatment on Plasma Levels of ADH in Hypertension-Induced laboratory rats

The ADH values for CPT, HCT, FAGE, CGE and CIGE treatments were compared with those for normal saline.

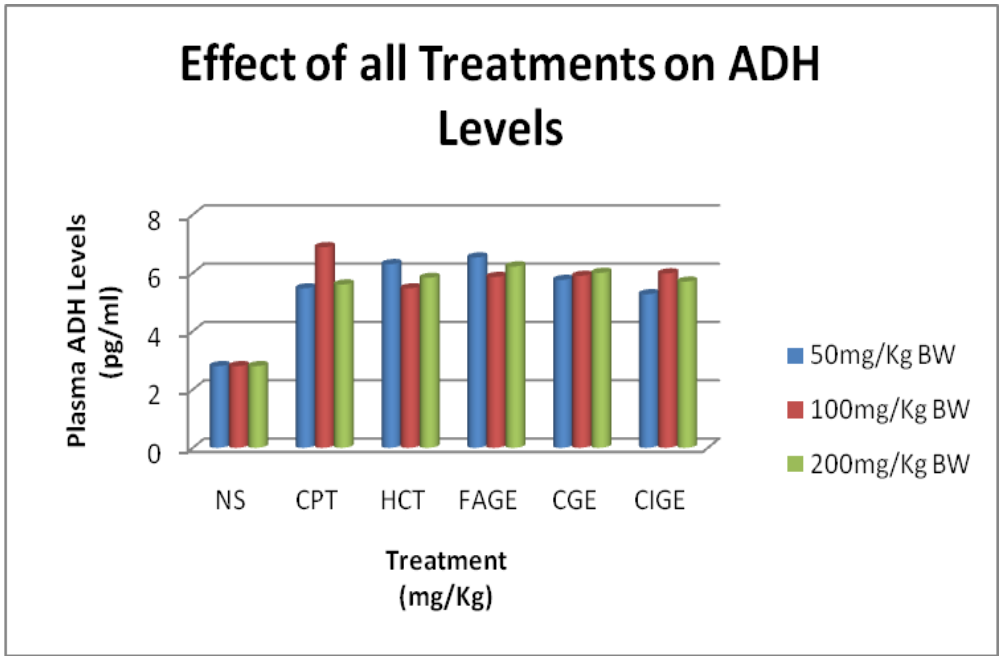
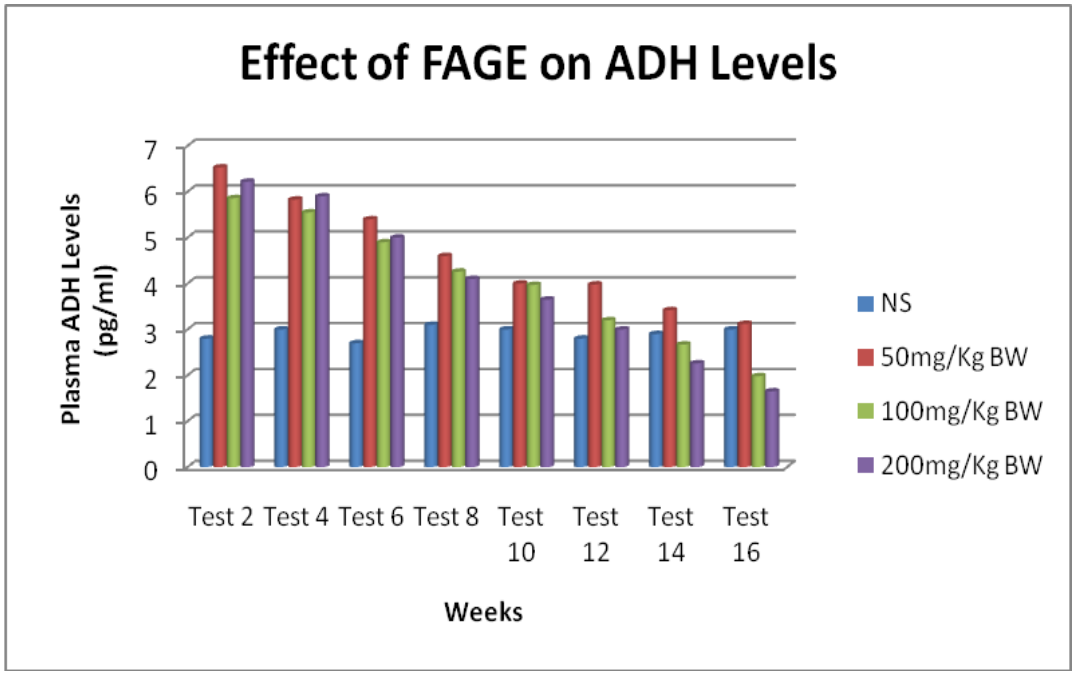


Figure 4.1.1a Effect of *Allium sativum* Extract Treatment on Plasma Levels of Antidiuretic Hormone (ADH) in Hypertension-Induce laboratory rats

The multiple comparison tests showed that though there were some differences in mean ADH levels at all concentration levels, these increments were not statistically significant across the different extract concentrations.

However, figures 4.1.1b shows a reduction in the Mean Plasma ADH levels following prolonged subsequent treatment of *Allium sativum* extract on the various groups for a period of 16 weeks recorded at intervals of two weeks.



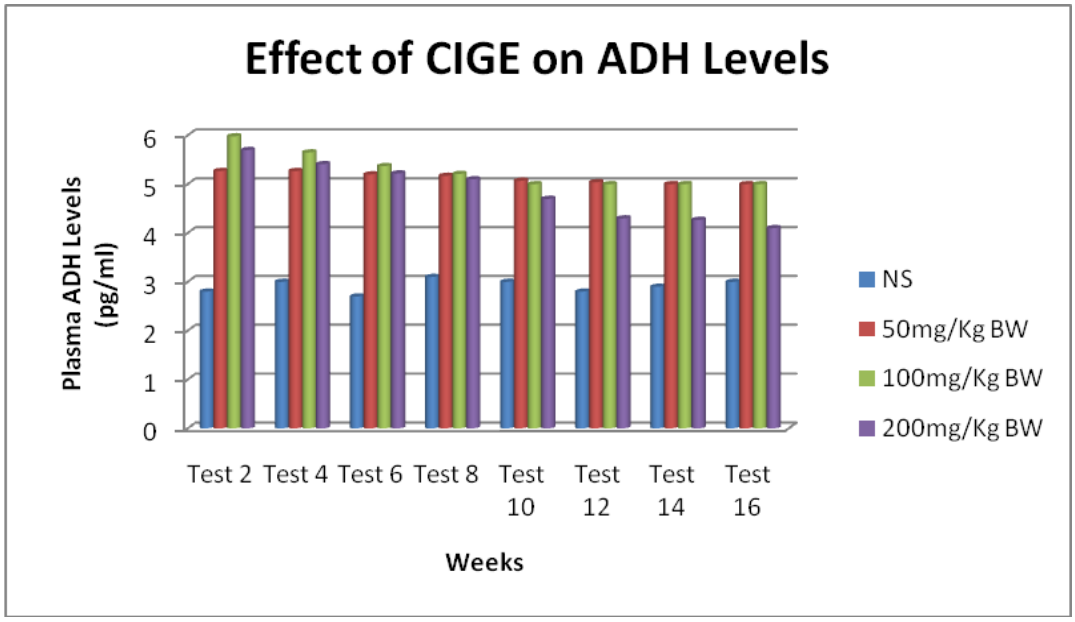


Figure 4.1.1b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of ADH for 16 weeks analyzed as 8 tests

4.1.2 Effect of *Allium sativum* Extract Treatment on Plasma Levels of B-type ANP in Hypertension-Induced laboratory rats

The results showed difference between treatment groups in comparison to the control, though the differences were not significant for all the groups compared with the control group.

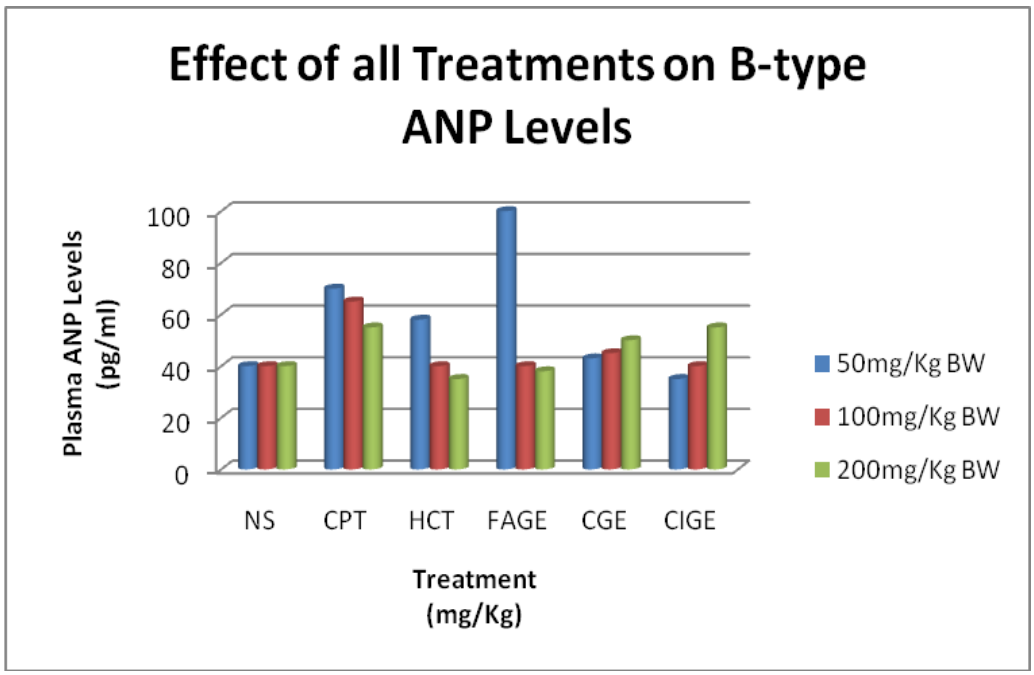
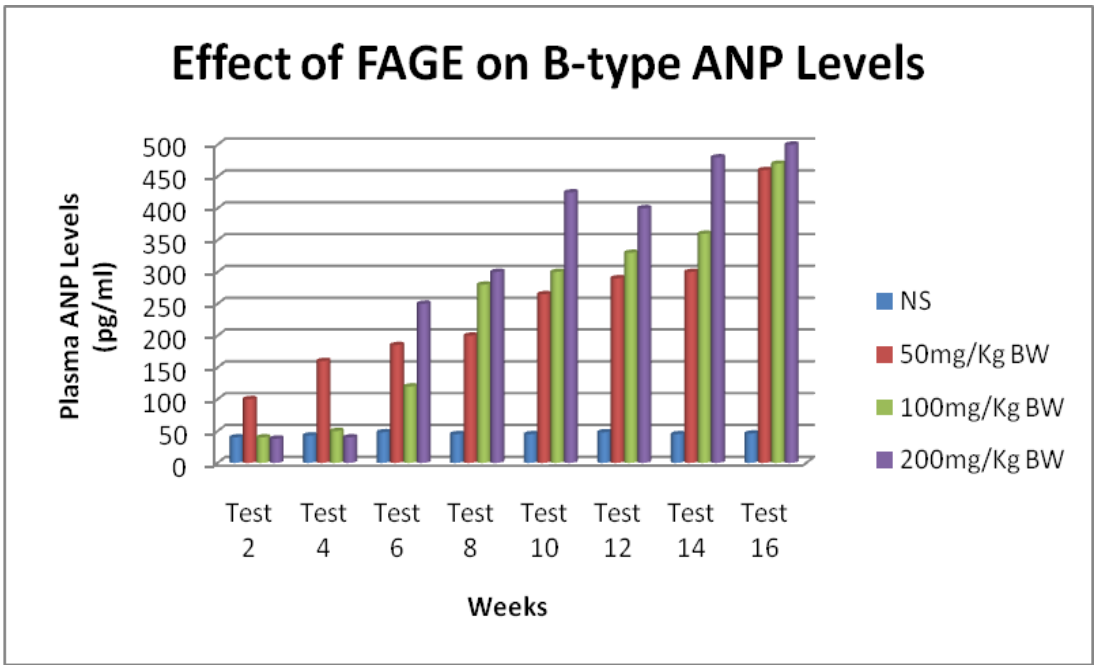


Figure 4.1.2a Mean Plasma levels of B-type Atrial Natriuretic Peptide following treatment of various concentrations of *Allium sativum* extracts

However, treatment group T1 that was treated with FAGE 200mg/Kg body weight (ΔM=-259.125, $p<.05$ = 29.5%); recorded a major significant increment in the Mean Plasma B-type ANP levels compared with the control group that was treated with normal saline.

Unlike in ADH where levels tend to decrease with number of tests, the levels in B-type ANP at the various concentration levels appeared to rise with increased number of testing as shown below in figure 4.1.2b.



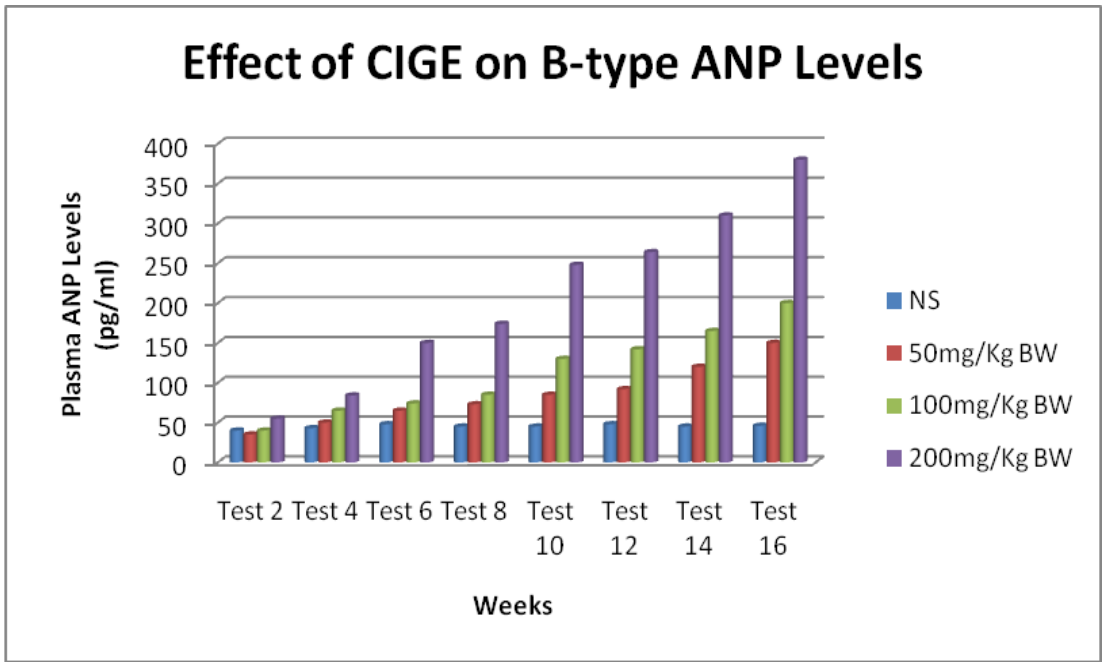


Figure 4.1.2b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of B-type ANP for 16 weeks analyzed as 8 tests

4.1.3 Test results of Effect of *Allium sativum* Extract Treatment on Plasma Levels of Angiotensin II (Ang-II) in Hypertension-Induced laboratory rats

The results showed Mean Plasma levels of Ang-II result with significant differences between treatment groups in comparison to the control. Ang-II reported significant increment in mean levels between NS and CPT 100mg/kg body weight (ΔM=-53.37500, p<.05); CPT 200mg/kg body weight (ΔM=-48.25000, p<.05); HCT 50mg/kg body weight (ΔM-54.75000, p<.05); HCT 100mg/kg body weight (ΔM=-50.75000, p<.05); CGE 100mg/kg body weight (ΔM=-68.25000, p<.05); CGE 200mg/kg body weight (ΔM=-58.37500, p<.05); CIGE 50mg/kg body weight (ΔM=-72.75000, p<.05); CIGE 100mg/kg body weight (ΔM=-79.87500, p<.05); and CIGE 200mg/kg body weight (ΔM=-74.0000, p<.05).

The mean plot revealed that there were wide differences between mean Plasma Ang-II levels at NS with those at most of the concentration levels.

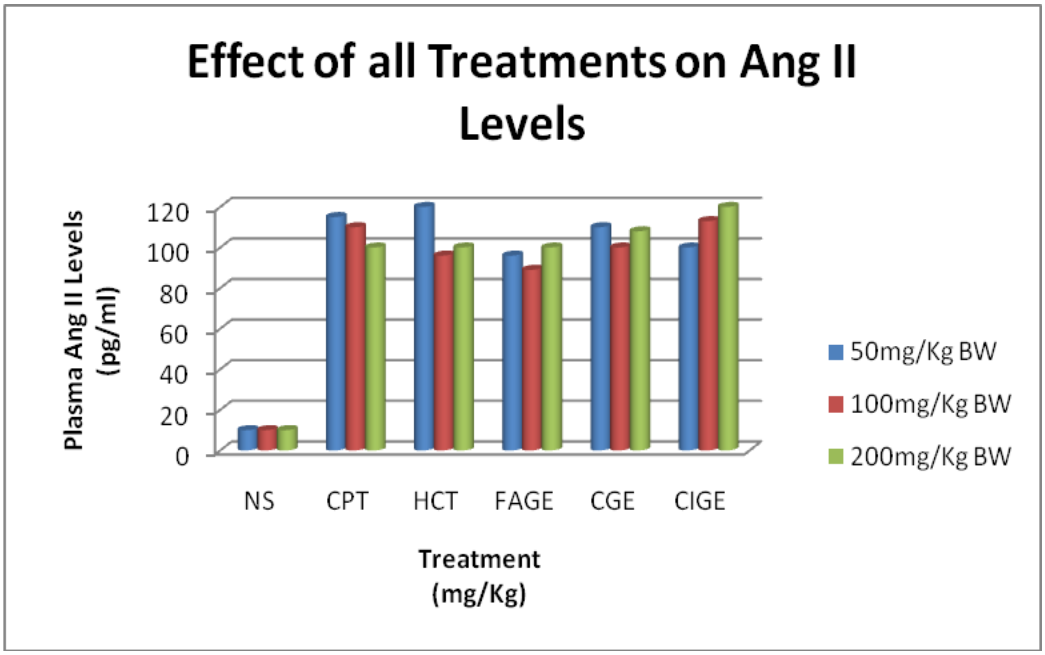
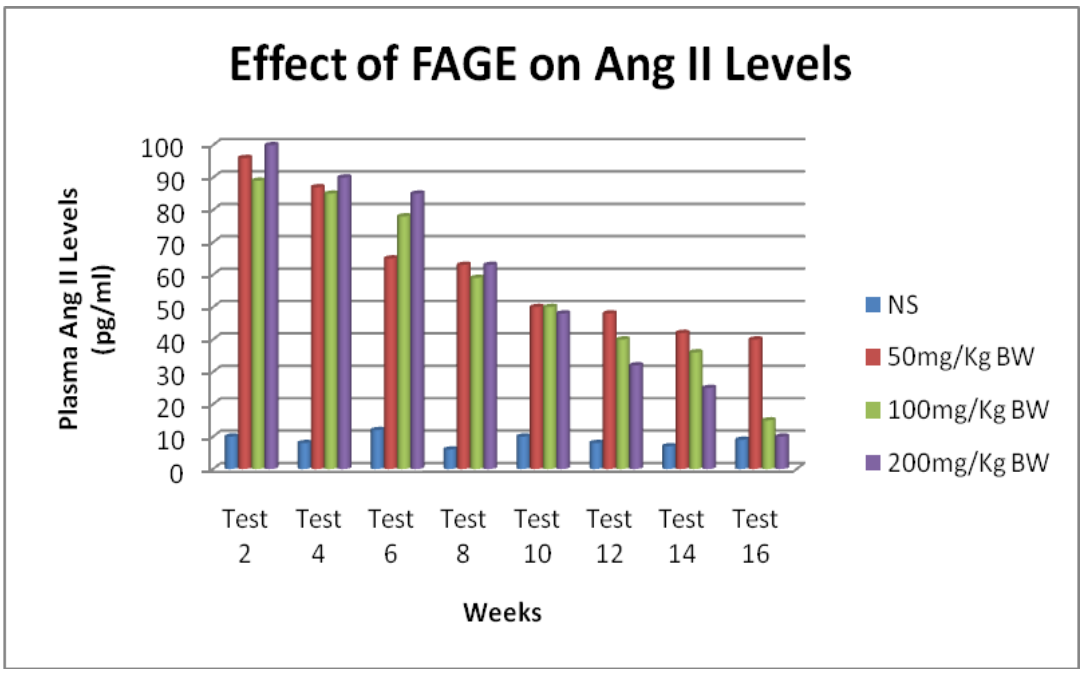


Figure 4.1.3a Mean Plasma levels of Angiotensin II following treatments of various concentrations of *Allium sativum* extracts

Besides, the Plasma Ang-II levels were noted to decrease with increasing frequency of testing as shown in the comparative linear graph below (Figures 4.1.3b).



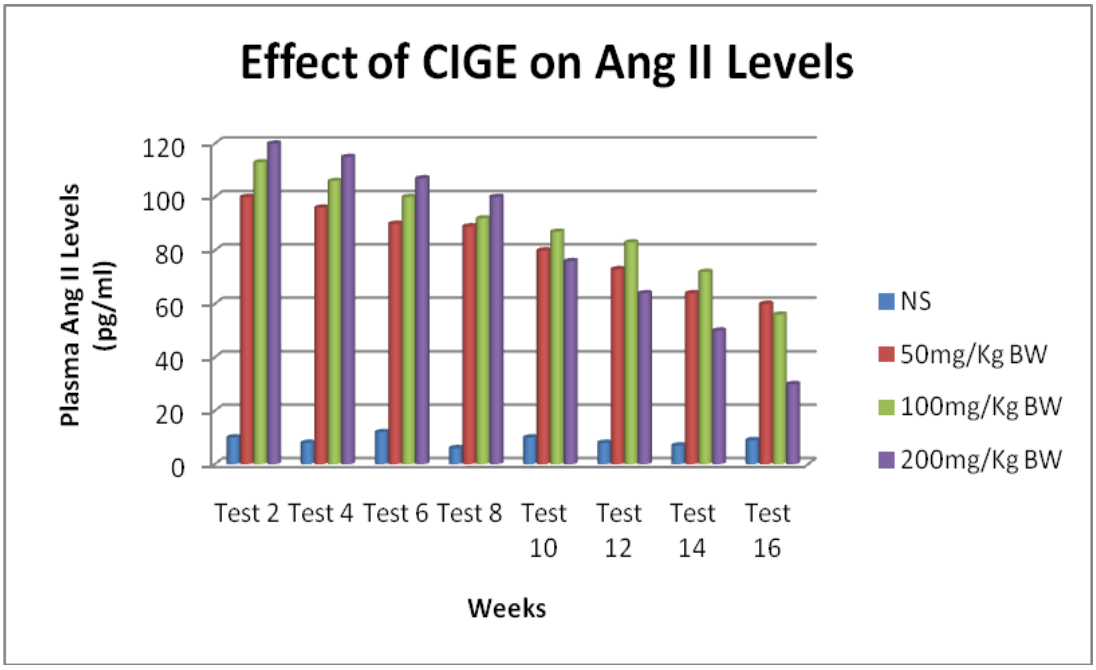


Figure 4.1.3b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Ang-II for 16 weeks analyzed as 8 tests

4.1.4 Effect of *Allium sativum* Extract Treatment on Plasma Levels of Aldosterone (Ald) in Hypertension-Induced laboratory rats

The results showed Mean Plasma levels of Ald with significant differences between treatment groups in comparison to the control. *Allium sativum* extract triggered significant differences in mean Plasma Ald levels at CGE 100mg/kg body weight (ΔM=-199.000, p<.05); CGE 200mg/kg body weight (ΔM=-192.12500, p<.05); CIGE 50mg/kg body weight (ΔM=-226.5000, p<.05); CIGE 100mg/Kg body weight (ΔM=-227.25000, p<.05); and CIGE 200mg/kg body weight (ΔM=-220.25000, p<.05). The rest of the treatment groups effect on Mean Plasma Ald levels were not statistically significant compared to the control group at p<0.05.

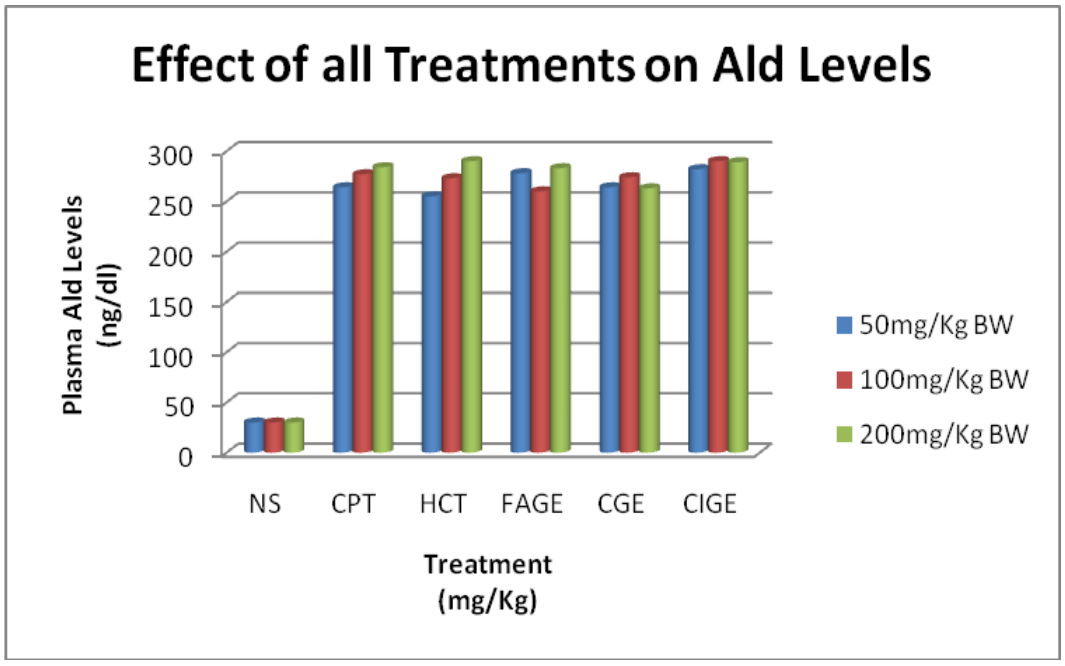
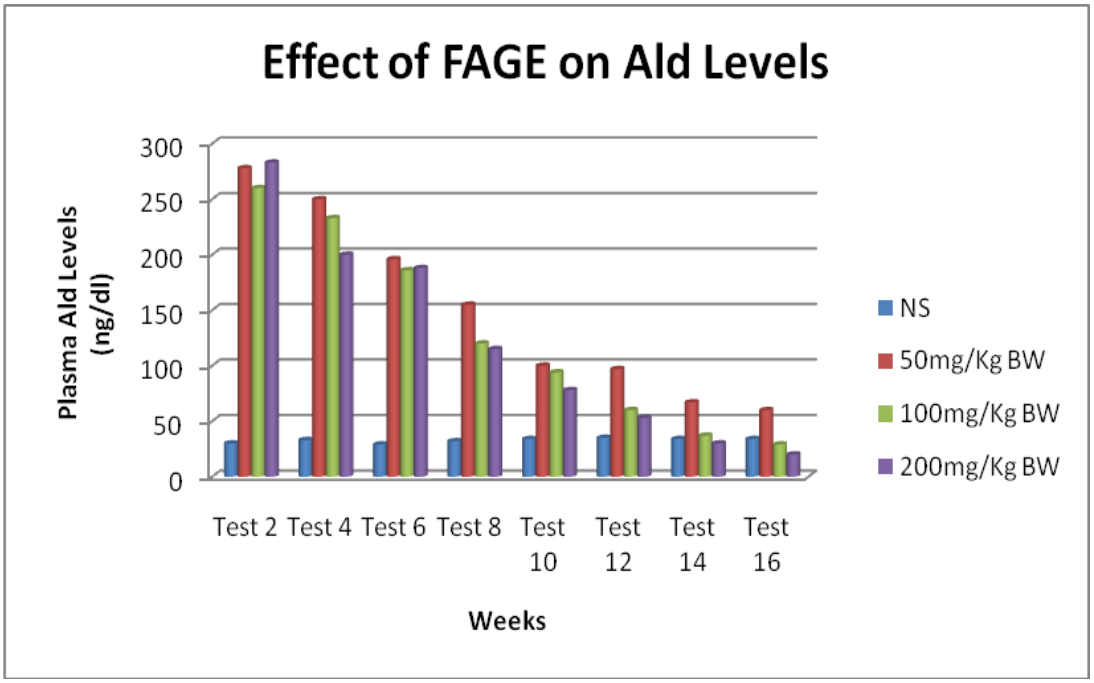


Figure 4.1.4a Mean Plasma levels of Aldosterone following treatments of various concentrations of *Allium sativum* extracts

However, a decline in Mean Plasma Ald levels at different concentrations was also realized when further tests were conducted and depicted by figures 4.1.4b below.



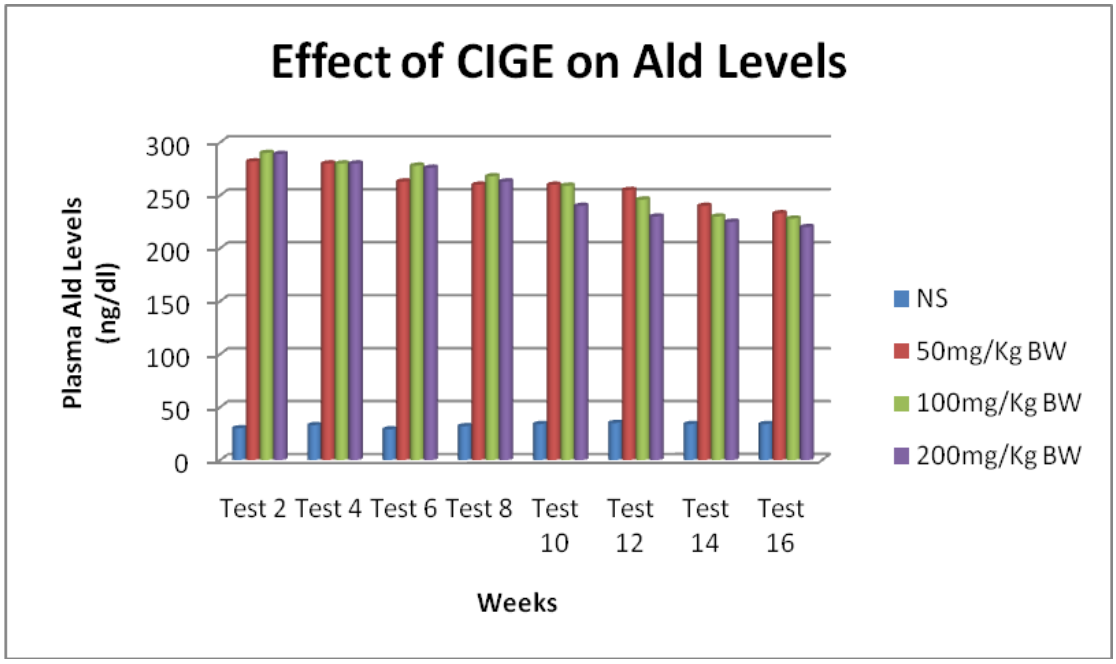


Figure 4.1.4b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Ald for 16 weeks analyzed as 8 tests

4.1.5 Test results of Effect of *Allium sativum* Extract Treatment on Plasma Levels of Renin in Hypertension-Induce laboratory rats

The results showed Mean Plasma levels of Renin with significant differences between treatment groups in comparison to the control. This implied that the *Allium sativum* treatment had a major impact on the hormone renin.

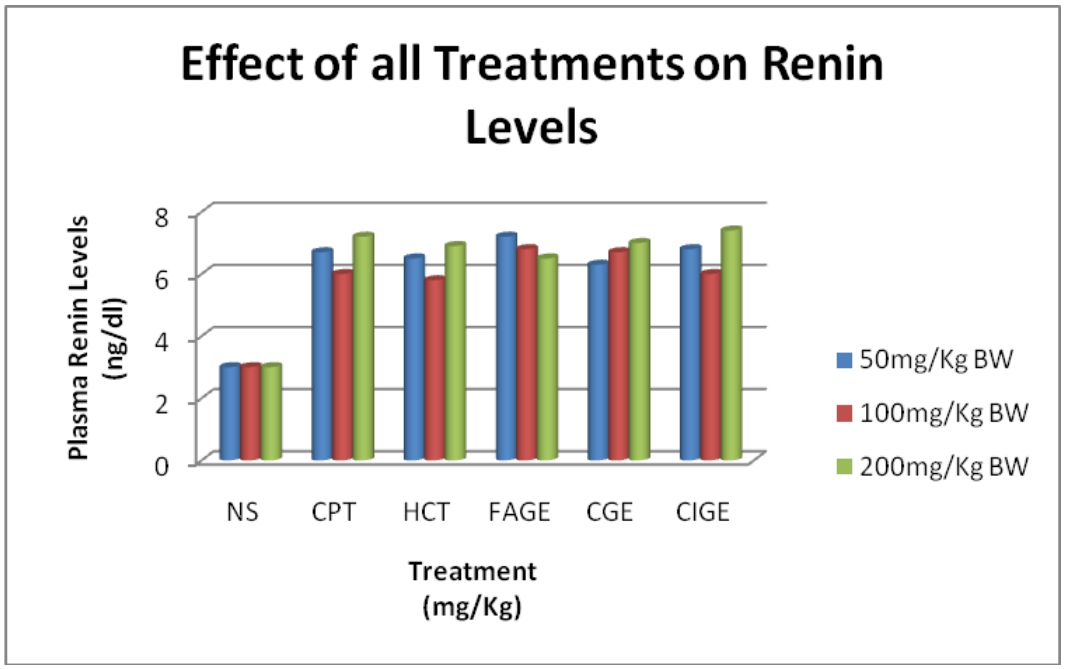
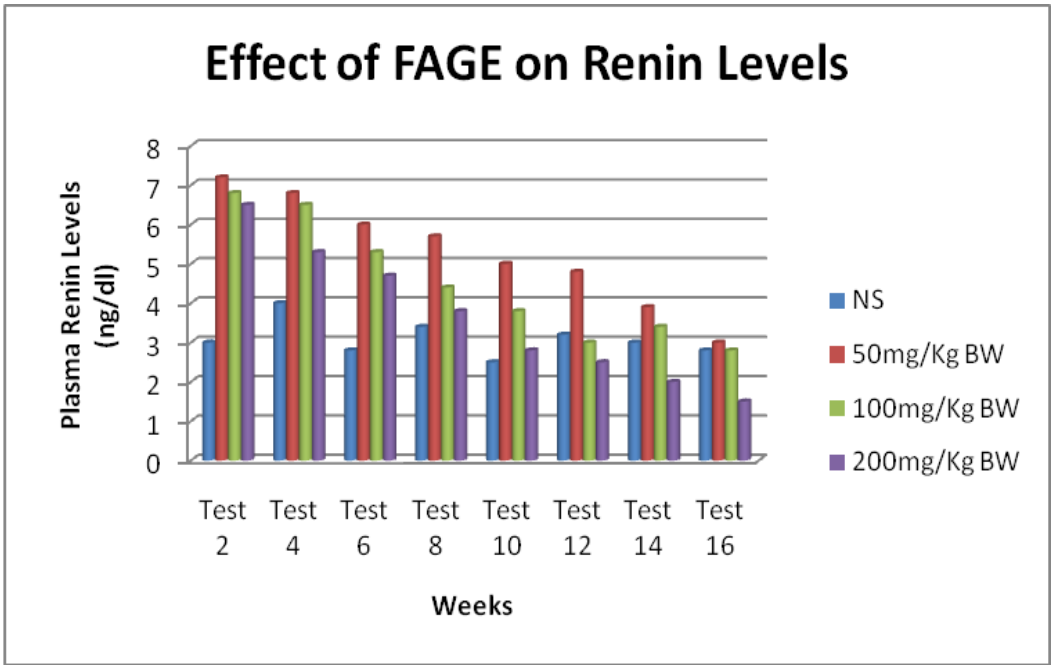


Figure 4.1.5a Mean Plasma levels of Renin following treatments of various concentrations of *Allium sativum* extracts

Due to the indications for significant differences depicted by the results above, levels of hormone renin were also found to be reducing with subsequent cumulative testing and hence decreased with frequency of testing as shown in the comparative bar graph below in figures 4.1.5b.



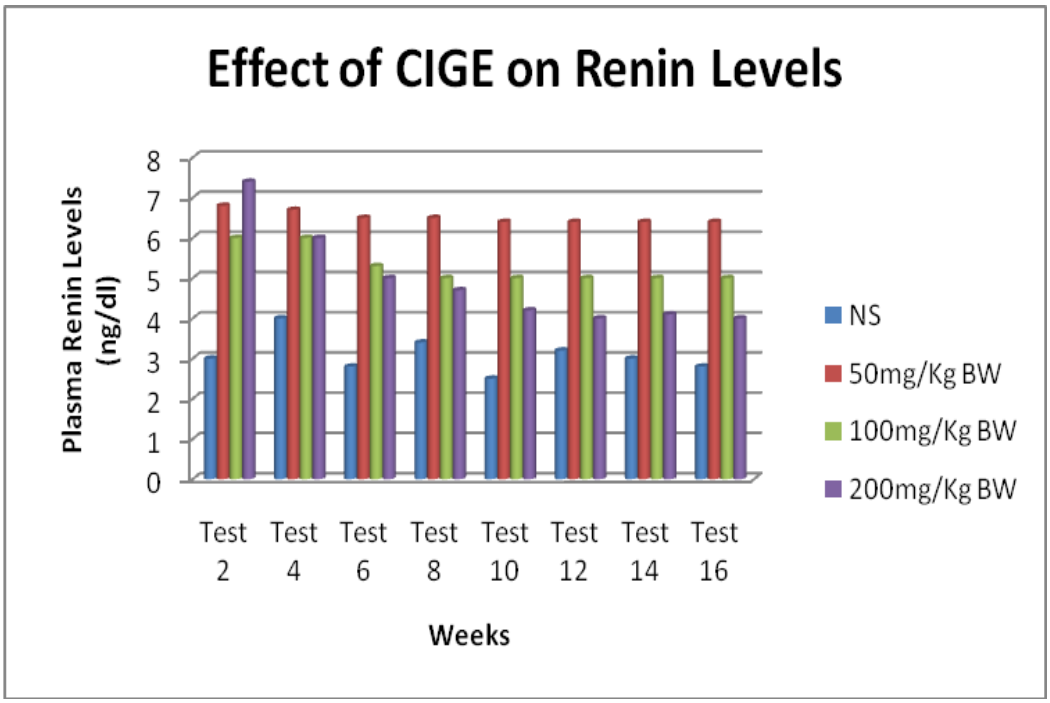


Figure 4.1.5b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Renin for 16 weeks analyzed as 8 tests

4.2 Serum levels of Electrolytes and metabolites in hypertension-induced laboratory rats following *Allium sativum* Extract Treatment

The effects of *Allium sativum* extract treatment on plasma levels of electrolytes were examined using four electrolytes namely Sodium (Na^+), Potassium (K^+), Chloride (Cl^-) and total ionic Calcium (iCa^{2+}).

3.7.5 Effect of *Allium sativum* Extracts Treatment on Serum Levels of Sodium (Na^+) ions in Hypertension-Induced laboratory rats

Allium sativum extract treatment was found to trigger a major significant difference in the mean levels of Na^+ ions ($p<0.05$) at all extract concentration levels between and among the treatment groups in comparison to the control group (C1) pre-treated with normal saline (NS).

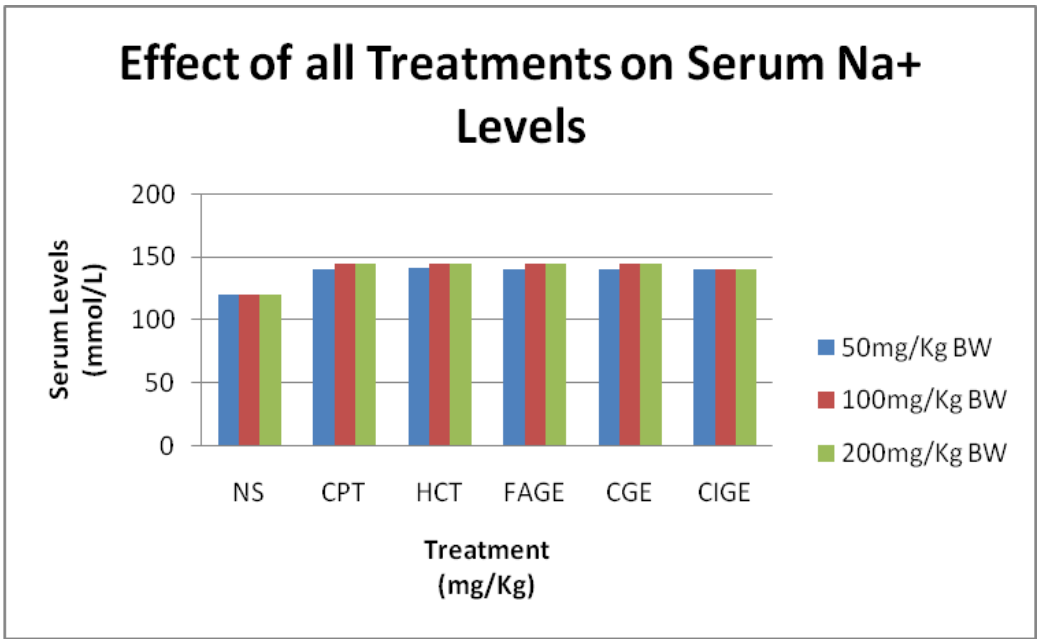
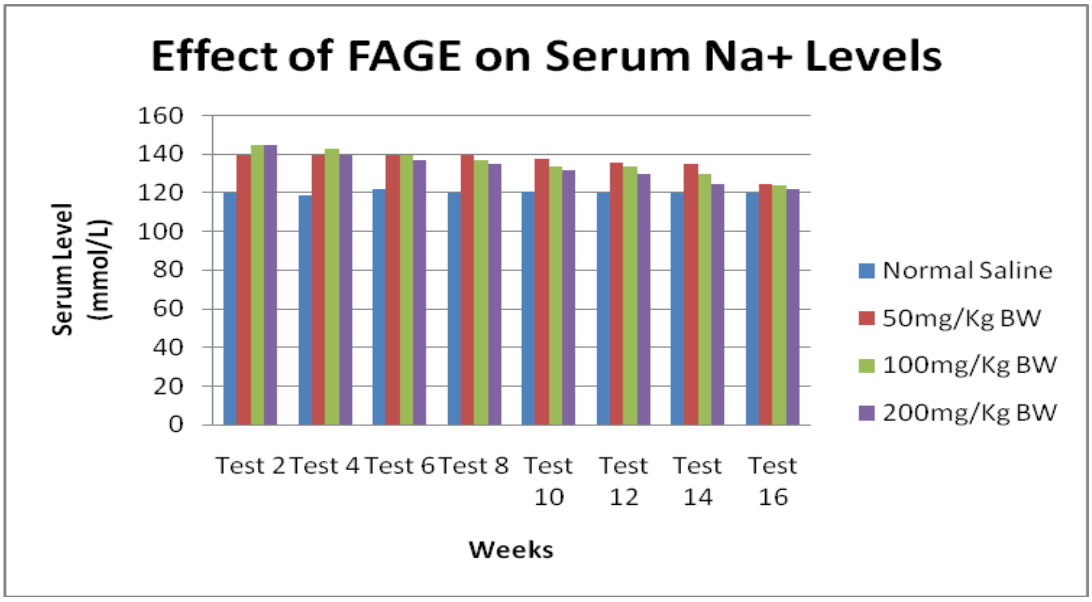


Figure 4.2.1a Mean serum levels of Na^+ ions following treatments of various concentrations of *Allium sativum* extracts

The highest difference being achieved at CGE 100mg/kg body weight (ΔM = -18.750, $p<0.05$ = 15.625%) and the least difference in mean Na^+ levels being achieved at HCT 200mg/kg body weight (ΔM= -12.750, $p<0.05$ = 10.625%).

Further, the comparative mean plots for mean Na^+ ion levels against test revealed that the concentration at all treatment levels reduced serum levels of Na^+ ions with increased testing as shown in figures 4.2.1b.



