EVALUATION OF EFFECTS OF AQUEOUS EXTRACTS OF GARLIC *(ALLIUM SATIVUM)* AND GINGER *(ZINGIBER OFFICINALE)* ON SOME PHYSIOLOGICAL PROFILES OF LABORATORY ANIMALS

BY

JOSHUA ADAMU TENDE

DEPARTMENT OF HUMAN PHYSIOLOGY
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA

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By

Joshua Adamu TENDE, B.Sc. (ABU) 1995; M.Sc. (ABU) 2004

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DEPARTMENT OF HUMAN PHYSIOLOGY
FACULTY OF MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA

FEBRUARY, 2015

DECLARATION

I declare that the work in this Dissertation entitled: 'Evaluation of effects of aqueous extracts of garlic (*Allium sativum*) and ginger (*Zingiber officinale)* on some physiological profiles of laboratory animals' has been carried out by me in the Department of Human Physiology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

Joshua Adamu TENDE

| --- | --- | --- |
| Name of Student | Signature | Date |

CERTIFICATION

This dissertation entiltled EVALUATION OF EFFECTS OF AQUEOUS EXTRACTS OF GARLIC (*ALLIUM SATIVUM*) AND GINGER (*ZINGIBER OFFICINALE*) ON SOME PHYSIOLOGICAL PROFILES OF LABORATORY ANIMALS by Joshua Adamu TENDE meets the regulation governing the award of Doctorate of Philosophy in Human Physiology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

Professor J.O Ayo — Signature ----------
Chairman, Supervisory Committee,
Department of Veterinary Physiology and Toxicology, — Date----------
Ahmady Bello University, Zaria

Dr. A. Mohammed — Signature----------
Member, Supervisory Committee
Department of Human Physiology, — Date----------
Ahmadu Bello University, Zaria

Dr. A.U Zezi — Signature----------
Member, Supervisory Committee
Department of Pharmacology and Therapeutics, — Date----------
Ahmadu Bello University, Zaria

Dr. A. Mohammed — Signature----------
Head of Department
Ahmadu Bello University, Zaria — Date----------

Professor A.Z Hassan — Signature…….
Dean, School of Postgraduate studies
Ahmadu Bello University, Zaria — Date……..

DEDICATION

This work is dedicated to God Almighty, by whose grace I am what I am.

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ABSTRACT

The aim of the study was to evaluate the effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on some heamato-biochemical parameters, blood pressure and their cardioprotective properties in laboratory animals. Wistar rats were assigned into seven (7) groups as mentioned below: Group 1: served as control group and received 1 ml/kg of distilled water, Group 2: received 20 mg/kg of garlic, Group 3: received 40 mg/kg of garlic; Group 4: received 20 mg/kg of ginger; Group 5: received 40 mg/kg of ginger; Group 6: received 10 mg/kg of garlic and 10 mg/kg ginger; Group 7: received 20 mg/kg of garlic and 20 mg/kg of ginger. All administrations were given orally once daily for a period of 28 days. At the end of the treatment, all animals were sacrificed; blood samples collected and the serum separated for determination of liver enzymes activities, lipid profile assay, biochemical and heamatological parameters. The liver tissue was excised and subjected to routine histological investigation. The effects of the various doses of both extracts on cat blood pressure, and isolated perfused rabbit heart were also studied. Results represented as mean ± SEM were compared using ANOVA. The study showed that the cholesterol (51.27 ± 5.29 versus 106.97 ± 2.41), Triglycerides (27.20 ± 7.16 versus 86.14 ± 4.15), Low density lipoprotein (13.49 ± 9.28 versus 64.16 ± 4.23), Na^+ (118.55 ± 5.24 versus 141.52 ± 118 mmol/L), Ca^{2+} (0.64 ± 0.027 versus 0.86 ± 0.038 mmol/L), Haemoglobin concentration (11.87± 0.23 versus 18.26 ± 0.58 g/dL), PCV (35.60 ± 0.68 versus 54.80 ± 1.77%), Mean arterial blood pressure (43.8 ± 2.53 versus 59.9 ± 0.84 mmHg), and force of contraction (87.60 ± 1.94 versus 91.80 ± 0.20 mV) were significantly lower ($P < 0.05$) in groups treated with garlic + ginger as compared with the control group. While the High density lipoprotein (62.73 ± 2.23 versus 21.57 ± 1.91), K^+ (7.94 ± 0.27 versus 4.68 ± 0.19 mmol/L) and Red blood cell (5.51 ± 0.079 x 10^{12} /L versus 4.21 ± 0.078 x 10^{12} /L) were significantly higher in groups treated with garlic + ginger as compared to control. The

combination of both extracts significantly decreased ($P < 0.05$) the leucocytes counts (4.31 ± 0.75 versus 12.03 ± 0.28), ALT (87.40 ± 9.39 versus 115.60 ± 7.84 IU/L), AST (26.0 ± 1.30 versus 41.80 ± 4.68 IU/L), and ALP (12.47 ± 0.96 versus 38.88 ± 2. 89 IU/L) as compared to the control. The combined effects of both extracts did not differ significantly ($P < 0.05$) from the single effect except for the liver enzymes and White blood cell count. The cholesterol and Low density lipoprotein lowering effect produced by both extracts is probably due to increased lipid oxidation and bile excretion, while the observed decrease in mean arterial blood pressure may be due to the effect of anaesthesia on the animals. These results suggest that the combination of garlic and ginger extracts may be cardioprotective but toxic to the liver and kidney.

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ABBREVIATIONS

µL	- -	Micro litre
5-HT	- -	5–Hydroxytryptamine
AGE	- -	Aged Garlic Extracts
AIDS	- -	Acquire Immune Deficiency Syndrome
ALP	- -	Alkaline Phosphate
ALT	- -	Alanine Aminotransferase
ANOVA	-	One Way Analysis of Variance
AST	- -	Aspartate Aminotransferase
ATP	- -	Adenosine Triphosphate
BSA	- -	Bovine Serum Albumin
Ca^{2+}	- -	Calcium
Ca-ATPase	-	Calcium Adenosine Triphosphatase
CAE	- -	Crude Aqueous Extract
Cl^-	- -	Chloride
CNS	- -	Central Nervous System
CP	- -	Crude Powder
CV	- -	Central Vein
DBP	- -	Diastolic Blood Pressure
EDTA	- -	Ethylenediaminetetraacetic Acid
EPG	- -	Eggs Per Gram
G	- -	Glomeruli
GC-MS	-	Gas Chromatography-mass Spectrometry

Gy	-	Gamma radiation
H & E stain	-	Hematoxylin and Eosin Stain
H	-	Hematocytes
HCO_3^-	-	Bicarbonate
HDL-C	-	High-Density Lipoprotein Cholesterol
Hg	-	Mercury
IP	-	Intraperitoneally
IP3	-	Inositol-1,4,5-Triphophatase
K^+	-	Potassium
L	-	Longitudinal
LD_{50}	-	Median Lethal Dose
LDL-C	-	Low-Density Lipoprotein Cholesterol
L-NAME	-	L-Nitro-Arginine Methyl Ester
MABP	-	Mean Arterial Blood Pressure
MAP	-	Mean Atrial Pressure
mg/dl	-	Milligram per deciliter
Mg^{2+}	-	Magnesium
Mg-ATP	-	Magnesium- Adenosine Triphosphatase
MLCK	-	Myosin Light-chain kinase
mm	-	Millimeter
mv	-	Millivolt
Na^+	-	Sodium
Na^+K^+-ATPase	-	Sodium-Potassium Adenosine Triphosphatase

NaCl	-	-	Sodium Chloride
NICE	-	-	National Institute for Health Excellence
NO	-	-	Nitric Oxide
PCV	-	-	Packed Cell Volume
PGE_2	-	-	Prostaglandin – E_2
PO_4^{2+}	-	-	Phosphate
RBC	-	-	Red Blood Cell
RBL-2H3	-		Rat Basophilic Leukemia
RT	-	-	Renal Tubules Epithelium
SA Node	-		Sino Atrial Node
SAC	-	-	S–allylcysteine
SBP	-	-	Systolic Blood Pressure
SEM	-	-	Standard Error of Mean
SERCA2a	-		Sarcoplasmic Reticulum Calcium
SO_4-		-	Sulphate Ion
SR	-	-	Sarcoplasmic Reticulum
STZ- type		-	Streptozocin-type 1 diabetes
T	-	-	Transverse
TBX_2	-	-	Thromboxane – B_2
TC	-	-	Total Cholesterol
TGL	-	-	Triacylglyceride
TNP	-	-	Trinitrophenyl
UV	-	-	Ultraviolet

WBC - - White Blood Cell

ZOE - - *Zingiber officinale* Extract

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Preamble

The development of medicinal plants in the domain of nutrition has unveiled their additional theraupeutic potentials (Ramaa *et al.,* 2006). In the same era, terms like functional foods, nutraceutical and pharma foods have taken hold of the nutrition market, mainly aiming to provide nutrition and healthy diets (Bar-Chen *et al.,* 2010). Many plants and their components have been extensively investigated for their health-promoting benefits, including antioxidant, cardio-protective, anti-cancer, antimicrobial and immune-modulatory activities (Huffman, 2003; Miller *et al.,* 2004; Hamlaoui-Gasmi *et al*., 2012).

Lifestyle modification such as improved dietary habit, increased physical activities, avoiding alcohol and smoking have been very beneficial in management of some cardiovascular diseases and diabetes mellitus (Colberg *et al.,* 2010). Potential health benefits of vegetables belonging to the *Allium* species; in particular, garlic (*Allium sativum*), have its origin in antiquity, but there is a need to unveil the details of these benefits (Galeone *et al.,* 2006). Garlic has been reputed to possess therapeutic properties, and is probably the most-widely investigated medicinal plant (Nrashan *et al.,* 2010). Several reports established that garlic has cardioprotective effects through lowering plasma cholesterol concentrations, inhibiting platelet aggregation and increasing fibrinolytic activity (Swaran *et al.,* 2009; Asdaq and Inamdar 2011a).

The mechanisms involved are far from clear but garlic contains a number of organosulphur compounds such as S-allyl cysteine, S-allyl mercaptocysteine, allicin, ajoene and diallyl

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sulphide, which are widely believed to be the active agents (Nrashan *et al.*,2010). Tremendous anecdotal evidence supports the invaluable role that garlic has played in the therapy of many diseases since time immemorial (Amagase, 2006). Increasingly, this role is being confirmed by basic and clinical research reports from around the world. Garlic is the best example of the philosophy that your medicine should be your food, and your food should be your medicine (Asdaq and Inamdar 2011b). Garlic has attracted particular attention in modern medicine because of its wide spread health use all over the world, and there is the cherished belief that it helps in maintaining good health, warding off illnesses and provides vitality (Ginter and Simko 2010).

Ginger (*Zingiber officinale*, Roscoe Zingiberaceae) is one of the most widely consumed spices worldwide. From its origin in Southeast Asia and its spread to Europe, it has a long history of use as herbal medicine to treat a variety of ailments including vomiting, pain, indigestion, and cold-induced syndromes (Wang and Wang *et al.*, 2005; White, 2007; Yiming *et al.*, 2012). More recently, it was reported that ginger also possessed anti-cancer, anticlotting, anti-inflammatory, and analgesic activities (Yiming *et al.*, 2012). A number of clinical trials have been undertaken with diverse products containing ginger and ginger products, with various results (Elrokh *et al*., 2010) such as gingerol (S) - (6) and (S) – (8) gingerols.

1.2 Statement of Research Problem

Previous studies have shown the separate effects of *Allium sativum* and *Zingiber officinale* on various cardiovascular such as antihypertensive effect, bradycardiac effect and vasodialatory effect (Ginter and Simko 2010) and heamatological parameters such as leukocytes, packed cell volume and platelet count (Nrashan et al., 2010), but no record of their combined effects. Garlic

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and Ginger are often consumed together, thus a study on their combined effect on some physiological profile will be of paramount significance to learning.

1.3 Justification

Garlic *(Allium sativum)* and ginger *(Zingiber officinale)* are widely consumed as spices in food and drink forms. Several studies have been carried out on these plants separately (Mikaili *et al*., 2013), especially on the heart and blood pressure, but there is paucity of information in the available literature on their combined effects. Although these plants are often consumed in combined form as food species, the beneficial effect of these mixture on physiological parameters have not been documented.

Separate consumption of either of these plants have shown beneficial effects as hypotensive effect, bradycardia, hypolipidemic effect, and improved leukocyte functions which have been widely reported (Yiming *et al.,* 2012); but whether the combination of these plants have the same beneficial effect as the separate effect is not well known. Do the combined effects on some of these parameters differ from the separate effect? These questions are yet to be answered.

Results of investigation into their combined effects on cardioprotective functions and heamatological effects may be beneficial in therapy and prophylaxis of cardiovascular diseases. Since the plants are often consumed in combined form

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1.4 Aim and Objectives

1.4.1 Aim

This study was designed to investigate the separate and combined effects of *Allium sativum* (garlic) and *Zingiber officinale (*ginger) on some cardiovascular, haematological and biochemical parameters of laboratory animals (cats, rats and rabbits).

1.4.2 Specific objectives

The specific objectives of the study were to:

1 Determine effects of separate and combined effects of *Allium sativum* (garlic) and *Zingiber officinale (*ginger) on isolated perfused rabbit heart.

2 Evaluate effects of separate and combined effects of *Allium sativum* (garlic) and *Zingiber officinale (*ginger) on blood pressure in normotensive cats.

3 Determine the combined effects of the extracts on haematological parameters of Wistar rats.

4 Determine effects of separate and combined effects of *Allium sativum* (garlic) and *Zingiber officinale (*ginger) on lipid profile, serum electrolyte concentration and activities of liver enzymes in Wistar rats

1.5 Hypotheses

1. Single administration of either garlic or ginger does not alter the cardiovascular, biochemical and haematological parameters in laboratory animals.

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2. There is no significant difference between the effects exerted by single or combined administration of garlic and ginger on the cardiovascular, biochemical and haematological parameters in laboratory animals.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical background of Garlic

The medicinal use of garlic has a long history. Garlic is probably one of the earliest known medicinal plants (Starek, 2001; Lewis and Elvin-Lewis, 2003). Its bulbs (cloves) had been used as a cure-all in ancient Egypt and are mentioned in the Ebers Papyrus, one of the earliest treatises on medicinal plants (Wichtl, 2004). The word *garlic* comes from Old English *garleac,* meaning *"spear leek."* Dating back over 6,000 years, garlic is native to Central Asia, and has long been a staple in the Mediterranean region, and a frequent seasoning in Asia, Africa and Europe (Starek, 2001). Over the centuries, garlic has acquired a special position in the folklore of many cultures as a formidable prophylactic and therapeutic medicinal agent. Garlic has been a traditional treatment in many countries, notably the Near East, China, and India. It has attracted particular attention of modern medicine because of its widespread use around the world and the cherished belief that it helps to maintain good health by warding off illnesses and providing more vigor (Mikaili *et al* 2013).

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Plate I: Garlic (Allium Sativum) plant (Adam 2010)

Plate II: Garlic (Allium Sativum) cloves (John 2013)

2.1.1 Medicinal uses of garlic

2.1.1.1 Garlic and cardiovascular disease

Garlic is a popular supplement, well-perceived as a healthy choice among people aiming at improving cardiovascular wellness. Approximately 4% of all cardiovascular disease patients and 30 % of cardiovascular patients, who use herbal supplements take, garlic (Yeh *et al.,* 2006). Known risk factors for cardiovascular disease include inflammation, high cholesterol, high homocysteine, hypertension, diabetes and dementia; especially its most common form, Alzheimer's disease. Indeed, as early as the 1920's and 1930's numerous studies highlighted the beneficial cardiovascular effects of garlic (Younis *et al.,* 2010). Garlic is a potent scavenger of oxidants: It increases the activities of superoxide dismutase, catalase glutathione peroxidase, and glutathione concentration. It inhibits lipid peroxidation and inflammatory prostaglandins (Yousuf, 2010).

Garlic reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA. It has been shown to inhibit low-density lipoprotein oxidation, platelet aggregation, and arterial plaque formation, decrease homocysteine and lowers blood pressure. It increases microcirculation which is important in diabetes, where microvascular changes increase heart disease and dementia risks. Garlic may also possess anti-inflammatory abilities to suppress the nuclear factor-kappa B-activation pathway (Madkor *et al.,* 2010).

2.1.1.2 Garlic and hypertension

Garlic has played an important dietary as well as medicinal role in human history (Chobanian *et al.,* 2003). Blood pressure reducing properties of garlic have been linked to its hydrogen sulphide

production (Benavides *et al.,* 2007), and allicin content (Banerjee *et al.,* 2003; Higdon and Lawson, 2005), which has angiotensin II inhibiting and vasodilating effects as shown in animal and human cell studies (Sharifi *et al.,* 2003; Al-Qattan *et al*., 2006; Benavides *et al.,* 2007). Das and co-workers (1995), found some evidences that suggested garlic reduces blood pressure by inhibiting platelet nitric oxide synthase. Nitric oxide is an important local vasodilator which controls several physiological functions of the cardiovascular system (Benavides *et al.,* 2007). Garlic has probably been most popularised as a complementary therapy for blood pressure control, as it is used by approximately 50% of patients who have hypertension (Capraz et *al.,* 2006). *In vitro* studies by Benavides and associates (2007) have confirmed the vasoactive ability of sulphur compounds contained in garlic. The study showed that erythrocytes convert garlic's organic polysulphides into hydrogen sulphide, a known endogenous cardio-protective vascular cell signaling molecule. In one pilot study, a high dose of garlic tablet (2400 mg, containing 31.2-mg allicin) had been used and overall blood pressures decreased at approximately 5 h after the dose, including a 16 mm drop in diastolic pressure (Asdaq and Inamdar, 2010a).

2.1.1.3 Anti-platelet or Anti-thrombic effect of garlic

Garlic and its components are known to possess anti-platelet activity which has been demonstrated mostly *in vitro* (Lawson *et al.,* 1998), and several platelet inhibitors have been isolated and characterized from garlic. The inhibitory effects of garlic extracts as well as allicin, ajoene and other individual garlic compounds on thrombus formation and platelet aggregation has been reported (Apiz-castro *et al.,* 1992). Cavagnavaro *et al.* (2007) studied the effect of cooking on garlic anti-platelet activity and its content in thiosulphates. Their results suggested that allicin and thiosulphates are responsible for *in vitro* anti-aggregatory activity. The study

carried out by Chang *et al.* (2004) showed that the alkenyl thiosulphate sodium 2-propenyl thiosulphate obtained from boiled garlic has the potential to prevent cardiovascular disease by inhibiting platelet aggregation in dogs and humans *in vitro* as these compounds are not volatile, these compounds are considered heat-stable platelet inhibitory factors.

2.1.1.4 Garlic and immune system modulation

Garlic has been suggested as a promising candidate for maintaining the homeostasis of immunomodulatory activity (Chandrashekar and Venkatesh, 2012). Several studies have been carried out on animal models to examine the effect of different garlic components and formulations on immunomodulatory activity (Kyo *et al.,* 2001). Aged garlic extract (AGE) has been shown to exert anti-allergic effect as it may directly and / or indirectly modify the functions of mast cells, basophils and lymphocytes, which play a leading role in the allergic cascade reactions, including inflammation. The pharmacological effect of AGE to inhibit the tumour cell growth through immune stimulation has also been described (Henieh *et al.,* 2012). Garlic appears to be effective for restoration of the immune suppression by different agents such as chemotherapy, UV irradiation and physical and psychological stress (Dwight *et al.*, 2006; Asdaq and Inamdar, 2010). Age-related deterioration of learning behavior and abnormal impairment of immune response, as occurs with acquired immunedeficiency syndrome (AIDS) have been reported to be improved by the immunomodulatory effect of garlic (Mikaili *et al*., 2013)

2.1.1.5 Garlic and lipid profile

Several *in vitro* studies have indicated that garlic and its constituents inhibit certain enzymes involved in the cholesterol and fatty acids biosynthesis in hepatocytes of humans (Liu and Yeh, 2001). It has also been shown that more water soluble compounds like S-allylcysteine (SAC) present in Aged garlic extract (AGE) are less cytotoxic and more efficient in inhibiting cholesterol biosynthesis than the lipid-soluble sulphur compound (Yeh and Liu, 2001). One survey mentioned that garlic has antihyperlipidemic, hypocholesterolaemic and hypotriacylglyceride activities (Campbell *et al.,* 2001). The hypolidemic effect was due to the organosulphur compound in garlic (Lii *et al.,* 2012). Also in another study, Ashraf *et al.* (2005) demonstrated that a dietary supplementation of garlic reduced the atherogenic properties of cholesterol and maintained the NO-mediated endothelial function in rats. The ogano-sulphur compounds are the main active constituents responsible for the hypolipidemic and hypocholesterolemic effects of garlic, as much as in humans as in experimental animals (Liu and Yeh, 2002).

2.1.1.6 Garlic and diabetes mellitus

A number of animal studies support the effectiveness of garlic in reducing blood glucose in streptozotocin-induced as well as alloxan-induced diabetes mellitus in rats and mice. Most of the studies showed that garlic can reduce blood glucose level in diabetic mice, rats and rabbits (Banerjees and maulik, 2004). One Iranian study evaluated oral administration of garlic extract for 14 days on the level of serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine in normal and streptozotocin-induced diabetic rats. Administration of the garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine

and activities of aspartate aminotransferase and alanine aminotransferase, while it increased serum insulin in diabetic rats. Interestingly, a comparison was made between the action of garlic extract and glibenclamide, a well-known antidiabetic drug. The antidiabetic effect of garlic extract was more effective than that observed with glibenclamide (Eidi *et al.*, 2006).

2.1.1.7 Garlic and homocysteine

Elevated plasma homocysteine concentrations have been associated with an increased risk of vascular disease as high homocysteine is known to inhibit endothelial-cell proliferation (Weiss *et al.*, 2006; Seyar, 2007) and contribute to atherothrombotic events. In one animal study by Yeh *et al*. (2006), the addition of garlic extract decreased plasma total homocysteine concentration by 30%.

2.1.1.8 Anti-allergic effect of garlic

A number of reports have revealed the relationship between foods and allergic responses. Tanaka *et al.* (1992) reported that *Allium* vegetables, including garlic, inhibit the release of β-hexosaminidase, which is correlated with histamine release, in rat basophilic leukemia cells (RBL-2H3), suggesting an anti-allergic effect. Usui and Suzuki (1996) reported that the anti-allergic activity of (*Z*)-ajoene might be due to its inhibition of chemical mediator release (Kyo *et al*., 2001). Histamine release in the rat basophil cell line, RBL-2H3 was induced by mouse anti-trinitrophenyl (TNP) monoclonal antibody and the TNP-bovine serum albumin (BSA) hapten carrier complex. AGE added to the culture medium at doses of 1.25, 2.5 and 5.0 g/100 g significantly inhibited the antigen-specific histamine release by 50, 80 and 90%, respectively. Oral administration of AGE, (10 mL/kg) also decreased 25 - 45% of the ear swelling, used as an

index of immunoglobulin (Ig)E–mediated skin reaction, which was caused by intravenous administration of anti-TNP Ig E antibody and subsequent picryl chloride painting on the ear in mice (Eikai *et al*., 2001).

2.2 Historical background of Ginger

Ginger (*Zingiber officinale*), Roscoe belonging to the Family *Zingiberaceae,* is a perennial herb with thick tuberous rhizomes. The erect leafy aerial stem grows up to approximately 1 meter in height and has purple flowers. Its roots are used as spice in cooking throughout the world. The ginger plant has a long history of cultivation. It originated in China and then spread to India, South East Asia, West Africa and the Caribbean (McGee, 2004; Wang and Wang, 2005;White, 2007).

It has been used in Unani, Ayurvedic and Chinese herbal medicines for a wide array of unrelated ailments that include arthritis, rheumatism, sprains, pains, muscular aches, sore throats, indigestion, vomiting, fever, hypertension, cramps, constipation, dementia, helminthiasis and infectious diseases **(**Ali *et al*, 2008**).** Ginger was known in China as early as 400BC. It was used as a spice by the Greeks and Romans who considered it an Arabian product because it was received from India by way of the Red Sea (Shariq *et al.,* 2011). Ginger was also known to Europeans as it was an important item in European Commerce with the East during the middle age (Evans, 2003).

Plate III: Ginger (*Zingiber officinale)* plant (Bradtke 2007).

Plate IV: Ginger (Zingiber officinale) Rhizome (Bradtke 2007)

2.2.1 Nutrient and active constituents of ginger

Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fibre and 12.3% carbohydrates (Yimming et al., 2012). The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C (Wang 2009). The composition of ginger varies with the type, variety, agronomic conditions, curing methods, drying and storage conditions (Rehman *et al.,* 2011). The active ingredients in *Zingiber officinale* are in volatile oil. The concentrations of active ingredients vary with growing conditions. The major active ingredients in ginger oil are the sesquiterpenes: bisapolene, zingiberene, and zingiberol. Ginger's active ingredients have a variety of physiologic effects such as antihypercholesterolaemic effect, hypotensive effect, decreased body weight gain, hypoglycemic, anticancer, anti-inflammatory and analgesic activities (Rehman *et al.,* 2011).

2.2.2 Uses of Ginger in traditional medicine and food industry

Ginger has been used by traditional Chinese and Indian medicine for over 25 centuries (Badreldin *et al.,* 2008). Ginger was brought to Mexico by the Spaniards and later introduced to Jamaica, the latter currently being one of the world's foremost producers of ginger (Wichtl, 2004). Ginger has been introduced into various tropical countries where diverse chemotypes have been developed (Yiming *et al.,* 2012). Ginger is recommended to relieve and prevent nausea, caused by motion sickness and morning sickness (Lien *et al.,* 2003). Apparently, this effect is not mediated through the central nervous system (CNS), but rather, ginger's active principles act directly on the gastrointestinal tract (Yiming *et al.,* 2012). It has been hypothesized that ginger ameliorates the nausea associated with motion sickness by preventing the

development of gastric dysrrhythmias and the elevation of plasma vasopressin (Lien *et al.*, 2003).

Ginger has been made into candy and used as flavoring for cookies, crackers and cakes as well as flavour in ginger ale-a sweet, carbonated, non-alcoholic beverage, ginger bread, ginger snaps, ginger cake and ginger biscuits (McGee, 2004). It is extensively used in preparation of dietaries for its aroma and flavour. Dry ginger is used in the manufacture of oil, oleoresin, essence and processed meat (Madkor et al., 2010).

Ginger contains up to 3% of an essential oil that causes the fragrance of the spice (Shalaby, and Hamowieh, 2010). The main constituents are sesquiterpenoids with *zingiberene* as the main component. Other components include β-sesquiphellandrene bisabolene and farnesene which are also sesquiterpenoids, (β-sesquiphellandrene, cineol and citral) (Rani, *et al.*, 2012). The pungent taste of ginger is due to nonvolatile phenylpropanoid – derived compounds, gingerols and shogaols. The shogaols are formed from gingerols when ginger is dried or cooked (Abdularaq *et al.*, 2011). It can be cooked as an ingredient in many dishes. They can be stewed in boiling water to make ginger tea, to which honey is often added as a sweetener. The juice of ginger roots is extremely potent and is often used as spice to flavour dishes such as seafood, mutton, snacks or stew (Yiming 2012).

2.2.3 Medicinal uses of Ginger

2.2.3.1 Effects of ginger on lipid profile

Studies on the effects of spices especially ginger (*Zingiber officinale* Roscoe) and their extracts on lipid profile have reported favourable outcomes with regards to cholesterol levels. For example, Bhandari *et al*. (2005), observed a significant lipid lowering activity of ethanol extract of *Zingiber officinale* in diabetic rats. Al- Amin *et al*. (2006) concluded that raw ginger possessed hypoglycaemic, hypocholesterolaemic and hypolipidaemic potential. Tanabe *et al*. (1993) isolated principles from ginger observed to exhibit an inhibitory effect on cholesterol biosynthesis.

2.2.3.2 Anti-inflammatory and analgesic activities of ginger

The anti-inflammatory properties of ginger have been reported (Afzal *et al.,* 2001; Grzanna *et al.,* 2005). Several lines of evidence have been provided, mostly in different animal models of inflammation, and to a much lesser extent in humans or human cells, of the effectiveness of either ginger or of compounds isolated there from against inflammation and its mediators (Charlier and Michaux, 2003; Martel-Pelletier *et al.,* 2003; Badreldin *et al.,* 2008). More recently, it has been shown that ginger (and some of its constituents) is effective against cytokines synthesized and secreted at sites of inflammation (Grzanna *et al.,* 2005). Cytokines are small proteins secreted at sites of inflammation by lymphocytes, macrophages, fibroblasts and other cells, and act as chemical messengers between cells involved in immune and inflammatory responses. Ginger was found to modulate some biochemical pathways activated in chronic inflammation (Grzanna *et al.,* 2005).

2.2.3.3 Anti-oxidant actions of ginger

The essential oil with volatile compounds (prepared by hydrodistillation) showed also a good antioxidant activity with its phenolic content (El-Ghorab *et al.*, 2010). Furthermore, antioxidant activity of both volatile and non-volatile fractions has been ascribed to the synergistic effects of phenolics (such as eugenol, shoagols, zingerone, gingerdiols, gingerols, *etc.*).

As confirmed by GC-MS, the most abundant terpenes in ginger essential oil are zingiberene, ar-turmerone, curcumene, tumerone and curlone (Singh *et al.*, 2008). Hydroethanolic extract of the rhizome ginger is known for its strong free-radical reducing efficacy (Ali *et al.*, 2008). It is mediated mostly by its phenolic constituents that may be divided into two groups: gingerol-, gingeron- and shogaol-related group and diarylheptanoids. A mixture of non-phenolics (sesquiterpene hydrocarbons, carbonyl compounds, monoterpene hydrocarbons and esters) contributes to the antioxidative activity and is responsible for the strong aroma of ginger in food, beverages and dietary supplements (Ali *et al.*, 2008).

Several authors have shown that ginger is endowed with strong in vitro and in vivo anti-oxidant properties. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of radiation (Jagetia *et al.*, 2003; Haksar *et al.*, 2006) and a number of toxic agents such as carbon tetrachloride and cisplatin (Amin and Hamza, 2006; Yemitan and Izegbu, 2006), and as an anti-ulcer drug (Siddaraju and Dharmesh, 2007). Kim *et al.* (2007) showed that 6-gingerol is endowed with strong anti-oxidant action both in vivo and in vitro, in addition to strong anti-inflammatory and anti-apoptotic actions

2.2.3.4 Tissue and radio-protective effects of ginger

Several extracts and fractions of *Z. officinale* have been shown to protect against chemically-induced tissue damage (Badreldin *et al.,* 2008). For example, it has been shown by Yemitan and Izegbu (2006) that pretreatment of rats with an ethanol extract of the rhizome of *Z. officinale* and oil extracted from the plant were effective in ameliorating carbon tetrachloride and acetaminophen or paracetamol-induced acute hepatotoxicity. The radio-protective effect of the hydroalcoholic extract of ginger rhizome was studied in mice given the extract at an intraperitoneal dose of 10 mg/kg, once daily for five consecutive days before exposure to 6–12 Gγ of gamma radiation, and were monitored daily up to 30 days post-irradiation for the development of signs of radiation sickness and mortality (Jagetia *et al.,* 2003). The protection of ginger against radiation lethality was confirmed by the same authors in a subsequent publication (Jagetia *et al.,* 2004). Pretreatment of mice with ZOE reduced the severity of radiation sickness and the mortality, and protected mice from gastrointestinal syndrome, as well as bone marrow syndrome. The dose reduction factor for ZOE was found to be 1.15. The optimum protective dose of 10 mg/kg ZOE was 1/50 of the LD_{50} (500 mg/kg). It has been shown by Sharma *et al.* (2005) that ginger extract mitigates the neuro-behavioral effects of gamma radiation-induced conditioned taste aversion in Sprague– Dawley rats. Administration of the extract 1 h before 2-Gy gamma irradiation was effective in blocking the saccharin avoidance response for five post-treatment observational days, both in a dose- and time-dependent manner, with 200 mg/kg i.p., being the most effective dose.

2.2.3.5 Effect of ginger on blood pressure

Afzal *et al.* (2001) have suggested that ginger exerts many direct and indirect effects on blood pressure and heart rate. Ghayur and Gilani (2005) reported that the crude extract of ginger induced a dose-dependent fall in the arterial blood pressure of anaesthetised rats. In guinea pig paired atria, the crude extract exhibited a depressant activity on the rate and force of myocardial spontaneous contractions. In rabbit thoracic aorta preparation, the crude extract relaxes the phenylephrine-induced vascular contraction at a dose 10 times higher than that required against K^+-induced contraction. Ca^{2+}- channel-blocking activity was confirmed when the crude extract shifted the Ca^{2+} - dose–response curves to the right, similar to the effect of verapamil (Badreldin *et al.*, 2008).

The extract also inhibited the phenylephrine control peaks in normal Ca^{2+}-plus and Ca^{2+}-free solutions, indicating that ginger extract acts at both the membrane-bound and the intracellular Ca^{2+} channels. When tested in endothelium- intact rat aorta, it again relaxed the K^+-induced contraction at a dose 14 times less than that required for relaxing the phenylephrine -induced contraction (Badreldin *et al.*, 2008). The vasodilator effect of the crude extract was endothelium-independent because it was not blocked by either L-NAME (a non-selective inhibitor of nitric oxide synthase used experimentally to induce hypertension) or atropine. The effect was also reproduced in the endothelium-denuded preparations in the same dose range. Data indicate that the blood pressure-lowering effect of ginger is mediated through the blockade of voltage-dependent calcium channels. In another study, Ghayur *et al.* (2005) concluded that the blood pressure-lowering action of aqueous ginger extract was exhibited via a dual inhibitory effect, mediated via stimulation of both muscarinic receptors and blockade of Ca^{2+} channels.