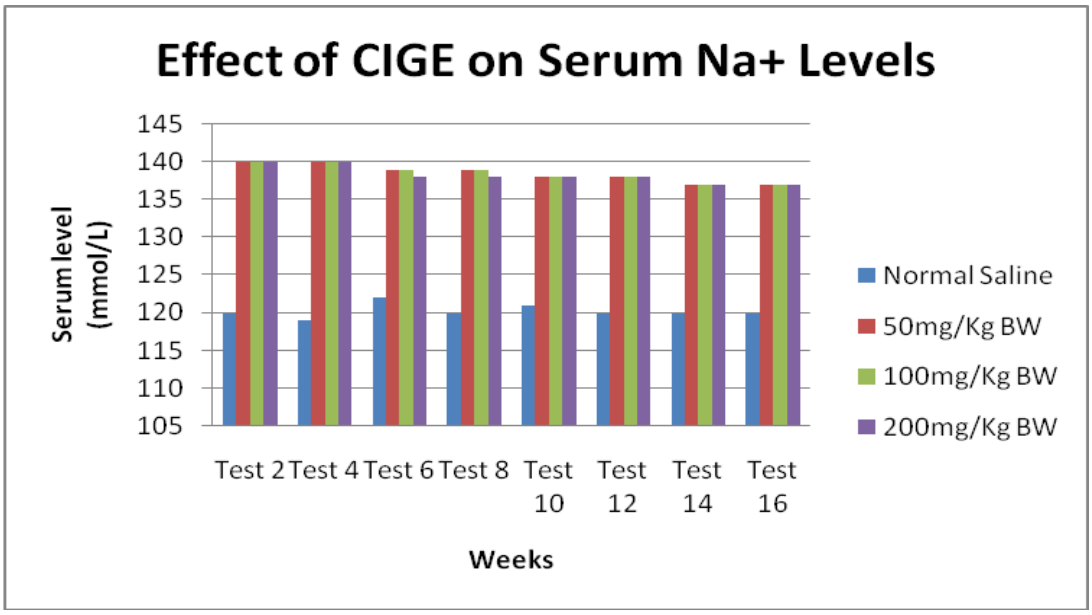


Figure 4.2.1b A comparative bar graph of mean Plot effect of *Allium sativum* extract treatment on Serum levels of Na^+ ions for 16 weeks analyzed as 8 tests

4.2.2 Effect of *Allium sativum* Extracts Treatment on Serum Levels of Potassium (K^+) ions in Hypertension-Induce laboratory rats

Results analyzed showed that *Allium sativum* extract triggered significant differences in the mean serum concentrations of K^+ ions between groups, except for control group C2 at CPT 50mg/kg body weight (ΔM=-1.488, $p>0.05$) and treatment group T2 at CGE 50mg/kg body weight (ΔM=-0.9125, $p>0.05$) which was not statistically significant at $p<0.05$.

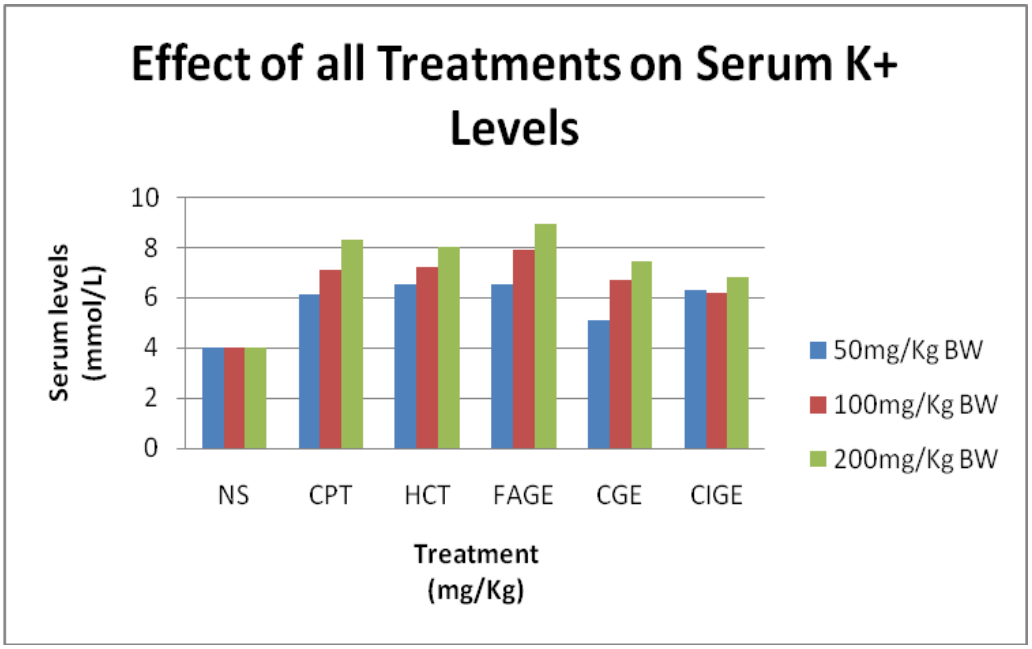
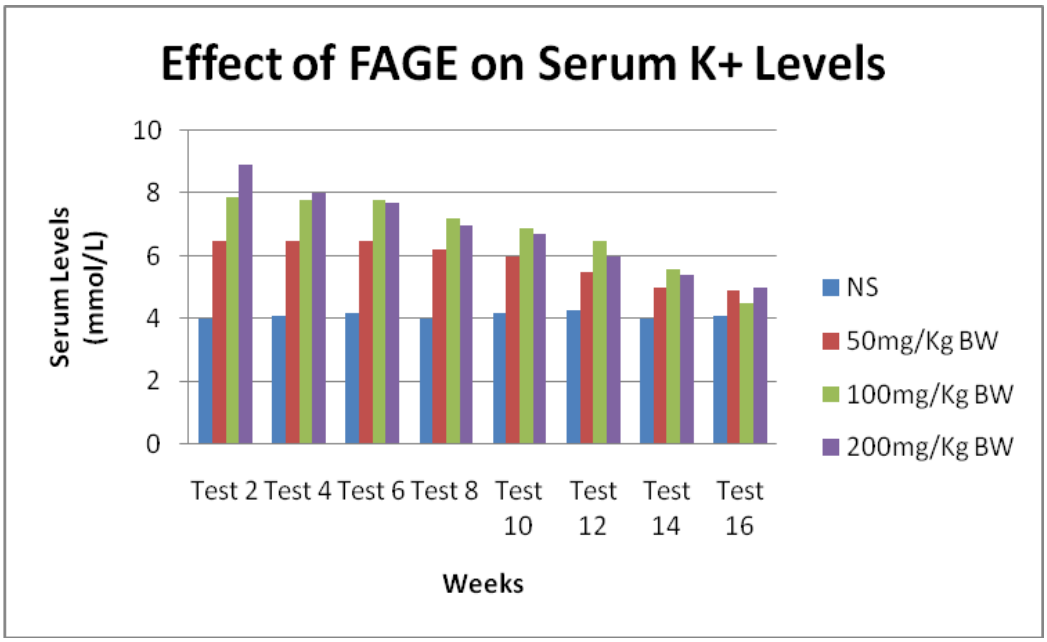


Figure 4.2.2a Mean serum levels of K^+ ions following treatments of various concentrations of *Allium sativum* extracts

The largest mean difference in serum levels of K^+ ions when compared with the control group C1 treated with NS was achieved by treatment group T1 at FAGE 100mg/kg body weight (ΔM=-2.66, $p<0.05$ = 79.72%) and the smallest significant difference was registered by control group C3 at HCT 50mg/kg body weight (ΔM=-1.663, $p<0.05$ = 12.16%). The rest were statistically different and significant from the control group.

The Comparative bar graphs (Figures 4.2.2b) of mean serum K^+ ions against subsequent tests for 16 weeks revealed a decrease in mean serum K^+ ion levels in successive tests. Seven out of 11 treatment concentrations (63.63% of all treatment groups) were statistically significant, and significantly lowered serum K^+ ions levels in hypertension-induced laboratory rats.



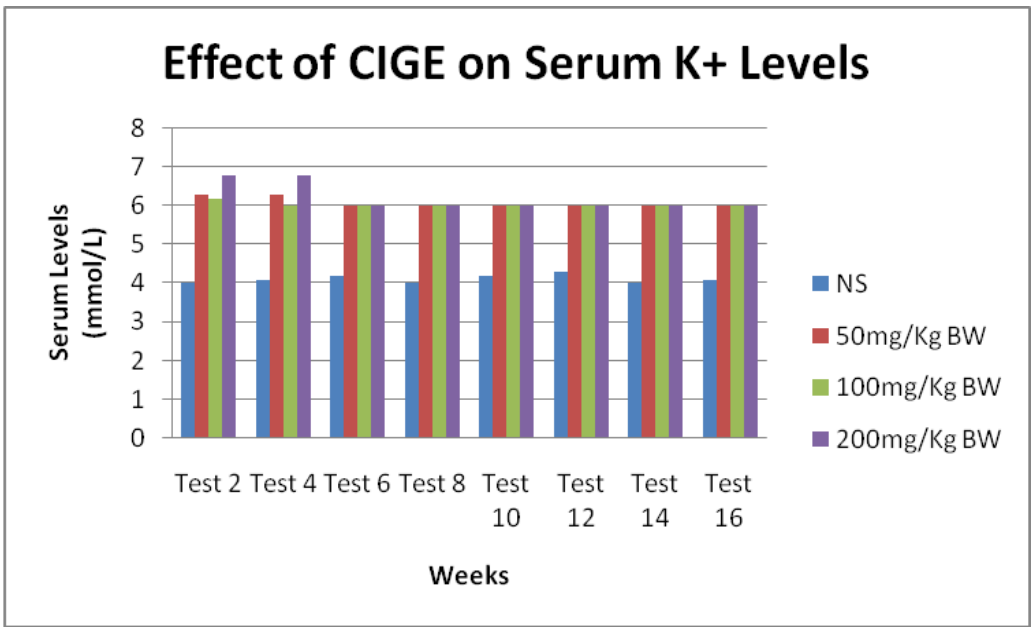


Figure 4.2.2b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Serum levels of K^+ ions for 16 weeks analyzed as 8 tests

4.2.3 Effect of *Allium sativum* Extracts Treatment on Serum Levels of Chloride (Cl^-) ions in Hypertension-Induce laboratory rats

Just as in the case of serum levels of Na^+ ions, *Allium sativum* extract triggered significant differences in serum levels of Cl^- ions at all extract concentrations. The largest mean difference in serum levels of Cl^- ions compared to control group C1 treated with normal saline was registered by treatment group T3 at CIGE 100mg/kg body weight (ΔM=-23.25, $p<0.05$ = 23.25%), while the least significant mean difference was registered treatment group T1 at FAGE 200mg/kg body weight (ΔM=-11.375, $p<0.05$ = 11.38%).

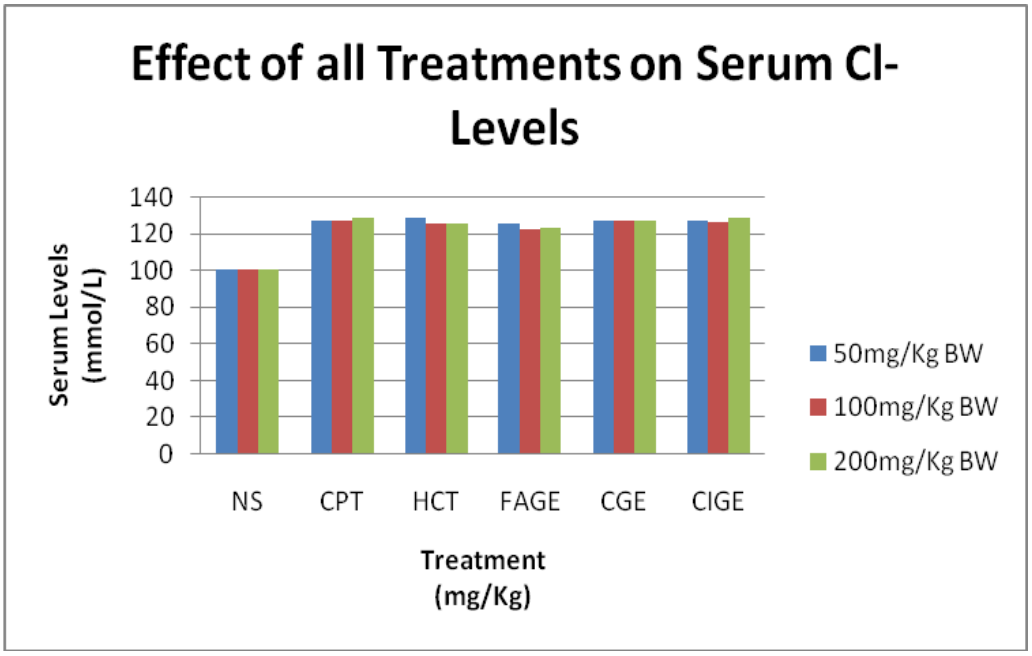
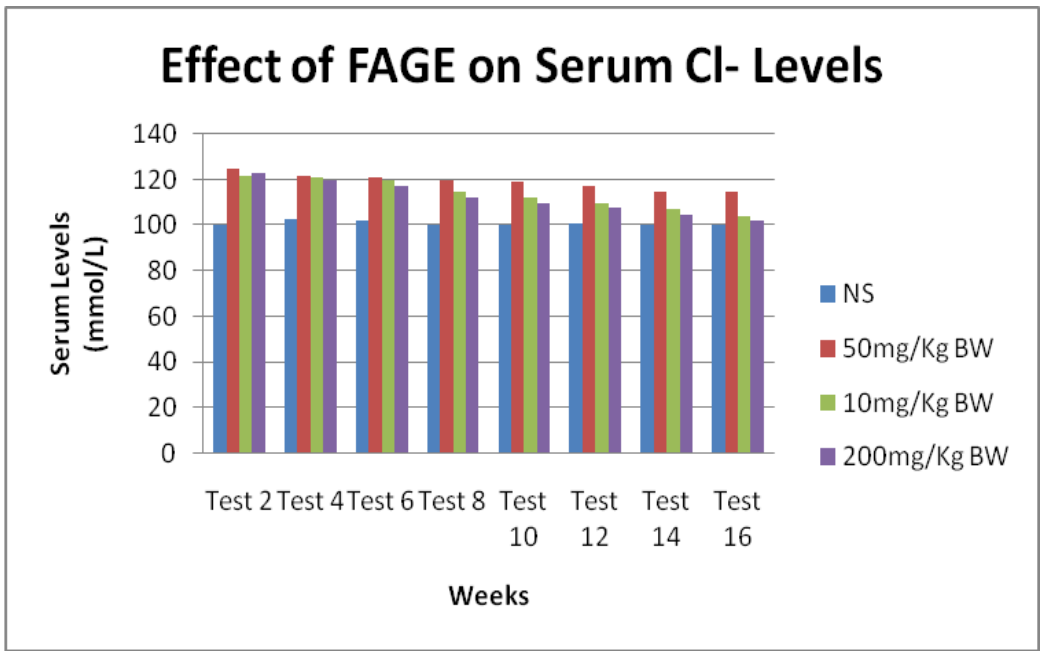


Figure 4.2.3a Mean serum levels of Cl^- ions following treatments of various concentrations of *Allium sativum* extracts

Further the comparative bar graph for concentration of serum Cl^- ions against subsequent testing for a period of 16 weeks revealed a reduction in Mean serum Cl^- ions with increased testing across all concentration levels other than NS as reflected by figures 4.2.3b.



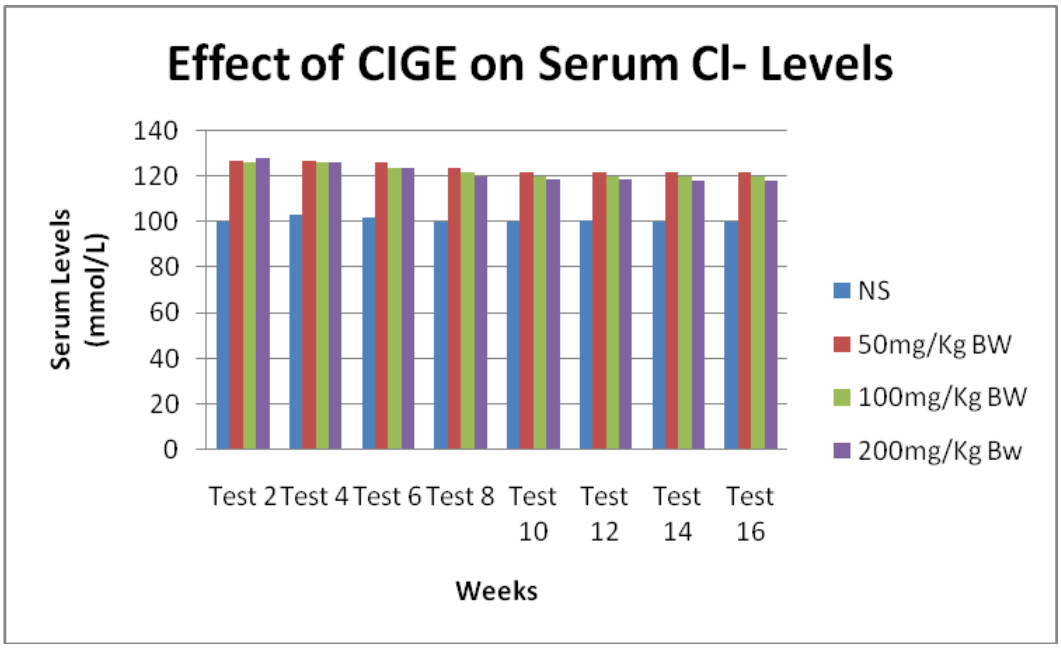


Figure 4.2.3b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Serum levels of Cl^- ions for 16 weeks analyzed as 8 tests

1.2.4. Effect of *Allium sativum* Extracts Treatment on Serum Levels of Ionic Calcium (iCa^{2+}) in Hypertension-Induce laboratory rats

Unlike for the other electrolytes, *Allium sativum* extract treatment did not trigger significant differences in Serum levels of total ionic Ca^{2+} across all concentrations. The few concentrations that elicited a difference were not statistically significant compared to the control group.

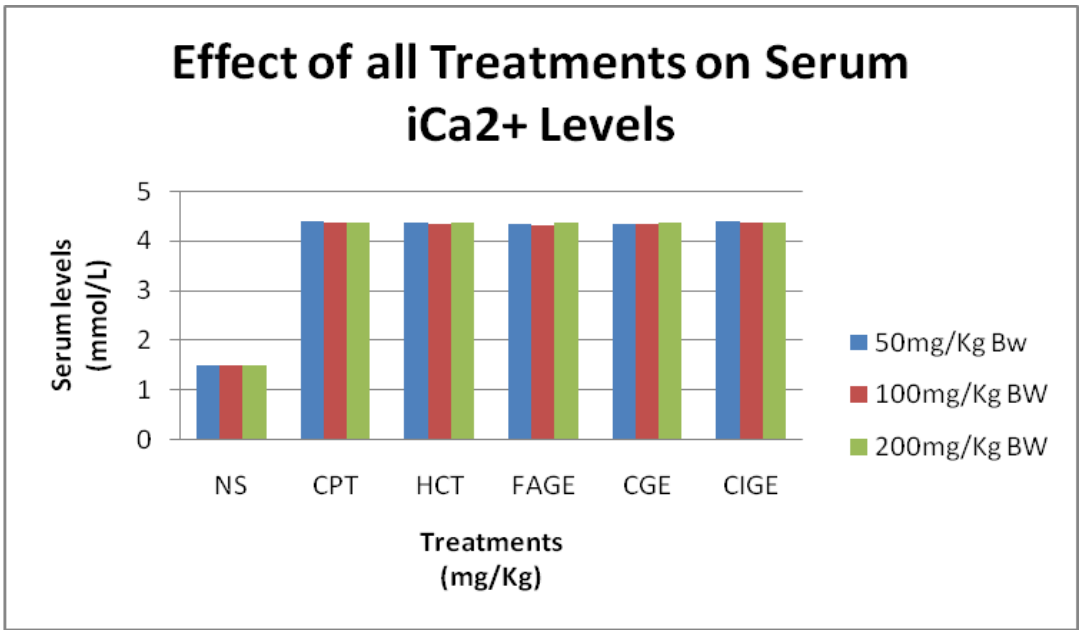
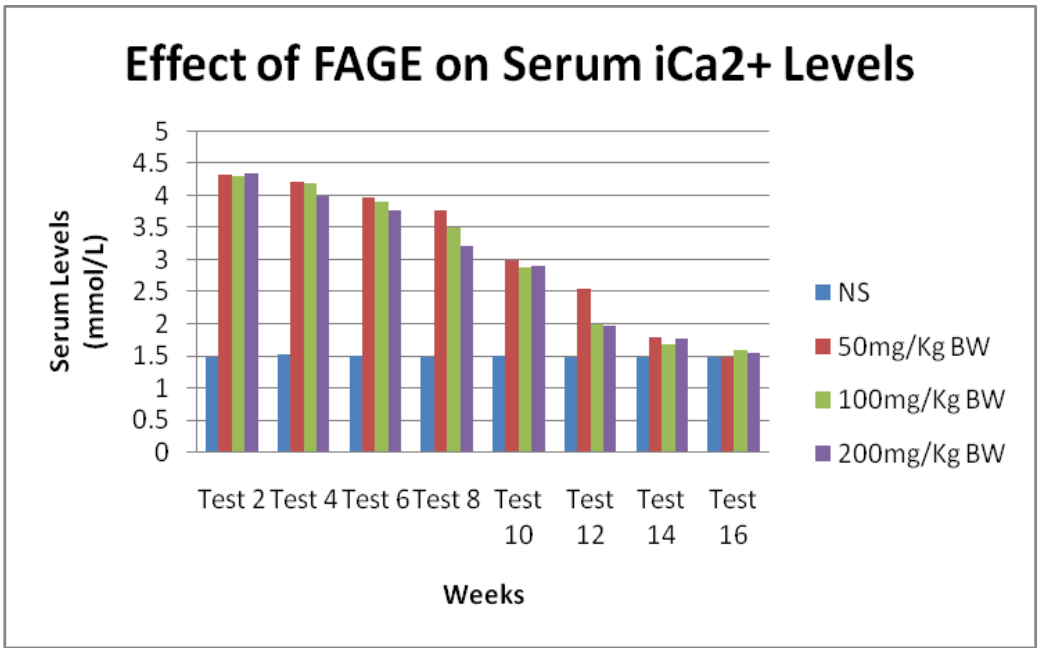


Figure 4.2.4a Mean serum levels of ionic Calcium (iCa^{2+}) following treatments of various concentrations of *Allium sativum* extracts

There were significant difference in control group C2 at CPT 50mg/kg body weight ($\Delta M=-1.599$, $p<0.05$), control group C3 at HCT 50mg/kg body weight ($\Delta M=-1.59$, $p<0.05$); treatment group T1 at FAGE 50mg/kg body weight ($\Delta M=-1.651$, $p<0.05$); treatment group T2 at CGE 50mg/kg body weight ($\Delta M=-2.649$, $p<0.05$); CGE 100mg/kg body weight ($\Delta M=-2.605$, $p<0.05$); CGE 200mg/kg body weight ($\Delta M=-2.596$, $p<0.05$); and treatment group T3 at CIGE 50mg/kg body weight ($\Delta M=-2.657$, $p<0.05$); CIGE

100mg/kg body weight ($\Delta M=-2.637$, $p<0.05$); and CIGE 200mg/kg body weight ($\Delta M=2.63, p<0.05$).

These differences are shown as peaks at various concentration levels shown in the Mean Plot graph depicted in figure 4.2.4a. Among the mean significant differences, the least significant difference from the control group C1 (NS) was by treatment group T1 FAGE 200mg/Kg body weight at peak 3.00mmol/L (= 75%) and the highest seen in treatment group T2 CGE 50mg/Kg body weight at peak 4.35mmol/L (= 45%) when tested short term. Once again, the Mean Serum iCa^{2+} levels tended to decrease with subsequent tests upon treatment of the animals with *Allium sativum* as shown in figures 4.2.4b.



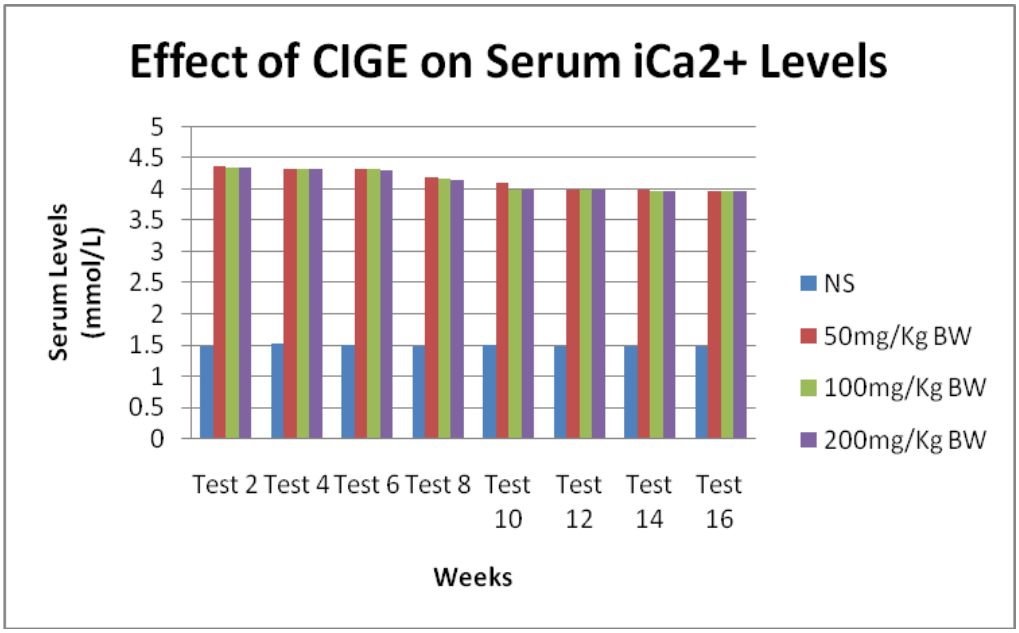


Figure 4.2.4b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Serum levels of iCa^{+} for 16 weeks analyzed as 8 tests

4.3 Statistic Analysis of the Effects of *Allium sativum* Extracts Treatment on Metabolites

Rats were randomly assigned to the control group (Normotensive) and three experimental group T1 – T3 (Hypertensive). The results present the Mean Plasma Metabolite levels of Aspartate Aminostraferase (pAST), Alanine Aminotransferase (pALT), Cardiac Troponin-I (cTi), Cardiac Troponin-T (cTt), Total Cholesterol and Creatine Phosphokinase Isoenzyme-MB (CK-MB) with various treatment concentration levels of *Allium sativum* extracts.

4.3.1 Effect of *Allium sativum* Extracts Treatment on Aspartate Aminostraferase (pAST) in Plasma in Hypertension-Induce laboratory rats

The *Allium sativum* activity of FAGE, CGE and CIGE extracts on pAST were compared with normal saline. However, despite the gradual increment in mean difference values of

pAST at different concentrations of FAGE, CGE, and CIGE, there were no significant differences in the mean pAST levels between the control group and individual treatment groups with all the three treatment levels. This is portrayed in the results in figure 4.3.1a.

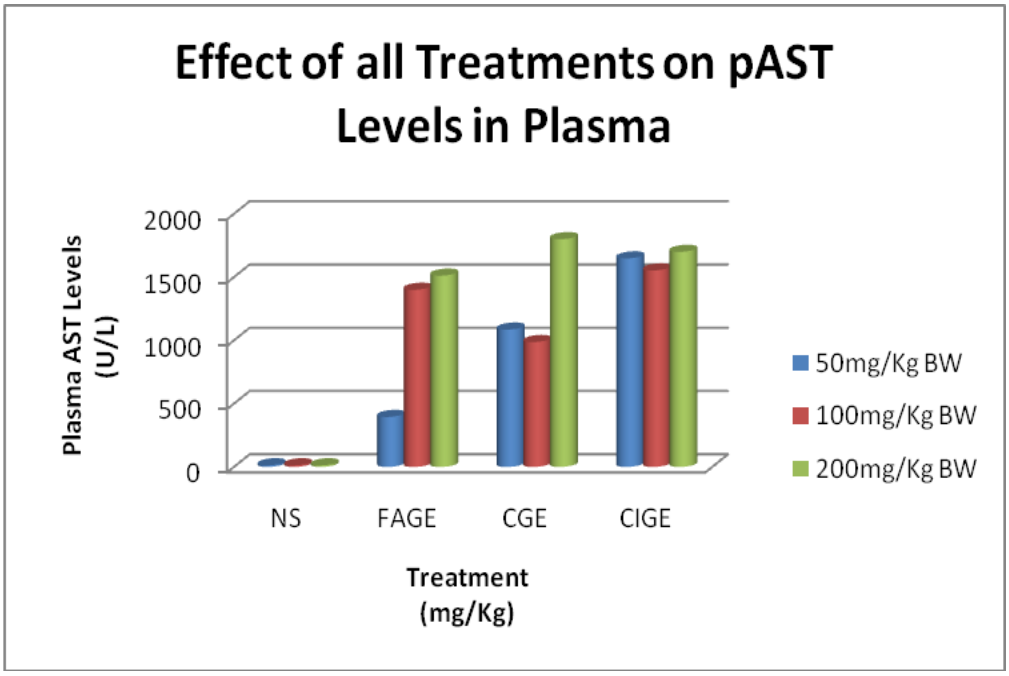
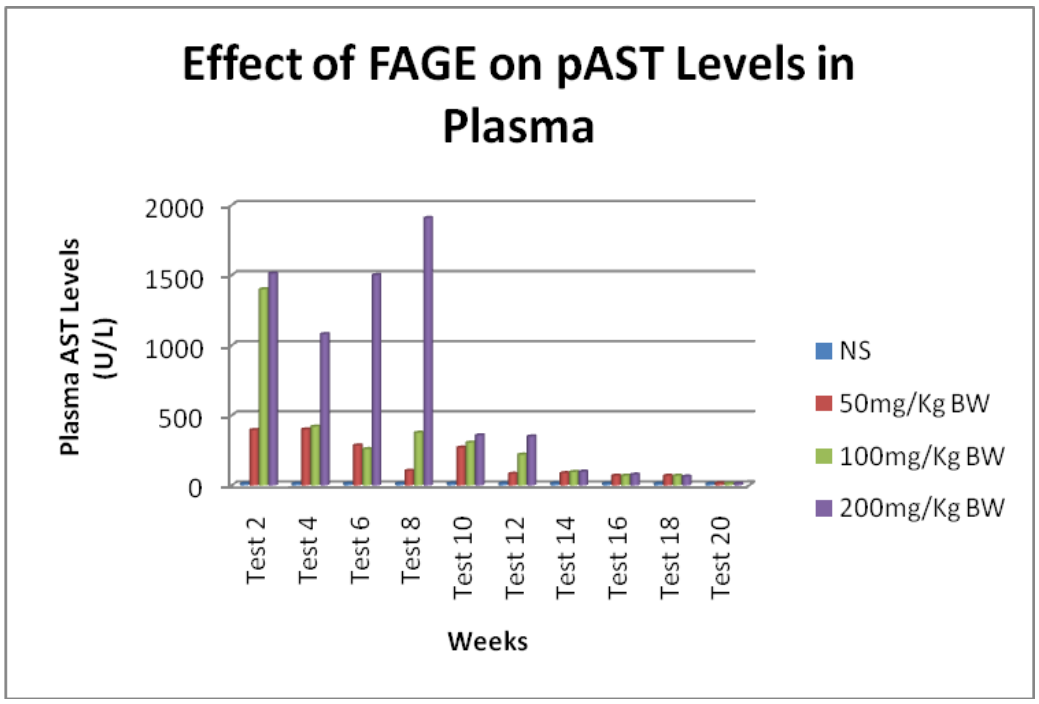


Figure 4.3.1a Mean plasma levels of Aspartate Aminostraferase (pAST) following treatments of various concentrations of *Allium sativum* extracts

The baseline values for all concentration levels were larger than values obtained in subsequent tests, though these increments were not statistically significant. Figures 4.3.1b show a decreasing trend of the Mean Plasma AST levels as treated with various concentrations of *Allium sativum* extract with successive tests.



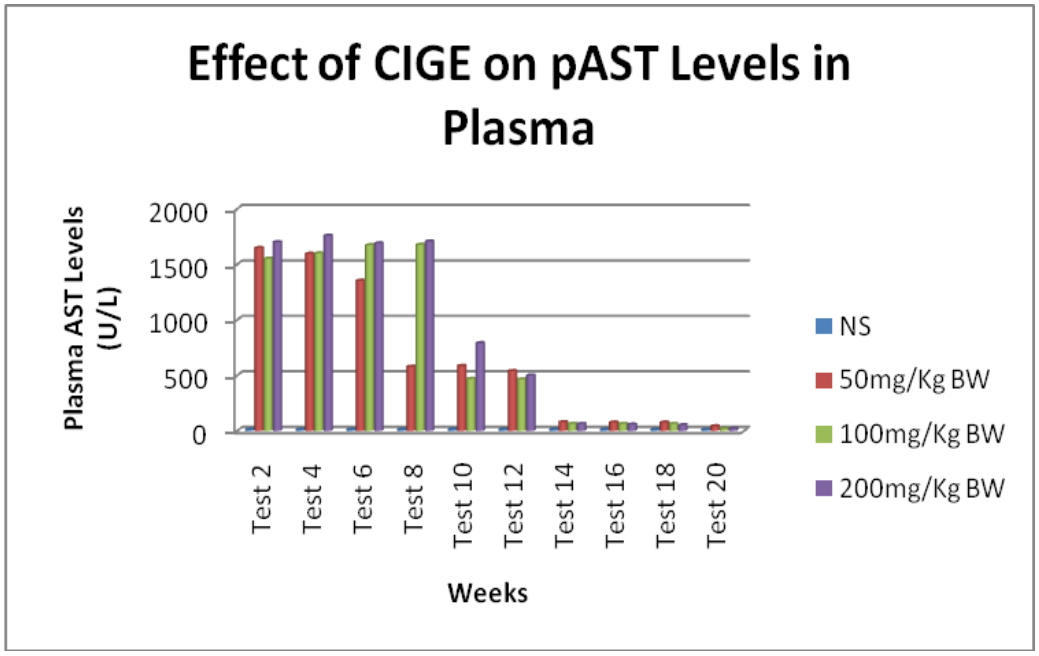


Figure 4.3.1b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Aspartate Aminostraferase (pAST) for 20 weeks analyzed as 10 tests

4.3.2 Effect of *Allium sativum* Extracts Treatment on Alanine Aminotransferase (pALT) in Plasma in Hypertension-Induce laboratory rats

The result shows a significance difference in the Mean Plasma ALT levels between the treatment groups (Figure 4.3.2a).

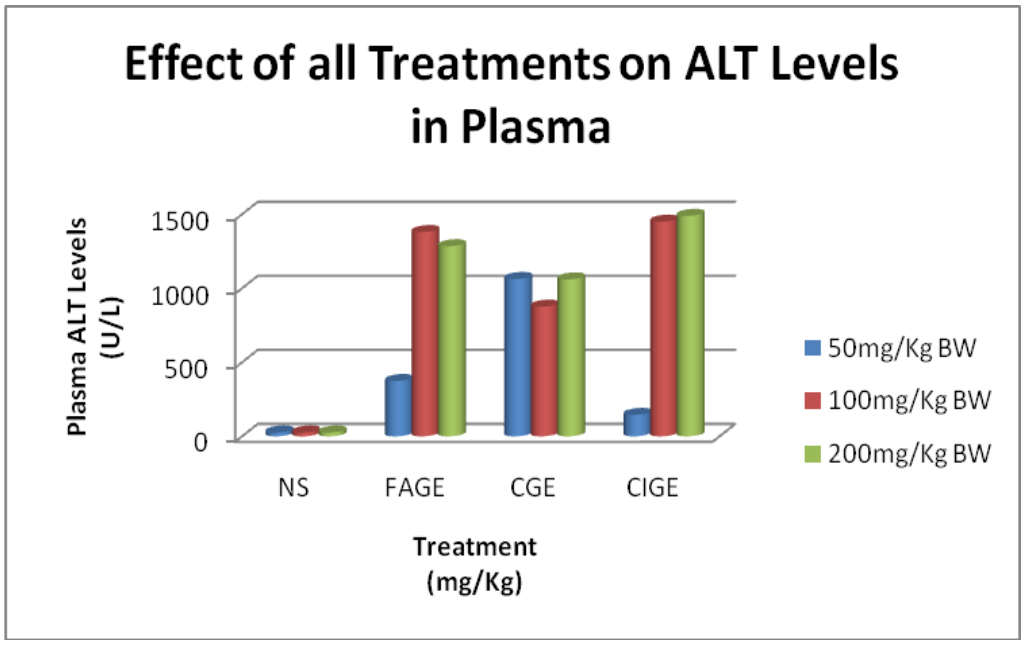
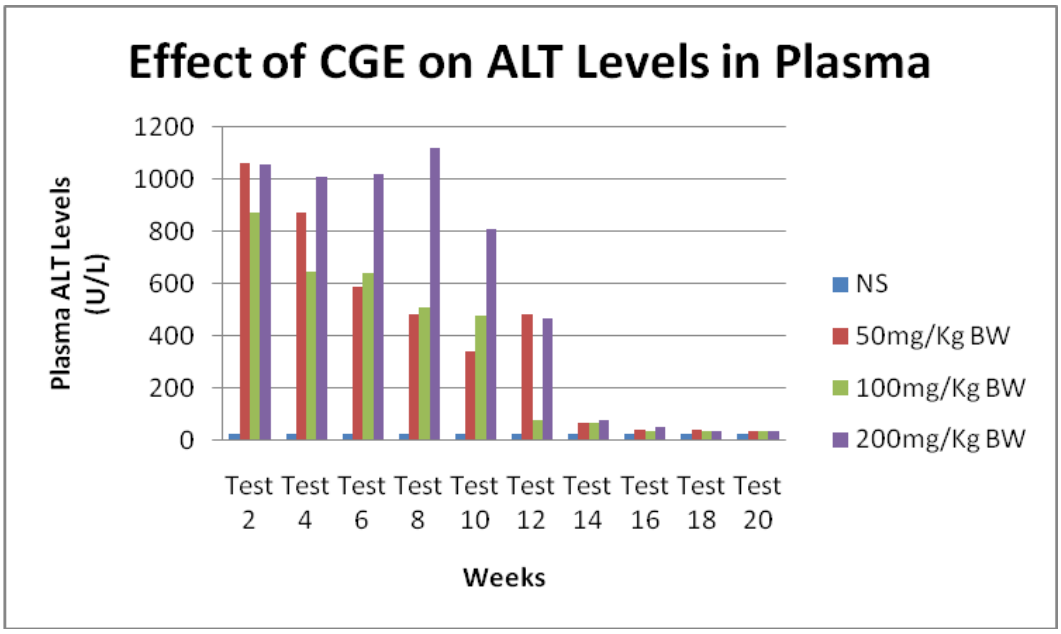


Figure 4.3.2a Mean plasma levels of Alanine Aminostraferase (pALT) following treatments of various concentrations of *Allium sativum* extracts

While there were no significant increments in mean Plasma levels of ALT treated with FAGE and CGE in all concentrations compared with control group (NS), there was significant increment in mean levels of pALT in treatment group T3 at CIGE 100mg/Kg body weight ($\Delta M=-726.73$, $p<0.05$) and CIGE 200 mg/kg body weight ($\Delta M=-740.82$, $p<0.05$) compared with the control group. There was a gradual increase in mean pALT from the control group to treatment group T1 at FAGE 200mg/kg body weight ($\Delta M=-444.6$U/L, $p>0.05$) then a dip by treatment group T3 at CIGE 50mg/kg body weight ($\Delta M=-176.6$U/L, $p>0.05$) before sharply increasing to ($\Delta M=-726.7$U/L, $p>0.05$) at CIGE

100mg/kg body weight and (ΔM=-740.8U/L, p>0.05) at CIGE 200mg/kg body weight, both of treatment group T3. Subsequent testing appeared to have an impact on concentrations of ALT with further testing reducing Plasma concentration levels as shown below in figures 4.3.2b.



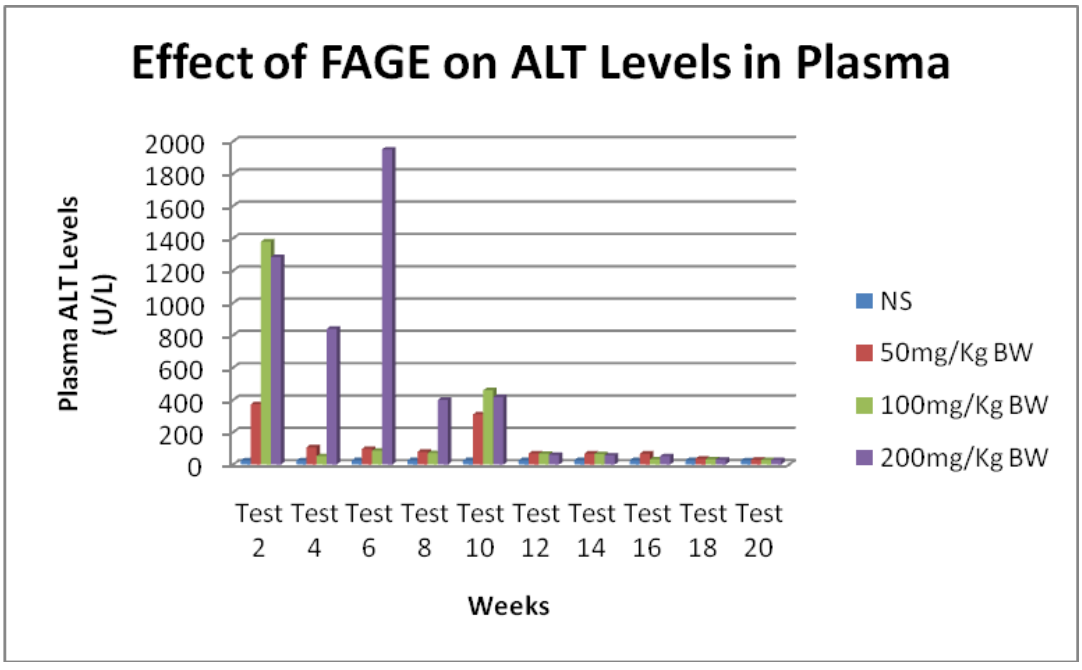


Figure 4.3.2b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Alanine Aminotransferase (pALT) for 20 weeks analyzed as 10 tests

4.3.3 Effect of *Allium sativum* Extracts Treatment on Cardiac Troponin-I (cTi) in Plasma in Hypertension-Induce laboratory rats

Figure 4.3.3a shows that there were significant increments in mean cTi at all treatment groups/concentrations compared to the control group (NS) following short term treatment. Thus in treatment group T1 FAGE 50mg/Kg body weight, significant increase was found at ($\Delta M=1.20$, $p<0.05$); in FAGE 100 mg/kg body weight, significant increment was found at ($\Delta M=1.21$, $p<0.05$); in FAGE 200mg/Kg body weight, the increment was at ($\Delta M=1.51$, $p<0.01$). Treatment group T2, CGE 50mg/kg body weight had a high significant increment at ($\Delta M=1.53$, $p<0.01$); in CGE 100mg/kg body weight, a significant increase was observed at ($\Delta M=1.29$, $p<0.05$) and in CGE 200mg/kg body weight, a highly significant increment was found at ($\Delta M=1.48$, $p<0.01$).

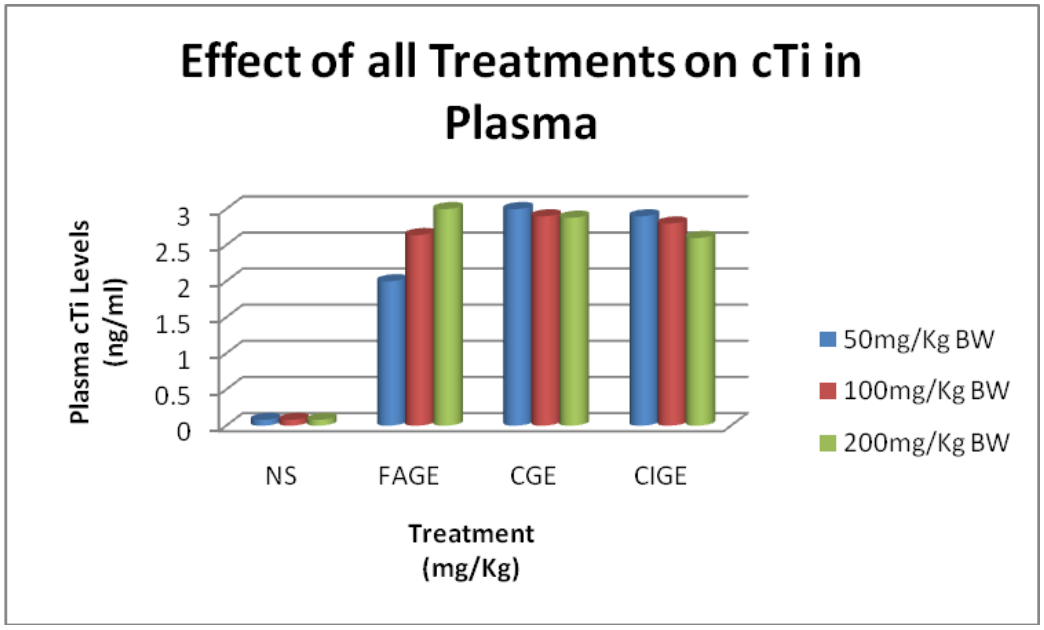
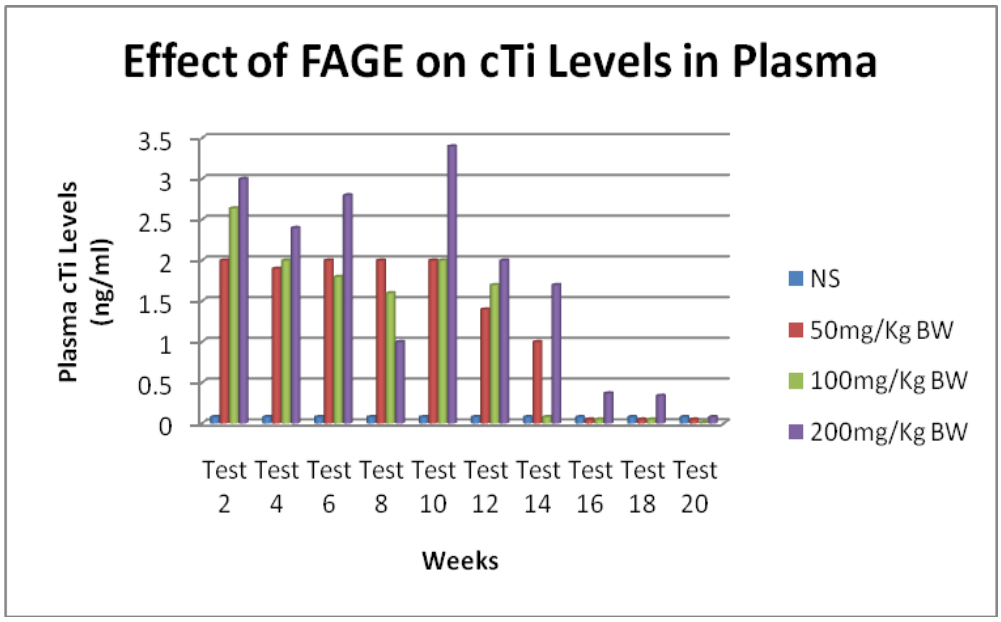


Figure 4.3.3a Mean plasma levels of Cardiac Troponin-I (cTi) following treatments of various concentrations of *Allium sativum* extracts

Treatment group T3 also exemplified similar significant increments. In CIGE 50mg/Kg body weight, a highly significant increment was observed at ($\Delta M=1.49$, $p<0.01$); in CIGE 100mg/kg body weight, significant increment was observed at ($\Delta M=1.32$, $p<0.01$); and in CIGE 200mg/kg body weight, significant increase was found at ($\Delta M=1.45$, $p<0.01$).

The mean plot graph for Plasma Cardiac Troponin-I (cTi) shown in figure 4.3.3a clearly shows a sharp increase from the control (0.1ng/ml) to the very first treatment concentration (1.3ng/ml) of treatment group T1 at FAGE 50mg/Kg body weight. The highest peaks were observed in treatment group T1 at FAGE 200mg/Kg body weight (1.6ng/ml) and treatment group T2 at CGE 50mg/Kg body weight (1.69ng/ml). This explains the significant differences observed at all treatment concentration levels.

The associated cTi against *Allium sativum* extract treatment plots reveal a decline in Plasma cTi with continued testing as shown in figures 4.3.3b.



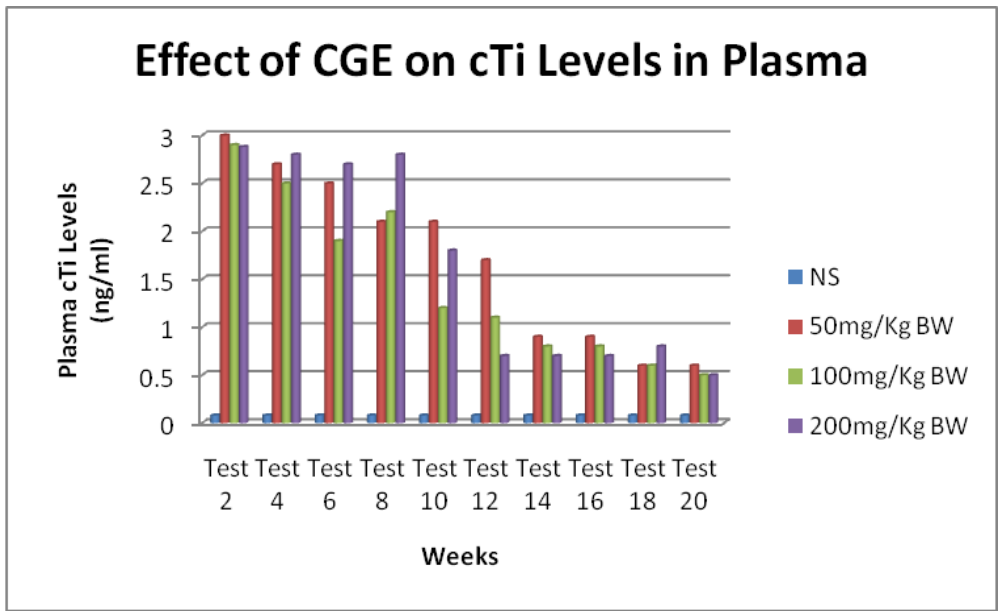


Figure 4.3.3b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Cardiac Troponin-I (cTi) for 20 weeks analyzed as 10 tests

4.3.4 Effect of *Allium sativum* Extracts Treatment on Cardiac Troponin-T (cTt) in Plasma in Hypertension-Induce laboratory rats

Figure 4.3.4a shows results for the significant difference in Mean Plasma cTt between the treatment groups and the control. *Allium Sativum* treatment on Cardiac Troponin–T (cTt) had a highly significant difference in mean Plasma cTt in treatment group T2 at CGE 50mg/kg body weight ($\Delta M=1.17$, $p<0.01$), a significant increase in treatment group T3 at CIGE 100mg/kg body weight (($\Delta M=1.04$, $p<0.05$) and a significant difference at CIGE 200mg/kg body weight ($\Delta M=.941$, $p<0.05$), compared to the control group.

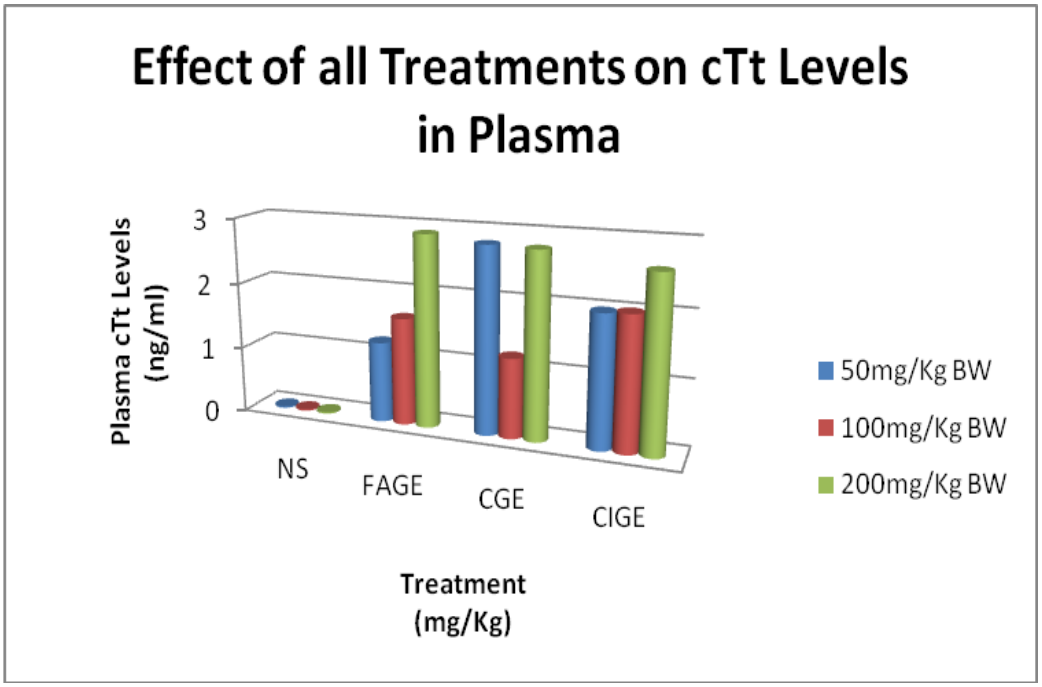
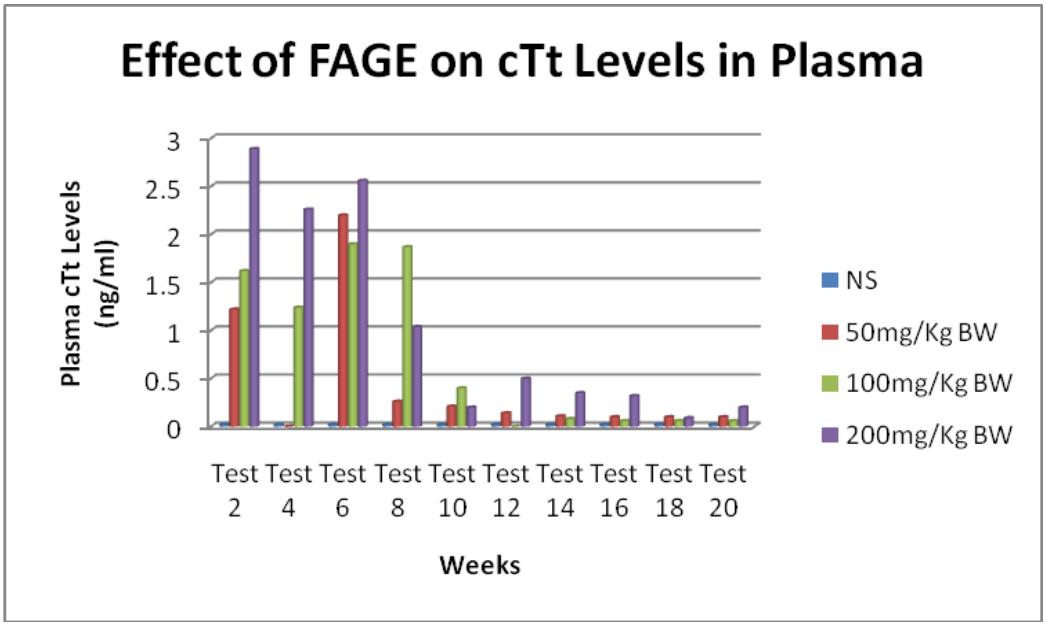


Figure 4.3.4a Mean plasma levels of Cardiac Troponin-T (cTt) following treatments of various concentrations of *Allium sativum* extracts

The mean plot graph for Plasma Cardiac Troponin-T (cTt) shown in figure 4.3.4a clearly shows a sharp increase from the control (0.1ng/ml) to the very first treatment concentration (0.75ng/ml) of treatment group T1 at FAGE 50mg/Kg body weight. Although there were increments, not all were significant. The highest peak was observed

in treatment group T2 at CGE 50mg/Kg body weight (1.2ng/ml), followed by treatment group T3 at CIGE 100mg/Kg body weight (1.12ng/ml). The lowest significant increment was noted in treatment group T2 at CGE 100mg/Kg body weight (0.7ng/ml). However, the figures below however shows a decline in Mean Plasma cTt values from baseline following subsequent ttreatment of *Allium sativum* extract to the 10th test



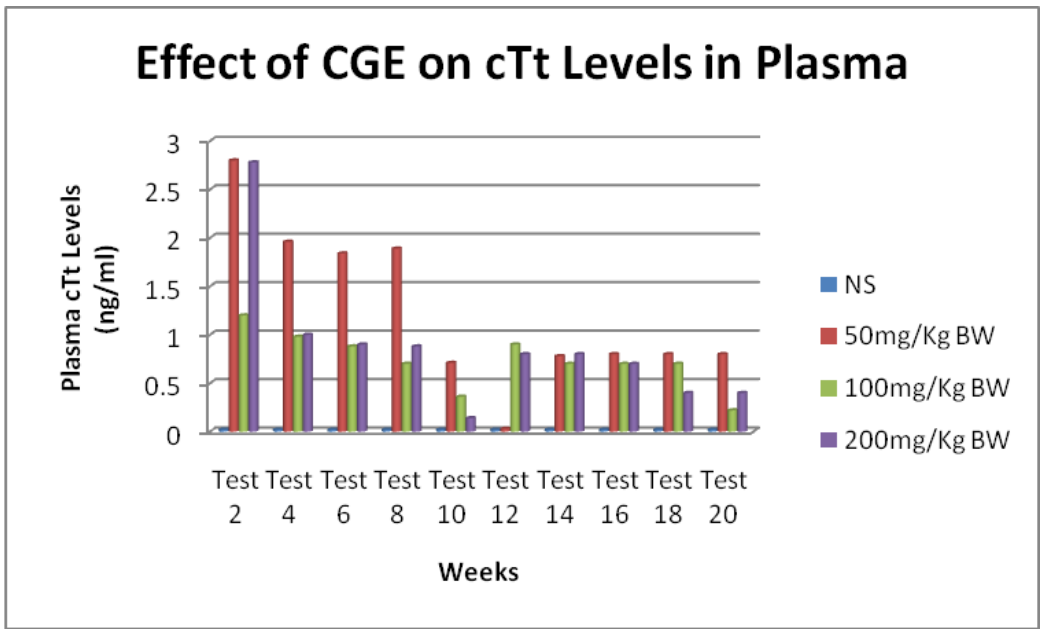


Figure 4.3.4b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Cardiac Troponin-T (cTt) for 20 weeks analyzed as 10 tests

4.3.5 Effect of *Allium sativum* Extracts Treatment on Total Cholesterol Levels in Plasma in Hypertension-Induced laboratory rats

Results in figures 4.3.5a on the effect of *Allium sativum* extract treatment on cholesterol levels in Plasma shows significant difference between groups.

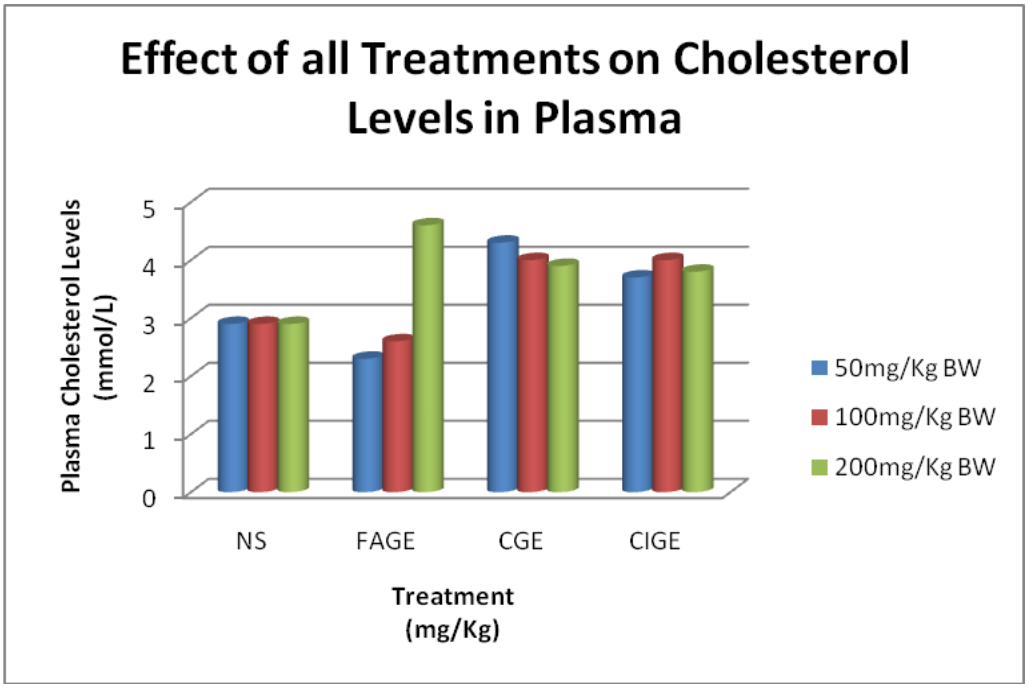


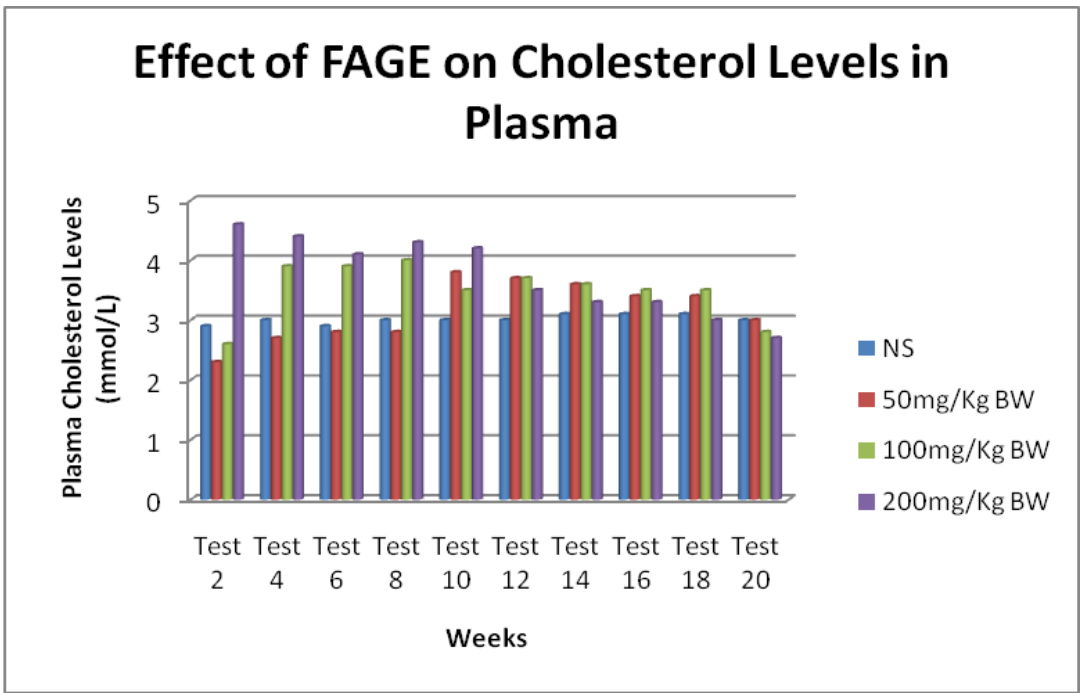
Figure 4.3.5a Mean plasma levels of Total Cholesterol following treatments of various concentrations of *Allium sativum* extracts

The figure further revealed that there were significant differences in total cholesterol mean levels in Plasma between the control group and treatment group T2 at CGE 200mg/kg body weight (ΔM=-0.809, $p<0.05$). Mean differences in Plasma total cholesterol levels were however highly significant in treatment group T3 at CIGE 50mg/kg body weight (ΔM =-1.209, $p<0.01$); CIGE 100mg/kg body weight (ΔM = -1.209, $p<0.01$) and CIGE 200mg/kg body weight (ΔM = -1.318, $p<0.01$). The Mean Plasma total cholesterol levels of other treatment groups T1, treatment group T2 at CGE 5mg/Kg body weight and CGE 100mg/Kg body weight were not statistically different from that of the control group.

There is a sharp increase in total Cholesterol from the control group (3.09mmol/L) to the very first treatment concentration of CGE 50mg/Kg body weight (3.75mmol/L), which is 21.36% rise. There is a characteristic steep rise in treatment group T3 at CIGE 100mg/Kg

body weight (3.59mmol/L) to 4.25mmol/L at CIGE 50mg/Kg body weight (18.38% rise), with the highest total Cholesterol mean level of 4.50mmol/L at CIGE 200mg/Kg body weight (25.35% rise). Although there were increments, not all were significant compared to the control group.

The comparative bar graph of Mean plot effects of *Allium sativum* extract treatment did not however yield any noticeable trend in Mean Plasma cholesterol levels with respect to subsequent testing as depicted by figures 4.3.5b



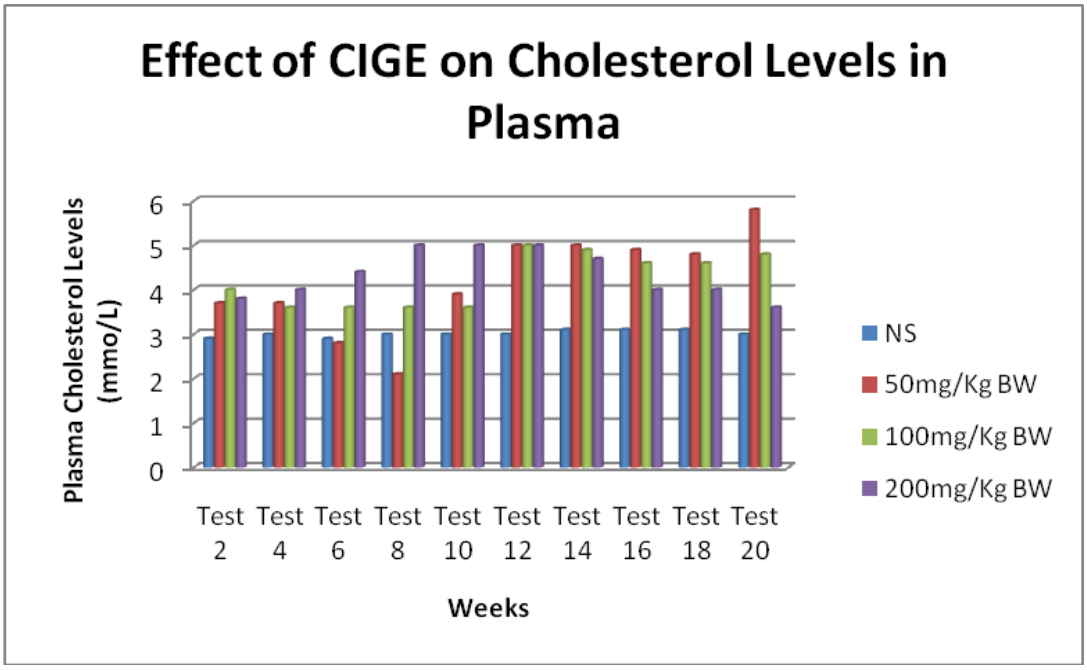


Figure 4.3.5b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Total Cholesterol for 20 weeks analyzed as 10 tests

4.3.6 Effect of *Allium sativum* Extracts Treatment on Plasma Creatine Phosphokinase Isoenzyme-MB (CK-MB) Levels in Hypertension-Induced laboratory rats

Allium sativum extract treatment triggered a mean significance differences on Plasma CK-MB as shown in figure 4.3.6a. The results gives significant differences in treatment group T2 at CGE 200mg/kg body weight concentration ($\Delta M=-1.500$, $p<0.05$) and in treatment group T3 at CIGE 200mg/Kg body weight concentration ($\Delta M=-1.891$, $p<0.01$) only.

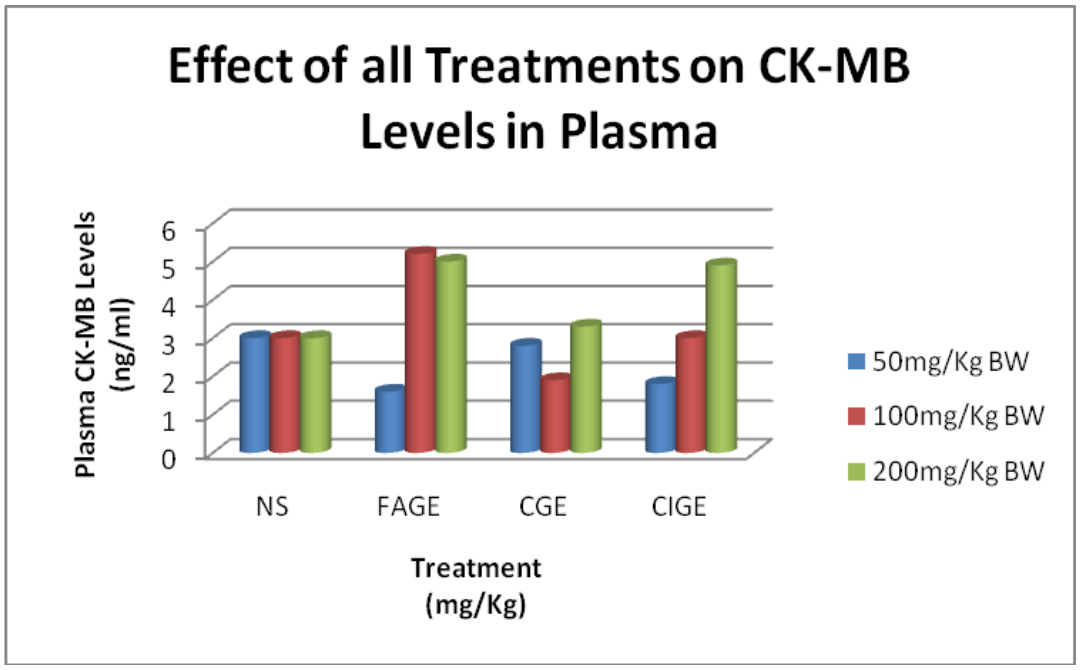
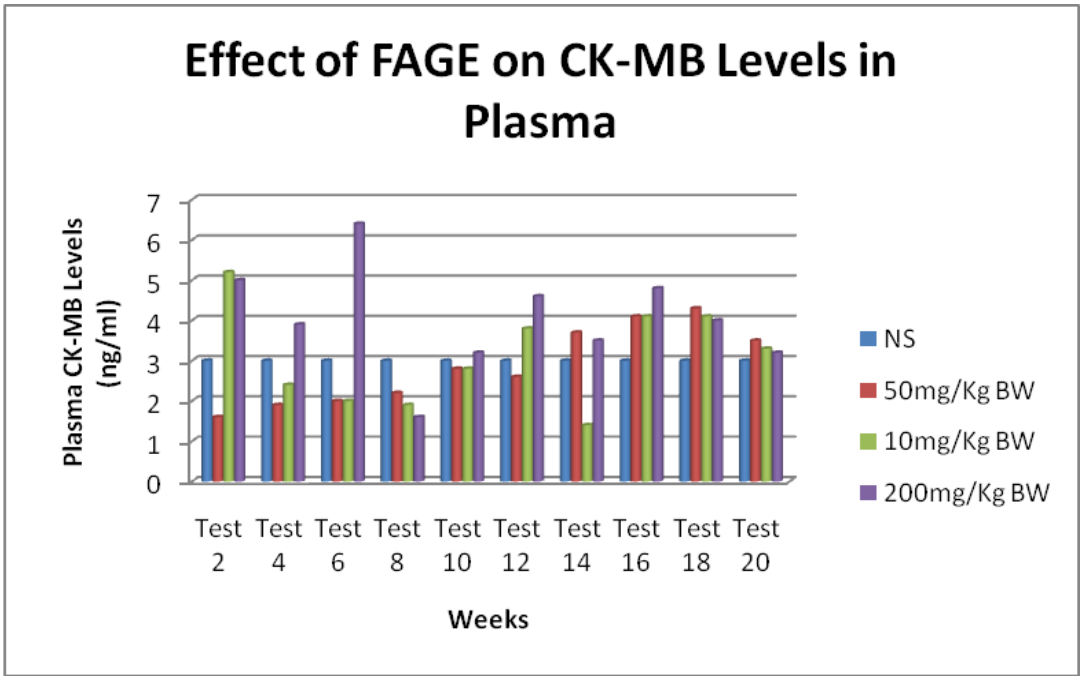


Figure 4.3.6a Mean plasma levels of Creatine Phosphokinase Isoenzyme-MB (CK-MB) following treatments of various concentrations of *Allium sativum* extracts

The mean plot graph in figure 4.3.6a confirms these results showing mean significant differences between the crests at CGE 200mg/Kg body weight (4.500ng/ml) (= 50% rise) of treatment group T2 and treatment group T3 at CIGE 200mg/Kg body weight (4.80ng/ml) (= 60% rise) compared to the control group with mean Plasma CK-MB of 3.0ng/ml. All the other treatments had some mean differences, but they were not statistically significant compared to the control. However, no clear pattern in CK-MB values in Plasma with respect to subsequent treatment of the groups with *Allium sativum* extract yielded discerned trends as shown in figures 4.3.6b.



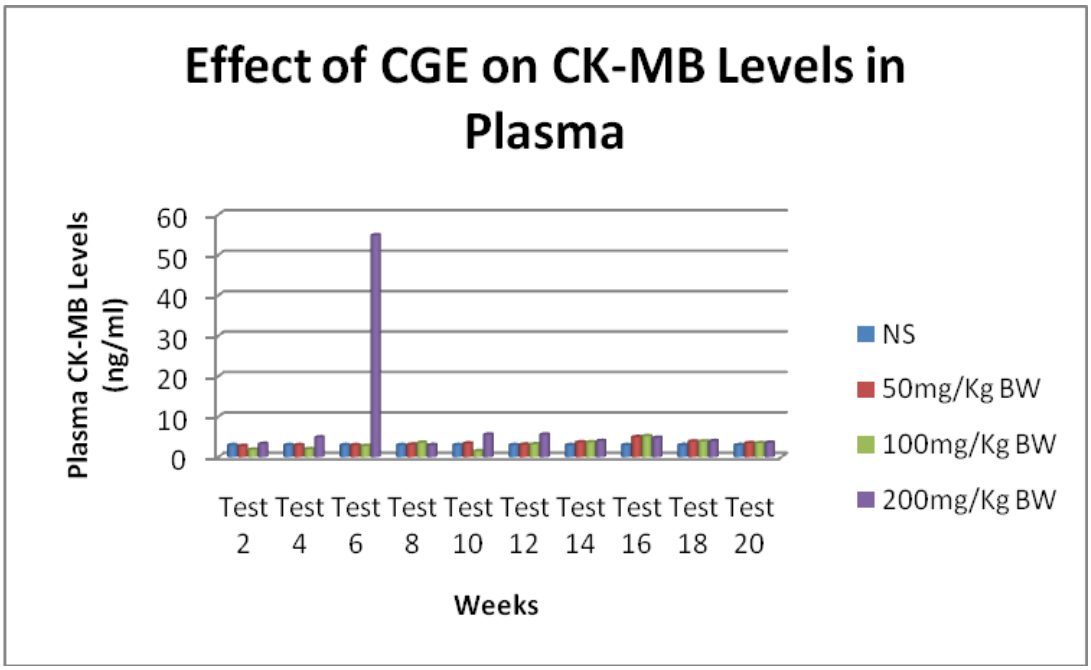


Figure 4.3.6b A comparative bar graph of Mean Plot effect of *Allium sativum* extract treatment on Plasma levels of Creatine Phosphokinase Isoenzyme-MB (CK-MB) for 20 weeks analyzed as 10 tests

4.4 Effects of *Alluvium sativum* Extract Treatment on the Electrical and Mechanical Activities of the Heart

The ANOVA output in Table 4.3.0 revealed existence of significant differences in mean levels of all the parameters at different concentration levels.

4.4.1 Effect of *Allium sativum* Extract Treatment on Heart RR-intervals of Hypertension-Induced Laboratory Rat from Real-Time ECG

The RR intervals for the treatment groups using CPT, HCT, FAGE, CGE and CIGE were compared with RR interval of the control group that was treated with normal saline. The multiple comparison tests in Table 4.3.1 showed that there were significant increments in mean RR intervals at all concentration levels except for treatment groups C3b at HCT 200mg/kg body weight and treatment group T1b at FAGE 200mg/kg body weight.

Significant increments in mean RR interval were reported in treatment groups C2a at CPT 100mg/kg body weight ($\Delta M=-26.61$, $p<0.05$); and C2b CPT 200mg/kg body weight ($\Delta M=-94.0$, $p<0.05$); treatment group C3a at HCT 50mg/kg body weight ($\Delta M=-25.14$, $p<0.05$) and C3b HCT 200mg/kg body weight ($\Delta M=-0.785$, $P<0.05$); treatment group T1a at FAGE100mg/kg body weight ($\Delta M=-56.971$, $P<0.01$,$P<0.05$); treatment group T2a at CGE 100mg/kg body weight ($\Delta M=-10.071$, $p<0.05$); and treatment group T3a at CIGE100mg/kg body weight ($\Delta M=-16.057$, $p<0.05$) and finally T3b CIGE 200mg/kg body weight ($\Delta M=-23.321$, $p<0.05$).

Interestingly, the mean RR interval for treatment group T2b at CGE 200mg/kg body weight dropped compared to that of the control group C1 at NS ($\Delta M=6.4$, $p<0.05$). There were no significant differences in mean RR intervals of treatment group C3b at HCT

200mg/kg body weight and at FAGE 200mg/kg body weight in treatment group T1b compared to NS levels of the control C1.

Figure 4.4.1 clearly shows the differences in mean RR interval of the control group at C1 NS with the other concentration levels. Compared to the control, C3b of HCT 200mg/Kg body weight, T1b of FAGE 200mg/Kg body weight and T2b of CGE 200mg/Kg body weight did not have any difference with the control group by maintaining their mean RR intervals lower and closer to that of the control group.

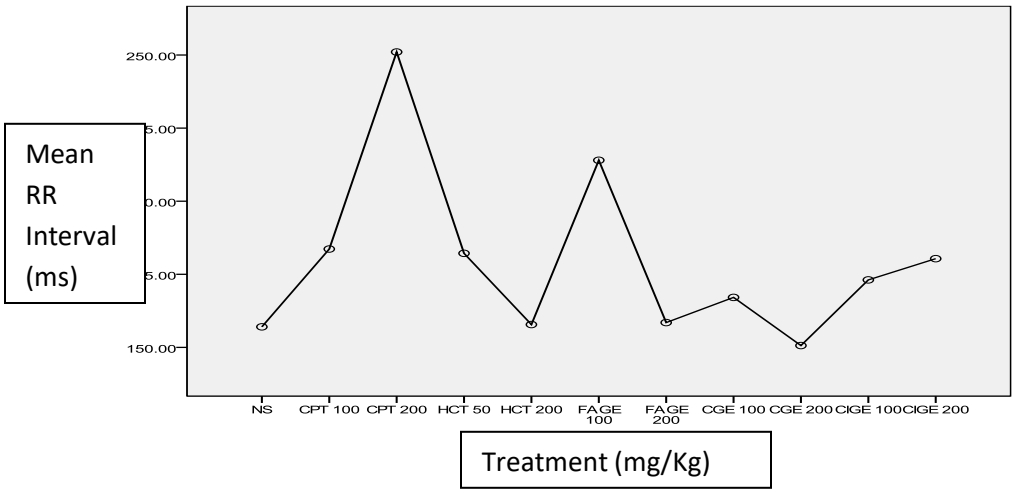


Figure 4.4.1 Mean Plot Effect of *Allium sativum* Extract Treatment on RR-Interval from a Real-Time ECG. Each peak represents a Mean of four animals per treatment group

4.4.2 Effect of *Allium sativum* Extract Treatment on the Heart Rate of Hypertension-Induced Laboratory Rat from Real-Time ECG

The heart rate at CPT, HCT, FAGE, CGE, and CIGE were compared with heart rate at NS. The ANOVA Table 4.3.0 shows the significance difference between groups. The

summary report of mean heart rates at the various treatment levels shows that although all the heart rates were within the normal heart rate interval of 250–450b/m, all treatment concentrations reported a drop in mean heart rate compared to NS, except at CGE where there was an increase in the mean heart rate. See Table 4.3.2a.

Turkey's multiple comparisons (Table 4.3.2b) revealed that the observed drops in mean heart rates were significant at all the concentrations except at C3b of HCT 200mg/kg body weight ($\Delta M=380.27$, $p<0.05$) and T1b of FAGE 200mg/kg body weight ($\Delta M=378.62$, $p<0.05$. The largest mean significant differences were reported at C2b of CPT 200mg/kg body weight ($\Delta M=239.01$, $p<0.05$). The increase in mean heart rates at CGE (T2a CGE 100mg/Kg body weight and T2b CGE 200mg/Kg body weight) had a mean difference of 16.228 compared to that of NS.

Figure 4.4.2 depicts this scenario that shows treatment group T2b of CGE 200mg/kg body weight triggering a heart rate clearly above that of the control group at NS. The lowest mean heart rate shown by C2b at CPT 200mg/Kg body weight followed by T1a at FAGE 100mg/Kg body weight.

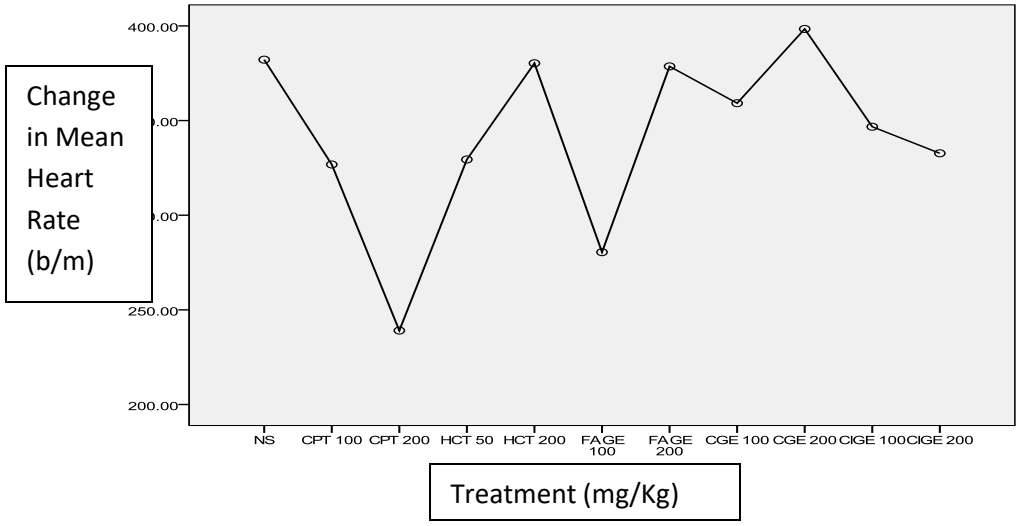


Figure 4.4.2 Mean Plot Effect of *Allium sativum* Extract Treatment on Heart Rate from a Real-Time ECG. Each point represents a Mean of four animals per treatment group

4.4.3 Effect of *Allium sativum* Extract Treatment on PR Intervals of Hypertension-Induced Laboratory Rat from Real-Time ECG

The mean PR intervals for CPT, HCT, FAGE, CGE, and CIGE were compared with those at Normal Saline. The ANOVA Table 4.3.0 shows the significance difference of PR Intervals between groups. Turkey's multiple comparison test results (Table 4.3.3) revealed that significant mean differences on PR intervals from the ECG recording were only at C2b at CPT 200mg/kg body weight ($\Delta M=46.678$, $p<0.05$); T1a at FAGE 100mg/kg body weight ($\Delta M =37.304$, $p<0.05$) and T2a at CGE 100mg/kg body weight ($\Delta M=26.385$, $p<0.05$). At CPT 200mg/kg body weight and FAGE 100mg/kg body weight, the mean PR interval was lower than of the control group C1 at NS. However, at CGE 100mg/kg body weight the mean PR interval was higher than at NS. The mean differences at all the other concentrations were not significant.

The mean plot shows clear dips in mean PR intervals at CPT 200mg/kg body weight and FAGE 100mg/kg body weight confirming the drop in PR intervals. A peak can also be

seen at CGE 100mg/kg body weight confirming the increments in mean PR interval. The rest of the concentrations were recorded as being around the control group at NS concentration as shown in the mean plot results in figure 4.4.3.

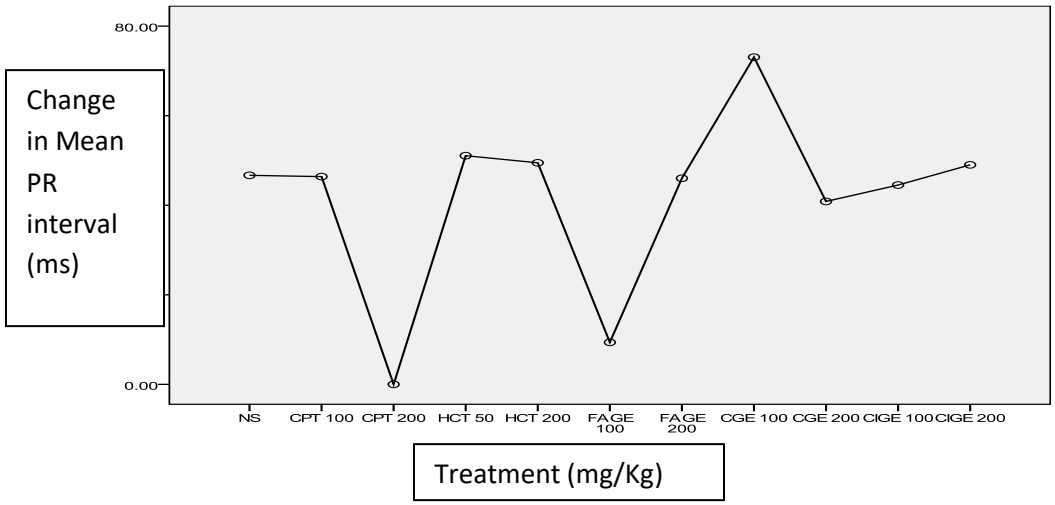


Figure 4.4.3 Mean Plot Effect of *Allium sativum* Extract Treatment on PR Interval from a Real-Time ECG. Each point represents a Mean of four animals per treatment group

4.4.4 Effect of *Allium sativum* Extract Treatment on P Duration of Hypertension-Induced Laboratory Rat from Real-Time ECG

Mean P duration of hypertensive rats treated with CPT, HCT, FAGE, CGE and CIGE *Allium sativum* concentrations were compared with mean P duration of the control group treated with NS. The ANOVA Table 4.3.0 showed the significance difference in P durations between groups. The ECG results revealed that treatment group C2b at CPT 200mg/kg body weight and treatment group T1a at FAGE 100mg/Kg body weight caused a significant decrease in mean P duration among hypertension induced laboratory rats ($\Delta M=16.915$, $p<0.05$) and ($\Delta M=13.575$, $p<0.05$) respectively. On the contrary,

treatment group T2a at CGE 100mg/kg body weight triggered a significant increase in mean P duration among the rats (ΔM=-26.315, $p<0.05$). All the other concentrations caused a decrease, but they had no significant effects on P duration. Table 4.3.4 shows the mean comparison test results of the effect of *Allium sativum* concentrations of the P duration.

The mean plot in figure 4.4.4 shows clear dips in mean P durations at CPT 200mg/kg body weight and FAGE 100mg/kg body weight confirming the drop in P duration. A peak can also be seen at CGE 100mg/kg confirming the increments in mean P duration. The rest of the concentrations were recorded as being around the control group at NS concentration as shown in the mean plot results.

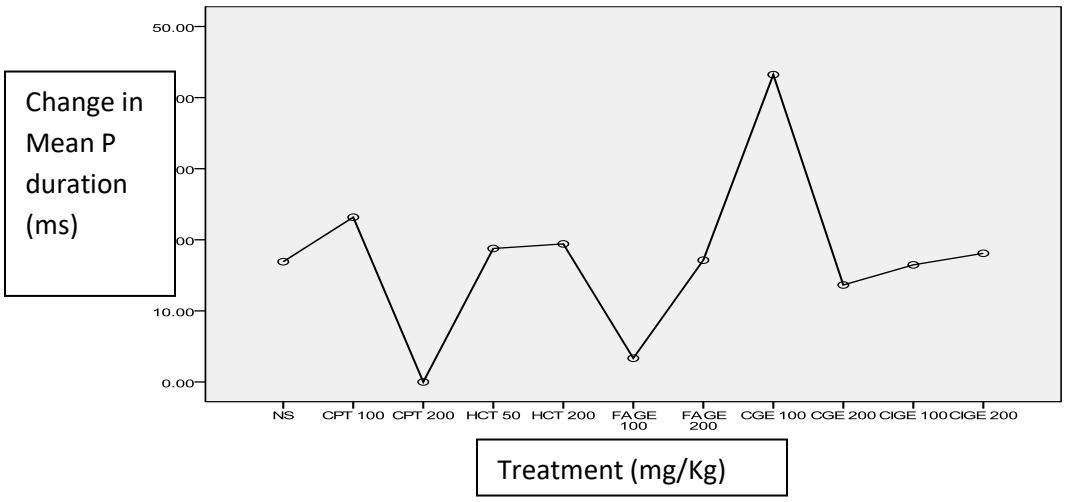


Figure 4.4.4 Mean Plot Effect of *Allium sativum* Extract Treatment on P Duration from a Real-Time ECG. Eac point represeents a Mean of four animals per treatment group

4.4.5 Effect of *Allium sativum* Extract Treatment on QRS Interval of Hypertension-Induced Laboratory Rat from Real-Time ECG

The effects of CPT, HCT, FAGE, CGE and CIGE concentrations on the Mean QRS intervals were compared with mean QRS intervals of the control group at NS. The ANOVA Table 4.3.0 shows the significance difference in QRS intervals between groups. Results of the multiple comparisons test Table 4.3.5 showed that the mean QRS interval increased significantly in treatment group C2b with CPT 200mg/kg body weight concentration (ΔM=-23.166, $p<0.05$), but dropped significantly in treatment group T1b at with FAGE 200mg/kg body weight concentration (ΔM=10.61, $p<0.05$). The other concentration levels triggered marginal increments about the mean in QRS interval but these increments were not statistically significant compared to the control.

The mean plot in figure 4.3.1.5 shows clear dips in mean QRS interval at HCT 200mg/kg body weight (ΔM=4.024, $p<0.05$) and FAGE 200mg/kg body weight (ΔM=10.61, $p<0.05$), confirming the drop in mean QRS intervals. Though CIGE 200mg/Kg body weight was also low, the reduction in mean QRS interval was not statistically significant compared to the control group. A peak can also be seen at CPT 100mg/kg body weight confirming the increments in mean QRS interval. The rest of the concentrations were recorded as being around the control group at NS concentration as shown in the mean plot results.