Interestingly, Ghayur *et al.* (2005) also suggested that the different constituents of ginger may have opposing actions on the reactivity of blood vessels. For example, an atropine-resistant and L-NAME- sensitive vasodilator activity are demonstrated for the ginger phenolic constituents (Badreldin *et al.*, 2008).

2.2.3.6 Effect of ginger on blood clotting

The effect of an aqueous extract of ginger on platelet thromboxane-B_2 (TBX_2) and prostaglandin-E_2 (PGE_2) production was examined after giving rats a raw aqueous extract of ginger daily for a period of 4 weeks, either orally or intraperitoneally (IP) (Thomson *et al.*, 2002; Badreldin *et al.*, 2008). A low dose of ginger (50 mg/kg) administered either orally or IP did not produce any significant reduction in the serum TBX_2 levels. However, ginger administered orally caused significant changes in the serum PGE2 at this dose. High doses of ginger (500 mg/kg) were significantly effective in lowering serum PGE_2 when given either orally or IP. However, TXB_2 levels were significantly lower in rats given 500 mg/kg ginger orally, but not IP. These results suggest that ginger could be used as an anti-thrombotic and anti-inflammatory agent (Thomson *et al.*, 2002; Badreldin *et al.*, 2008).

2.2.3.7 Effects of ginger on blood glucose level

A number of studies have examined the efficacy of ginger in hyperglycaemia control *in vitro* and *in vivo* on cell and animal models and in clinical trials. Investigation of the active principles and mechanism of action have also been undertaken (Yiming *et al.*, 2012). Results from animal studies have shown that oral administration of an ethanolic extract of ginger (800 mg/kg) significantly decrease fasting blood glucose level after 1 hour treatment in an STZ-type 1 diabetic rat model. The effect peaked after 4 hours, with ginger producing a 24% to 53%

reduction in blood glucose at doses ranging from 100 to 800 mg/kg (Ojewole, 2006). Another study showed that a single dose of ginger juice prevented 5-hydroxytryptamine-(5-HT-) induced acute hyperglycemia, suggesting the involvement of 5-HT receptors in the glycaemic control (Akhani *et al.*, 2004)

2.3 The Serum Electrolytes

Electrolytes are vital to health, and act as chemical messengers in the body, carrying electrical impulses from the nerves to control all tissue functions including movement. An imbalance of any of the electrolytes can lead to serious disruptions in physiologic functions (Thomas 2011).

Many processes in the body are highly dependent on serum electrolytes. They include primarily heart and nerve functions, muscle coordination and control, and maintenance of the body fluid levels. Electrolytes are found throughout the body tissues. The blood, plasma and interstitial fluid that bathe the cells is high in sodium (Na^+) and chloride (Cl^-), which is similar to common table salt, or sodium chloride (NaCl). Electrolytes are not evenly distributed within the body, and is that allows many important metabolic reactions to occur. Sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl^-), phosphate (PO_4^{2-}), bicarbonate (HCO_3^-), and sulphate (SO_4^-) ions are important electrolytes in humans (Sharif 2003).

2.3.1 Sodium

Sodium affects how much urine the kidney produces, and is involved in the transmission of nerve impulses and muscle contraction (Rebholz et al., 2012). Too high a concentration of sodium in the blood causes hypernatraemia. Causes of hypernatraemia include excessive water loss (for example, severe diarrhoea), restricted water intake, untreated diabetes (which causes

water loss), kidney disease, hormonal imbalances and excessive salt (NaCl) intake (Carbone *et al*., 2005).

Symptoms include signs of dehydration such as extreme thirst, dark urine, sunken eyes, fatigue, irregular heartbeat, muscle twitching, seizures and coma (Rebholz *et al*., 2012). Too low a concentration of sodium in the blood causes hyponatraemia. This is one of the most common electrolyte imbalances, and occurs in about 1% of hospitalized individuals. It can result from vomiting, diarrhoea, severe burns, taking certain drugs that cause the kidneys to selectively excrete sodium, inadequate salt intake, water intoxication, hormonal imbalances, kidney failure, and liver damage. Symptoms include nausea, vomiting, headache, tissue swelling (oedema), confusion, mental disorientation, hallucinations, muscle trembling, seizures, and coma (Kenney 2004).

2.3.2 Potassium

Potassium ions play a major role in regulating fluid balance in cells, the transmission of nerve impulses and in muscle contraction (Hawkins, 2006). Too high a concentration of potassium causes a condition called hyperkalaemia that is potentially life-threatening. The most common cause is kidney failure. It can also result from severe burns or injury (excess potassium released from injured cells), inadequate adrenal hormones (Addison's disease), the use of certain medications such as excessive use of potassium supplements (Krishnamurti & Shyamala 2001). Some times hyperkalaemia occurs in conjunctions with hypernatraemia. Symptoms include nausea, diarrhoea, weakness, muscle pain, and irregular heartbeat, and coma and / or death may ensue. Abnormally low concentrations of potassium cause hypokalaemia. Hypokalaemia can result from excess adrenal hormones (Cushing's disease), kidney disease, and long-term use of

certain diuretic drugs, laxative abuse and bulimia and kidney failure. Symptoms include increased production of urine, muscle pain, paralysis, irregular heartbeat and low blood pressure (Hawkins, 2006).

2.3.3 Calcium

Calcium is needed to build and maintain bones. It is involved in nerve impulse transmission and muscle contraction. Excess calcium results in hypercalcaemia (Loren 2012). Hypercalcaemia can be caused by excess parathyroid hormone, certain cancers, some genetic disorders, and excessive use of antacids, containing calcium in rare cases (Ghayur & Gilani 2005). Symptoms include bone and muscle pain, mental changes such as depression and confusion, increased urine production, fatigue, nausea and vomiting. Abnormally-low concentrations of calcium cause hypocalcaemia (Rebholz *et al*., 2012). Hypocalcaemia can be caused by too little parathyroid hormone, kidney failure and vitamin D deficiency. Vitamin D is necessary for the body to absorb calcium. Symptoms include muscle twitches and spasms, convulsions, mental changes such as depression and irritability, dry skin, and brittle nails (Loren, 2012).

2.3.3.1 Role of calcium in the blood and the calcium pump

Heart disease is caused in great part by free radicals causing damage to individual cells inside the arteries. One of the sites where such damage takes place is the "calcium pump" inside cells in the arteries, particularly the cells within the middle layer of the artery (Niedziocha, 2011). When the free-radical bombardment stops, the damage can be reversed. The amount of calcium in the blood stream is a critical factor in controlling the beat of the heart. If there is too much or too little calcium in the blood stream, the heart can simply stop beating (Loren, 2012). Calcium

controls the contraction of the heart as it is pumping blood through the body. Magnesium controls the relaxing of the heart, when no blood is being pumped out. If either one or the other starts to increase in quantity, the body has some very well established mechanisms to bring the balance back to normal. This is why the consumption of a large amount of calcium (as a supplement) does not impair the heart beat. The large and sudden increase of calcium (or magnesium) flood through the blood stream increases the potential for sudden calcium imbalance and cardiac arrest (Loren, 2012).

2.3.3.2 Role of calcium and the heart beat

It has been reported that failing hearts do not handle calcium properly (Krishnamurti & Shyamala 2001). The contraction of heart muscle cells – like all muscle cells – is controlled by cycling levels of calcium, which is stored in the sarcoplasmic reticulum (SR). In response to the electrical impulses that control heart beat, calcium is released from the SR into the cytosol stimulating the cell to contract. After contraction, calcium moves back from the cytosol into the SR via a molecular calcium pump, the SERCA2a protein. In heart failure, the return of calcium into the SR is diminished. With this disruption to the normal cycling of calcium, the muscle cells cannot respond appropriately to the heartbeat impulses. They contract weakly, and the heart's pumping activity decreases. Animal studies also had shown that increasing production of SERCA2a improved heart function, both in individual cells and in living animals (Hawkins, 2006). Free radicals also cause tissue calcification by damaging the integrity of cell membranes, causing leaks in cell walls, and by damaging enzymatic cell-wall transport pumps. If the calcium pump is weakened or if cell wall integrity is damaged, the calcium pump is unable to remove

calcium as it leaks in. Intracellular calcium accumulates, causing malfunction and eventually cell death (Nielziocha, 2011).

2.3.3.3 Calcium-channel blockers

Once the calcium pump is damaged; for instance, by free radicals, a class of drugs called calcium-channel blockers are often administered to the patients (Loren, 2012). The toxic metals that cause the free radicals which damage the calcium pump are better removed. For example, calcium-channel blockers are widely used for angina, arrhythmia and hypertension. However, the initial damage to the calcium pump has multiple dietary and lifestyle causes, including pesticides and other xenobiotic exposures, transfatty acids and nutritional deficiencies. Studies confirm that mere poisoning of the calcium channels with calcium-channel blockers leads to an accelerated rate of cancers and other diseases, resulting also in impairment of other membrane functions (Loren, 2012).

Calcium has many different functions inside the body. Apart from building the bones, it is involved in the beating of the heart and its normal contraction. The heart beats through a spontaneous excitation of specialized cardiac cells called SA node. Depolarisation of the cells results in a contraction that prompts the atrioventricular node and ventricles of the heart to contract in rhythm (Rebholz et al., 2012). The resting state of cardiac cells is -90 mV, as depolarisation occur the cells increase up to zero mV. Positive sodium ions begin to slowly seep into the sinoatrial node cells, which opens the gates for positive calcium ions to rush in. Calcium helps the beginning of a heartbeat, but it is also a mediator in initiating the other cardiac cells to beat. Once the SA node depolarises, slow calcium channels open to the other cardiac cells, and calcium triggers the contraction of the muscle fibres (Karppanen 1991).

2.3.3.4 Hypercalcaemia and hypocalcaemia

Hypercalcaemia is caused by an excess of calcium. Excess calcium adversely affects the heart causing bradycardia (Campbell, 2012). Hypocalcaemia occurs when there is too little calcium in the body and results in tachycardia. These two disorders affect the heart rate by influencing the contraction strength of the heart beat. Fortunately, even with a moderately healthy diet, calcium imbalances are rare. Significant and independent associations occur between high dairy products and dietary calcium intakes and reduced blood pressure among middle-aged men. Dietary calcium is an important factor in the control of blood pressure via its effects on calcium metabolism (Hawkins 2006).

2.3.4 Chloride

Chloride is involved in regulating blood pressure. High concentrations of chloride, called hyperchloraemia, can be caused by kidney failure, kidney dialysis, and an overproduction of parathyroid hormone. Symptoms include weakness, headache, nausea, and vomiting (Kenney, 2004). In people with diabetes mellitus, hyperchloraemia impairs the control of blood glucose levels. Hypochloraemia often occurs along with hyponatraemia or hypokalaemia, and is caused by excessive fluid loss (for example, diarrhoea). Serious deficiencies of chloride decrease blood acidity, resulting in metabolic alkalosis. Symptoms of severe hypochloraemia include confusion, paralysis and dyspnoea (Rebholz *et al*., 2012).

2.4 Neural Control of Blood Pressure

Blood pressure is a function of vascular resistance and cardiac output, two variables that are controlled by the autonomic nervous system. In turn, cardiac output is dependent on three regulated variables: end-diastolic volume, myocardial contractility and heart rate. End-diastolic volume is the volume reached by the ventricular chamber before contraction. It is determined by venous pressure, which is related to blood volume and venous smooth muscle tone, both of which are under sympathetic control. Myocardial contractility and heart rate are regulated by both the sympathetic and parasympathetic divisions of the autonomic nervous system. On a short-time scale (seconds to hours), the autonomic nervous system adjusts the circulation in keeping with behaviour (for example, feeding and exercise), the environment (for example, thermoregulation) and emotions (for example, fright). These circulatory changes are components of more global autonomic response patterns that are elaborated in large portions of the central nervous system in long-term blood pressure control, which is not well understood (Cowley, 1992; Osborn, 2005).

2.4.1 Regulation of blood pressure

The maintenance of blood pressure is multifactorial, as a result many factors are implicated in the cause of hypertension. There are physiological variations that affect the blood pressure such as age, gender, body mass index, anxiety and exercise. The regulation of blood pressure is by three major mechanisms: nervous, hormonal and renal mechanism. (Seyer et al., 2007).

2.4.2 Endogenous regulation of blood pressure

The endogenous regulation of arterial pressure is not completely understood, but the following mechanisms of regulating arterial pressure have been well-characterized:

1. Baroreceptor reflex: Baroreceptors in the high pressure receptor zones, mainly in the aortic arch and carotid sinus detect changes in arterial pressure. These baroreceptors send signals ultimately to the medulla of the brain stem, specifically to the rostral ventrolateral medulla. The medulla, via the autonomic nervous system, adjusts the mean arterial pressure by altering the force, speed of the heart's contractions, and the total peripheral resistance. The most important arterial baroreceptors are located in the left and right carotid sinuses and in the aortic arch.
2. Renin-angiotensin system: This system is generally known for its long-term adjustment of arterial pressure. It allows the kidneys to compensate for loss in blood volume or drops in arterial pressure by activating angiotensin II.
3. Aldosterone release: This steroid hormone is released from the adrenal cortex in response to angiotensin II or high serum potassium levels. Aldosterone stimulates sodium retention and potassium excretion by the kidneys. Since sodium is the main ion that determines the amount of fluid in the blood vessels by osmosis, aldosterone increases fluid retention, and indirectly, arterial pressure.
4. Baroreceptors: In low pressure receptor zones (mainly in the *venae cavae* and the pulmonary veins, and in the atria) result in feedback by regulating the secretion of antidiuretic hormone (vasopressin), renin and aldosterone. The resultant increase in blood volume results in an increased cardiac output by the Frank–Starling law of the heart, increasing in turn the arterial blood pressure (Rebholz *et al.*, 2012).

2.4.3 Ion channels in the control of blood pressure

Resnick (1999) has proposed a unifying 'ionic' hypothesis, taking into consideration cytosolic free calcium and magnesium concentrations in a variety of cells and tissues. The hypothesis also considers a variety of clinical conditions in relation to the concentrations of these ions in normal subjects. Calcium and magnesium contribute to the final common pathways, mediating not only blood pressure, but also the major metabolic processes in all cells. Resnick (1999) provides a cellular explanation for the heterogeneity of hypertension as well as the co-existence of several clinical conditions such as hypertension, obesity and non-insulin dependent diabetes mellitus. Steady-state elevation of cytosolic free calcium is associated with a suppression of cytosolic free magnesium in many tissues (Loren 2012). The concomitant changes alter many cellular functions. In blood vessels, this causes vasoconstriction, arterial stiffness and hypertension. The ionic changes result in hypertrophy in the heart, aggregation and thrombosis in platelets and activation of the central nervous system. The alterations in cellular ionic steady state become critical in defining the pathophysiology shared by hypertension, cardiac hypertrophy and dyslipidaemia, including obesity, non-insulin dependent diabetes mellitus and vascular disease. This may, as detailed later, also explain the heterogeneity of hypertension (Whelton *et al*., 1997). Vascular plasma membrane depolarization through decreased K^+ channel activation or stretch produces opening of the voltage-dependent T and L Ca channels or 'leak' channels, with subsequent calcium current representing influx into the cytosol of extracellular calcium. This increase in intracellular calcium induces activation of specific ryanodine-sensitive Ca-release channels in the sarcoplasmic reticulum and magnifies the initial localised increase in Ca^{2+}more generally throughout the cytosol. At the contractile site, the increase in calcium initiates the cascade of molecular rearrangements of calmodulin and myosin light-chain kinase (MLCK),

resulting in myofilament shortening and vasoconstriction. Muscle relaxation is a reversal of the ionic balance through the sarcoplasmic reticulum-mediated reuptake of calcium into intracellular storage as well as the plasma membrane Ca-ATPase and Na/Ca exchange-mediated calcium egress from the cell. These would result in reducing Ca^{2+}. In electrically non-excitable tissues, intracellular calcium is augmented primarily through stimulation of the phospholipase C-mediated inositol-1, 4, 5-triphosphate (IP3) pathway (Ghayur & Gilani 2005). Some tissues have both voltage/receptor-mediated and intracellular mobilization pathways to augment intracellular calcium. The end-result of this may include processes such as hormone secretion and renal ion secretion. The role of calcium in regulating cellular processes, including vascular tone, is dependent upon (and is modified by) cellular magnesium is a co-factor for all phosphotransferase reactions, in all ATP-dependent reactions, where Mg-ATP is the substrate and in all thiamine triphosphate-dependent reactions. Thus, it participates in intermediate metabolism of lipids, carbohydrates and amino acids is required for (and modifies) calcium entry and egress and other processes that use plasma membrane or endoplasmic reticulum Ca-ATPase and $Na^{+}K^{+}$-ATPase. Other processes in which magnesium is required for calcium influx and efflux include L-channel Ca current, calcium binding to calmodulin, Ca-activated K^{+} current and IP3-mediated Ca release from cellular stores. The steady-state concentrations of calcium and magnesium are reciprocally related. The net result is to maintain 'steady state cell metabolism' (Hawkins 2006), and buffer cell response to calcium dependent stimuli. A cellular magnesium deficiency, by decreasing the function of a wide range of enzymes, membrane pumps and ion pumps, may exaggerate Ca-induced cell stimulation in all cells and tissues. In the vasculature, it may results in vasoconstriction. Elevation of Ca^{2+} with a reciprocal decrease in magnesium is consistently observed in subjects with hypertension, obesity and non-insulin dependent diabetes mellitus.

These same changes are also observed in cells from healthy elderly persons. There is a strong correlation between cellular ionic steady state in individual subjects and the pathophysiological changes in the tissues of these individuals (Loren 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1. Animal materials

- Four Cats
- Four Rabbits and
- Thirty five Wistar Rats

3.1.2. Plant Materials

- Fresh Garlic Cloves
- Fresh Ginger Rhizomes

3.1.3. Drugs/Chemicals

- Adrenaline
- Propranolol
- Heparin
- Normal Saline
- Distill Water
- Acetylcholine
- Atropine
- Ringer Locke's Solution

3.1.4 Equipments

- Recording Microdynanometer
- Recording Physiograph
- Microscope
- Organ Bath
- 5ml Syringe

3.1.5 Management of experimental animals

Thirty five (35) Wistar rats of both sexes between the ages of 12 - 16 weeks' old and weighing between 150-200 g were procured from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were kept in well aerated laboratory cages in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. The rats were given access to standard animal feeds and drinking water *ad libitum* during the experimental period.

3.1.6 Collection and preparation of plant extracts

The fresh plants of garlic bulbs and fresh rhizomes of ginger were purchased from Samaru market in Zaria (11^0 10′ N, 07^0 38′ E) Nigeria. The plant bulbs and rhizomes where taken to the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where they were identified by a Taxonomist Mallam Musa Mohammed, and the voucher numbers 050913 for garlic and 0250913 for ginger were deposited. They were dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder of garlic (150

g) and ginger (208 g) was macerated in cold water at room temperature for 24 hours. This was then filtered using a filter paper (Whatmann size No. 1) and the filtrate was evaporated to dryness in water bath at 30 ^{0} C to a dry residue of garlic (24 g) and ginger (34 g). The powder was kept in an air-tight bottle until it was reconstituted for administration.

3.2 Methods

3.2.1 Acute toxicity studies of garlic bulbs and ginger rhizome extract on Wistar rats

The median lethal dose (LD_{50}) of the plant extracts was carried out in order to determine a suitable dose for its evaluation. This was done in two phases according to the method described by Lorke (1983) using 12 rats. In the initial phase, rats were divided into three groups of three rats each, and were treated with 10 mg, 100 mg and 1000 mg of the extract per kg body weight orally, respectively. The rats were observed for 24 h for signs of toxicity, including death. Based on the results of phase one, three fresh rats were divided into three groups of one rat each, and were treated with 1600, 2,900 and 5,000 mg of the extract per kg body weight respectively. The rats were also observed for 24 h for signs of toxicity, including death. The LD_{50} was then calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is, the geometric mean of the consecutive doses, for which 0 and 100% survival rates were recorded.

3.2.2 Preliminary qualitative phytochemical screening of the plant extract

Preliminary qualitative phytochemical screening of the plant was carried out as described by Trease and Evans (1996s).

Procedure for phytochemical screening.

Half of the crude extract was partitioned with petroleum either benzene, chlorofoam and ethyl acetate. The residue left was dissolved in methanol and the various fractions obtained were all subjected to phytochemical screening employing the standard screening test (Evans and Trease, 1996)

3.2.2.1 Test for carbohydrate

- Molisch test

To a small portion of the extract in a test tube, few drops of Molisch reagent was added and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish coloured ring at the interphase indicates presence of Carbohydrates (Evans 1996).

- Fehling test

To a small portion of the extract in a test tube, 5ml of an equal mixture of Fehling solution A and B was added and boiled on a water bath, brick red precipitate indicate presence of reducing sugar (Evans., 1996).

3.2.2.2 Test for glycosides

To a portion of the extract, 5ml of dilute sulphuric acid was added and bolied on water bath for 10-15 minutes. This was then cooled and neutralized with 20% KOH. I t was then divided in two portions:

- To the first portion, 5ml of a mixture of Fehling's solution A and B was added and boiled: a brick red precipitate shows the release of reducing sugar as a result of hydrolysis of glycoside (Evans., 1996)
- To a second portion, about 3ml of Ferric chloride solution was added; a green to blue color was produced due to release of phenolic aglycones due to the hydrolysis. (Evans., 1996)

3.2.2.3 Test for anthracene derivatives (Bontrager's test)

To a portion of the extract in a dry test tube, 5ml of chlorofoam was added and was shaken for at least 5 minutes. This was then filtered and the filtrate shaken with equal volume of 10% ammonia solution, bright pink xolor in the aqueous (upper) layer indicates the presence of free anthraquinones (Evans. 1996).

3.2.2.4 Test for combined anthracene derivatives (Modified Bontrager's Test)

Small portion of the extract in a test tube was boiled with 5ml of 10% hydrochloric acid for 2-3 minutes. This would hydrolyse the glycosides to yield aglycones, which are soluble in hot water. This was then filtered and the filtrate was cooled and extracted with 5ml of benzene. The benzene layer was pipette off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide. If the lower ammonia layer becomes rose pink to cherry red, the drug contains anthraquinones derivative (free or in combined state). (Evans. 1996)

3.2.2.5 Test for unsaturated steroid and triterpenes (Liebermann-Bucchard Test)

To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Color changes were observed immediately and over a period of one hour. Blue to Blue-green color

in the upper layer and a reddish pink or purple color indicates the presence of tripertene (Evans. 1996)

3.2.2.6 Salkowski test for unsaturated sterols

To a small portion of the extract, 2-3 drops of concentrate sulphuric acid was added at the side of the test tube. Note any immediate color change at the interphase of the extract and sulphuric acid. Note immediate color changes over one hour period (cherry red color usually indicates the presence of unsaturated sterols (Evans. 1996)

3.2.2.7 Test for cardiac glycoside

- Keller-Killani test

A portion of the extract was dissolved in 1ml of glacial acetate acid containing traces of ferric chloride solution. This was then transferred in to a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observe carefully at the interphase for purple-brown color. This indicates the presence of deoxy sugars and a pale green color in the upper acetic acid layer indicates the presence of cardiac glycosides. (Evans. 1996)

3.2.2.8 Test for saponin glycoside

- Frothing test

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for10-15 minutes indicates presence of saponins (Evans. 1996)

3.2.2.9 Test for tannins

- Ferric chloride test

To a portion of the extract, 3-5 drops of ferric chloride solution was added. A greenish-black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate (Evans. 1996)

3.2.2.10 Test for flavonoids

- Shinoda test

A portion of the extract was dissolved 1-2ml of 50% methanol in the heat. Metallic magnesium chips and a few drops of concentrated hydrocholic acid were added. Appearance of red color indicates presence of flavonoids.

- Sodium hydroxide test

Few drops of 10% sodium hydroxide were added to the extract. Yellow coloration indicates presence of flavonoids (Evans. 1996)

3.2.2.11 Test for alkaloids

- Dragendoff's test

To a portion of the extract, few drops of Dragendoff reagent were added. A reddish brown precipitate indicates presence of alkaloids (Evans. 1996)

3.2.3 Animal grouping and experimental design

In the present study, Wistar rats divided into seven groups were used. Each group consisted of five Wistar rats (n = 5), treated for a period of four (4) weeks as follows:

Group 1: Served as control and were administered with 1 ml/kg of distilled water orally.

Group 2: Garlic 20 mg/kg body weight

Group 3: Garlic 40 mg/kg body weight

Group 4: Ginger 20 mg/kg body weight

Group 5: Ginger 40 mg/kg body weight

Group 6: Garlic and ginger, 10 mg/kg body weight each

Group 7: Garlic and ginger, 20 mg/kg body weight each

3.2.4 Collection and preparation of serum samples for biochemical assays

Four weeks after the treatment period, all animals were fasted for 12 hours and thereafter sacrificed after mild anaesthesia with chloroform. Blood sample (5 ml) was drawn from the heart of each sacrificed rat by cardiac puncture. Blood samples were collected in EDTA bottles for the determination of haematological parameters. Another blood sample (5 ml) was collected from each rat in plain tubes and allowed to clot. The serum was separated by centrifugation using Denley BS400 centrifuge (England) at 2000 × g for 10 minutes. The serum collected was used to determine lipid profile, concentration of liver enzymes and serum electrolytes.

3.2.5 Determination of haematological parameters

The total and differential WBC counts, RBC count, PCV, haemoglobin concentration were determined as described by Dacie and Lewis (1991).

3.2.5.1. Determination of total red blood cell count

Total red blood cell counts were determinmed by haemocytometer method as described by Dacie and Lewis (1991). The tip of a clean and dry RBC pipette was placed at the edge of a drop of blood. Blood was then drawn to the mark 0.5; sucked Hayem's fluid to the mark 101, and the mixture mixed thoroughly for 3-4 minutes. After the blood has been well mixed with the diluents, the tip of the pipette was carefully wiped and quickly placed on the floor piece, its tip just touching the edge of the cover slip at an angle 30° to the horizontal. The diluted blood was allowed to flow by capillarity action evenly and slowly under the cover slip. The cells were allowed to settle down for 2-3 minutes and the chamber carefully placed on the microscope. The cells were then counted under the desire magnification.

3.2.5.2 Determination of packed cell volume

Non-EDTA acid capillary tubes were filled with the blood collected in bottles containing anticoagulant from the wistar rats. About 15 mm of the capillary tubes were left unfilled and the open end of each of the tubes was carefully sealed with flame from a microburner. The tubes were then loaded into a microhaematocrit centrifuge (Hawksley, England) and centrifuged at 1000 x g for 10 min. A haematocrit reader (Hawksley, England) was used to read the PCV value of each tube (Schalm *et al*., 1975).

3.2.5.3 Determination of haemoglobin concentration (Hb)

The haemoglobin concentration was determined using cyamethaemoglobin method of Barker and Silverton (1985). 20 µl of blood sample was pipetted into a tube and diluted with 5 mL of Drabkin's fluid and was allowed to stand for 3 minutes and the absorbance of the mixture read using a spectrophotometer (Beckman Coulter®, Model BU20, Australia) at a wavelength of 540 nm against the reagent blank. The haemoglobin concentration was calculated using the formula:

Hb = T x C x D (g/dl)/A x 1000

Where:

T = Absorbance

A = Standard absorbance

C = the concentration of cyanomethaemoglobin

D = Dilution factor (1: 250)

1000 converts the Hb concentration from mg/dl to g/ dL.

3.2.5.4. Determination of total and differential leucocyte counts

Absolute and differential leucocyte counts were determinmed as described by Dacie and Lewis (1991). The absolute counts were determined using a haemocytometer. The tip of a clean and dry WBC pipette was placed at the edge of a drop of blood. Blood was then drawn to the mark 0.5; sucked Turk's fluid to the mark 11, and the mixture mixed thoroughly for 3-4 minutes. The first two drops of fluid was discarded, after which the haemocytometer was charged with the diluted blood. The cells were allowed to settle for 3-4 minutes and the chamber placed under the

microscope. For differential count, a drop of blood was placed on one end of a glass slide (UNESCOPE®, England) and a blood film was made using a spreader. It was then allowed to air-dry. Leishman stain was poured over the film to cover it for 2 minutes. Thereafter, an equal number of drops of distilled water was added to the stain without allowing it to spill over. The diluted stain was allowed to remain on the slide for 4-6 minutes. The diluted stain was flushed–off in a gentle stream of water for 30 seconds and left on a rack for about a minute, with the last wash covering them. The slides were drained and put in an inclined position against a support. The stained smear appears bluish pink. It was then mounted on a microscope, and the cells were counted under a microscope using oil immersion at x 1000.

3.2.6 Determination of serum electrolytes

Serum sodium and potassium ions were measured by flame photometry (Model 410 Flame Photometer, Sherwood Scientific Ltd, England) using the method of Vogel (1960). Bicarbonate ion was determined according to the titration method of Segal (1955). Chloride ion was analyzed using the method of Schales and Schales (1941). Calcium ion was determined according to laboratory procedures of Randox Laboratories Limited kits, England, United Kingdom (Teitz, 1995).

3.2.6.1. Sodium (Na^+)

Sodium and potassium were measured by the flame photometry method of Vogal (1960).

Procedure:

The instrument was allowed to warm up for 5-10 minutes. Distilled water was fed to the instrument. The element Na was selected by turning the selector “Elementwahl”. The outer knob “Messbereich” was turned into position “100”. The “Kompensaton I” knob was pulled slightly

out and adjusts readout to 0. The “Kompensation I” knob was pressed back. The reading 0 was readjusted with “Kompensation II”. The most concentrated standard solution was aspirated and adjusted readout to approximately 350 (on uppermost scale) using inner “Messbereich” knob. Distilled water was aspirated–the instrument should read 0. Standard solutions no. 1, 2, 3, test solution were aspirated, and then standards 4, 5, 6. Results were then recorded. The procedure was repeated 3-7 for solutions of potassium. Distilled water was aspirated for at least 5 minutes to clean the system.

3.2.6.2 Chloride (Cl^-)

Chloride ion was measured using the method of Schales and Schales (1941).

Procedure:

This entire procedure was best carried out directly in the spectrophotometer tubes.

0.5 ml of serum was transfer to each of two spectrophotometer tubes. To one tube 15.0 mL. of color reagent was added; to the second tube add 15.0 ml. of reagent blank. The tubes were shaken as the reagents were added. A slight precipitation was form, but this disappeared on standing. 10 mins was allowed for the color to develop and also for the precipitate to dissolve. The spectrophotometer was set at zero absorhance at 480 m with the blank and then obtain the absorbance of the unknown. The concentration of the unknown was obtained from the precalibrated card or standard curve, whichever is preferred.

3.2.6.3. Bicarbonate (HCO_3^-)

Bicarbonate was determined using the fame photometry method of Segal (1955). Bicarbonate reacts with phosphoenolpyruvate (PEP) in the presence of PEPCase to produce oxaloacetate and phosphate. This reaction occurs in conjunction with the transfer of a hydrogen ion from NADH to oxaloacetate using malate dehydrogenase. The resultant formation of NAD caused a decrease in absorbance in the UV range (320–400 nm). The change in absorbance was directly proportional to the concentration of bicarbonate in the sample being assayed.

Procedure:

After clot formation, blood samples were centrifuged immediately to obtain clear unhemolyzed serum. 0.1 mL. of serum was pipetted into the specially designed centrifuge tubes and stoppered. During the remainder of the procedure, contact of the solution with the atmospheric air is kept to a minimum by rapid replacement of the stopper after each manipulation. 0.9 mL of distilled water from a Machlett burette was added, and the solution in the tube is mixed by gentle tapping. At the same time, a blank was prepared containing 1 mL of the distilled water. From this point, both the blank and the sample are treated identically. 0.3 mL. of saturated $Ba(OH)_2$ reagent was now added, and the solution was again mixed gently. After less than 1 minute, precipitation takes place; 0.5 mL of ethanol-water wash solution, was added, and the solution is mixed again. After centrifuging the samples for 10 minutes at 2000 r.p.m., the supernatant fluid was aspirated slowly by means of the Nz aspiration apparatus. This was accomplished without disturbing the precipitate at the bottom or touching the sides of the tube with the capillary. The precipitate was re-suspended in the small volume of fluid remaining and washed first with 1 mL. of the

ethanol-water wash solution reagent and finally with 1 mL of freshly boiled deionized water. After each washing, the tubes were centrifuged, and the supernatant fluid was aspirated over nitrogen . At this point, the rubber stoppers were removed, and the tubes were dried at 110^{o}c for approximately 20 minutes. After cooling, 0.5 ml of 2 M HCl was added to each tube and placed in a boiling water bath for 1 minute. The tubes were diluted to 5 mL with distilled water, stoppered, mixed, and then centrifuged. The samples were now ready for flame photometry. With the phototube shutter open, with water as a blank, the zero reading was adjusted with the dark current knob. Other adjustments comprise hydrogen gas pressure at the control panel, 4.5 pounds per sq. in; oxygen gas pressure, 15 pounds per sq. in. ; monochromator sensitivity, maximum counter-clockwise; slit width, approximately 0.1 mm.; wave-length, 493.4 rnp; photomultiplier sensitivity, 4; zero suppression. By means of the working standard $BaCl_2$ solution, the percent transmission was set to 50 with the slit adjustment. As a result, no calculation of volume percent CO_2 was necessary. Percent transmission equals volume percent CO_2. With water as the blank, each sample was then analyzed by frame photometry.