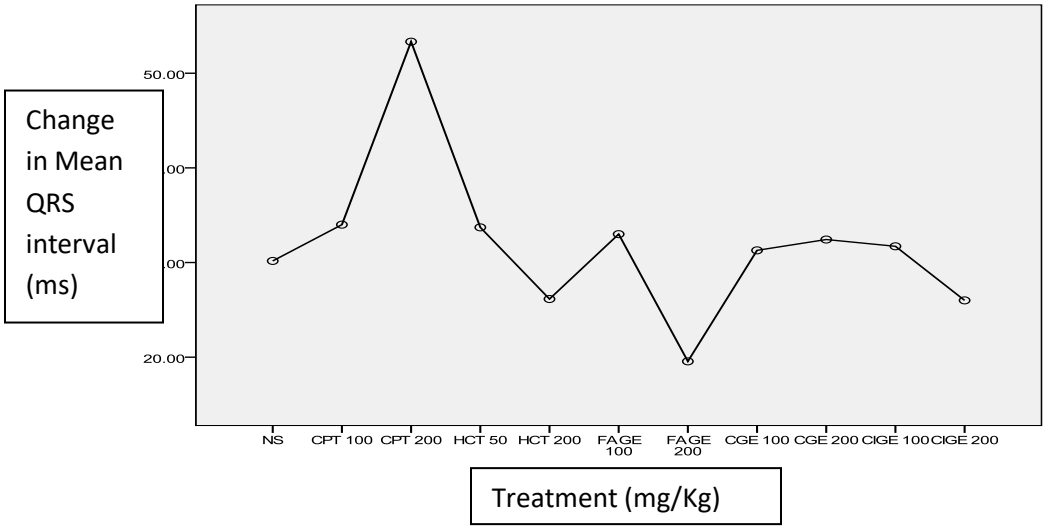


Figure 4.4.5 Mean Plot Effect of *Allium sativum* Extract Treatment on QRS Intervals from a Real-Time ECG. Each point represents a Mean of four animals per treatment group

4.4.6 Effect of *Allium sativum* Extract Treatment on QT Interval of Hypertension-Induced Laboratory Rat from Real-Time ECG

The mean QT interval for CPT, HCT, FAGE, CGE and CIGE of various treatment groups were each compared with the mean QT interval for the control group at NS. The ANOVA Table 4.3.0 shows the significance difference in QT intervals between groups. Results showed that *Allium sativum* extract triggered a significant increase in QT interval in treatment group C2b at CPT 200 mg/kg body weight (Δ=-31.606, $p<0.05$). Treatment groups T1a and T1b significantly showed a decreases in QT interval at FAGE 100mg/kg body weight (ΔM=22.45, $p<0.05$) and at FAGE 200mg/kg body weight (ΔM = 30.634, $p<0.05$). Additionally, treatment group T3b at CIGE 200mg/kg body weight (ΔM=20.944, $p<0.05$) was also statistically significant. Major differences in mean QT interval were registered at CPT 200mg/kg body weight and at FAGE 200mg/kg body weight as recorded by the multiple comparison test Table 4.3.6 below.

The mean plot in figure 4.3.1.6 shows clear dips in mean QRS interval at CPT 100mg/kg body weight (ΔM=21.63, p<0.05), FAGE 100mg/Kg body weight (ΔM=22.45, p<0.05), and FAGE 200mg/kg body weight (ΔM=30.63, p<0.05), confirming the drop in mean QT intervals. Though CIGE 200mg/Kg body weight was also low, the reduction in mean QT interval was not statistically significant compared to the control group. A peak can also be seen at CPT 200mg/kg body weight at (ΔM=31.61, p<0.05), confirming the increments in mean QT interval. The rest of the concentrations were recorded as being around the control group at NS concentration as shown in the mean plot results.

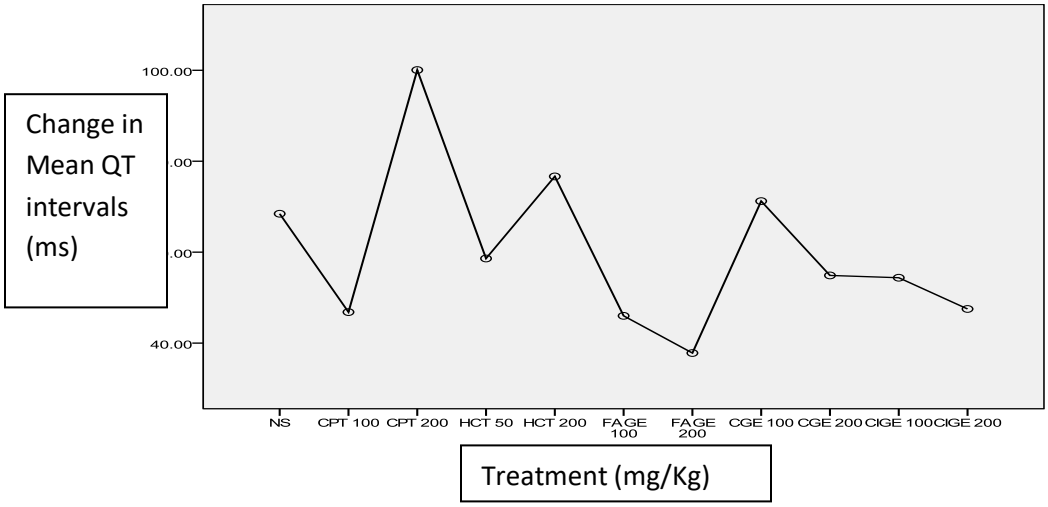


Figure 4.4.6 Mean Plot Effect of *Allium sativum* Extract Treatment on QT Intervals from a Real-Time ECG. Each point represents a Mean of four animals per treatment group

4.5 Effect of *Allium sativum* Extract Treatment on Blood Pressure of Hypertension-Induced Laboratory Rat from Real-Time ECG Recording

The ANOVA output (Table 4.3.7a) for both systolic and diastolic blood pressure revealed that there were significant differences in mean systolic and diastolic blood pressures at different concentrations of *Allium sativum* extracts treatment.

4.5.1 Effect of *Allium sativum* Extract Treatment on Systolic Blood Pressure (SBP) from a Real-Time ECG Recording of a Hypertensive-Induced Laboratory Rat

Allium sativum extract treatment was found to have no statistically significant effect on the Mean systolic blood pressure of hypertension-induced laboratory rats. The following mean systolic blood pressure values were just at the borderline of the highest systolic pressure value. The norm systolic blood pressure is about 90 – 145mmHg.

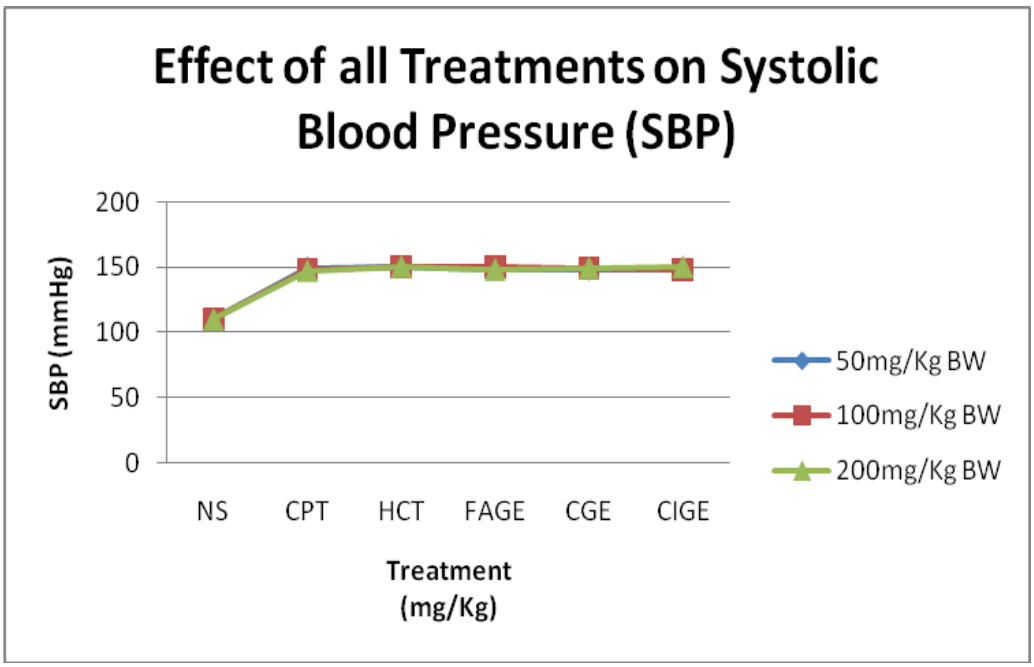


Figure 4.5.1a Mean Plot Effect of *Allium sativum* extract treatment on Systolic Blood Pressure (SBP)

No significant differences were therefore noted at concentrations of treatments. Though the treatment of CPT 200mg/Kg body weight (SP = 134.64) and FAGE 200mg/Kg body

weight (SP = 135.0) showed the lowest Mean systolic blood pressure values compared with the rest, as captured in Table 4.3.7b.

Figure 4.5.1b clearly depicts the events of treatment groups T2 and T3 in various concentrations having significant increments after induction of hypertension and commencement of treatments above the normal mean Systolic blood pressure with the highest mean Systolic blood pressure increment registered at concentrations of CIGE 50mg/Kg body weight followed by CGE 50mg/Kg body weight. However, these increments are not statistically significant, but they are noted as changes in Mean systolic blood pressure.

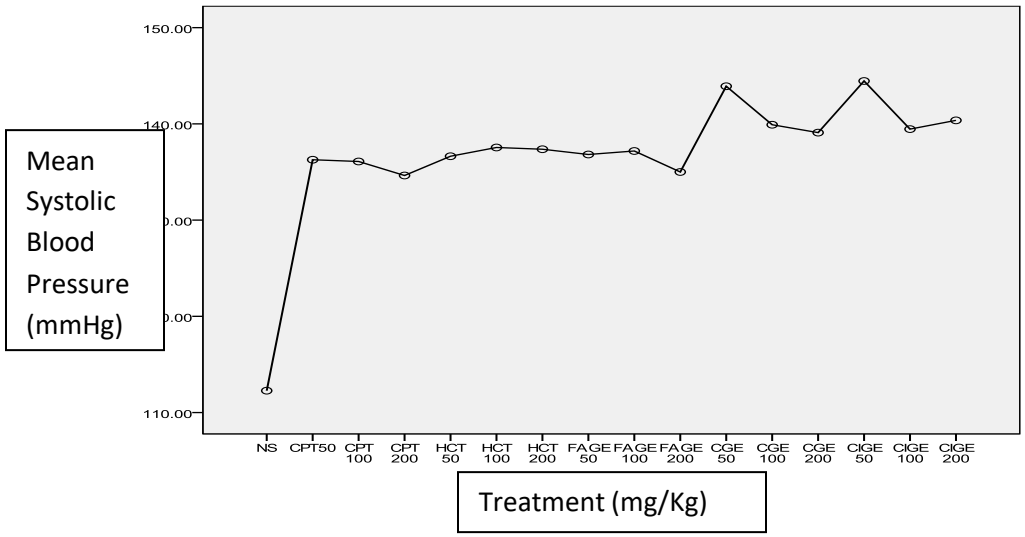
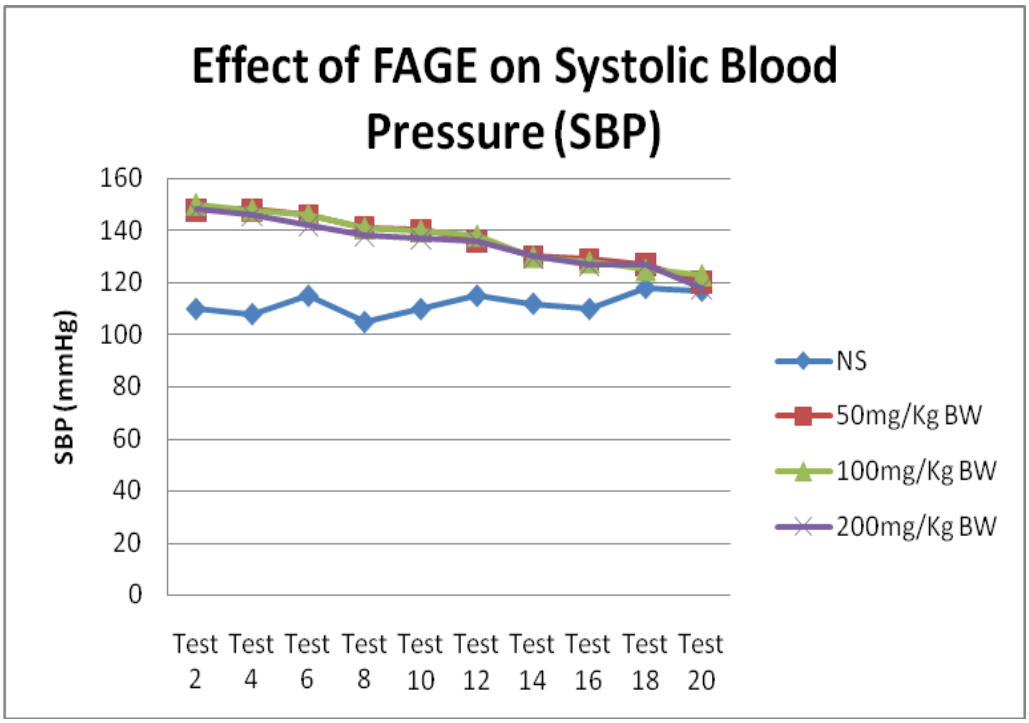


Figure 4.5.1b Mean Plot Effect of *Allium sativum* Extract Treatment on Systolic Blood Pressure from a Real-Time ECG recoding. Each point represent a Mean of four animals per treatment group

But upon further assessment, the comparative linear functions of Mean systolic blood pressures at different concentrations against time with subsequent cumulative treatment of *Allium sativum* extracts revealed that Mean systolic blood pressure reduced after

induction of hypertension in the treatment groups from week 1 to week 20 as depicted in figure 4.5.1c.



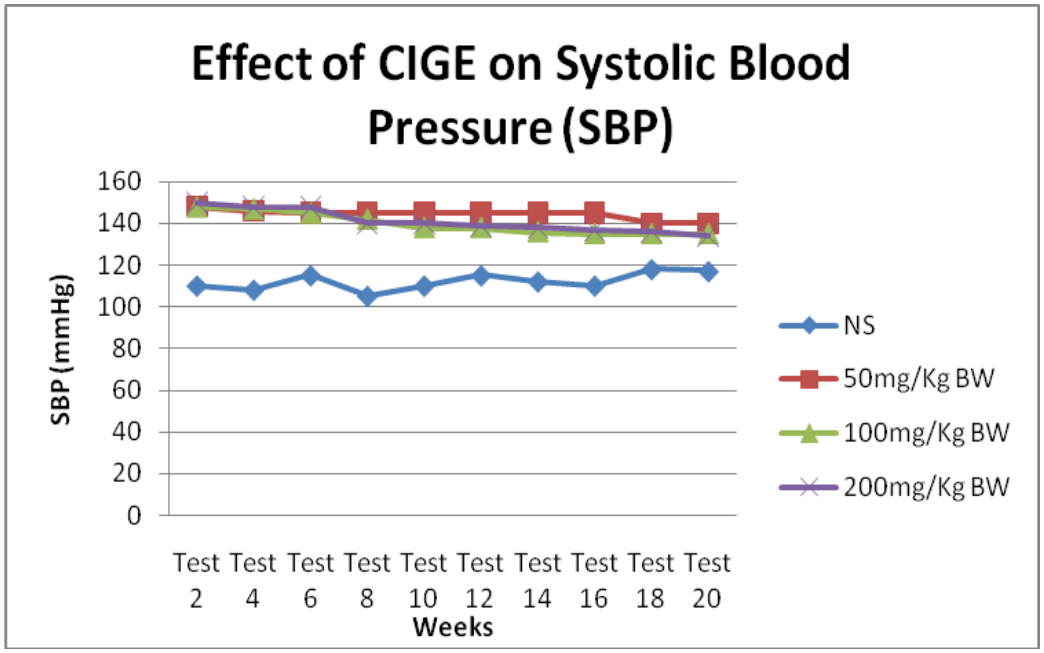


Figure 4.5.1c A Comparative linear graph of Mean Plot Effect of *Allium sativum* extract treatment on Systolic Blood Pressure for 20 weeks

Table 4.3.7c shows an examination of the multiple comparisons test results of Mean Systolic blood pressure of treatment group T2 with CGE and T3 with CIGE concentrations with that of the control group at NS revealed highest increments in the Mean systolic blood pressure at the start of the treatments. The largest differences in mean systolic blood pressure were registered at CIGE 50mg/kg body weight (ΔM=-32.181, $p<0.05$) which is 43.91% increment, CGE 50mg/kg body weight (ΔM=-31.636, $p<0.05$) which is 41.48% increment and at CIGE 200mg/kg body weight (ΔM=-28.091, $p<0.05$) which is 25.62% increment, compared with the baseline of the control group at NS (ΔM=-22.36, $p<0.05$).

4.5.2 Effect of *Allium sativum* Extract Treatment on Diastolic Blood Pressure (DBP) form a Real-Time ECG Recording of a Hypertensive-Induced Laboratory Rat

Allium sativum extract treatment was also found to trigger a decrease and maintained constancy in the normal diastolic blood pressure of hypertension-induced laboratory rats within the reference range for Wistar rat (55-66mmHg).

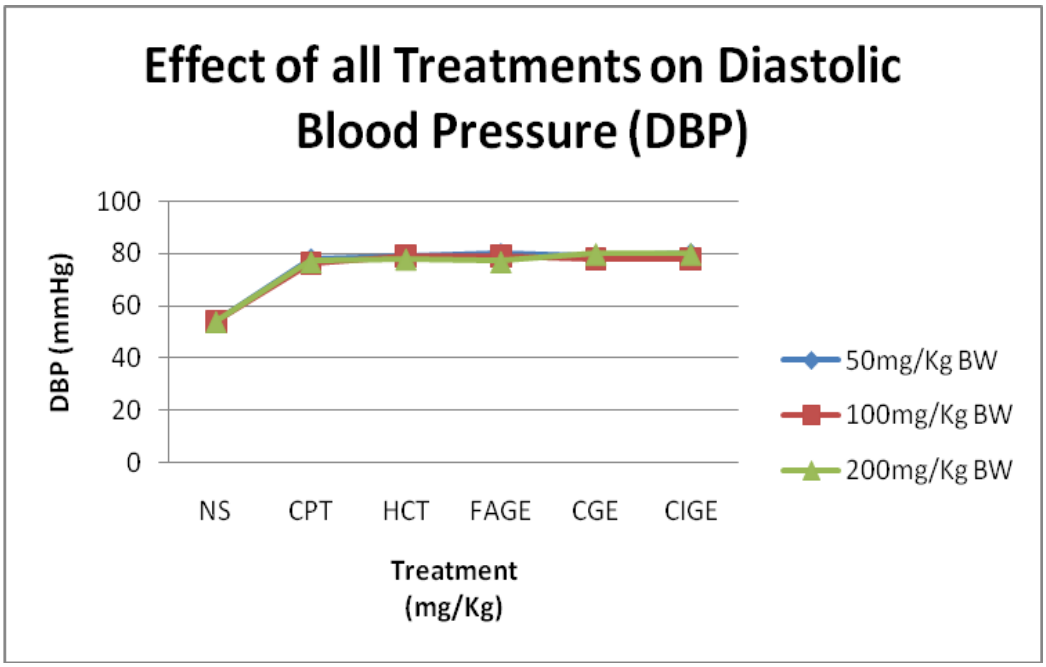


Figure 4.5.2a Mean Plot Effect of *Allium sativum* extract treatment on Diastolic Blood Pressure (DSP)

Though within normalcy, the least registered mean diastolic blood pressure was 63.00mmHg registered at treatment group C2b at a concentration of CPT 200mg/kg body weight, C3b at HCT 200mg/Kg body weight and treatment group T1b at a concentration of FAGE 200mg/kg body weight (= 10.7% decrement) compared to the control group with 56.91mmHg. The largest Mean diastolic blood pressure was 74.8182 recorded by treatment group T3 at a concentration of CIGE 50mg/kg body weight (= 31.47% decrement) as shown by Table 4.3.8a.

Figure 4.5.2b clearly depicts the events of treatment groups T2 and T3 in various concentrations having significant decrements compared to the normal mean Diastolic blood pressure with the highest mean Diastolic blood pressure decrement registered at concentrations of CIGE 50mg/Kg body weight followed by CGE 50mg/Kg body weight.

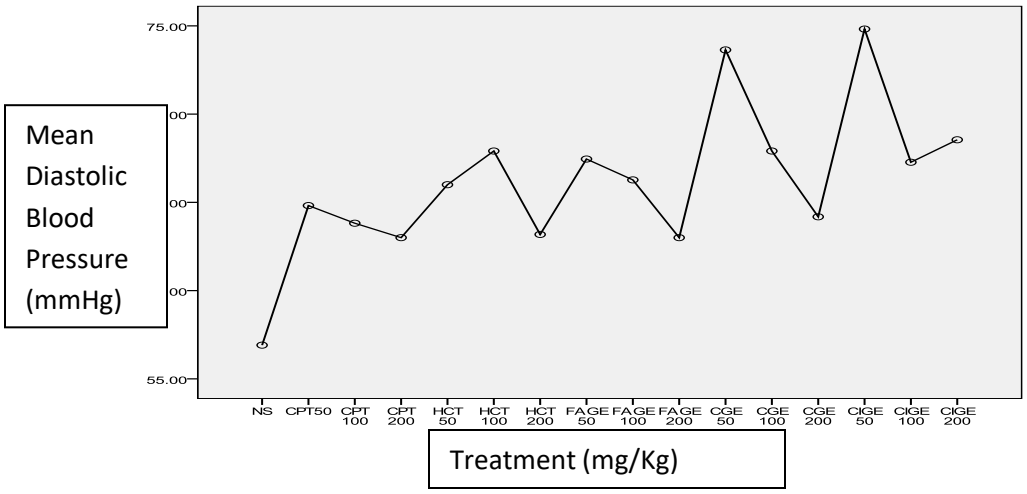
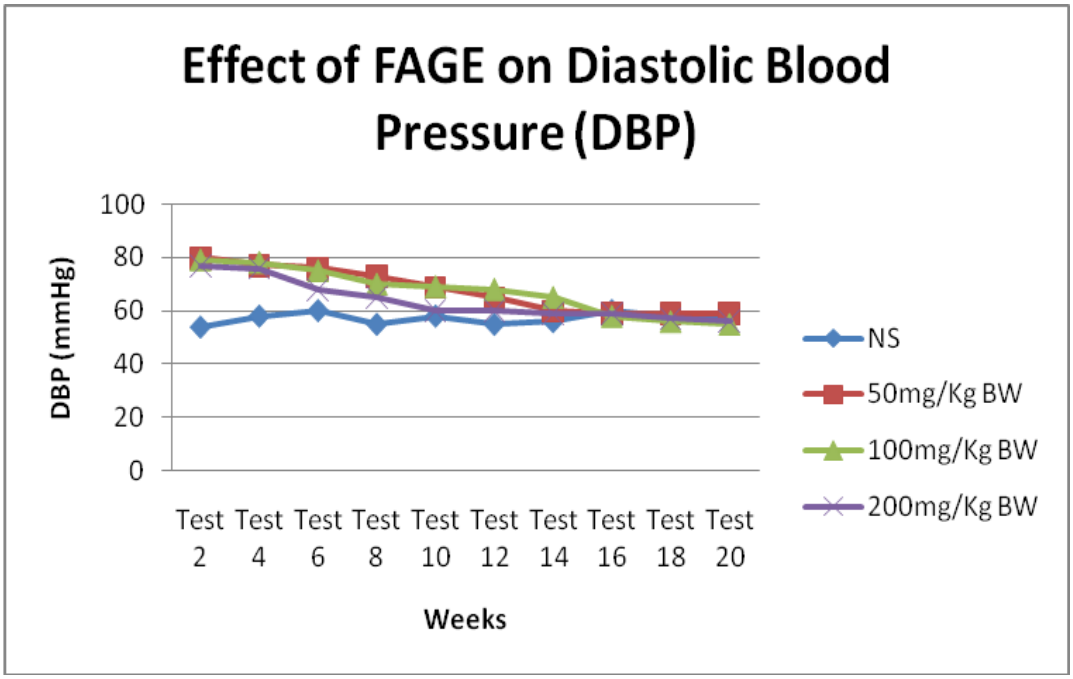


Figure 4.5.2b Mean Plot Effect of *Allium sativum* Extract Treatment on Diastolic Blood Pressure from a Real-Time ECG recoding. Each point represents a Mean of four animals per treatment group

The linear functions also depicted a decline in the mean diastolic blood pressure at different extract concentrations with subsequent cumulative exposure to the treatments over time for a period of 20 weeks as shown in figure 4.5.2c.



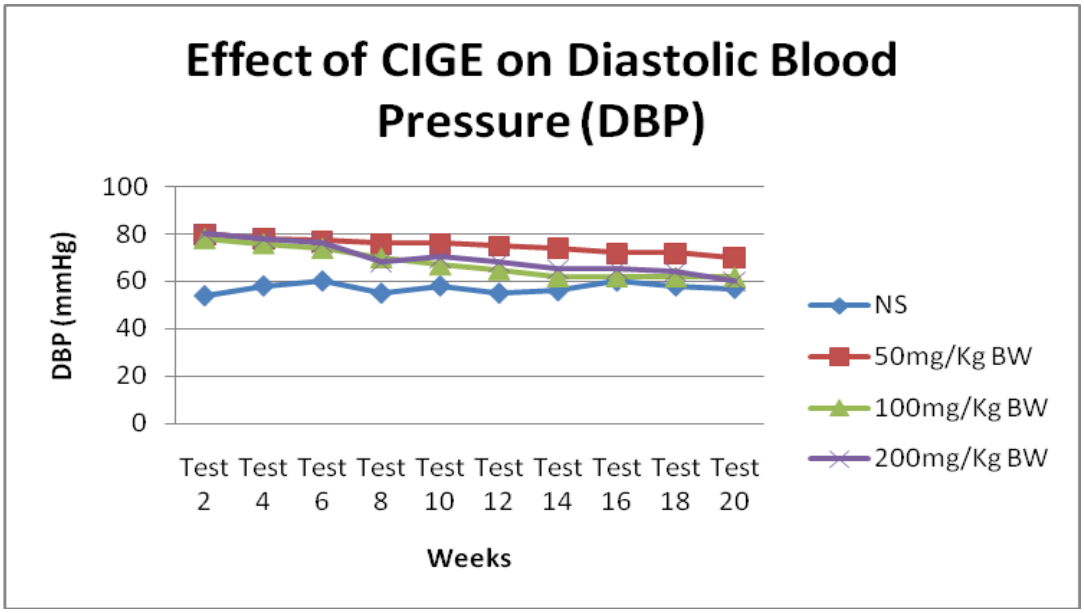


Figure 4.5.2c A Comparative linear graph of Mean Plot Effect of *Allium sativum* Extract Treatment on Diastolic Blood Pressure (DBP) for 20 weeks

Analysis of the multiple comparisons Table 4.3.8b revealed that after induction of hypertension, the following treatment groups and concentrations were able to lower or maintain the mean diastolic blood pressure within normal ranges and therefore they were found to be statistically significant compared with the control group that was treated with NS. Treatment groups C3b at HCT 100mg/kg body weight (ΔM=-11.00, $p<0.05$; = 19.32%); treatment group T2 at CGE 50mg/kg body weight (ΔM=-16.727, $p<0.05$; = 29.37%); CGE 100mg/kg body weight (ΔM=-11.000, $p<0.05$; = 19.32%) and treatment group T3 at CIGE 50mg/kg body weight (ΔM=-17.909, $p<0.05$; = 31.47) and at CIGE 200mg/kg body weight (ΔM=-11.636, $p<0.05$; = 20.45%). The highest mean diastolic blood pressure lowered was recorded in treatment T3 at a concentration of CIGE 50mg/kg body weight (ΔM=-17.909, $p<0.05$; = 31.47%).

4.6 Effects of *Allium sativum* Extract Treatment on the Histology of the Heart in Hypertension-Induced Laboratory Rats

Table 4.4.1 shows the experimental tissue results after treatment of the hearts with different *Allium sativum* extract treatments (T1 – T3) in various concentrations (50, 100 and 200mg/Kg body weight), compared to effects of known antihypertensive drugs (C2 – CPT and C3 - HCT) as positive controls groups with a normotensive control group pretreated with normal saline (NS) (C1) post hypertension period.

Figures 4.6 Heart Tissues mounted on slides for microscopic analysis from 11 selected experimental rats.

The myocardium was sectioned along different planes to establish the effect of prolonged hypertension on the heart, followed by establishment of the effect of post-hypertension treatment with the various concentrations of *Allium sativum* extracts. The analysis was recorded as seen in Table 4.4.1 and 4.4.2 below.

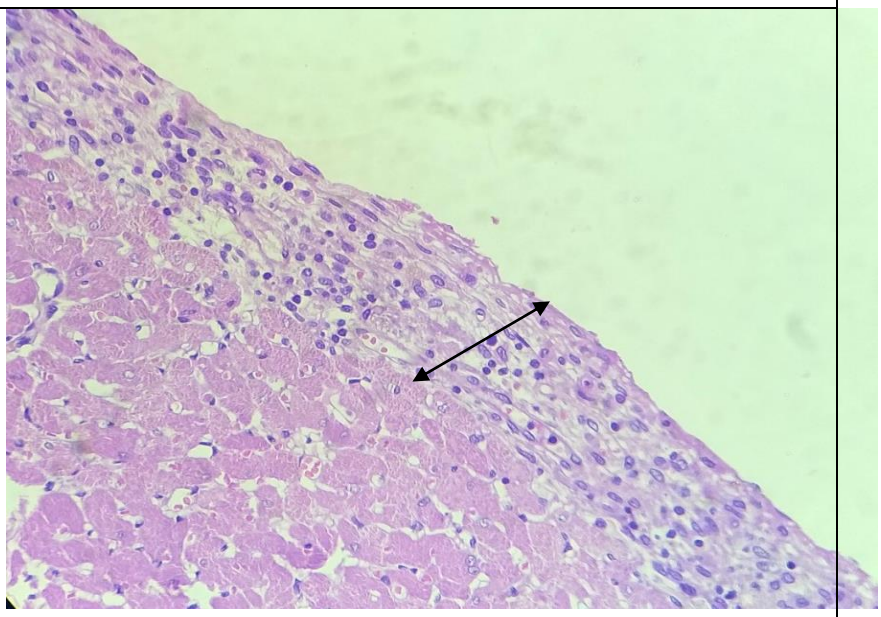
Table 4.6.1 Findings of the Effects of *Allium sativum* Extract Treatment on the Histology of the Heart in Hypertension-Induced Laboratory Rats

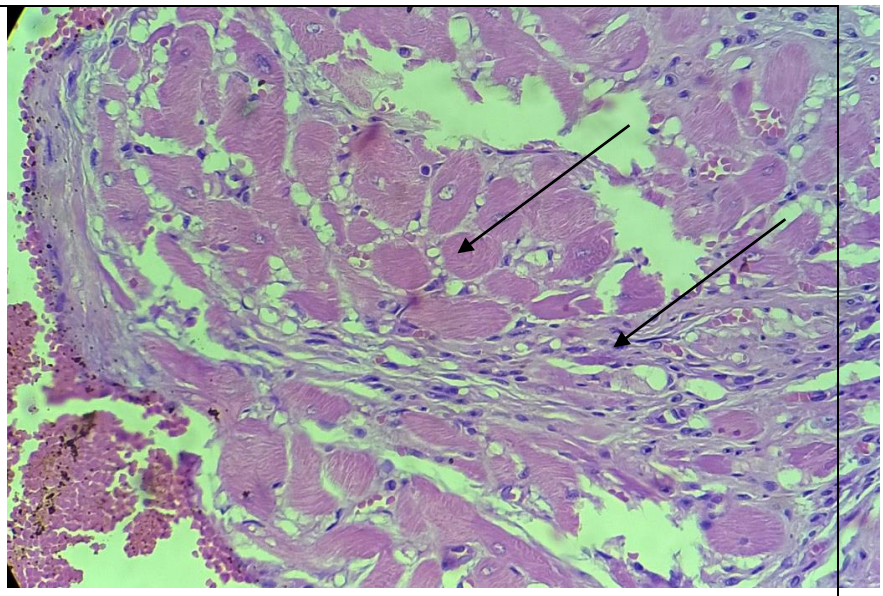
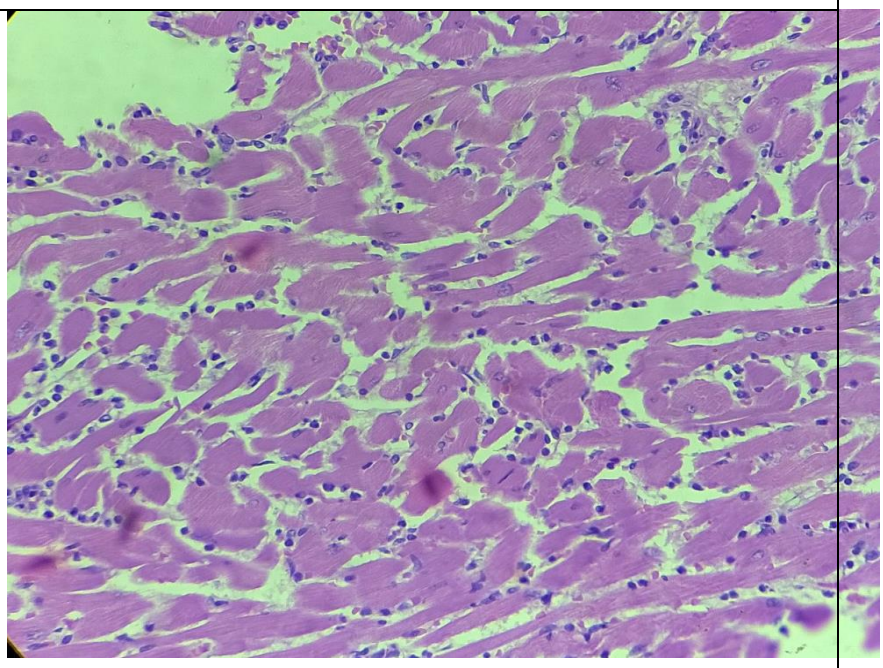
Rat	Case Number	Dimensions	Gross appearance	Left ventricular thickness	Microscopic appearance	Comment
Rat 1	T_1FAGE_{100}	11 x 11 x 9mm	Globular heart	3mm	The heart muscle and vessels appear normal. No degenerative or inflammatory changes were seen.	Normal heart
Rat 2	C_2CPT_{200}	9 x 10 x 8mm	Medium Heart	3mm	There were numerous intramuscular mast cells. Marked pericardial inflammation was noted associated with haemosiderin-laden macrophages	Inflammation
Rat 3	C1NS	8 x 10 x 8mm	Small Heart	2mm	The heart muscle and vessels appeared normal. No degenerative or inflammatory changes were seen.	Normal
Rat 4	C_3HCT_{200}	10 x 11 x 8mm	Medium Heart	3mm	Features of acute and chronic endocardial inflammation seen. There were infiltrations of the endocardium and papillary muscles by lymphocytes and neutrophils. Focal endomyocardial fibrosis was noted. Mast cells were noted.	Hypertrophy
Rat 5	T_1FAGE_{200}	11 x 12 x 9mm	Globular heart	3mm	The heart muscle and vessels appear normal. No degenerative or inflammatory changes were seen.	Normal
Rat 6	C_2CPT_{100}	10 x 11 x	Medium Heart	2mm	Mild perivascular inflammation	Hypertrophy

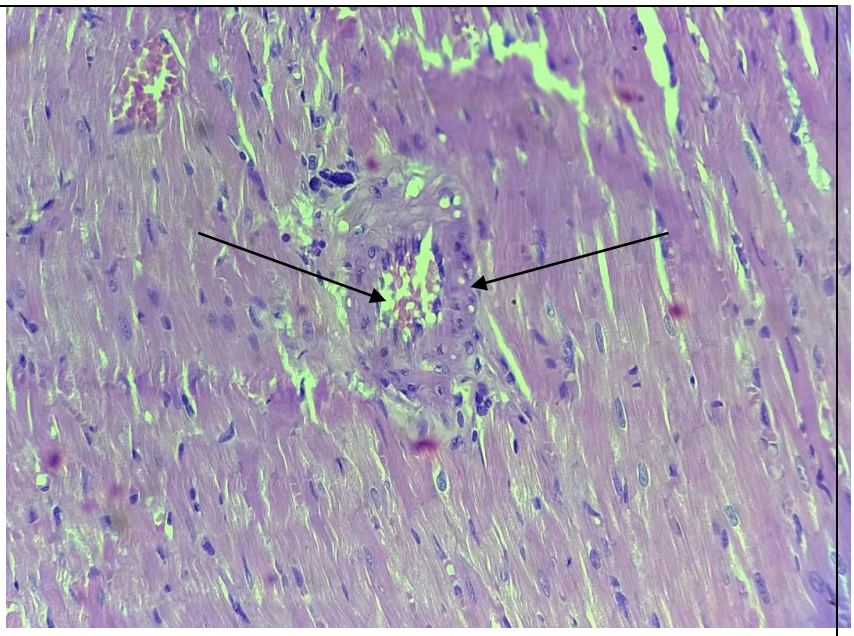
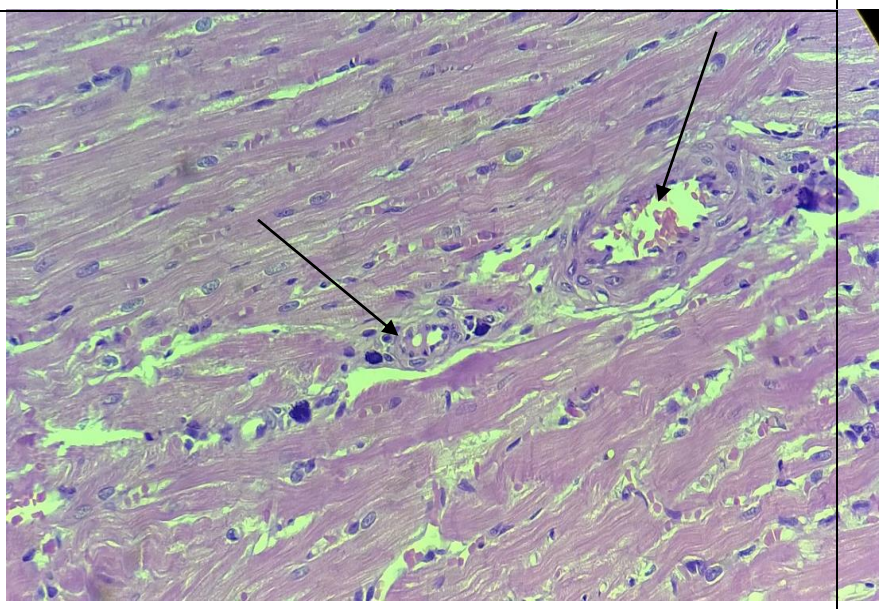
		8mm			noted	
Rat 7	C_3HCT_{50}	9 x 10 x 9mm	Medium Heart	3mm	Infiltration of the endocardium and papillary muscles by lymphocytes was noted.	Hypertrophy
Rat 8	T_3CIGE_{200}	10 x 11 x 8mm	Globular Heart	3mm	Features of acute and chronic endocardial inflammation seen. There were infiltration of the endocardium and papillary muscles by white cells. Focal endomyocardial fibrosis was noted. Mast cells were noted.	Major Hypertrophy
Rat 9	T_2CGE_{100}	8 x 10 x 8mm	Small Heart	2mm	The heart muscle and vessels appear normal. No degenerative or inflammatory changes were seen.	Normal
Rat 10	T_3CIGE_{100}	11 x 11 x 8mm	Globular Heart	3mm	Features of acute and chronic endocardial inflammation seen. There were infiltration of the endocardium and papillary muscles by white cells. Focal endomyocardial fibrosis is noted. Mast cells were noted.	Major Hypertrophy
Rat 11	T_2CGE_{200}	10 x 11 x 8mm	Globular Heart	3mm	Features of acute and chronic endocardial inflammation seen. There were infiltration of the endocardium and papillary muscles by white cells. Focal endomyocardial fibrosis was noted. Mast cells were	Major Hypertrophy

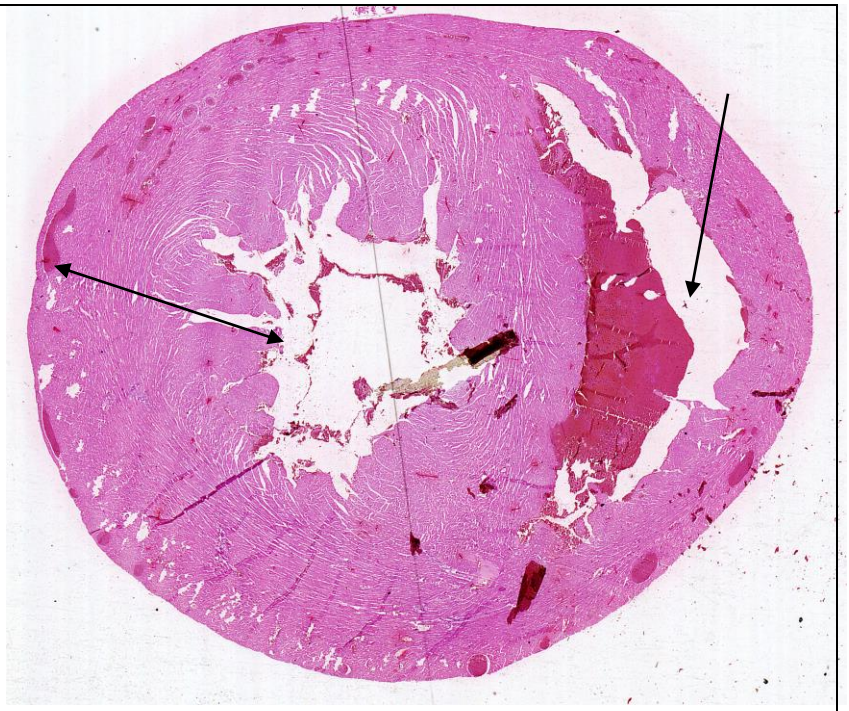
noted.

Table 4.6.2 Micrographs with descriptions showing Histological findings of the Heart following Prolonged Hypertension and treatment with *Allium sativum* Extracts

No 1	Finding	**Plate 1:** Photomicrograph of a section of the Aortic Root of the Rat heart administered with CPT_{200}. Captoten had no effect Post-Hypertension. H & E stained. X400
	Rat 2 Pericardial inflammation with haemosiderin-laden macrophages	
2.	**Rat 8**	**Plate 2:** Photomicrograph of a section of the Left Ventricle of the Rat heart administered with $CIGE_{200}$. *Allium sativum* extract had no effect Post-Hypertension. H & E stained. X400

	Acute and chronic endocardial inflammation Endomyocardial fibrosis Major Left Ventricular Hypertrophy	
3.	**Rat 4**	**Plate 3:** Photomicrograph of a section of the Left Atrial wall of the Rat heart administered with HCT_{200}. Hydrochlorothiazide had no effect on the myocardium Post-Hypertension. H & E stained. X400
	Endomyocarditis Infiltrations of the endocardium and papillary muscles by lymphocytes and neutrophils	
4.	**Rat 6**	**Plate 4:** Photomicrograph of a section of the Septal wall of the Rat heart administered with $FAGE_{200}$. Fresh Aqueous Garlic Extract had minimal effect on the myocardiocytes post-hypertension. H & E stained. X400

	Mild Perivascular inflammation	
5.	**Rat 10**	**Plate 5:** Photomicrograph of a section of the Left Atrial wall of the Rat heart administered with CGE_{100}. Crude Garlic Extract had minimal effect on the myocardiocytes Post-Hypertension. H & E stained. X400
	Focal endomyocardial fibrosis is noted. Mast cells were noted	
6	**Rat 3**	**Plate 6:** Photomicrograph of a section of the Left Ventricular wall of the Normotensive Rat heart administered with Normal Saline (NS). NS had no effect on the myocardiocytes Pos. H & E stained. X400

	The heart muscle and vessels appeared normal. No degenerative or inflammatory changes were noted.	

CHAPTER FIVE

DISCUSSION

5.1 Discussion

5.1.1 Plasma Hormones and Enzymes

The findings from this study showed a decrease in ADH levels when garlic extract was used for sixteen weeks. Garlic extract inhibits the secretion of ADH from the posterior pituitary gland. This could also be explained by a complementary feedback mechanism to the supraoptic nuclei in the hypothalamus to inhibit the synthesis of ADH as explained by Ried and Frank (2010). Consequently, this decreased water retention and blood osmolality, which is associated with a decline in blood volume, hence decreasing blood pressure. These findings were in agreement with a 2010 double-blind, parallel, randomized, placebo-controlled trial, involving 50 patients whose routine clinical records in general practice documented treated but uncontrolled hypertension (Ried and Frank, 2010).

The hypertensive group recorded a major significant increment in the mean plasma B-type ANP levels compared with the control group that was treated with normal saline. This was probably so due to excess stretch and over activity of the ventricular walls during hypertension. More-so, histological assessment conducted on the very myocardium confirmed myocardial injuries possibly caused by prolonged hypertension as observed in the histology micrographs. However, the hypertensive groups treated with garlic extract showed a reduction in mean plasma B-type ANP levels, since garlic tends to aid in wound healing and repair process of injured myocardial tissues and coronal arteries, hence improved contractility and reduction in blood pressure. Allicin, the active component of *A. sativum*, has been shown to activate fibroblast leading to a more

organized and rapid wound repair (Alhashim and Lombardo, 2018). A study carried out on anesthetized dogs, showed that gastric administration of encapsulated garlic powder induced dose-dependent natriuretic and diuretic responses which reached a maximum 30-40 minutes after garlic administration and decreased to basal levels of B-type ANP in plasma after 100-150 minutes. A simultaneous decrease in arterial blood pressure was observed which continued past the 250-minute mark (Pantoja *et al.,* 1996). ANP is a hormone usually released by the cardiac muscles from the atrial granules when the heart muscles are structurally damaged due to stretch, excess pressure, overload or other causes of damage. It produces natriuresis, diuresis and a modest decrease in blood pressure, while decreasing plasma renin and aldosterone (Pantoja *et al.,* 1996). Natriuretic peptides also alter synaptic transmission from the osmoreceptors (Rashid, 1985). Patients with arterial hypertension have left ventricular hypertrophy as the wall of the left ventricle participates in the secretion of ANP.

Initial induction of hypertension in the treatment groups T1, T2, T3, C2 and C3 for the first four weeks was directly proportional to increase in blood pressure and the mean plasma Ang-II levels. This rise was probably caused by increased secretion of Ang-II. Studies have reported that high angiotensin II concentrations suppress renin secretion via a negative feedback loop (Williams *et al.,* 2014). Angiotensin II acts on specific angiotensin AT_1 and AT_2 receptors causing smooth muscle contraction (i.e vasoconstriction) which increases blood pressure (Williams *et al.,* 2014). An increase in Ang-II also leads to increased release of aldosterone, which increase Sodium ion (Na^+) retention hence further increase in blood pressure. Mean plasma Ang-II levels were noted to decrease with increased frequency of *A. sativum* extract treatment during a 16 week-

period. These findings suggested that the long duration of garlic extract treatment on the hypertensive groups had a cumulative reduction effect on the synthesis and secretion of Ang-II. These findings are in agreement with the findings in a research conducted on garlic's effects on arterial blood pressure in normotensive and hypertensive animals (Mayorov, 2011), which showed a decrease in arterial blood pressure in hypertensive animals continuously treated with garlic extract. The results of this study are also in agreement with the results of Sharifi *et al.,* (2003), which showed that fresh garlic can inhibit Goldblatt hypertension. Angiotensin II is the main product of renin-angiotensin-aldosterone system (RAAS), which affects adrenal cortex, brain, cardiovascular system and kidneys (Ganong, 1999). The RAAS system is a long-term regulator of blood pressure (Gao *et al.,* 2014). Previous studies have shown that the levels of NO production in vascular endothelium affect the activity of Angiotensin converting enzyme (ACE) and receptors of angiotensin II (Bauersach and Widder, 2008; Higashi *et al.,* 2005). In *vitro* experiments have shown that isolated strips of canine carotid arteries and in isolated rat aorta, garlic exerted direct vasodilating effects (Korotkov, 1996; Maulik and Siddiqui, 1981). Garlic also activated the synthesis of nitric oxide, which is a potent endogenous vasodilator (Das *et al.,* 1995). Other animal findings showed that garlic extracts reduce blood pressure in a dose-response manner i.e the more the increase in garlic extract concentration, the more the reduction in blood pressure (Maulik and Siddiqui, 1981; Korotkov, 1996; Rashid and Khan, 1985 and Martin *et al.,* 1992). Angiotensin II has been cited as a factor in the pathogenesis of hypertension, myocardial infarction, sudden death and end-stage heart disease (Nickering *et al.,* 2006). Angiotensin receptor blocker losartan displace Ang-II from the angiotensin I receptor and lower the blood pressure by

antagonizing Ang-II-induced vasoconstriction, aldosterone release, adrenaline and nor-adrenalin release, ADH release, water intake and increased heart mass (Burnier, 2001).

No *A. sativum* effect was noted on mean plasma aldosterone (Ald) levels in treatment groups C2, C3 and T1 both in short term (4 weeks) and prolonged treatment (16 weeks). However, *A. sativum* extract significantly reduced mean plasma Ald levels in treatment group T2. The results correlate directly with the effects of *A. sativum* extracts treatment on plasma levels of Ang-II discussed earlier. The initial rise of Ang-II was directly proportional to the initial rise of Ald. Ang-II directly influences the synthesis and secretion of Ald. But after 16 weeks of *A. sativum* extract treatment to the hypertensive groups, the levels of aldosterone in plasma were significantly decreased proportionally to mean plasma Ang-II levels, though each one of them have their own individual effects which would not give similar values. A decrease in Ang-II also led to decreased release of aldosterone, which consequently decreased Sodium ion (Na^+) retention hence further lowering blood pressure in the hypertensive groups. These results were in agreement with the results reported by Aroor *et al.*, 2013, on the role of tissue renin-angiotensin-aldosterone system in the development of endothelial dysfunction and arterial stiffness.

There was a significant decrease in plasma renin activity in the hypertensive group compared to the non-treated hypertensive group at the end of the study; hence the decrease in plasma levels of aldosterone. These findings suggest that garlic extract either reduces the secretion of renin by the kidneys or reduces the activity of the RAAS system, much in agreement with the report by Shouk *et al.* (2014). In addition, the reduction of plasma levels of aldosterone is directly connected to mechanisms that inhibit or block Ang-II production. These observations were in agreement with other potential renin-

angiotensin-aldosterone system reports and the effects of garlic on hypertension. A study on cell culture and animal studies proposed that garlic acts on hypertension by blocking angiotensin-II production by inhibiting angiotensin-converting-enzyme (ACE) (Sharifi *et al.*, 2003). ACE is a component in the renin–angiotensin–aldosterone system, and inhibitors of ACE are used as standard BP-controlling pharmaceuticals (Shouk *et al.*, 2014). However, animal and cell culture experiments were mainly conducted with fresh garlic compounds, containing allicin (*S*-allyl-cysteine sulfoxide), which has a very low sustained bioavailability in human tissues (Lawson and Gardner, 2005). Angiotensin I is converted to an active octapeptide, angiotensin II by the angiotensin-converting enzyme (ACE) (Gao *et al.,* 2014). Though the renin–angiotensin system is widespread in the body, the main source of renin is the juxtaglomerular apparatus of the kidney (Williams *et al.*, 2014). This apparatus senses the renal perfusion pressure and the potassium concentration in the distal tubular fluid. In addition, renin release is stimulated by β- and decreased by α-adrenoceptor stimulation (Izzo and Weir, 2011). As much as a high angiotensin II concentration suppresses renin secretion via a negative feedback loop, *A. sativum* reduced plasma renin by inhibiting stimulatory activity in the juxtaglomerular apparatus, so did it reduce plasma Ang-II, which directly reduced plasma aldosterone hence lowering blood pressure, since lowered vasoconstriction followed by reduced Na^+ ion retention lowers blood pressure. Plasma renin activity also affects the activity of constitutive NOS (cNOS), which reduces secondary renin angiotensin system activity after cNOS reinforcement (Aroor *et al.,* 2013). The renin–angiotensin–aldosterone system plays an important role in the control of blood pressure including the sodium (Na^+) ion balance (Harrison-Bernard, 2009).

5.1.2 Plasma Electrolytes

Allium sativum extract treatment was found to trigger a major significant reduction in the mean levels of plasma Sodium (Na^+), chloride (Cl^-) and calcium (Ca^{2+}) ions at all extract concentration levels between and among the hypertensive treatment groups (n = 4) in comparison to the normotensive control group (C1) that was treated with normal saline (NS) ($p<0.05$). However, results showed that *A. sativum* concentrations at all treatment levels reduced plasma levels of Na^+ ion with increased treatments. This is in agreement with the previous results gathered from the results on plasma ang-II, aldosterone and renin levels. After inducing high blood pressure, it was established from this study that *A. sativum* reduced plasma renin and plasma Ang-II, which directly reduced plasma aldosterone, reduction in plasma Na^+ ion retention hence further lowering blood pressure. Sodium and water retention are associated with an increase in blood pressure. It is postulated that sodium, via the sodium–calcium exchange mechanism, causes an increase in intracellular calcium in vascular smooth muscle resulting in increased vascular tone (McMahon and Vargas, 1993). The renin–angiotensin–aldosterone system plays an important role in the control of blood pressure including the sodium balance. The comparative linear function for mean plasma levels of Cl^- ions against subsequent testing for a period of 16 weeks showed a significant reduction in mean plasma Cl^- ions with increased testing across all concentration levels towards that of the normotensive group ($p<0.05$). This result is in agreement with the results by Schales and Schales (1941), on studies on simple and accurate methods for the determination of chloride ions in biological fluids. Alnaqeeb *et al* (1996), observed a declining effect of chloride ions in plasma when rats were treated with garlic extracts as they analyzed the histopathological

effects of garlic on liver and lung of rats. The mean plasma ionic calcium (iCa^{2+}) levels decreased with subsequent tests upon treatment of the hypertensive animals with *A. sativum* extracts. It is postulated that sodium, via the sodium–calcium exchange mechanism, causes an increase in intracellular calcium in vascular smooth muscle resulting in increased vascular tone (Martin *et al.,* 1992). Based on this argument, *A. sativum* extract could have utilized the same Sodium-Calcium exchange mechanism to inhibit release of intracellular calcium in vascular smooth muscles resulting in reduction of plasma ionic calcium. Martin *et al.,* (1992) results are in agreement with a study on effects of garlic extract on smooth and cardiac muscles of rabbits and guinea pig tested in *vitro* using isolated segments of aorta, trachea and small intestines and isolated rabbit hearts by (Zanchetti *et al.,* 1985). It was reported that garlic extract inhibited the contractions of rabbit and guinea pig aortic rings induced by norepinephrine in Ca^{2+}-free and Ca^{2+}-containing Krebs-Henseleit solutions. Garlic extract inhibited the contractions of rabbit and guinea pig tracheal smooth muscles induced by acetylcholine and histamine, respectively, in both Ca^{2+}-free and Ca^{2+}-containing Krebs-Henseleit solutions.

Furthermore, garlic extract inhibited the spontaneous movements of rabbit jejunum and guinea pig ileum and inhibited the force of contraction of isolated rabbit hearts in a increasing concentration-dependent manner. All inhibitions were reversible (Martin *et al.,* 1993). Martin *et al.,* (1997) suggested that, the negative ionotropic effect of garlic dialysate was related to calcium ion availability. They suggested that there is a possibility that restriction of intracellular calcium contributes to this negative ionotropic effect. The hypotensive action of garlic extract is due to a direct relaxant effect on smooth muscles. This indicated that as *A. sativum* extract reduced plasma level of renin,

Ang-II, aldosterone, ADH and Na^+ ions, but increased K^+ ions reabsorption in the kidneys, since the actions of the RAAS system on plasma Na^+ ions are inversely proportional to its actions on plasma K^+ ions. For 16 weeks, the study revealed a statistically significant reduction ($p<0.05$) ($n =4$), and significantly lowered plasma K^+ ion levels in hypertension-induced laboratory rats (63.63% of all treatment groups). These results are in agreement with the study by Oluwole (2001). He found out that the serum levels of sodium decreased and potassium increased progressively, significantly with both doses and long-term (30days) of garlic treatment when compared with the control ($p<0.05$). This finding is suggestive of mild hyperkalaemic and hyponatriemic effects of hypertension by allicin components of garlic, which may partly be explained by its secondary effect on possible increase in renal blood flow which enhances renal excretion and reabsorption of basic electrolytes such as sodium and potassium respectively for maintenance of electrolyte (McMahon and Vargas (1993). McMahon and Vargas study in 1993 demonstrated that *A. sativum* extract administration can provide an effective protection against myocardial damage in heart tissue induced by prolonged hypertension in rats, since *A. sativum* extract was able to ameliorate plasma iCa^{2+}, Na^+, K^+, Cl^- ions levels in Wistar rats.

5.1.3 Plasma Metabolites

The results showed that *A. sativum* extract had no effect on aspartate aminotransferase (pAST) and alanine aminotransferase (pALT). AST/ALT measurements are used in the diagnosis and treatment of certain types of liver and heart disease (Rahman and Lowe, 2006). Elevated levels of the transaminases can signal myocardial infarction, hepatic

disease, muscular dystrophy, or organ damage (Asdoq and Inamdar, 2010; Anoush *et al.*, 2009). Since the results in this study showed that *A. sativum* had no effect on pAST and pALT, it can be inferred that the level of transaminase enzyme in plasma was not significantly affected by hypertension. These results were in agreement with the results of Reitman and Frankel (1957) on their studies on colorimetric determination of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) activity in hypertensive rats. In another study conducted to assess the protective effect of garlic extract against isoprenaline-induced myocardial infarction in rats, they found out that garlic extract significantly reduced isoprenaline activity by inhibiting the damaging effects of isoprenaline on myocardial tissue, hence reducing serum levels of AST compared to the control (Vibha *et al.*, 2011). Isoprenaline activity may not necessarily be as a result of a hypertensive heart. They concluded that garlic extract possesses potential to ameliorate the myocardial damage induced by isoprenaline in rats. The myocardial damages done by isoprenaline are similar to those caused by prolonged high blood pressure (Farvin *et al.*, 2010; Pinelli *et al.*, 2004).

After the induction of hypertension, all the hypertensive treatment groups initially showed significant increment in plasma levels of cardiac troponin-I (cTi) above reference values of 0.10 – 0.14ng/ml at $p<0.05$ (Mockel, M *et al*.; 2004). But following a 20 week *A. sativum* extract treatment to the hypertensive groups, statistically significant decrements in plasma cTi were recorded with subsequent tests. On the other hand, *A. sativum* treatment had no effect on cardiac troponin–T (cTt). The state remained the same even after subsequent test treatments. These findings probably suggest that as much as there were myocardial tissue injuries due to prolonged hypertension, the damage did not

cause myocardial infarction. Plasma cardiac biomarker troponin-T (cTt) is used to test levels of myocardial infarction. These results are in agreement with those recorded by Priscilla and Prince (2009), when they evaluated the protective effect of garlic acid on cardiac marker enzymes, Troponin-T, LDH isoenzymes pattern, lipid peroxidation products and antioxidant status in isoproterenol-induced myocardial infarction in male Wistar rats. ISO-induced myocardial damage was indicated by increased activities of marker enzymes such as creatine kinase, creatine kinase-MB, aspartate transaminase, alanine transaminase and lactate dehydrogenase in serum and no effect on levels of Troponin-T in the serum. Garlic extract pre-treatment showed significant protective effect on all the biochemical parameters studied. Histopathological findings of garlic acid pre-treated myocardial infarcted heart confirmed the biochemical findings of that study. Thus, they concluded that garlic acid protects the myocardium against isoproterenol-induced oxidative stress.

A. sativum extract significantly decreased the mean plasma total cholesterol in the hypertensive group. These findings would probably suggest that active constituents of sulphides in *A. sativum* extract oxidatively increased the formation of low-density lipoproteins (LDLs) from the digested fats which do not over-load the heart. LDLs are easily transported within plasma in circulating blood to the rest of the body cells for metabolism and utilization, and hence do not stick and thicken the walls of the myocardium. Several studies have shown that garlic contains active hypocholesterolemic components, known as diallyl disulfide and dipropyl disulfide (Bordia and Bansel, 1973; Jain and Vyas, 1975; Bordia *et al*., 1975; Jain, 1977). It has also been reported that garlic supplements in human subjects lead to the increased resistance of low density lipoprotein

to oxidation and may be one of the powerful mechanisms accounting for the antioxidative and anti-atherosclerotic properties of garlic (Munday *et al*., 1999; Borek, 2001; Lau, 2001).

Studies on the effects of short-term supplementation with oily garlic formulation on lipid metabolism (Augusti, 1977; Sodimu *et al*., 1984) and glucose level (Banerjee *et al*., 2002) reported hypolipidemic effects. A study on antioxidant status in 70 patients suffering from primary arterial hypertension, analyzed garlic preparation was found to significantly lower lipid level and the level of lipid peroxidation products in the blood (Ou *et al*., 2003; Thomson *et al*., 2006). The results of those studies reported that the garlic preparation may tentatively be used as an adjunct agent in treatment of arterial hypertension because of its hypolipidemic and antioxidant properties (Grazyna *et al*., 2008). Some reports have indicated that garlic preparations can correct lipid abnormalities and lower blood pressure in patients with hyperlipidemia and arterial hypertension (Durka, *et al.*, 2004; Rahman and Billington, 2000).

Creatine Kinase (CK) (molecular weight 86 kilodaltons (KD)) is an enzyme responsible for the conversion of creatine into phosphocreatine, the energy source for muscle contraction. Since CK is found in all muscle tissue, elevations of the total activity of this enzyme are not specific for cardiac damage. In heart muscle CKMM is the predominant form, but CK-MB makes up about 20% of the CK activity, whereas in skeletal muscle CK-MB is generally about 1% of the CK activity. The third form of CK, which is not used as a cardiac marker, is CKBB (Remiao et al., 2001). With respect to subsequent treatment of the hypertensive groups with *A. sativum* extract, no clear pattern yielded discerned trends of plasma CK-MB values. The results in this study were in agreement

with the results of Omnia *et al.,* (2014) on the effect of Isoproterenol injection on serum cardiac biomarkers CK-MB, Lactate dehydrogenase in control group. The result indicated the effect of garlic oil in *A. sativum* extract as responsible for prevention against cardiac tissue oxidative damage. Results from other studies by Sabeena *et al.,* (2004) and Gurgun *et al.,* (2008), reported that *A. sativum* extract reduced levels of CK-MB released into the blood stream and served as a marker of the myocardial membrane damage. Extent of cardioprotection offered by the drug is associated with significant attenuation of plasma creatine kinase (Gao *et al.,* 2000) and LDH levels (Hung *et al.,* 2001). These results are also in agreement with Senthilkumar *et al* (2010). They recorded a significant decrease in serum levels of CK, CK-MB, LDH and AST in treatment groups were indicative of the fact that *A. sativum* extract had a significant cardioprotective effect and maintains myocardial membrane integrity, reducing the myocardial damage restricting the increase in these enzymes levels (Senthilkumar *et al.,* 2010).

5.1.4 Electrical and Mechanical Activity of the Heart

A. sativum extract decreased RR interval at all concentrations compared to the normotensive group at which point the RR interval of hypertension-induced laboratory rats were shown to increase after induction of hypertenson. This indicated that *A. sativum* extracts had effects similar to those of CPT and HCT at lower concentrations and hence able to lower blood pressure by utilizing mechanisms that reduce the mean RR interval (160 – 150m/s, = 6.67% reduction at $p<0.05$). The heart rates (HR) in various treatment groups were initially increased as a more efficient compensatory blood perfusion mechanism to systemic tissues and aids venous return, hence reducing blood pressure.

These results were in agreement with Nwokocha *et al.*, (2011) in their study on antihypertensive properties of *A. sativum* on normotensive and two kidney one clip hypertensive rats (Hypertension was induced to the hypertensive rats/group by surgically and temporarily clipping the both kidneys together). They noted that aqueous garlic extract caused a dose dependent reduction in the systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) of the animals. After the cumulative effect of *A. sativum* extract, the heart rate was eventually significantly reduced ($p<0.05$) from 406 ± 6 to 250 ± 5 beats/min (61.57% reduction), but pulse pressure increased from 30 ± 3 to 40 ± 5. The reduction was not severe to cause hypotension, but reduced toward normal blood pressure (normal range for wistar rat - SBP: 84-134 mm/Hg and DBP: 55-60mmHg), compensated by the renal mechanisms of blood pressure regulation. Various mechanisms for antihypertensive effect of garlic have been reported to include vasorelaxation through H_2S production (Benavides *et al.*, 2007), inhibition of angiotensin-converting enzyme *in vitro* (Rietz *et al*., 1995), endothelium-derived relaxing factors (Sendl *et al*., 1992; Fallon *et al*., 1998), and beta-adrenoceptor blocking action (Martín *et al*., 1992). Part of the inhibitory actions of garlic on the blood pressure could be explained by myocardial mechanisms as blood pressure is known to be influenced by changes in cardiac contractility when total peripheral vascular resistance remains fairly unchanged (Nwokocha *et al.*, 2011). It is likely that the negative chronotropic effect of aqueous garlic extract is due to other mechanisms such as direct effect on the heart and blood vessels. Janssen *et al.*, (2000) had reported that MAP and heart rates were influenced by cholinergic function, although the blood vessels contain muscarinic receptors.

M_2 receptors in the heart mediate reduced heart rate but more profoundly affected by adrenergic beta-1 stimulation. It was noted that a significant reduction in systolic blood pressure, diastolic blood pressure and heart rate values in both the positive controls (groups C2 and C3) and hypertensive groups. In a study by Martin *et al.,* (1997), they reported that *A. sativum* extract reduced heart rate in a dose-dependent manner, in conjunction ventricular rhythm. They explained the findings by a depressant effect on automaticity and tension development in the heart, suggesting a beta-adrenoceptor blocking action produced by the garlic dialysate. Force of contraction of the heart also decreased with higher dose of the garlic extract. The results suggested that garlic extract had beneficial effect on heart modulating the rate, rhythm and force of contraction positively but very high doses may cause tetany, tachycardia and arrhythmias (Raj and Nar, 2004). This indicated that *A. sativum* extracts had no or very limited effects on the PR intervals in hypertensive induced rats. Only FAGE given at 100mg/Kg body weight and CGE given at 100mg/Kg body weight had effects on the hypertensive groups, a similar action to that of the positive control group C2 given CPT at 200mg/Kg body weight.

Data from the present study show that *A. sativum* extract triggered a significant decrease in mean QT intervals (70 – 40m/s; = 57.14% reduction; $p<0.05$) towards normal (30 – 50m/s). Earlier reports of ECG recordings in normal mice of different strains demonstrated cycle lengths and intervals similar to those of this study, but with the exception of the QT interval. The electrophysiological data obtained from C57BL/6J mice also were comparable to those of previous studies evaluating mouse cardiac conduction; although the basal heart rates and timing intervals were somewhat slower than reports in studies by Dalkara *et al.,* (1995); Hartley *et al.,* (1995); Meijler, (1985)

and Wang *et al.,* (1995), where they observed a reduced cardiac conduction in rats. The onion family like *A. cepa*, *A. sativum*, shallots and leeks as reviewed, have been found to normalize the electrocardiographic changes occurring after isoprenaline intoxication (Kramer *et al.,* 1993), since they contain similar effective active sulphides. Several researchers have reported that ST segment elevation, prolongation of QT interval, reduction of P-wave and R-R interval have returned to conventional values after treatment with *A. sativum* extracts (Hartley *et al.,* 1995; Meijler, 1985 and Wang *et al.,* 1995). The findings of this study show that, there is a possibility that *A. sativum* extracts reduced blood pressure by mechanisms similar to those utilized by pharmacological antihypertensive drugs Captoten (CPT) and Hydrochlorothiazide (HCT) at lower concentrations through mechanisms that independently regulate the different phases of the cardiac cycle. *A. sativum* extracts did not have significant effects on the other electrophysiological ECG intervals. These results are in agreement with Asdaq and Inamdar's results in 2011, on their study on the potential benefits of a garlic and hydrochlorothiazide combination as antihypertensive and cardioprotective in rats.

Initially, *A. sativum* extract had minimal effect on the mean systolic blood pressure in hypertension-induced laboratory rats when high blood pressure was induced during the 4 weeks period. This was so probably because the rats had not yet started responding to the *A. sativum* extracts at lower threshold. The physiological basis for this observation would be inadequate stimulation of receptors and respective signal transduction pathways which *A. sativum* extract active chemical molecules would use to elicit cellular response (Auer *et al*., 1990). However, there was significant reduction in mean systolic blood pressure during subsequent treatments. This was suggestive of the cumulative effect of *A. sativum*

extract active chemical molecules following treatment for a longer period (20 weeks). These findings were in agreement with a trial investigation on 47 hypertensive patients, who showed garlic significantly decreased the mean systolic blood pressure by 12 mmHg and the mean supine diastolic blood pressure by 9 mmHg versus placebo given to the control group having no effect ($p<0.05$) (Auer *et al*., 1990). In a study by Kandziora (1988), 200mg of garlic powder given three times daily, in addition to hydrochlorothiazide-triamterene (HCT) baseline therapy produced a mean reduction of systolic blood pressure by 10-11 mmHg and of diastolic blood pressure by 68 mmHg versus the control group ($p<0.05$) having no effect. A study on men with hypercholesterolemia indicated that garlic extract decreased systolic blood pressure compared to the control group ($p<0.05$) (Steiner *et al.,* 1996). *A. sativum* active ingredients are documented for its beneficial effects on major cardiovascular risk factors, including blood pressure. A study by Amitai *et al.,* (2013) on effects of purified allicin on the cardiovascular system showed that allicin had no effect on body weight whereas it reduced SBP significantly from 190 ± 7.5 mmHg to 168 ± 5.7 ($P < 0.0001$) and triglyceride levels from 96 ± 25 mg/dl to 71 ± 19 ($P =0.009$). They concluded that allicin lowered blood pressure by converting amounts of triglycerides into LDLs in hypertensive rats. This effect was not mediated through weight loss.

Although after the induction of high blood pressure, some treatment groups (30%) did not respond immediately to the *A. sativum* extract (or as used short-term of 4 weeks) and therefore maintained constancy in mean DBP within normal ranges for wistar rat (55-66mmHg). However, the other hypertensive group (60%), when treated with *A. sativum* extracts for a period of 16 weeks, significantly decreased mean diastolic blood pressure

by 10.7% toward the normotensive control group with 56.91mmHg ($p<0.05$) treated with NS to 56mmHg = 24mmHg = 42.86%) during the 20 week treatement period comapred with that of the control group (54mmHg) at $p<0.05$, which did not lead to hypotension. In a research carried out on a group of patients with high diastolic blood pressure, garlic powder consumption for 12 weeks decreased diastolic blood pressure compared to control (Auer *et al.,* 1990). Banerjee and Maulik (2002) reported results of studies where oily garlic preparations produced hypotensive effect in hypertensive individuals. Oily garlic has higher concentrations of the active sulphides molecules which are perceived to easily cause hypotension if incorrectly used in garlic extract studies as reported by Durak *et al.,* (2004) and Dhawan and Jain, (2004). In addition, a meta-analysis study by Hai-Peng *et al.,* (2015) on the effect of garlic (Garlic supplements) on DBP also showed a significant difference between garlic and control groups, with the garlic group more effective in reducing DBP than placebo (given to the control group ($p<0.01$) by 3.39 mm Hg (95% CI, 4.14 to 2.65; $P<.001$), especially in hypertensive patients.

5.1.5 Histology of the Heart

The normotensive heart (C1NS) showed normal myocardium with normal coronary vessels, no degenerative or inflammatory changes (Plate 6). The rats in the normotensive group were treated with normal saline and the myocardium had normal atrioventricular and semilunar valves, normal myocardial septum, a thicker left ventricle than the right ventricle and normal pericardial layers. The positive control group (C2) treated with an antihypertensive drug CPT 200mg/Kg body weight had numerous intramuscular mast cells, marked pericardial inflammations associated with haemosiderin-laden macrophages

as was observed in Plate 1. The observed lesions are positively due to the overload and hypercontractilty effects of hypertension. Positive control group C3 were hypertensive wistar rats treated with an antihypertensive drug HCT 200mg/Kg body weight had features of chronic endocardial inflammation, infiltrations of the endocardium and papillary muscles by lymphocytes and neutrophils with focal endomyocardial fibrosis and presence of mast cells as shown by Plate 3. This was an indication of left ventricular hypertrophy due to overload and excess demand stretched by prolonged hypertension in order to compensate for the cardiac output required to perfuse the systemic circulation.

There were no signs of tissue repair in the groups treated with the antihypertensives CPT and HCT after prolonged period of hypertension. However, the hypertensive group T1 which initially had myocardial damage and then treated with FAGE 200mg/Kg body weight for 20 weeks, was observed to be normal with no degenerative or inflammatory changes as shown in Plate 4. The inference from this group showed that *A. sativum* extract contain properties that enhanced tissue repair and ability to reinstate of normal myocardial functionality following a period of damage due to hypertension and hence was able to mediate lowering effects of most cardiovascular parameters that were analyzed in this study, compared to the hypertensive groups that were treated with CGE and CIGE despite slight decrease in SBP and DBP observed.

Fresh *A. sativum* extract (FAGE) was found to play a key role in wound healing reversing functionally damaged myocardium to regain effective myocardial contractility post-hypertension. Allicin, the active component of *A. sativum,* has been shown to have antimicrobial and anti-inflammatory properties and has been used historically by many cultures to heal wounds. Animal studies have shown *A. sativum* extract increase wound

healing process and decrease the rate of infection. Alhashim and Lombardo (2018) in their study on mechanism of action on topical garlic on wound healing showed that fibroblasts are activated by allicin, leading to more organized and rapid wound repair. There were more proliferating fibroblasts in previously hypertensive myocardial tissues treated with *A. sativum* extract than in other micrographs. The lesions and tissue inflammations initially noted were no more.

FAGE reversal ability to myocardial damage and restoration of myocardial functionality could possibly be utilizing mechanisms used by HCT more than CPT to mediate its actions on cardiac and renal regulation of blood pressure, especially in the reduction of systolic and diastolic blood pressure. Thiazide diuretics: e.g Hydrochlorothiazide (HCT - microzide) and chlorthalidone sometimes called water pills are medications that act on the kidneys to help the body eliminate sodium and water, reducing blood volume. Thiazide diuretics are often the first, but not the only, choice in high blood pressure medications. Diuretics or calcium channel blockers may work better for older people than do angiotensin-converting enzyme (ACE) inhibitors alone.

The heart tissues from wistar rats in group T2, hypertensive and treated with CGE 100mg/Kg body weight for 20 weeks were normal, histologically similar to the ones treated with FAGE (Plate 5). From this group, CGE was noted to have minor reversal effects on the myocardial damage, hence slight blood pressure lowering effect. CGE could possibly utilize mechanisms used by CPT more than HCT to mediate its actions on cardiac and renal regulation of blood pressure, especially in the reduction of systolic and diastolic blood pressure. Angiotensin-converting enzyme (ACE) inhibitors, are medications — such as captopril (capoten - CPT), lisinopril (Zestril) and benazepril

(Lotensin) — help relax blood vessels by blocking the formation of a natural chemical that narrows blood vessels. People with chronic kidney disease may benefit from having an ACE inhibitor as one of their medications.

The heart tissues from wistar rats in group T3, treated with CIGE 200mg/Kg body weight for 20 weeks had features of acute endocardial inflammation, infiltration of the endocardium and papillary muscles by white cells. Focal endomyocardial fibrosis and mast cells were noted. The heart exhibited major cardiac left ventricular hypertrophy as shown in Plate 2. Despite slight reduction in SBP and DBP observed in groups treated with CIGE, conclusive inability to reversal myocardial damage was noted.

These findings signify that *A. sativum* extract (garlic), use modulatory mechanisms that majorly target cardiac and renal function to ameliorate the damaging effects and regulation of high blood pressure.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the findings on the effect of *A. sativum* extract treatment on cardiac and renal regulation of blood pressure were as follows:

1. As far as hormone analysis is concerned:

A) *A. sativum* extract reduced plasma levels of antidiuretic hormone (ADH).

B) *A. sativum* increased mean plasma B-type atrial natriuretic peptide (B-type ANP).

C) Mean plasma Ang-II levels were noted to decrease with increased frequency of *A. sativum* extract treatment during a 16 week-period. These findings suggested that the long duration of garlic extract treatment on the hypertensive groups had a cumulative reduction effect on the synthesis and secretion of Ang-II.

D) *A. sativum* effect on Aldosterone though not significant both short-term and long-term, a decrease in Ang-II above also leads to decreased release of aldosterone, which decreased Sodium ion (Na^+) retention hence further decrease in blood pressure.

E) *A. sativum* significantly reduced plasma renin activity. A decrease in Ang-II above is a negative loop stimulus for reduced plasma renin concentration.

2. As far as electrolyte and enzyme analysis is concerned:

A) *A. sativum* did not have any significant effect on electrolytes. However, the findings showed slight decrease in plasma sodium (Na^+), chloride ions (Cl^-) and

ionic calcium (iCa^{2+}) following cumulative treatment with garlic extract, but with significant increase in retention of potassium ions (K^{+}) in plasma.

B) *A. sativum* had no effect on levels of aspartate aminotransferase (pAST) and alanine aminotransferase (pALT) in plasma. Plasma cardiac troponin-I (cTi) level was significantly decreased cumulatively over time, while there was no effect on cardiac troponin-T (cTt) level in plasma. *A. sativum* extract significantly decreased total cholesterol levels in plasma. However, no clear pattern yielded discerned trends of creatine phosphokinase isoenzyme-MB (CK-MB) levels in plasma.

3. With reference to selected cardiovascular parameters to analyze electrical and mechanical activity of the heart, *A. sativum* significantly decreased the systolic blood pressure, diastolic blood pressure, heart rate, the RR-interval and the QT-interval in the hypertensive wistar rats. However, *A. sativum* extract had no significant decrements on the P-duration, the QRS complex and the PR-intervals as recorded and analyzed by the real-time electrocardiogram (ECG).

4. Fresh *A. sativum* extract (FAGE) was found to play a key role in wound healing reversing functionally damaged myocardium to regain effective myocardial contractility post-hypertension as shown by the histological micrographs. Allicin, the active component of *A. sativum*, has been shown to activate fibroblast leading to a more organized and rapid wound repair. There were more proliferating fibroblasts in previously hypertensive myocardial tissues treated with *A. sativum* extract than in other micrographs.

6.2 Recommendations

Further studies should be carried out to identify the specific plasma membrane proteins/receptors utilized by *A. sativum* extract to effect the observed changes.

A. sativum extract should be chemically characterized to identify the specific chemical compounds and their effect on Ca^{2+} transporter mechanisms within the myocardial tissues.

It will also be significant to determine whether *A. sativum* extracts stimulates the recovery and repair of damaged kidney cells.

A study should be carried out to establish the cellular mechanisms influenced by *A. sativum* extract to effect hypotensive changes.

REFERENCES

Ademiluyi A.O, Oboh G, Owoloye T.R and Agbebi O.J. (2013). Modulatory effects of dietary inclusion of garlic (*Allium sativum*) on gentamycin–induced hepatotoxicity and oxidative stress in rats. *Asian Pac J Trop Biomed*, **3**: 470-475.

Alhashim, M. and Lombardo, J. (2018). Mechanism of Action of Topical Garlic on Wound Healing. *Dermatol. Surg*. **44(5):** 630-634.

Al-Qattan, K.K., Alnaqeeb, M.A. and Ali, M. (2006). The antihypertensive effect of garlic (*Allium sativum L.*) in the rat two-kidney-one-clip Goldblatt model. *J. Ethnopharmacol*. **66:** 217– 222.

Al-Qattan, K. K, Khan, I, Alnaqeeb, M.A and Ali, M. (2003). *US National Library of Medicine National Institutes of Health*. **69(4):**217-22.

Al-Qattan K. K, Khan I, Alnaqeeb MA, Ali M. (2001). Thromboxane-B2, prostaglandin-E2 and hypertension in the rat 2-kidney 1-clip model: a possible mechanism of the garlic induced hypotension. *Prostaglandins Leukot Essent Fatty Acids.* **64**(1):5–10

Agarwal, K.C. (1996). Therapeutic action of garlic constituents. *Medical Research Review.* **16:** 111-124.

Alnaqeeb, M.A, Thomson, M and Bordia T. (1996). Histopathological effects of garlic on liver and lung of rats. *Toxicol. Lett*. **85:** 157-164.

Alnumair, K.S, Chandramohan, G and Alsaif, M. A. (2012). Preatment with morina flavonoid, ameliorates adenosine triphosphates and glycoproteins in isoproterenol induced myocardial infarction in rats. *J. Nat. Med.* **1 (95):** 95-101.

Alwan A. (1993). Cardiovascular disease in Eastern Mediteranean region. *WHO Statisties quarteryly.* **46**:237–40.

Amitai, E, Edna, P, Ehud, G, Zehava, S and Yehonatan, S. (2013). Effects of Allicin in cardiovascular risk factors in Spontaneously hypertensive rats. *AMAJ*. **39:** 102-107.

Anouch, M, Eghbal, M.A, Fathiazad, F, Hamzery, H and Kouzehkonani, N.S. (2009). The protective effects of garlic extracts against acetaminophen – induced oxidative stress and glutathione depletion. Park .*J. Biol .Sci.* **12(10):**765-771.

Aroor, A.R, Demarco, V.G, Jia G, Sun Z, Nistala R, Meininger GA, et al. (2013). The role of tissue Renin-Angiotensin-aldosterone system in the development of endothelial dysfunction and arterial stiffness. . *Front Endocrinol.* **4**

Asdaq, S.M and Inamdar, M.N. (2011). The potential benefits of a garlic and hydrochlorothiazide combination as antihypertensive and cardioprotective in rats. *J. Nat. Med.* **65**: 81-88.

Asdaq, S.M and Inamdar, M.N. (2010). Pharmacodynamic interaction of captopril with garlic in isoproterenol induced myocardial damage in rat. *Phytother. Res*. **24:** 720-725.

Augusti, K.T. (1977). Hypocholesterolemic effect of garlic, *Allium sativum* Linn. *Ind. J. Exp. Biol.,* **15:** 489-490.

Auer W, Eiber A, Hertkorn E, Hoehfeld E, Koehrle U, Lorenz A, Mader F, Merx W, Otto G, and Schmid-Otto B. (1990). Hypertension and hyperlipidaemia: garlic helps in mild cases. *Br J Clin Pract Suppl*, **69**: 3-6.

Avicenna A. (1988). Al Qanoon Fil Tib. Translated by Sharafkandi, S. and Soroosh Press, IV vol., PP.122-178, Tehran, Iran.

Aviello, G. Abenavoli L, Borrelli F, Capasso R, Izzo AA, Lembo F, Romano B and Capasso F. (2009). Garlic: empiricism or science? *Nat Prod Commun*, **4**: 1785-1796.

Bakhshi, M. Taheri JB, Shabestari SB, Tanik A and Pahlevan R. (2012). Comparison of therapeutic effect of aqueous extract of garlic and nystatin mouthwash in denture stomatitis. *Gerodontology*, **29**: e680-684.

Banerjee. S.K., M. Maulik, S.C. Mancahando, A.K. Dinda, S.K. Gupta and S.K. Maulik. (2002). Dose-dependent induction of endogenous antioxidants in rat heart by chronic administration of garlic. *Life Sci.*, **70**: 1509- 1518.

Banerjee, S.K and Maulik, S.K. (2002). Effect of garlic on cardiovascular disorders: a review. *J Nutr*. **1:** 1–14.

Bauersachs, J, Widder, J.D. (2008). Endothelial dysfunction in heart failure. *Pharmacol Rep.* **60**(1):119-26.

Bayan, L. Koulivand P.H and Gorji A. (2014). Garlic: a review of potential therapeutic effects. *Avicenna J Phytomed*, **4(1)**: 1-14.

Bell, C. and Mchachlan, E.M. (1979). Dependence of Deoxycorticosterone/Salt Hypertension in the Rat on the Activity of Adrenergic Cardiac Nervers. *Clin. Sci. (Land).* **57 (2):** 203-210.

Benavides G.A, Squadrito G.L, Mills R.W, Patel H.D, Isbell T.S, Patel R.P, Darley-Usmar V.M, Doeller J.E, Kraus D.W. (2007). Hydrogen sulfide mediates the vasoactivity of garlic. *Proc Natl Acad Sci U S A.* **104(46):**17977-17982.

Bhave, G. and Neilson, E. G. (2011). Body fluid dynamics: back to the future. *J. Am. Soc. Nephrol.* **22**: 2166–2181.

Bohr D.F, Dominiczak A.F and Webb R.C (1991) Pathophysiology of the vasculature in hypertension. *Hypertension 18* (**111**):S69–S75.

Bordia, A. and H.C. Bansal. (1973). Essential oil of garlic in prevention of atherosclerosis. *Lancet*, **2:** 1491- 1492.

Bordia, A.K., S.K. Sodhya, A.S. Rathore and N. Bhu (1975). Essential oil of garlic on blood lipids and fibrinolyitc activity in patients of coronary artey disease. *J. Asso. Physicians Ind.,* **26:** 327-331.

Borek, C. (2001). Antioxidant health effects of aged garlic extract. *J. Nutr.*, **131**: 1010S-1015S.

Brace, L.D. (2002). Cardiovascular benefits of garlic (*Allium sativum* L). *J. Cardiovasc Nurs.* **16:** 33–49.

Bravo E.L, Tazari R.C and Dunstan, H.P. (1983). The changing clinical spectrum of primary aldosteronism. *Ann J Med* **74**:641-51.

Burnier, M. (2001). Angiotensin II type I receptor blockers. *Circulation.* **103:** 904-912.

Burnier, M., Biollaz, J., Magnin, J. L., Bidlingmeyer, M. & Brunner, H. R. (1994). Renal sodium handling in patients with untreated hypertension and white coat hypertension. *Hypertension* **23:** 496–502.

Cain, A.E, Khalil, R.A. (2002). Pathophysiology of essential hypertension: role of the pump, the vessel, and the kidney. Semin Nephrol. **22:** 3–16

Chan J.Y, Yuen A.C, Chan R.Y and Chan S.W. (2013). A review of the cardiovascular benefits and antioxidant properties of allicin. *Phytother Res.* **27**: 637-646.

Chanderkar A.G and Jain P.K (1973). Analysis of hypotensive action of *Allium sativum* (garlic). *Ind J Physiol Pharmacol*, **17**: 132-133.

Chobanian A.V. (2009). The Hypertension Paradox - More Uncontrolled Disease despite Improved Therapy. *N Engl J Med*. **361**:878-887.

Chung, L.Y. (2006). The antioxidant property of garlic compounds: Allyl cysteine, alliin, allicin and allyldisulfide. *J Med Foods*. **9:**205-213.

Colín-González A.L, Santana R.A, Silva-Islas C.A, Chánez-Cárdenas M.E, Santamaría A, and Maldonado P.D. (2012). The antioxidant mechanisms underlying the aged garlic extract- and S-allylcysteine-induced protection. *Oxid Med Cell Longev*, 2012: 907162.

Conn, J.W, Knopf, R.F and Nesbit, R.M. (1964). Clinical characteristics of primary aldosteronism from an analysis of 145 cases. *Am J Surg* **107**:159-172.

Crofton, J.T and Share, L. (1997). Gonadal hormones modulate Deoxycorticosterone-salt in Male and Female rats. *Hypertension.* **29:** 494-499.

Dalkara, T, Irijura K, Huang Z, Panahian N and Moskowitz, M.A. (1995). Cerebrovascular responses under controlled and monitored physiological condition in the anesthetized mouse. *J Cereb Blood Flow Metab*. **15:**631-638.

Dannesteter J. (2003). AVESTA: VENDIDAD: Fargard 20: The origins of medicine. Translated from Sacred Books of the East, *American Edition*, New York

Das I, Khan, N.S and Sooranna, S.R. (1995). Potent activation of nitric oxide synthase by garlic: a basis for its therapeutic applications. *Curr Med Res Opin*. **13:**257-63

De Jong W (1996). Experimental and genetic models of hypertension. In: Birkenhger W, Reid JL, editors. Handbook of hypertension, vol 4. Amsterdam, New York, Oxford: Elsevier, **1984**:1–549.

Deng, L.Y and Schiffrin, E.L. (1991). Morphological and functional alterations of mesenteric small resistance arteries in early renal hypertension in rats. *Am J Physiol.* **261**(4 Pt 2):H1171–7.

Dhawan, Vand Jain, S. (2004). Effect of garlic supplementation on oxidized low density lipoproteins and lipid peroxidation in patients of essential hypertension. *Mol Cell Biochem.* **266:** 109–115.

DiBona, G. F. (2005). Physiology in perspective: the wisdom of the body. Neural control of the kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289:** R633–R641.

Drury, P.L. (1985). Disorders of mineralcorticoid activity. *Clin Endocrinol Metab.* **14**:175-202.

Durak I, Kavutcu M, Aytac B, Avci A, Devrim E, Ozbek H, Ozturk HS: (2004). Effects of garlic extract consumption on blood lipid and oxidant/antioxidant parameters in humans with high blood cholesterol. *J Nutr Biochem.* **15,** 373–377.

Durak, I, Aytac B, Atmaca Y, Devrim E, Avci A, Erol C, Oral, D. (2004). Effects of garlic extract consumption on plasma and erythrocyte antioxidant parameters in atherosclerotic patients. *Life Sci*. **75:** 1959–1966.

Dzau V.J, Gibbons G.H, Kobilka B.K, Lawn R.M, and Pratt R.E (1995) Genetic models of human vascular disease. *Circulation* **91**:521–531.