3.2.7 Determination of lipid profile

Lipid profile was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, United Kingdom) as follows:

3.2.7.1 Determination of serum total cholesterol

The serum concentration of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by Stein (1987). Briefly, 1000 µL of the reagent was added to each of the sample and standard, incubated for 10 minutes at 20 ^{0}C after mixing. The

absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The concentration of total cholesterol in the serum was expressed in mg/dL as follows:

Total cholesterol concentration = A *sample* /A *standard x* 195.0 mg/dL

3.2.7.2 Determination of serum triglyceride

The serum triglyceride concentration was determined after enzymatic hydrolysis of the sample with lipases according to the method of Tietz (1990). Briefly, 1000 µL of the reagent was added to each of the sample and standard, incubated for 10 minutes at 20 ^{0}C after mixing. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The concentration of triglyceride in the serum was expressed in mg/dL.

Triglyceride concentration = A *sample*/A *standard x* 196.0 mg/dl

3.2.7.3 Determination of serum high-density lipoprotein cholesterol

The serum concentration of HDL-C was measured by the method of Wacnic and Albers (1978). Briefly, low-density lipoproteins, very low-density lipoprotein and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature, and centrifuged for 10 minutes at 2,000 × g. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in mg/dL

3.2.7.4 Determination of serum low-density lipoprotein cholesterol

The serum level of low-density lipoprotein was measured according to the protocol of Friedewald *et al*. (1972) using the relationship below:

LDL-C = TC-TGL/5 + HDL-C

Where LDL-C = Low-density lipoprotein cholesterol,

TC = total cholesterol,

TGL = triglyceride

HDL-C = High-density lipoprotein

The value was expressed in mg/dL.

3.2.8 Determination of activities of serum liver enzymes

The activities of serum enzymes, alanine aminotransferase (ALT) was determined spectrophotometrically, using enzymatic colometric assay kits using the method of Berg *et al.* (2006), while aspartate aminotransferase (AST) was carried out by the method described by Bergmeyer *et al.* (1978) and alkaline phosphatase (ALP) was estimated by the method described by Bowers and Mc Comb (1966).

3.2.8.1. Alanine aminotransferase and aspartate aminotransferase

The Alanine aminotransferase and aspartate aminotransferase activities were determined by the method of Reitman and Frankel (1957) that uses a coupled enzyme assay to measure ALT and AST activities in serum or plasma. AST catalyses the transfer of an amino group from L-

aspartate to 2-oxoglutarate to form oxaloacetate and L-glutamate. Oxaloacetate spontaneously decarboxylates to form pyruvate under the strongly acidic conditions. ALT catalyses the transfer of an amino group from L-alanine to 2-oxoglutarate to form pyruvate and L-glutamate. An increase in pyruvate concentration corresponds with the levels of AST and ALT activities. The pyruvate concentration was determined spectrophotometrically in the form of hydrazone, which was produced by reaction with 2, 4-dinitrophenylhydrazine in an alkaline medium. The pyruvate hydrazone absorbs at 510 nm more than 2-oxoglutarate hydrazone.

Procedure:

2.0 mL of reaction medium was added to 0.2 mL of 2-oxoglutarate. After preincubation for 15 minutes of 0.3 mL serum, without or with supplementary pyridoxal-5'-phosphate, the reaction was started by adding serum or serum + coenzyme to the otherwise complete reaction medium. The decrease in absorhance at 340 nm was measured every minute for 10 minutes. Enzyme activities were calculated from the mean absorbance change from the fourth to the ninth minute.

3.2.8.2**.** Alkaline phosphatase

Serum alkaline phosphate activity was assayed as described by Bassey *et al*., (1946). In the presence of magnesium ions, *p*-nitrophenylphosphate is hydrolyzed by phosphatases to phosphate and *p*-nitrophenol. The rate of *p*-nitrophenol liberation was proportional to the ALP activity and measured photometrically.

Procedure:

Serum samples (5 c.mm) were transferred to the bottom of 6 X 50 mm tubes in a wire rack. The rack was immersed in a shallow pan of ice water and to each tube 50 c.mm of ice-cold Reagent C was rapidly added, with a constriction pipette. Each tube ws mixed by

tapping with the finger. Care was taken not to warm the tube by so doing. The whole rack of tubes was now immersed in a water bath at 38°c at 2 depth sufficient to cover the bottom half of the tubes. After exactly 30 minutes the rack was again placed in the pan of ice water and 0.5 ml. of 0.02 N NaOH was added to each tube with sufficient force to mix the sample. This addition stops the reaction and dilutes the samples which was now transferred to calorimeter tubes and read at 400 to 420 rnp = R. After the initial reading, 2 to 4 c.mm of concentrated HCI was added with a 0.1 ml. graduated pipette (drawn out tip) and a second reading, R1, was made. RI and R2 are converted into optical densities (- log transmission or 2 - log per cent transmission) = D1 and D,. Then D, - D, = D, the corrected density. Standards and blanks were provided by treating 5 c.mm. volumes of the standards and of distilled water exactly as though they were serum samples. The corrected densities (DC) were used to construct a standard curve from which the serum values were calculated. Since sera and standards undergo the same dilution, it is unnecessary to take into account the exact volumes of the various pipettes. It is to be noted that D, is the density corrected for possible residual absorption after acid addition but is not corrected for the D, of the blank analysis. This second necessary correction is automatically provided by the standard curve. A"millimole unit" is defined as the phosphatase activity which will liberate 1 mM of nitrophenol per liter of serum per hour. Therefore, since the standard incubation time is only 30 minutes, the 1, 2, 4, and 6 rnM standards are equivalent to sera with activities of 2, 4, 8, and 12 mM units.

3.2.9 Studies on isolated perfused rabbit heart

Four (4) rabbits were used for this experiment. This was carried out as described by Bardenheuer and Schrader (1983). Two milliliters of 10 mg of heparin was injected into the ear vein rabbit,

weighing 1.5 kg before sacrificing the animal to prevent clots forming inside the heart. The rabbit was sacrificed by jugular vein venisection. The heart was removed quickly and placed in a Ringer-Locke's solution. The preparation was rinsed several times when first placed in the Ringer-Lock's solution to remove as much blood as possible. The aorta was dissected free, and all other vessels connected to the heart were trimmed. The aorta was cut just below the point where it divides, and the heart was transferred to the perfusion apparatus, where it was tied unto a glass cannula. Care was taken to ensure that air bubbles did not enter the aorta. Threads were attached to the ventricle by the hook and to the auricle by a small spring dip. The threads were connected to spring lever to ensure the record of the contraction of the muscle on a kymograph (Kymograph model No.7050) at speed of 24 mm/min. The rate of beating was recorded on a tracing with duration of sixty (60) seconds each. The base-line or steady-control level was determined before the commencement of the experiment. The effect of 0.9% normal saline on the contraction was used as the control responses, and then the effects of various standard drugs and extract were tested.

The effects of various concentration of the extract (1, 10, 20, 40 and 100mg/ml) on the perfused rabbit heart were recorded after the baseline was established. The concentration was given one at a time. Additional concentration was given until the preparation had fully recovered to the former (control) level or settled down at a new control level. Each effect was washed off 2 minutes before the next. First, the effect of *Allium sativum* and then that of *Zingiber officiale* and finally their combined effects were studied. Doses used were 10mg/kg, 20mg/kg, and 100mg/kg.

3.2.10 Interaction between the standard drugs, extracts and perfused rabbit heart

The effectiveness of the blockers of β-adrenergic, cholinergic, and histaminergic receptors were first established by the following procedure:

1. Effective concentrations for methoxamine (500 µg/kg), adrenaline (10 mg/kg) and acetycholine (2 mg/kg), respectively were administered and the effects were recorded.
2. The various blockers were then administered and the effectiveness of each blocker was recorded (Phentolamine 2 mg/kg) was administered, then the agonist, methoxamine (10 µg/kg) was administered immediately. The same was repeated for other drugs as follows:
Atropine 2 mg/kg, acetylcholine 2 µg/kg; and propronalol 2 mg/kg and Adrenaline 10 µg/kg

Secondly, the possible interaction between the extract and the various receptors were examined by the administration of various pharmacological concentrations of the antagonists, and followed by an effective concentration of the extracts. Each test dose was followed by concentration of 20mg/ml; of garlic and ginger. Each test concentration was administered only after the preparation returned to the control level. The force and rate of contraction of the isolated heart perfused were then recorded as described by Bardenheuer and Schrader (1983)

3.2.11 Studies on effect of extract on blood pressure of the cat (*in vivo* studies)

Four cats were used for this study. They were anaesthetised by injection of 40 mg/kg of pento barbitone sodium intraperitioneally. After induction of anaesthesia in the animals (about 30 minute post-injection), they were secured to the dissecting board and cannulated as below:

i. Their carotid arteries and vagus nerve (in the neck) were exposed by dissection. Two short ligatures were placed wider than the carotid arteries and the peripheral one was tied tightly.

ii. The carotid artery was temporarily occluded centrally with a lip. A canular was inserted into the artery, and then connected to a pressure transducer coupled to a recording microdynamometer.

iii. Heparin (10 mg/ml) in normal saline was injected to prevent clotting.

iv. The record of the blood pressure began immediately after the occlusion.

v. The left femoral vein was exposed, tied off with a peripheral ligature.

There was a cannula connected to a 2 ml syringe, containing normal saline for flushing of injected drugs. The next administration was inserted into the femoral vein and tied securely with a second ligature. The various test drugs (garlic and ginger) were administered beginning from a very low dose of 1 mg/ml. Other doses are 10, 20, 30, 40 and 100 mg/kg. The effect of different dose of the extract on the blood pressure was studied. No additional dose was given until the preparation had fully recovered to the former (control) level or a new control level. Each effect was flushed by administering 2 ml of distill water. This procedure was done for *A. sativum* and *Z. officinale* separately, and then for their combined form.

3.2.12 Pharmacological antagonist studies

Similar antagonistic studies as those of isolated perfused rabbit heart, described earlier, were carried out. Atropine was administered, then 20mg/kg of garlic and ginger was administered. Garlic and ginger (20 mg/kg) was also administered alongside adrenaline. The blood pressure

was calculated from the tracing and the changes in mean arterial pressure (MAP), estimated as the difference between steady MAP before and the maximum change in MAP after injection of drugs were obtained. The MAP was calculated from the diastolic and systolic blood pressure using the formula:

MAP= Diastolic pressure + 1/3 Pulse Pressure

Pulse Pressure = (Systolic – diastolic) pressure

3.2.13 Histopathological studies

Four weeks after the period of treatment of all animals from each group were sacrificed after anaesthesia with chloroform soaked in cotton wool and placed in an anaesthetic box covered with lid. Liver and kidney tissues were dissected out and washed on ice-cold saline immediately. A portion of each tissue was fixed in 10% neutral formal-saline fixative solution for histological studies. After fixation, tissues were embedded in paraffin and solid sections were cut at the thickness of 5 µm. Various sections were stained with haematoxylin and eosins as described by Galozhger and Kocloff (1971). The slides were viewed at the magnification of X 250 and pictures taken for the preparation of photomicrographs.

3.3 Statistical analysis

Values were expressed as mean ± SEM. The data obtained were statistically analysed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post-hoc tests to determine the level of significance between control and experimental groups. Values of $P < 0.05$

were considered significant (Duncan *et al*., 1997). Graphpad prism statistical package, San Diego, California, U.S.A was used.

CHAPTER FOUR

4.0 Results

4.1 Results of phytochemical screening

Phytochemical screening of garlic shows the presence of carbohydrates, cardiac glycosides and alkaloids. While that of ginger shows carbohydrate, cardiac glycosides, saponin glycosides and alkaloids.

4.2 LD_{50} and results of acute toxicological studies

From the results of the acute toxicological screening, the oral LD_{50} was calculated and found to be: 120 mg/kg for garlic and 100 mg/kg for ginger.

4.3 Effects of garlic and ginger on serum lipid profile

There was a significant ($P < 0.05$) reduction of serum total cholesterol, triglyceride and low-density lipoprotein levels in rats, administered with single or combined doses of the extract, compared to the control group. The extract at single and combined doses significantly ($P < 0.05$) elevated serum high-density lipoprotein level in the treated animals, when compared to the control group (Table 4.1).

4.4 Effects of garlic *(Allium sativum)* and ginger (*Zingiber officinale*) on serum electrolyte concentration in Wistar rats

The mean values of electrolytes in control and experimental groups, administered with graded doses of garlic and ginger and their combination as presented in Table 4.2 shows that the serum sodium concentration decreased significantly ($P < 0.05$) and serum potassium concentration rose

significantly ($P < 0.05$) in garlic and ginger-extract treated groups, when compared to the corresponding concentrations obtained in the control group. Garlic administered at the dose of 20 or 40 mg/kg increased serum chloride ion concentration significantly ($P < 0.05$) when compared to that of control group. The serum bicarbonate ion concentration in all groups administered with different doses of garlic and ginger extract did not differ when compared to that of the control group. There was a significant reduction ($P < 0.05$) in serum concentration of both ionized and total calcium in the extract-treated groups, when compared to the value obtained in the control group.

Table 4.1: Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Serum Lipid Profile (mg/dL) in Wistar Rats (n = 5)

Group	Treatment Given	Serum total cholesterol	Serum triglyceride	Serum low-density lipoprotein	Serum high-density lipoprotein
I	Normal + Distilled Water	106.97 ± 2.41	86.14 ± 4.15	64.16 ± 4.23	21.57 ± 1.91
II	Garlic 20 mg/kg	62.49 ± 4.09 [a]	48.09 ± 5.96 [a]	22.63 ± 2.24 [a]	31.64 ± 3.40 [a]
III	Garlic 40 mg/kg	72.64 ± 4.65 [a]	49.54 ± 4.31 [a]	19.84 ± 1.77 [a]	42.89 ± 4.27 [a]
IV	Ginger 20 mg/kg	59.24 ± 4.09 [a]	27.66 ± 2.63 [a]	11.81 ± 1.13 [a]	41.97 ± 3.56 [a]
V	Ginger 40 mg/kg	58.17 ± 5.83 [a]	31.24 ± 5.67 [a]	15.06 ± 1.44 [a]	37.40 ± 3.51 [a]
VI	Garlic 10 mg + Ginger 10 mg/kg	51.27 ± 5.29 [a]	30.00 ± 6.45 [a]	13.49 ± 9.28 [a]	31.79 ± 2.54 [a]
VII	Garlic 20 mg + Ginger 20 mg/kg	81.86 ± 12.35 [a]	27.20 ± 7.16 [a]	13.69 ± 2.28 [a]	62.73±2.23 [a]

Data are presented as mean ± standard error of mean
a = $p < 0.05$ is significant

Table 4.2: Effects of Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) on Serum Electrolyte Concentration (mmol/L) in Wistar Rats (n = 5)

Groups	Treatment Given	K^+ (mmol/L)	Na^+(mmol/L)	Cl^-(mmol/L)	HCO_3^-(mmol/L)	Ionised Ca^{2+} (mmol/L)	Non-ionised Ca (mmol/L)
I	Normal saline	4.68 ±0.19	141.52 ± 1.18	103.36±2.36	17.80±0.86	0.86±0.038	1.096±0.067
II	Garlic 20 mg/kg	6.20±0.18[a]	119.03±1.70[a]	88.34±3.62 [a]	22.03±0.74[ns]	0.61±0.033[a]	1.014±0.023[ns]
III	Garlic 40 mg/kg	6.96 ± 0.23[a]	119.03 ±1.69 [a]	87.13±2.16[a]	23.82±1.43[ns]	0.64±0.027 [a]	0.878±0.078[a]
IV	Ginger 20 mg/kg	6.87 ± 0.26[a]	125.79±4.90 [a]	90.35±4.26[ns]	21.18±0.79 [ns]	0.60±0.038 [a]	0.842±0.062[a]
V	Ginger 40 mg/kg	6.68 ± 0.28[a]	122.22±2.97 [a]	93.70±2.06[ns]	23.17±1.71[ns]	0.59±0.035 [a]	0.844±0.074[a]
VI	Garlic 10 mg + Ginger 10 mg/kg	7.44 ± 0.28[a]	118.55±5.24 [a]	92.36±2.42[ns]	22.97±1.64[ns]	0.64±0.027 [a]	0.854±0.093[a]
VII	Garlic 20 mg + Ginger 20 mg/kg	7.94 ± 0.27[a]	94.19±4.01 [a]	90.58±3.44[ns]	25.93±1.25[ns]	0.64 ± 0.070[a]	0.890 ±0.086 [a]

Data are presented as mean ± standard error of mean.
a = $p < 0.05$ is significant
ns = non significant

4.5 Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Erythrocyte Indices in Wistar Rats

The mean values of control and experimental groups administered with graded doses of garlic, ginger and their combination as presented in table 4.3 shows that there was a significant ($P < 0.05$) reduction in PCV and haemoglobin concentration in garlic and ginger-extract treated groups, when compared to the corresponding values recorded in the control group. The red blood cell count did not show a significant change ($P > 0.05$).

4.6 Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Leucocyte Indices in Wistar Rats

There was significant ($P < 0.05$) change in total white blood cell count and neutrophil count in the groups treated with 20 or 40 mg/kg b w of garlic extract. There was a significant difference ($P < 0.05$) in total white blood cell counts in the groups that received 20 mg/kg body weight of ginger extract and their combination with garlic at various doses and neutrophil count in those given 40 mg/kg bw of ginger and its combination when compared to the control group. The lymphocyte count was significantly ($P < 0.05$) higher in rats administered with 40 mg/kg bw of garlic and its combination; and in rats treated with garlic 20 plus ginger 20 mg/kg bw. The monocyte count was higher ($P < 0.05$) than that of the control group as presented in table 4.4.

Table 4.3: Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Packed Cell Volume, Erythrocyte Count and Haemoglobin Concentration in Wistar Rats (n = 5)

Group	Treatment Given	PCV (%)	RBC (10^{12}/L)	Hb (g/dL)
I	Normal saline	54.80 ± 1.77	4.21 ± 0.078	18.26 ± 0.58
II	Garlic 20 mg/kg	43.00 ± 1.79 [a]	4.99±0.268 [ns]	14.34 ± 0.59[a]
III	Garlic 40 mg/kg	36.40 ± 2.292[a]	5.11 ± 0.194 [ns]	12.14 ± 0.76[a]
IV	Ginger 20 mg/kg	36.60 ± 2.16 [a]	5.29 ± 0.077 [ns]	12.19 ±0.72[a]
V	Ginger 40 mg/kg	37.40 ± 1.25 [a]	5.24 ±0.066 [ns]	12.45 ± 0.41[a]
VI	Garlic 10 mg + Ginger 10 mg/kg	33.20 ± 1.11 [a]	5.27 ± 0.107 [ns]	11.04 ± 0.37[a]
VII	Garlic 20 mg + Ginger 20 mg/kg	35.60 ± 0.68 [a]	5.51 ± 0.079 [ns]	11.87 ± 0.23[a]

Data are presented as mean ± standard error of mean
a = $p < 0.05$ is significant
ns = non significant

Table 4.4: Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Total and Differential Leucocyte Counts in Wistar rats (n = 5)

Groups	Treatment Given	WBC $\times 10^9$/L	Neutrophils ($\times 10^9$/L)	Lymphocytes ($\times 10^9$/L)	Monocytes ($\times 10^9$/L)	Eosinophils ($\times 10^9$/L)	Basophils ($\times 10^9$/L)
Group I	Normal saline	12.03 ± 0.28	3.41 ± 0.34	6.86 ± 0.85	0.68 ± 0.22	-	-
Group II	Garlic 20 mg/kg	10.29 ± 0.67	3.95 ± 1.00	6.50 ± 0.96	0.66 ± 0.23	-	-
Group III	Garlic 40 mg/kg	9.27 ± 1.22	3.03 ± 1.24	4.26 ± 0.33[a]	0.43 ± 0.13	-	-
Group IV	Ginger 20 mg/kg	6.38 ± 0.27[a]	2.17 ± 0.81	2.58 ± 0.95	0.36 ± 0.20	-	-
Group V	Ginger 40 mg/kg	5.98 ± 0.36[a]	2.33 ± 0.90[a]	2.79 ± 1.11	0.30 ± 0.30	-	-
Group VI	Garlic 10 mg + Ginger 10 mg/kg	5.39±0.38[a]	1.91 ± 0.74[a]	3.13 ± 1.37[a]	0.30 ± 0.13	-	-
Group VII	Garlic 20 mg + Ginger 20 mg/kg	4.31 ± 0.75 [a]	1.39 ± 0.43	2.80 ± 1.98[a]	0.29 ± 0.25[a]	-	-

Data are presented as mean ± standard error of mean
a = $p < 0.05$ is significant

4.7 Effects of Garlic and Ginger on Activities of Serum Liver enzymes in Wistar Rats

There was a significant ($P < 0.05$) increase in the concentrations of serum ALT and AST in the rats administered with the combination of ginger and garlic, when compared to those of the control group (Figures 4.1 and 4.2). There was also no significant ($P > 0.05$) change in serum activities of ALP in the group that received 20 and 40 mg/kg of garlic, respectively, when compared to the control group. The serum activities of ALP,ALT and AST in the groups treated with various doses of garlic and ginger separately did not differ significantly from the control ($P > 0.05$).

4.8 Microscopic Findings on the Liver Tissues of Rats administered with Garlic and Ginger Extract

The histological features of the liver of control and the experimental groups administered with garlic and ginger extract are shown in Plate V-XI. The histological findings showed that the plant extract did not adversely affect the morphology of liver tissues in all groups, treated with various doses of garlic and ginger extract, at the single doses. However, the combined doses of garlic and ginger altered the liver and kidney morphology. The liver tissues showed cords of hepatocytes that were well preserved, essentially normal and arranged in fairly radial position in relation to the central vein. The cytoplasm was not vacuolated, the sinusoids were well demarcated and no areas of necrosis, in the single dose of garlic and ginger. The combined doses showed congested central vein on the liver morphology.

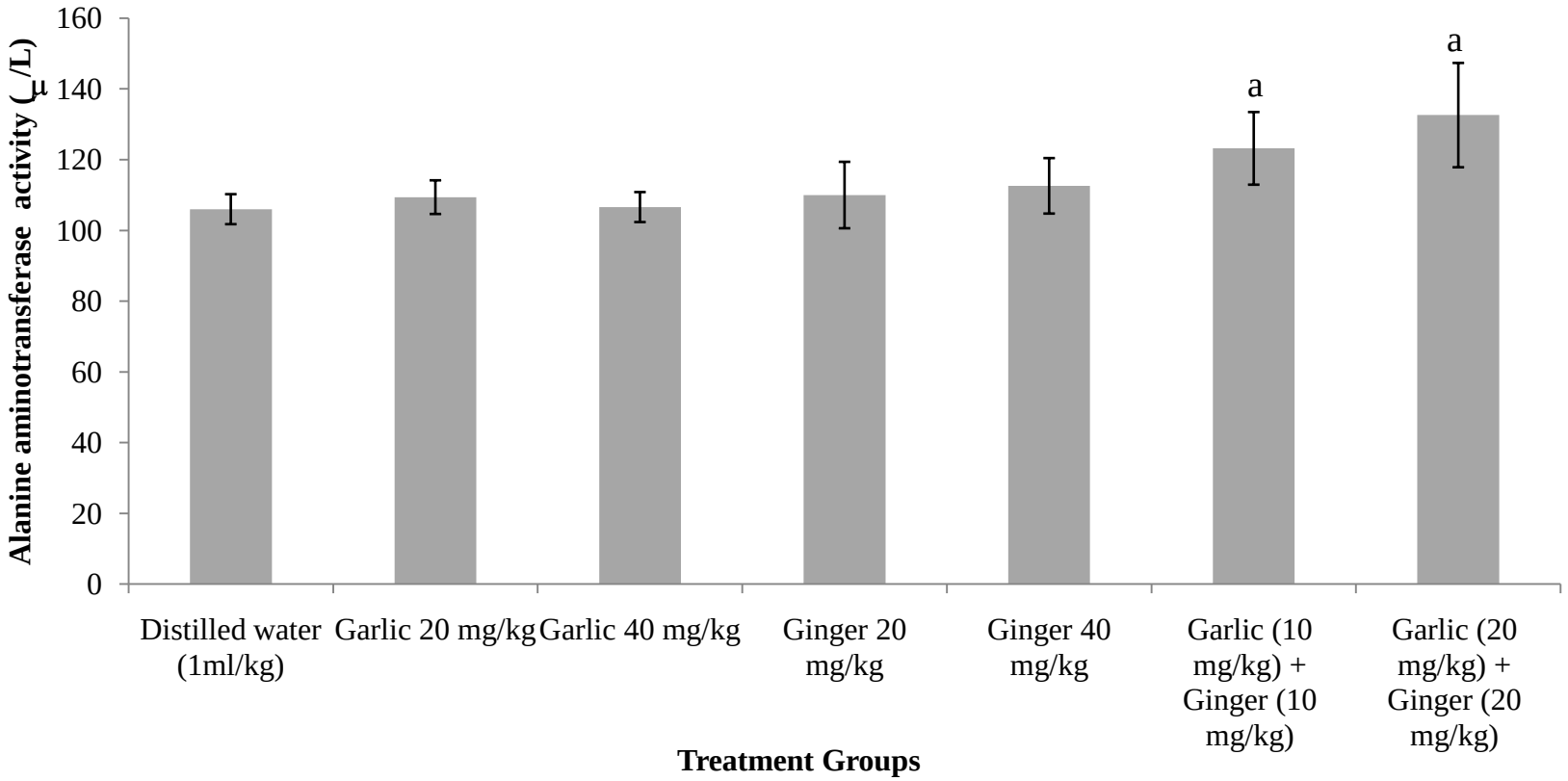


Figure I: Effects of Garlic and Ginger on Activities of Serum Alanine Aminotransferase in Wistar rats.

Data are presented as mean ± standard error of mean
a = $p < 0.05$ is significant

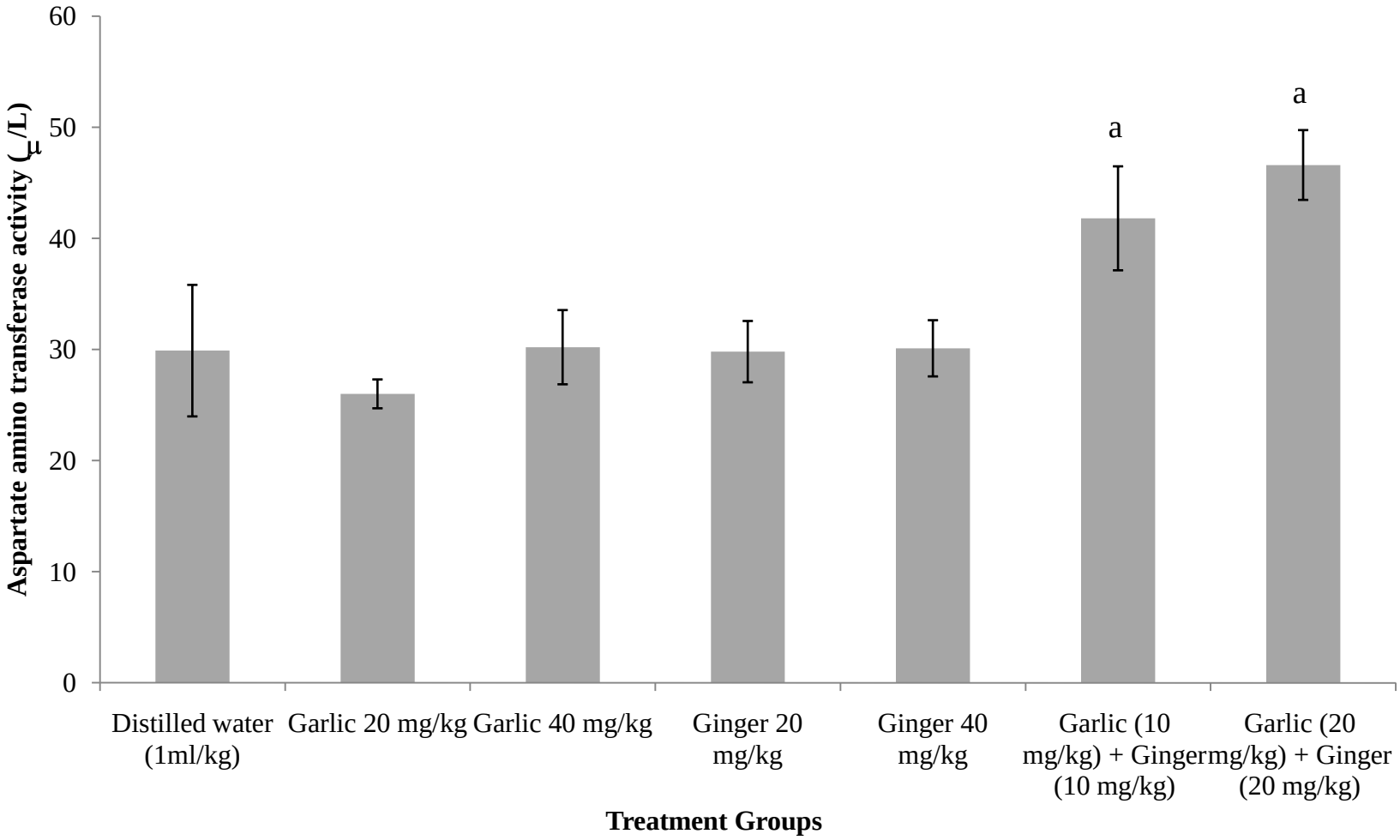


Figure II: Effects of Garlic and Ginger on Activities of Serum Aspartate Aminotransferase in Wistar rats.

Data are presented as mean ± standard error of mean
a = $p < 0.05$ is significant

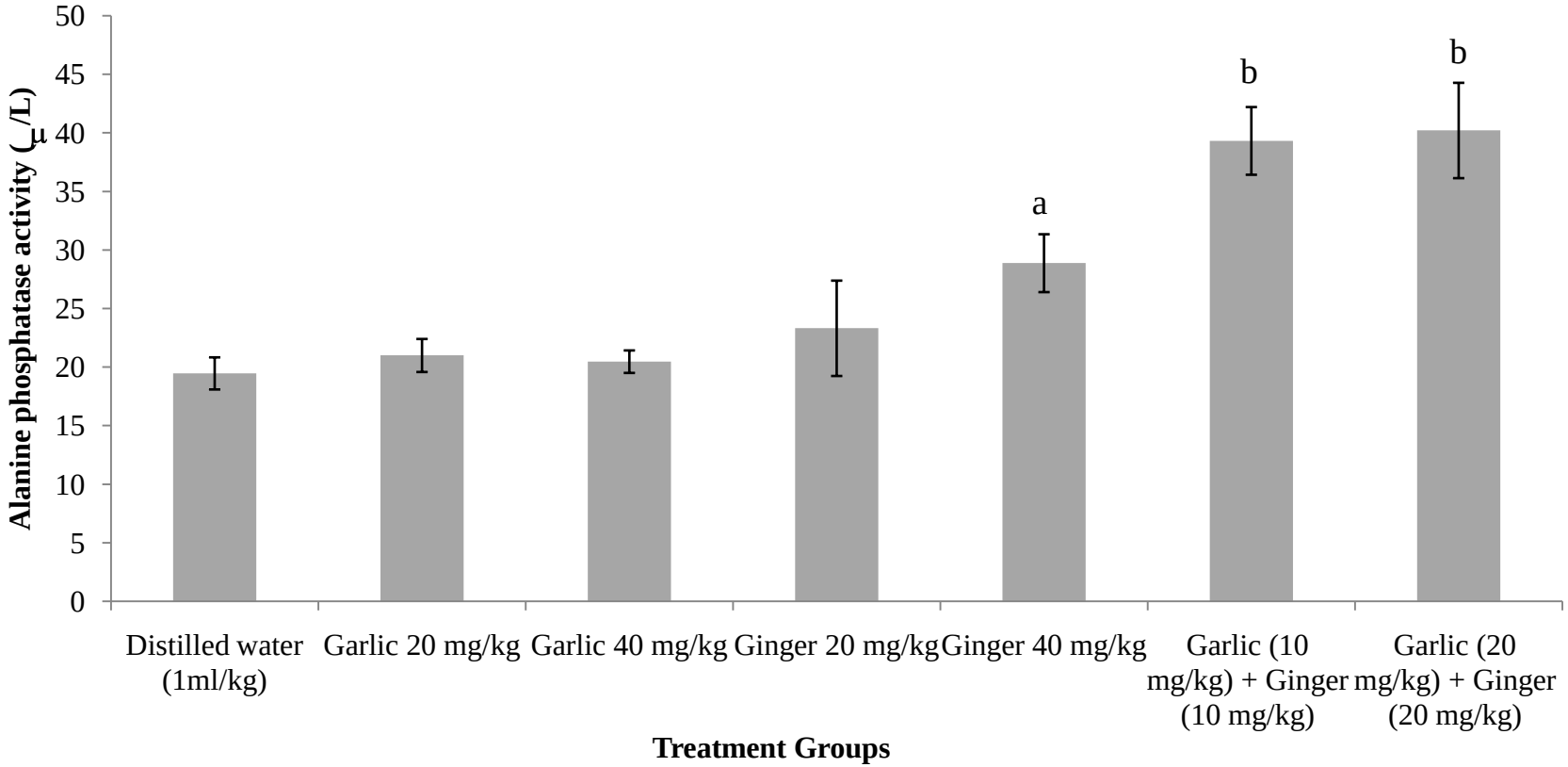


Figure III: Effects of Garlic and Ginger on Activities of Serum Alanine Phosphatase in Wistar rats.

Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant

4.9 Microscopic Findings on Kidney Tissues of Rats administered with Garlic and Ginger Extract

The histological features of kidney tissues of control and experimental groups administered with garlic and ginger extract are shown in Plates XII-XVIII. Histological findings showed that the plant extract did not exert adverse effects on the morphology of kidney tissues in all groups, treated with various doses of garlic and ginger extract separately. The kidney tissues showed intact and normal architecture of glomeruli, renal tubules and epithelium (H & E Stained X250), while the combination of garlic and ginger showed glomerula atrophy as seen in plate XVIII.

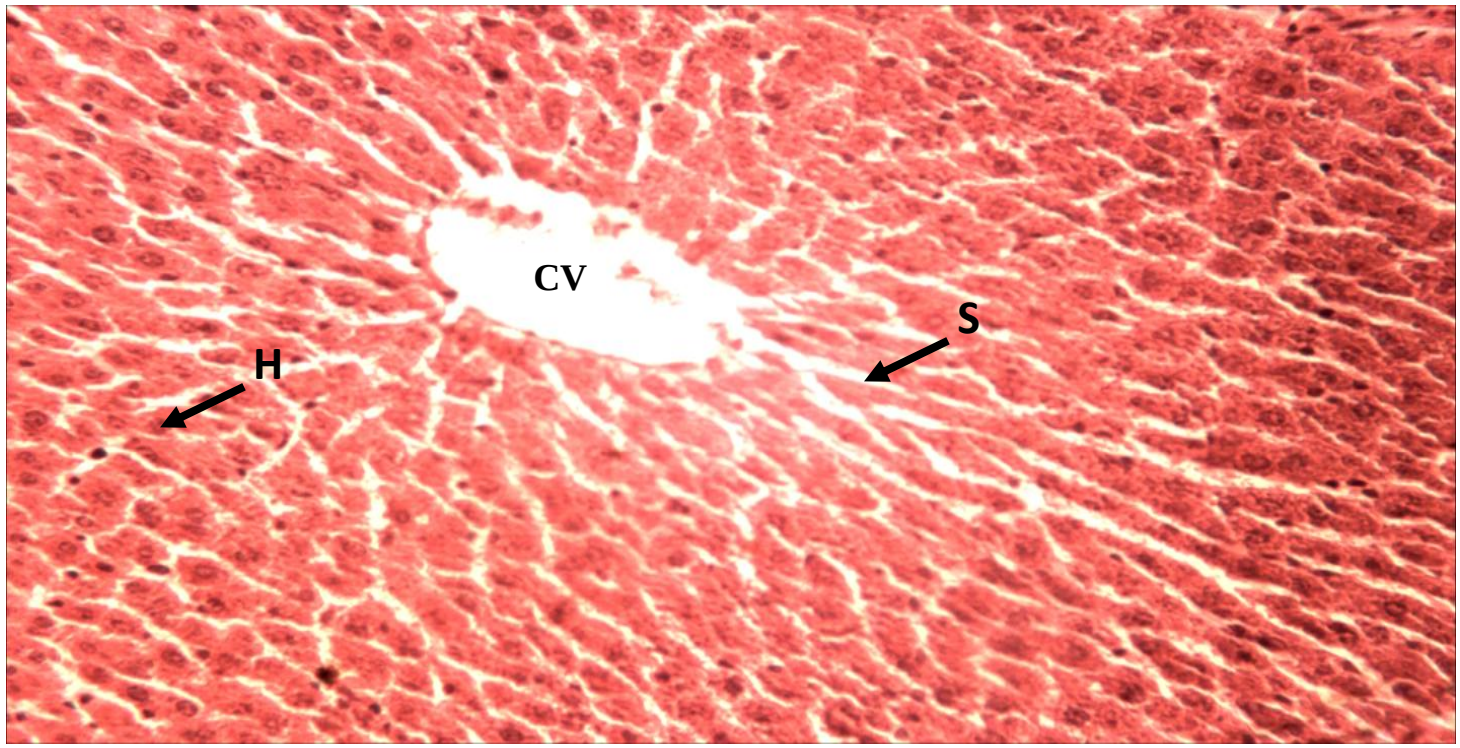


Plate V: Section of the liver of control showing normal histoarchitecture of liver of Wistar rats: Central vein (**CV**); Hepatocyte (**H**); Sinusoid (**S**). H&E (Mag x 250).

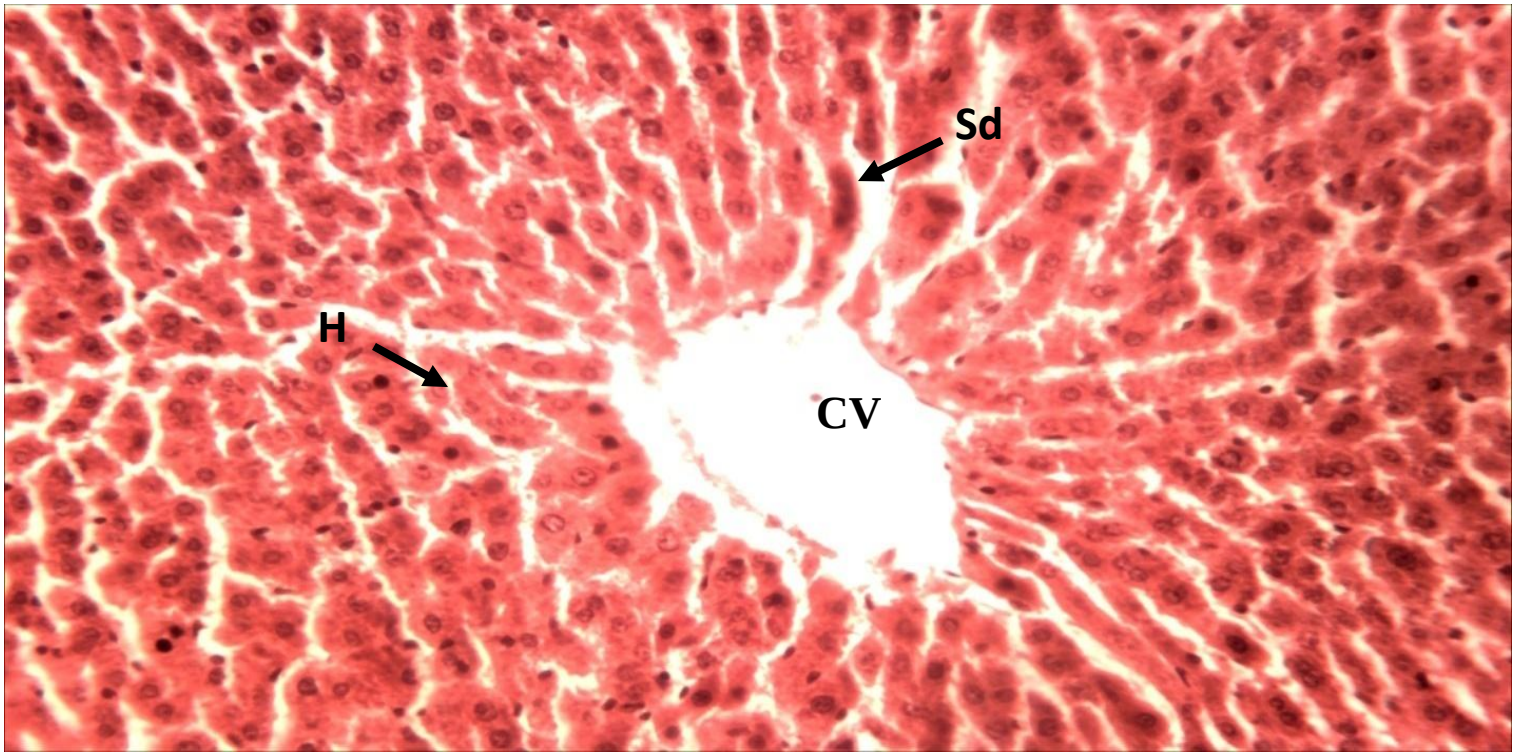


Plate VI: Section of the liver of treatment group that received 20 mg/kg of garlic showing mild histoarchitectural distortion of the liver of Wistar rat. Central vein (**CV**); Hepatocyte (**H**); Sinusoidal dilatation (**Sd**). H&E (Mag x 250).

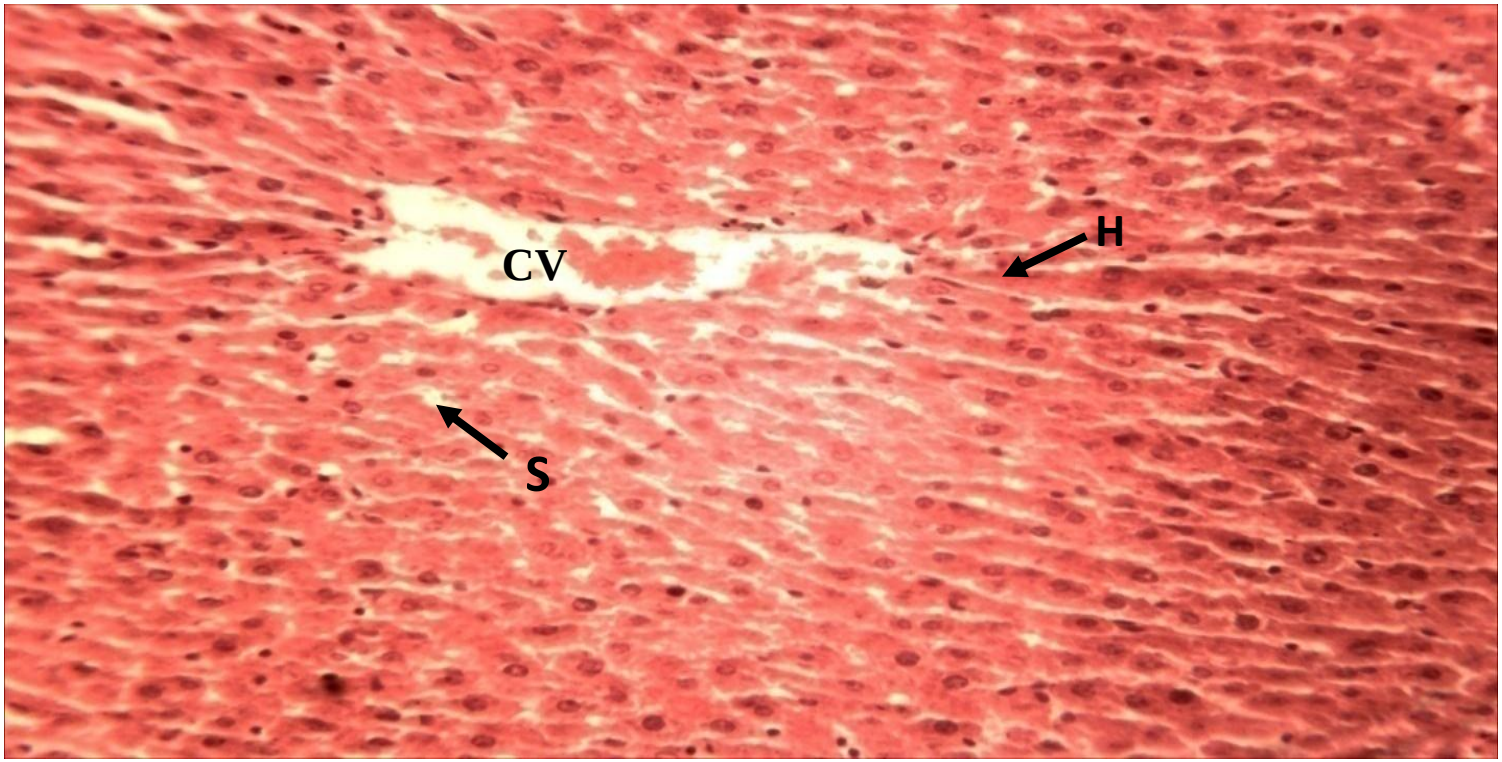


Plate VII: Section of the liver of treatment group that received 40 mg/kg of garlic showing mild histoarchitectural distortion of the liver of Wistar rat. Congested central vein (**CV**); Hepatocyte (**H**); Sinusoid (**S**). H&E (Mag x 250).

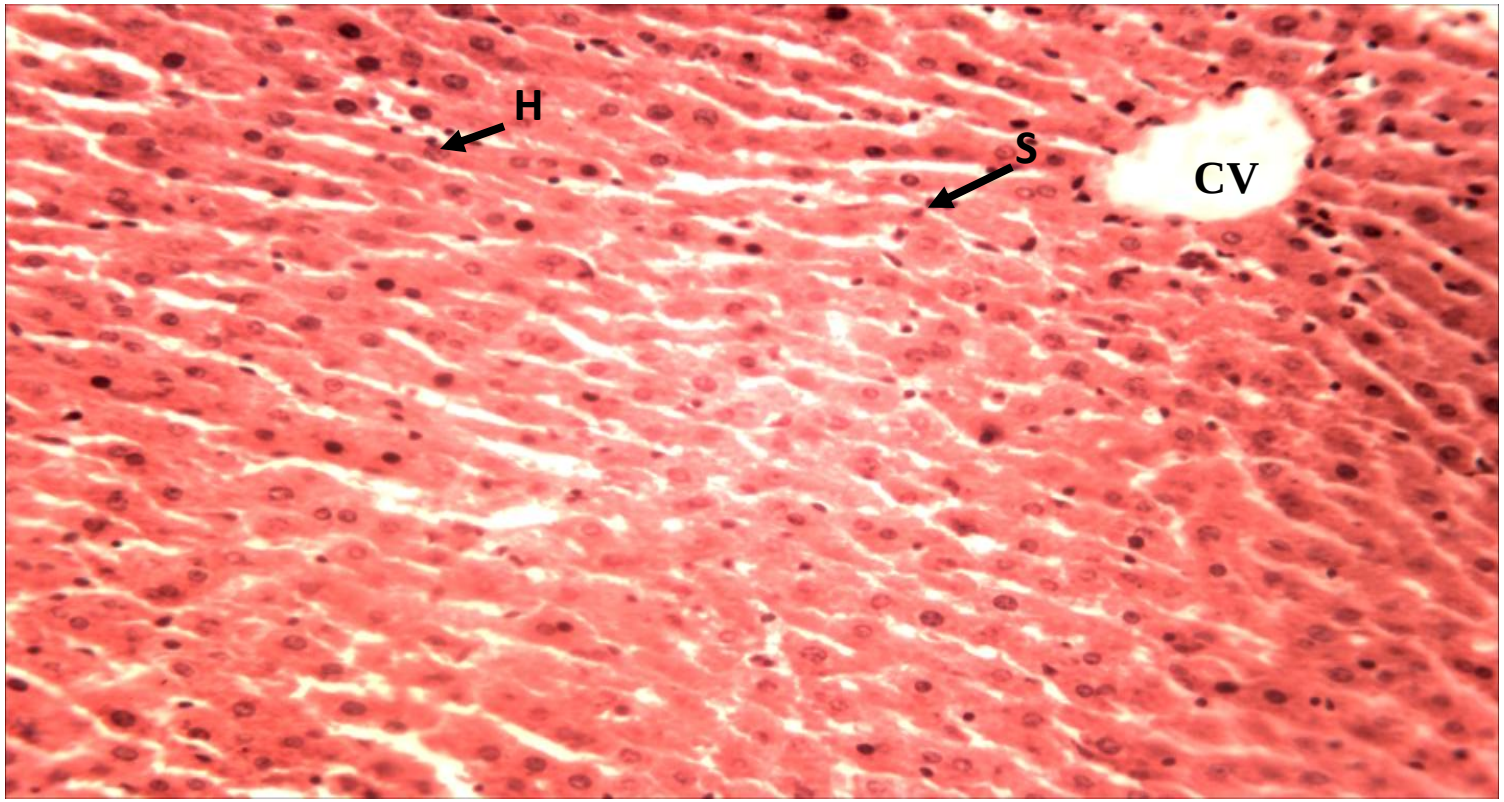


Plate VIII: Section of the liver of treatment group that received 20 mg/kg of ginger showing normal architecture of liver of Wistar rats. Central vein (**CV**); Hepatocyte (**H**); Sinusoid (**S**). H&E (Mag x 250).

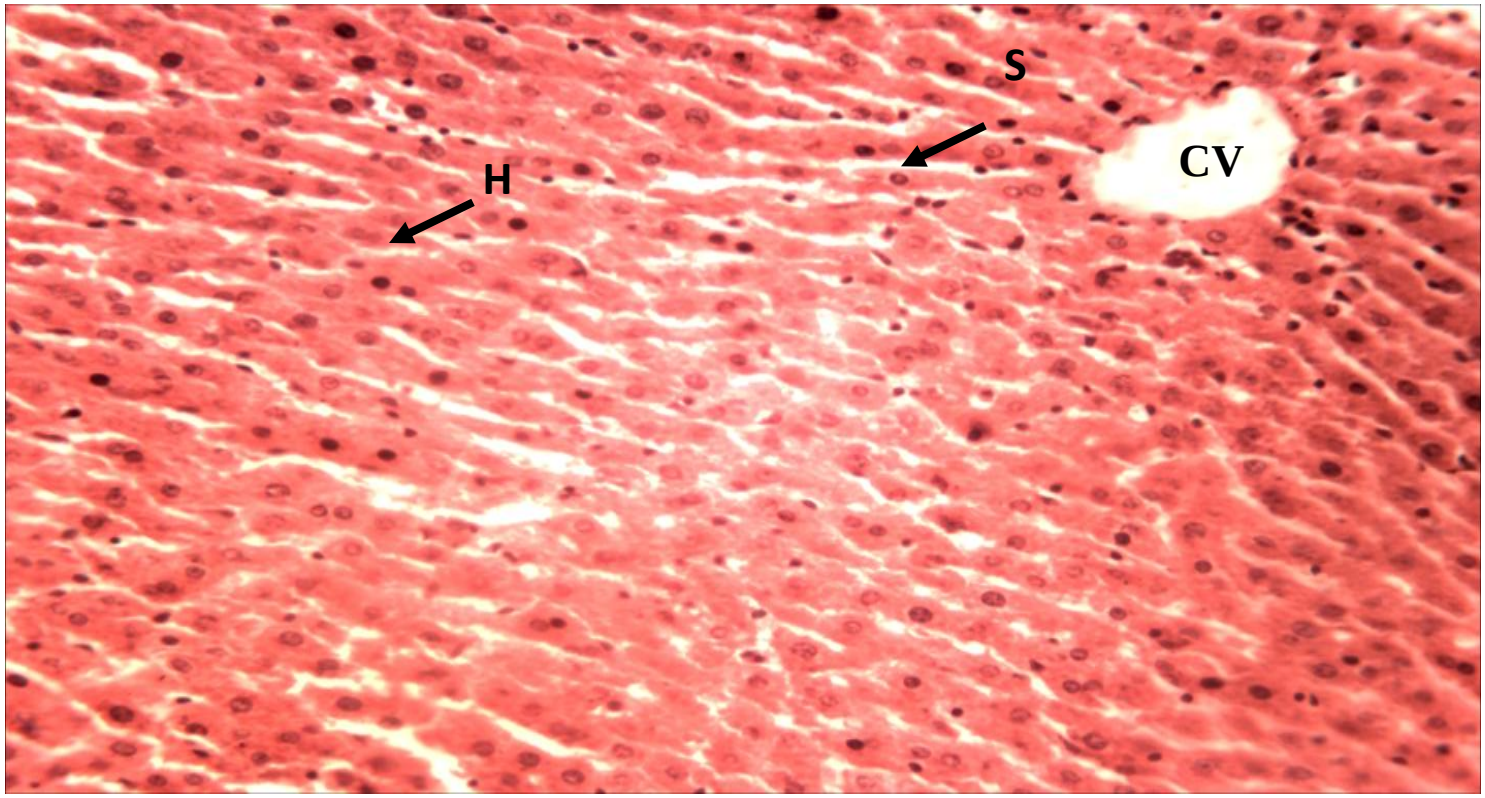


Plate IX: Section of the liver of treatment group that received 40 mg/kg of ginger showing normal architecture of liver of Wistar rats; central vein (**CV**); Hepatocyte (**H**); Sinusoid (**S**). H&E (Mag x 250).

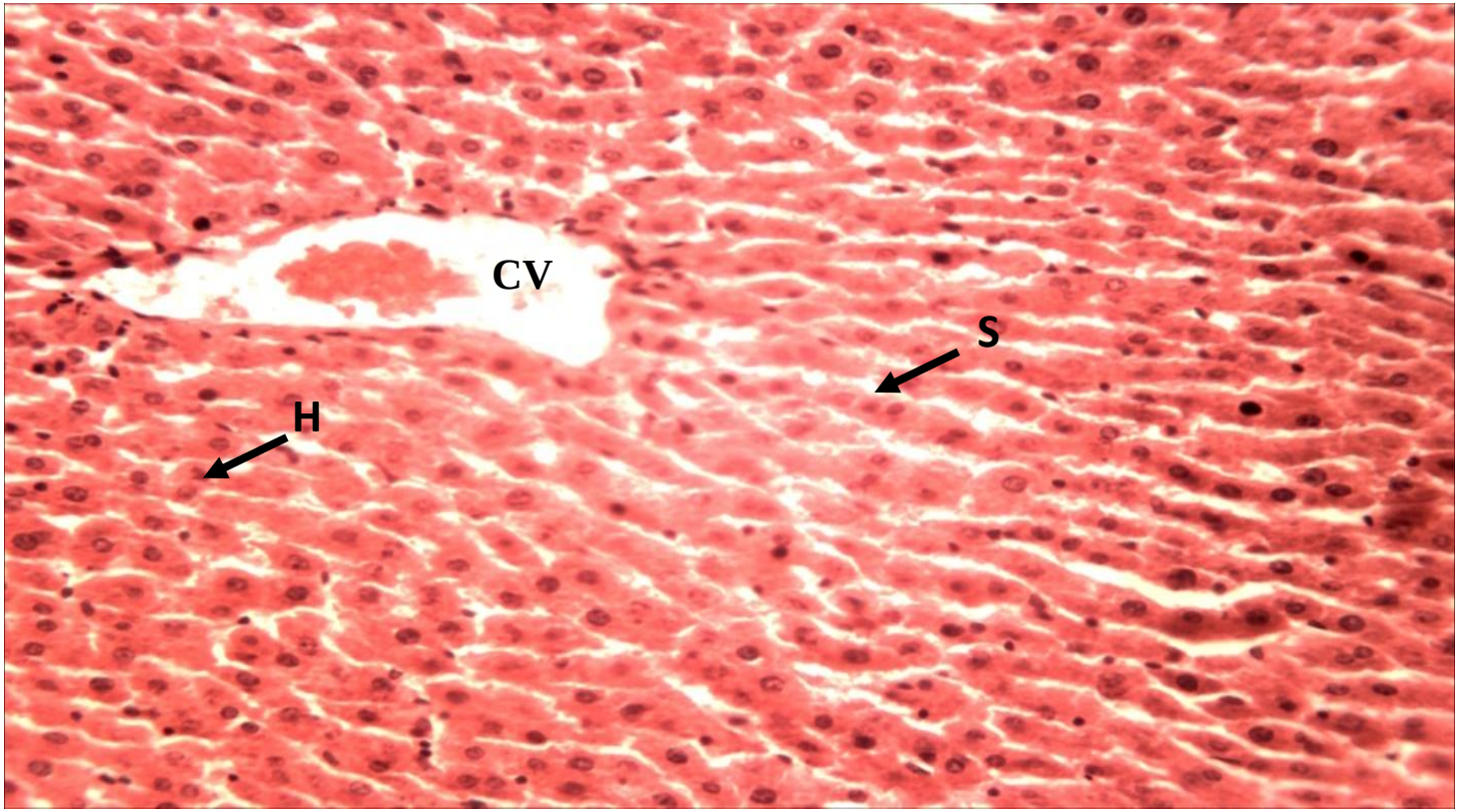


Plate X: Section of the liver of treatment group that received 10 mg/kg of garlic and ginger showing mild histoarchitectural distortion of the liver of Wistar rat. Congested central vein (**CV**); Hepatocyte (**H**); Sinusoid (**S**). H&E (Mag x 250).

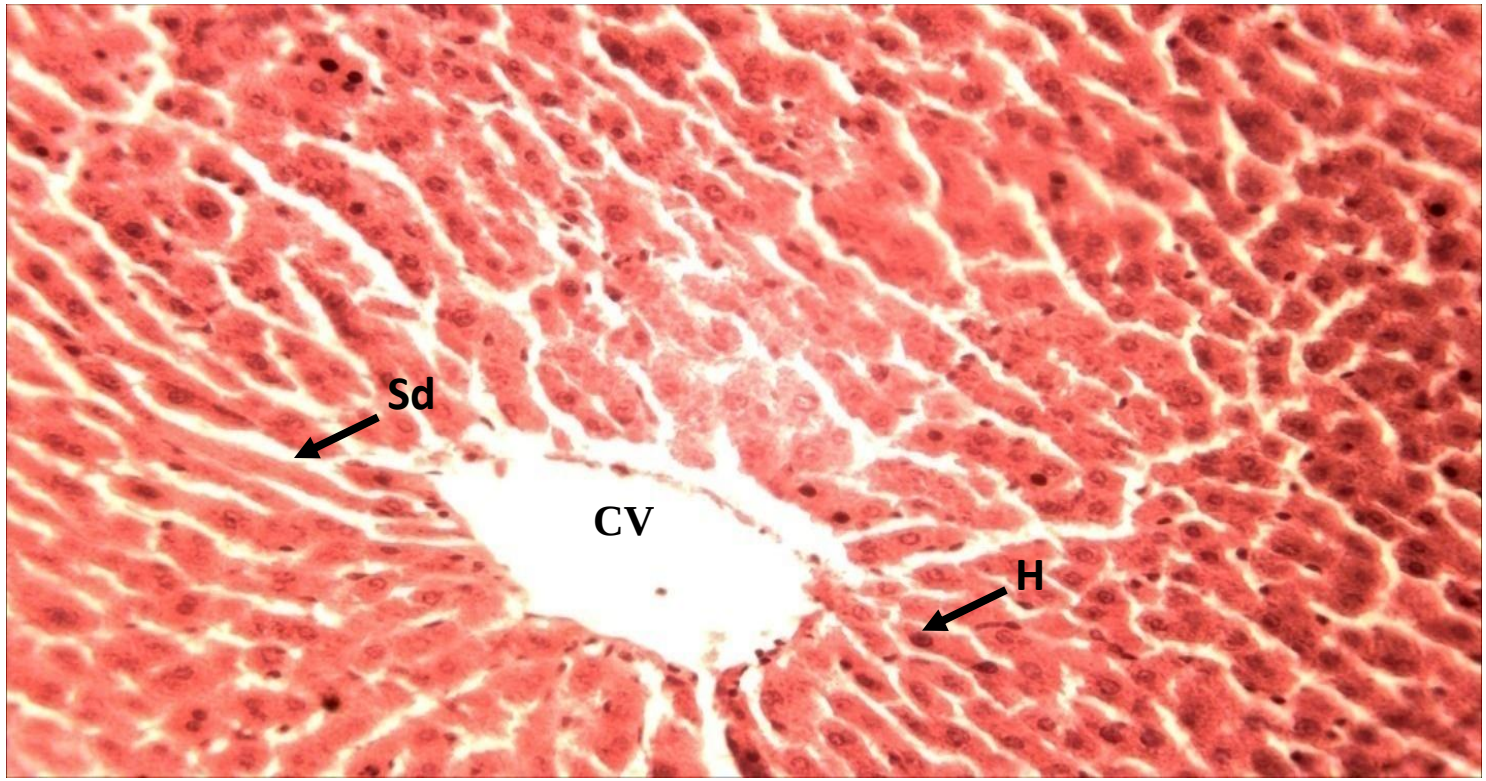


Plate XI: Section of the liver of treatment group that received 20 mg/kg of garlic and ginger showing mild histoarchitectural distortion of the liver of Wistar rat. Central vein (**CV**); Hepatocyte (**H**); Sinusoidal dilatation (**Sd**). H&E (Mag x 250).

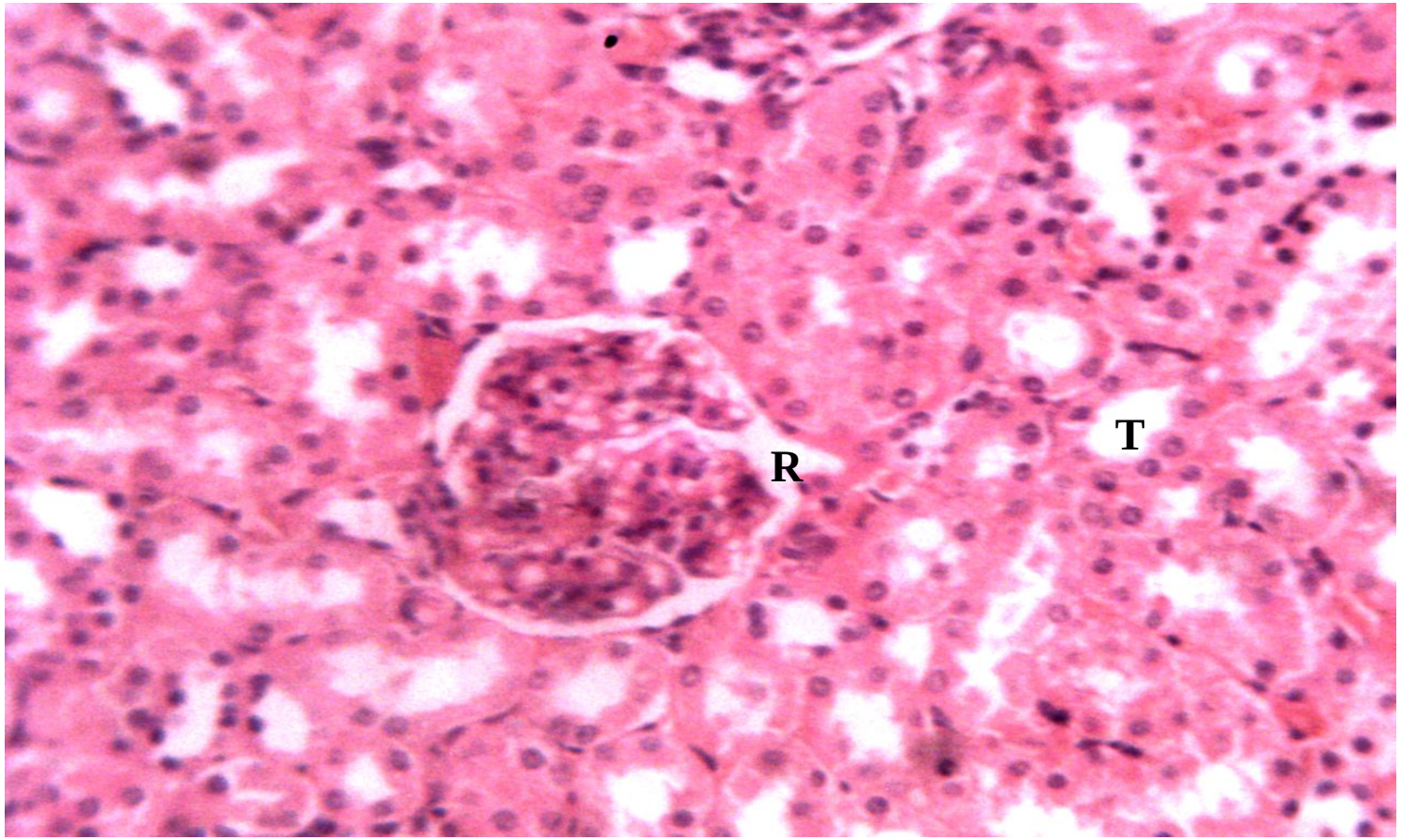


Plate XII: Section of the kidney of Wistar rats of control group showing renal corpuscle (R), renal tubules (T). H&E (Mag X250)

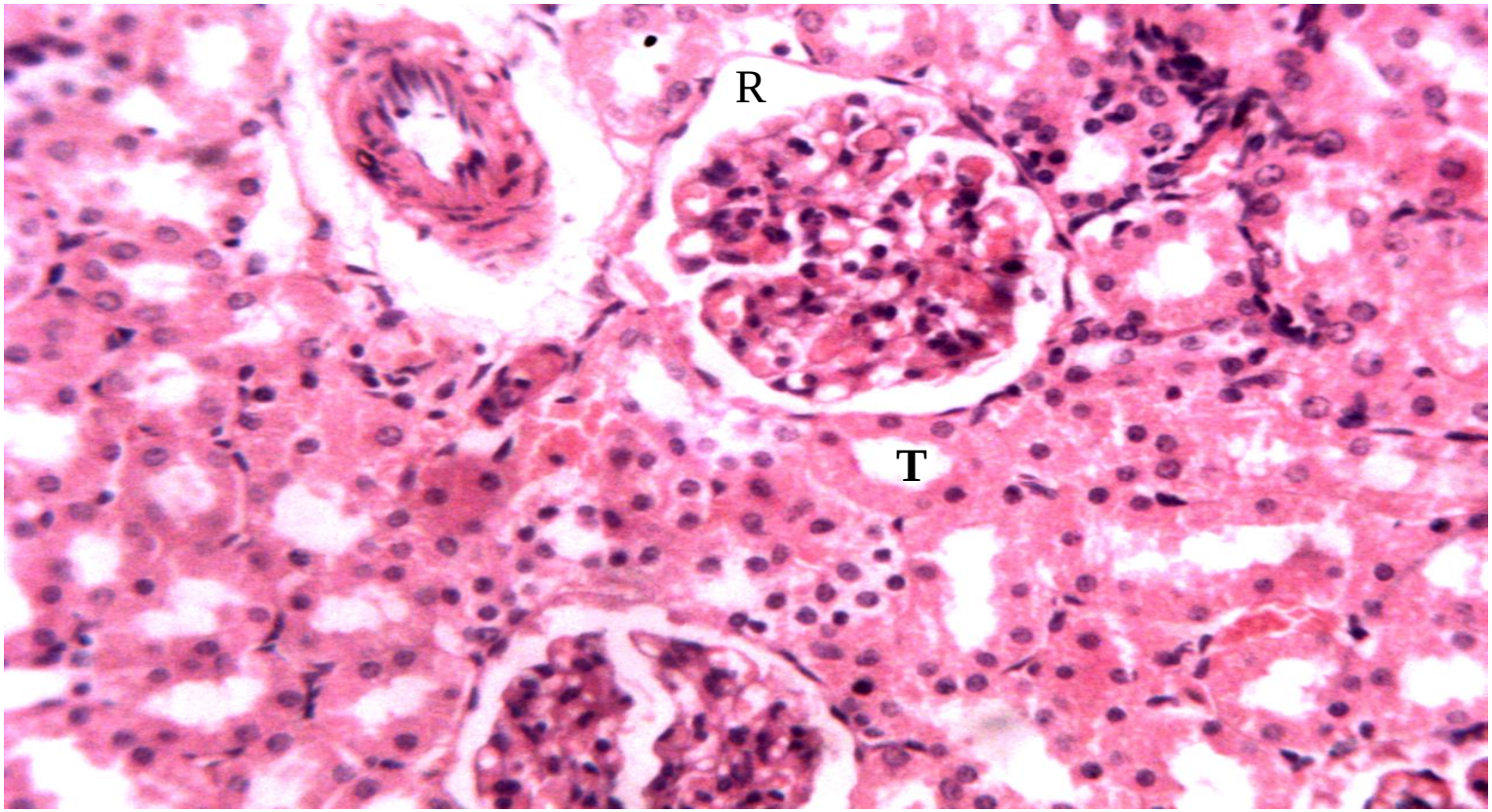


Plate XIII: Section of the kidney of Wistar rats of treatment group that received 20 mg/kg of garlic showing renal corpuscle (**R**), renal tubules (**T**). H&E (Mag x 250).