Elkayam, A. D, Mirelman, E, Peleg, M, Wilcheh, T, Miron, A, Rabinkov, M, Oron-Henman and Rosenthal, T. (2003). The effects of allicin on weight in fructoseinduced hyperinsulinemic, hyperlipidemic, hypertensive rats. *Am. J. Hypertension*. **16:** 1053-1056

Esler, M., Lambert, E. and Schlaich, M. (2010). Point: Chronic activation of the sympathetic nervous system is the dominant contributor to systemic hypertension. *J. Appl. Physiol.* **109:** 1996–1998.

Fallon, M.B, Abrams GA, Abdel-Razek TT, Dai J, Chen SJ, Chen YF, Luo B, Oparil S, Ku DD. (1998). Garlic prevents hypoxic pulmonary hypertension in rats. *Am. J. Physio.* **275(2 pt 1):** L283-L287.

Farvin, K.H, Surendraraj, A and Anandan, R. (2010). Protective effects of squalene on certain lysosomal hydrolases and free amino acids in isoprenaline induced myocardial infarction in rats. *Int.J. pharmacol*. **6:** 97-103.

Frank J. (2008). Managing hypertension using combination therapy. *Am Fam. Physician*. **77**:1279-1286.

Franklin, S.S, Khan SA, Wong ND, Larson MG and Levy D. (1999). Is pulse pressure useful in predicting risk for coronary heart disease? The Framingham heart study. *Circulation.* **100:** 354–60.

Foëx, P. and Sear, J.W. (2004). Hypertension, Pathophysiology and Treatment. *Contin Educ Anaesth Crit Care Pain*. **4 (3):** 71-75.

Ganong, W. (1999). *Review of medical physiology.*San Francisco; pp 142.

Gao, J, Marc Y, Iturrioz X, Leroux V, Balavoine F, Llorens-Cortes C. (2014). A new strategy for treating hypertension by blocking the activity of the brain renin-angiotensin system with aminopeptidase A inhibitors. *Clin Sci (Lond).* **127**(3):135–48.

Gao, F, Chen, J, Lopez, B.L., Christopher, T.A., Gu, J., Lysko, P., Ruffolo, R.R.J.R., Ohlstein, E.H., Ma, X.L., Yue, T.L. (2000). Comparison of bisoprolol and carvedilol, cardioprotection in a rabbit ischemia and reperfusion model. *Eur. J. Pharmacol*. **406(1):** 109 -16.

Gardner C.D, Chattejee L.M, Carlson J.J. (2001). The effect of a garlic preparation on plasmalipid levels in moderately hypercholesterolemic adults. *Atherosclerosis*, **154**: 213-220.

Gebhardt R, Beck H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte culhue. *Lipids*, **31**: 1269-1276.

Gorinstein, S. H, Leontowicz, M, Leontowicz, J. Drzewiecki, K. Najman, E. Katrrich, D. Garasch, K. Yamamoto and S. Trakhtenberg, (2006). Raw andboiled garlic enhances plasma antioxidant activity and improves plasma lipid metabolism in cholesterol-fed rats. *Life Sciences*. **78:** 655-663.

Grazyna, D, Joanna, S and Danuta, P. (2008). Effects of short-term garlic supplementation on lipid metabolism and antioxidant status in hypertensive adults. *Pharmacology*. **60:** 163-170.

Gurgun, C, Ildizli, M., Yavuzgil, O., Sin, A., Apaydin, A., Cinar, C., Kultursay, H. (2008). The effects of short term statin treatment on left ventricular function and inflammatory markers in patients with chronic heart failure. *Inter. J. Cardiology*, **123:** 102-107.

Guyton, A. C. (1990). Renal function curves and control of body fluids and arterial pressure. *Acta Physiol. Scand. Suppl*. **591**: 107–113.

Guyton, A.C, Jones, C.E and Coleman, T.G. (1973). Circulatory Physiology: Cardiac Output and its Regulation. 2nd Ed. Philladelphia. WB Saunders.

Guyton, A. C. (1961). Physiologic regulation of arterial pressure. *Am. J. Cardiol*. **8:** 401–407.

Guide to Community Preventive Services. Cardiovascular disease prevention and control: team-based care to improve blood pressure control. PP 167.

Hai-Peng, W, Jing, Y, Li-Qiang, Q and Xiang-Jun, Y. (2015). Effect of garlic on Blood pressure: A Meta-Analysis. *J. Clin. Hypertens. (Greenwich).* 17: 223-231.

Hartley, CJ, Michael LH and Entman M.L. (1995). Noninvasive measurement of ascending aortic blood velocity in mice. *Am J Physiol*. **268**:H499-H505.

Hansson, L, Zanchetti, A, Carruthers SG, *et al*. (1998). Effects of intensive blood- pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. *Lancet.* **351:** 1755–62

Hanes D.S, Wier M.R and Sower J.R. (1996). Gender considerations in hypertension pathophysiology and treatment. *Am J Med,* **101**:10S–21S.

Hani M. W. and Stephen C. (2012). Division of Nephrology and Hypertension, Department of Medicine, Mayo Clinic Rochester, 200 First Street SW, Rochester, MN 55905, USA

Harauma A, Moriguchi T: (2006). Aged garlic extract improves blood pressure in spontaneously hypertensive rats more safely than raw garlic. *J Nutr*. **136:** 769S–773S.

Harrison-Bernard LM. (2009). The renal renin-angiotensin system. *Adv Physiol Educ*. **33**(4):270-274.

Harvey, J.M and Beevers, D.G. (1990). Biochemical investigation of hypertension. *Ann Clin Biochem* **7**:287-296

Health, United States (2011). End-stage renal disease patients, by selected characteristics: *Centers for Disease Control and Prevention* website

Heidenreich P.A, Trogdon JG, and Khavjou OA, (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation*. **123**:933–44.

Hiss, R.G and Lamb LE. (1962). Electrocardiographic findings in 122,043 individuals. *Circulation*. **25**:947-952.

Horie, T., T. Murayama and T. Mishima, (1989). Protection of liver microsomal membranes from lipid peroxidation by garlic extract. *Planta. Medica*., **55:**506-508

Howard, C. G. and Mitchell, K. D. (2012). Renal functional responses to selective intrarenal renin inhibition in Cyp1a1-Ren2 transgenic rats with ANG II-dependent malignant hypertension. *Am. J. Physiol. Renal Physiol.* **302**: F52–F59.

Hung, L.M, Chen, J.K., Lee, R.S., Liang, H.C., Su, M.J. (2001). Beneficial effects of astringininarevertrol analogue, on the ischemia and reper Fusion damage in rat heart. *Free Radic Biol Med.* **30(8):** 877-830.

Iciek M, Kwiecień I and Włodek L. (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environ Mol Mutagen*, **50**: 247-265.

Izzo JJ, Weir MR. (2011). Angiotensin-converting enzyme inhibitors. *J Clin Hypertens (Greenwich).* **13**(9):667-75.

Jain R.C. (1977). Effect of garlic on serum lipids, coagulability and fibrinolyhc activity of blood. *Am J Clin Nutr*, **30**: 1380-1381.

Jain, R.C. and C.R. Vyas. (1975). Garlic and alloxan-induced diabetic rabbits. *Am. J. Clin. Nutr.* **28:** 684-685.

James PA, Oparil S, Carter BL, et al. (2014). Evidence-based guideline for the management of high blood pressure in adults. *The Journal of the American Medical Association.* **12:** 135-139.

Jang, H. R. *et al*. (2012). The origin and the clinical significance of urinary angiotensinogen in proteinuric IgA nephropathy patients. *Ann. Med.* **44**: 448–457.

Janssen, B.J., Leenders, P.J. and Smits J.F. (2002). Short-term and long-termblood pressure and heart rate variability in the mouse, *Am. J. Physiol., Regul. Integr. Comp. Physiol*. **278:** 215–225.

Kamanna V.S and Chandrasekhara N. (1982). Effect of garlic on serum lipoproteins cholesterol levels in albino rats rendered hypercholesteremic by feeding cholesterol. **Lipids**, **17**: 483-488.

Kandlikar, S.S and Fink, G.D. (2011). Mild DOCA-Salt Hypertension: Sympathetic System and role of Renal Nerve. *Am. J. Physiol. Heart Cir. Physiol.* **300:** H1781- 1787.

Kandziora J. (1988). Blutdruck- und lipidsenkendeWirkung eines Knoblauch- Preparates in Kombination mit einem Diuretikum (Study 1) [in German]. *Aerztliche Forschung*, **35**: 3-8.

Kang, J. J. *et al*. (2008). The collecting duct is the major source of prorenin in diabetes. *Hypertension*. **51**: 1597–1604.

Kaye, A.D, De-Witt, B.J, Anwar, M, Smith, D.E, Feng, C.J, Kadowitz, P.J and Nossaman, B.D. (2000). Analysis of responses of garlic derivatives in the pulmonary vascular bed of the rat. *J. Appl. Physiol.* **89:** 353-358.

Kearney, P. M. *et al.* (2005).. Global burden of hypertension: analysis of worldwide data. *Lancet* **365**: 217–223.

Khalid, S and Al-Numair. (2009). Hypocholesteremic and Antioxidant Effets of Garlic extract in Rats fed High Cholesterol Diet. *Pakistan journal of Nutrition*. **8(2):** 161-166.

Kim-Park, S and Ku, D.D. (2000). Garlic elicits a nitric-oxide-Dependent relaxation and inhibits hypoxic pulmonary vasoconstriction in rats. *Clin. Exp. Pharmacol Physiol.* **27:** 780-786.

Kiran, V.U, Rajaiah, N.V, Krishna, D.R and Reddy, Y.N. (2010). Effect of Losartan and ramipril on oxidative stress and antioxidant status in South Indian hypertensive patients. *Int. J. Pharmacol*. **6:** 916-920.

Kobori, H., Nangaku, M., Navar, L. G. & Nishiyama, A. (2007). The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol. Rev*. **59:** 251–287.

Kobori, H., Nishiyama, A., Harrison-Bernard, L. M. & Navar, L. G. (2003). Urinary angiotensinogen as an indicator of intrarenal angiotensin status in hypertension. *Hypertension.* **41**: 42–49.

Kokubo Y, Kamide K. (2009). High-normal blood pressure and the risk ofcardiovascular disease. *Circ J*. **73**:1381-1385.

Korotkov, V.M. (1966). The effect of garlic juice on blood pressure. *Vrach Delo*. **6**:123

Koscielny, J., D. Klüßendorf, R. Latza, R. Schmitt, H. Radtke, G. Siegel and J. Kieswetter, (1999). The antiatherosclerotic effect of *Allium sativum*. *Atherosclerosis*. **144:** 237-249.

Kramer, K, van Acker, S.A, Voss, H.P, Grimbergen, J.A, vander Vijgh, W.J and Bast A. (1993). Use of telemetry to record electrocardiogram and heart rate in freely moving mice. J Pharmacol Toxicol Methods. **30**:209-215.

Lanzotti V. (2006). The analysis of onion and garlic. *J Chromatogr A*, **1112**: 3-22.

Lau, B.H. (2001). Suppression of LDL oxidation by garlic. *J. Nutr.*, **131:** 985S-988S.

Law M.R, Morris JK, Wald NJ. (2009). Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ*. **338**:b1665.

Lawson, L.D and Gardner, C.D. (2005). Composition, stability, and bioavailability of garlic products used in a clinical trial. *J Agric Food Chem*. **53(16):**6254–6261

Lawson L.D and Bauer R. (1998). Garlic: a review of its medicinal effects and indicated active compounds. In: Phytomedicines of Europe. Chemistry and Biological Activity. Series 691, *American Chemical Society*. Washington DC, 176-209.

Luley C, Lehmann-Leo W, Moller, B, Martin T and Schwartzkopff W. (1986). Lack of efficacy of dried garlic in patients with hyperlipoproteinemia. *Arzneimittelforschung 1 Drug Res*, **36**: 766-768.

Manrique C, Lastra G, Gardner M, Sowers JR. (2009). The renin angiotensin aldosterone system in hypertension: roles of insulin resistance and oxidative stress. *Med Clin North Am*. **93**:569-582.

Mark, A. L. (1996). The sympathetic nervous system in hypertension: a potential long-term regulator of arterial pressure. *J. Hypertens. Suppl.* **14:** S159–S165.

Martín, N, Bardisa L, Pantoja C, Román R, Vargas M. (1992). Experimental cardiovascular depressant effects of garlic (Allium sativum) dialysate. *J Ethnopharmacol*. **37(2):**145-9.

Martin, N, Bardisa L, Pantoja, C, Barra E, Demetrio C, Valenzuela J, Barrios, M and Sepulveda, M.J. (1997) Involvement of calcium in the cardiac depressant actions of a garlic dialysate, *J Ethnopharmacol*, 55 113.

Malik, Z.A and Siddiqui, S. (1981). Hypotensive effect of freeze-dried garlic (Allium Sativum) sap in dog. *JPMA J Pak Med Assoc*. **31**:12-3.

Mayorov, D.N. (2011). Brain angiotensin AT1 receptors as specific regulators of cardiovascular reactivity to acute psychoemotional stress. *Clin Exp Pharmacol Physiol.* **38**(2):126–35.

McMahon, F.G and R.Vargas. (1993). Can garlic lower blood pressure? A pilot study. *Pharmacotheraphy*. **13(4)**: 406.

Meijler, F.L. (1985). Atrioventricular conduction versus heart size from mouse to whale. *J Am Coll Cardiol*. **5**:363-365.

Mockel, M. And Danne, O. (2004). Reference Values for Cardiac Troponins I and T in a Goal-Oriented Concept of Health: Cardiac Marker Values in a Series of Out-patients without Acute Coronary Syndromes. *Clin chim. Acta*. **342(1-2):** 83-86.

Mohamadi, A, Jarrell ST, Shi SJ, Andrawis NS, Myers A, Clouatre D. (2000). Effects of wild versus cultivated garlic on blood pressure and other parameters in hypertensive rats. *Heart Dis.* **2**(1):3–9.

Morihara, N, Sumioka I, Moriguchi T, Uda N, Kyo E. (2002). Aged garlic extract enhances production of nitric oxide. *Life Sci.* **71**(5):509-17.

Mozaffarian D, Fahimi S, Singh G.M. (2014). Global Sodium Consumption and Death from Cardiovascular Causes. *N Engl J Med.* **371:**624-634.

Mozzafarian D, Benjamin E.J and Go A.S. (2015). Heart Disease and Stroke Statistics- Update: a report from the American Heart Association. *Circulation*. **2015**; e29-322.

Munday, J.S., K.A. James, L.M. Ray, S.W. Kirkawood and K.G. Thompson. (1999). Daily supplementation with aged garlic, but not raw garlic, protects low density lipoprotein against *in vitro* oxidation. *Atherosclerosis*, **1453:** 399-404.

Navar, L. G., Prieto, M. C., Satou, R. & Kobori, H. (2011). Intrarenal angiotensin II and its contribution to the genesis of chronic hypertension. *Curr. Opin. Pharmacol*. **11**: 180–186.

Nature Reviews Nephrology, (2012) *J. Hypertension*. **8**:602-609

National Kidney Foundation (2002). K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am. J. Kidney Dis*. **39** (Suppl. 1), S1–S266.

National Heart, Lung, and Blood Institute website. (Accessed December 20, 2013). www.nhlbi.nih.gov/health/health-topics/topics/hbp . Updated August 2, 2012.

National Institute of Diabetes and Digestive and Kidney Diseases. *United States Renal Data System* (2012) *Annual Data Report: Volume 2: Atlas of End-Stage Renal Disease in the United States.* Washington, D.C.: U.S

Nickering, G, Ostergren, J and Struijker-Boudier, H. (2006). Clinical evidence for the cardiovascular benefits of angiotensin receptor blockers. *JRAAS*. **7:** S1-S7.

Nwankwo T, Yoon S.S, Burt V and Gu Q. (2013). Hypertension among adults in the US: National Center for Health Statistics, Centers for Disease Control and Prevention, US Dept of Health and Human Services.

Nwokocha, C.R, Ozulua, R.I, Owu, D.U, Nwokocha, M.I and Ugwu, A.C. (2011). Antihypertensive properties of *Allium sativum* (garlic) on normotensive and two kidney one clip hypertensive rats. *Niger. J. Physiol. Sci*. **26**: 213-218.

O'Gara, E.A, Hill, D.J. and Maslin, D.J. (2000). Activities of Garlic Oil, Garlic Powder and their Diallyl Constituents against Helicobacter Pylori. *Appl. Environ. Microbiol*. **66:** 2269 – 2273.

Oluwole, F.S. (2001). Effects of garlic on some haematological and biochemical parameters. *Afr. j. biomed. Res.* **4**: 139 - 141).

Omar S.H. (2013). Garlic and Cardiovascular Diseases. *Natural Products: Springer*, 3661-3696.

Omnia, M.A, Bahgat, H. Rania, H.M and Abou-Elnaga. (2014). Biochemical effect of garlic oil administration in heart necrosis induced experimentally in rats. *Benha Vet. Med. J.* **27(2):** 264-276.

Ou, C.C., S.M. Tsao, M.C. Lin and M.C. Yin. (2003). Protective action on human LDL against oxidation and glycation by four organosulfur compounds derived from garlic. *Lipids*, **38:** 219-224.

Padwal R.S, Hemmelgarn B.R, Khan N.A. (2008). The 2008 Canadian Hypertension Education Program recommendations for the management of hypertension: Part 1 - blood pressure measurement, diagnosis and assessment of risk. *Can J Cardiol.* **24**:455-463.

Palar K and Sturm R. (2009). Potential societal savings from reduced sodium consumption in the U.S. adult population. *Am J Health Promot*. **24**:49–57.

Pantoja, C.V, Chiang, L.C, Norris, B.C and Concha, J.B. (1991). Diuretic, natriuretic and hypotensive effects produced by Allium sativum (garlic) in anaesthetized dogs. *J Ethnopharmacol*. **31:**325-31.

Pantoja, C.V, Norris, B.C and Contreras, C.M. (1996). Diuretic and natriuretic effects of chromatographically purified fraction of garlic (Allium sativum). *J. Ethnopharmacol*. **52:**101-5.

Patschan, O., Kuttler, B., Heemann, U., Uber, A. & Rettig, R. (1997). Kidneys from normotensive donors lower blood pressure in young transplanted spontaneously hypertensive rats. *Am. J. Physiol.* **273:** R175–R180.

Patel, V. Upaganlawar, A., Zalawadia, R and Balaraman, R. (2010). Cardioprotective effect of melatonin against isoproterenol induced myocardial infarction in rats: a biochemical, electro cardiographic and histoarchitectural evaluation. *European J. Pharmacology*, **644(1-3):** 160-168.

Plantinga, L. C. *et al*. (2009). Blood pressure control among persons without and with chronic kidney disease: US trends and risk factors 1999–2006. *Hypertension.* **54**: 47–56.

Pedraza-Chaverri, J and Tapia, E. (1998). Garlic prevents hypertension induced by chronic inhibition of nitric oxide synthesis. *Life Sci.* **62**(6): Pg 71–7.

Petkov V. (1979). Plants and hypotensive, antiatheromatous and coronarodilatating action: *Am J Chin Med*, **7**: 197-236.

Pinelli, A.S, Brenna, E, Accinithi, R and Trivulzio, L. (2004). Isoproterenol-Induced myocardial infarction in rabbits protection by propranolol or labetalol a proposed non-invasive procedure. *Eur. J. Pharma. Sci*. **23:** 277-285.

Prieto-Carrasquero, M. C. *et al*. (2004). Enhancement of collecting duct renin in angiotensin II-dependent hypertensive rats. *Hypertension.* **44**: 223–229.

Priscilla, D.H and Prince, P.S. (2009). Effect of garlic acid on cardiac marker enzymes, troponin-T, LDH-isoenzyme pattern, lipid peroxidation products and antioxidant status in isoproterenol (ISO)-induced myocardial infarction in male Wistar rats. *Chem. Boil. Interact*. **15:** 179(2-3).

Pope, J. E., Anderson, J. J. & Felson, D. T. (1993). A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. *Arch. Intern. Med.* **153:** 477–484.

Rafiq, K. *et al*. (2012). Renal sympathetic denervation suppresses *de novo* podocyte injury and albuminuria in rats with aortic regurgitation. *Circulation* **125:** 1402–1413.

Rahman K and Lowe G.M. (2006). Significance of garlic and its constituents in cancer and cardiovascular disease. *J Nutr*, **136**: 736S-740S.

Rahman, K., (2001). Historical perspective on garlic and cardiovascular disease. *J. Nutr.,* **131:** 977S-979S.

Rahman, K and Billington, D. (2000). Dietary supplementation with aged garlic extract inhibits ADP-induced platelet aggregation in humans. *J Nutr*, **130**: 2662–2665.

Raj Kumar Yadav and Nar Singh Verma . (2004). Effects of garlic *(Allium sativum)* extract on the heart rate, rhythm and force of contraction in frog: A dose-dependent study. *Indian Journal of Experimental Biology*. pp. 628-631.

Rashid A and Khan H.H. (1985). The mechanism of hypotensive effect of garlic extract. *J Pak Med Assoc*, **35**: 357-362.

Richards, A.G, Simonson, E and Visscher, M.B. (1953). Electrocardiogram and phonocardiogram of adult and newborn mice in normal conditions and under the effect of cooling, hypoxia, and potassium. *Am J Physiol*. **174***:*293-*298.*

Ried K, Frank O.R and Stocks N.P. (2013a). Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. *Eur J Clin Nutr*. **67**: 64-70.

Ried K, Toben C and Fakler P. (2013b). Effect of garlic on serum lipids: an updated meta-analysis. *Nutr Rev*, **71**: 282-299.

Ried, K, Frank, O. R and Stocks, N. P. (2010). "Aged garlic extract lowers blood pressure in patients with treated but uncontrolled hypertension: a randomised controlled trial". *Maturitas* **67** (2): 144–150.

Reitman, S., Frankel, S. (1957). Colorimetric determination of GOT and GPT activity. American J. Clinical Pathology. Pp 26-56.

Rietz, B, Belagyi J, Torok B, Jacob R. (1995). The radical scavenging ability of garlic examined in various models. *Bolletino Chimico Farmaceutico* , **134:**69-76.

Remiao, F., Carmo, H., Caruahlo, F., Bastos, M.L. (2001). Copper enhance. isoproternol toxicity in isolated rats cardiomyocytes: effects on oxidative stress. *Cardiovasc Toxicol* **1(3):** 195-204.

Rivlrn R.S. (1998). Patient with hyperlipidemia who received garlic supplements. Lipid management. *Report from the Lipid Education Council*, **3**: 6-7.

Sabeena, F.K.H., Anandan, R., Kumar, S.H., Shiny, K.S., Sankar, T.V., Thankappan, T.K. (2004). Effect of squalene on tissue defense system in isoprotemol induced myocardial infarction in rats. *Pharma. Cological. Res.* **50:** 231-236.

Sacks F.M, Campos H. 2(010). Dietary therapy in hypertension. *N Engl J Med.* **362**:2102-2112.

Sarafidis, P. A. *et al*. (2008). Hypertension awareness, treatment, and control in chronic kidney disease. *Am. J. Med*. **121**: 332–340.

Saravanan, G and Prakash, J. (2004). Effect of garlic (Allium sativum) on Lipid peroxidation in experimental myocardial infarction in rats. *J. Ethnopharm*. **94:** 155-158.

Schales, P and Schales, S.S. (1941). A simple and accurate method for the determination of chloride in biological fluids. *J. Biol Chem*. **140**: 879 – 884.

Schenk, J. and McNeill, J.H. (1992). The Pathogenesis of DOCA-Salt hypertension. *J. Pharmacol. Toxical Methods.* **27:** 161-167.

Sendl, A, Elbl, G, Steinke, B, Redl, K, Breu, W and Wagner, H. (1992). Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. *Planta Medica.* **58:** 1-7.

Senthilkumar, G.P., Moses, M.F, Sengottuvelu, S.T., Rajarajan, T., Saravanan, G. (2010). Cardioprotective activity of garlic (allium sativum) in isoproternol – induced rat myocardial necrosis. *International journal of pharmaceutical sciences and nanotechnology*. **2(4):** 797-784.

Sharifi, A.M, Darabi, R and Akbarloo, N. (2003). Antihypertensive mechanism of garlic in two-kidney-one-clip (2K1C) hypertensive rat. *J Ethnopharmacol.* **86(2-3)**: 219-24.

Shouk, R, Abdou A, Shetty K, Sarkar D, Eid A. (2014). Mechanisms underlying the antihypertensive effects of garlic bioactives. *Nutr Res*. **34:**106–115.

Sial A.Y and Ahmed SJ. (1982). Study of the hypotensive action of garlic extract in experimental animals. *J Pak Med Assoc*, 32:237-239.

Simões E Silva, A. C. and Flynn, J. T. (2011). The renin-angiotensin-aldosterone system in hypertension and chronic kidney disease. Pediatr. Nephrol.

Silagy, C.A. and H.A. Neil, (1994). A meta-analysis of the effect of garlic on blood pressure. *J. Hypertens.,* **12:** 463-468.

Smith-Spangler C.M, Juusola J.L, Enns E.A, Owens D.K, Garber AM. (2010). Population strategies to decrease sodium intake and the burden of cardiovascular disease. *Ann Intern Med. Ann Intern Med*. **152**:481-487.

Sodimu, O., P.K. Joseph and K.T. Augusti. (1984). Certain biochemical effects of garlic oil on rats maintained on high fat- high cholesterol diet. *Experiential*, **40:** 78-80.

Stabler S.N, Tejani A.M, Huynh F and Fowkes C. (2012). Garlic for the prevention of cardiovascular morbidity and mortality in hypertensive patients. *Cochrane Database Syst Rev*, 8.

Stampfer M.J, Colditz G.A and Willett W.C. (1991). Postmenopausal estrogen therapy and cardiovascular disease: ten-year follow-up from the nurses health study. *New Engl J Med* **325**:756–762.

Steiner, M. and W. Li, (2001). Aged garlic extract, a modulator of cardiovascular risk factors a dose-finding study on the effects of AGE on platelet functions. *J. Nutr.,* **31:** 980S-984S.

Thethi, T., Kamiyama, M. and Kobori, H. (2012). The link between the renin-angiotensin-aldosterone system and renal injury in obesity and the metabolic syndrome. *Curr. Hypertens. Rep*. **14**: 160–169.

Thomson, M., K.K. Al-Qattan, T. Bordia and M. Ali. (2006). Including garlic in the diet may help lower blood glucose, cholesterol and triglycerides. *J. Nutr.*, **136:** 800S-802S.

Udani, S., Lazich, I. and Bakris, G. L. (2011). Epidemiology of hypertensive kidney disease. *Nat. Rev. Nephrol*. **7**: 11–21.

US Renal Data System. (2011). 2011 Annual Data Report.

Upaganlawar, A, Gandhi, H and Balaraman, R. (2011). Isoproterenol induced myocardial infarction: protective role of natural products. *J. Pharmacol. Toxicol*. **6:** 1-7.

Vahidinia, A.A, Zarei M, Heshmatian B, Salehi I. (2007). The study of garlic effects on hypertention in the goldblatt's rats model. *Physiology and pharmacology*; Mashhad, Iran. pp. 26–30.

Vibha, L, Asdaq, S.M, Napal, S and Rawri, R.K. (2011). Protective effect of medicinal garlic against isoprenaline induced myocardial infarction in rats. *Int. J. Pharmacol*. **7(4):** 510-515.

Wang, S.B, Tian, S., Yang, F., Yang, H.G., Yang, X.Y.A., Du, G. (2009). Cardioprotective effect of salvianolic acid A on isoproterenol–induced myocardial infarction in rats. *Eur. J. Pharmacol*. **615**: 125-132.

Wang, C. T., Chin, S. Y. & Navar, L. G. (2000). Impairment of pressure-natriuresis and renal autoregulation in ANG II-infused hypertensive rats. *Am. J. Physiol. Renal Physiol.* **279:** F319–F325.

Wang, Q, Shen J, Splawski I, Atkinson D, Li Z, Robinson JL, Moss AI, Towbin JA and Keating, M.T. (1995). SCN5A mutation associated with an inherited cardiac arrhythmia: long QT syndrome. *Cell.* **80**:805-811.

Wang, E., Yayama, K., Takano, M. & Okamoto, H. (1994). Sexual dimorphism of urine angiotensinogen excretion in the rat. *Jpn J. Pharmacol*. **64**: 243–250.

Weinberger, M. H. (1993). Sodium sensitivity of blood pressure. *Curr. Opin. Nephrol. Hypertens.* **2:** 935–939.

Weindruch R. (1995). Animal models. In: Masoro EJ, editors. Handbook of physiology, section 11: Aging. New York, Oxford: Oxford University Press, **120**: 37–52.

Williams, B, Poulter, N.R and Brown MJ. (2004). British Hypertension Society guideline for hypertension management. *Br J Med.* **328:** 634–40.

Williams, S.F, Nicholas SB, Vaziri ND, Norris KC. (2014). African Americans, hypertension and the renin angiotensin system. *World J Cardiol.* **6**(9):878–89.

Xue, C. and Siragy, H. M. (2005). Local renal aldosterone system and its regulation by salt, diabetes, and angiotensin II type 1 receptor. *Hypertension.* **46**: 584–590.

Yusuf S, Sleight, P, Pogue J, Bosch J, Davies R and Dagenais G. (2000). Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *New Engl J Med.*; 342: 145–53

Yu-Yan Y and Liu L. (2001). Cholesterol lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. *J Nutr*, **131**: 989- 993.

Zanchetti A, Stella A, Golin R. (1985). Adernergic sodium handling and the natriuretic action of calcium antagonists. *Journal of Cardiovascular Pharmacology* **6(7):**194-198.

Zeng T, Zhang C.L, Zhao X.L, Xie K.Q. (2013)**.** The roles of garlic on the lipid parameters: a systematic review of the literature. *Crit Rev Food Sci Nutr*, **53**: 215-230.

Ziaei, S, Hantoshzadeh S, Rezasoltani P and Lamyian M. (2001). The effect of garlic tablet on plasma lipids and platelet aggregation in nulliparous pregnants at high risk of preeclampsia. *Eur J Obstet Gynecol Reprod Biol*, **99**: 201-206.

Zipes, D.P. (1992). Specific arrhythmias: diagnosis and treatment. In: Braunwald E, ed. *Heart Disease: A Textbook of Cardiovascular Medicine.* 4th ed. Philadelphia, Pa: WB Saunders. 667-725.

APPENDICES

Appendix I: Reference data and graphs

Table 4.0.2 A summary of ANOVA output for the hormone levels following the effect of *Allium sativum* Extract Treatment in hypertension-induced laboratory rats

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
ADH	Between Groups	42.523	15	2.835	2.282	.007
	Within Groups	139.136	112	1.242		
	Total	181.660	127			
ANP	Between Groups	588973.930	15	39264.929	2.374	.005
	Within Groups	1852804.625	112	16542.898		
	Total	2441778.555	127			
AngII	Between Groups	41946.219	15	2796.415	3.726	.000
	Within Groups	84055.000	112	750.491		
	Total	126001.219	127			
Ald	Between Groups	505922.867	15	33728.191	6.650	.000
	Within Groups	568039.125	112	5071.778		
	Total	1073961.992	127			
Renin	Between Groups	22431.926	15	1495.462	8.974	.000
	Within Groups	18664.969	112	166.652		
	Total	41096.895	127			

Table 4.0.3b Multiple comparison test results of the Effects of *Allium sativum* Extract Treatment on Plasma Levels of ADH using Turkeys HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
NS	CPT50	-0.55750	.55729	1.000	-2.5110	1.3960
	CPT 100	-0.66000	.55729	.998	-2.6135	1.2935
	CPT 200	-0.67375	.55729	.998	-2.6273	1.2798
	HCT 50	-0.84375	.55729	.979	-2.7973	1.1098
	HCT 100	-0.86250	.55729	.974	-2.8160	1.0910
	HCT 200	-0.95250	.55729	.940	-2.9060	1.0010
	FAGE 50	-0.83875	.55729	.980	-2.7923	1.1148
	FAGE 100	-0.53250	.55729	1.000	-2.4860	1.4210
	FAGE 200	-1.36375	.55729	.517	-3.3173	.5898
	CGE 50	-1.77000	.55729	.122	-3.7235	.1835
	CGE 100	-1.72375	.55729	.150	-3.6773	.2298
	CGE 200	-1.66000	.55729	.195	-3.6135	.2935
	CIGE 50	-1.75125	.55729	.133	-3.7048	.2023
	CIGE 100	-1.56125	.55729	.284	-3.5148	.3923
	CIGE 200	0.02375	.55729	1.000	-1.9298	1.9773

The mean difference is significant at the 0.05 level.

Table 4.0.4 Multiple comparison test results of the Effects of *Allium sativum* Extract Treatment on Plasma Levels of B-type ANP using Turkeys HSD

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-155.00000	64.30960	.544	-380.4297	70.4297
	CPT 100	-171.25000	64.30960	.368	-396.6797	54.1797
	CPT 200	-178.75000	64.30960	.296	-404.1797	46.6797
	HCT 50	-142.87500	64.30960	.680	-368.3047	82.5547
	HCT 100	-181.87500	64.30960	.269	-407.3047	43.5547
	HCT 200	-221.25000	64.30960	.060	-446.6797	4.1797
	FAGE 50	-200.00000	64.30960	.144	-425.4297	25.4297
	FAGE 100	-198.75000	64.30960	.151	-424.1797	26.6797
	FAGE 200	-259.12500*	64.30960	.009	-484.5547	-33.6953
	CGE 50	-68.50000	64.30960	.999	-293.9297	156.9297
	CGE 100	-111.87500	64.30960	.931	-337.3047	113.5547
	CGE 200	-139.87500	64.30960	.712	-365.3047	85.5547
	CIGE 50	-38.75000	64.30960	1.000	-264.1797	186.6797
	CIGE 100	-67.62500	64.30960	1.000	-293.0547	157.8047
	CIGE 200	-163.12500	64.30960	.453	-388.5547	62.3047

*. The mean difference is significant at the 0.05 level.

Table 4.0.5 Multiple comparison test results of the Effects of *Allium sativum* Extract Treatment on Plasma Levels of Ang-II using Turkeys HSD

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-60.62500	13.69755	1.000	-53.8901	42.1401
	CPT 100	-53.37500*	13.69755	.002	-108.6401	-12.6099
	CPT 200	-48.25000*	13.69755	.015	-101.3901	-5.3599
	HCT 50	-54.75000*	13.69755	.048	-96.2651	-.2349
	HCT 100	-50.75000*	13.69755	.011	-102.7651	-6.7349
	HCT 200	-47.12500	13.69755	.027	-98.7651	-2.7349
	FAGE 50	-47.12500	13.69755	.060	-95.1401	.8901
	FAGE 100	-47.75000	13.69755	.018	-100.6401	-4.6099
	FAGE 200	-47.75000	13.69755	.053	-95.7651	.2651
	CGE 50	-47.87500	13.69755	.052	-95.8901	.1401
	CGE 100	-68.25000*	13.69755	.000	-124.8901	-28.8599
	CGE 200	-58.37500*	13.69755	.000	-116.2651	-20.2349
	CIGE 50	-72.75000*	13.69755	.004	-106.3901	-10.3599
	CIGE 100	-79.87500*	13.69755	.000	-120.7651	-24.7349
	CIGE 200	-74.00000*	13.69755	.000	-127.8901	-31.8599

*. The mean difference is significant at the 0.05 level.

Table 4.0.6 Multiple comparison test results of the Effects of *Allium sativum* Extract Treatment on Plasma Levels of Ald using Turkeys HSD

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-7.00000	35.60821	1.000	-131.8204	117.8204
	CPT 100	-116.62500	35.60821	.095	-241.4454	8.1954
	CPT 200	-117.00000	35.60821	.092	-241.8204	7.8204
	HCT 50	-116.37500	35.60821	.096	-241.1954	8.4454
	HCT 100	-100.50000	35.60821	.272	-225.3204	24.3204
	HCT 200	-93.62500	35.60821	.390	-218.4454	31.1954
	FAGE 50	-87.50000	35.60821	.510	-212.3204	37.3204
	FAGE 100	-117.75000	35.60821	.087	-242.5704	7.0704
	FAGE 200	-94.75000	35.60821	.369	-219.5704	30.0704
	CGE 50	-88.25000	35.60821	.495	-213.0704	36.5704
	CGE 100	-199.00000*	35.60821	.000	-317.1954	-67.5546
	CGE 200	-192.12500*	35.60821	.000	-323.8204	-74.1796
	CIGE 50	-226.50000*	35.60821	.000	-316.9454	-67.3046
	CIGE 100	-227.25000*	35.60821	.000	-351.3204	-101.6796
	CIGE 200	-220.25000*	35.60821	.000	-352.0704	-102.4296

*. The mean difference is significant at the 0.05 level.

Table 4.0.7 Multiple comparison test results of the Effects of *Allium sativum* Extract Treatment on Plasma Levels of Renin using Turkeys HSD

(I)	(J)	Mean Difference			95% Confidence Interval	
treatment	treatment	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-45.53750*	6.45468	.000	-68.1636	-22.9114
	CPT 100	-40.03750*	6.45468	.000	-62.6636	-17.4114
	CPT 200	-40.03750*	6.45468	.000	-62.6636	-17.4114
	HCT 50	-47.28750*	6.45468	.000	-69.9136	-24.6614
	HCT 100	-39.91250*	6.45468	.000	-62.5386	-17.2864
	HCT 200	-36.91250*	6.45468	.000	-59.5386	-14.2864
	FAGE 50	-49.91250*	6.45468	.000	-72.5386	-27.2864
	FAGE 100	-41.91250*	6.45468	.000	-64.5386	-19.2864
	FAGE 200	-33.28750*	6.45468	.000	-55.9136	-10.6614
	CGE 50	-54.66250*	6.45468	.000	-77.2886	-32.0364
	CGE 100	-55.16250*	6.45468	.000	-77.7886	-32.5364
	CGE 200	-47.53750*	6.45468	.000	-70.1636	-24.9114
	CIGE 50	-62.03750*	6.45468	.000	-84.6636	-39.4114
	CIGE 100	-49.78750*	6.45468	.000	-72.4136	-27.1614
	CIGE 200	-46.16250*	6.45468	.000	-68.7886	-23.5364

Table 4.2.1a A summary of the effect of *Allium sativum* extract treatment on Plasma electrolyte levels as Means and Standard Errors for the four electrolytes at various treatment concentration levels.

Treatment	**Mean ± SEM**			
	Na^+	K^+	Ca^{2+}	Cl^-
NS	120.25±.313	4.113±.0398	1.5075±.00412	100.75±.412
CPT50	136.75±1.677	5.600±.2360	3.1063±.40436	120.38±1.558
CPT 100	137.00±2.053	6.363±.3959	2.9538±.41151	116.50±2.928
CPT 200	136.13±2.460	6.750±.5593	2.9313±.41026	115.75±3.161
HCT 50	136.50±2.062	5.775±.2852	3.0975±.39999	120.12±1.695
HCT 100	135.50±2.563	6.488±.4159	2.9550±.41044	115.13±2.936
HCT 200	133.00±2.854	6.538±.5189	2.9125±.40098	112.88±2.918
FAGE 50	136.75±1.820	5.888±.2371	3.1588±.38329	119.25±1.236
FAGE 100	135.88±2.445	6.775±.4267	3.0025±.40140	113.88±2.386
FAGE 200	133.25±2.698	6.750±.5268	2.9413±.382	112.13±2.601
CGE 50	138.38±.680	5.025±.0164	4.1563±.06245	120.62±1.375
CGE 100	139.00±1.452	6.225±.1333	4.1125±.06606	120.38±1.546
CGE 200	138.25±1.800	6.700±.2196	4.1038±.07385	118.00±2.244
CIGE 50	138.50±.423	6.075±.0491	4.1650±.05898	124.00±.824
CIGE 100	138.50±.423	6.025±.0250	4.1450±.06262	122.25±.959
CIGE 200	138.25±.412	6.200±.1309	4.1375±.0607	121.50±1.389
Total	135.74±.582	6.080±.0971	3.3366±.09547	117.09±.682

Table 4.1.1b ANOVA Table for Sodium ion (Na^+)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2427.867	15	161.858	5.881	.000
Within Groups	3082.625	112	27.523		
Total	5510.492	127			

Table 4.1.2 Multiple comparison test results of the effect of *Allium sativum* extract treatment on Plasma levels of Na^+ ions using Turkeys HSD

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-16.500*	2.623	.000	-25.70	-7.30
	CPT 100	-16.750*	2.623	.000	-25.95	-7.55
	CPT 200	-15.875*	2.623	.000	-25.07	-6.68
	HCT 50	-16.250*	2.623	.000	-25.45	-7.05
	HCT 100	-15.250*	2.623	.000	-24.45	-6.05
	HCT 200	-12.750*	2.623	.000	-21.95	-3.55
	FAGE 50	-16.500*	2.623	.000	-25.70	-7.30
	FAGE 100	-15.625*	2.623	.000	-24.82	-6.43
	FAGE 200	-13.000*	2.623	.000	-22.20	-3.80
	CGE 50	-18.125*	2.623	.000	-27.32	-8.93
	CGE 100	-18.750*	2.623	.000	-27.95	-9.55
	CGE 200	-18.000*	2.623	.000	-27.20	-8.80
	CIGE 50	-18.250*	2.623	.000	-27.45	-9.05
	CIGE 100	-18.250*	2.623	.000	-27.45	-9.05
	CIGE 200	-18.000*	2.623	.000	-27.20	-8.80

*. The mean difference is significant at the 0.05 level.

Table 4.1.3a ANOVA Table for Potassium ions (K^+)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	60.827	15	4.055	4.907	.000
Within Groups	92.554	112	.826		
Total	153.381	127			

Table 4.1.3b Multiple comparison test results of the effect of *Allium sativum* extract treatment on Plasma levels of K^+ using Turkeys HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT50	-1.4875	.4545	.095	-3.081	0.106
	CPT 100	-2.2500*	.4545	.000	-3.843	-0.657
	CPT 200	-2.6375*	.4545	.000	-4.231	-1.044
	HCT 50	-1.6625*	.4545	.032	-3.256	-0.069
	HCT 100	-2.3750*	.4545	.000	-3.968	-0.782
	HCT 200	-2.4250*	.4545	.000	-4.018	-0.832
	FAGE 50	-1.7750*	.4545	.014	-3.368	-0.182
	FAGE 100	-2.6625*	.4545	.000	-4.256	-1.069
	FAGE 200	-2.6375*	.4545	.000	-4.231	-1.044
	CGE 50	-0.9125	.4545	.816	-2.506	0.681
	CGE 100	-2.1125*	.4545	.001	-3.706	-0.519
	CGE 200	-2.5875*	.4545	.000	-4.181	-0.994
	CIGE 50	-1.9625*	.4545	.003	-3.556	-0.369
	CIGE 100	-1.9125*	.4545	.005	-3.506	-0.319
	CIGE 200	-2.0875*	.4545	.001	-3.681	-0.494

*. The mean difference is significant at the 0.05 level.

Table 4.1.4a ANOVA Table for Cl^- ions

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3760.047	15	250.670	7.338	.000
Within Groups	3792.000	111	34.162		
Total	7552.047	126			

Table 4.1.4b Multiple comparison test results of the Effects of *Allium sativum* extract treatment on Plasma Levels of Cl^- using Turkeys HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT50	-19.625*	2.915	.000	-29.84	-9.41
	CPT 100	-15.750*	2.915	.000	-25.97	-5.53
	CPT 200	-15.000*	2.915	.000	-25.22	-4.78
	HCT 50	-19.375*	2.915	.000	-29.59	-9.16
	HCT 100	-14.375*	2.915	.000	-24.59	-4.16
	HCT 200	-12.125*	2.915	.006	-22.34	-1.91
	FAGE 50	-18.500*	2.915	.000	-28.72	-8.28
	FAGE 100	-13.125*	2.915	.002	-23.34	-2.91
	FAGE 200	-11.375*	2.915	.014	-21.59	-1.16
	CGE 50	-19.875*	2.915	.000	-30.09	-9.66
	CGE 100	-19.625*	2.915	.000	-29.84	-9.41
	CGE 200	-17.250*	2.915	.000	-27.47	-7.03
	CIGE 50	-23.250*	2.915	.000	-33.47	-13.03
	CIGE 100	-21.500*	2.915	.000	-31.72	-11.28
	CIGE 200	-20.750*	2.915	.000	-30.97	-10.53

*. The mean difference is significant at the 0.05 level.

Table 4.1.5a ANOVA Table for iCa^{2+}

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	65.883	15	4.392	5.979	.000
Within Groups	82.274	112	.735		
Total	148.156	127			

Table 4.1.5b Multiple Comparison Test results of the Effects of *Allium sativum* extract Treatment on Plasma levels of iCa^{2+} using Turkeys HSD

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	-1.59875*	.42854	.025	-3.1009	-0.0966
	CPT 100	-1.44625	.42854	.072	-2.9484	0.0559
	CPT 200	-1.42375	.42854	.084	-2.9259	0.0784
	HCT 50	-1.59000*	.42854	.027	-3.0922	-0.0878
	HCT 100	-1.44750	.42854	.072	-2.9497	0.0547
	HCT 200	-1.40500	.42854	.094	-2.9072	0.0972
	FAGE 50	-1.65125*	.42854	.017	-3.1534	-0.1491
	FAGE 100	-1.49500	.42854	.052	-2.9972	0.0072
	FAGE 200	-1.43375	.42854	.078	-2.9359	0.0684
	CGE 50	-2.64875*	.42854	.000	-4.1509	-1.1466
	CGE 100	-2.60500*	.42854	.000	-4.1072	-1.1028
	CGE 200	-2.59625*	.42854	.000	-4.0984	-1.0941
	CIGE 50	-2.65750*	.42854	.000	-4.1597	-1.1553
	CIGE 100	-2.63750*	.42854	.000	-4.1397	-1.1353
	CIGE 200	-2.63000*	.42854	.000	-4.1322	-1.1278

*. The mean difference is significant at the 0.05 level.