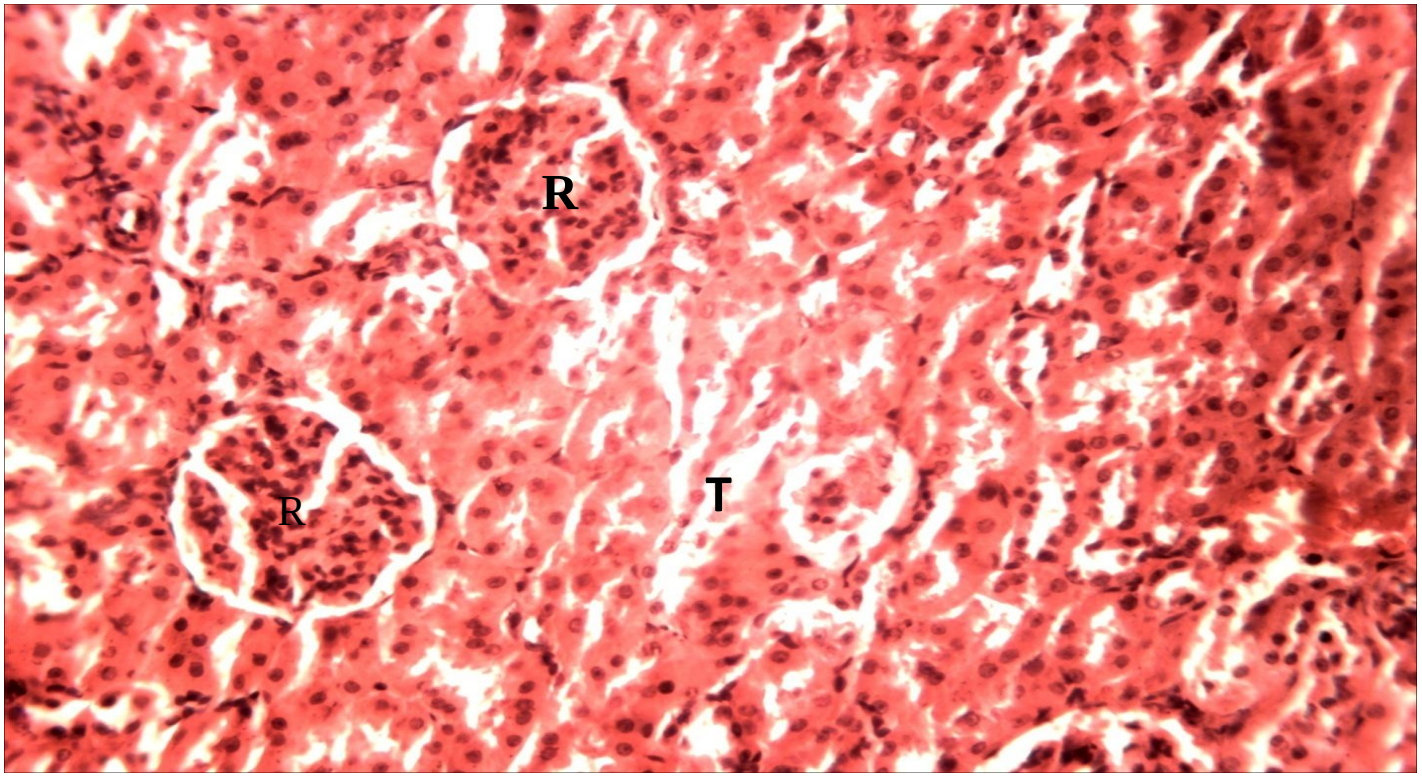


Plate XIV: Section of the kidney of Wistar rats of treatment group that received 40 mg/kg of garlic showing renal corpuscle (**R**), renal tubules (**T**). H&E (Mag x 250).

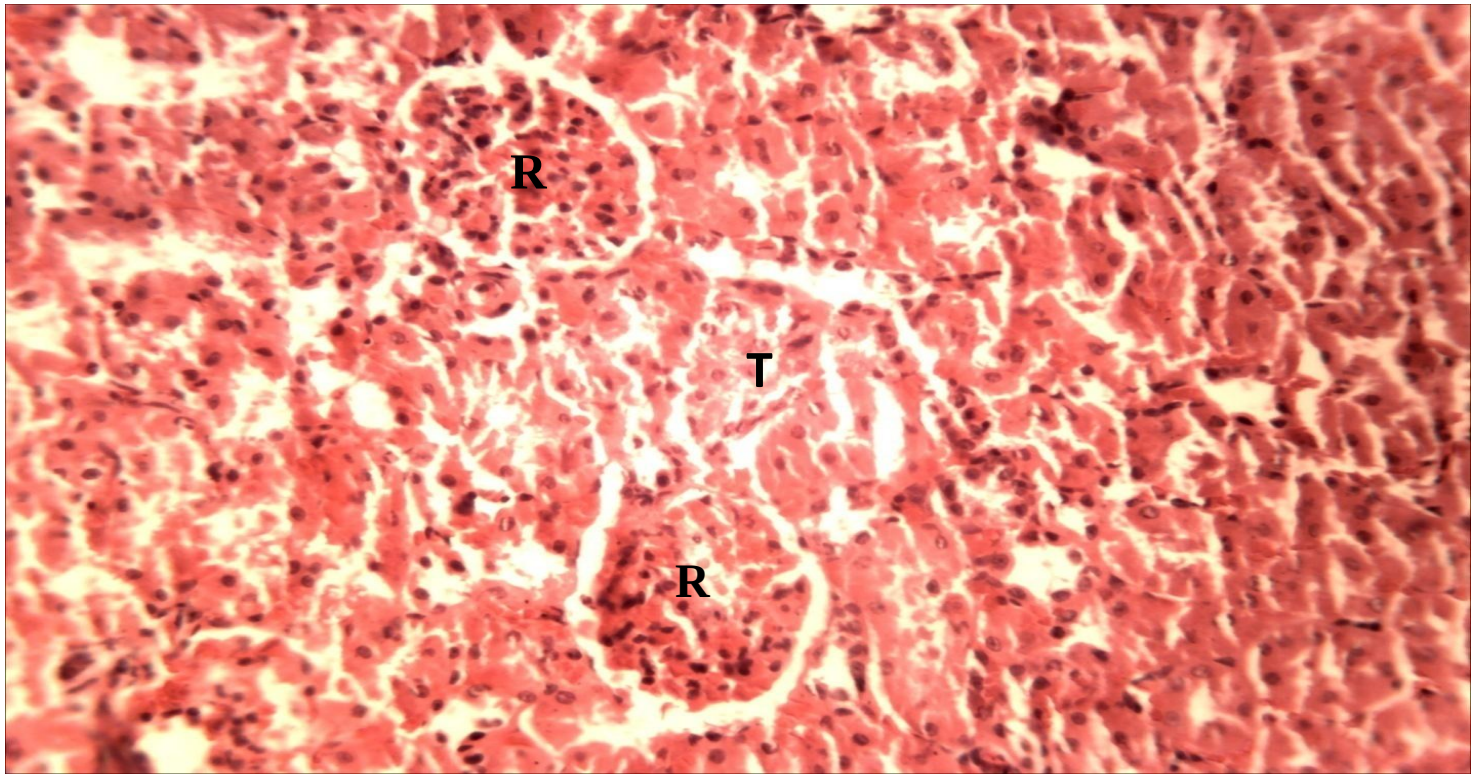


Plate XV: Section of the kidney of Wistar rats of treatment group that received 20 mg/kg of ginger renal corpuscle (**R**), renal tubules (**T**); H&E (Mag x 250).

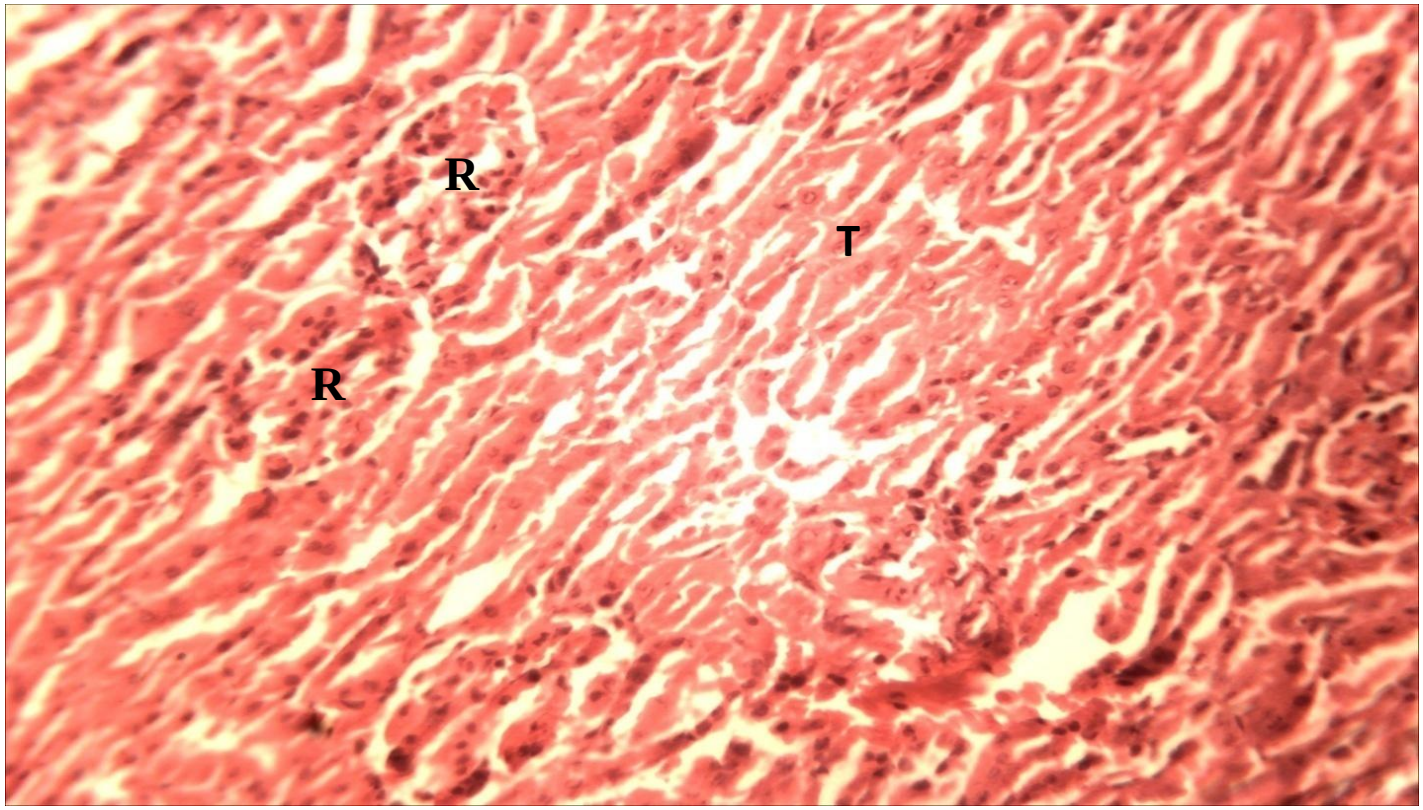


Plate XVI: Section of the kidney of Wistar rats of treatment group that received 40 mg/kg of ginger renal corpuscle (**R**), renal tubules (**T**); H&E (Mag x 250).

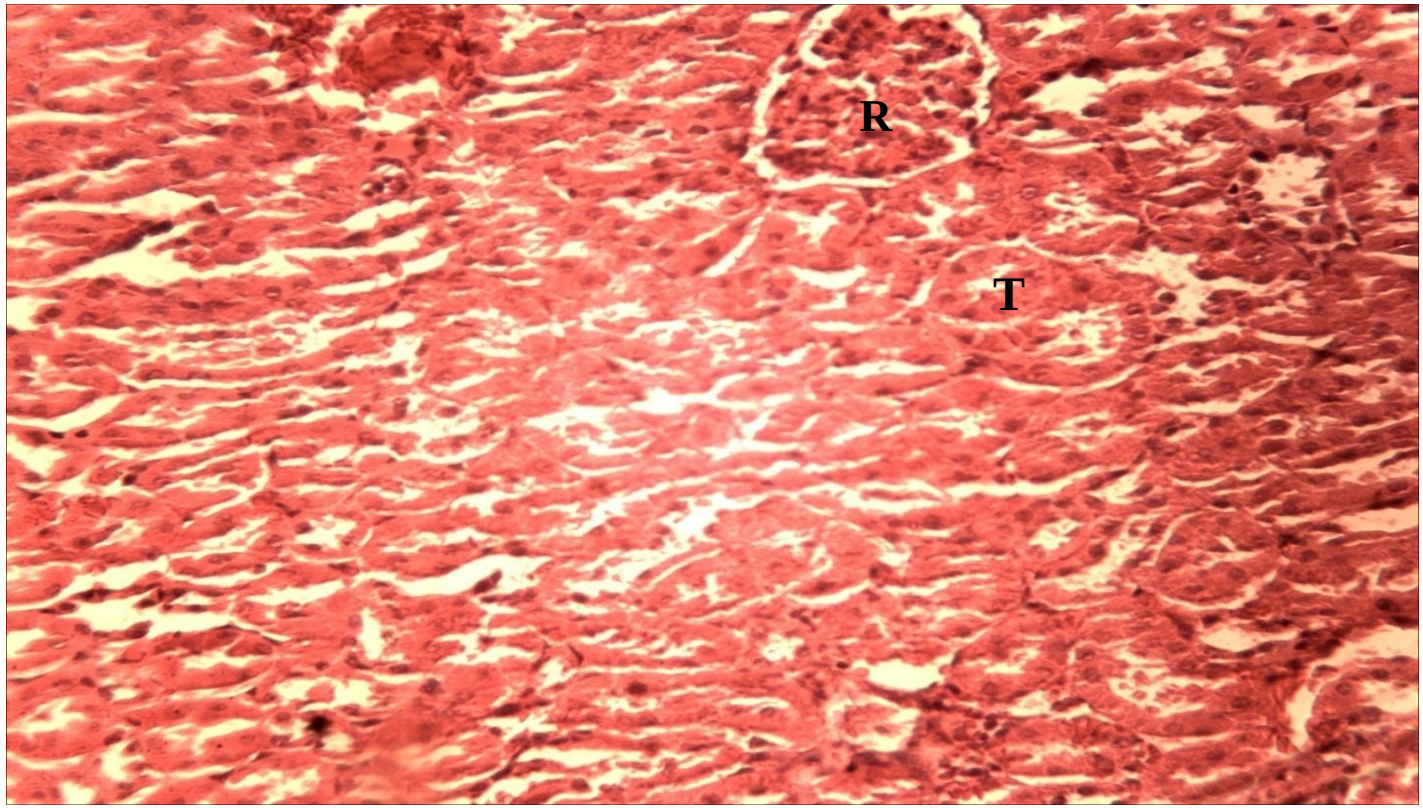


Plate XVII: Section of the kidney of Wistar rats of treatment group that received 10 mg/kg of ginger and garlic renal corpuscle (**R**), renal tubules (**T**); H&E (Mag x 250).

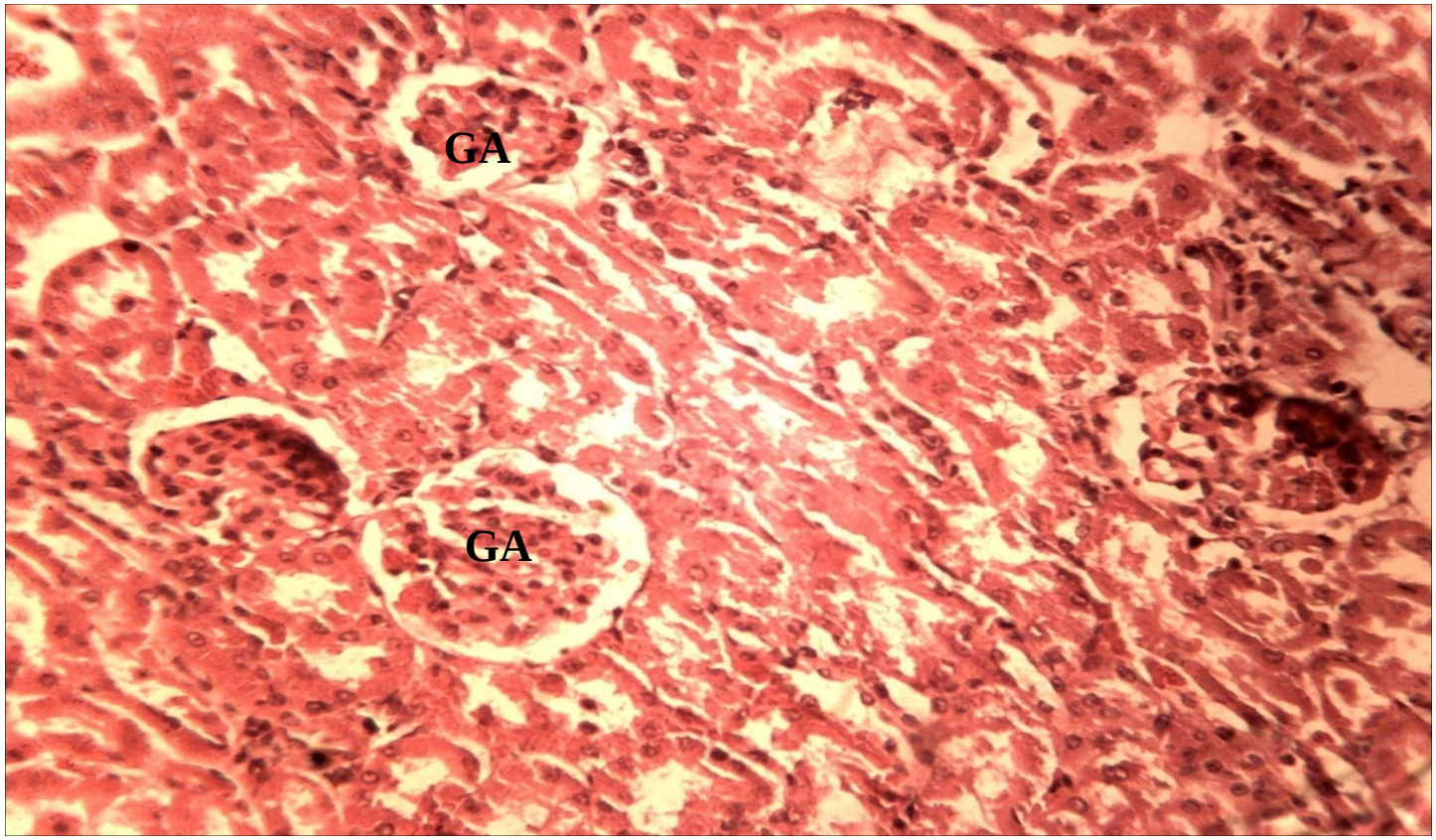


Plate XVIII: Section of the kidney of Wistar rats of treatment group that received 20 mg/kg of ginger and garlic showing glomeruli atrophy (GA). H&E (Mag x 250).

4.10 Effects of Ginger and Garlic on Blood Pressure Parameters in Cats

The separate and combined doses of garlic and ginger extracts significantly ($p<0.05$) decrease the systolic, diastolic and mean arterial blood pressure as presented in table 4.5. However, there is no significant difference between the separate effect and combined effect of the extract.

4.11 Effects of Garlic and Ginger on the Force and Rate of Contraction of Rabbit Heart

Both garlic and ginger separately decreased the force and rate of rabbit heart significantly ($p < 0.05$) as shown in figure 4.4 and figure 4.5 respectively. The combination of garlic and ginger also significantly decreased both the force of contraction and the rate of contraction of rabbit heart. Garlic and ginger when administered with adrenalin showed no significant difference from the control ($p > 0.05$), while atropine when administered with garlic and ginger showed a significant difference ($p < 0.05$) from the control as seen in figure 4.4 and 4.5.

Table 4.5: Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Blood Pressure Parameters in Cats

Dose /concentrations given	SBP (mm/Hg)	DBP (mm/Hg)	Pulse pressure (mm/Hg)	MABP (mm/Hg)
Normal saline	75.75±0.85	64.3±1.09	11.45±0.82	68.11±0.84
Garlic (0.1 mg/ml)	54.3±1.32 [a]	34.5±1.55[a]	19.8±0.25 [a]	41.1±1.46 [a]
Garlic (1 mg/ml)	54.0±0.41 [a]	35.8±0.48[a]	18.3±0.25 [a]	41.8±0.44[a]
Garlic (10 mg/ml)	55.8±1.12 [a]	36.8±0.95[a]	19.0±0.71[a]	43.1±0.96 [a]
Garlic (20 mg/ml)	59.0±2.48[ns]	42.8±0.63[a]	16.2±0.75 [ns]	48.2±2.53 [a]
Ginger (0.1 mg/ml)	63.5±2.50[ns]	51.5±3.50[ns]	12.0±1.00[ns]	55.5±3.20 [a]
Ginger (1 mg/ml)	66.2±3.13[ns]	56.0±2.97[ns]	10.2±0.31[a]	59.4±3.10 [a]
Ginger (10 mg/ml)	56.5±3.52[a]	45.0±4.71[a]	11.5±1.55 [a]	48.6±4.45 [a]
Ginger (20 mg/ml+)	46.6±0.93[a]	38.8±0.80[a]	7.8±0.73 [a]	41.4±0.76[a]
Garlic + Ginger (0.1 mg/ml)	60.5±0.65[ns]	38.8±2.59[a]	21.7±1.88	46.0±1.68 [a]
Garlic + Ginger (1 mg/ml)	59.0±2.48[a]	36.3±2.59[a]	22.75±0.75 [a,b]	43.8±2.53 [a]
Garlic + Ginger (10 mg/ml)	62.8±1.75[a]	44.5±2.63[a]	18.25±1.11 [ns]	50.6±2.32 [a]
Garlic + Ginger (20 mg/ml)	63.5±2.75[a]	43.0±2.79[a]	20.50±0.28 [a,b]	49.8±2.78 [a]

Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant
ns= non significant

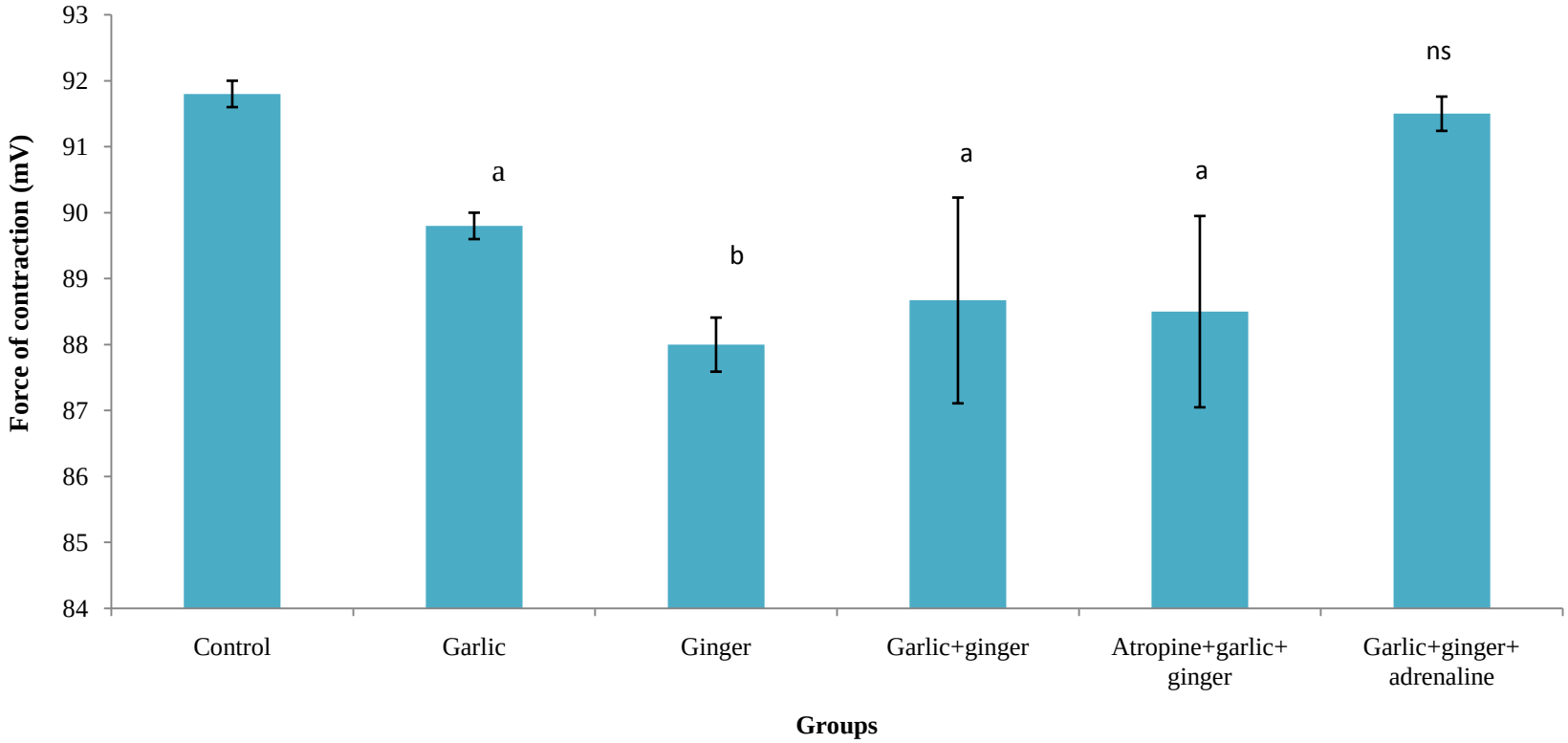


Figure IV: The Effect of Garlic and Ginger on the Force of Contraction of Rabbit Heart

Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant
ns = non significant

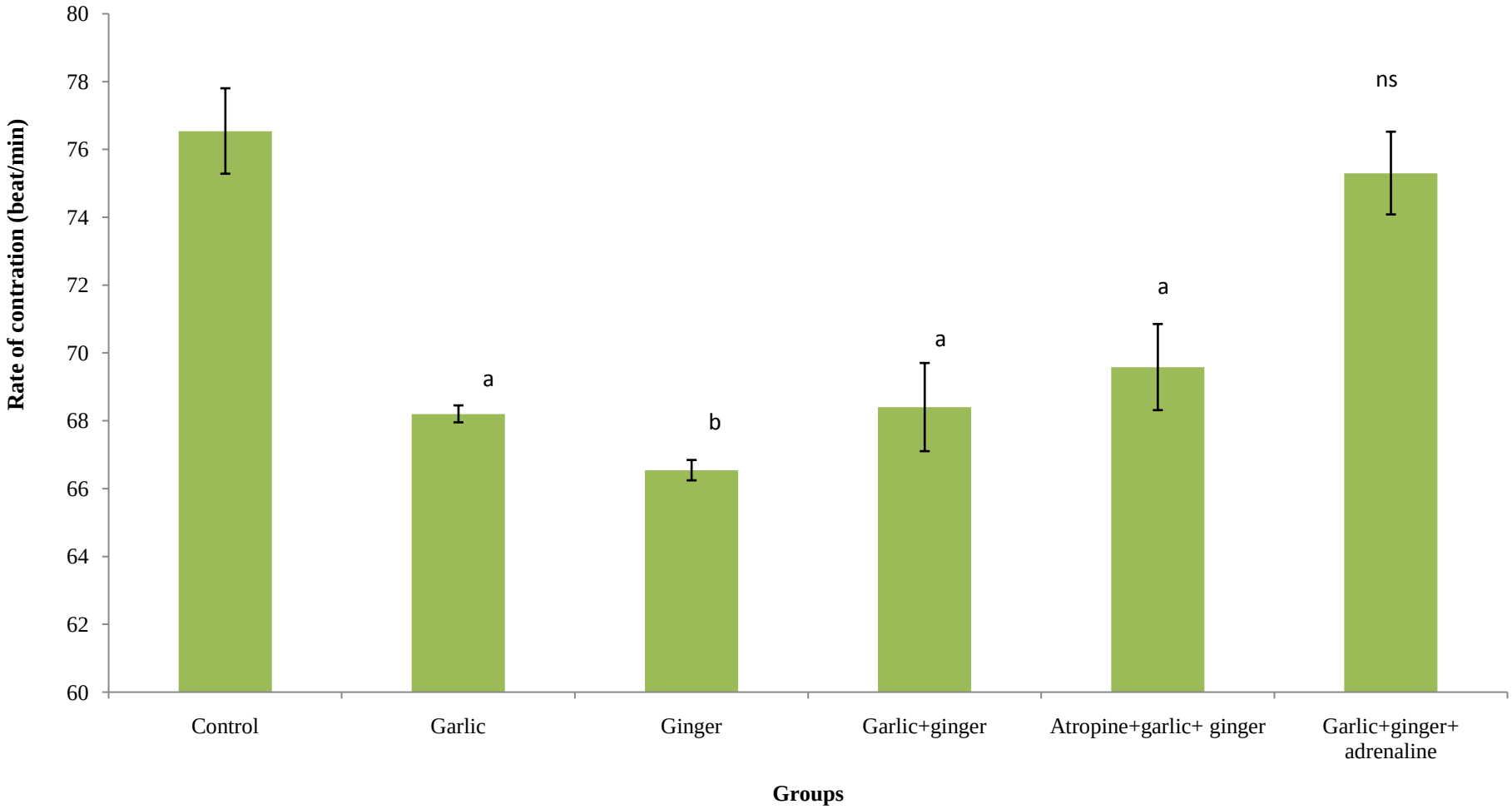


Figure V: The Effect of Garlic and Ginger on the Rate of Contraction of Rabbit Heart

Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant
ns= non significant

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of Garlic *(Allium Sativum)* and Ginger *(Zingiber officinale)* on serum electrolytes concentration in Wistar rats

The present study investigated the biochemical and haematological changes induced by garlic and ginger extracts singly and their combination in animals. A marked decrease in serum sodium concentration observed during the present study may be due to a change in glomerular filteration rate and/or renal blood flow (Asdaq and Inamdar, 2011) or interference with aldosterone secretion and/or aldosterone action on the distal tubules. It may also be as result of interference with adrenergic sodium handling (Asdaq and Inamdar, 2010) caused by garlic and ginger administration as well as their combination. There was also significant elevation in serum potassium concentration in the groups, administerred with garlic and ginger extract, when compared to the control group. The present finding suggests that the rise in the concentration of potassium may be due to the alteration in potassium transport produced by the extracts.

5.2 Effects of Garlic *(Allium Sativum)* and Ginger *(Zingiber officinale)* on erythrocyte and leucocyte indices in Wistar rats

The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds toxins, chemicals and plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as

enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs (Reddy *et al.,* 2012). In the present study, the effects of garlic and ginger extract on haematogical parameters such as packed cell volume, haemoglobin concentration and red cell count, total and differential white blood cells were assessed. The study showed that daily administration of animals with various doses of garlic and ginger significantly decreased packed cell volume and haemoglobin concentration revealing that garlic and ginger are toxic to red blood cells. This finding is in agreement with the works of Banerjee and Maulik (2004), who showed that prolonged feeding of rats with garlic and ginger may result in anaemia, weight loss and growth retardation in rats, although the weight of the animals was not determined in the present study. This may result in hypoxia, which then stimulates red blood cell production in the bone marrow. There was a significantly increased neutrophil and lymphocyte counts in animals fed with garlic 40 mg/kg and ginger 40 mg/kg and its combination. The monocyte count was also significantly elevated in rats administered with 20 mg/kg of garlic and ginger 20 mg/kg and its combination. This observation supports the finding of Bjarnsholt *et al.,* and Chandrashekar and Venkatesh (2012), who demonstrated that garlic stimulates cell-mediated immune functions in rats.

5.3 Effects of Garlic and Ginger on serum lipid profile

The results showed that there was a statistically significant reduction of serum total cholesterol concentration in the animals administered with both single and combined doses of aqueous garlic and ginger extract, when compared to the control group. Allicin has been proposed as the active compound produced by garlic responsible for its hypocholesterolaemic effect. Garlic and ginger

have been shown to modify lipid metabolism by inhibiting cellular cholesterol biosynthesis, increasing bile acid biosynthesis to eliminate cholesterol from the body and

increasing faecal cholesterol excretion; therefore, cholesterol synthesis is reduced by up to 75% (Gu and Zhu, 2011). Al-Amin, 2006; Singh and Poter, 2006; Matsuda *et al.*, 2009, (Drobiova, 2011). The cholesterol lowering effects of garlic has also been attributed not only to its organosulphur constituents, but also to a variety of steroidal saponins present in garlic extract (Lanzotti, 2012). There was also significant decrease in serum triglyceride and low- density lipoprotein concentrations at all single and combined doses of the aqueous garlic and ginger administered to animals, when compared to the control group. However, the extract at single and combined doses significantly elevated serum high density lipoprotein concentration in the treated animals when compared the control group. These findings are in agreement with those of Ali *et al.* (2000) who demonstrated that administration of garlic to rats is effective in decreasing total cholesterol and triglycerides. However, the mechanism for triglyceride lowering effect of garlic is not well understood. However, Yeh and Yeh (1994) demonstrated that the rate of acetate incorporation into fatty acid was reduced in hepatic cell culture treated with garlic extract. Thus, the triglyceride lowering effect of garlic may be due to the inhibition of synthesis of fatty acids. The result of the present study agree with the finding of Elshater *et al.* (2009) who showed that ginger extract administered to diabetic rats produced significant reduction in the levels of plasma cholesterol, triglycerides and low-density lipoprotein cholesterol, and significant elevation in the high-density lipoprotein cholesterol when compared with the control group. Elrokh *et al.* (2010) also demonstrated that ginger exerted significant lipid lowering effect in hyperlipidaemic patients. The reduction in low-density lipoprotein cholesterol by garlic obtained in the present study may be due to allicin, an active compound contained in garlic. Allicin reduces the

production and release of low-density lipoprotein cholesterol by the liver and promotes the activity of low-density lipoprotein receptors in the liver cells, which helps the liver to clear the circulating low-density lipoprotein cholesterol (Madkor *et al.,* 2010). Brousseau *et al.* (2004) reported that the increase in HDL-c level is usually attributed to allicin, which alters the distribution of cholesterol among high-density lipoprotein and low-density lipoprotein subclasses by a mechanism apparently due to a reduction of very low-density lipoprotein level. This reduction is associated with a secondary decrease in apo D activity, which results in less transfer of high-density lipoprotein cholesterol to low-density lipoprotein rceptor particles. Hyperlipidaemia is the underlying pathophysiology of the number one killer, atherosclerotic coronary artery disease (Lavu *et al.,* 2011).

5.4 Effects of Garlic and Ginger Extract on Isolated Rabbit Heart

The present study revealed that administration of the extract, especially at the combined doses, produced a reduction on the rate and force of contraction, respectively, when compared with the base-line control level. The interaction between the extract and the standard drug (adrenaline) revealed that the extract completely blocked the action of adrenaline when co-administered, hence reducing both the rate and force of contraction. This finding suggests that the extracts may act via β_1-adrenergic receptor, located on the myocardium. The blockade of β_1-receptors has been shown to cause negative inotropic and chronotropic effect, which in turn brings about a decrease in cardiac work and output; and these effects are useful in the treatment of myocardial infarction, cardiac arrhythmias and angina pectoris (Lai *et al* 2012). Recent *in vitro* studies by Benavides *et al.* (2007) have confirmed the vasoactive ability of garlic's sulphur compounds, whereby red blood cells convert garlic's organic polysulphides into hydrogen sulphide, a known

endogenous cardio-protective vascular cell signaling molecule. Ghayur and Gilani (2005) reported that the crude extract of ginger induced a dose-dependent decrease in the arterial blood pressure of anaesthetized rats. In guinea pig paired atria, the crude extract was shown by Ghayur and Gilani (2005) to exhibit a cardio-depressant activity on the rate and force of spontaneous contractions.

5.5 Effects of Garlic and Ginger Extract on serum liver enzymes activities

The liver is the main target organ of acute toxicity where exposed to the foreign substances being absorbed in intestines and metabolised to other compounds, which may or may not be hepatotoxic to the rats (Rhiouania *et al.,* 2008). In this study, the liver histology revealed evidence of normal hepatocytes and did not cause any alteration to the structure of the liver cells. Also there was no necrosis, inflammatory reaction, fibrosis or local fatty degeneration in the liver of the control group (Plate 1) and the garlic and ginger extract treated animals (plate 2-7) when compared to control group. The observed non-significant change in activities of the liver enzymes as well as the preserved the structural integrity of liver tissues in all groups of animals fed with single and combined doses of garlic and ginger suggest the non-deleterious effects of the plant extract. Therefore, the extracts are hence safe for consumption especially in humans (Banerjee *et al.*, 2001).

5.6 Effects of Garlic *(Allium Sativum)* and Ginger (*Zingiber officinale*) on Blood Pressure Parameters in Cats

The study assessed the effect of garlic and ginger on blood pressure parameters in cats. The study revealed that the administration of only garlic at doses of 10 and 20 mg/kg produced a significant decrease ($P < 0.05$) in the systolic blood pressure, when compared to normal control

group. On the other hand, there was a statistically significant decrease ($P < 0.05$) diastolic blood pressure in all single doses of garlic as well as the combined doses of garlic and ginger extract administered when compared to control group. The result obtained also showed that administration of only single doses of ginger produced a significantly reduced ($p < 0.05$) pulse pressure, when compared to normal control group. In regards to mean arterial blood pressure, administration of single doses of garlic (0.1 and 1 mg/ml) produced a significant reduction in mean arterial pressure when compared to control group. Nitric oxide (NO) is an important mediator of blood pressure homeostasis. It has been reported that pharmacologically- reducing the bioavailability of NO can lead to hypertension in normal rats (Ribeiro *et al.,* 1992). Garlic appears to exert blood pressure reducing activity by modulating the activity of several mechanisms that are vital in blood pressure homeostasis, which include the prostaglandin system (Al-Qattan *et al.,* 2001), rennin-angiotensin system (Sharif *et al.,* 2003), and renal tubular transport mechanisms Al-Qattan *et al.,* 2003). Another possible mechanism by which garlic might induce its blood pressure effect may be through the direct and indirect vasodilatory actions of NO (Asdaq and Inamdar 2011). Garlic was reported to contain arginine and enhance the synthesis of NO (Kim *et al.,* 2001; Asdaq and Inamdar 2011).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

The findings in the present work can be summarized as follows:

i. The combination of garlic and ginger lowered the mean arterial blood pressure, sodium concentration and lipid profile, hence may have cardio protective properties and decreased the risk of cardiovascular diseases.

ii. Garlic and ginger separately are non toxic to the liver and kidney, but a combination of both garlic and ginger may be harmful to the liver and kidney.

iii. The combination of garlic and ginger decreased the packed cell volume and haemoglobin concentration.

iv. The mechanism of action of the combination of garlic and ginger on the cardiovascular system is probably through adrenergic receptors.

v. There was a significant difference between the effects exerted by single administration of garlic and ginger on the haematological and biochemical parameters as compared with the combined effect of garlic and ginger.

vi. Single administration of either garlic or ginger altered the cardiovascular biochemical and haematological parameters in laboratory animals.

vii. The combination of ginger and garlic decreased the leucocytes counts but increased the serum liver enzymes.

viii. The combination of ginger and garlic also produced significant changes in the activities of serum liver enzymes as revealed by the histological findings. Thus, the plant extract adversely affect the morphology of liver and kidney tissues in groups treated with combined doses of the extracts. While, the single effect of either garlic or ginger did not alter the morphology of the liver and kidney.

6.2 CONCLUSION

Garlic and ginger extracts lowered the blood pressure, decreased force and rate of contraction of the heart, decreased the lipid profile and thus may have very important cardioprotective functions. The combined effect of garlic and ginger does not differ significantly from the single dose of either garlic or ginger. However, the combined effect may be toxic to the liver and white blood cells.

6.3 RECOMMENDATIONS

i. Further studies should be carried out on fractions of these extracts on the physiological profiles
ii. The consumption of garlic and ginger together should be discouraged, since the combination may be toxic to the liver and kidney.
iii. Studies on long-term effect of garlic and ginger should be done.
iv. Prolonged consumption of these plants should be discouraged, since the combination may be toxic to the liver and kidney.
v. Toxicity study of the combination is suggested.

REFERENCES

Abdulrazaq, B.M., Cho, M.M., Win, N.N., Zaman, R. and Rahman, T.M. (2011). "Beneficial effects of ginger (Zingiber officinale) on carbohydrate metabolism in streptozotocin-induced diabetic rats," *British Journal of Nutrition,* 108(7): 1194–1201.

Achenbach, S., Ropers, D., Pohle, K., Leber, A., Thilo, C., Knez, A., Daniel, W. G., (2002). Influence of lipid-lowering therapy on the progression of coronary artery calcification: a prospective evaluation. *Circulation,* 106: 1077–1082.

Afzal, M., Al-Hadidi, D., Menon, M., Pesek, J. and Dhami, M.S. (2001). Ginger: an ethnomedical, chemical and pharmacological review. *Drug Metabolism and Drug Interaction,* 18:159–190.

Aggarwal, B.B. and Shishodia, S.(2004). Suppression of the nuclear factor-kappaB activationpathway by spice-derived phytochemicals: reasoning for seasoning. *Annals of the New York Academy of Sciences,* 1030: 434-441.

Akhani, S. P.,Vishwakarma, S. L. and Goyal, R. K. (2004). "Antidiabetic activity of *Zingiberofficinale* in streptozotocininduced type I diabetic rats," *Journal of Pharmacy and Pharmacology,* 56(1): 101–105.

Akita, S. (2003). Effect of the dietary approaches to stop hypertension (DASH) diet on the pressure–natriuresis relationship. *Hypertension,* 42: 8–13.

Al-Amin, Z.M., Thomson, M. Al-Qattan, K.K.,Peltonen- Shalaby, R.and Ali, M. (2006). Anti diabetic and hypolipidaemic properties of ginger (*Zingiber officinale*) in streptozotocin induced diabetic rats. *British Journal of Nutrition,* 96: 660–666.

Ali, M., Al-Qattan, K.,Al-Enezi, F., Khanafe, R. and Mustafa, T. (2000). Effect of allicin from garlic powder on serum lipid and blood pressure in rats fed high cholesterol diet. *Prostaglandins, Leukotrienes Essential Fatty acids,* 62(4): 253–259.

Ali, B.H., Blunden, G., Tanira, M.O. and Nemmar, A.(2008). Some phytochemicalpharmacological ant toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food and Chemical Toxicology,* 46:409–420.

Alizadeh, R.F., Roozbeh, F., Saravi, M., Pouramir, M., Jalali, F. and Moghadamnia, A. A. (2008). Investigation of the effect of ginger on the lipid levels. A double blind controlled clinical trial. *Saudi Medical Journal,* 29(9): 1280–1284.

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

Al-Qattan, K.K., Khan, I., Alnaqeeb, M.A. and Ali, M. (2003). Mechanism of garlic (AlliumSativum) induced reduction of hypertension in 2K–1C rats: a possible mediation of Na/H exchanger isofrom-1. *Prostaglandins, Leukotrienes Essential Fatty Acids,* 69: 217–39.

Al-Qattan, K. K.,Thomsom, M., Al-mutawa'a, S., Al-Hajeri, D., Drobiava, H. and Ali, M. (2006). Nitric oxide mediates the blood pressure lowering effect of garlic in the rat two kidney, one-clip model of hypertension. *The Journal of Nutrition,* 136:774-776.

Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. *The Journal of Nutrition,* 136:716S–725S

Amin, A. and Hamza, A.A. (2006). Effects of roselle and ginger on cisplatin-induced reproductive toxicity in rats. *Asian Journal of Andrology,* 8:607–612.

Amira, O. C. and Okubadejo, N. U. (2007). Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care centre in Nigeria. *Complementary* and *Alternative Medicine*, 7(30): 1–5.

Apiz-castro, R., Badimon, J.J and Badimon, L. (1992). Effect of ajoene, the major antiplatelet compound from garlic on platelet thrombus formation. *Thrombosis Research,* 68:145–155

Asami, A., Shimada, T., Mizuhara, Y., Asano, T., Takeda, S., Aburada, T., Miyamoto, K. and Aburada, M. (2010). "Pharmacokinetics of [6]-shogaol, a pungent ingredient of Zingiber officinale Roscoe (Part I),"*Journal of Natural Medicines*, 64(3): 281–287.

Asdaq, S.M., Inamdar, M.N. and Asad, M. (2009). Effect of conventional antihypertensive drugs on hypolipidemic action of garlic in rats. *Indian Journal of Experimental Biology*, 47: 176-181.

Asdaq, S.M. and Inamdar, M.N. (2010). Potential of garlic and its active constituent, S-allyl cysteine, as antihypertensive and cardioprotective in presence of captopril. *Phytomedicine,* 17: 1016-1026.

Asdaq, S.M. and Inamdar, M.N. (2011a). The potential benefits of a garlic and hydrochlorothiazide combination as antihypertensive and cardio-protective in rats. *Journal of Natural Medicine,* 65: 81-88.

Asdaq, S.M. and Inamdar, M.N. (2011b). Pharmacodynamic and Pharmacokinetic Interactions of Propranolol with Garlic (Allium sativum) in Rats. *Evidence-Based Complementary and Alternative Medicine,* 824042.

Ashrif, M. Z., Hussain, M. E. and Fahim, M. (2005). Antiatherosclerotic effect of dietary supplementation of garlic and turmeric restoration of endothelial function in rats. *Life Sciences*, 77:837–857.

Badreldin, H.A., Gerald, B., Musbah, O. and Tanira, A.N. (2008). Some phytochemical,pharmacological and toxicological properties of ginger *(Zingiber officinale Roscoe)*: A review of recent research. *Food and Chemical Toxicology,* 46: 409–420.

Banerjee, S. K., Maulik, M., Manchanda, S. C., Dinda, A. K. Das, T. K. and Maulik, S. K. (2001). Garlic-induced alteration in rat liver and kidney Morphology and associated changes in endogenous antioxidant status, *Food and Chemical Toxicology,* 39:793–798.

Banerjee, S. K., Mukherjee, P. K. and Maulik, S.K. (2003). Garlic as an antioxidant: the good, the bad and the ugly. *Phytotherapy Research,* 17:97–106.

Banerjee, S.K. and Maulik, S.K. (2004). Effect of garlic on cardiovascular disorders: a review. *Nutrition Journal*.1(4): 1-14

Bar-Chen, W., Golan, T., Peri, I., Ludmer, Z. and Schwartz, B. (2010). Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2. *Nutrition and Cancer*, 62: 947-957.

Bardenheuer, H. and Schrader, J. (1983) Relationship between myocardial oxygen consumption coronary flow, and adenosine release in an improved isolated working heart preparation of guinea pigs. *Circulation Research,* 51:263–271

Barrett, M. (2004). Handbook of Clinically Tested Herbal Remedies. Volume 2. New York: Haworth Herbal Press. Pp. 493-545.

Becker, P.M., van Wikselaar, P.G., Mul, M.F., Pol, A., Engel, B., Wijdenes, J.W., Stockhofe-Zurwieden, N., … (2012). Actinobacillus pleuropneumoniae is impaired by the garlic volatile allyl methyl sulfide (AMS) in vitro and in-feed garlic alleviates pleuropneumonia in a pig model. *Veterinary Microbiology,* 154: 316-324.

Benavides, G.A., Squadrito, G.L., Mills, R.W., Patel, H.D., Isbell, T.S., Patel, R.P., and Kraus, D.W. (2007). Hydrogen sulfide mediates the vasoactivity of garlic. *Proceedings of the National Academy of Sciences of the United States of America,* 104(46):17977–17982.

Berg, J.M., Tymoczko, J.L. and Stryer, L. (2006). *Biochemistry*. W.H. Freeman and company, New York 5th edition. pp. 656–660.

Bergmeyer, H.U., Scheibe, P. and Wahlefeld, A.W. (1978). Optimization of methods for aspartate aminotransferase and alanine aminotransferase. *Clinical Chemistry* 24(1): 58–73.

Bhandari, U., Kanojia, R. and Pillai, K.K. (2005). Effect of Ethanolic Extract of *Zingiber officinale* on dyslipidaemia in diabetic rats. *Journal of Ethnopharmacology,* 28(97): 227 –230.

Bjarnsholt, T., Ostrup, J.P., Rasmussen, T.B., Christophersen, L., Calum, H., Hougen, H.P., Gisvkovs, N. M., … (2005). Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa reniore*. *Microbiology,* 151: 3878–3880.

Bowers, G.N.J. and Mc-Comb, R.B. (1966). A continuous spectrophotometric method for measurement of the activity of serum alkaline phosphatase. *Clinical Chemistry* 12: 70–73

Brankovic, S., Radenkovic, M., Kitic, D., Veljkovic, S., Ivetic, V., Pavlovic, D., Miladinovic, B. (2011). Comparison of the hypotensive and bradycardic activity of ginkgo, garlic, and onion extracts. *Clinical and Experimental Hypertension*, **33:**95-99

Brousseau, M.E., Schaefer, E. J., Wolfe, M. L., Bloedon, L. D., Digenio, A. G., Clark, R. W. and Rader, D. T. (2004). Effect of inhibitor of cholysteryl ester transfer protein on HDL cholesterol. *New England Journal of Medicine,* 8(15): 1491–1494.

Campbell, J. H., Efendy, J. L., Smith, N. J. and Campbell, G. R. (2001). Molecular basis by which garlic suppresses atherosclerosis. Recent advances on the nutritional effects associated with the use of garlic supplement. *The Journal of Nutrition,* 131: 1120–1123.

Campbell, D. (2012). Calcium Supplement "Double Risk of Heart Attack". In: *Medical Journal of The Heart*. 15: 30–37.

Capraz, M., Dilek, M. and Akpolat, T. (2006). Garlic, hypertension and patient education. *International Journal of Cardiology*, 15:45–50.

Carbone, L.D., Barrow, K.D., Bush, A.J., Boatright, M.D., Michelson, J.A., Pitts, K.A., Watsky, M.A., … (2005).Effects of a low sodium diet on bone metabolism. *Journal of Bone Mineral Metabolism*, 23(6):506–513.

Cavagnavaro, P. F., Camargo, A., Galmarini, C. R. and Simon, P.W. (2007). Effect of cooking on garlic *(Allium sativum L.)* antiplatelet activity and thiosulphates content. *Journal of Agicultural and Food Chemistry,* 55:1280–1283.

Chakraborty, D., Mukherjee, A., Sikdar, S., Paul, A., Ghosh, S. and Khuda-Bukhsh, A.R. (2012). "[6]-Gingerol isolated from ginger attenuates sodium arsenite induced oxidative stress and plays a corrective role in improving insulin signaling in mice,"*Toxicology Letters*, 210(1): 34-43.

Chandrashekar, P.M. and Venkatesh, Y.P. (2012). Fructans from aged garlic extract produce a delayed immunoadjuvant response to ovalbumin antigen in BALB/c mice. *Immunopharmacological Immunotoxicology*, 34: 174-180.

Chang, H. S., Yamato, O., Saki, Y., Yamasaki, M. and Maede, Y. (2004). Acceleration of superoxide generation in polymorphnuclear leuckocytes and inhibition of platelet aggregation by alkenyl thiosulphates derived from onion and garlic in dogs and humans. *Prostaglandins, Leukotrieness and Essential Fatty acids,* 70: 77–83.

Charlier, C. and Michaux, C., (2003). Dual inhibition of cyclooxygenase-2 (COX-2) and 5lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. *European Journal of Medicinal Chemistry,* 38, 645–659.

Chavan, S.D., Shetty, N.L. and Kanuri, M. (2010). Comparative evaluation of garlic extract mouthwash and chlorhexidine mouthwash on salivary Streptococcus mutans count - an in vitro study. *Oral Health and Preventive Dentistry*, 8: 369-374.

Chobanian, A. V., Bakris, G.L., Black, H.R., Cushman, W.C., Green, L.A., Izzo, J.L., … and Wright, T.T., (2003). The Seventh Report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure: the JNC report. *The Journal of the American Medical Association*, 289: 2560–2572.

Choi, Y.H. and Park, H.S. (2012). Apoptosis induction of U937 human leukemia cells by diallyl trisulfide induces through generation of reactive oxygen species. *Journal of Biomedical Sciences*, 19:50.

Colberg, S.R., Sigal, R.J., Fernhall, B (2010). Exercise and type-2 diabetes. The American College of Sports Medicine and the American Diabetes Association: Joint position statement executive summary. *Diabetes Care*. 33:2692-2696

Cowley, A. W. (1992). Long-term control of arterial blood pressure. *Physiological Review,* 72: 231–300.

Dacie, J.V. and Lewis, S. (1991). Practical Hematology. 7th Edition., Churchhill Livingstone, New York, 50-56.

Danaei, G., Finucane, M.M., Lu, Y., Singh, G.M., Cowan, M.J., Paciorek, C.J., Ezzati, M., …, (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants, *The Lancet*, 378(9785): 31–40.

Das, I., Khan, N.S. and Sooranna, S.R. (1995). Potent activation of nitric oxide synthase by garlic: A basis for its therapeutic applications. *Current Medical Research and Opinion* 13: 257–263.

Dattner, A.M. (2003). From medical herbalism to phytotherapy in dermatology: back to the future. *Dermatologic Therapy,* 16:106–113.

Drobiova, H., Thomson, M., Al-Qattan, K., PeltonenShalaby, R., Al-Amin, Z. and Ali, M. (2011). Garlic increases antioxidant levels in diabetic and hypertensive rats determined by a modified peroxidase method. *Evidence-Based Complementary and Alternative Medicine,* 7(10): 30-49.

Duncan, R.C., Knapp, R.G. and Miller, M.C. (1977). Test of hypothesis in population means. In: *Introductory Biostatistics for the health sciences*. John Wiley and Sons Inc. NY Pg 71-96.

Dwight, M., Luczy-Bachman, N.G., Min, P., Chang, M. S. and Amagase, H. (2006). Effects of aged garlic extract on the immunosuppressive effect of stress. *Brain, Behaviour and Immunity,* 20:50–51.

Eidi, A., Eidi, M. and Esmaeili, E. (2006). Antidiabetic effect of garlic *(Allium sativum)* in normal and streptozotocin-induced diabetic rats. *Phytomedicine,*13(9-10): 624–629.

Eikai, K., Naoto, U., Shigeo, K. and Yoichi, I. (2001). Immunomodulatory effects of aged garlic extract. *Journal of Nutrition,* 131:(3) 1075–1079.

El-Ghorab, A.H., Nauman, M., Anjum, F.M., Hussain, S. and Nadeem, M. (2010). A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *Journal of Agricultural and Food Chemistry,* 58:8231–8237.

Elrokh, E.S.M., Yassin, N.A.Z., El-Shenawy, S.M.A. and Ibrahim, B.M.M. (2010). Antihypercholesterolaemic effect of ginger rhizome (*Zingiber officinale*) in rats, *Inflammopharmacology*, 18(6): 309–315.

Elshater A.E., Salman, M. A. and Moussa, M. A. (2009). Effect of Ginger Extract Consumption on levels of blood Glucose, Lipid Profile and Kidney Functions in Alloxan Induced Diabetic Rats. *Egyptian Academic Journal of Biological Sciences,* 2(1): 153–162.

Evans, W.C. (1996) Trease and Evan's Pharmacognosy. 15th edition., WB Saunders Company Ltd. London, UK, pp. 52,277-280,454,481.

Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Summary of the second report of the National Cholesterol Education Program (NCEP) (1993). Expert on Detection Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel). *Journal of the American Medical Association,* 269: 3015-23.

Ficker, C., Smith, M.L., Akpagana, K., Gbeassor, M., Zhang, J., Durst, T., Arnason, J.T., … (2003a). Bioassay-guided isolation and identification of antifungal compounds from ginger. *Phytotherapy Research,* 17, 897-902.

Ficker, C.E., Arnason, J.T., Vindas, P.S., Alvarez, L.P., Akpagana, K., Gbassor, M., Smith, M.L., … (2003b). Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. *Mycoses,* 46:29-37.

Fong, H. H. (2002). Integration of herbal medicine into modern medical practices: issues andprospects. *Integrative Cancer Therapies,* 1:287–293.

Friedewald, W. T., Levy, R. and Fradrickson, D. S. (1972). Estimation of concentration of Low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifugation. *Clinical Chemistry,* 19: 449-452.

Galeone, C. Pelucchi, C., Levi, F., Negri, E., Franceschi S., Talamini, R., Giacosa, A. and La Vecchia C. (2006). Onion and garlic use and human cancer. *American Journal of Clinical Nutrition,* 84:1027–32.

Galozhger, A.E. and Kocloff, E.N. (1971). *Essential Practical Microtechnique*. 2nd edition. Lee and Febizer, Philadelphia, pp: 77.

George, C., Sivadasan, R., Devakaran, D. and Sreekumari, K.P. (1989). Spices Board, Cochin and International Trade Centre, Geneva, p.28.

Ghayur, M.N. and Gilani, A. H. (2005). Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. *Journal of Cardiovascular Pharmacology,* 45:74–80.

Ghayur, M.N., Gilani, A.H., Afridi, M.B. and Houghton, P.J. (2005). Cardiovascular effects of ginger aqueous extract and its phenolic constituents are mediated through multiple pathways. *Vascular Pharmacology,* 43: 234–241.

Ginter, E. and Simko, V. (2010). Garlic (*Allium sativum*) and cardiovascular diseases. *Bratislava Medical Journal,* 111: 452-456.

Go, A.S., Mozaffarian, D., Roger, V.L., Benjamin, E.J., Berry, J.D., Blaha, M.J., Turner, M.B., … (2014) on behalf of the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2014 update: a report from the American Heart Association. *Circulation* 129: e28–e292.

Gouvea, S.A., Moyses, M.R., Bissoli, N.S., Pires, J.G.P., Cabral, A.M. and Abreu, G.R. (2003). Oral administration of L-arginine decreases blood pressure and increases renal excretion of sodium and water in renovascular hypertensive rats. *Brazilian Journal of Medical Biology and Research*, 36: 943–949.

Grzanna, R., Lindmark, L. and Frondoza, C.G. (2005). Ginger - an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food*, 8: 125–132.

Gu, X. and Zhu, Y.Z. (2011). Therapeutic applications of organosulfur compounds as novel hydrogen sulfide. *Expert Review of Clinical Pharmacology,* 4: 123-133.

Haffner, S.M., Miettinen, H., Gaskill, S.P. and Stern, M.P. (1992*b*). Metabolic precursors of hypertension. *Archives of Internal Medicine*, 156: 1994–2001.

Hajjar, I. and Kotchen, T.A. (2003).Trends in prevalence, awareness, treatment, and control of hypertension in the United States, *Journal of the American Medical Association,* 290(2):199–206.

Haksar, A., Sharma, A., Chawla, R., Kumar, R., Arora, R., Singh, S., Sharma, R.K., … (2006). *Zingiber officinale* exhibits behavioral radioprotection against radiation. *Pharmacology, Biochemistry and Behaviour,* 84: 179–188.

Hamlaoui-Gasmi, S., Mokni, M., Limam, N., N'guessan, P., Carrier, A., Limam, F., Amri, M., Aouani, E., and Marzouki, L. (2012). Grape seed and skin extract mitigates garlic-induced oxidative stress in rat liver. *Canadian Journal of Physiology and Pharmacology,* 90: 547-556.

Hanieh, H., Narabara, K., Tanaka, Y, Gu, Z., Abe, A. and Kondo, Y. (2012). Immunomodulatory effects of Alliums and *Ipomoea batata* extracts on lymphocytes and macrophages functions in White Leghorn chickens: in vitro study. *Journal of Animal Science*, 83: 68-76.

Hawkins, W. (2006). Rex. Eat Right—Electrolyte: A Nutritional Guide to Minerals in Our Daily Diet. Amherst, NY: Prometheus Books. 110-256.

Heidenreich, P.A., Trogdon, J.G., Khavjou, O.A., Butler, J., Dracup, K., Ezekowitz, M.D., Woo, Y.J, … (2011). Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. *Circulation.* 123(8): 933–44.

Heimes, K., Feistel, B. and Verspohl, E.J. (2009). "Impact of the 5-HT3 receptor channel system for insulin secretion and interaction of ginger extracts," *European Journal of Pharmacology,* 624(1–3): 58–65.

Higdon, J. and Lawson, L. (2005). Garlic and Organosulfur Compounds. Micronutrient Information Center. Linus Pauling Institute, Oregon State University. [http://lpi.oregonstate.edu/infocenter/phytochemicals/garlic/#table1]. accessed 16 February 2012.

Hiyasat, B., Sabha, D., Grotzinger, K., Kempfert, J., Rauwald, J.W., Mohr, F.W. and Dhein S. (2009). Antiplatelet activity of *Allium ursinum* and *Allium sativum*. *Pharmacology* 83: 197-204.

Holzgartner, H., Schmidt, U. and Kuhnu, H. (1992). Comparison of the efficacy and tolerance of a garlic preparations VS benzafibrate . *Arzneimittelforschung.,* 42: 1473–1477.

Huffman, M. A. (2003). Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. *Proceedings of the Nutrition Society* 62: 371–381.

Iqbal, Z., Lateef, M., Akhtar, M.S., Ghayur, M.N. and Gilani, A. H. (2006). *In vivo* antihelmintic activity of ginger against gastrointestinal nematodes of sheep. *Journal of Ethnopharmacology,* 106: 285–287.

Islam, S.M. and Choi, H. (2008). "Comparative effects of dietary ginger (*Zingiber officinale*) and garlic (*Allium sativum*) investigated in a type 2 diabetes model of rats," *Journal of Medicinal Food*, 11(1): 152–159.

Ismail-Beigi, F, Craven, T., Banerji, M.A., Basile, J., Calles, J., Cohen, R.M., Hramiak, I., …, (2010). "Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial," *The Lancet*, 376(9739): 419–430.

Jagetia, G.C., Baliga, M.S., Venkatesh, P. and Ulloor, J.N. (2003). Influence of ginger rhizome *(Zingiber officinale Rosc.)* on survival, glutathione and lipid peroxidation in mice after whole-body exposure to gamma radiation. *Radiation Research*, 160: 584–592.

Jagetia, G., Baliga, M. and Venkattesh, P. (2004). Ginger (*Zingiber officinale Rosc.)*, a dietary supplement, protects mice against radiation-induced lethality: mechanism of action. *Cancer Biotherapy Radiopharmaceuticals*. 19: 422–435.

Kalu, I.G., Ofoegbu, U., Eroegbusi, J., Nwachukwa, C.U. and Ibeh, B. (2010). Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. *Journal of Medicinal Plants Research*, 4:496-498.

Karppanen, H. (1991). Minerals and blood pressure. *Annals of Medicine*, 2: 299–305.

Katzung, B. G. (2007). Adrenoceptor antagonist drugs. In: Basic and Clinical Pharmacology, 10th edition. Hoffman, B.B. (Editor). McGraw Hill, Singapore Pp. 141–143.

Kenney, L. (2004). Dietary Water and Sodium Requirements for Active Adults. *Sports Science Exchange* 92 (17): 1-10.

Kianoush S, Balali-Mood M, Mousavi SR, Moradi V, Sadeghi M, Dadpour B, Rajabi, O., Shakeri, M.T. (2012). Comparison of therapeutic effects of garlic and d-Penicillamine in patients with chronic occupational lead poisoning. *Basic Clinical Pharmacology and Toxicology*, 110: 476-481.