Table 4.5.1 A Summary of the Effect *of Allium sativum* Extract Treatment on levels of Plasma Metabolites as Means and SEM.

Metabolic Parameters

Treatment	AST	Cholesterol	ALT	CK-MB	cTi	cTt
Normal	13.18 ± .182	3.02 ± .023	26.73 ± .141	3.00 ± .000	.078 ± .001	.018 ± .001
FAGE 50	168.2 ± 42.6	3.17 ± .145	115.9 ± 34.8	3.06 ± .333	1.28 ± .250	.76 ± .258
FAGE100	300.0 ± 117.6	3.50 ± .132	212.7 ± 122.4	3.26 ± .382	1.29 ± .250	.798 ± .220
FAGE200	638.8 ± 216.0	3.69 ± .195	470.3 ± 192.4	3.96 ± .376	1.59 ± .367	.937 ± .329
CGE 50	396.6 ± 121.3	3.76 ± .116	369.8 ± 110.2	3.49 ± .189	1.61 ± .277	1.19 ± .244
CGE 100	314.5 ± 88.4	3.54 ± .069	368.0 ± 96.4	3.2 ± .326	1.37 ± .257	.702 ± .089
CGE 200	618.3 ± 201.7	3.83 ± .070	520.6 ± 146.0	4.50 ± .289	1.56 ± .311	.786 ± .220
CIGE 50	604.9 ± 192.0	4.23 ± .330	203.4 ± 65.7	3.49 ± .423	1.57 ± .177	.876 ± .133
CIGE100	701.5 ± 192.1	4.23 ± .185	753.4 ± 196.8	3.96 ± .410	1.40 ± .167	1.05 ± .150
CIGE200	763.6 ± 238.2	4.34 ± .153	380.8 ± 46.6	3.68 ± .112	1.33 ± .084	.809 ± .069

Table 4.5.2a ANOVA Table for Plasma AST levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6197608.945	9	688623.216	2.316	.021
Within Groups	2.973E7	100	297322.867		

Table 4.5.2a ANOVA Table for Plasma AST levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6197608.945	9	688623.216	2.316	.021
Within Groups	2.973E7	100	297322.867		
Total	3.593E7	109			

Table 4.5.2b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Aspartate Aminotransferase (pAST) using Turkeys HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	FAGE 50	-155.000	232.505	1.000	-907.45	597.45
	FAGE 100	-286.818	232.505	.965	-1039.27	465.63
	FAGE200	-625.636	232.505	.192	-1378.09	126.81
	CGE50	-383.455	232.505	.820	-1135.91	369.00
	CGE100	-301.273	232.505	.952	-1053.72	451.18
	CGE 200	-605.091	232.505	.230	-1357.54	147.36
	CIGE50	-591.727	232.505	.258	-1344.18	160.72
	CIGE100	-688.273	232.505	.103	-1440.72	64.18
	CIGE200	-750.364	232.505	.051	-1502.81	2.09

Table 4.5.3a ANOVA Table for Plasma ALT

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6287036.365	9	698559.596	3.532	.001
Within Groups	1.978E7	100	197794.887		
Total	2.607E7	109			

Table 4.5.3b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Alanine Aminotransferase (pALT) using Turkeys HSD

Multiple Comparisons (ALT)

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	FAGE 50	-89.1818	189.6384	1.000	-702.904	524.540
	FAGE 100	-186.0000	189.6384	.993	-799.722	427.722
	FAGE200	-443.6000	189.6384	.374	-1057.322	170.122
	CGE50	-343.0909	189.6384	.728	-956.813	270.631
	CGE100	-341.2727	189.6384	.734	-954.995	272.449
	CGE 200	-493.8545	189.6384	.230	-1107.576	119.867
	CIGE50	-176.6364	189.6384	.995	-790.358	437.086
	CIGE100	-726.7273*	189.6384	.008	-1340.449	-113.005
	CIGE200	-740.8182*	189.6384	.006	-1354.540	-127.096

*. The mean difference is significant at the 0.05 level.

Table 4.5.4a ANOVA Table for cTi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20.599	9	2.289	3.628	.001
Within Groups	63.095	100	.631		
Total	83.695	109			

Table 4.5.4b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Cardiac Troponin-I (cTi) using Turkeys HSD

Multiple Comparisons (cTi)

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	FAGE 50	-1.20455*	.33870	.020	-2.3007	-.1084
	FAGE 100	-1.20727*	.33870	.019	-2.3034	-.1111
	FAGE200	-1.50727*	.33870	.001	-2.6034	-.4111
	CGE50	-1.53091*	.33870	.001	-2.6270	-.4348
	CGE100	-1.29455*	.33870	.008	-2.3907	-.1984
	CGE 200	-1.48364*	.33870	.001	-2.5798	-.3875
	CIGE50	-1.49455*	.33870	.001	-2.5907	-.3984
	CIGE100	-1.32182*	.33870	.006	-2.4180	-.2257
	CIGE200	-1.44909*	.33870	.002	-2.5452	-.3530

*. The mean difference is significant at the 0.05 level.

Table 4.5.5a ANOVA Table for cTt

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.804	9	1.089	2.340	.019
Within Groups	46.553	100	.466		
Total	56.357	109			

Table 4.5.5b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Cardiac Troponin-T (cTt) using Turkeys HSD

Multiple Comparisons

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	FAGE 50	-.74182	.29093	.256	-1.6834	.1997
	FAGE 100	-.78000	.29093	.196	-1.7215	.1615
	FAGE200	-.91909	.29093	.062	-1.8606	.0224
	CGE50	-1.17455*	.29093	.004	-2.1161	-.2330
	CGE100	-.68364	.29093	.367	-1.6252	.2579
	CGE 200	-.76727	.29093	.215	-1.7088	.1743
	CIGE50	-.85818	.29093	.106	-1.7997	.0834
	CIGE100	-1.03636*	.29093	.019	-1.9779	-.0948
	CIGE200	-.94182*	.29093	.050	-1.8834	-.0003

*. The mean difference is significant at the 0.05 level.

Table 4.5.6a ANOVA Table for Total Cholesterol in Plasma

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	19.596	9	2.177	7.428	.000
Within Groups	29.311	100	.293		
Total	48.907	109			

Table 4.5.6b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Total Cholesterol using Turkeys HSD

Multiple Comparison for Plasma Total Cholesterol

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Control	FAGE 50	-0.1545	.2309	1.000	-0.902	0.593
	FAGE100	-0.4818	.2309	0.541	-1.229	0.265
	FAGE200	-0.6727	.2309	0.115	-1.420	0.074
	CGE50	-0.7364	.2309	0.057	-1.483	0.011
	CGE100	-0.5182	.2309	0.434	-1.265	0.229
	CGE 200	-0.8091*	.2309	0.023	-1.556	-0.062
	CIGE50	-1.2091*	.2309	0.000	-1.956	-0.462
	CIGE100	-1.2091*	.2309	0.000	-1.956	-0.462
	CIGE200	-1.3182*	.2309	0.000	-2.065	-0.571

*. The mean difference is significant at the 0.05 level.

Table 4.5.7a ANOVA Table for Plasma CK-MB levels

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	39.801	9	4.422	3.985	.000
Within Groups	110.975	100	1.110		
Total	150.776	109			

Table 4.5.7b Multiple Comparison Test Results of the Effects of *Allium sativum* Extract Treatment on Plasma Creatine Phosphokinase Isoenzyme-MB (CK-MB)

Multiple Comparisons for Plasma CK-MB

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Control	FAGE 50	-.0545	.4492	1.000	-1.508	1.399
	FAGE 100	-.2636	.4492	1.000	-1.717	1.190
	FAGE200	-.9545	.4492	.515	-2.408	.499
	CGE50	-.4909	.4492	.984	-1.945	.963
	CGE100	-.2000	.4492	1.000	-1.654	1.254
	CGE 200	-1.5000*	.4492	.037	-2.954	-.046
	CIGE50	-.4909	.4492	.984	-1.945	.963
	CIGE100	-.9545	.4492	.515	-2.408	.499
	CIGE200	-1.8909*	.4492	.002	-3.345	-.437

*. The mean difference is significant at the 0.05 level.

Table 4.3.0 ANOVA Tables for Electrical and Mechanical Parameters Analyzed

		Sum of Squares	Df	Mean Square	F	Sig.
RR Interval	Between Groups	62305.494	10	6230.549	3449.188	.000
	Within Groups	124.640	69	1.806		
	Total	62430.134	79			
Heart Rate	Between Groups	162207.899	10	16220.790	2887.923	.000
	Within Groups	387.557	69	5.617		
	Total	162595.456	79			
PR Interval	Between Groups	22492.378	10	2249.238	28.885	.000
	Within Groups	4594.299	59	77.869		
	Total	27086.677	69			
P Duration	Between Groups	7444.909	10	744.491	11.279	.000
	Within Groups	3894.517	59	66.009		
	Total	11339.426	69			
QRS Interval	Between Groups	4694.972	10	469.497	22.907	.000
	Within Groups	1373.246	67	20.496		
	Total	6068.218	77			
QT Interval	Between Groups	12857.746	10	1285.775	14.979	.000
	Within Groups	3776.828	44	85.837		
	Total	16634.574	54			

Table 4.3.1 Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on Heart RR-intervals from Real-Time ECG using Turkeys HSD

Multiple Comparisons of RR-Intervals (ms)

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT 100	-26.61429*	.71841	.000	-29.0059	-24.2227
	CPT 200	-94.00000*	.71841	.000	-96.3916	-91.6084
	HCT 50	-25.14286*	.71841	.000	-27.5344	-22.7513
	HCT 200	-.78571	.71841	.990	-3.1773	1.6059
	FAGE 100	-56.97143*	.71841	.000	-59.3630	-54.5799
	FAGE 200	-1.46032	.67732	.543	-3.7151	0.7945
	CGE 100	-10.07143*	.71841	.000	-12.4630	-7.6799
	CGE 200	6.40000*	.71841	.000	4.0084	8.7916
	CIGE 100	-16.05714*	.71841	.000	-18.4487	-13.6656
	CIGE 200	-23.32143*	.69559	.000	-25.6371	-21.0058

*. The mean difference is significant at the 0.05 level.

Table 4.3.2a Summary Report of the Mean Heart Rates of Hypertension-Induced Laboratory Rats from Real-Time ECG

Mean Heart Rates (b/m)

Treatment	Mean	Std. Error of Mean
NS	382.1629	.42129
CPT 100	326.7857	.19040
CPT 200	239.0457	.23251
HCT 50	329.4229	.79395
HCT 200	380.2657	.24370
FAGE 100	280.4686	1.48350
FAGE 200	378.6222	.50004
CGE 100	359.1543	1.25139
CGE 200	398.3914	.55777
CIGE 100	346.7357	1.83598
CIGE 200	332.6988	.54268
Total	342.0778	5.07219

Table 4.3.2b Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on Heart Rate from Real-Time ECG using Turkeys HSD

Multiple Comparisons of Heart Rate (b/m)

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT 100	55.37714*	1.26680	.000	51.1600	59.5943
	CPT 200	143.11714*	1.26680	.000	138.9000	147.3343
	HCT 50	52.74000*	1.26680	.000	48.5228	56.9572
	HCT 200	1.89714	1.26680	.916	-2.3200	6.1143
	FAGE 100	101.69429*	1.26680	.000	97.4771	105.9115
	FAGE 200	3.54063	1.19435	.125	-.4354	7.5166
	CGE 100	23.00857*	1.26680	.000	18.7914	27.2257
	CGE 200	-16.22857*	1.26680	.000	-20.4457	-12.0114
	CIGE 100	35.42714*	1.26680	.000	31.2100	39.6443
	CIGE 200	49.46411*	1.22658	.000	45.3808	53.5474

*. The mean difference is significant at the 0.05 level.

Table 4.3.3 Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on PR Intervals from Real-Time ECG using Turkeys HSD

Multiple Comparisons for PR Intervals (ms)

(I) Treatment	(J) Treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT 100	0.28633	5.34342	1.000	-17.6033	18.1760
	CPT 200	46.67833*	5.34342	.000	28.7887	64.5680
	HCT 50	-4.38595	4.90943	.998	-20.8226	12.0507
	HCT 200	-2.79310	4.90943	1.000	-19.2298	13.6436
	FAGE 100	37.30433*	5.34342	.000	19.4147	55.1940
	FAGE 200	0.66262	4.90943	1.000	-15.7740	17.0993
	CGE 100	-26.38595*	4.90943	.000	-42.8226	-9.9493
	CGE 200	5.83167	5.09475	.986	-11.2255	22.8888
	CIGE 100	2.16976	4.90943	1.000	-14.2669	18.6064
	CIGE 200	-2.34042	4.76570	1.000	-18.2959	13.6151

*. The mean difference is significant at the 0.05 level.

Table 4.3.4 Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on P Duration from Real-Time ECG using Turkeys HSD

(I) Treatment	(J) Treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT 100	-6.26100	4.91968	.970	-22.7320	10.2100
	CPT 200	16.91500*	4.91968	.039	0.4440	33.3860
	HCT 50	-1.85929	4.52010	1.000	-16.9925	13.2739
	HCT 200	-2.50643	4.52010	1.000	-17.6396	12.6268
	FAGE 100	13.57500	4.91968	.200	-2.8960	30.0460
	FAGE 200	-0.21786	4.52010	1.000	-15.3511	14.9153
	CGE 100	-26.31500*	4.52010	.000	-41.4482	-11.1818
	CGE 200	3.27333	4.69073	1.000	-12.4311	18.9778
	CIGE 100	0.44071	4.52010	1.000	-14.6925	15.5739
	CIGE 200	-1.18375	4.38777	1.000	-15.8739	13.5064

*. The mean difference is significant at the 0.05 level.

Table 4.3.5 Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on QRS Interval from Real-Time ECG using Turkeys HSD

Multiple Comparison for QRS Intervals (ms)

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT 100	-3.83333	2.51874	.907	-12.226	4.559
	CPT 200	-23.1667*	2.61382	.000	-31.876	-14.456
	HCT 50	-3.54762	2.51874	.942	-11.940	4.845
	HCT 200	4.02381	2.51874	.878	-4.369	12.417
	FAGE 100	-2.83333	2.51874	.988	-11.226	5.559
	FAGE 200	10.61111*	2.38608	.002	2.659	18.562
	CGE 100	-1.11905	2.51874	1.000	-9.512	7.274
	CGE 200	-2.26190	2.51874	.998	-10.655	6.131
	CIGE 100	-1.54762	2.51874	1.000	-9.940	6.845
	CIGE 200	4.16667	2.44501	.828	-3.980	12.314

*. The mean difference is significant at the 0.05 level.

Table 4.3.6 Multiple Comparison Test Results of Effect of *Allium sativum* Extract Treatment on QT Interval from Real-Time ECG using Turkeys HSD

Multiple Comparison for QT Intervals (ms)

(I) Treatment	(J) Treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT 100	21.63333	7.07613	.111	-2.3759	45.6426
	CPT 200	-31.60667*	7.07613	.002	-55.6159	-7.5974
	HCT 50	9.84500	8.02357	.976	-17.3789	37.0689
	HCT 200	-8.20667	7.07613	.984	-32.2159	15.8026
	FAGE 100	22.45143*	5.80704	.014	2.7482	42.1547
	FAGE 200	30.63375*	5.67352	.000	11.3835	49.8840
	CGE 100	-2.77500	6.55122	1.000	-25.0032	19.4532
	CGE 200	13.56714	5.80704	.429	-6.1361	33.2704
	CIGE 100	14.07500	5.98042	.418	-6.2165	34.3665
	CIGE 200	20.94375*	5.67352	.023	1.6935	40.1940

*. The mean difference is significant at the 0.05 level.

Table 4.3.7a ANOVA Table for Systolic Blood Pressure and Diastolic Blood Pressure

		Sum of Squares	df	Mean Square	F	Sig.
systolic	Between Grps	8304.176	15	553.612	9.808	.000
	Within Groups	9031.636	160	56.448		
	Total	17335.813	175			
diastolic	BetweenGrps	2983.614	15	198.908	3.936	.000
	Within Groups	8085.273	160	50.533		
	Total	11068.886	175			

Table 4.3.7b Summary Report of Mean and SEM of Systolic Blood Pressure in Hypertension-Induced Laboratory Rats

Systolic

Treatment	Mean	Std. Error of Mean
NS	112.2727	1.22170
CPT50	136.2727	2.80937
CPT 100	136.0909	2.39490
CPT 200	134.6364*	2.69772
HCT 50	136.6364	2.92722
HCT 100	137.5455	2.62977
HCT 200	137.3636	2.84213
FAGE 50	136.8182	2.79551
FAGE 100	137.1818	2.82141
FAGE 200	135.0000*	2.69005
CGE 50	143.9091	1.13981
CGE 100	139.9091	1.71864
CGE 200	139.0909	1.72919
CIGE 50	144.4545	.71812
CIGE 100	139.4545	1.54545
CIGE 200	140.3636	1.72823
Total	136.6875	.75023

Table 4.3.7c Multiple Comparison Test Results of Effects of *Allium sativum* Extract Treatment on Systolic Blood Pressure in Hypertension-Induced Laboratory Rats using Turkeys HSD

Multiple Comparison for Systolic Blood Pressure (mmHg)

(I) treatment	(J) treatment				95% Confidence Interval	
		Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
NS	CPT50	24.00000	3.20363	.000	-35.1534	-12.8466
	CPT 100	23.81818	3.20363	.000	-34.9715	-12.6648
	CPT 200	22.36364	3.20363	.000	-33.5170	-11.2103
	HCT 50	24.36364	3.20363	.000	-35.5170	-13.2103
	HCT 100	25.27273	3.20363	.000	-36.4261	-14.1194
	HCT 200	25.09091	3.20363	.000	-36.2443	-13.9376
	FAGE 50	24.54545	3.20363	.000	-35.6988	-13.3921
	FAGE 100	24.90909	3.20363	.000	-36.0624	-13.7557
	FAGE 200	22.72727	3.20363	.000	-33.8806	-11.5739
	CGE 50	-31.63636*	3.20363	.000	-42.7897	-20.4830
	CGE 100	-27.63636*	3.20363	.000	-38.7897	-16.4830
	CGE 200	-26.81818*	3.20363	.000	-37.9715	-15.6648
	CIGE 50	-32.18182*	3.20363	.000	-43.3352	-21.0285
	CIGE 100	-27.18182*	3.20363	.000	-38.3352	-16.0285
	CIGE 200	-28.09091*	3.20363	.000	-39.2443	-16.9376

*. The mean difference is significant at the 0.05 level.

Table 4.3.8a Summary Report of Mean and SEM of Diastolic Blood Pressure (DBP) in Hypertension-Induced Laboratory Rats

Report

Diastolic

Treatment	Mean	Std. Error of Mean
NS	56.9091	.62457
CPT50	64.8182	2.37341
CPT 100	63.8182	2.19428
CPT 200	63.0000	2.21975
HCT 50	66.0000	2.57258
HCT 100	67.9091*	2.22570
HCT 200	63.1818	2.42644
FAGE 50	67.4545*	2.40592
FAGE 100	66.2727	2.71055
FAGE 200	63.0000	2.29228
CGE 50	73.6364*	1.85508
CGE 100	67.9091*	2.03360
CGE 200	64.1818	2.36573
CIGE 50	74.8182*	.95173
CIGE 100	67.2727*	1.87854
CIGE 200	68.5455*	2.06866
Total	66.1705	.59948

Table 4.3.8b Multiple Comparison Test Results of Effects of *Allium sativum* Extract Treatment on Diastolic Blood Pressure in Hypertension-Induced Laboratory Rats using Turkeys HSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
NS	CPT50	-7.90909	3.03114	.400	-18.4619	2.6438
	CPT 100	-6.90909	3.03114	.639	-17.4619	3.6438
	CPT 200	-6.09091	3.03114	.816	-16.6438	4.4619
	HCT 50	-9.09091	3.03114	.181	-19.6438	1.4619
	HCT 100	-11.00000*	3.03114	.032	-21.5528	-.4472
	HCT 200	-6.27273	3.03114	.781	-16.8256	4.2801
	FAGE 50	-10.54545	3.03114	.050	-21.0983	.0074
	FAGE 100	-9.36364	3.03114	.146	-19.9165	1.1892
	FAGE 200	-6.09091	3.03114	.816	-16.6438	4.4619
	CGE 50	-16.72727*	3.03114	.000	-27.2801	-6.1744
	CGE 100	-11.00000*	3.03114	.032	-21.5528	-.4472
	CGE 200	-7.27273	3.03114	.551	-17.8256	3.2801
	CIGE 50	-17.90909*	3.03114	.000	-28.4619	-7.3562
	CIGE 100	-10.36364	3.03114	.060	-20.9165	.1892
	CIGE 200	-11.63636*	3.03114	.016	-22.1892	-1.0835

*. The mean difference is significant at the 0.05 level.

Table 1: Electrical Analysis and Mechanical Activity of Normotensive group (C1) treated with Normal Physiological Saline (NS) extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM*)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
0.9	157.5	380.95	42.49	12.72	32	70.6	55.19	11.06	252.97
1.5	157.3	381.55	45.11	15.78	30		40.57	18.34	217.64
2.2	157.3	381.55	45.08	14.13	31	67.4	56.28	27.58	219.06
2.8	157.3	381.55	45.09	15.45	29	67.9	55.16	14.44	221.28
3.5	156.5	383.38	57.03	27.48	30		-39.27	15.89	220.74
4.1	156.3	384.00	45.27	15.93	29	67.9	57.33	26.93	227.73
5.0	157.0	382.16							

*Normal Cardiac rate/Min = 250 – 450b/m

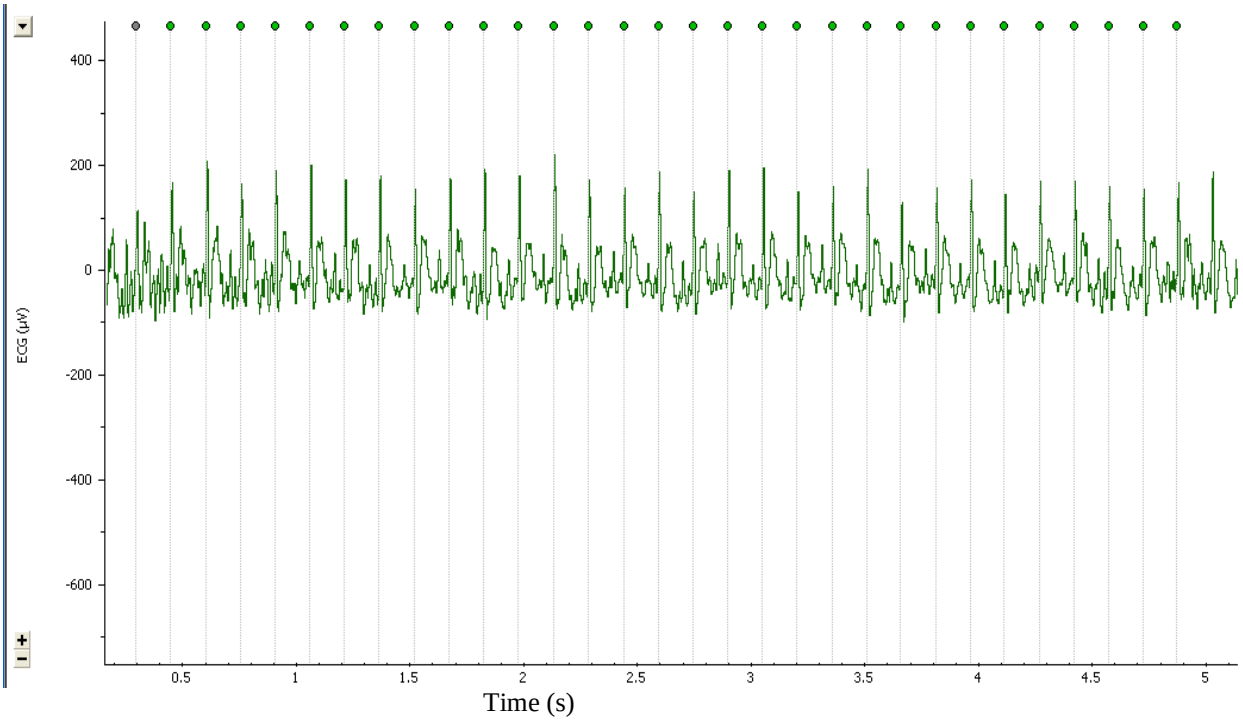


Figure 1: A corresponding ECG graph of Electerical and Mechanical Activity for the Normotensive group (C1) in Table 1 treated with Normal Physiological Saline (NS)

Table 2a: Electrical Analysis and Mechanical Activity of Hypertensive group (C2a) treated with Captoten (CPT) 100mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
8.5	183.5	326.98	52.84	19.23	34	47.17	24.70	3.98	309.27
9.0	183.8	326.53	51.43	20.53	34		26.53	2.34	303.55
9.5	183.8	326.53			34			-2.46	305.57
10.0	184.0	326.09	51.45	41.40	34	46.84	17.27	-3.95	306.61
10.5	183.8	326.53	24.56	14.77	34		-31.79	-3.67	306.13
11.1	183.3	327.42			34			-3.27	299.91
11.5	183.3	327.42	51.68	19.95	34	46.44	24.60	-3.52	309.20

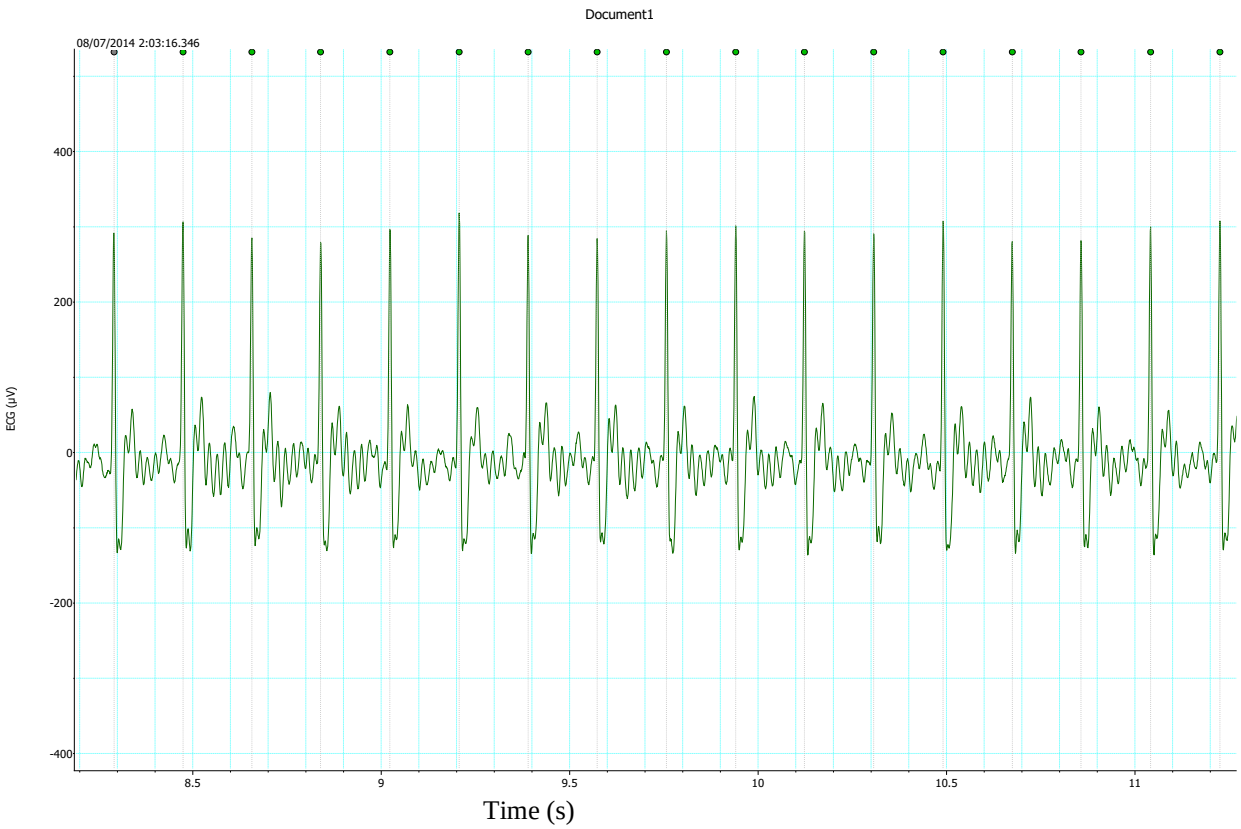


Figure 2a: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (C2a) in Table 2a treated with Captoten (CPT) 100mg/Kg body weight

Table 2b: Electrical Analysis and Mechanical Activity of Hypertensive group (C2b) treated with Captoten (CPT) 200mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
2.30	250.0	240.00			55	99.26		6.13	174.25
2.42	250.8	239.28			53			5.67	172.37
2.45	250.5	239.52			50			6.99	171.18
2.50	251.3	238.81			55	101.25		4.16	167.55
2.60	251.3	238.81			53			10.57	173.42
2.70	251.3	238.81			54	99.66		10.80	160.66
2.80	252.0	238.09							

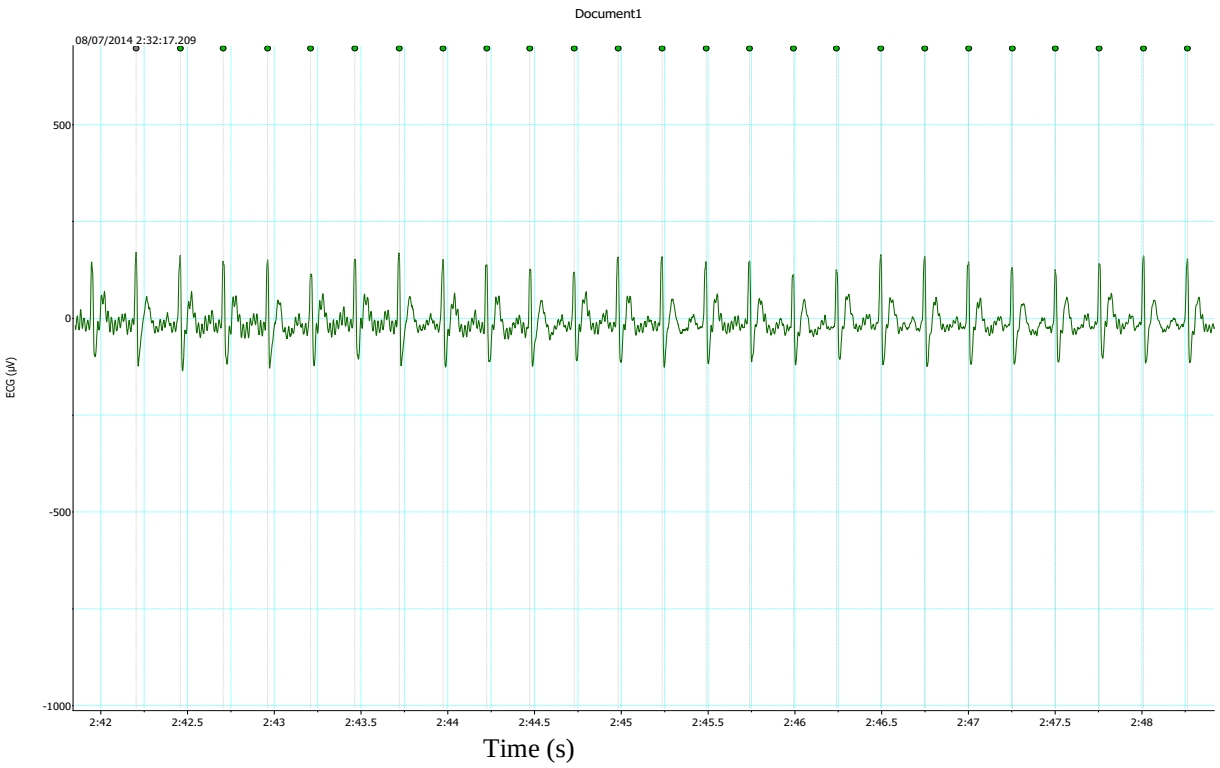


Figure 2b: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (C2b) in Table 2b treated with Captoten (CPT) 200mg/Kg body weight

Table 3a: Electrical Analysis and Mechanical Activity of Hypertensive group (C3a) treated with Hydrochlorothiazide (HCT) 50mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
12.8	183.3	327.42	52.30	21.39	33		37.02	-8.86	214.42
13.5	181.5	330.58	51.53	17.32	33	64.29	54.16	-1.58	208.02
14.3	183.3	327.42	54.12	20.42	33		46.13	-6.56	206.68
15.0	183.5	326.98	53.07	20.44	33		47.61	-6.12	220.01
15.7	181.8	330.12	43.84	16.15	39	52.92	44.51	-9.08	200.71
16.5	181.0	331.49	51.80	18.20	32		61.26	3.05	208.37
17.2	180.8	331.95	50.79	17.50	33		62.17	2.10	205.99

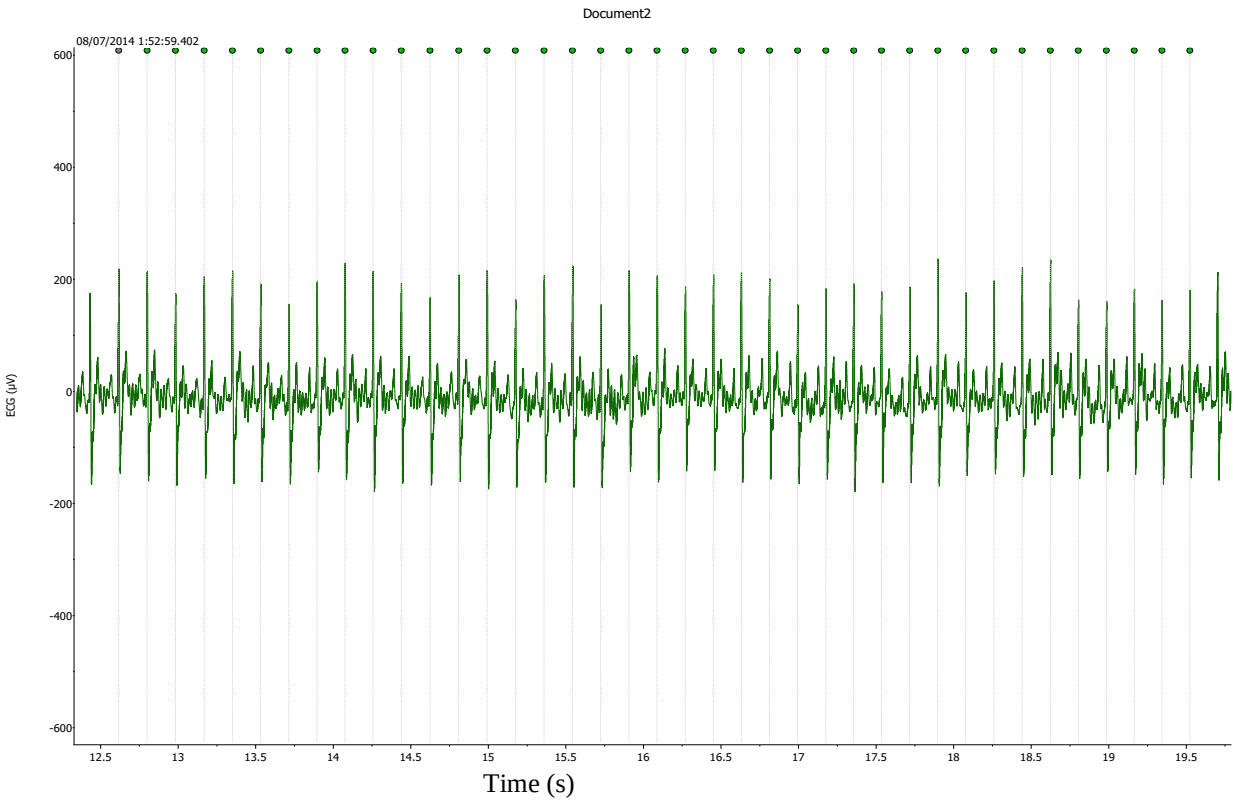


Figure 3a: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (C3a) in Table 3a treated with Hydrochlorothiazide (HCT) 50mg/Kg body weight

Table 3b: Electrical Analysis and Mechanical Activity of Hypertensive group (C3b) treated with Hydrochlorothiazide (HCT) 200mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (μV)	Q Amplitude (μV)	R Amplitude (μV)
0.6	157.3	381.56	50.35	30.70	31	80.99	91.72	17.85	346.98
1.3	157.8	380.35	47.18	15.39	19		90.67	-1.32	345.22
2.0	157.8	380.35	51.78	15.68	31	51.09	92.98	21.00	339.50
2.5	158.0	379.75	49.40	28.01	17		92.34	25.45	333.77
3.2	158.0	379.75	49.45	15.80	33	97.89	88.90	12.88	340.77
3.8	158.0	379.75	48.78	14.57	18		93.52	17.88	333.45
4.4	157.8	380.35	49.36	15.80	34		88.00	11.91	344.81

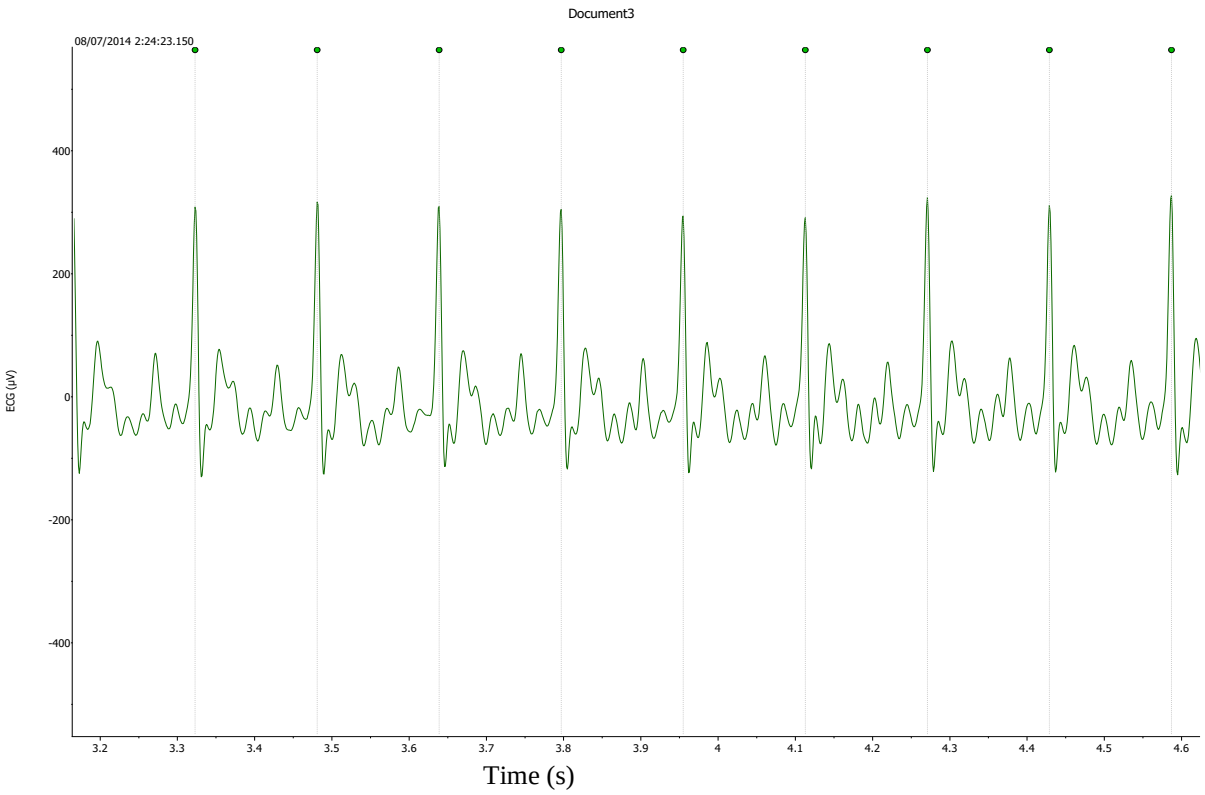


Figure 3b: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (C3b) in Table 3b treated with Hydrochlorothiazide (HCT) 200mg/Kg body weight

Table 4a: Electrical Analysis and Mechanical Activity of Hypertensive group (T1a) treated with Fresh Aqueous Garlic Extract (FAGE) 100mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
0.9	208.8	287.43			28	39.46		-35.75	127.95
1.7	219.0	273.97			33	45.82		-2.55	179.75
2.6	213.8	280.70			34	47.06		2.13	191.16
3.4	214.8	279.40	46.87	16.70	34	46.83	27.74	6.15	184.50
4.3	213.5	281.03			34	47.68		5.12	198.24
5.1	214.3	280.05			34	46.87		-1.41	181.15
6.0	213.8	280.70			34	48.27		3.15	191.95

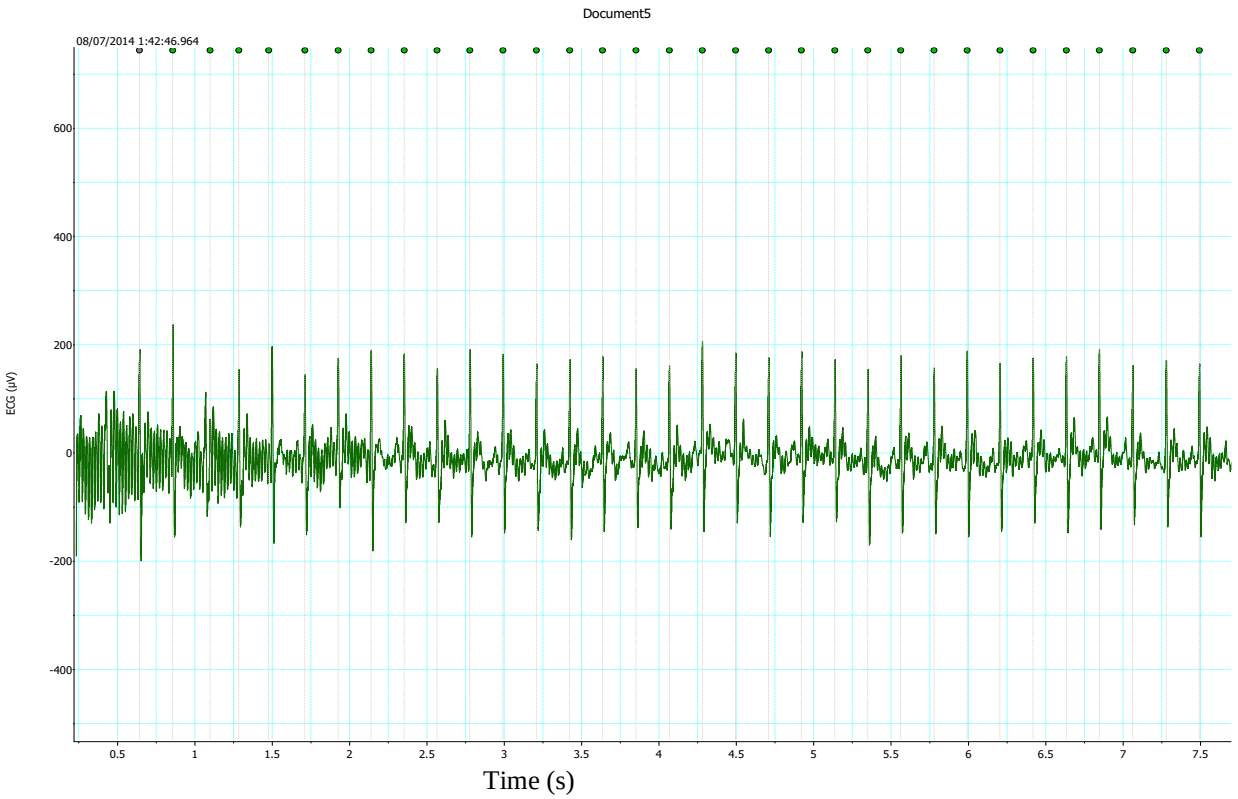


Figure 4a: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (T1a) in Table 4a treated with Fresh Aqueous Garlic Extract (FAGE) 100mg/Kg body weight

Table 4b: Electrical Analysis and Mechanical Activity of Hypertensive group (T1b) treated with Fresh Aqueous Garlic Extract (FAGE) 200mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
0.6	158.0	379.74	43.74	15.62	33	67.78	44.61	10.68	205.32
1.2	158.3	379.15	48.05	17.94	15	32.50	26.97	12.07	179.89
1.8	159.5	376.18			15	33.24		13.70	181.61
2.5	159.5	376.18	46.84	19.58	16	33.56	20.46	1.07	180.03
3.1	158.5	378.55			15	33.21		18.27	191.91
3.7	158.3	379.15	45.82	16.48	16	33.95	34.20	10.23	187.98
4.4	158.5	378.55	45.84	17.44	34		32.80	12.63	205.67
5.0	158.0	379.75	46.61	16.48	16	34.03	29.98	11.78	190.05
5.6	157.8	380.35	45.21	16.39	16	34.26	34.59	14.90	189.27