Kim, J.K., Kim, Y., Na, K.M., Surh, Y.J. and Kim, T.Y. (2007). (6)-Gingerol prevents UVB induced ROS production and COX-2 expression in vitro and in vivo. *Free Radical Research*, 41: 603–614.

Kim, K.M., Chun, S.B., Koo, M.S., Choi, W.J., Kim, T.W., Kwon, Y.G., Billiar, T.R., … (2001). Differential regulation of NO availability from macrophages and endothelial cells by the garlic component S-Allyl cysteine. *Free Radical Biology and Medicine*, 7:747–756.

Kim, Y.C. (2002). Effect of heat treatments on antimicrobial activities of garlic (*Allium sativum*). *Journal of Microbiology and Biochemistry,* 12(2): 331–335.

Kim-Park, S. and Ku, D.D. (2000). Garlic elicits a nitric oxide-dependent relaxation and inhibitshypoxic pulmonary vasoconstriction in rats. *Clinical Experimental, Pharmacology and Physiology,* 27: 780–786.

Krishnamurti, D. and Shyamala, D. (2001). Blood pressure regulation and Micronutrients. *Nutrition Research Reviews,* 14:3–43.

Krumm, P., Giraldez, T., Alvarez de la Rosa D, Clauss, W.G., Fronius, M. and Althaus, M. (2012). Thiol-reactive compounds from garlic inhibit the epithelial sodium channel (ENaC). *Bioorganic and Medicinal Chemistry*, 20: 3979-3984.

Kuo, C.F., Jao, Y.C. and Yang, P. (2008). Downregulation of hepatic lipoprotein assembly in rats by fermented products of Monascus pilosus. *Nutrition*, 24:477-483.

Kyo, E., Uda, N., Kasuga, S. and Itakura, Y. (2001). Immunomodulatory effects of aged garlic extract. *The Journal of Nutrition,* 131:1075–1079.

Lai, K.C., Kuo, C.L., Ho, H.C., Yang, J.S., Ma, C.Y., Lu, H.F., Chung, J.G., ... (2012) Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colon 205 human colon cancer cells *in vitro* and *in vivo*. *Phytomedicine*, 19: 625-630.

Lamm, D.L. and Riggs, D. R. (2001). Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. *The Journal of Nutrition,* 131: 1067–1070.

Lanzotti, V., Barile, E., Antignani, V., Bonanomi, G.and Scala, F. (2012). Antifungal saponins from bulbs of garlic, Allium sativum L. var. Voghiera. *Phytochemistry,* 78: 126-34.

Lavu, M., Bhushan, S. and Lefer, D.J. (2011). Hydrogen sulfide mediated cardioprotection: mechanisms and therapeutic potential. *Clinical Science,* 120: 219-229.

Lawson, L. D., Berthold, H. K., Sudhop, T. and Von-Bergmann, K. (1998). Effect of garlic on serum lipids. *The Journal of the American Medical association,* 280:1568–1578.

Lewis, W., Elvin-Lewis, M. (2003). Medical Botany: Plants Affecting Human Health. 2nd edition. New York: Wiley.

Li, X.H., McGrath, K.C.Y., Nammi, S., Heather, K.A. and Roufogalis, D.B. (2012). "Attenuation of liver pro-inflammatory responses by *Zingiber officinale* via inhibition of NF-kappa B activation in high-fat diet-fed rats," *Basic and Clinical Pharmacology and Toxicology,* 110(3): 238–244.

Li, Y., Tran, V.H., Duke, C.C. and Roufogalis, D.B. (2012). "Gingerols of Zingiber officinale enhance glucose uptake by increasing cell surface GLUT4 in cultured L6 myotubes," *Planta Medica,* 78(14): 1549–1555.

Lien, H.C., Sun, W.M., Chen YH, Kim, H., Hasler, W. and Owyang, C. (2003). Effects of ginger on motion sickness and gastric slow-wave dysrhythmias induced by circular vection. *Ameriacan Journal of Physiology: Gastrointestinal Liver Physiology,* 284(3):481–489.

Lii, C.K., Huang, C.Y., Chen, H.W., Chow, M.Y., Lin, Y.R., Huang, C.S. and Tsai C.W. (2012). Diallyl trisulfide suppresses the adipogenesis of 3T3-L1 preadipocytes through ERK activation. *Food and Chemical Toxicology*, 50: 478-484.

Liu, L. and Yeh, Y. Y. (2001). Water soluble organosulphur compounds of garlic inhibit fatty acid and triglyceride synthesis in cultured rat hepatocytes. *Lipids*, 36: 395-400.

Liu, L. and Yeh, Y. Y. (2002). S-alk(en)yl cysteines of garlic inhibit cholesterol synthesis by deactivating HMG-coA reductase in cultured rat hepatocytes. American Society for Nutritional Sciences. *The Journal of Nutrition,*132:1129–1134.

Liu, S., Sun, Y., Li, W., Yu, H., Li, X., Liu, Z., Zeng, J., Zhou, Y., Chen, C. and Jia, J. (2010). The antibacterial mode of action of allitridi for its potential use as a therapeutic agent against Helicobacter pylori infection. *FEMS Microbiology Letters*, 303: 183-189.

Liu, Z., Li, M., Chen, K., Yang, J., Chen, R., Wang, T., Liu, J., Yang, W. and Ye, Z. (2012). Sallylcysteine induces cell cycle arrest and apoptosis in androgen-independent human prostate cancer cells. *Molecular Medicine Report*, 5: 439-443.

Londhe, V.P., Gavasane, A.T., Nipate, S.S., Bandawane, D.D. and Chaudhari, P.D. (2011). Role of garlic (Allium sativum) in various diseases: An overview. *Journal of Pharmaceutical Research & Opinion*, 1: 129 -134.

Loren, K. (2012). Calcium and blood pressure. *Journal of Vibrant Life,* 8(18): 558–570.

Lorke, D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, 275–287.

Madkor, R.H., Mansour, W.S. and Ramadan, G. (2010). Modulatory effects of garlic, ginger, turmeric and their mixture on hyperglycaemia, dyslipidaemia and oxidative stress instreptozotocin-nicotinamide diabetic rats, *British Journal of Nutrition*, 105(08): 1210–1217.

Magalhaes, P., Appell, H. and Duarte, J., (2008). Involvement of advanced glycation end products in the pathogenesis of diabetes complication: the protective role of regular physical activity. *European Review of Aging Physical Activity,* 5(1): 17–29.

Martel-Pelletier, J., Lajeunesse, D., Reboul, P. and Pelletier, J.P. (2003). Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti inflammatory drugs. *Annals of the Rheumatic Diseases*, 62:501–509.

Martinez-Velazquez, M., Rosario-Cruz, R., CastilloHerrera, G., Flores-Fernandez, J.M., Alvarez, A.H. and LugoCervantes, E. (2011). Acaricidal effect of essential oils from Lippia graveolens (Lamiales: Verbenaceae), Rosmarinus officinalis (Lamiales: Lamiaceae), and Allium sativum (Liliales: Liliaceae) against Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). *Journal of Medical Entomology*, 48: 822-827.

Matsuda A., Z. Wang, H., Takahashi, S. H., Tokuda, T., Miura, N. and Hasegawa, J. (2009). Up-regulation of mRNA of retinoid binding protein and fatty acid binding protein by cholesterol enriched-diet and effect of ginger on lipid metabolism. *Life Sciences,* 84(25-26):903–907.

McGee, H. (2004). On food and cooking. The science and lore of the kitchen. 2nd Edition. Harold McGee (Ed). New York.pp.425–426.

McMahon, F.G. and Vargas, R. (1993). Can garlic lower blood pressure? A pilot study. *Medicine,* 26: 15–22.

Meriga, B., Mopuri, R. and MuraliKrishna, T. (2012). Insecticidal, antimicrobial and antioxidant activities of bulb extracts of Allium sativum. *Asian Pacific Journal of Tropical Medicine,* 5: 391-395.

Mervaala, E. (1995). A potassium-, magnesium-, and l-lysineenriched mineral salt. Cardiovascular and renal effects and interactions with antihypertensive drugs in the rat. Academic Dissertation, University of Helsinki. ISBN 952-90-7197-3.

Miller, K. L., Liebowitz, R. S., and Newby, L. K. (2004). Complementary and alternative medicine in cardiovascular disease: a review of biologically based approaches. *American Heart Journal 147: 401-411*

Mikaili, P., Maadirad, S., Moloudizargari, M., Aghajanshakeri, S, Sarahroodi, S (2013). Therapeutic Uses and Pharmacological Properties of Garlic, Shallot, and their Bilogically Active Compounds. *Iranian Journal of Basic Medical Sciences*, 16: 1031-1048

Morihara, N., Sumioka, I., Moriguchi, T., Uda, N. and Kyo, E. (2002). Aged garlic extracts enhance production of nitric oxide. *Life Sciences*, 71: 509–517.

Mousa, S.A. (2010). Antithrombotic effects of naturally derived products on coagulation and platelet function. *Methods in Molecular Biology*, 663: 229-240.

Moyers, S. (1996). Garlic in Health, History and World Cuisine. Suncoast Press, St Petersburg, FL : 1–36.

Nammi, S., Sreemantula, S. and Roufogalis, D.B. (2009). "Protective effects of ethanolic extract of Zingiber officinale rhizome on the development of metabolic syndrome in high-fat diet-fed rats," *Basic and Clinical Pharmacology and Toxicology*, 104(5): 366–373.

Nammi, S., Kim, S.M., Gavande, S.N., Li, O.G. and Roufogalis, D.B. (2010). "Regulation of low-density lipoprotein receptor and 3-hydroxy-3- methylglutaryl coenzyme A reductase expression by Zingiber officinale in the liver of high-fat diet fed rats," *Basic and Clinical Pharmacology and Toxicology*, 106(5): 389–395.

Narkiewicz, K. (2006). Diagnosis and management of hypertension in obesity. *Obesity Review*,7(2):155–62.

Niedziocha, L. (2011). Calcium, magnesium and phosphorus heallths. (online). Avaiable: http://www.livestrong.com/article/368873-calcium-magnesium-and-phosphorus-healthbenefits/ [22 Febuary 2010]

Noipha, K., Ratanachaiyavong, S. and Ninla-aesong, P. (2010). "Enhancement of glucose transport by selected plant foods in muscle cell line L6" *Diabetes Research and Clinical Practice*, 89(2): 22–26.

Nrashan , T. S., Kumar, D. Kewal L.S., Raisuddin, A. and Sahu, P. (2010). Adverse health effects due to arsenic exposure, modification by dietary supplemnation of jaggey in mice *Toxicology and Applied Pharmacology* 242(3): 247–255.

Nwokocha, C.R., Ozolua, 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. *Nigerian journal of physiological sciences*, 26: 213-218.

O'Hara, M., Keifer, D., Farrel, K. and Kemper. K. (1998). A review of 12 commonly used medicinal herbs. *Archives of Family Medicine*, (7): 523–536.

Ojewole, J. A. O. (2006). " Analgesic, antiinflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (Zingiberaceae) in mice and rats," *Phytotherapy Research*, 20(9): 764–772.

Osborn, J. W. (2005). Hypothesis: set-point s and long-term control of arterial pressure. A theoretical argument for a long-term arterial pressure control system in the brain rather than the kidney. *Clinical and Experimental Pharmacolgy and Physiology.* 32: 384–393.

O'Shaughnessy, K.M. and Karet, F.E. (2006). Salt handling and hypertension. *Annual Review of Nutrition*, 26: 343-65.

Otsuka, F., Ogura, T., Kataoka, H., Kishida, M., Takahashi, M., Mimura, Y., Makino, H., … (2000). Differential effect of chronic inhibition of calcium channel and angiotensin II type-1 receptor on aldosterone synthesis in spontaneously hypertensive rats. *Journal of Steroid Biochemistry and Molecular Biology*, 74(3): 125–136.

Palaksha, M.N., Ahmed, M. and Das, S. (2010). Antibacterial activity of garlic extract on streptomycin-resistant Staphylococcus aureus and Escherichia coli solely and in synergism with streptomycin. *Journal of Natural Science, Biology and Medicine*, 1:12-5.

Peeters, A.C., Van Aken, B.E., Blom, H.J., Reitsma, P.H. and Den Heijer, M. (2007). The effect of homocysteine reduction by B-vitamin supplementation on inflammatory markers. *Clinical Chemistry and Laboratory Medicine.* 45(1):54-58

Pezzin, L.E., Feldman, P.H., Mongoven, J.M., McDonald, M.V.,Gerber, L.M.and Peng, T.R. (2011). Improving Blood Pressure Control: Results of Home-based Post-acute Care Interventions. *Journal of General Internal Medicine,* 26(3): 280–286.

Priya Rani, M., Padmakumari, P.K., Sankarikutty, B., Lijo Cherian, O., Nisha, V.M. and Raghu, G.K. (2011). "Inhibitory potential of ginger extracts against enzymes linked to type 2 diabetes, inflammation and induced oxidative stress," *International Journal of Food Sciences and Nutrition*, 62(2): 106–110.

Pszczola, D.E. (2002). Antimicrobials setting up additional hurdles to ensure food, safety. *Food and Technology*, 56: 99–107.

Pundir, R.K., Jain, P. and sharma, C.H. (2010). Antimicrobial activity of ethanolic extracts of syzygium aromaticum and Allium sativum against food associated bacteria and fungi. *Ethnobotan Leaflets*, 14: 344-360.

Rahman, K. (2001). Historical Perspective of Garlic and Cardiovascular Diseases. *Journal of Nutrition*, 131: 977-979

Rahman, K. and Lowe, G.M. (2006). Garlic and cardiovascular disease: a critical review. *Journal of Nutrition*, 136: 736–740.

Ramaa, C. S., Shirode, A. R., Mundada, A. S., and Kadam, V. J. (2006). Nutraceuticals an emerging era in the treatment and prevention of cardiovascular diseases. *Current Pharmaceutical Biotechnology*, 7:15–23.

Ramudu, S.K., Korivi, M., Kesireddy, N., Lee, L.C., Cheng, I.S, Kuo, C.H. and Kesireddy, S.R. (2011). "Nephroprotective effects of a ginger extract on cytosolic and mitochondrial enzymes against streptozotocin (STZ)-induced diabetic complications in rats," *The Chinese Journal of Physiology*, 54: 79–86.

Rani, P.M., Krishna, S.M., Padmakumari, P.K., Raghu, G.K. and Sundaresan, A. (2012). "Zingiber officinale extract exhibits antidiabetic potential via modulating glucose uptake, protein glycation and inhibiting adipocyte differentiation: an *in vitro* study,"*Journal of the Science of Food and Agriculture*, 92(9): 1948–1955.

Ranilla, G.L., Kwon, I.Y., Apostolidis, E. and Shetty, K. (2010). "Phenolic compounds, antioxidant activity andin vitro inhibitory potential against key enzymes relevant for

hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America,"*Bioresource Technology*, 101(12): 4676–4689.

Ray, K.K., Seshasai, S.R., Wijesuriya, S., Sivakumaran, R., Nethercott, S., Preiss, D., Erqou, S. and Sattar, N. (2009). "Effect of intensive control of glucose on cardiovascular outcomes and death in patients with diabetes mellitus: a meta-analysis of randomised controlled trials," *The Lancet*, 373(9677): 1765–1772.

Rebholz, C.M., Gu., D., Chen, J., Huang, J.F., Cao, J., Chen J.C., He, J., … (2012). Physical activity reduces salt sensitivity of blood pressure: the Genetic Epidemiology Network of Salt Sensitivity Study. *American Journal of Epidemiology*. 176(7): 106–113.

Reckelhoff, J.F. (2001) Gender differences in the regulation of blood pressure. *American Heart Association,* 37(5): 119–208.

Reddy, G.D., Reddy, A.G., Rao, G.S. and Kumar, M.V. (2012). Pharmacokinetic interaction of garlic and atorvastatin in dyslipidemic rats. *Indian Journal of Pharmacology,* 44: 246-252.

Rehman, R., Akram, M., Akhtar, N., Jabeen, Q., Saeed, T., Shah, A., Asif, H.M., … (2011). *Zingiber officinale* Roscoe (pharmacological activity). *Journal of Medicinal Plants Research*, 5(3): 344–348.

Resnick, L.M., Militianu, D.M. and Cunnings, A.J. (1997). Direct magnetic resonance determination of aortic distensibility in essential hypertension: relation to age, abdominal visceral fat, and *in situ* intracellular free magnesium. *Hypertension* 30: 654–659.

Resnick, L.M. (1999). The cellular ionic basis of hypertension and allied clinical conditions. *Progress in Cardiovascular Diseases* 42: 1–22.

Rhiouania, H., El-Hilalya, J., Israili, Z. H., and Lyoussia, B. (2008). Acute and subchronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. *Journal of Ethnopharmacology*, 118: 378–386.

Ribeiro, M.O., Antunes, E., De Nucci, G., Lovisolo, S.M. and Zatz, R. (1992). Chronic inhibition of nitric oxide synthesis: a new model of arterial hypertension. *Hypertension,* 20: 298–303.

Rong, X., Peng, G., Suzuki, T., Yang, Q., Yamahara, J. and Li, Y. (2009). "A 35-day gavage safety assessment of ginger in rats," *Regulatory Toxicology and Pharmacology*, 54(2): 118–123.

Rose, P.,Whiteman, M., Moore, P. K. and Zhu, Y. Z. (2005). Bioactive S-alk(en)yl cysteine sulphoxide metabolites in the genus Allium: the chemistry of potential therapeutic agents. *Natural Product Reports,* 22: 351–368.

Saraswat, M., Reddy, Y.P., Muthenna, P. and Reddy, B.G. (2009). "Prevention of non-enzymic glycation of proteins by dietary agents: prospects for alleviating diabetic complications," *British Journal of Nutrition*, 101(11): 1714–1721.

Saraswat, M., Suryanarayana, P., Reddy, Y.P., Patil, A.M., Balakrishna, N. and Reddy, B.G. (2010). "Antiglycating potential of Zingiber officinalis and delay of diabetic cataract in rats," *Molecular Vision*, 16: 1525–1537.

Schales, O. and Schales, S. (1941). A simple and accurate method for the determination of chloride in biological fluids. *Journal of Biochemistry*, 140: 879–884.

Segal, M.A. (1955). A rapid electrotitimetric method for determining CO_2 combining power in plasma or serum. *American Journal of Clinical Pathology*, 25(10): 1212–1216.

Seyar, N., Terzi, S., Bilsel, T., Yilmaz, H.Y., Orhan, L., Cakmak, N., Yesilcimen, K., … (2007). Plasma homocysteine concentration in improves homocysteine-induced endothelial dysfunction in macro-and microcirculation. *Journal of Nutrition,*136 (3): 750–754.

Shalaby, A.M. and Hamowieh, R.A. (2010). "Safety and efficacy of Zingiber officinale roots on fertility of male diabetic rats,"*Food and Chemical Toxicology*, 48(10): 2920–2924.

Shamseer, L., Charrois, T. L., and Vohra, S. (2006). Complementary, holistic, and integrative medicine: Garlic. *Pediatrics Review*, 27: 77–80.

Shanmugam, K.R., Mallikarjuna, K., Kesireddy, N. and Sathyavelu, R. K. (2011). "Neuroprotective effect of ginger on antioxidant enzymes in streptozotocin-induced diabetic rats," *Food and Chemical Toxicology*, 49(4): 893–897.

Shanmugam, K.R., Ramakrishna, C.H., Mallikarjuna, K. and Reddy, K.S. (2009). "The impact of ginger on kidney carbohydrate metabolic profiles in STZ induced diabetic rats," *Asian Journal of Experimental Sciences*, 23(1): 127–134.

Shanmugam, K.R.K., Mallikarjuna, K., Nishanth, C., Kuo, H. and Reddy, K.S. (2011). "Protective effect of dietary ginger on antioxidant enzymes and oxidative damage in experimental diabetic rat tissues," *Food Chemistry*, 124(4): 1436–1442.

Sharif, A.M., Darabi, R. and Akbarloo, N. (2003). Investigation of antihypertensive mechanism of garlic in 2K1C hypertensive rat. *Journal of Ethnopharmacology,*86: 219–24.

Shariq, S., Tajuddin, S.H. and Afaq, S.H. (2011). Spice and medicine: *Zingiber officinale. The International Journal of Applied Biology and Pharmaceutical Technology* 1(3): 968–973.

Sharma, A., Haksar, A., Chawla, R., Kumar, R., Arora, R., Singh, S., Sharma, R., … (2005). *Zingiber officinale Rosc.* modulates gamma radiation induced conditioned taste aversion. *Pharmacology Biochemistry and Behaviour,* 81: 864–870.

Shih, P.C., Kuo, C.H., Juang, J.Y., Liu, C.H., Hsu, L. and Liu, C.T. (2010). Effects of garlic oil on the migration of neutrophil-like cell studied by using a chemotactic gradient Labchip. *Journal of Biomedicine and Biotechnology*, 31:9059.

Siddaraju, M.N. and Dharmesh, S.M. (2007). Inhibition of gastric H^+, K^+- ATPase and Helicobacter pylori growth by phenolic antioxidants of *Zingiber officinale*. *Molecular Nutrition and Food Research,* 51: 324–332.

Singh, D. K. and Porter, T. D. (2006). Inhibition of sterol 4alpha-methyl oxidase is the principal mechanism by which garlic decreases cholesterol synthesis. *Journal of Nutrition*, 136: 759–764.

Singh, G., Kapoor, I.P.S., Singh, P., De Heluani, C.S., De Lampasona, M.P. and Catala, C.A.N. (2008). Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food and Chemical Toxicology*, 46:3295–3302.

Starek, S. (2001). Enciclopedia de Plantas Medicinales, Aromáticas y Culinarias. Madrid: Servilibro Ediciones.

Stein, E. A. (1987). Lipids, lipoproteins and Apolipoproteins. In: Treitz, N. W. (Ed). Fundamentals of Clinical Chemistry, 3rd Edition., W.B Sauders Philadelphia, pp; 470-479.

Sumiyoshi, H. (1997). New pharmacological activities of garlic and its constituents (Review). *Folia Pharmacological Japonica,*110 (Suppl 1): 93–97.

Swaran, J., Flora, S., Ashish, M., and Richa G. (2009). Prevention of arsenic – induced hepaticapoptosis by concomitant administration of garlic extracts in mice. *Chemico-Biological Interactions* 177(3): 227–233.

Tanabe, M., Chen, Y. D., Saito, K. and Kano, Y. (1993). Cholesterol biosynthesis inhibitory component from *Zingiber officinale Roscoe. Chemical and Pharmaceutical Bulletin,* 41(4): 710–713.

Tanaka, Y., Kataoka, M., Konishi, Y., Nishimune, T. and Takagaki, Y. (1992). Effects of vegetable foods on ß-hexosaminidase release from rat basophilic leukemia cells (RBL2H3). *Japanese Journal of Toxicology Environmental Health*, 38:418–424.

Tariq, H., Abdullah, M. D., Kandil, O., Elkadi, A. and Carter, J. (1988). Garlic revisited: therapeutic for the major diseases of our times? *Journal of the National Medical Association*, 80(4): 439–445

Tedeschi, P., Leis, M., Pezzi, M., Civolani, S., Maietti, A. and Brandolini, V. (2011). Insecticidal activity and fungitoxicity of plant extracts and components of horseradish (Armoracia rusticana) and garlic (Allium sativum). *Journal of Environmental Science and Health*, 46: 486-490.

Thomas, H. (2011). Management of Hypertension. *American Journal of Hypertension.* 24: 898–903.

Thomson, M., Al-Qattan, K.K., Al-Sawan, S.M., Alnaqeeb, M.A., Khan, I. and Ali, M. (2002). The use of ginger *(Zingiber officinale Rosc.)* as a potential anti-inflammatory and antithrombotic agent. *Prostaglandins Leukotrienes and Essentential Fatty Acids*, 67:475–478.

Tietz, N.W. (1990). Clinical Guide to Laboratory Test, Second Edition W.B. Saunders Company,Philadelphia, U.S.A. 554–556.

Tietz, N.W. (1995) Clinical Guide to Laboratory Tests, 3rd Edition, W.B. Saunders, Philadelphia, PA 554–556.

Trease, G.E. and Evans, M.C. (1983). Text book of pharmacognosy, 13th edition, Bailler Tindal, London. pp.247–762.

Tsai,Y.,Cole, L. L. and Davis, L. E. (1985). Anti-viral properties of garlic: In vitro effects on influenza B, herpes simplex I, and cocksackie viruses. *Planta Medica*; 5:460-461.

Usui, T.and Suzuki, S. (1996). Isolation and identification of antiallergic substance from garlic *(Allium sativum). Nature Medicine,* 50: 135–137.

Vazquez-Prieto, M.A., González, R.E., Renna, N.F., Galmarini, C.R. and Miatello, R.M. (2010). Aqueous garlic extracts prevent oxidative stress and vascular remodeling in an experimental model of metabolic syndrome. *Journal of Agricultural and Food Chemistry,* 58: 6630-6635.

Vidyashankar, S., Sambaiah, K. and Srinivasan, K. (2009). Dietary garlic and onion reduce the incidence of atherogenic diet-induced cholesterol gallstones in experimental mice. *British Journal of Nutrition,* 101: 1621-1629.

Vogel, A.I. (1960). *A Textbook of Quantitative Inorganic Analysis*. Longman Group Ltd. London. 3rd Edition, Pp. 882-885.

Wacnic, R. G. and Albers, J. J. (1978). A comprehensive evaluation of the heparin manganese precipitation procedure for estimating high density lipoprotein cholestsrol. *Journal of Lipid Research,* 19: 65–76.

Wagner, B. (1985). Intervention in the Aging Process, editorial. *Human Pathology,* 16(7): 653–758

Wang, W. H. and Wang, Z. M. (2005). "Studies of commonly used traditional medicine-ginger," *Zhongguo Zhongyao Zazhi, China Journal of Chinese Materia Medica* 30(20): 1569–1573.

Wang, W., Li, Y.C., Wen, D.X., Li, P. and Qi, W.L. (2009). "Simultaneous determination of 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol in rat plasma by liquid chromatography-mass spectrometry: application to pharmacokinetics," *Journal of Chromatography B,* 877(8-9): 671–679.

Wang, X., Jiao, F., Wang, Q.W., Wang, J., Yang, K., Hu, R.R., Liu, H.C., Wang, H.Y. and Wang, Y.S. (2012). Aged black garlic extract induces inhibition of gastric cancer cell growth in vitro and in vivo. *Molecular Medicine Report,* 5: 66-72.

Weiss, N., Ide, N., Abahji, T., Nill, L., Keller, C. and Hoffmann, U. (2006). Aged garlic extract With Cardiovascular Disease. *The American Journal of Cardiology,* 98(5): 673–680.

Westfall, R.E. (2004). Use of anti-emetic herbs in pregnancy: women's choices, and the question of safety and efficacy. *Complementary Therapies in Nursing Midwifery,*10(1):30–36.

Whelton, P.K., He, J., Cutler, J.A., Brancati, F.L., Appel, L.J., Follmann, D. and Klag, M.J. (1997). Effects of oral potassium on blood pressure, meta-analysis of randomized controlled clinical trials. *JAMA* 277: 1624–1632.

White, B. (2007) "Ginger: an overview," *American Family Physician,* 75(11): 1689–1691.

Wichtl, M. (2004). Herbal Drugs and Phytopharmaceuticals 3rd edition. Boca Raton, FL: CRC Press; pp. 653–656.

Widgren, B.R., Herlitz, H. and Wikstrand, J. (1992). Increased waist/hip ratio, metabolic disturbances, and family history of hypertension. *Hypertension* 20: 563–568.

World Health Organization (2002). Diet, nutrition and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation. WHO Technical report series 916. Geneva, Switzerland.

Yang, Y., Chan, S.W., Hu, M., Walden, R. and Tomlinson, B. (2011). Effects of some common food constituents on cardiovascular disease. *ISRN Cardiology,* 397136.

Yeh, Y. Y. and Liu, L. (2001). Cholesterol lowering effects of garlic extracts and organosulphur compounds: Human and Animal studies. *The Journal of Nutrition,* 131: 989S–993S.

Yeh, Y.Y and Yeh, S. M. (1994). Garlic reduce plasma lipid by inhibiting hepatic cholesterol and triglyceride synthesis. *Lipid,* 29: 189–193.

Yeh, Y.Y. and Yeh, S. M. (2006). Homocysteine-lowering action is another potential cardiovascular protective factor of aged garlic extract. 136(3 Suppl): 745S–749S.

Yeh,G.Y., Davis, R.B. and Phillips, R.S.(2006). Use of Complementary Therapies in Patients With Cardiovascular Disease. *The American Journal of Cardiology,* 98(5): 673–680.

Yemitan, O.K. and Izegbu, M.C. (2006). Protective effects of *Zingiber officinale (Zingiberaceae)* against carbon tetrachloride and acetaminopheninduced hepatotoxicity in rats. *Phytotherapy Research,* 20: 997–1002.

Yiming, L., Van, H. T., Colin, C. D., and Basil, D. R. (2012). Preventive and Protective Properties of *Zingiber officinale* (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. *Evidence-Based Complementary and Alternative Medicine,* 2012(516870): 1–10

Young, H.Y., Luo, Y.L. and Cheng H.Y. (2005). Analgesic and anti-inflammatory activities of (6) - gingerol. *Journal of Ethnopharmacology,* 96(1-2):207–210.

Younis, F., Mirelman, D., Rabinkov, A. and Rosenthal, T. (2010). Sallyl-mercapto-captopril: a novel compound in the treatment of Cohen-Rosenthal diabetic hypertensive rats. *Journal of Clinical Hypertension*, 12: 451-455.

Yousuf, S., Ahmad, A., Khan, A., Manzoor, N. and Khan, L.A. (2010). Effect of diallyldisulphide on an antioxidant enzyme system in Candida species. *Canadian Journal of Microbiology*, 56: 816-821.

Yu, Y., Zick, S., Li, X., Zou, P., Wright, B. and Sun, D. (2011). "Examination of the pharmacokinetics of active ingredients of ginger in humans," *BioMed Central Biology*, 13(3): 417–426.

Yu, C.S., Huang, A.C., Lai, K.C., Huang, Y.P., Lin, M.W., Yang, J.S. and Chung, J.G. (2012). Diallyl trisulfide induces apoptosis in human primary colorectal cancer cells. *Oncology Reports*, 28: 949-954.

Zanchetti, A. and Leonetti, G. (1985). Natriuretic effect of calcium antagonists, *Journal of Cardiovascular Pharmacology,* 4: 33–37.

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

Zhu, Z., Mao, S. and Zhu, W. (2012). Effects of ruminal infusion of garlic oil on fermentation dynamics, fatty acid profile and abundance of bacteria involved in biohydrogenation in rumen of goats. *Asian Australasian Journal of Animal Sciences,* 25: 962–970.

Zimmer, H.G. (1998). The isolated perfused heart system and its pioneers. *New Physiological Science,* 13: 203–210.

APPENDIX I

Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Activities Serum Liver Enzymes in Wistar Rats

Treatment Given	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Normal saline	115.60 ± 7.84	41.80 ± 4.68	38.88 ± 2.89
Garlic 20mg/kg	109.40 ± 4.76 ^{ns}	38.20 ± 1.32 ^{ns}	40.21 ± 4.07 ^{ns}
Garlic 40mg/kg	132.60 ± 14.75 ^{ns}	46.60 ± 9.14 ^{ns}	39.32 ± 2.47 ^{ns}
Ginger 20mg/kg	123.20 ± 10.27	29.80 ± 2.76 ^{ns}	19.47 ± 1.37^{b}
Ginger 40mg/kg	106.60 ± 4.24 ^{ns}	30.20 ± 3.34 ^{ns}	23.96 ± 3.34^{a}
Garlic 10mg + Ginger 10mg/kg	87.40 ± 9.39 ^{ns}	26.0 ± 1.30 ^{ns}	21.00 ± 1.41^{a}
Garlic 20mg + Ginger 20mg/kg	109.0 ± 9.77 ^{ns}	29.89 ± 9.92^{ns}	12.47 ± 0.96^{b}

Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant
ns = non significant

APPENDIX II

Effects of Garlic *(Allium sativum)* and Ginger (*Zingiber officinale*) on Force of Contraction of Perfused Rabbit Heart (n = 3)

Treatment groups and dose given	Force of contraction (mV)
Normal saline	91.80 ± 0.20
Garlic (1 mg/ml)	88.00 ± 0.31[a]
Garlic (10 mg/ml)	89.80 ± 0.20 [ns]
Garlic (20 mg/ml)	90.50 ± 0.29 [ns]
Ginger (1 mg/ml)	88.20 ± 0.37[a]
Ginger (10 mg/ml)	88.20 ± 0.38[a]
Ginger (20 mg/ml)	88.00 ± 0.41[a]
Garlic (1 mg/ml) +Ginger (1 mg/ml)	87.60 ± 1.94 [a]
Garlic (10 mg/ml)+Ginger (10 mg/ml)	88.67 ± 1.56 [a]

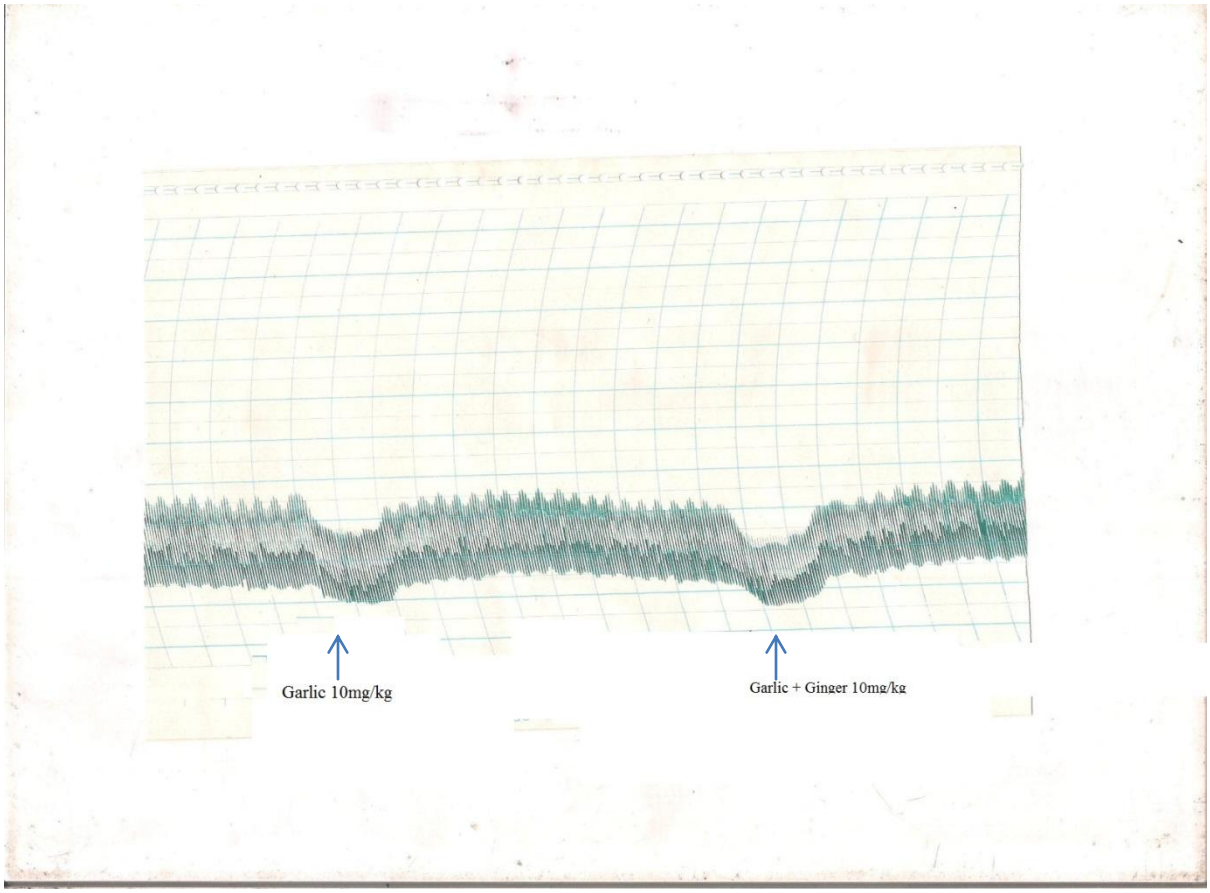
Data are presented as mean ± standard error of mean
a,b = means with different superscript letters are significantly different.
a = $p < 0.05$ is significant
b = $p < 0.01$ is significant

APPENDIX III

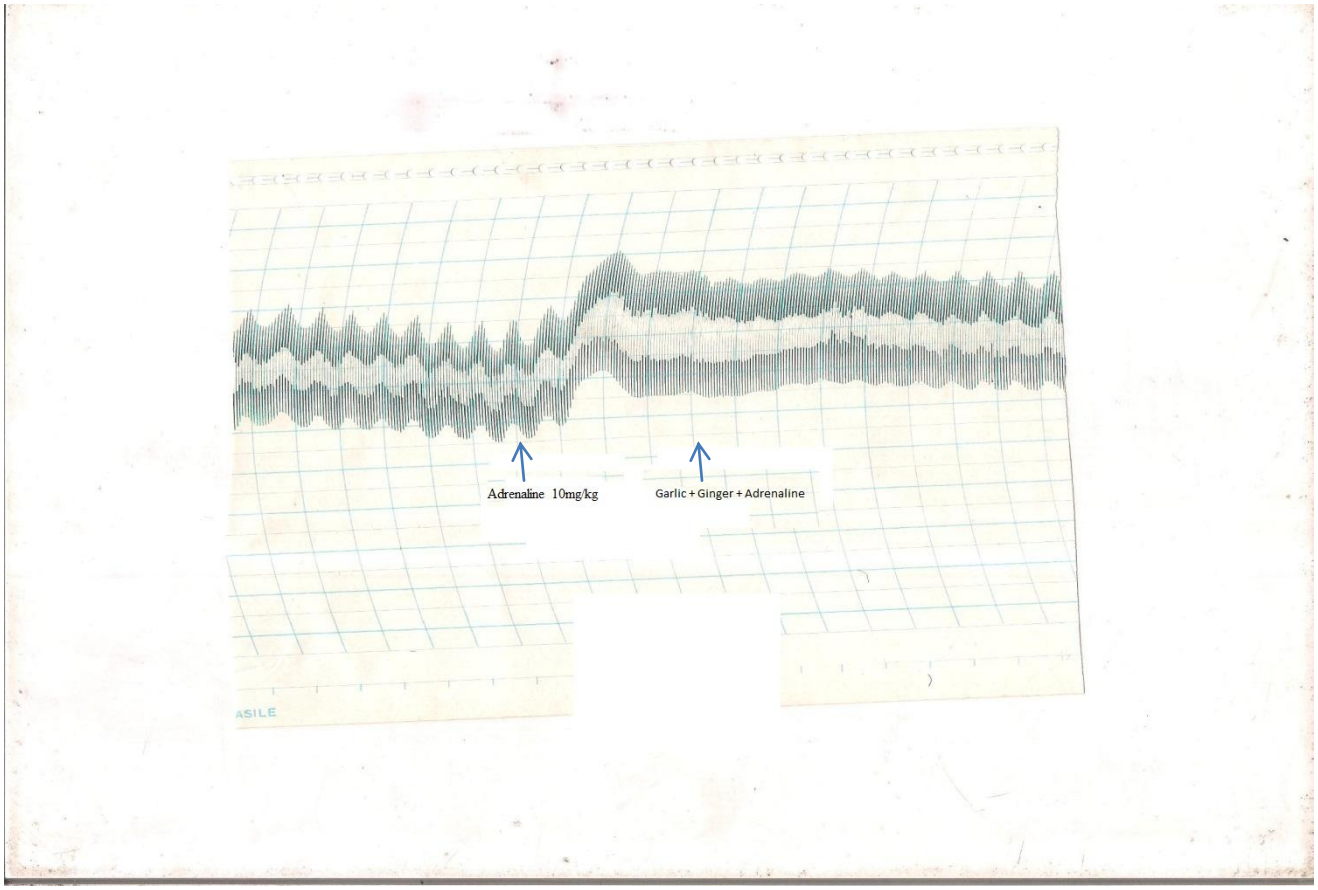
Phytochemical Screening

Compounds	Garlic	Ginger	Tests
Carbohydrate	Positive	Positive	Molisch
Unsaturated Steroid and triterpenes	Negative	Negative	Liebermann-Bucchard test
Unsaturated Sterols	Negative	Negative	Salkowski
Cardiac Glycoside	Positive	Positive	Keller-Kiliani
Saponin Glycoside	Negative	Positive	Frothing test
Test for Tannins	Negative	Negative	Ferric Chloride solution: 3-5 drops
Flavonoids	Negative	Negative	Sodium hydroxide test + Shinoda test
Alkaloids	Positive	Positive	Dragendoffs test
Free Anthraceae Derivative	Negative	Negative	Bontragers test

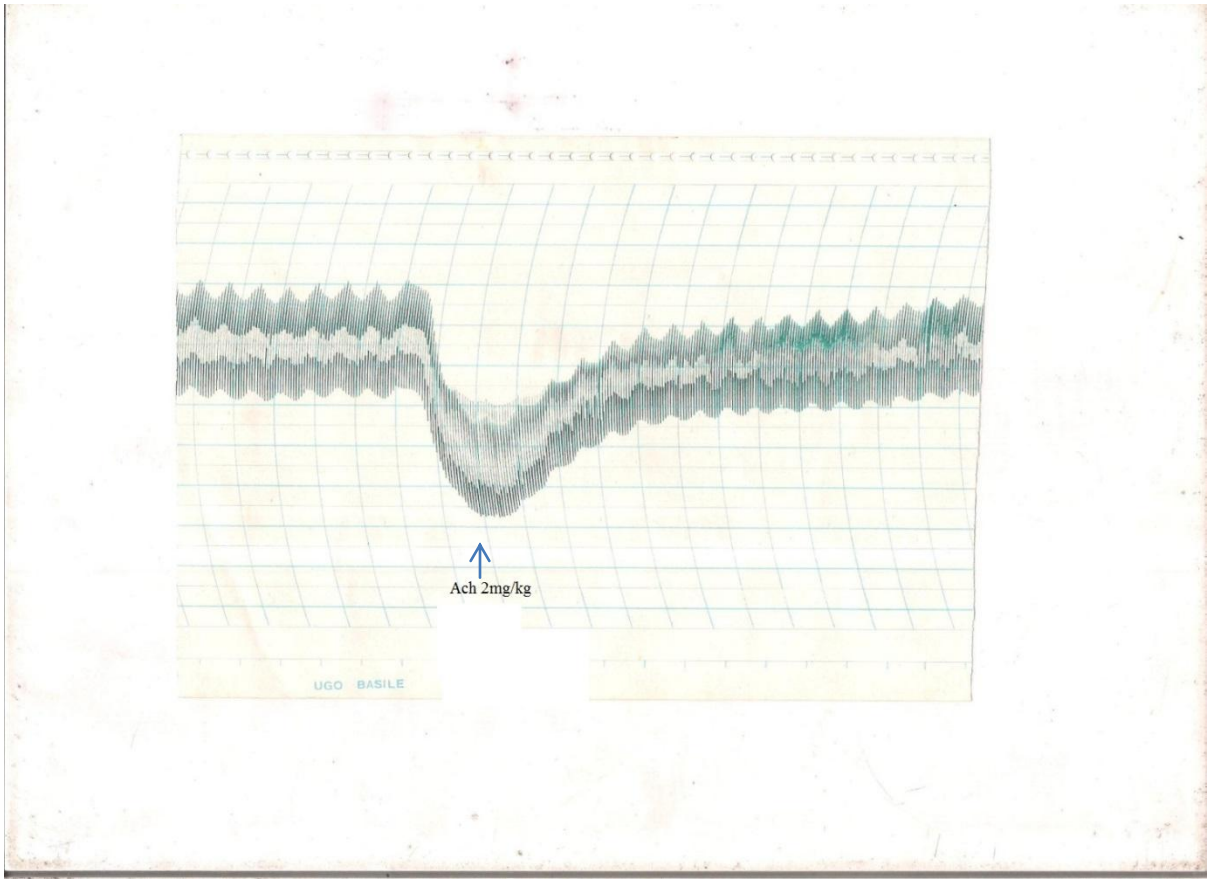
The Effect of Garlic + Ginger on Nomotensive Cat Blood Pressure



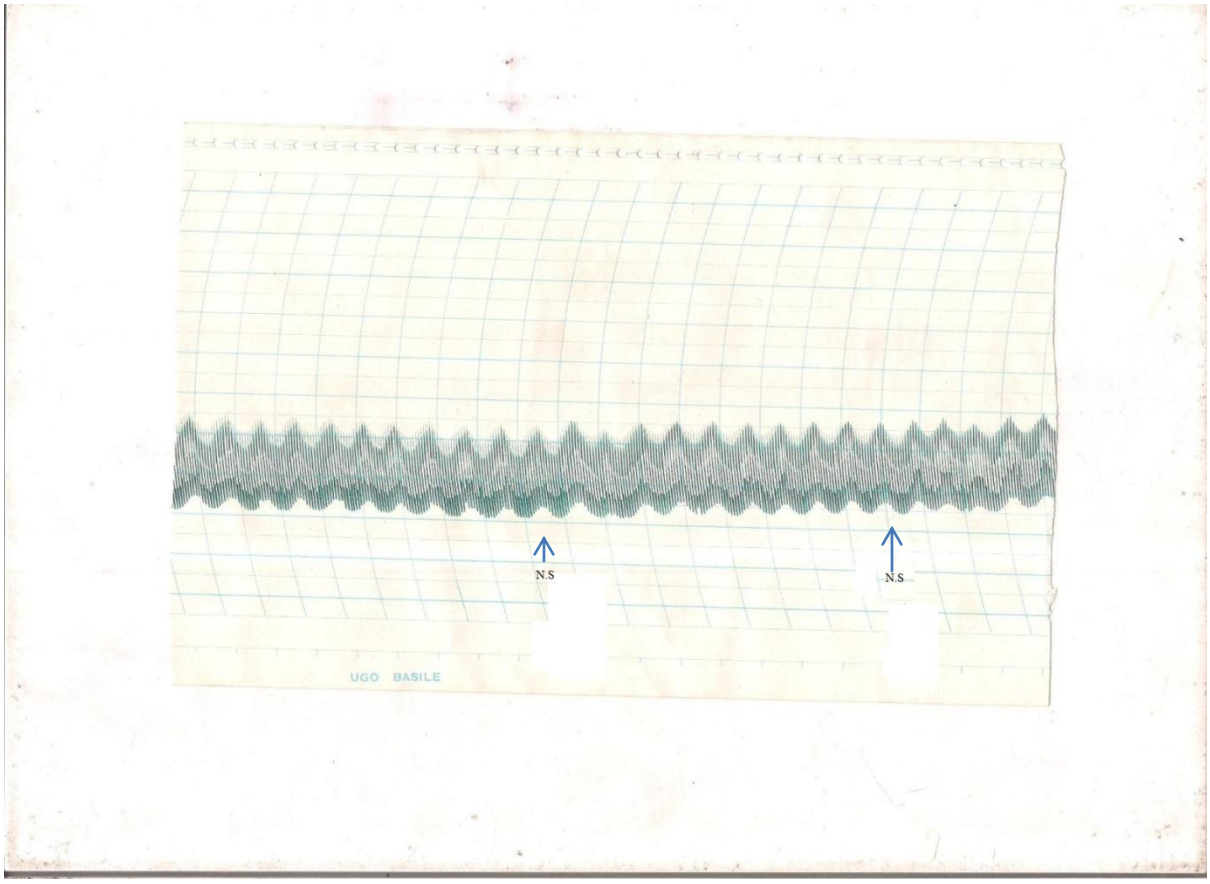
The Effect of Adrenaline, Garlic + Ginger on Nomotensive Cat Blood Pressure



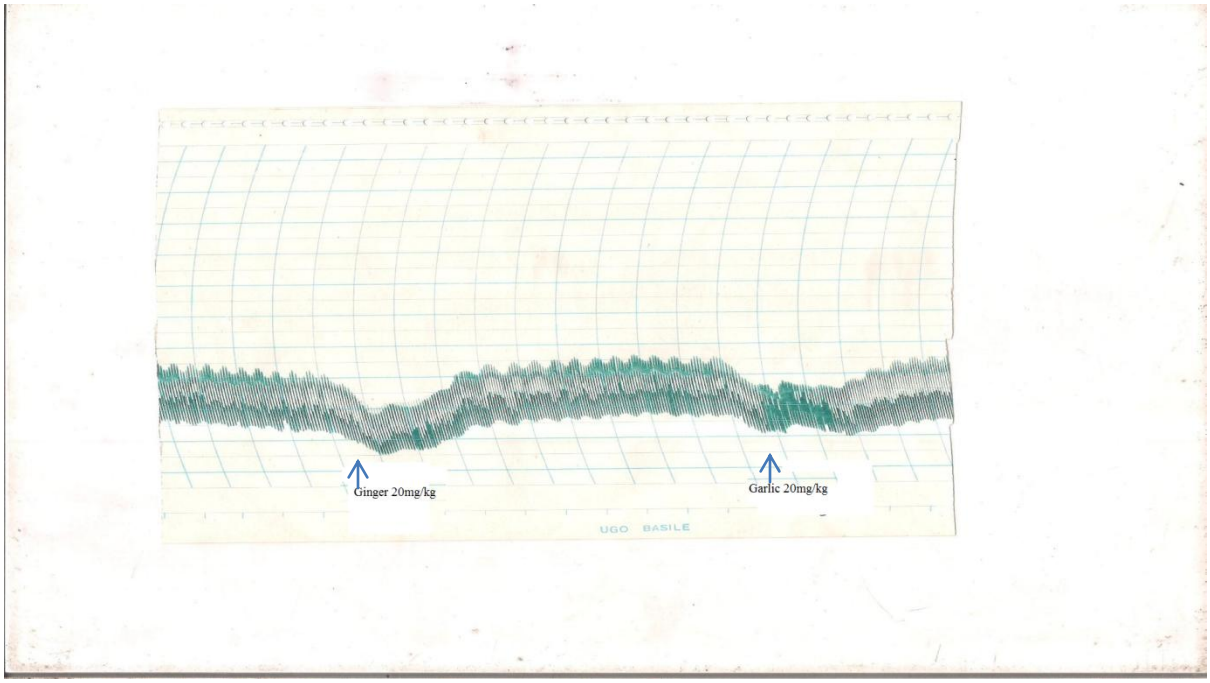
The Effect of Acetycholine on Nomotensive Cat Blood Pressure



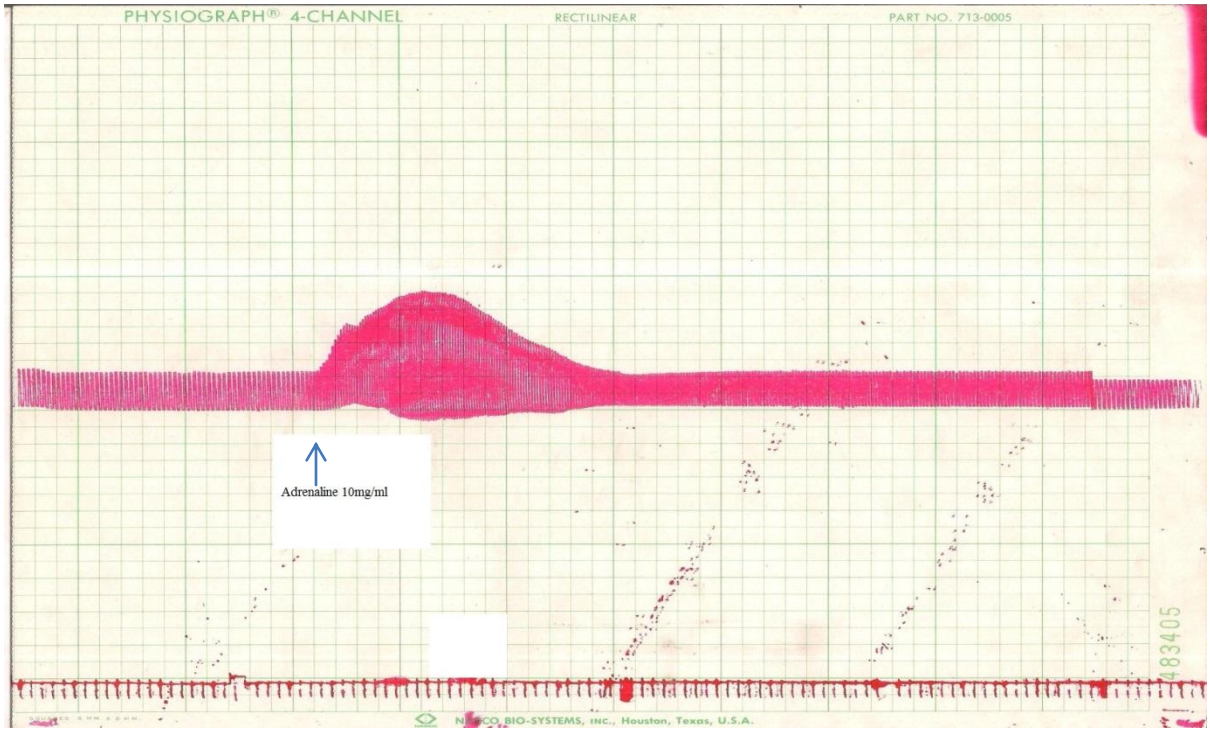
The Effect of Normal Saline



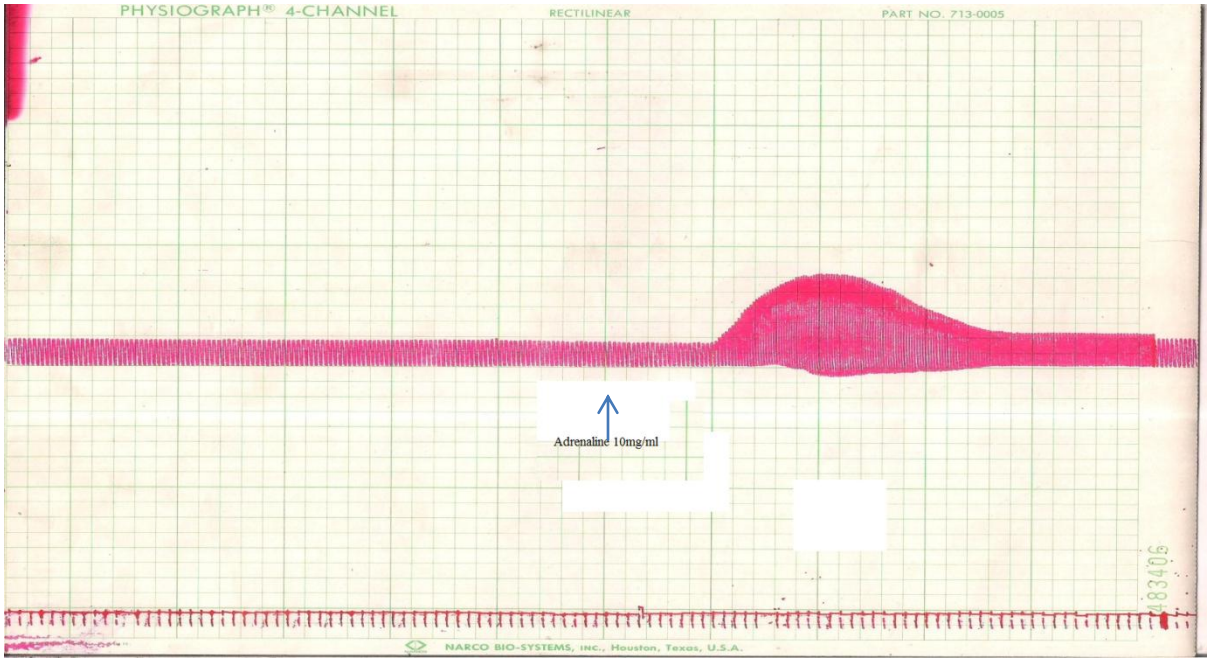
The Effect of Garlic and ginger on Nomotensive Cat Blood Pressure



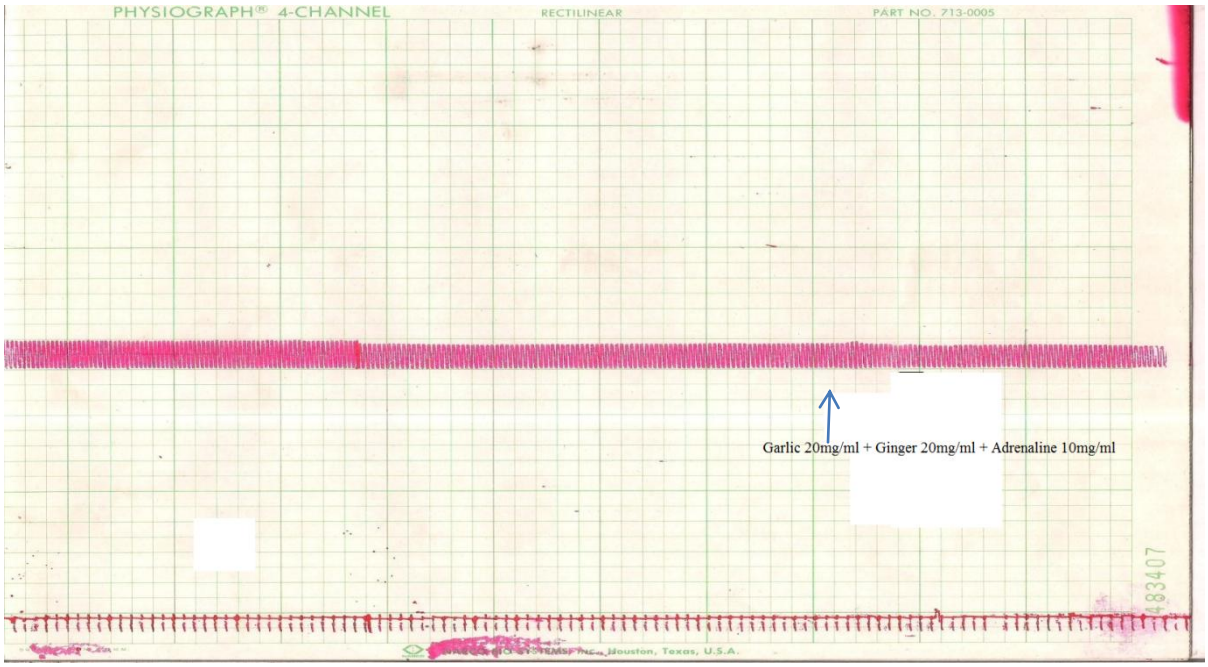
Effect of Adrenaline on Isolated Perfused Rabbit Heart



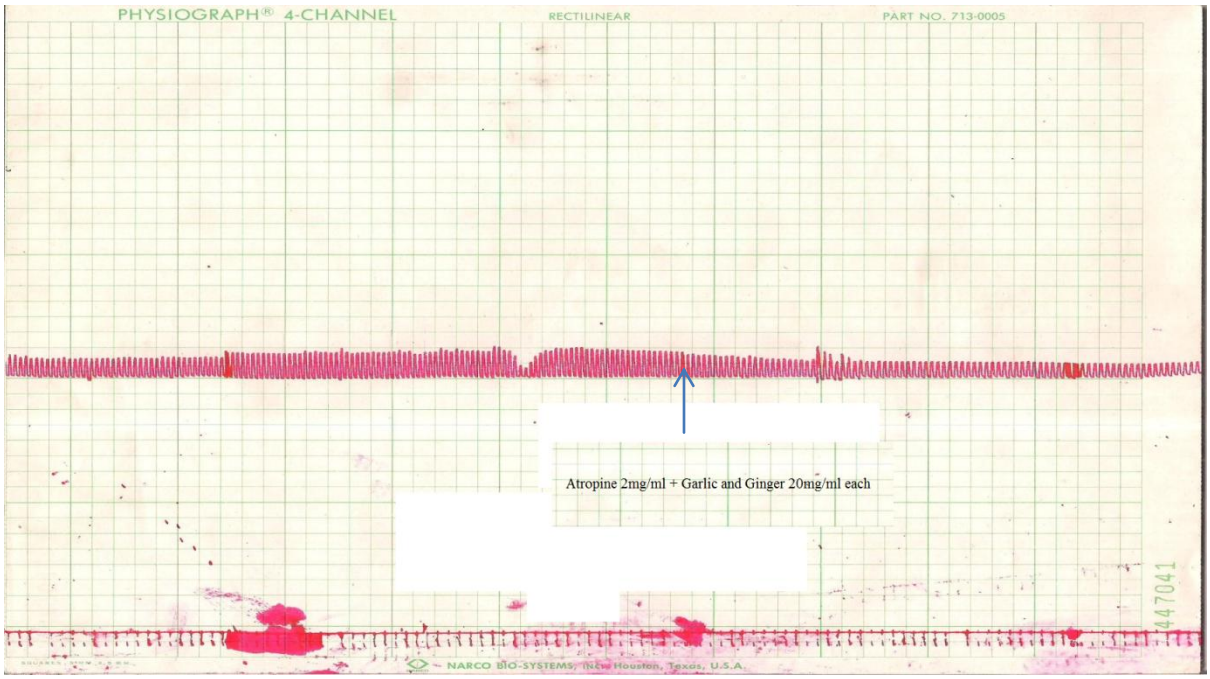
Effect of Adrenaline on Isolated Perfused Rabbit Heart



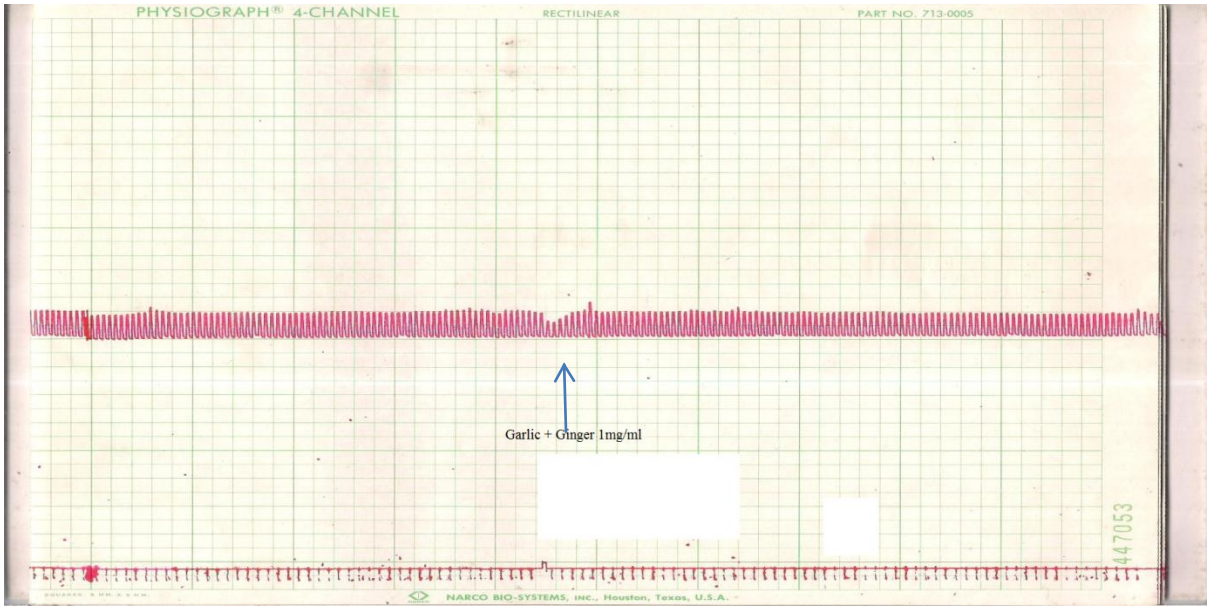
Effect of Garlic, Ginger and Adrenaline on Isolated Perfused Rabbit Heart



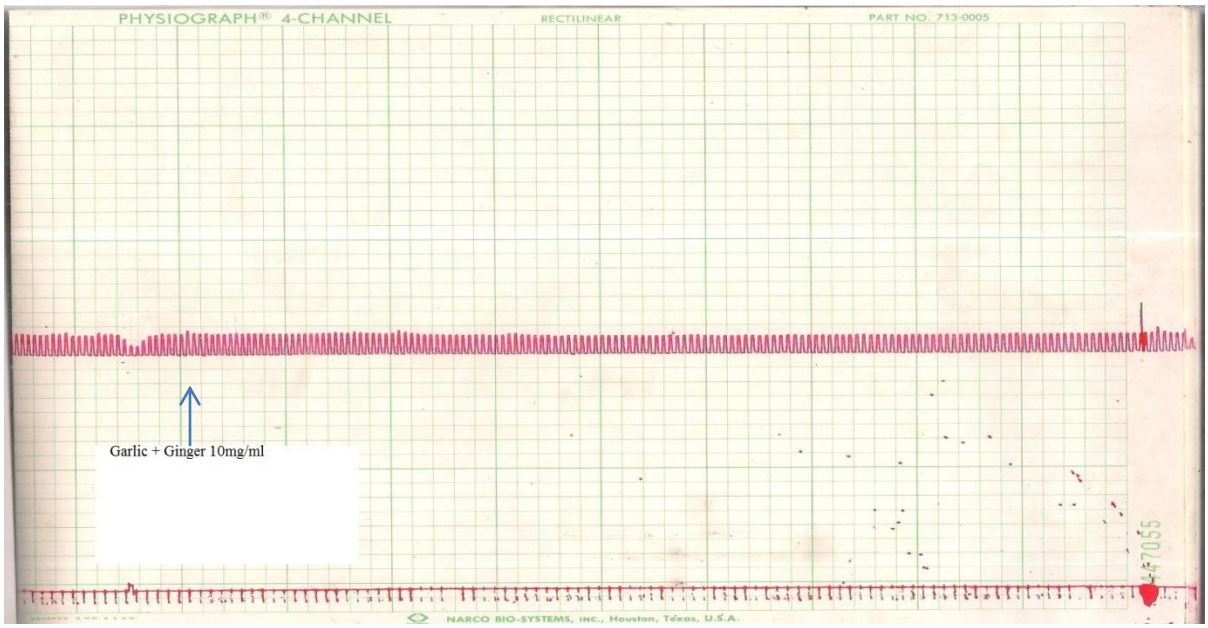
Effect of Atropine, Garlic and Ginger on Isolated Perfused Rabbit Heart



Effect of Extracts (Garlic and Ginger) on Isolated Perfused Rabbit Heart



Effect of Extracts (Garlic and Ginger) on Isolated Perfused Rabbit Heart



Effect of Extracts (Garlic and Ginger) on Isolated Perfused Rabbit Heart