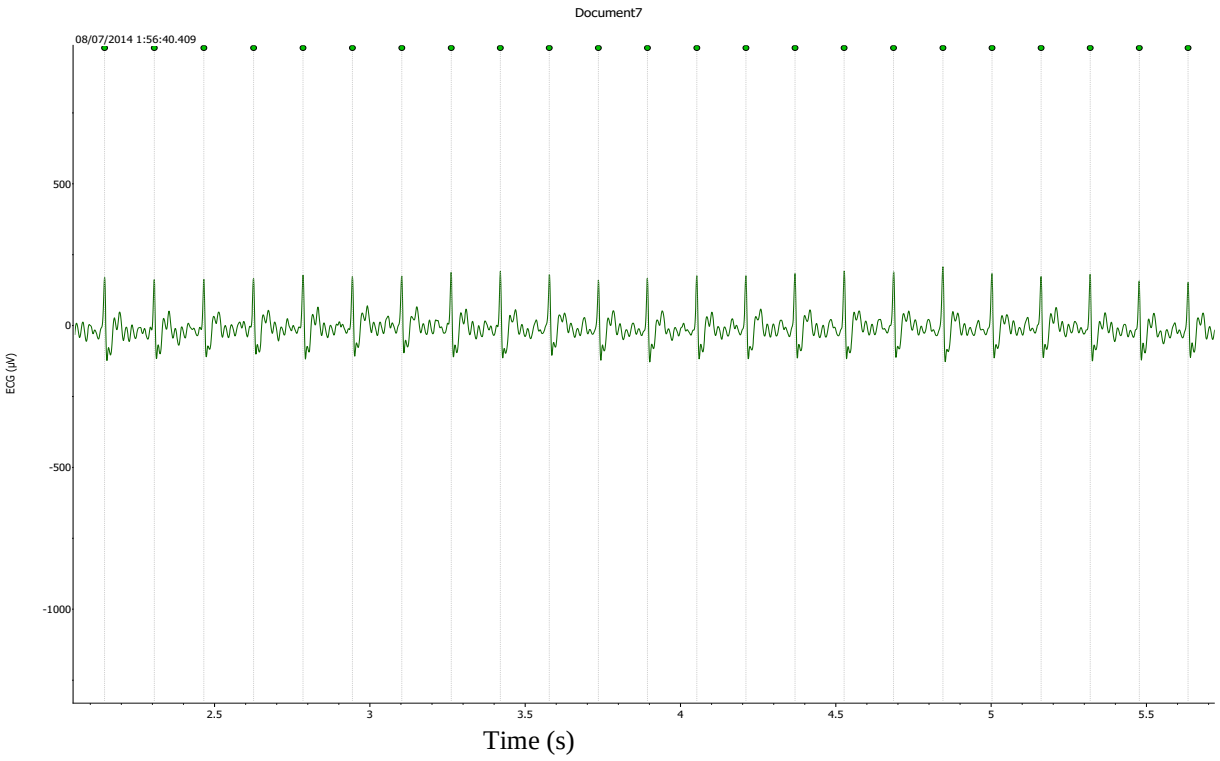


Figure 4b: A corresponding ECG graph of Electerical and Mechanical Activity for the Hypertensive group (T1b) in Table 4b treated with Fresh Aqueous Garlic Extract (FAGE) 200mg/Kg body weight

Table 5a: Electrical Analysis and Mechanical Activity of Hypertensive group (T2a) treated with Crude Garlic Extract (CGE) 100mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (μV)	Q Amplitude (μV)	R Amplitude (μV)
0.6	164.8	364.19	63.62	34.00	30		45.47	-13.09	241.77
1.2	166.8	359.82	102.29	79.12	38	70.31	40.84	-26.60	255.73
1.9	165.8	361.99	61.46	29.67	30		49.05	5.70	239.34
2.6	167.0	359.28	57.45	25.99	30		49.28	-3.62	247.72
3.2	167.5	358.21	68.03	37.20	30	64.85	66.22	-5.23	258.32
3.9	169.3	354.51	70.61	39.39	30	85.66	55.50	-1.63	253.88
4.6	168.5	356.08	87.99	57.24	31	64.08	57.18	-3.57	250.11

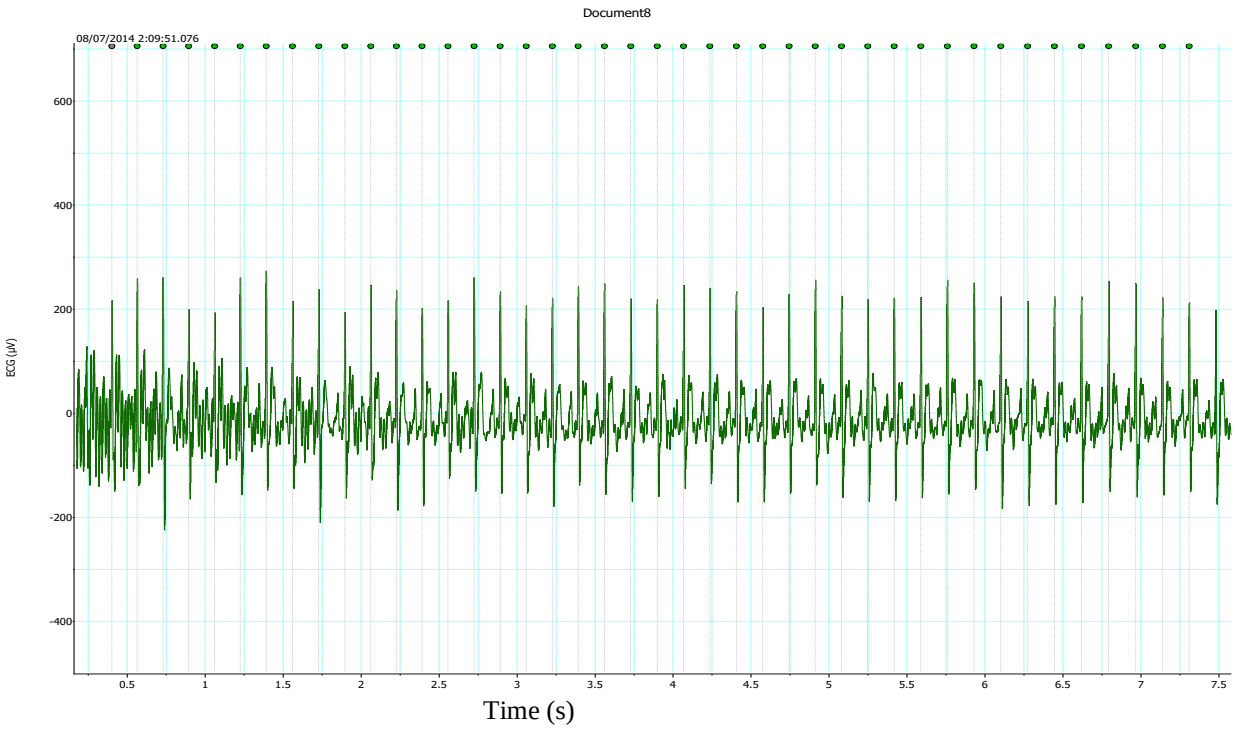


Figure 5a: A corresponding ECG graph of Electerical and Mechanical Activity for Hypertensive group (T2a) in Table 5a treated with Crude Garlic Extract (CGE) 100mg/Kg body weight

Table 5b: Electrical Analysis and Mechanical Activity of Hypertensive group (T2b) treated with Crude Garlic Extract (CGE) 200mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (μV)	Q Amplitude (μV)	R Amplitude (μV)
0.5	150.0	400.00	40.70	12.70	32	51.19	43.15	32.24	430.28
1.1	149.8	400.67			33	47.83		-6.61	397.45
1.7	150.5	398.67	41.03	15.54	32	49.19	47.18	0.48	408.96
2.3	151.0	397.35	40.94	14.72	33	50.68	50.78	9.18	424.94
2.9	151.0	397.35	41.04	12.53	32	83.72	46.06	20.04	430.47
3.5	151.3	396.69	41.70	13.70	33	50.94	51.61	9.89	423.40
4.1	150.8	398.01	39.67	12.66	32	50.63	36.55	13.38	438.78

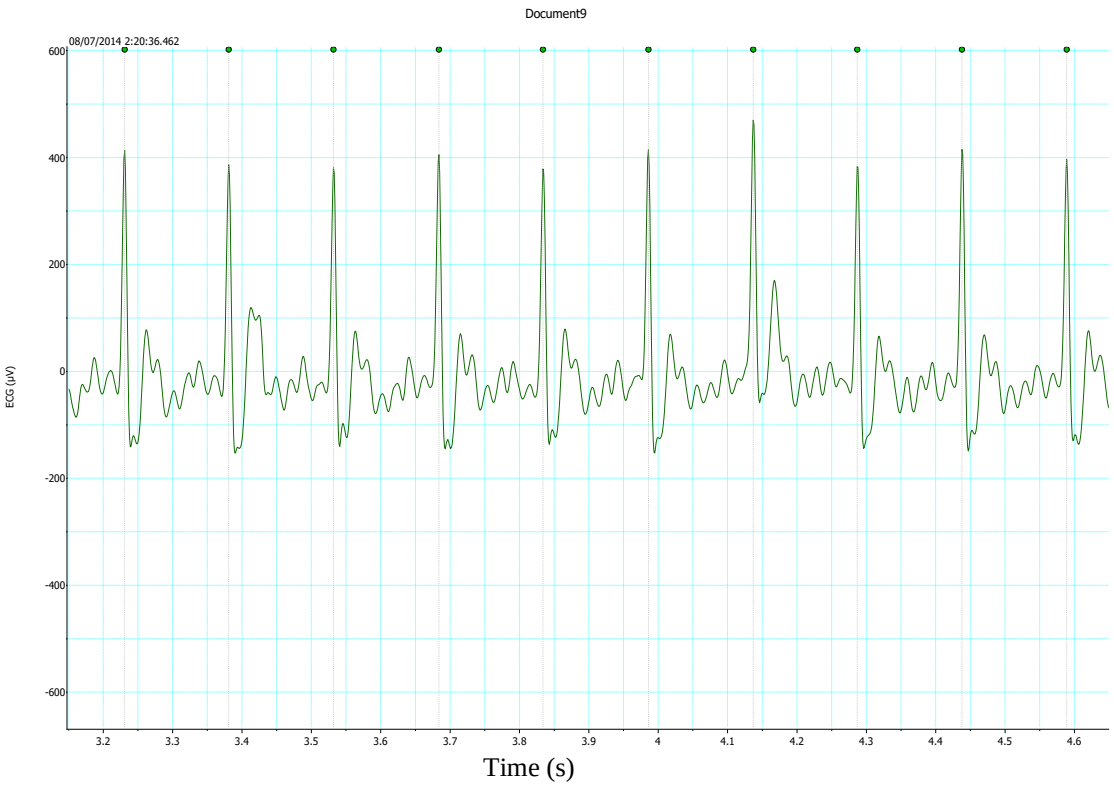


Figure 5b: A corresponding ECG graph of Electerical and Mechanical Activity for Hypertensive group (T2b) in Table 5b treated with Crude Garlic Extract (CGE) 200mg/Kg body weight

Table 6a: Electrical Analysis and Mechanical Activity of Hypertensive group (T3a) treated with Crude Industrial Garlic Extract (CIGE) 100mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
0.6	170.3	352.42	31.19	12.05	41	64.38	42.84	-17.89	163.77
1.2	170.8	351.39	43.60	13.98	31	51.98	53.21	3.07	137.65
1.9	171.0	350.88	56.85	18.00	19		19.98	-5.37	124.86
2.6	173.5	345.82	47.86	18.93	31	51.08	32.61	1.62	155.84
3.3	174.5	343.84	44.88	16.79	32	51.46	34.09	4.13	134.20
4.0	175.0	342.86	39.08	13.39	36	57.08	29.40	-2.76	128.00
4.7	176.5	339.94	48.10	22.18	32	50.27	34.75	2.12	130.71

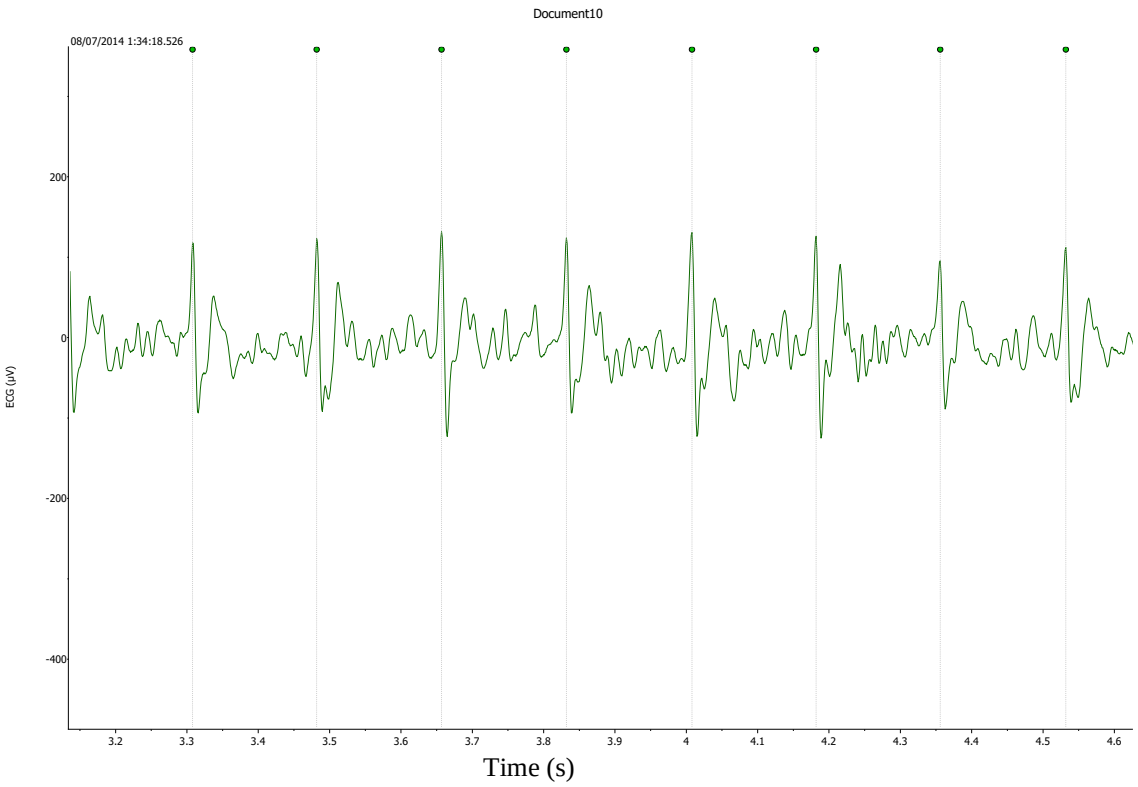


Figure 6a: A corresponding ECG graph of Electerical and Mechanical Activity for Hypertensive group (T3a) in Table 6a treated with Crude Industrial Garlic Extract (CIGE) 100mg/Kg body weight

Table 6b: Electrical Analysis and Mechanical Activity of Hypertensive group (T3b) treated with Crude Industrial Garlic Extract (CIGE) 200mg/Kg body weight extracted from a Real-time Electrocardiogram (ECG)

Time (s)	RR Interval (ms)	Heart Rate (BPM)	PR Interval (ms)	P Duration (ms)	QRS Interval (ms)	QT Interval (ms)	P Amplitude (µV)	Q Amplitude (µV)	R Amplitude (µV)
0.6	180.0	333.30	47.78	40.72	31	48.29	30.54	-63.97	229.06
1.3	179.5	334.26	46.61	14.63	29	48.62	57.37	-31.90	227.95
2.0	179.5	334.26	46.42	13.30	28	48.91	72.23	-1.30	238.47
2.7	180.5	332.41	45.56	17.19	29	53.77	61.55	-13.80	235.77
3.5	181.0	331.49	50.88	15.20	22	45.97	65.33	11.75	224.24
4.2	181.3	331.03	51.59	14.46	21	44.43	71.48	5.64	206.51
4.9	181.5	330.58	52.71	15.16	26	43.94	66.76	4.13	205.27
5.6	179.5	334.26	50.60	14.13	22	46.12	69.07	8.57	208.20

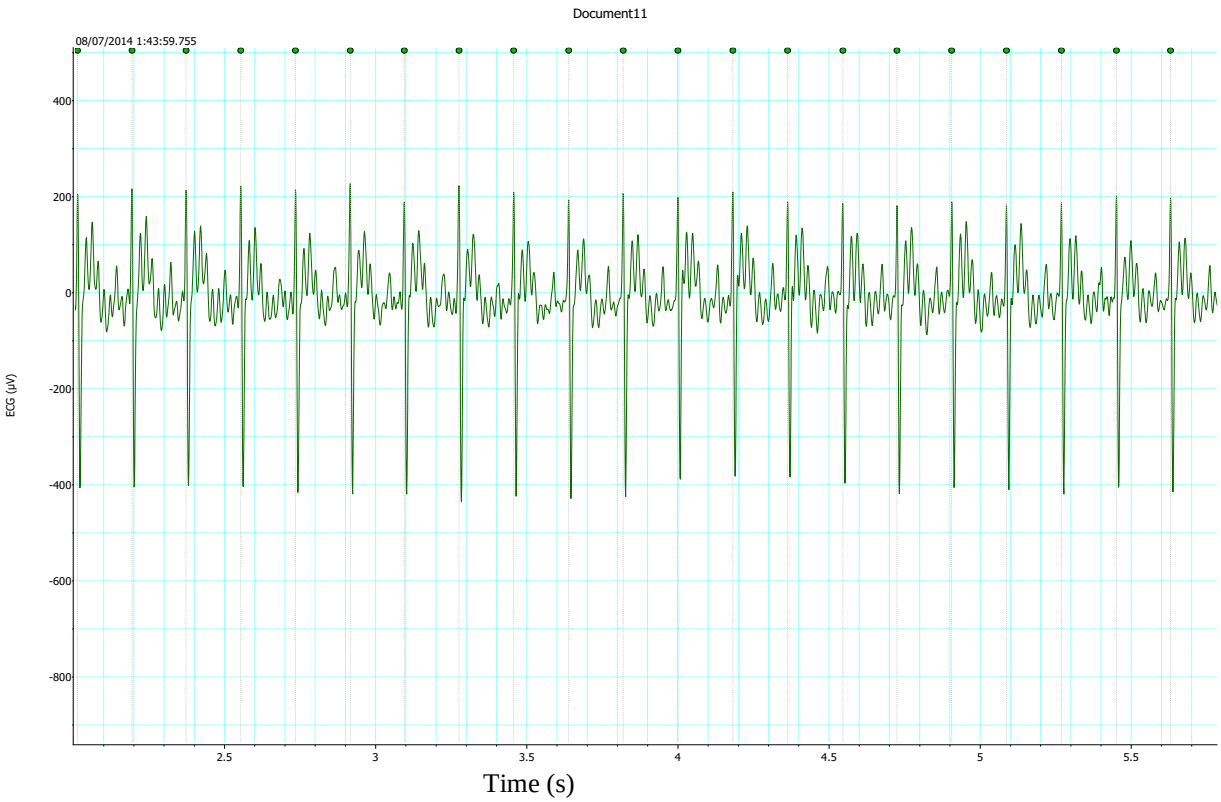
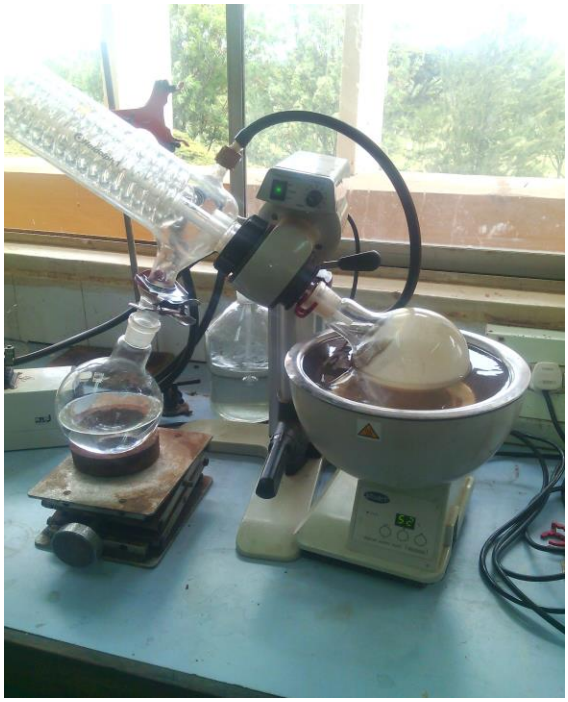
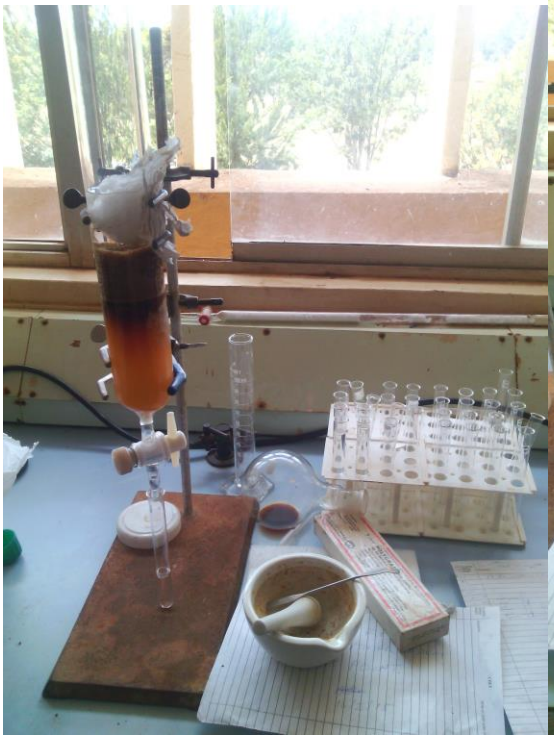


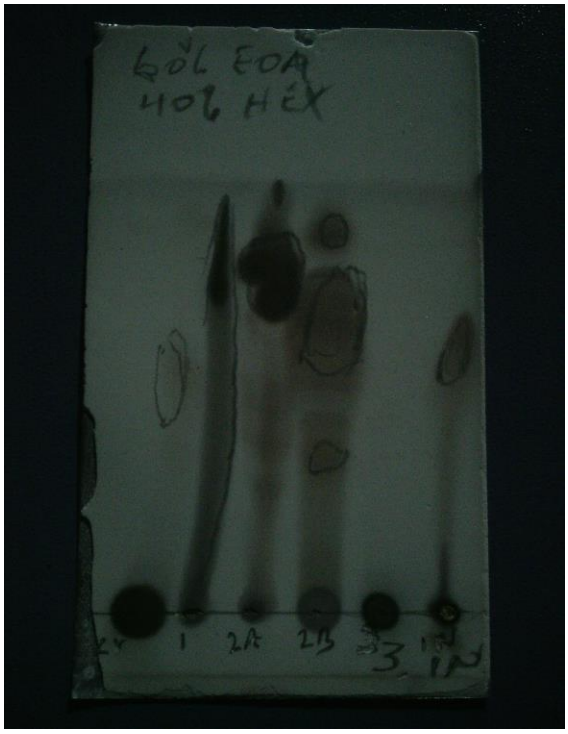
Figure 6b: A corresponding ECG graph of Electerical and Mechanical Activity for Hypertensive group (T3b) in Table 6b treated with Crude Industrial Garlic Extract (CIGE) 200mg/Kg body weight

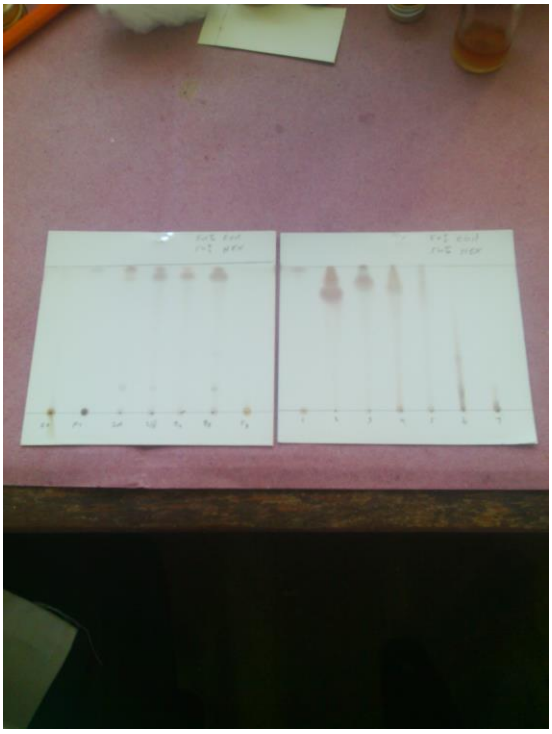
APPENDIX II

PLATE 1: Preparation of Garlic (*Allium sativum*) Extracts from the drying, Ethanolic extraction, Purification, Crude samples of FAGE, CGE and CIGE. Site: Department of Chemistry and Biochemistry, University of Eldoret.






40% HEX
1
3



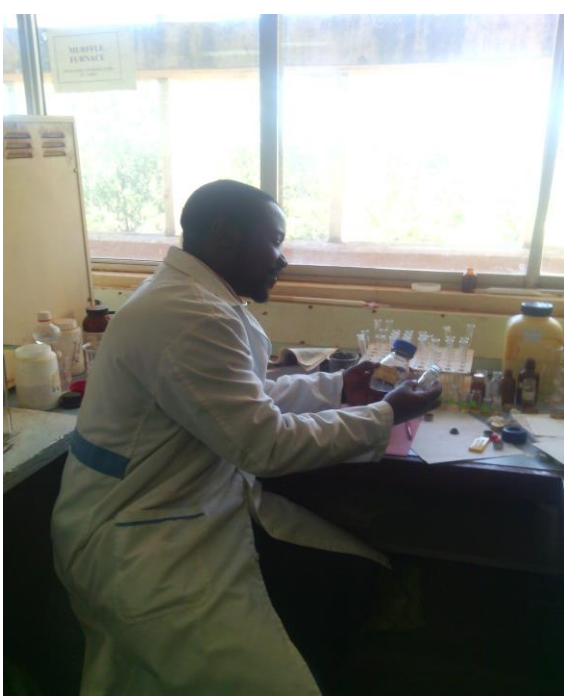


PLATE 2: The Male DOCA-salt Wistar rats, Animal welfare, Feeding and Housing at the University of Eldoret

PLATE 3: Animal House management

PLATE 4: Phlebotomy: Collection of Blood samples for the Biochemical Analysis

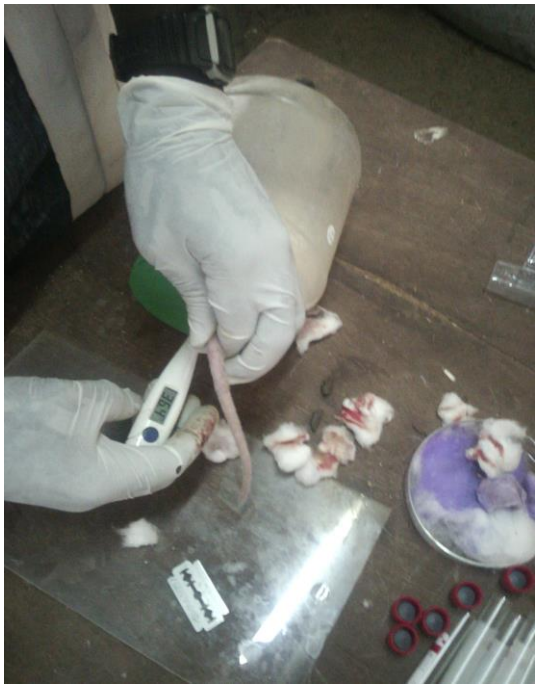
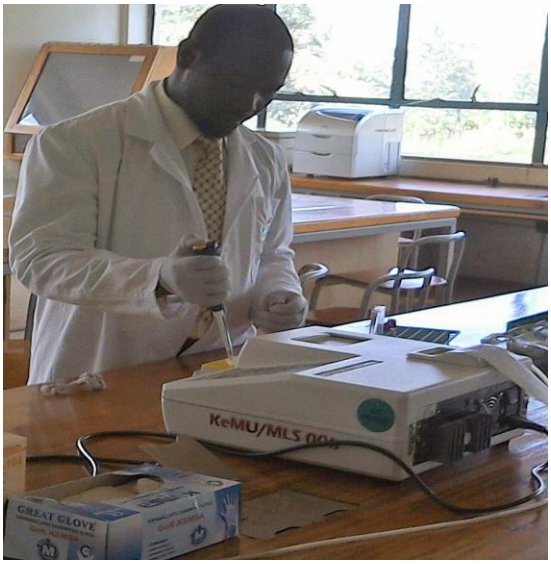


PLATE 5: Analysis of Plasma Hormones, Metabolites and Electrolytes at Nairobi Laboratory Annexe in collaboration with the Kenya Methodist University


KeMU/MLS 004
GREAT GLOVE


KeMU/MLS 00
GREAT GLOVE


KeMU/MLS 00


KeMU/MLS 00
GREAT GLOVE


Atlas Medical
HDL Cholesterol Kit
(Precipitating Method)
Kit Component
Cat: 8.05.26.0.0100
Lot: 13062484
Exp: 25/12/2014
Size: 2X50ml
Store at: 2-8 C
Erba
Atlas Medical
Cholesterol Liquid
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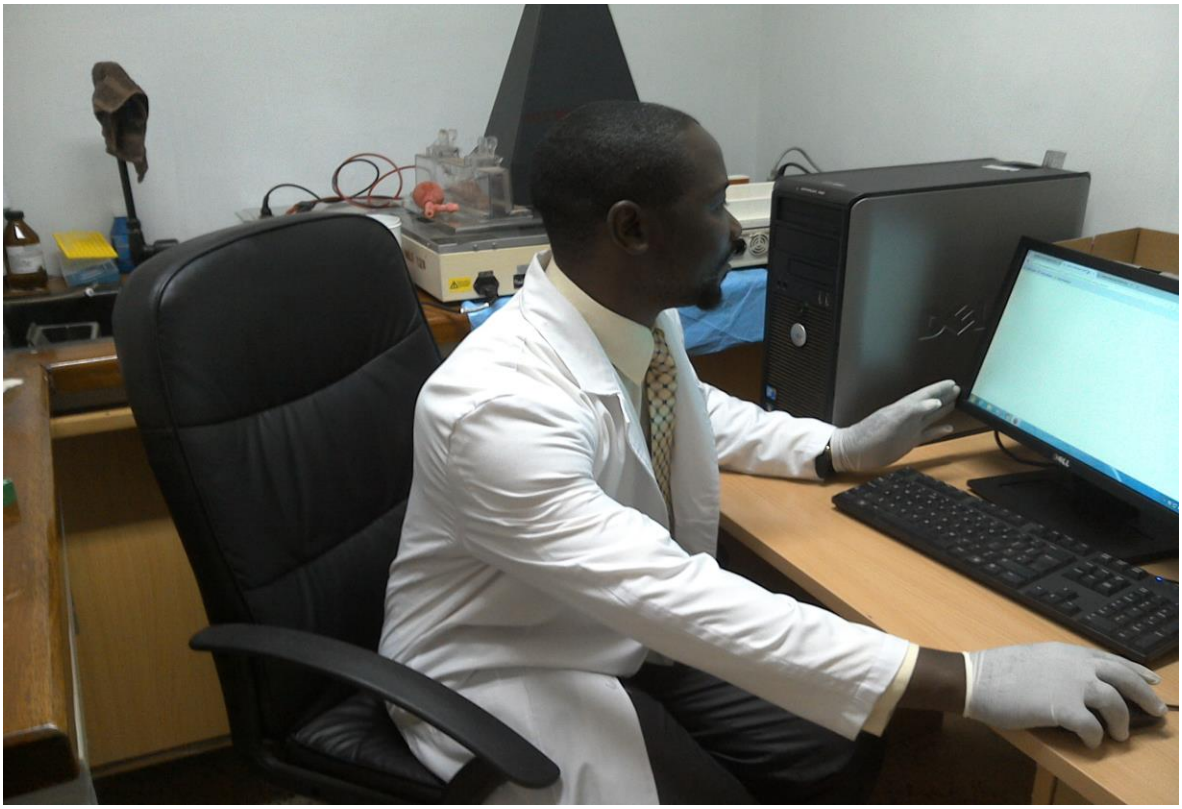
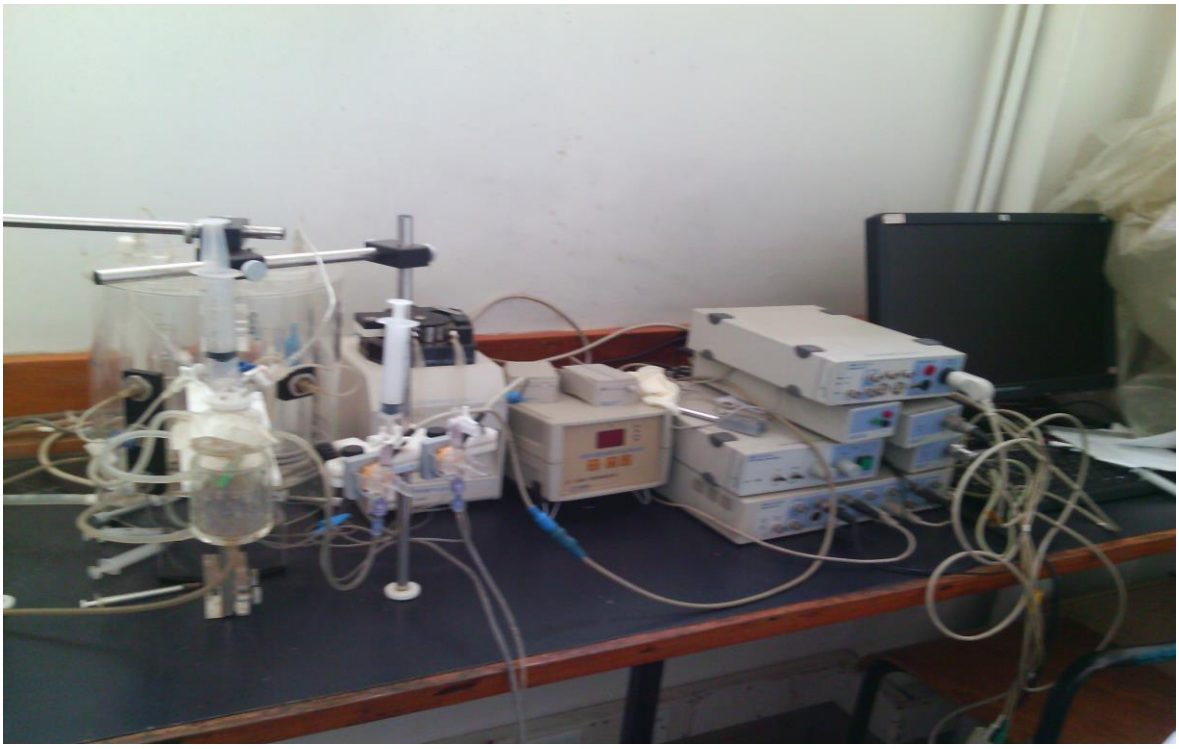
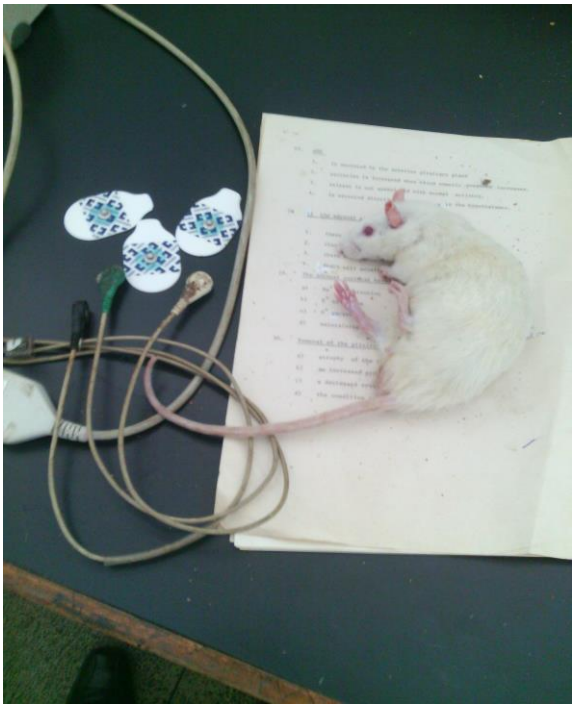
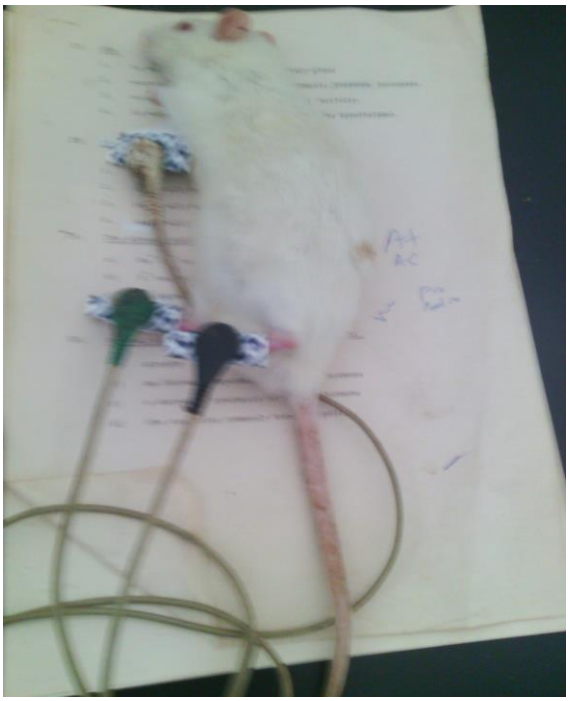


PLATE 6: Analysis of the Electrical and Mechanical activities of the Hearts from 11 selected DOCA-salt Wistar rats using a Real-time Electrocardiogram PowerLab machine. Site: Department of Medical Physiology Laboratory, University of Nairobi.







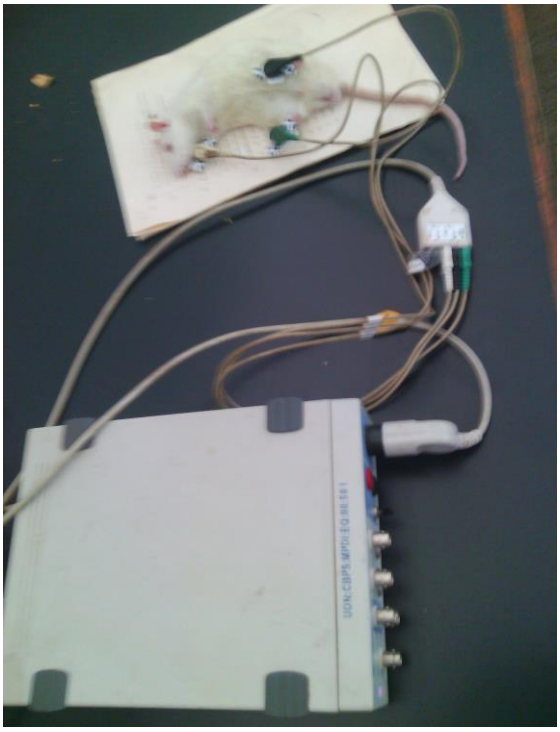
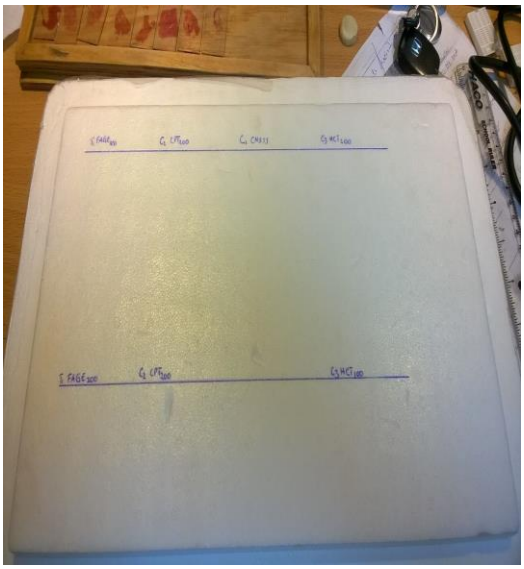
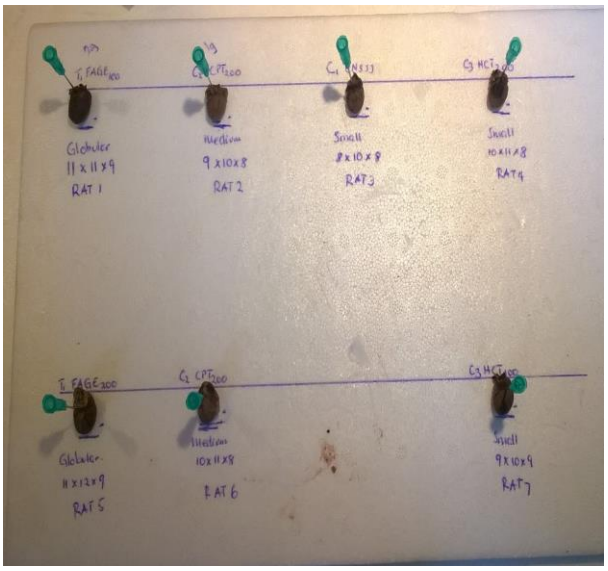
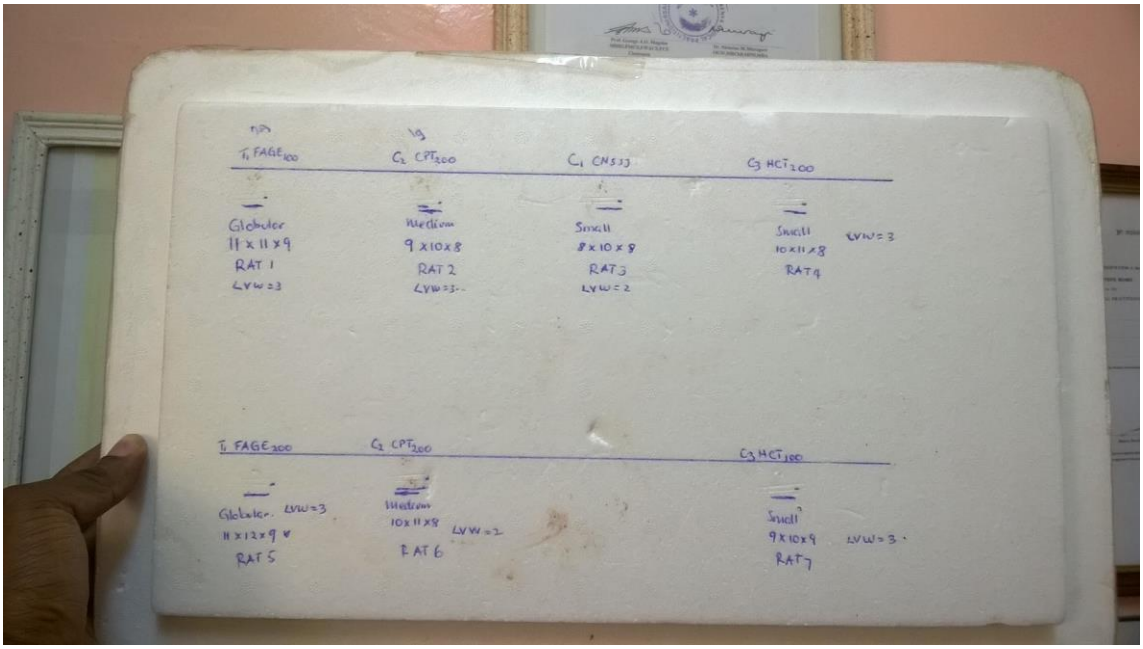


PLATE 7: Histological Analysis: Dissection of the selected rat Hearts, making of tissue slides, staining and Microscopic analysis using Photomicrographs




Globular
11 x 11 x 9
RAT 1
9 x 10 x 8
RAT 2
Small
8 x 10 x 8
RAT 3
10 x 11 x 8
RAT 4
Globular
11 x 12 x 9
RAT 5
10 x 11 x 8
RAT 6
9 x 10 x 9
RAT 7


T1 FAGE100
C2 CPT200
C1 CNS33
C3 HCT200
Globular
11 x 11 x 9
RAT 1
LVW=3
Medium
9 x 10 x 8
RAT 2
Small
8 x 10 x 8
RAT 3
LVW=2
Small
10 x 11 x 8
RAT 4
LVW=3
T1 FAGE200
C2 CPT200
C3 HCT100
Globular
11 x 12 x 9
RAT 5
LVW=3
Medium
10 x 11 x 8
RAT 6
LVW=2
Small
9 x 10 x 9
RAT 7
LVW=3


RAT 1
RAT 2
RAT 3
RAT 4
RAT 5
RAT 6
RAT 7

APPENDIX III: SIMILARITY REPORT

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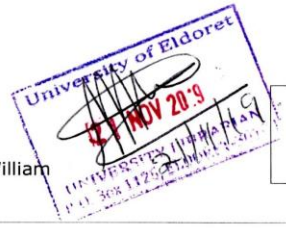

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